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Modelling and Simulation Based Assessment in Sustainable Bioprocess Development

A thesis submitted to the University of Cape Town
in fulfilment of the requirements for the degree of
Master of Science in Engineering (Chemical Engineering)

by

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ABSTRACT

Modelling and simulation enhance our insight and understanding of chemical processes and aid in identifying bottlenecks and potential improvements. A simplified simulation package, providing a reasonable estimate of material and energy usage and process emissions is often valuable in very early stages of process development, when temporal and financial limitations do not allow for more detailed estimates. Environmental burdens are an increasing concern in industrial processes and various methodologies and tools have been developed for gathering and analysis of process information to enhance understanding of the process system and inform decision makers. The systems nature of these approaches is aimed at mitigation of environmental burdens through improved technologies, sustainable resource consumption and screening of process alternatives. Ideally, the process design team should bring together these tools in early stages of development when design flexibility is greatest.

In the present study, such a simplified approach to bioprocess design is demonstrated using a case study for the large-scale production of citric acid. A generic flow sheet simulation tool was used for the first estimation of material and energy balance calculations and capital and operating cost estimation. The results of the case study were compared to data presented in the literature on the production of citric acid using maize starch as the main raw material. The material and energy inventory was used as the input for a ‗cradle-to-gate‘ Life Cycle Assessment (LCA) of the production process. The LCA was compared to an LCA generated from literature-based inventory data. Further, the case study compared the production of citric acid from alternative substrates beet molasses and maize starch as the main raw material input. Capital and operating costs were determined by variant-based cost estimation using order-of-magnitude estimates. The overall task aimed to combine various tools for sustainable bioprocess assessment in early stages of process development

The results from the generic flowsheet simulation for the production of citric acid from starch were in good agreement with the literature case study. Environmental impacts were mostly common for both starch and molasses processing routes, largely as a result of the large electricity and steam requirements and the production of maize starch and beet molasses.

Capital and operating costs were estimated for the production of citric acid. The total capital investment was $175 million and $188 million for the starch and molasses based process respectively. The cost estimate for the starch based process was in agreement with the literature case study. The economic assessment gave meaningful insight into the cost of the producing citric acid. Further, by comparing costs of the two processing routes a basis for decision making was established. Substitution of mixing vessels and raw materials for an ultrafiltration membrane used in the starch process demonstrated how these decisions may be implemented. The high capital cost for stirred tank reactors provided incentive to investigate alternative bioreactor technology or already depreciated equipment.

A generic model for generating first-estimate inventory data provided a basis for environmental and economic assessments. The time period for conducting the assessment could be significantly reduced and a wider range of alternatives considered. Using a first-approximation process simulation as the basis for environmental and economic assessments, a valuable contribution can be made to sustainable bioprocess development and optimisation.

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LIST OF ACRONYMS

ABP	Aspen Batch Plus
AC	Activated Carbon
BOD	Biochemical Oxygen Demand
CeBER	Centre for Bioprocess Engineering Research
CFD	Computational Fluid Dynamics
CIP	Cleaning In Place
CML	Institute of Environmental Sciences
CMS	Condensed Molasses Solubles
COD	Chemical Oxygen Demand
CS	Carbon Steel
CV	Coefficient of Variation
DFC	Direct Fixed Capital
DOT	Dissolved Oxygen Tension
EF	Environmental Factors
EI	Environmental Indices
EIA	Environmental Impact Assessment
FT	Filtration Unit
GEI	General Effect Index
GWP	Global Warming Potential
ICI	Impact Category Indices
IE	Ion Exchange
IG	Impact Groups
IRR	Internal Rate of Return
ISO	International Organization for Standardization
LCA	Life Cycle Assessment
LCC	Life Cycle Costing

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LCI	Life Cycle Inventory
LCI	Life Cycle Impact Assessment
LCM	Life Cycle Management
MI	Mass Indices
MOC	Material of Construction
MS	Microsoft
NPV	Net Present Value
ODP	Ozone Layer Depletion
PBR	Packed Bed Reactor
PDC	Direct Plant Costs
PEC	Purchased Equipment Cost
PIC	Plant Indirect Costs
ROI	Return on Investment
RX	Reactor
SS316	Stainless Steel
SAIC	Scientific Applications International Corporation
SD	Standard Deviation
SETAC	Society of Environmental Toxicology and Chemistry
SS	Suspended Solids
STR	Stirred Tank Reactor
TCA	Tricarboxylic Acid Cycle
TPC	Total Plant Cost
UASB	Upflow Anaerobic Sludge Blanket
UCT	University of Cape Town
UCTE	Union for the Coordination of Transmission of Electricity
UV	Ultraviolet
VIS	Visual Interactive Simulation
VLE	Vapour–Liquid Equilibrium

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NOMENCLATURE

A_r	aeration rate (vvm)
BOD	biochemical oxygen demand (kg)
COD	chemical oxygen demand (kg)
c_s	substrate concentration (g/L)
$c_{s,in}$	initial substrate concentration (g/L)
c_x	biomass concentration (g/L)
$c_{x,in}$	initial biomass concentration (g/L)
$c_{x,final}$	final biomass concentration (g/L)
DO_2	dissolved oxygen (%)
CFC^{-11} eq	chlorofluorocarbon equivalent (kg)
C_2H_4 eq	ethylene equivalent (kg)
CO_2 eq	carbon dioxide equivalent (kg)
1,4-DB eq	1,4-dichlorobenzene equivalent (kg)
Sb eq	antimony equivalent (kg)
SO_2 eq	sulphur dioxide equivalent (kg)
PO_3^{-4} eq	phosphate equivalent (kg)
kg/ton P	kilogram per ton product
k_La	oxygen transfer coefficient (1/h)
K_s	limiting nutrient concentration (g/L)
kW/m^3	kilowatt per cubic metre
η	agitation efficiency (%)
OTR	oxygen transfer rate (mol/L h)
ppm	parts per million
P_r	reactor pressure (bar)
P_v	agitation power per unit volume (kW/m^3)
t	time
tpa	tons per annum

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T_a	ambient temperature (°C)
t_b	batch time (h)
T_c	cooling medium temperature (°C)
T_r	reactor temperature (°C)
μ	specific growth rate (1/h)
μ_{max}	maximum specific growth rate (1/h)
v/v	volume per volume (m^3/m^3)
vvm	volume per volume per minute (m^3/m^3 min)
$Y_{p/s}$	product on substrate yield coefficient (g/g)
$Y_{x/o}$	biomass on oxygen yield coefficient (g/g)
$Y_{x/s}$	biomass on substrate yield coefficient (g/g)

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CHAPTER 1

INTRODUCTION

1.1 Sustainability in Bioprocess Development

1.1.1 Assumptions Surrounding Bioprocess Sustainability

The biomanufacturing industry is capable of generating enormous value from the vast array of products and services produced. Bioprocess designs are increasingly adopted over traditional chemical production systems due to advocated benefits of biological products, renewable feedstocks, reduced emissions and favourable operating conditions. Further, economic benefits realised from processes relying on renewable feedstocks, reduced costs for emission mitigation and increased demand for ―natural" products, are seemingly well justified (Herman *et al.*, 2007; Herman & Patel, 2007; Lynd 2008). The development of new processes within the bioprocessing industry are thus automatically assumed to be more beneficial in terms of environmental impact and economic performance in comparison to traditional chemical systems (Sheehan *et al.,* 2004; Botha & von Blottnitz, 2006); this is often not the case (Anex, 2003; Geigrich, 2003). There is a need to assess the performance of these processing routes, especially to improve the criteria with which to select new process designs or technology alternatives.

The remainder of this chapter provides a context for the thesis and introduces the literature and application of systematic performance assessment. The chapter provides a brief overview of the challenges facing the biomanufacturing industry, specifically with respect to the integration of quantified process assessment in the initial design phases. The chapter also introduces current methodologies, tools and approaches for process performance assessment and highlights where improvement is required.

1.1.2 Challenges in Sustainable Design and Development

While many businesses and manufacturers have adopted sustainability goals, such as reduced greenhouse gas emissions and lower energy consumption (Kurdikar *et al.*, 2001; Dornburg *et al.,* 2003; Roes *et al.,* 2007), the development of systems and tools for multi-criteria performance assessment and implementation are not as advanced (Batterham, 2004). Generally, the design and development of current industrial processes was not guided by systematic quantification of environmental, economic and social performance. The growing interest in biotechnology and the possibilities it offers, requires that increased focus be shifted towards providing tools to support process selection and improved development by considering trade-offs between environmental burdens and economic performance.

In an attempt to improve process performance, modifications to unit operations and retro-fitting with new technology can enable biomanufacturing facilities to improve energy efficiency, reduce net water consumption and reduce waste generation. This approach to improved process development can ensure a more environmentally benign process by reducing greenhouse gas emissions and natural resource consumption. It is however equally, if not more important, to design more environmentally and economically sustainable processes in very early stages of process development. This is increasingly motivated by considering that opportunities for design modifications for improving economic and environmental performance are mostly available in the beginning stages of process development (Cano-Ruiz & McRae, 1998; Stewart *et al.,* 2003). The additional cost for redesign to solve overlooked issues rises as the level of the development stage increases (Li & Kraslawski, 2003).

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Consideration of alternative process technologies in early-stages of process design and development requires analysis of trade-offs between multiple performance measures. These multi-criteria trade-offs typically effect the entire process life cycle and an integrated approach to design requires multiple, systematic support tools to ensure sufficient data is available for generating performance measures to compare alternatives. The development of these tools requires a multi-dimensional approach, incorporating knowledge from a number of fields, including, amongst others, systems thinking, engineering process design, environmental assessment and project finance. Benyahia (2000) has emphasised that without sufficient quality input data and a good chemical engineering background to judge results objectively and critically, results from assessment tools can be misleading. Chemical engineers and process design practitioners thus form an important part of developing and using such tools. The science of systems analysis, material and energy balances and process modelling form part of the fundamental competencies of chemical engineering practice. Although chemical engineering provides the theoretical framework and practice for developing these tools, there is a need for improved understanding, including:

1. An understanding of approaches to generating early-stage process material and energy data, forming the quantitative basis of process development or assessment;
2. An understanding of appropriate environmental and economic performance metrics and assessment methodologies and how material and energy balance data forms a basis for these assessments;
3. An understanding of the value and limitations of the performance assessments and metrics and how they inform improved process design and development.

1.1.3 Approaches to Quantified Sustainability Assessment

Numerous systematic methodologies and tools have been developed for the characterisation and quantification of the potential impacts of chemical processes and products on biological, physical and socio-economic environments. Some of the common methodologies include Ecological Footprinting (Holmberg *et al.,* 1999), Environmental Impact Assessment (EIA 1982; Wilson, 1998; Petts, 1999), Environmental Impact Indices (Baasel 1985; Stephan *et al.*,1994; Elliot *et al.*, 1996; Biwer & Heinzle, 2004), Ecoefficiency (WBCSD, 2000; Saling *et. al.*, 2002; Huppes & Ishikawa, 2005), Carbon Footprinting (IPIECA 2003; ISO 14064: 2006) and Life Cycle Assessment (ISO, 14040, 14044: 2006). Many studies available in the literature have demonstrated the application of these environmental assessment methodologies in quantifying environmental burdens, comparing process alternatives and directing conceptual process design and development. Studies are however often limited to considering a few impacts; mostly greenhouse gas emissions and energy consumption (Dornburg *et al.,* 2003; Patel, 2003; Roes *et al.,* 2007). The limitations are due primarily to a lack of reliable process material and energy data, which is typical at early stages of process design. It is thus critically important that methods for generating holistic process data be developed to ensure rigorous environmental performance assessment of process designs (Gasafi *et al.,* 2003, Biwer & Heinzle, 2004a; Harding, 2008).

Similarly to the environmental methodologies presented above, the integration of economic metrics into holistic process assessment has received considerable focus due to increased emphasis on sustainable process design. A number of studies have demonstrated cost and profitability analysis as a measure to compare economic sustainability of large-scale bioprocesses (Sonesson *et al.,* 1999; Gonzalez & Smith, 2003; Biwer *et al.*, 2004; Dornburg *et al.*, 2006; Roes *et al.*, 2007; Heinzle *et al.,* 2008; Kloepffer, 2008;). However, many of these studies are limited to only considering costs associated with resource and utility

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consumption and transport costs (Sonesson *et al.*, 1999, Roes *et al.*, 2007; Heinzle *et al.*, 2008). It is insufficient to only consider a few measures of economic performance. Concerns such as capital costs, financing requirements and equipment depreciation are critical in considering the long term financial sustainability of the design. In order to provide a basis for robust economic evaluation of process options both capital and operating costs should be considered.

The argument proposes that environmental impacts should be identified and mitigated as early as possible in process development to avoid unnecessary expenses and delays at later stages when re-design is typically more complex and difficult to implement. Similarly, process designs that are not economically viable should be substituted for more profitable process designs as early as possible in the design process. Considering the trade-offs between these two performance measures early in design process, the effectiveness of process screening is improved by reducing the demand on finite resources for generating detailed process data for multiple alternatives, while simultaneously contributing to the selection of more sustainable processes.

1.2 A Possible Solution

1.2.1 Early-Stage Simulation as a Basis for Quantified Assessment

In addressing the need for early-stage process assessment, a number of practitioners have focused on generating early-stage process data using various methods. Techniques such as ultra-scale-down approaches (Boychyn *et al.*, 2000; Boulding *et al.*, 2001; Neal *et al.*, 2002) and process simulation (Rouf *et al.*, 2001; Biwer *et al.*, 2004; Lim *et al.*, 2004; Chhatre *et al.*, 2007a; Harding, 2008) for early stage process design and evaluation have been developed. Commercially available, ‗off-the-shelf' simulation packages, have been commonly applied to simulate process flowsheets and generate detailed material and energy balance data (Rouf *et al.,* 2001; Varga *et al.,* 2001; Chang *et al*., 2002; Biwer *et al.,* 2004; Chhatre *et al.,* 2007b). Although commercially available software packages provide detailed information, they are at times complex (Shanklin *et al.,* 2000; Harding, 2008), difficult to use and time consuming to set up. Further, it is often required that the practitioner have extensive experience in detailed mathematical analysis and simulation of chemical process design, using the software. Application of such tools by practitioners with non-specialist knowledge of chemical engineering theory and practice (e.g. life science or environmental practitioners) results in extended lead times for learning and understanding. This has provided scope for development of simplified simulation tools for use in the very early stages of process design and development. Additionally, there is scope for the development of tools that allow non-specialist design and assessment practitioners to generate material and energy balance data. Simplified simulation tools should provide a reasonable estimate of material and energy usage and process emissions, which is valuable in early stages of process development, when temporal, data and financial limitations do not allow for a more detailed design estimate.

In work previously conducted in the Centre for Bioprocess Engineering Research (CeBER), within the UCT Department of Chemical Engineering (Harding, 2008), such a simplified generic flowsheet for bioprocesses simulation was developed. The spreadsheet model, deployed in Microsoft® Excel, is used to generate a first estimate material and energy balance for early decision making in bioprocess development. The material and energy balance data from the model provides a basis for inventory data for environmental assessments of specific bioprocesses. The tool was developed primarily in response to the need for simplified bioprocess modelling during conceptual design and development phases, when limited process data are available for environmental assessments (Harding, 2008).

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Harding (2008) demonstrated the tool by determining a sufficient data set to perform a rigorous assessment of bioprocesses from an environmental perspective. Since the simulation tool was developed to provide a basis for environmental assessment, there is scope for identifying appropriate methodologies for quantifying additional process performance measures (i.e. costing, profitability) using first-estimate simulation data as a basis. The assessments can enhance our understanding of the process, identify potential problems and highlight areas where improvements are needed. Decisions can be made on whether improvements should be implemented, process development should be stopped because it is not economically or environmentally viable, or development of the process concept into an industrial application can continue as intended. This provides the foundation and scope for the initiation of this thesis.

1.2.2 Quantification of Process Performance Measures

Current approaches to quantified assessment of process performance from an environmental or economic perspective are typically based on well-established methodologies and principles. Environmental and economic performance assessment can serve as a valuable decision supporting tool to improve sustainable process development. Previous studies have shown that early-stage process simulation and subsequent first estimate environmental and economic assessments are able to improve and guide sustainable process development (Rouf *et al.*, 2001; Biwer *et al.*, 2004; Mustafa, *et al.*, 2004; Chhatre *et al.*, 2007a; Harding, 2008). The numerous methodologies available and actively applied are expanded upon in the thesis.

As described above, there are numerous methods for the characterisation and quantification of environmental impacts from process emissions and resource consumption. Included, is the well-established and well accepted method of Life Cycle Assessment (LCA). Life Cycle Assessment is used to quantify and assess the environmental impacts of a product or service system. The main objective of the LCA is to select the best product and process, with the least effect on human health and the environment (SAIC, 2006). The results thereof can contribute to the integration of the various elements required for decision making in sustainable bioprocess development. In conjunction with reviewing literature for a number of the aforesaid methodologies for environmental assessment, the thesis aims to assess the suitability of LCA as a method for quantifying environmental performance of a bioprocess system, based on first-estimate simulation data. A more detailed description of LCA, associated limitations and reasons for its use are provided in the thesis.

Similar to systematic environmental assessment, a first-estimate economic analysis of process alternatives can give valuable insight into the economic viability of the conceptual process alternative. The economic viability of a process has often been the primary focus when considering design options within a commercial setting, but due to a shift of emphasis on other sustainability criteria it is increasingly important that trade-offs between these measures be considered. An evaluation of both environmental and economic performance in initial process development can improve decision making toward a more sustainable option. In early stage of process development order-of-magnitude and factorial estimation techniques are commonly employed. These methods are accurate typically to within 30-40% of the detailed design (Peters and Timmerhaus, 1991, Couper, 2003). The thesis presents methods to estimate economic metrics that are most appropriate in early stages of process development.

The case presented above for quantified environmental and economic assessment as a support tool for sustainable process development, using first-estimate simulation data, provides the necessary basis for the research. This thesis is focused on contributing to the development of a systematic method of providing first-estimate data for environmental and economic assessment as independent metrics. The overall result is an ability to take both the environmental and economic assessments and associated trade-offs into

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account in process design and screening. Using the review presented above as a context for this thesis, the main objectives, key questions and scope of the work are established.

1.3 Thesis Objectives and Key Questions

The overall objectives of this dissertation are to provide an approach to assess the environmental and economic performance of large-scale bioprocesses in early stages of process development as a basis for decision making. In the initial phase of the work (Part A), the objectives are focused on drawing key conclusions from relevant literature with regard to bioprocess development, simulation, and assessment. The suitability of the generic flowsheet model, developed by Harding (2008), is to be evaluated as a tool for early stage bioprocess assessment.

Owing to its early stage application, functioning on reduced input data, the model has to meet certain criteria to be of value as a bioprocess simulation tool. The model should be user-friendly and give the user the ability to generate early-stage input data for an environmental and economic performance assessment of a large-scale bioprocess. Following from the work of Harding (2008), the objectives aim to improve the user-interface of the model and extend the flowsheet to provide the necessary data and calculation procedures for the economic assessment of the process as a first estimate.

In the second phase of the project (Part B), the overall task is aimed at demonstrating the application of the generic flowsheet. The flowsheet is to be used to provide input data for an environmental and economic performance assessment of a bioprocess design. The performance assessments should provide a basis for decision making during preliminary stages of bioprocess development. A case study aims to compare the results of the assessments against results obtained from a commercially available simulation package. Finally, the thesis aims to evaluate whether the assessment results obtained from the first-estimate inventory data provide a suitable basis for environmental and economic assessment of process alternatives.

A literature base is established, within which to position the objectives of the study, using the following questions:

1. What is the current state of the art with regards to sustainability assessment and its requisite process simulation for bioprocess development?
2. What elements of sustainability are required to form a basis for early-stage process assessment?

Using the literature, certain key questions are addressed in terms of a specific case study:

1. Are the results from a first-estimate generic flowsheet tool, sufficient to provide comparable mass and energy balance information to more detailed simulation tools as input data for process performance assessment?
2. Can an early-stage assessment tool, functioning on reduced input data, provide material and energy balance data to identify process ‗hot-spots' and compare process options?
3. Can Life Cycle Assessment (LCA) and order-of-magnitude cost and profitability performance measures support decision making when only first-estimate material and energy data are available? If so, what are the benefits and limitations of these measures?

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1.4 Thesis Structure

The thesis is structured as two parts. In *Part 1 (Chapters 2 to 5)* the literature for bioprocess development, process simulation and sustainability assessment is presented. *Chapter 5* presents the research focus, hypothesis and methodology. In *Part 2 (Chapters 6 to 10)* the theory is applied to a case study for large-scale citric acid production. *Chapter 10* concludes the thesis.

Chapter 2 presents an overview of bioprocess development and synthesis, including stages in process development, opportunities for redesign, and the role of process simulation.

Chapter 3 provides an overview of bioprocess modelling and simulation. An overview of the necessary components of a simulation model, model development and the choice of software is presented.

Chapter 4 discusses process sustainability within the context of early-stage bioprocess development. A review of literature case studies using various methods for environmental and economic process assessment is provided. The chapter concludes with a discussion of the integration of environmental and economic assessments as a measure of process sustainability.

Chapter 5 draws on key conclusions highlighted in the literature to develop the research hypothesis and methodology.

Introducing the case study in *Part 2*, *Chapter 6* presents a literature review of industrial citric acid production. The current state of the art is discussed including production techniques, raw materials, unit operations and new developments.

Chapter 7 presents the process model and simulation of citric acid using starch and molasses as alternative raw material inputs. The simulation models include input data for upstream, bioreaction and downstream unit operations.

Chapter 8 presents the environmental assessment of the production process using a cradle-to-gate life cycle assessment methodology. The assessment includes goals and scope, life cycle inventory data (LCI) and impact assessment.

Chapter 9 presents the economic analysis of the citric acid process options. The analysis includes the goal and scope and process design basis. A summary of major equipment sizing and estimates for capital and operating costs is presented. The capital and operating cost estimates are used as the basis for a process profitability assessment.

Chapter 10 is the final chapter of the dissertation and presents a summary and conclusions with regards to the overall project objectives as well as the citric acid case study.

1.5 Scope and Limitations

The thesis does not provide all information and tools necessary to engage in quantitative process development, but rather serves to highlight the most important aspects of sustainable bioprocess development, while concurrently providing a quantitative approach to early stage assessment in sustainable bioprocess development. The scope of the simulation model, environmental assessment and economic analysis is aligned with the overall objectives of the project and is outlined in the relevant sections of the project report.

1.6 Significance of this Thesis

Early-stage assessment of environmental and economic performance of bioprocesses has potential for substantial improvement of process design, but is often difficult to achieve owing to limited availability of

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input data. This thesis is positioned to contribute to the latter through the development of a generic simulation tool which provides early-stage input data for environmental and economic performance assessment.

The thesis contributes to the research efforts of the Centre for Bioprocess Engineering Research (CeBER) within the Department of Chemical Engineering, University of Cape Town. Previous research within CeBER initiated the development of a simplified process flowsheet model, implemented in MS-Excel, for first estimation of material and energy balance calculations. The overall objectives of this research project aim to contribute to the previous research work and development of the process flowsheet model. The objectives include improving understanding of early stage process simulation and subsequent environmental and economic assessment of large-scale bioprocesses. This understanding supports decision making in process design and selection of process alternatives. The generic flowsheet model has been extended to include unit sizing and costing as well as process profitability assessment. This improves the practitioners‘ ability to obtain process data as a basis for both environmental and economic assessment of large-scale bioprocesses in early stages of process design.

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PART 1

LITERATURE REVIEW AND RESEARCH METHODOLOGY

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CHAPTER 2

BIOPROCESS DESIGN AND DEVELOPMENT

2.1 Developing the Bioprocess Industry

It is expected that the development of the biomanufacturing industry can contribute to achieving clean industrial products and processes (OECD, 2001; Herman *et al.*, 2007; Herman & Patel, 2007; Lynd 2008). Bioprocesses are economically competitive across a number of industries (e.g. pharmaceuticals, bulk commodity products, fine chemicals) and have the potential to address several local and global challenges such greenhouse gas emissions, fossil fuel reserves and limited feedstock resources. Although many bioprocessing routes offer the potential to reduce the environmental impact of large-scale industry on natural systems (Sheehan *et al.,* 2004; Botha & von Blottnitz 2006), it is important that environmental performance be optimised and aligned with economic performance during process development. Some of the major challenges include relatively low product concentrations and productivities, the assumption that the environmental performance of biomanufacturing is superior compared to traditional chemical synthesis (Anex, 2003; Geigrich, 2003) and the competition of bio-based feedstock with food production. There is thus a strong need to assess the relative performance of new bioprocessing technologies to ensure the most environmentally benign and economically viable process options are selected. To support the discussion in this chapter, an extensive review of biocatalysts, products, unit operations and separation techniques commonly used in the biomanufacturing industry is provided in Appendix A.

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2.1.1 Stages in Process Development

The task of process development is to extrapolate a conceptual idea or discovery to an industrial scale; taking into consideration economic, safety and ecological boundary conditions (Vogel, 2005). The basis for design and development is formulated from research and development agendas and an associated plan that focuses effort on the most pertinent problems and most promising opportunities (Biwer *et al.,* 2006). Quite often there are a number of process options for producing the same product. Selecting from these options, the design engineer needs to consider technical, economic, and more recently, environmental performance of the operations involved. Once the most suitable process options have been identified the project is ready for the development steps. The nature and sequence of the design and costing steps in the development process, shown in Table 2.1, are often typical, but are by no means obligatory. It is necessary to establish a basic framework however, which can be applied during process design and development. The values for design accuracy quoted in the literature (Peters & Timmerhaus, 1991; Perry *et al.*, 1997; Coulson *et al.,* 1999; Couper, 2003) are typically associated with equipment cost estimates and process economics.

The degree of accuracy of the design improves as process development progresses from the conceptual design phase to the detailed plant design. The improved level of accuracy needs to be supported by more detailed process data as the development process progresses. The accuracy of the input data (i.e. unit operation parameters) and the process model, determine the degree to which the performance of process options can be compared. Therefore, consideration of the accuracy of the design phase estimate is important. In light of this, a key aspect of the thesis is to compare early-stage process data and estimation techniques to more detailed data and methods.

Table 2.1 Process design estimation at different design phases (Peters *et al.,* 2003)

Design Phase	Estimation	Requirements	Accuracy
Conceptual	Order-of-Magnitude Estimate	Process cost data for a similar process	40-45%
Project Planning	Study Estimate	Major material flows; Major equipment costing	20-30%
Preliminary Design	Preliminary Estimate	Preliminary material & energy balance; Process design and development costs	6-12%
Detailed Engineering	Detailed Estimate	Detailed material & energy balances; detailed P&ID drawings; detailed process cost	2-3%

2.1.1.1 Conceptual Design Phase

The initiative for design and development typically originates from a conceptual idea or discovery in a laboratory, research and development within an existing process, or the need for an alternative processing scheme to meet current and future market trends. If the initial analysis of the concept, idea, or discovery indicates that there may be possibilities for development into a worthwhile project, a preliminary investigation program is initiated. If the development process is to continue from this point, certain economic, technical, environmental and social criteria need to be fulfilled.

A review of relevant literature and patents is required once the initial process concept has been finalised. The review is used to establish whether a similar product is already produced or being developed and to identify potential competitors. If the product is already commercially produced, information regarding the production process should be gathered. A patent review is necessary to establish intellectual property ownership and protection. Some of the major difficulties during the conceptual development phase include:

1. Selection of an appropriate biocatalyst;
2. Selection of reactor configuration;
3. Medium and reaction conditions;
4. Configuration and scheduling of separation processes;

Once a suitable enzyme or microorganism is found to catalyse the formation of the desired product it has to be optimized to obtain an economically viable product yield and concentration. Nutrient medium and reaction conditions require adjustment to ensure optimal performance of the bioreaction system. Various compositions and concentrations of the medium, such as different carbon and nitrogen sources can be investigated. The impact of the bioreaction product composition on downstream separation and purification steps should be considered. As prescribed by Biwer *et al.*, (2006), decisions should be made while taking into account the entire production supply chain e.g. the availability of sufficient raw material at an acceptable quality and price. Reaction conditions (temperature, oxygen supply, pH) and configuration (batch, semi-batch, continuous) should be selected to provide the optimal environment for the biocatalyst and product yield. Suitable parameters have to be found for agitation, aeration rate and aeration medium (e.g. air, pure O_2, CO_2). Following optimisation of the bioreactor conditions, the appropriate separation and purification steps can be selected. The overall objective in selecting separation steps is the trade-off between unit operation cost and product recovery. The environmental impact of the

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selected units should also be considered. Once the potentialities of the process are well established and the necessary information assembled, the initial version of the process can be designed. Process versions should be considered in the initial design phase and analysed by the design team. The advantages and disadvantages of each variation should be considered and the most feasible design chosen. Deciding on the optimum version of the process requires engineering knowledge and experience, creativity and an appropriate set of decision making criteria.

2.1.1.2 Intermediate Process Development

Once the initial design phase has been completed and accepted, an industrial plant can be designed, on the basis of information gathered in the initial development phase. The industrial process design is usually scaled down and a pilot plant or miniplant constructed. The miniplant approach is generally preferred due to the high cost of pilot plants and long times required for construction (Vogel, 2005). Concomitant mathematical modelling of the industrial plant, integrated with the miniplant design, allows the design team to investigate individual units and obtain valuable data to be used in mathematical simulation of the process steps. The synergistic approach between the miniplant and mathematical modelling allows for scale up with a degree of confidence similar to that obtained from a pilot plant study. A pilot plant may be required if the scale up risk is too large, stages cannot be accurately modelled, novel technology is used, or representative product quantities are required (Palluzi, 1991).

2.1.1.3 Project Execution and Start-Up

Once the project study has been completed, process development is for the most part concluded. A positive decision by the project investors or board of directors to continue with the project on the basis of the information made available through process development initiates project execution. The detailed project documents are prepared as a basic skeleton for plant plans, contract writing and equipment ordering. Once the plant has been constructed it is the responsibility of the commissioning stage to transfer the mechanically complete plant into a state ready for start-up.

2.1.2 Opportunities in the Development Process

An integral part of improved design and development is the consideration that opportunities for re-design are mostly available in initial phases of process development (Cano-Ruiz & McRae, 1998; Li & Kraslawski, 2003; Stewart *et al.,* 2003). At successive stages in the process design the degree of complexity is increased to improve the accuracy of the design, until a detailed estimate is obtained. At this point a final design can be realised and the plant can be constructed. The additional costs for redesign to solve overlooked issues rise as the level of the development stage increases. Heinzle & Hungerbuhler (1997) have incorporated this understanding across the entire development process, shown in Figure 2.1. The freedom of development is most prevalent in the conceptual phases of project development. This freedom decreases rapidly as the detailed design and engineering phases are initiated. The ability to determine the actual economic and environmental performance of the process increases as development progresses and more detailed data is available. The knowledge and cost for redesign increases similarly however, as development progresses. The cost of preparing an estimate increases from about 0.1 percent of the total project cost for ±30 percent accuracy, to about 2 percent for a detailed estimate with an accuracy of ±5 percent (Coulson *et al.,* 1999). It is therefore important to the gain as much understanding of the actual future production process as early as possible, to avoid additional and unnecessary costs.

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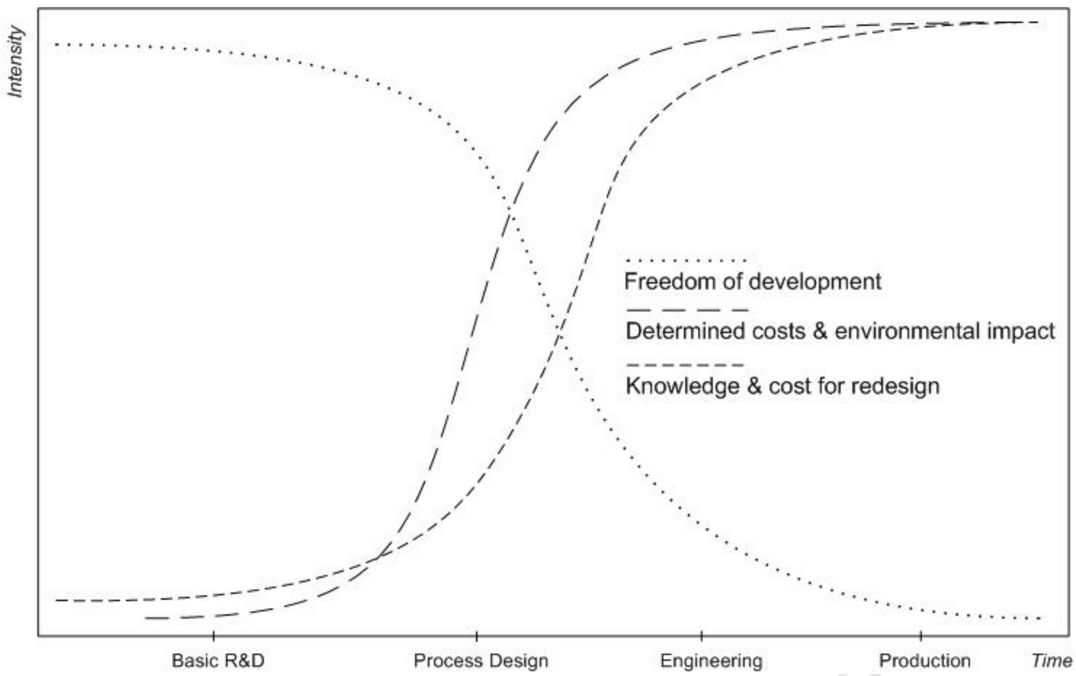

Figure 2.1 Knowledge and freedom of decision in process development (Heinzle & Hungerbuhler, 1997)

As prescribed by longstanding design literature (Peters & Timmerhaus, 1991; Perry *et al.*, 1997; Coulson *et al.,* 1999), criteria for project success mostly include the technical feasibility of the discovery, a clear definition of the product (purity, quality etc.) and the identification of a potential market for the product. As described by Coulson *et al.,* 1999: ―*Chemical plants are built to make a profit, and an estimate of the investment required and the cost of production are needed before the profitability of a project can be assessed*.” Similarly, Peters & Timmerhaus (1991) prescribes that in selecting from possible process alternatives: ―*a general survey of the possibilities for a successful process is made considering the physical and chemical operations involved as well as the economic aspects."* These criteria are however limited to technical and economic feasibility. In light of current local and global environmental challenges, there is increased focus on including environmentally conscious decision criteria for process selection, especially in early-stages of process development (Cano-Ruiz and McRae, 1998; Stewart *et al.*, 2003; Biwer *et al.,* 2006; Harding, 2008). Stewart *et al.* (2003) support the inclusion of quantified environmental assessment in early design phases by reiterating the understanding that opportunities for meaningful improvements in process performance are rapidly reduced as the design engineer progresses through the design phases. This makes a strong case for considering environmental performance evaluation with technical and economic criteria at early phases of development.

In addition to the challenges associated with increased complexity as development progresses, the bioprocess design engineer is typically faced with multiple choices throughout the process design phases. This includes selection of appropriate raw materials, comparison of competing bioreaction technologies and selection and sequencing of separation processes. Decisions have to be based on estimates of costs and potentials for process ‗hot-spots‘ in the process schedule. The accuracy of an estimate depends on the amount of design detail available: the accuracy of the cost data available; and the time spent on preparing the estimate. In early stages of the project only an approximate estimate is required, and justified, by the amount of information available. Insufficient data and uncertainty in available data with which to compare alternatives is often a primary concern (Heinzle & Hungerbuhler, 1997).

As demonstrated by numerous studies in the literature (Biwer *et al.*, 2004; Chhatre *et al.*, 2007a; Harding *et al.*, 2007a; Harding *et al.*, 2007b), modelling and systematic assessment of the process design help improve understanding during process development. This provides motivation for the use of modelling to provide additional data with which to assess both process economics and environmental performance during development phases.

2.2 The Role of Computer Modelling in Process Development

In the development of a chemical or biological process, modelling and simulation can be used to enhance our understanding and compare process alternatives (Steffens *et al.,* 1999; Rouf *et al.,* 2001; Harding *et al.,* 2007b), accelerate process development (Zhou *et al.*, 1997) and highlight areas for improvement (Groep *et al.,* 1997). A detailed discussion of process modelling and simulation in the bioprocessing industry is provided in Chapter 3.

Process modelling and simulation thus act as quantitative tools in early stages of development to provide information, used in conjunction with experimental data, as a basis for decision making. As described in the previous section, one of the primary concerns in process development is data quality, or the lack thereof. Uncertainty in data results in an incomplete representation of the expected large-scale process. Process modelling can fill this gap and provide the design engineer with a more robust basis for decision making. Acquisition of this data often requires that discrete unit operation models be combined into an integrated flowsheet model. A study by Zhou *et al.* (1997) demonstrated this integrated approach using computer simulation to predict the performance of downstream protein recovery and purification. The study demonstrated that optimisation of process performance cannot be achieved by simply optimising individual unit operations in isolation. An integrated approach to design must be adopted in order to achieve acceptable designs. Biwer *et al.* (2006) provides a representation (Figure 2.2) of the manner in which process modelling and simulation can form part of integrated design and assessment. As shown in Figure 2.2, process models should be developed alongside the design process. The simulation results are used to evaluate the process design and guide further development toward the most sustainable process option. The methods used for sustainability assessment are presented in Chapter 4. As demonstrated in the study by Zhou *et al.* (1997), the goal is not to optimise single unit operations, such as the bioreaction step, but rather, identify the most economically and environmentally feasible process.

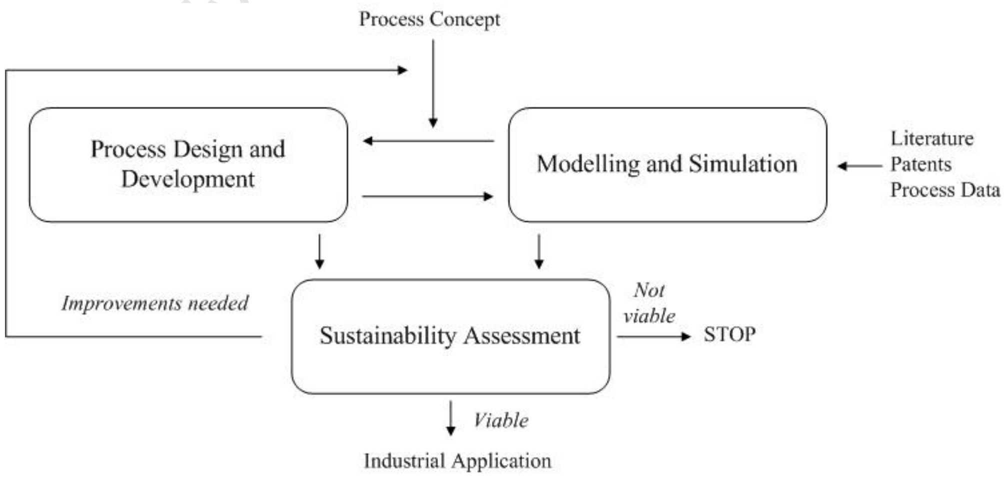

Figure 2.2 Integrated process development (Biwer *et al.,* 2006)

2.3 Conclusions

The modelling and assessment process is iterative and requires interdisciplinary knowledge. The integrated approach is able to highlight potential problems and opportunities earlier in the development process, avoiding costs for additional research and re-design at later stages. Although there is intrinsic uncertainty with this approach, the uncertainty should be quantified to allow for clarification of the performance of the design alternatives. The approaches adopted to quantify uncertainty and applying a value judgement are explored in Chapter 3.

As stated in the introductory chapter, the development of modelling and simulation tools requires a multi-dimensional approach, incorporating knowledge from a number of fields, including, amongst others, systems thinking, engineering process design, environmental assessment and project finance. Chapter 2 has identified various stages in process development and how modelling and simulation may be used to fill certain data gaps, especially at early stages of process design, when lack of important data is typical. This provides the design engineer with clearer ‗picture' of the overall design process on which to base decision making. Chapter 3 of the thesis explores the explicit requirements of the simulation model and the way by which the model can be implemented in the design process. Specifically, the chapter explores the application of modeling and simulation in the bioprocess industry.

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CHAPTER 3

MODELLING AND SIMULATION OF BIOPROCESSES

3.1 Bioprocess Modelling Requirements

Bioprocesses, like all chemical processes, involve a number of material and energy inputs, specific unit operations and ancillary requirements (e.g. labour; utilities; consumables). Additionally, bioprocesses are typically divided into three main sections, namely; upstream operations, bioreaction and downstream operations. All three sections and associated operations need to be analysed and implemented into the flowsheet model. It is important to specifically define the model boundaries and intended application, so that the necessary components to meet the intended model outcomes are included. The specific simulation method used depends on the purpose of the simulation. The first task in developing a flowsheet model is to gather the necessary process information. This is implemented into a specific model structure designed to suit the process scheme.

3.1.1 Model Development

3.1.1.1 Goal Definition and Model Boundaries

In the goal definition stage, the purpose of the study is stated, usually in response to certain questions. The systems to be evaluated are determined and various capacity, production and time parameters are set. A realistic production capacity should be defined. This can be based on market demand, technical feasibility, or a strategic decision by the business. A definition of the product (e.g. nature, purity etc.) is essential, since the specific unit operations are determined by the product characteristics. Streamlining of the system definition can only be done once the system has been completely examined.

3.1.1.2 Data Acquisition

Sufficient data for raw materials use, energy use, product outputs and environmental releases must be quantified for each of the specific processes within the defined system. In the case that data is not available for the specific purpose, values can be estimated from similar operations and extrapolated for the intended use. It is critical that data quality be defined (Harding *et al.,* 2007b). As an important focus of the thesis, quality of input and output data is explored in detail in subsequent chapters. Data not available from literature or experimental work can be sourced from industry if made available. Typical data required for process development includes:

1. Material and physicochemical data;
2. Ecological and toxicological data;
3. Raw materials, intermediates and end products specification and cost data;
4. Energy and equipment specification and cost data;

3.1.1.3 Unit Operations

Once the necessary process data has been collected, the individual unit operations can be defined. The process is typically expanded from the bioreactor outwards. The material and energy inputs for the

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reaction are firstly determined. The reaction parameters are defined including residence time, operating volume, ratio of products to co-products, and yields. In each case, data validity should be specified, assumptions defined and the degree of accuracy estimated. Once the bioreaction operation has been adequately defined, the remaining unit operations and associated parameters can be sequentially implemented. Sequential flowsheet development is typically iterative in nature and unit designs and material and energy inputs often need to be updated (Shanklin *et al*., 2000).

3.1.2 Choice of Software and Model Implementation

Process simulation software applies a series of algorithms, where inputs and detailed transformation functions are combined and mathematically modelled for each unit process. The model simulates the activity in each operation and the movement of materials, parts, etc. across the operation steps. Fundamental physical and chemical properties, standardised unit operation modules and other primary data are required in the database of the models.

3.1.2.1 Mathematical and Spreadsheet Models

Process performance is often dependent on a few key unit operations. It is valuable to investigate the design and performance characteristics of these units by mathematical modelling, to gain additional insight into potential process improvement. Software tools used typically include Microsoft® Excel, MATLAB (Mathworks; Natick, Massachusetts) and MathCAD (Mathsoft; Cambridge, Massachusetts) (Gosling, 2005). Although the software packages are generally used for individual unit modelling, it is possible to model complete flowsheets. Microsoft® Excel is commonly applied in process design and modeling and numerous studies have used demonstrated spread-sheet based models for process simulation and assessment, especially in early stage process development. A number of these studies are reviewed in Section 3.2. Although flowsheet modelling using spreadsheet based methods is extensively applied, models consisting of more than a few unit operations, often results in complex calculation procedures. Bearing this in mind, the flowsheet developer should design the user interface to be easily navigated and understood by a first-time user.

3.1.2.2 Detailed Simulation Packages

A number of more-targeted simulation packages have been developed and are commercially available. The packages contain a library of common unit operation models. Some support the development of customized models. In setting up a process simulation, icons representing the unit operations are placed in a process flowsheet window. The parameters of the individual units are specified and the flowsheet is completed by specifying the input streams and connecting the units. The final technical model results in the material and energy balances of the process. The modelling process is usually highly iterative and many ‗runs' are usually required for the setup of a realistic model. It is also suggested that the results are regularly checked with order-of-magnitude calculations (Biwer *et al.*, 2006). Examples of some of these simulation programs include Aspen Plus (Aspen Technology, Inc., Massachusetts, USA) and SuperPro Designer™ (Intelligen, Inc., New Jersey, USA). A summary of typical applications of commercial software packages is given in Table 3.1. Although the mathematical software applications (e.g. Microsoft® Excel, MATLAB) can be specified to provide material and energy balance data, these packages are not purpose-built for process flowsheeting, as in the case of Aspen (Batch) Plus and SuperPro Designer™. Although detailed flowsheeting packages are powerful tools, able to provide unique solutions, Gosling (2005) suggests that detailed simulation should only be used to tackle complex problems where solutions are not obvious and where the investment is justifiable. This leaves scope for ‗non-specialist' mathematical software where a less rigorous design or solution is acceptable or desirable.

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Table 3.1 Commercial software for process modelling and simulation (Adapted from Gosling, 2005)

	MS Excel	MATLAB MatchCAD	Aspen Batch (Plus)	SuperPro
Material Balance	✓	✓	✓	✓
Material dn Energy Balance	✓	✓	✓	✓
Detailed Decription	✓	✓	✓	✓
Rate-Based Models		✓	✓	✓
CFD & Rate Based Models			✓	✓

A study by Shanklin *et al.* (2000), reviewed the capability of ‗off-the-shelf' bioprocess simulation software to integrate all aspects of an industrial biotechnology process, from unit operation modelling, to managing equipment and expenses. At the time of the investigation, only Aspen Batch Plus v1.2 (ABP) and SuperPro Designer^{TM} v3.0 (ISP) were able to provide a complete, integrated-process simulation. Both simulation packages were well suited to perform basic material and energy balances, answer scheduling problems, explore equipment/facility changes and perform economic analyses. It was found that the software applications lacked rigorous, predictive unit-operations models however, limiting their ability to predict unit operation scale-up or optimize operating conditions within 5 percent accuracy of the actual process. The study by Shanklin *et al.* (2000) shows that predicting accurate scale-up and yield of the actual production process is difficult to achieve without reliable large-scale process data. The majority of the unit-operation models used yield data from pilot-scale studies and were not a function of the equipment and operating parameters changed to reflect the operating scale. The results were nonetheless, mostly within 10% of the actual process data. This is deemed acceptable in early stages (or even intermediate) stages of process development however, where estimations are typically within 20-40% of actual values. It may be concluded that application of detailed simulation in early stage process development provides a valuable insight into scaled-up process performance.

Although the simulation packages, reviewed above, are able to provide detailed information regarding the process material and energy balances, they are typically complex, difficult to use and time consuming to set up. The packages are also relatively expensive, with prices ranging from $5,000-$10,000 per copy, per year (Shanklin *et al.,* 2000). It is thus important to establish the required outcomes of the process simulation, the level of accuracy required and the intended use and user before deciding on these packages. A simplified flowsheet tool, requiring less time and effort for specification, which provides results within 35-45% of actual process data, is likely to be similarly (or more) useful in early design phases.

3.1.3 Simulation Based Scenario Analysis

Scenario analysis is used to interrogate variations in the process flow scheme, especially in early process development, to compare potential routes to the same product. The most favourable process scheme can be chosen and more detailed process development implemented. There are many outcomes that may occur for given assumptions in a common system. Firstly, a process model should be constructed and the factors around which the scenario is built should be identified. Factors may include variations in availability and cost of feedstock materials, change in product selling price, or change in specific process emissions due to variations in technology or any combination of these. The second is to consider the number of scenarios for each factor. Although additional scenarios may be more realistic, it may become difficult to gather the necessary input data and differentiate between scenarios (Zhu & Jin, 2000). Finally, a probability should be attached to each scenario. The accuracy of the probability is most often dependent on the practitioner's knowledge of the industry. A typical approach to scenario analysis is the use of Monte Carlo Simulation.

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A number of case studies have demonstrated modelling and simulation based scenario analysis as a decision making methodology (Huijbregts *et al.,* 2003; Cooney *et al.,* 2004; Benedetti *et al.,* 2010). Cooney *et al.* (2004) demonstrated this by analysing Penicillin V production and the uncertainties propagated through the system that affected key economic and environmental outcomes. Bioreaction parameters such as yield and maintenance coefficients had a high impact on the environmental performance of the process. Production costs were mostly affected by downstream yield and raw material costs. The case study was useful in better understanding Penicillin V production and the impact of both technical and market variance. This allowed for identification of stochastic variables that were critical to process efficiency and provided potential starting points for process improvements. The study proved that robust scenario analysis based on calculation based modelling can be used as a general methodology for multi-parameter uncertainty analysis.

Scenario analysis is especially useful in early stages of process development for improved decision-making by allowing consideration potential outcomes and associated implications (Cooney *et al.,* 2004; Harding *et al.,* 2007a). This may assist focusing further development of processes that result in mitigation of adverse environmental effects and improved cost savings. It can be used to test the reliability of results produced by the model and identify parameters which require special attention. This can help guide further process development and optimise parameters that have the greatest influence on the process (e.g. energy requirements, product yields, return on investment etc.).

3.2 Simulation Case Studies in the Bioprocess Industry

Process simulation software has been traditionally applied in petroleum and chemical industries. Development of simulation software began in the 1950‘s, developed in-house for companies‘ custom applications (Biegler, 1989). It has since then been developed extensively and applied to various unit operations including reaction, distillation, adsorption and vapour–liquid equilibrium (VLE) models. Although the simulation of chemical processes is relatively advanced, the application of simulation and modelling to bioprocess operations is relatively underdeveloped (Potera, 1998; Lim, 2004). The development and application of large-scale bioprocess simulation, although not wide-spread, is becoming an increasingly important part of process development. Large-scale bioprocesses have been studied extensively in an attempt to better understand their applicability and usefulness; process simulation is able to form an integral part of the analysis.

The majority of the focus to date with respect to bioprocess design has been on simulating individual unit operations using methods such visual interactive simulation (VIS) and computational fluid dynamics (CFD) (Chau & Bell, 1994; Neal *et al.,* 2002). Although these approaches are valuable in optimising individual unit operations, an integrated holistic approach is more valuable in early stages of process design to select design options. As discussed in Chapter 2, opportunities for addressing design concern are mostly available in initial phases of process development. In combination with existing unit optimisation, an integrated approach serves as a tool with which to consider process alternatives and eliminate non-viable options as quickly as possible. The level of detail required however, is highly dependent on the intended outcome of the study and the stage of development at which the simulation model is applied.

3.2.1 Early-Stage Bioprocess Simulation

In response to the need for rapid process assessment and typical challenges facing the biomanufacturing industry such as rising costs, extended time periods to meet regulatory requirements, the importance of time-to-market, and strong market competition (Chhatre *et al.*, 2007a), techniques such as ultra-scale-down approaches (Boychyn *et al.*, 2000; Boulding *et al.*, 2001; Neal *et al.*, 2002) and process simulation

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(Rouf *et al.*, 2001; Biwer *et al.*, 2004; Lim *et al.*, 2004; Farid *et al.,* 2005; Chhatre *et al.*, 2007a; Harding, 2008;) for early stage process design and evaluation have been developed.

An ultra-scale-down approach presented by Neal *et al.* (2002) describes the development of a model for the recovery of a therapeutic antibody from an analysis of individual unit operations. The approach used both CFD analysis and millimeter quantities of process material to define the important physical properties of a full-scale flowsheet. The results of the study provided a basis for predicting the overall performance of the process, allowing engineers to assess process options rapidly. A similar investigation was presented by Boychyn *et al.* (2000) for the recovery and dewatering of protein precipitates. The studies both demonstrated the ability of early-stage process modelling to guide large-scale process development and optimisation. A study presented by Lim *et al.* (2004) highlights the need for decision-support tools in the biotechnology industry. A case study was used to demonstrate the application of such a tool to integrate manufacturing tasks, including resource management, mass balance analysis and in-process testing and costing. The methodology was developed as a hierarchical task-oriented system, linking key operational tasks. The case study demonstrated the ability of bioprocess simulations to serve as a tool for process management, resource utilisation, cost analysis, mass balance assessments, unit operation characterisation and ultimately early planning of process development. The approach presented by Lim *et al.* (2004) provides a basis for decision making in terms of management functions, but lacks explicit material and energy balance quantification, which is critical in providing an adequate basis for process assessments, demonstrated by a number of studies (Biwer *et al.*, 2004; Chhatre *et al.*, 2007a; Harding *et al.*, 2007a; Harding *et al.*, 2007b). As discussed by Chhatre *et al.* (2007a), bioprocess simulations often fail to integrate both rigorous material balance models and dynamic resource constraints such as staff and equipment availability. A simulation based on both rigorous material balance equations and dynamic process constraints is able to provide a more robust basis for process optimisation and consideration of process options. Using this approach as a conceptual framework, Chhatre *et al.* (2007a) developed a prototype software methodology, implemented in Microsoft® Excel, for screening out inferior process options based on technical performance. The methodology is comprised of a three-layer framework where the complexity of the model and decision criteria is increased from one layer to the next. Inferior process options are eliminated at each layer and the options showing the greatest potential are investigated in greater detail in the successive layer. The methodology was demonstrated using a case study for the separation of rattle-snake anti-venom antibodies in ovine serum from contaminating albumin. Process alternatives were modelled and various improvements were made to the process. The methodology serves as a valuable approach to evaluate processing options for any biotherapeutic product and provides a basis for decision-making and optimal flowsheet development. Importantly, the study demonstrated the suitability of spreadsheet software (Microsoft® Excel) for implementation of a process screening and optimisation methodology.

A similar case study presented by Chhatre *et al.* (2007b) for the manufacture of polyclonal F_{AB} for the treatment of rattle-snake envenomation attempted to use mathematical simulation to assess developmental and manufacturing metrics simultaneously. The impacts of various production scenarios were evaluated and the most desirable alternatives to the current operation identified. Although the approach allowed for rapid assessment of process alternatives, the process model was developed for the specific case study for which an existing manufacturing process was in operation. A study by Rouf *et al.* (2001) on the production of tissue plasminogen activator (t-PA) showed that simulation of large scale bioprocesses can help to quantify the anticipated gain by considering process alternatives. The study made use Bioprocess Simulator™ and SuperPro Designer® to quantify the material and energy balances and perform an economic evaluation. A varying degree of data was required by the respective models and although most of the data was available from literature and built-in correlations, experimental data was required.

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Deliberation that detailed simulation packages require significant time and effort to provide a basis for comparison of flowsheets, has prompted development of simplified computer based tools for process assessment and optimisation. These tools are developed primarily to act as simplified support tools in early stages of process development (Elliot *et al.,* 1996; Farid, 2005; Harding, 2008). Elliott *et al.* (1996) demonstrated the use of spreadsheet based assessment by developing an environmental impact index using MS-Visual Basic ™ that could be directly linked to any spreadsheet-based process model. The study demonstrated that a simple computer based tool was able to assess the overall relative environmental impact of a process design. Farid *et al.,* (2005) developed a decision-support tool, SimBioPharma, for assessing different manufacturing strategies for the production of biopharmaceuticals. The tool architecture was designed to combine interactive graphics, animation and dynamic simulation to create a more flexible environment for modeling than that found in conventional process software tools. The tool was applied to a case study comparing disposable components as opposed to stainless steel for clinical trial material preparation. Although the case study only considered process economics, it was shown that simplified simulation studies can help determine ranking of alternatives under different scenarios and hence provide key support to strategic decision-makers.

Although the case studies presented above are used to interrogate process options at early stages of development they are limited in approach and do not allow sufficiently for environmental and economic performance assessment for bioprocess screening. In an attempt to improve early-stage process simulation and subsequent environmental assessment, a simplified generic flowsheet for bioprocesses simulation, introduced in Chapter 1, was developed by Harding (2008). Development and application of the tool is core to this thesis and the subsequent section provides a description of the flowsheeting tool and its application.

3.2.2 Generic Flowsheet Model (Harding, 2008)

In work previously conducted in the Centre for Bioprocess Engineering Research (CeBER), within the UCT Department of Chemical Engineering (Harding, 2008), a generic flowsheet model for bioprocesses was developed. The spreadsheet model was developed to generate first-estimate material and energy balance inventory data for Life Cycle Assessment of bioprocesses. Harding (2008) initiated the development of the model under the premise that these data are often not easily obtained or not available at all, at early stages of process development.

The material and energy balance data from the model provides a basis for inventory data for Life Cycle Assessment of specific bioprocesses. The flowsheet was developed to meet three desirable features of an early-stage generic simulation tool:

1. The flowsheet should act as a first-estimate bioprocess simulation tool;
2. The tool should provide all relevant data for a comprehensive Life Cycle Assessment (LCA);
3. The flowsheet should require minimum input data to yield a first stage estimate which can be refined on availability of more comprehensive data;

3.2.2.1 Model Description

Harding (2008) developed the generic flowsheet model to generate first-estimate material and energy balance data of selected bioprocess designs. The flowsheet is implemented in Microsoft® Excel (MS-Excel) (MS-Office 2008). Development of model features and calculations procedures are based primarily on first principles and appropriate literature data. The model is designed as six sequential steps that define a typical large-scale bioprocess flowsheet. These six steps and the most important features of each step are shown in Table 3.2. A simplified process flow diagram, shown in Figure 3.1, demonstrates the sequential

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selection of optional unit operations in each step as the user defines the process. The tool allows for batch or continuous microbial growth with intra- or extracellular product formation. Material and energy requirements of the process are based on a specified amount of product. Sterilisation, inoculation, microbial growth and product formation are followed by solid liquid separation, cell disruption and further purification. Downstream processing is specified by selecting from a number of unit operations commonly found in bioprocesses. Downstream unit operations are limited to six concentration or purification steps, followed by a final formulation step. Recycle of process streams is not taken into account in downstream operations.

Although process parameters may be specified for individual unit operations, default values commonly encountered in bioprocesses, are provided for select units. Default values include yield coefficients, material compositions and densities, operating temperatures and pressures and recovery values. A detailed discussion of unit operation theory, calculation procedures and default values is provided by Harding (2008). Since the model is developed for assessment in early stages of process development it requires minimal inputs to obtain reasonable estimates for material and energy balance data.

Table 3.2 Input requirements and simulation outputs from generic flowsheet (Harding, 2008)

Operation	**User Inputs**	**Simulation Outputs**
Overall process	Option for batch or continuous operation	Material and energy requirements
	Option for solid or liquid and intra- or extracellular product	Categorises waste materials
	Option for anaerobic or aerobic biomass growth	Product purity and recovery
	User defined quantity of product	
Bioreaction	Requires yield coefficients, growth rates, biomass concentrations	Predicts microbial growth
	Option to include maintenance calculations	Agitation and antifoam requirements
Sterilisation	Media sterilisation	Material and energy requirements
	Option for steaming out vessels, backing steam and space heating	
	Option for preheating during sterilisation i.e. heat integration	
Cooling	Include relevant cooling (bioreactor-, post bioreactor cooling)	Material and energy requirements
Downstream	Specify downstream units from built-in models	Material and energy requirements
	Addition of reacting and non-reacting chemicals at each step	
	Input parameters (e.g. temperature, %recovery, wash ratio)	
Waste treatment	Option for waste treatment	Chemical oxygen demand (COD)

3.2.2.2 Application of the Generic Flowsheet

The default model data is based primarily on unit operation data commonly encountered in bioprocesses. The default values give the user the ability to specify a process flowsheet when limited empirical or actual process data is available. Although previous studies have presented scaled-down approaches for simplified process simulation (Neal *et al.*, 2002; Boulding *et al.*, 2001; Boychyn *et al.*, 2000; Biwer *et al.*, 2004; Chhatre *et al.*, 2007a) the studies have relied heavily on available literature and actual process data. Similarly, the model developed by Harding (2008) was also developed using literature and actual process data. However, by combining process data for a number of bioprocess flowsheets and using a single default data set for similar process designs, the time and effort required for a reasonable material and energy balance estimate is greatly reduced. The input data however, is aggregated over a number of bioprocess designs and not process or geographically specific. This may result in significantly varied results to the actual process design, especially where the default data differs substantially from the location specific data e.g. the use of natural gas in a region where coal is the dominant fossil fuel for power generation. Further, the temporal advantage gained by using non process specific default data may

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also result in reduced accuracy of the material and energy balance results in comparison to the actual process. However, there is the option to use either use non-specific default data or design-specific literature data.

Harding (2008) applied the generic flowsheet in a number of case studies to generate first-estimate material and energy balance data for specific bioprocess designs. Case studies included penicillin V, cellulase and poly-β-hydroxyburate production. The results of the case study for the production of penicillin V were within 20% of literature results. The process model however, was not fully specified using default data and certain critical process data was provided from literature studies specific to penicillin V production (Nielson, 2001; van Nistelrooij *et al.*, 1998; Falbe & Regnitz, 1999; Lowe, 2001). The use of process specific literature data for certain critical parameters is discussed in the subsequent section. The material and energy balance results obtained from the flowsheet were used to complete a Life Cycle Assessment of the process. The results of the LCA were in good agreement with results of an LCA using inventory data generated using SuperPro Designer^{TM} (Biwer *et al.*, 2006). The case studies investigating the production of cellulase and the production of poly-β-hydroxyburate were completed in a similar manner to the penicillin study. The results of case study investigating the production cellulase were mostly within 33% of literature results obtained predominantly from the detailed cellulase production models of Biwer *et al.* (2006) and Zhuang *et al.* (2007). A number of differences are evident in comparing the Harding (2008) process model to the literature models. The biomass reaction model of the generic flowsheet included a more detailed approach to nutrient utilisation for biomass formation. The literature study assumed yeast extract and urea do not react in the biomass reaction step, whereas this was included in the generic model. Cleaning-in-place water, included by Zhuang *et al.* (2007), was not included in the generic flowsheet development and thus not accounted for in the cellulase model by Harding (2008).

Comparing the Harding (2008) for poly-β-hydroxyburate to the detailed process flowsheet, presented by Harrison (1990), approximately 50% of the material balance values of the Harding (2008) model were within 12% of literature values. Significant deviations from the literature values were evident however for water consumption (50% lower), electricity requirements (30% higher), and natural gas (100% higher). Harding (2008) justified these deviations, in part, due to the comprehensive spray dryer model used in the literature study by Harrison (1990). Harding (2008) attempted to further justify the differences by claiming that certain setups in the generic flowsheet were unable to adequately describe the literature model. A large number of trace elements were included in the literature model and trace elements were not accounted for in the generic flowsheet development. Further, the generic flowsheet is unable to model physical limitation and ammonium sulphate, the limiting nutrient in the Harrison (1990) model, was supplied in excess in the Harding (2008) model.

3.2.2.3 Model Sensitivity from Input Parameter Variation

Although able to provide a basis to for Life Cycle Assessment, the results for the case studies investigated by Harding (2008) were not obtained using only default flowsheet data. Unit operation parameters were partially specified using process specific literature data. Although default parameter values provided in the simulation tool allow the user to obtain a reasonable estimate with limited process specific data, the model is most appropriate when certain critical input data are specified. Harding (2008) investigated the sensitivity of the flowsheet to input data in a case study for the production of Penicilin V. The process simulation was fully specified using input data found in literature. Since detailed process data are not always available, especially in early stages of process design, successive scenarios assumed that less process specific data was available and the input data was progressively reduced. A summary of typical

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input data is shown in Table 3.3 for three scenarios. Scenario 2 and scenario 3 used approximately 45% and 30% of the original input data respectively.

Table 3.3 Input parameter variation for Penicillin V production (Harding, 2008)

Input Parameter	Scenario 1	Scenario 2	Scenario 3	Units
Bioreaction				
Maintenance coefficient	0.022	0.022	0.022	h
Time for maintenance	106	106	[10]	h
Final biomass concentration	45	45	[16.7]	g/l
Yield coefficients: Yx/s	0.45	[0.43]	[0.43]	g/g
Yp/s	0.81	[0.64]	[0.64]	g/g
Yx/o	1.56	[1.35]	[1.35]	g/g
Filtration				
Solid fraction removed	100	[95]	[95]	%
Liquid fraction retained	91	91	[70]	%
Additive: Sulphuric acid	0.028	0.028	0.028	%v/v
Precipitation and Crystallisation				
Outlet temperature	6	[40]	[40]	°C
Residence time	12	[2]	[2]	h
Power per unit volume	0.6	[0.8]	[0.8]	kW/m3
Additive: Acetone	12.3	12.3	12.3	%v/v

[ ] Default values used in the simulation model when no explicit inputs are given

A fully specified flowsheet showed the material and energy balance results were within 20% of the literature values. The results of the scenario 3, using approximately 30% of the original process data, were within 55% of literature values. The deviations resulted from model sensitivity to critical inputs, especially those affecting system volume (e.g. final biomass concentration, separation efficiencies). The sensitivity study showed that the model was appropriate if critical data were provided. When using minimal set of inputs, as in scenario 3, the flowsheet was still able to provide an order-of-magnitude estimate for the material and energy balance results. Relying completely on default simulation data however, should be carefully considered, since a high degree of deviation from the actual process data is expected. This is an especially important when the results are used for additional calculations, such as process costing, where the first-estimate costing methods are typically accurate to within 20-30% of the actual values (Vogel, 2005; Peters & Timmerhaus, 1991). Harding (2008) extended the sensitivity analysis to consider the effect of simulation inputs on Life Cycle Assessment (LCA) scores. The aim was to identify key variables, to which the LCA results were most sensitive. Although the analysis was focused at assessing the sensitivity of the LCA scores, valuable insight was given into simulation variables that had the most significant influence on material and energy balance results. Critical inputs, required to obtain acceptable first-estimate simulation results, were identified. A summary of the critical inputs is given in Table 3.4.

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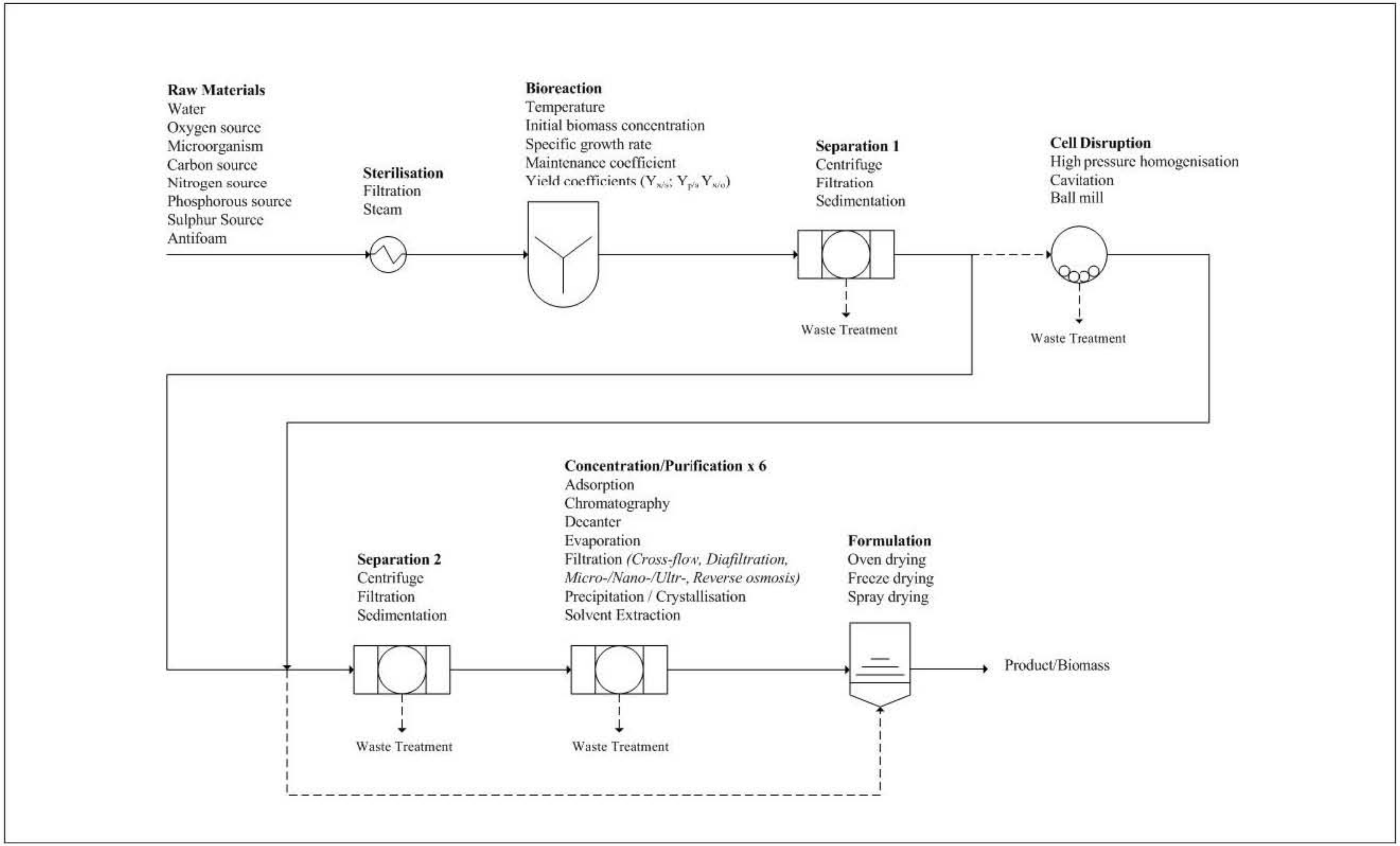

Figure 3.1 Process flow diagram accommodated in the generic flowsheet model (Harding, 2008)

Table 3.4 Critical process inputs for generic flowsheet simulation

Input Parameter	Symbol	Unit
Bioreaction		
Product to biomass ratio	-	-
Final biomass concentration	$C_{x,final}$	g/l
Biomass on substrate	Yx/s	g/g
Product on substrate	Yp/s	g/g
Biomass on oxygen	Yx/o	g/g
Oxygen Supply		
Aeration rate	Ar	vvm
Compression Pressure	-	kPa
Downstream Processing		
Separation efficiencies	-	%

3.2.2.4 Value of the Generic Model

Although the generic flowsheet by Harding (2008) provides default values based on literature and common industry values for input parameters, certain critical default values should be compared to process specific literature or plant data to ensure minimal deviation from the actual process. Critical variables were identified in the sensitivity analysis for Pencillin V production. The material and energy balance results of the generic flowsheet are especially sensitive to variations in these critical variables. The variations ultimately affect the Life Cycle Assessment (LCA) scores and careful consideration of these variables is necessary to ensure results are within an accuracy similar to that of a first-estimate cost study i.e. 30-50% (Peters & Timmerhaus, 1991).

The model provides a fast and reliable means of generating first-estimate material and energy balance data of large-scale bioprocess. The model allows for continuous or batch production by anaerobic or aerobic bioreaction to produce either intra- or extracellular products. A limited number of downstream unit operations are available for selection. An extensive database of physical process data and common operating parameters is used in the model to allow for a simplified approach to process simulation. The material and energy balance results of the model were shown to be within acceptable accuracy when compared to literature data. The results provided sufficient inventory data for Life Cycle Assessment of the process designs when process specific literature data was used. Even with limited process specific data, the key features of the LCA were retained. Harding‘s (2008) approach to process simulation as a basis for Life Cycle Assessment (LCA) ultimately reduced the time and effort required to obtain a LCA of the process design compared to detailed process simulation. This allows LCA to be included in very early stages of process development and decision making.

3.2.2.5 Model Limitations

Although the model developed by Harding (2008) is able to reduce the time and effort for simulating large-scale bioprocess, a number of limitations exist. Future modifications could include additional unit design models; more rigorous unit operation modelling as well as the inclusion of recycle streams. It is also important the model requires the necessary basis for economic considerations of both capital and operating expenses. The most important limitations of the model developed by Harding (2008) are presented below.

i. User Interface

Since the model was developed in Microsoft® Excel the user interface is somewhat tedious to navigate. The sequential nature of the flowsheet results in a large worksheet interface, requiring extensive scrolling

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to move through process steps. A more user-friendly interface would give new users the ability to easily format the software which can then be widely distributed. Flowsheeting packages such as Aspen Plus, Bioprocess Simulator^{TM} and SuperPro Designer$^{®}$, using icons to represent unit operations, are excellent references with which to implement these changes. Extensive knowledge of software application design and implementation would however be required. This would likely be a joint task between the design engineer and software developer.

ii. Unit Sizing and Economic Calculations

The simulation tool does not allow for unit sizing, unit costing and profitability assessment of the process design. Scope for further development exists in extending the generic flowsheet to provide the necessary data for an economic assessment of the process as a first estimate. Continuing with the design philosophy of the generic flowsheet by Harding (2008), this extension should include the necessary default data, input requirements, calculations and outputs. The extension should allow for sizing of main equipment, capital cost estimation, operating incomes and expenses and profitability analysis. Together with the process Life Cycle Assessment (LCA), this will allow for improved decision-making by providing a multi-criteria basis for comparing process designs.

iii. Design Calculations

Specific design constraints of the model limit the ability to simulate a broader range of process designs and products. The model does not allow for enzyme catalysed reactions within, or following the bioreactor. This is especially important for substrate pre-treatment in a number of important industrial processes (Pandey *et al.*, 2000; van Maarel *et al.,* 2001; Sivaramakrishnan *et al.,* 2006). Further, the model only allows for the production of a single product and intermediate or multiple product formation is not possible. The model also does not include recycle loops and cleaning-in-place (CIP) in downstream processing. The functionality and overall applicability of the model may be greatly enhanced if these design calculations are included. Harding (2008) does view the inclusion of recycle loops and multiple product formation as an opening for future development, owing especially to the importance of optimising water use in bioprocesses.

iv. Downstream Operations

The number of downstream unit operations is limited to six concentration, purification or formulation steps. This is mostly due to the limitations imposed by developing the flowsheet on a single Microsoft$^{®}$ Excel worksheet, where repetitive tasks are difficult to setup. By allowing the user to specify an unlimited number of downstream unit operations, far greater flexibility and value would be added to the flowsheet.

3.3 Conclusions

Simulation tools have been applied extensively for simulating bioprocess flowsheets and comparing process alternatives. Considering of various methodologies reported in the literature it can be concluded that in the development of an industrial bioprocess, modelling and simulation can form an integral part of decision making, process optimisation and sustainable process development. Simulations can provide early estimates for assessments and assist in identifying new and previously overlooked problems. Decisions can then be made on whether improvements should be implemented, process development should be terminated due to economic or environmental concerns, or development of the process concept into an industrial application can continue.

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There is however scope for developing simplified simulation tools to be applied in early stages of process development when rigorous process simulation is not justified. The tool developed by Harding (2008) is an example, but improvements to the model are desirable. Further simulation tools should be developed primarily to provide a basis for technical, economic and environmental assessment with which to compare process alternatives. There is scope for identifying appropriate methodologies for quantifying process sustainability measures using first-estimate simulation data as a basis. Chapter 4 provides a context and overview of sustainability assessment and appropriate methodologies used to systematically quantify process performance.

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CHAPTER 4

PROCESS SUSTAINABILITY ASSESSMENT

4.1 Sustainability in Context

Since the 1970s, when Stiglitz (1974) provided a contemporary definition of sustainability as: ―*the optimal growth path that maintains economic development while protecting the environment and optimizing the social conditions with the boundary of relying on limited, exhaustible natural resources*", numerous definitions of sustainability have been proposed. It was not until the 1980s however, that global dialogue around the concept of sustainability was initiated by the Brundtland report. The report was primarily in response to increasing awareness of the adverse impact of human development on local and global natural systems. A widely accepted definition of sustainability, given by the Brundtland Commission (1987), is: ―*Sustainable development meets the needs of the present without compromising the ability of future generations to meet their own needs."*

These definitions concede that change is an intrinsic feature of natural systems. Sustainability needs to form part of that change and should not focus on preservation, but instead, strive to induce responsible human development. The WCED (1987) defines the so-called ‗triple bottom line‘ as the three primary pillars of sustainable development. These pillars provide a framework for focusing efforts in bringing about the need for ‗responsible human development‘. The pillars, shown in shown in Figure 4.1, are concerned with economic viability, environmental performance, and social responsibility. The Dow Jones Sustainability Indices were subsequently started in 1999, defining sustainability as a business approach that creates long-term shareholder value by utilising opportunities and managing risks associated with economic, environmental, and social development.

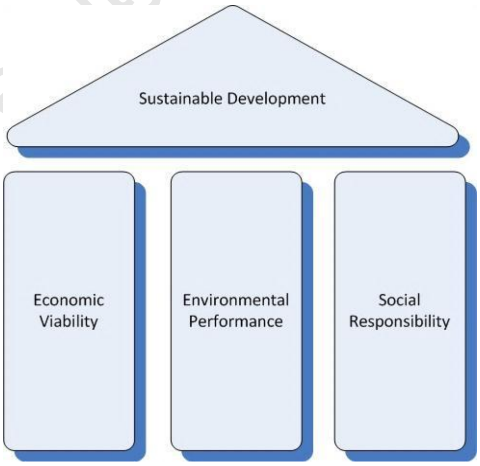

Figure 4.1 Three pillars of sustainability (Adapted from WCED, 1987)

The three pillars of sustainability have been represented often as a synergistic relationship (Clift, 1995; Cowell *et al.,* 1997), shown in Figure 4.2 as a Venn diagram. In the context of sustainable process design and development (a core theme of the thesis), these dimensions can be more specifically defined. Techno-centric concerns are principally coupled to applying scientific principles to optimise the use of materials and energy localised to the production process. Eco-centric concerns are mostly related to optimising the

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use of material and energy on a global scale. The socio-economic dimension is related to concerns with social expectations and socio-economic development directly and indirectly affected by the production or service process. Defining *thermodynamics* as the study of energy and material flows, Clift (1998), refers to these three approaches as *micro-thermodynamics*, *macro-thermodynamics*, and *macro-economics*. The partial overlap of the dimensions produces a set of conditions that perpetuates sustainability, inclusive of economic, environmental and social considerations.

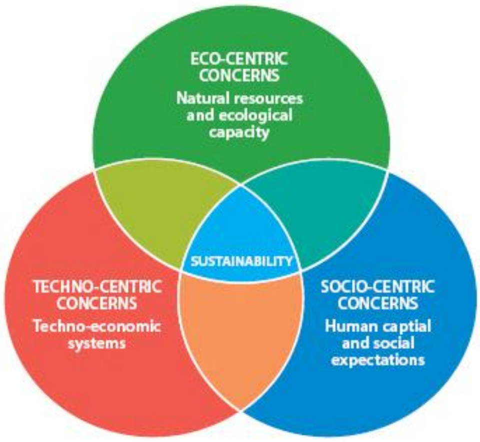

Figure 4.2 Synergistic relationship of sustainability elements (Clift, 1998)

Mebratu (1998) suggested that although the partially overlapping synergistic representation of sustainability has been widely advocated, the three pillars of sustainability cannot be viewed as independent segments. Mebratu (1998) proposes that the elements should be integrated into a single system, shown in Figure 4.3, whereby the economic and social elements are considered subsets of the natural system. Adapted from a definition by Robert *et al.* (2001), the natural system can be defined loosely as the full space between the lithosphere and the outer limits of the atmosphere. The argument implies that the dimensions are intrinsically interdependent and the synergistic model fails to adequately represent the interlinked systems nature of the three aspects of sustainability.

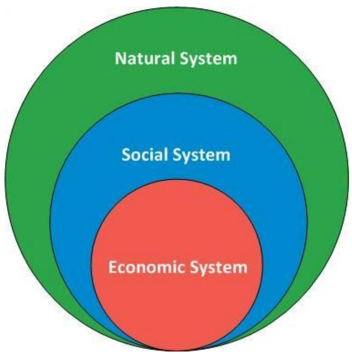

Figure 4.3 Integrated relationship of sustainability elements (Mebratu, 1998)

Although this theoretical stand-point by Mebratu (1998) provides an intuitively sound interpretation of the interaction of sustainability elements, it is somewhat unrealistic in approach to achieving sustainability goals. Although a sustainability framework should include the most important issues pertaining to the interaction of the economic, environmental, and social aspects, the tools and methodologies necessary to adequately assess and implement such an all-inclusive framework for assessing the actual sustainability of a process, as suggested by Mebratu (1998), are not currently available. This is a view supported by

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Gonzalez & Smith (2003), who suggest that a unified, all-inclusive methodology is most likely an unattainable objective. The Sustainability Integrated Guidelines for Management (SIGMA, 2007) provides a model of sustainability that partially encompasses elements of the two afore mentioned models. SIGMA provides guiding principles with which to achieve a sustainable organisation. The guidelines include two core elements, namely, holistic management of five different types of capital that reflect an organisation‘s overall impact on wealth; and the exercise of accountability to stakeholders and regulations. The five different types of capital are defined as:

Natural capital – the environment
Social capital – social relationships and structures
Human capital – people
Manufacturing capital – fixed assets
Financial capital – profit and loss, sales, shares, cash etc.

SIGMA prescribes that natural capital encompasses the other capitals as natural resources and ecological systems are the basis for life, on which all organisations depend. The model suggests that financial capital is simply derived from the value that the other four capitals provide. Further, all the capitals are heavily interlinked and there is some overlap between them. The whole system is encircled by the principle of accountability, representing the relationship between the organisation and the outside world. Although the model provides a framework for discrete elements of sustainability with interdependency and partial overlap, the financial capital is solely derived from the other elements. This suggests an integrated model, similar to that prescribed by Mebratu (1998).

Comparing the above frameworks for sustainability, it is clear that the natural system in which sustainable development is to be applied is tremendously complex. Defining the principles of sustainability requires a comprehensive description of the principles governing the natural system. As a result, an all-inclusive framework for sustainability is complex and difficult to define. However, Robert *et al.* (2001) maintain that the primary objective for sustainable development is not to study the principles of the natural system, but rather to discover the mechanisms by which it is destroyed. It is the task of sustainable development to purge these mechanisms from human development. The description of the natural system can then be restricted to a limited set of principles that are relevant to sustainable development, such as material and energy conversion, the laws of thermodynamics, and the inability of the biosphere to sustain excess resource consumption. Building on this, metrics can be used to qualify and quantify the anthropogenic activities so that we may align them with the principles of sustainable development. Robert (2000) emphasises that most methodologies for assessing process sustainability address the issues relating to ecological and economic performance metrics. These methods include for instance cost-benefit analysis, life cycle assessment (LCA), ecological footprinting (EF), and eco-efficiency (EE). This suggests that the overlapping relationship of the elements of sustainability, presented in Figure 4.2, is well suited as a framework with which to apply assessment of sustainable process development.

Considering the current challenges facing the biomanufacturing industry, there is a need to adopt a definition of sustainable processes that provides a realistic framework within which appropriate tools can be developed. Robert (2000) emphasises that an environmental management system like ISO 14001 (2006) should systematically align a firm‘s specific outcomes, activities and metrics with a general framework for sustainability. The approach to aligning sustainability principles as defined by Robert *et al.* (2001) and the definition of corporate sustainability thus provides a sound framework in which to position the thesis. The overall objective of developing industrial bioprocesses is to maximise economic, environmental and social benefit for society as a whole, while mitigating the risks associated with the

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potential for adverse impacts on economic, natural and social systems. In light of the scope of the thesis, objectives are focused on developing a generic tool for early stage quantification of environmental and economic performance of process flowsheet alternatives. Although social aspects are vitally important, the thesis aims to consider quantifiable process performance measures using simulated material and energy data. The current approaches to the assessment of economic and environmental performance are based on well-established methodologies and principles and provide a robust basis for improvements on both a process level and a global scale. In the following sections, methods to assess sustainability with respect to these two dimensions are presented. Although presented as discrete sections, there are interdependencies and multiple interactions between them. The chapter also provides an overview of the combination of assessment methodologies and interpretation of their interactions.

4.2 Environmental Assessment

4.2.1 Purpose and Approach of Environmental Assessment

As echoed by Robert (2000), one of the primary functions of sustainable development assessment is to highlight the areas of the process with greatest potential of environmental burden. A core focus of the thesis is use quantitative methods to assess the impact of biological processes on the environment. The discussion in Section 4.1 has highlighted that the primary purpose of the environmental assessment is to address eco-centric concerns, related to optimising the use of material and energy on a global scale. The assessment aims to quantify the environmental impact of a process option and identify process ‗hot spots' in early stages of process development. It should draw attention to the materials and process steps that cause most of the environmental burden. Alternative processing routes can be compared and mitigation of environmental burdens can be achieved by comparing and quantifiably supporting decision making toward a more environmentally benign process.

A number of systematic methodologies have been developed for the characterisation and quantification of the potential impacts of chemical processes and products on biological, physical and socio-economic environments. These methods include Ecological Footprinting (Holmberg *et al.,* 1999), Environmental Indices (Elliot *et al.*, 1996; Biwer & Heinzle, 2004a; Jia *et al.,* 2004), Eco-Efficiency (Saling *et. al.*, 2002; Huppes & Ishikawa, 2005) and Life Cycle Assessment (Harding *et al.,* 2007a; Harding 2008; Kim & Dale, 2008; Kloepffer, 2008). Many methodologies have been developed specifically for environmental assessment in early stages of process design, where opportunities for redesign are mostly available and extensive material input data is not available. The subsequent sections provide an overview of some common ways in which environmental performance information is generated and communicated, especially during early stages of process design.

4.2.2 Environmental Impact Indices

Two approaches to environmental impact indices are typically used (Golonka, 1996). The first approach bases the indices on the mass ratio of pollutant to the product (Jones, 1992; Stephan *et al.*, 1994; Luper, 1996; Moser, 1996). As discussed by Golonka & Brennan (1996), these methods quantify the waste emissions within a reasonable time during early stages of process development and provide a sound basis for decision making, but fail to characterise the relative impacts of the wastes. The method by Stephan *et al.* (1994) is used to compare the pollution generated by the original with that from the modified or replacement product. Although the methodology categorises the pollution prevented (e.g. human health, use impairment impacts) as a consequence of the redesign and provides information of the specific classes of pollution prevented (e.g. toxic organics, heavy metals) the methodology does not provide a measure for relative process performance with which to compare alternatives. Burgess & Brennan (2001) further highlight that these methods fail to account for indirect environmental effects such as resource depletion.

The second approach bases the indices on a number of parameters which are assigned weighting factors according to relative environmental effects (Elliot *et al.*, 1996; Heinzle *et al.*, 1998; Koller *et al.*, 2001; Biwer & Heinzle, 2004a; Jia *et al.,* 2004). In most environmental impact indices, component impact scores are grouped into categories for air, water and land. These impact categories are in turn aggregated to obtain an overall impact index. The general method structure for an environmental indices approach is shown in Figure 4.4. The inventory analysis of the production process forms the basis for the evaluation. The data required for the inventory analysis is obtained from the various design and simulation phases performed in preliminary process development. The process model is used to generate the necessary material and energy balances and an inventory of the material and energy input and output streams can be generated. The mass index (MI) is used to state how much of material component is consumed or formed to produce a unit amount of final product. Secondly, a weighting factor, based on a number of impact categories, is derived for every input and output component. The amount of each component together with its weighting factor is then combined into a number of indices. The indices are used to identify the most environmentally relevant components, the overall environmental performance of the process and impact categories that are affected the most by the process.

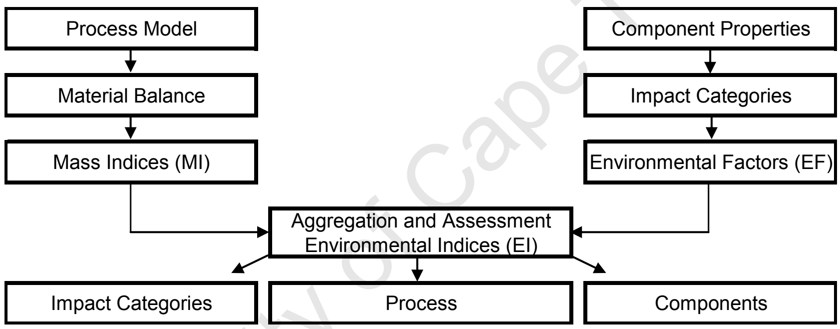

Figure 4.4 Structure of evaluation method (adapted from Biwer & Heinzle, 2004a)

An early-stage assessment method proposed by Biwer & Heinzle (2004a) was developed to evaluate the environmental, health and to a limited degree, the safety aspects of a process within a reasonable time. The methodology has a relatively simple structure and is based on data that is relatively easily accessible. Although the approach is liable to miss small differences in material or process system, the method is specifically designed to reduce the time and detail required for the assessment. A method presented by Elliot *et al.* (1996) uses an index to perform an integrated analysis of the relative environmental impact of a process by incorporating the distribution of pollutants between air, water and soil. Although the method combines component flows into individual impact scores for air, water and soil, the impact of component flows in each category are retained for analysis. The index tool was developed to compare the relative impact of design alternatives of a given process to reduce the number of acceptable alternatives for a more detailed evaluation. A similar approach presented by Jia *et al.* (2004) considers the procedure for environmental performance comparison of process alternatives as a multi-criteria decision making (MCMD) problem. An integrated environmental index (IEI) is used to combine resource conservation, energy consumption and potential environmental impacts associated with releases.

The methods presented above are essentially aimed at accounting for most types of environmental impacts (e.g. ecotoxicity, ozone layer depletion, global warming potential). However, allocating these categories to impact groups and finally deriving a single environmental factor from the groupings, is likely to obscure the relative contributions of the impact categories. Burgess & Brennan (2001) further highlight that there is difficulty in applying weighting factors using ‗expert panels', which often results in the weighting factors being highly debatable. In an attempt to mitigate the subjective approach to generating weighting factors, Biwer & Heinzle (2004a) developed a relatively complex system for generating weighting factors based on the classifications in the impact categories. Although the method does avoid subjectivity in calculating the weighting/environmental factors (EF), there is subjective allocation of components materials to impact groups based on a high, medium, or low contribution, ranking system. A discussion of environmental impact indices presented above demonstrates that these weighted index methods are able to include most types of environmental impacts. However, the relative contributions may be obscured once the overall index has been formed, since the aggregation method includes subjective evaluations of the relative importance of the different impacts. This view is supported by Burgess & Brennan (2001). Biwer & Heinzle (2004a) themselves recognise that the aggregation method is not possible on an exclusively scientific, objective basis.

4.2.3 Eco-Efficiency Indicators

Eco-efficiency was first introduced in 1992 at the United Nations Conference on Environment and Development (UNCED), Rio de Janeiro, Brazil, as part of the efforts to measure sustainability. Since introduction, eco-efficiency has received significant attention and undergone considerable development. The World Business Council for Sustainable Development (WBCSD, 2000) has subsequently defined eco-efficiency as: ―*the delivery of competitively priced goods and services that satisfy human needs and bring quality of life while progressively reducing ecological impacts and resource intensity, through the life cycle, to a level at least in line with the Earth's estimated carrying capacity".* The WBCSD defined three main objectives to be achieved using the eco-efficiency performance assessment methodology:

1. Reduce resource consumption;
2. Reduce the impact on nature;
3. Increase product or service value;

Considerable work has been completed in developing a quantified eco-efficiency methodology for performance assessment as a tool for addressing the objectives stated by the WBCSD (Dahlström & Ekins, 2005; Huppes & Ishikawa, 2005). Quantified eco-efficiency analysis is concerned with the relative trade-off between economic benefit of a good or service and the adverse environmental impact of producing the product or service. This quantified trade-off is shown in Equation 4.1.

$$eco-efficiency = \frac{economic\,value\,of\;good\,or\,service}{environmental\,impact} \qquad 4.1$$

In a similar way that eco-efficiency may be applied on a micro scale to specific process performance assessment, eco-efficiency may be used at a macro level to examine, for example, national economic performance and alternate government policies (Cha *et al.,* 2007). Eco-efficiency is thus a valuable holistic tool for achieving the objectives outlined by the WBCSD.

In contrast to other quantified assessment methodologies, eco-efficiency simultaneously considers environmental performance and the economic benefit of a product or service system. The performance

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measures are combined, providing a single metric with which to compare system alternatives. Further, eco-efficiency may be applied as multi-criteria metric, considering multiple environmental effects, or alternatively as an aggregated metric, using ‗weighting factors' to combine multiple environmental and economic values into a single score. There is still considerable divergence with regard to the appropriate application of eco-efficiency in terms of multi-criteria or aggregate performance assessment (Korhonen, 2007). Although single score normalization of eco-efficiency values is common in multi-criteria performance assessment (Norris, 2001b), Huppes & Ishikawa (2005) demonstrate that combining the economic and environmental scores into a single measure omits specific information necessary for optimal analysis.

A number of eco-efficiency indicators have been published, most notably by the WBCSD and Muller and Sturm (2001), which provide a framework for eco-efficiency assessment and reporting. Although both frameworks aim to achieve an accepted standard for eco-efficiency reporting, both recommend that eco-efficiency indicators should be included in the company environmental reports and not presented as stand-alone reports. Erkko (2003) expands on this by demonstrating that eco-efficiency indicators are mostly included in reporting due to common practice of relating process emissions or energy use to production as opposed to the adoption of the eco-efficiency concept. Further, the study shows that comprehensive adoption of eco-efficiency indicators and reporting is limited to a few case studies. Ehrenfeld, (2005) argues that eco-efficiency is only meaningful in the context of economic sustainability and does not adequately consider the finite limitations on natural resources. Thus, if eco-efficiency is to be a valuable method for evaluating process alternatives it must be coupled with other indicators and tools enabling a more holistic consideration of process sustainability.

Saling *et al.* (2002) provide a review of eco-efficiency application for improved decision-making and product development by BASF chemical company. The study provides clear evidence for the application of eco-efficiency in process and product development. There is however significant resistance to adopt eco-efficiency as an accepted measure of sustainability assessment. As described by van Berkel (2007), a lack of consistency in terminology, aims, objectives and means is the primary reason for this reluctance by industry and government.

A conceptual overview of eco-efficiency application by Huppes & Ishikawa (2005) demonstrates a number of ways eco-efficiency methodology may be applied at both micro and macro levels. However, the study does not provide supporting evidence of this in the form of a case study. The study demonstrates that practical measures for eco-efficiency are required, but an explicit methodology for eco-efficiency analysis is somewhat underdeveloped. Further, primarily due to a lack of sufficient process data, quantified eco-efficiency scores will be possible only at a certain nearly final stages of design.

The literature reviewed above provides supporting evidence that quantified eco-efficiency measures may be used for multi-criteria assessment of process systems by considering environmental and economic performance. The methodology has however been limited in its application for process screening and assessment in early stages of process development. Quantified eco-efficiency methodology has not been sufficiently developed to explicitly conclude that the approach is suitable for comparing early-stage design alternatives based on environmental and economic performance metrics.

4.2.4 Life Cycle Assessment (LCA)

The use of LCA as tool for systematic evaluation of the environmental performance of a manufacturing or service system has gained wide acceptance. The methodology provides a holistic assessment approach to include all the environmental impacts associated with the defined system. LCA thus provides a method to

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support decision making in the context of environmental performance. As described by the International Organization for Standardization (ISO, 2006), the assessment framework consists of four main phases, namely (1) goal and scope definition; (2) inventory analysis (i.e. materials and energy used, emissions, effluents); (3) impact assessment (i.e. evaluation of inventory effects on the environment); and (4) interpretation (i.e. assessment of the results). A detailed description of LCA methodology is provided in Appendix B.

The system boundaries for the assessment are commonly defined as ‗cradle-to-grave‘ or ‗cradle-to-gate‘. The ‗cradle-to-grave‘ approach incorporates the entire product life cycle i.e. from resource extraction to product production, recycle and disposal. The ‗cradle-to-gate‘ approach only includes the system boundaries to the point that the product is transferred to an intermediate producer or end-user. While the ‗cradle-to-gate‘ approach carries the risk of shifting potential burdens down the product life cycle, the former approach is often too data intensive to be performed in an acceptable time frame. The cradle-to-gate approach is thus considered suitable for this thesis.

Although a single methodology is not suitable for all situations, life cycle assessment (LCA) has been increasingly adopted in the assessment of product systems and chemical process design (Clift, 1998; Burgess & Brennan, 2001; Bauer, 2004; Azapagic, 2006; Weiss, 2006; Kloepffer, 2008). Although many life cycle assessment studies have focused on products (Burgess & Brennan, 2001), it is becoming more widely accepted as a tool for process selection and optimisation (Azapagic & Clift, 1999). A number of studies demonstrating the application of LCA in this regard have been published (Azapagic & Clift, 1999; Harding *et al.*, 2007a; Nielson *et al.,* 2007; Harding, 2008; Roes & Patel, 2007). The adoption of LCA in process selection and optimisation is mostly due to its ability to function as an integrated approach to include environmental issues in decision-making from the beginning of process development. This observation has been supported by numerous case studies presented in the literature. A core focus of this thesis is the use of LCA for comparing alternative bioprocess flowsheets in early stages of process development and the literature is discussed accordingly.

A study by Harding *et al.* (2007a) demonstrated the application of life cycle assessment to compare inorganic (NaOH) and biological catalysis (lipase) for the production of biodiesel by transesterification. The case study provided a good example of using LCA to compare biological processing routes to traditional chemical synthesis. The study used Aspen Plus® (AspenTech, 2008) to model five flowsheet options, each specified for a different combination of the catalyst and alcohol. The process flowsheets were compared using a ‗cradle-to-gate‘ life cycle assessment using SimaPro v6 (Pré Consultants B.V.). The material and energy inventory data for the biological catalyst (lipase) were not available in the literature and a generic flowsheet model (described in Chapter 3) was used to generate suitable data. Midpoint impact scores provided insight into the production process via the alternative processing routes. The study found that enzyme catalysed biodiesel production had advantages over the chemical route due to avoided use of chemical catalyst, neutralising acid, and utilities for heating. The results could be used to prompt research into optimising the production of lipase as a biological catalyst. A number of studies applying a similar approach to environmental assessment were performed by Harding (2008), including the production of penicillin V; comparison of cellulase production from three alternative routes; and comparison of PHA biopolymer production to traditional polypropylene production. Similarly to the case study comparing inorganic and biological catalysis for biodiesel production (Harding, 2007a), the studies identified major contributors to life cycle impact categories and could be used to assess the relative performance of alternative process options by directly comparing midpoint impact categories of process alternatives.

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In contrast to the ‗mostly theoretical' studies performed by Harding (2008), Nielson *et al.* (2007) used a cradle-to-gate LCA to compare the environmental impact of producing five representative enzyme products by Novozymes in Denmark. The LCA methodology used included an inventory analysis and midpoint impact assessment. The results of the study allowed for identification of the main sources of environmental impacts. The fermentation processes and production of ingredients were the main contributors to the impact categories. The impact scores of the enzyme products varied by a factor of ten due to differences in the use of carbohydrates, fermentation time, formulation type, yield, and final product concentration. These results provided a basis for decision making to improve the environmental implications of using enzymes in modern industrial processes. This was demonstrated by Nielsen & Wenzel (2006) using the results of the LCA study to develop a novel approach using Ronozyme P 5000CT (Enzyme C) as an additive to pig feed in order to release natural phosphate in grains as an alternative to supplementing inorganic phosphate from external sources.

In a study performed by Roes & Patel (2007), LCA was used to compare risks related to the production of organic chemicals by petrochemical processes versus bio-based based processes. The assessment included the total process chain for both petrochemical and bio-based products and was applied to five plastics: polytrimethylene terephthalate (PTT), polyhydroxyalkanoates (PHA), polyethylene terephthalate (PET), polyethylene (PE), and ethanol. The overarching results of the study showed that conventional risks related to bio-based products are lower than those of petrochemical products. This was due primarily to lower energy use in bio-based production faclilties. There was however significant uncertainty associated with the input data, incomplete coverage by the impact method (EPS, 2000), and uncertainty associated with specific assumptions concerning the duration of accidents and illness. Due to the uncertainty the authors recommended further research was needed to reduce uncertainty associated with the results. Although mainly inconclusive, the study was valuable in demonstrating the application of LCA as a flexible method to analyse risks associated with comparative production processes.

A study by Kim & Dale (2007) investigated the environmental performance of fuel ethanol, derived from corn grain via dry milling, used in a compact passenger vehicle. The purpose of the ‗cradle-to-gate' analysis was to identify practices that will help ensure that a renewable fuel, such as ethanol, may be produced in a sustainable manner. Inventory data was obtained from eight counties in seven Corn Belt states as corn farming sites, in the United States. The functional unit was defined as bioethanol derived from corn grain used in an E10 fuelled vehicle, and the reference flow was defined as 1 kg of ethanol. The study found that using ethanol derived from corn grain dry milling would reduce non-renewable energy and greenhouse gas emissions, but would increase acidification, eutrophication and photochemical smog, compared to using gasoline as liquid fuel. The LCA results also provided insight into the relative performance of the counties. Counties mostly varied in performance due to differences in the energy source used for dry milling (i.e. coal and natural gas). Coal was found to contribute more to impact scores, prompting the investigation alternative energy sources for the corn mills. Further, it was determined that the dominant contribution to the impact scores was from nitrogen fertiliser and crop residues. The planting of winter crops was able to reduce the overall environmental impact due to a reduction in fertiliser leaching and hence lower fertiliser requirements. These insights could be applied at other sites around the Unites States. The study is a valuable example of ability of LCA inform both local and global decision making.

Although the numerous studies presented above clearly demonstrate the benefits of LCA as tool for systematic process screening and optimisation, there are also limitations in the LCA approach. Burgess and Brennan (2001) provide an insightful discussion surrounding the limitations associated with LCA, of which most are still subject of on-going debate. These are well-acknowledged limitations within the LCA

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community and it is not in the scope of this thesis to discuss these limitations at length. However, some of the most apparent limitations include the need to use a quantified functional unit related to the impact scores; the lack of regional or temporal specification; problems encountered with allocation of environmental burdens; and the lack of impacts associated with water use and soil erosion. Although the impact of land use is similarly lacking, Udo de Haes (2008) argues that this can be ‗smoothly' included in the assessment. Similarly, Kloepffer (2008) argues that considerable effort has been made in overcoming the regional and temporal specification limitations. In contrast, Harding (2008) argues that the lack of geographical and temporal specification allows the methodology to be applied across a wide scope of industries and processing routes. This is most likely only applicable in early stage assessment when intrinsic inaccuracy of the assessment results is likely to reduce the inherent differences associated with geographical and temporal differences. This view is indirectly supported by Guinee *et al.* (1993), arguing that site-specific assessment is not practical in LCA. The LCA can however be ‗tuned' for geographic conditions depending on the source and availability of input data. This is most likely achievable for a specific process plant but difficult to achieve in early stage of process development. The spatial representativeness of the process should, however, be specified in the goal and scope. Further, the problems encountered with the allocation of burdens to co-products can be overcome mainly by allocating on a mass basis and assigning all the environmental impacts to the main product as prescribed by Stormberg *et al.* (1997). This approach is however not appropriate if significant co-products are generated (e.g. molasses and cane residue in cane sugar production). Biwer *et al.* (2006) further argue that life cycle assessment (LCA) can be time-consuming and complex and may be unnecessary in early stages of process development where simpler methods can be used.

Although LCA faces a number of practical limitations and simpler methods may be specifically developed for early stage process evaluation (Biwer & Heinzle, 2004a), LCA provides a partly standardised (ISO, 2006) and systematic approach to explore impact potentials and process environmental assessment on a relative basis. A systematic and well developed method is required to ensure a reliable basis on which to identify ‗hot spots' and compare process alternatives. There is also a need to facilitate convergence and standardisation of corporate sustainability management approaches to avoid confusion among stakeholders, typically arising through proliferation of different assessment approaches (EC, 2002; Azapagic, 2003). The complexity of the LCA study is largely dependent on the complexity in obtaining the process material and energy inputs and outputs, which is likely to be the case irrespective of the environmental assessment method used. Further, early stage assessment using LCA forms a basis for more detailed assessments at later stages of process development. It is thus an important aim of the present thesis to develop the application of LCA in early stage environmental assessment.

4.3 Economic Assessment

In development of a new process, optimisation of an existing process, or screening from a number of options, the economic viability of each scenario needs to be considered and has often been regarded as the most important decision criteria. In order for a new process or technology to be implemented on a commercial scale it needs to be economically, environmentally and socially sustainable. Broadly defined, economic viability is the difference in the value of the finished product and the cost required to manufacture the product. The metrics most typical used to measure this value include *gross margin (GM)*, *earnings before interest and taxation (EBIT)*, *net present value (NPV)* and *return on investment (ROI)*. The basis for these metrics, in the context of a bioprocess, includes total capital investment (e.g. equipment; land; start-up), process material costs (e.g. raw material, consumables, detergents) and process operating costs (e.g. utilities, labour, rent). A comparative assessment of the effect of process alternatives,

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new technologies and process optimisation on the metrics can give valuable insight into the economic viability of the process. Further, by integrating the assessment into the initial process development stages the basis for decision making can be greatly improved and sustainable process development can be enhanced. A study by Rouf *et al.* (2001) demonstrated the method by comparing two process flowsheets for the production of tissue plasminogen activator (t-PA) on the basis of return on investment and gross margin. The results of the study highlighted differences in capital and operating costs and aided in quantifying the relative gain of the process options.

Although process profitability has been regarded as the most important criterion when considering design options within a commercial setting, the integration of economic metrics into holistic process assessment methodologies is increasingly important due to the greater emphasis on other sustainability criteria. A number of studies have presented methodologies that included economic analysis as a measure to compare sustainability of large-scale bioprocesses (Sonesson *et al.,* 1999; Gonzalez & Smith, 2003; Biwer *et al.*, 2004; Dornburg *et al.*, 2006; Roes *et al.*, 2007; Heinzle *et al.,* 2008; Kloepffer, 2008;). The studies by Heinzle *et al.* (2008), Sonesson *et al.* (1999) and Roes *et al.* (2007) were limited in their approach and only included typical costs associated with resource and utility consumption and transport costs. The extensive study by Dornburg *et al.* (2006) included an economic investigation of a polylactic acid (PLA) bio-refinery system. The study included an analysis of production costs and investment cost requirements of producing the PLA from alternative feedstocks. The study did not specify investment costs and simply compared economies of scale using a scaling factor on the total investment cost of an existing facility. Although the authors themselves noted the limitations of the study in terms of detailed investment cost analysis, the study demonstrated that capital costs such as auxiliary production equipment and land have a significant effect on the viability of the process.

In order to provide a basis for robust economic evaluation of process options both capital and operating costs should be considered. This thesis focuses on assessing economic performance in early stage of process development. A review of methods typically used to estimate economic metrics in early stages of process design and development and the accuracy associated with such estimates is presented in Appendix B. The estimates are typically based on data obtained by conceptual process design or by means of process modelling or simulation. As demonstrated by the case studies above, the assessments can form a basis for decision making in consideration of process alternatives, ‗trade-offs' between economic and environmental metrics, and implementation of new and improved technologies.

4.4 Combined Economic and Environmental Evaluations

In evaluating the results of the environmental and economic assessments, it is important to consider the results in conjunction with one another rather than independent sets of decision making criteria. By integration of the sustainability assessments, the domain of application of each can be enhanced as decision making tools (Huppes, 1996). In recent times a number of studies have presented approaches toward integration of environmental and economic assessments (Gonzalez & Smith, 2003; Biwer & Heinzle, 2004a; Dornburg *et al.*, 2006; Kim & Dale, 2008; Kloepffer, 2008). The approaches evaluate process performance by integrating the environmental impact assessments and process cost estimations.

4.4.1 Integration of Index Assessment Methodologies

Kim & Dale (2008) assessed the overall environmental and economic performance of corn-based ethanol production in a dry mill. The analysis used a cradle-to-grave LCA of fuel ethanol including corn cultivation, transportation, milling processes and distribution and use in ethanol fuelled vehicle operation. The case study also considered an economic assessment of the process. In an attempt to combine the

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results of both the environmental assessment and economic analysis an eco-efficiency metric was defined. An environmental index was defined as the ratio of the environmental impact from alternative product systems to that of the corn-ethanol product system. Similarly an economic index was defined as the ratio of value added by the ethanol production system (market value of product and co-products) to the operating cost of process plant. The two index values were plotted on independent axes to show the economic and environmental performance in a single plot. Process options could be plotted on a single chart for each environmental impact category so that relative performances of the process could be compared. The analysis allowed for direct comparison of the economic benefits derived from different geographical locations depending on the local economic conditions.

Similarly, integration of economic and environmental assessment metrics is demonstrated in a case study for the production of penicillin V by Biwer *et al.* (2004). The case study aimed to quantify and evaluate uncertainty associated with variance in process parameters created during decision making. A large-scale-simplified process model, based primarily on literature data, was developed using process simulation software and formed the basis for environmental and economic assessments. The environmental assessment method (Biwer & Heinzle, 2004a) aggregates a range of environmental impacts into two performance figures, namely input (EI_{in}) and output (EI_{out}). The performance figures can be compared to appropriate economic assessment measures and uncertainty associated with the environmental impacts investigated. The authors stated that the single score approach for inputs and outputs is more appropriate for a direct comparison of economic and environmental metrics than complex environmental assessment methodologies such as life cycle assessment. Further, the study showed that assessment modelling coupled with parameter uncertainty analysis (Monte Carlo simulation) can be used as a methodology for multi-parameter uncertainty analysis. Although using single scores is able to quantify environmental performance values, these metrics contained inherent subjective weighting which may distort environmental performance indicators. A specific process may in reality emit a specific compound, but due to gaps in the data the product system will appear to have no such emission. This bias will in most cases result in an underestimation of the actual impact. Single scores may also result in biased comparison when comparing similar process options where different environmental assessment methods have been applied. Although methods have been presented to address issues with data gaps (Huijbregts *et al.,* 2001, Suh *et al.,* 2004, Heijungs *et al.,* 2007), the methods are not widely accepted (EC, 2002; Azapagic *et al.,* 2006) and single score methodologies should be avoided for direct process comparisons.

4.4.2 Integrating Life Cycle Assessment and Life Cycle Cost Analysis

In an attempt to increase assessment integration, approaches using analogous environmental and economic assessment methodologies, such as life cycle costing (LCC) have been applied (Rebitzer *et al.*, 2003; Roes *et al.*, 2007). Life cycle cost refers to all the costs associated with the defined life cycle system. In addition to the cost of the physical process and associated material and energy flows, the methodology aims to include, amongst others, labour costs, overhead costs (marketing, patents, R&D) and costs for information management and exchange. Analogous to life cycle assessment (LCA), the LCC methodology is used to compare the costs of alternatives, identify drivers and quantify trade-offs between products (Rebitzer *et al.*, 2003). The major differences between LCA and LCC are summarised in Table 4.1. The methodologies are closely linked and the information necessary is complementary. This includes material and energy flows, transport, product use and waste disposal. Additional elements not contained in the LCA (e.g. R&D) are included in the LCC as separate items. The LCA-based life cycle costing provides an integrated environmental and economic assessment methodology with which product options can be compared. This provides decision-makers with a more holistic basis for product development.

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A number of studies have demonstrated the integration of life cycle assessment (LCA) and life cycle costing (LCC) in product and process assessment (Norris, 2001a; Rebitzer, 2002; Nakamura & Kondo, 2005; Roes *et al.*, 2007). Roes *et al.* (2007) applied life cycle assessment and life cycle costing in a case study for the manufacture of polypropylene nanocomposite. The study aimed to investigate the life cycle of products manufactured from nanocomposite and from conventional materials. The standard LCA (ISO-14040) methodology was used for the environmental assessment and a LCC methodology for the economic assessment. The results gave valuable insight into the environmental impacts associated with products manufactured from nanocomposite in comparison to conventional materials. Further, insight was gained into the economic advantages of products from nanocomposite as well as the constraints on the advantages. Although still requiring development, the integration of LCA and LCC proved to be a valuable method for assessing the potential economic and environmental impacts of process options.

Table 4.1 Comparison of LCA and LCC methodologies (Norris, 2001a)

	LCA	**LCC**
Purpose	Compare relative environmental performance of product systems for meeting the same end-use function, from a broad, societal perspective	Determine cost-effectiveness of alternative investments and business decisions, from the perspective of an economic decision maker
Activities considered part of the Life Cycle	All processes connected to the physical life cycle of the product; including the entire pre-usage supply chain; use and the processes supplying use; end-of-life and the processes supplying and-of-life steps	Activities causing direct costs or benefits to the decision maker during the economic life of the investment, as a result of the investment
Flows considered	Emissions, resources, and inter-process flows of materials and energy	Cost and benefit monetary flows directly impacting decision maker
Units for flows	Physical units (e.g. mass, energy, volume)	Monetary units (e.g., dollars, euro, etc.)
Time treatment and scope	The timing of processes and their release or consumption flows is traditionally ignored; impact assessment may address a fixed time window of impacts (e.g., lO0-year time horizon for assessing global warming potentials) but	Timing is critical. Present valuing (discounting) of costs and benefits. Specific time horizon scope is adopted, and any costs or benefits occurring outside that scope are ignored

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CHAPTER 5

RESEARCH FOCUS, HYPOTHESIS AND METHODOLOGY

5.1 Research Focus

The objectives of the dissertation use a generic flowsheet tool for early stage quantification and evaluation of process sustainability. The evaluation is to provide a basis for improved decision making, process screening and process design. This is demonstrated by a case study application of a generic flowsheet tool, Life Cycle Assessment (LCA) and order-of-magnitude economic estimation techniques. These tools are brought together for assessing the environmental and economic performance of a large-scale biomanufacturing process and support decision making in early stages of process development.

5.1.1 The Need for Early Stage Simulation

Literature cited in Chapter 3 has affirmed the necessity for rapid process assessment to meet challenges facing the biomanufacturing industry, such as rising costs, extended time periods to meet regulatory requirements and strong market competition (Chhatre *et al.*, 2007a). Although previous studies have focused on generating process data using ―off-the-shelf" simulation packages (Rouf *et al.,* 2001, Varga *et al.,* 2001; Chang *et al*., 2002; Biwer *et al.,* 2004; Chhatre *et al.,* 2007b), there is scope for development of simplified simulation tools, as demonstrated by Harding (2008), for very early stages of process design and development. While rigorous software packages are able to provide detailed information regarding the process material and energy balances, they are at times complex, difficult to use and time consuming to set up. Further, many studies are limited in their approach and typically consider greenhouse gas emissions and energy consumption only (Gerngross & Slater, 2000; Kurdikar *et al.*, 2001; Dornburg *et al.,* 2003; Roes *et al.*, 2007). Studies by Patel (2003) and Akiyama *et al.* (2003) demonstrated the environmental benefits of certain bioprocess technologies, but the analyses were limited to few environmental categories. The lack of holistic process assessment is due primarily to a lack of reliable process material and energy data to support such an assessment.

It is an obvious conclusion that to ensure rigorous comparison of process options as a screening mechanism, reliable, holistic process data is required, especially at an early stage of process design. This is a view that has been echoed by a number of environmental practitioners (Gasafi *et al.,* 2003, Biwer & Heinzle, 2004a; Harding*,* 2008) The assessments can enhance our insight and understanding of the process, identify potential problems and highlight areas for improvements. Decisions can then be made on whether improvements should be implemented, process development should be stopped because it is not economically or environmentally sustainable, or development of the process concept into an industrial application can continue as before. The argument above provides the basis for the first hypothesis of the research:

Hypothesis 1: *A first-estimate flowsheet tool can provide holistic inventory data of sufficient reliability for process performance assessment and support decision-making in large-scale bioprocess design.*

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5.1.2 LCA and Economic Assessment as a Measure of Sustainability

Chapter 4 demonstrates that current approaches to assessment of process sustainability are based on well-established methodologies and principles and provide a systematic basis for improvements on both a process level and a global scale. Environmental and economic performance assessment can serve as a decision support tool to improve process performance. Previous studies have shown that simplified early-stage process simulation and subsequent first estimate environmental and economic assessments are able to improve and guide sustainable process development (Rouf *et al.*, 2001; Biwer *et al.*, 2004; Mustafa, *et al.*, 2004; Chhatre *et al.*, 2007a; Harding, 2008). Using early stage process data as a basis for these assessments however, does not account for all resource consumption, process emissions, market conditions and profitability scenarios. Hence, an early-stage assessment approach serves as a valuable support tool to existing approaches, methodologies and tools. Further, combined economic and environmental analysis provides valuable insight into process interdependencies, informing holistic decision making (Biwer *et al.,* 2004; Basson & Petrie, 2007; Kloepffer, 2008; Benedetti *et al.,* 2009). Ultimately, this contributes to improved process sustainability assessment.

A number of environmental assessment methodologies have been applied in screening process options e.g. Ecological Footprinting (Holmberg *et al.,* 1999), Environmental Indices (Elliot *et al.*, 1996; Biwer & Heinzle, 2004a; Jia *et al.,* 2004), Eco-Efficiency (Saling *et. al.*, 2002; Huppes &Ishikawa, 2005) and Life Cycle Assessment (Harding *et al.,* 2007a; Nielson *et al.* 2007; Roes & Patel, 2007; Harding 2008; Kim & Dale, 2008). As discussed in Section 4.2.4, Life cycle assessment (LCA) offers potential as a tool for quantitative assessment of the environmental performance of large-scale processes. While time-consuming and complex, life cycle assessment (LCA) methodology is not location specific, allows for direct comparison of process alternatives and is supported by a strong literature base (ISO 14040, 2006; ISO 14044, 2006). Although LCA includes recognised standards there is significant methodological choice with regard to the impact assessment methods, interpretation and weighing. It is thus up to the LCA practitioner and stakeholders to consider the relative trade-offs between environmental impacts and socio-economic factors and make decisions based on these trade-offs.

Similarly, a first-estimate economic analysis of process alternatives, new technologies and process optimisation strategies can give valuable insight into the economic viability of the process. By integrating the assessment into initial process development decision making can be greatly enhanced. A number of key economic metrics, presented in Appendix B, provide the quantified basis for ‗early-stage' decision making. This approach for process performance assessment is demonstrated by Sonesson *et al.* (1999); Dornburg *et al.* (2006); Roes *et al.* (2007) and Heinzle *et al.* (2008). The studies are, however, limited in their approach and it may be concluded that in providing a basis for robust economic evaluation of process options, both capital and operating costs should be considered. Citing these studies, there is an obvious need for integration of a systematic approach to generating study estimates for process costs and profitability in initial stages of process screening and development.

Considering the arguments for an early-stage assessment of environmental and economic performance of the process, a systematic and well developed method is required as a basis on which to identify impact potentials, hot-spots and compare process alternatives. Further, convergence and standardisation of corporate sustainability management approaches is needed to avoid confusion among stakeholders, typically arising through proliferation of different assessment approaches (Azapagic, 2003; EC, 2002). This thesis aims to contribute to this convergence and standardisation by developing a tool to support early-stage process sustainability assessment. A number of approaches combining environmental and economic measure into integrated metrics, such as Cost-Benefit Analysis (CBA), Life Cycle Costing (LCCA) and single score index values have been presented from literature (Dornburg *et al.*, 2006;

Gonzalez & Smith, 2003; Kloepffer, 2008). However, at the outset of this research, there was no standardised and widely-accepted method by which to accomplish this. This thesis applies a systematic method to provide first-estimate data for environmental and economic assessment as independent metrics. It is ultimately left to the practitioner, stakeholders or regulatory authorities to determine the relative trade-off between these performance measures.

Further, early stage environmental assessment using LCA forms a basis for more detailed analysis at later stages of process development. Similarly, economic considerations of process options need to be evaluated. The overall result is an ability to account for environmental and economic performance assessments and associated trade-offs in process design and screening. The case presented above for the use of Life Cycle Assessment (LCA) and first-estimate cost and profitability analysis as a support tool for process sustainability assessment provides the necessary basis for the second hypothesis for the research:

Hypothesis 2: *Life Cycle Assessment and a study estimate of costs and profitability provide a method that supports systematic sustainability assessment with which to compare bioprocess design alternatives.*

5.2 Research Methodology

5.2.1 Research Approach

The research approach and methodology is formulated to test the hypotheses presented above and address key questions presented in Chapter 1. The generic flowsheet model developed by Harding (2008) is a valuable tool for early stage assessment of bioprocess flowsheets and forms the basis of the research design. Firstly, building on the work by Harding (2008) and using certain key questions to inform the methodology, the research aims to address important model limitations by further developing the first-estimate generic flowsheet tool. The hypotheses are then tested by means of a case study. A case study approach demonstrates the actual application of the methods while contributing to expanding the academic theory of the process design and applied concepts (Biwer *et al.,* 2006). Research literature cited throughout this dissertation, using case studies to demonstrate novel approaches to environmental and economic assessment, gives testament to this. The case study demonstrates the value of a first estimate process simulation tool to generate inventory data for environmental and economic assessment of the process.

A summary of the research design steps, associated scope and applied methodology, shown in Table 5.1, provides the necessary roadmap to complete the research. The methodology for each step is deduced from the key questions presented in Chapter 1, with the overall objective of testing the research hypothesis. The *problem formulation* step aims to provide the necessary basis for the research to be completed. The problems addressed include important limitations of the generic flowsheet by Harding (2008), process screening by comparison of results of flowsheet options and comparison of results from the generic flowsheet to data from detailed simulation packages and literature. These problems are informed primarily from the main key questions concerning flowsheet feature requirements, model accuracy and the ability of the flowsheet to function as a screening tool for process options.

An appropriate case study is used to compare flowsheet options. Selection of the case addresses the key question relating to the type of process that represents a typical large-scale bioprocess. Alternatives of the case study flowsheet are analysed and compared by material and energy balance, LCA mid-point values and specific economic metrics. This provides the necessary results to address the fundamental key questions relating to the ability of first-estimate inventory data to inform environmental and economic assessments and the ability of an early-stage assessment tool to identify process ‗hot-spots' and compare

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process options. A critical evaluation of these observations is necessary to identify limitations, and formulate recommendations for future work.

Table 5.1 Research design steps, scope and methodology

Research Design Step	Design Scope	Methodology
Problem formulation	Generic flowsheet development	Generic flowsheet evaluation and design
	Process alternatives screening	Process flowsheet comparison
	Assessment methodology	Comparison to detailed literature data
Consideration of alternatives	Bioprocess case study	Appropriate selection criteria
	Process alternatives	Process technology; Process flowsheet
Analysis of alternatives	Process simulation	Material and energy balance
	Environmental assessment	LCA mid-point indicators
	Economic assessment	Order-of-magnitude economic metrics
Critical Reflection	Methodology and Results	Objective Evaluation

5.2.2 Generic Flowsheet Model Development

Opportunity exists to address specific limitations of the generic flowsheet developed by Harding (2008). Part of the objectives developed for this thesis involves evaluating and addressing these shortcomings. The specific shortcomings identified in Section 3.2.2, are broadly categorised as limitations pertaining to *user interface, unit sizing and economic calculations, design calculations* and *downstream operations*. In using the tool, each aspect was critically assessed and, where necessary, modifications were incorporated to improve the model robustness. The focus of the resources available for the thesis work is placed on developing critical feature shortcomings and improving overall flowsheet usability. The basis for the flowsheet development is established by physical inspection of the model, collating the limitations identified in the case studies presented by Harding (2008) and comparing the model features with features of commercial simulation packages (SuperPro Designer™, Aspen Plus). Since software problem-solving and design is typically completed by a number of development experts, the end-result of the generic model interface is not likely to be an end-product on par with commercially available software packages. Rather, the development provides a primer for further work and ensures the flowsheet meets the requirements to achieve the research objectives. The flowsheet has to meet certain criteria to be suitable for use as bioprocess simulation tool and development should be an iterative and on-going process. Limitations discussed in Chapter 3 are based on the results of the case studies by Harding (2008) and additional limitations are likely to arise with application of the flowsheet to new case studies.

5.2.2.1 User-Interface

Since the model was developed in Microsoft® Excel, shown in Figure 5.1, the user interface is somewhat tedious to navigate and there is opportunity to improve the architecture of the software. Elliott *et al.* (1996) emphasised the need for a graphical user interface (GUI) that could be used by design engineers, as well as those with minimal knowledge of Microsoft® Excel software and environmental analysis. Similarly, Bo (1997) and Benyahia (2000) stressed that improved GUI design makes flowsheeting packages a great deal more ‗user-friendly' and increased the ‗intuitive' learning component of mastering flowsheet packages. This ultimately eases simulation chores in process flowsheeting and optimisation. In developing a more user-friendly interface, the generic flowsheet model is compared to Aspen Plus and SuperPro Designer® flowsheeting packages. These ―off-the-shelf" applications have been expertly

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developed and the user- interface is based on the design framework of commonly encountered simulation packages.

The user-interfaces are intuitive and provide well designed input screens for process data and operating parameters. An example of such an input screen is shown in Figure 5.2 (SuperPro Designer®). Although these detailed simulation packages can be tedious and time consuming in comparison to the generic flowsheet developed by Harding (2008), it is necessary that development of any software interface adheres to intuitive design architecture.

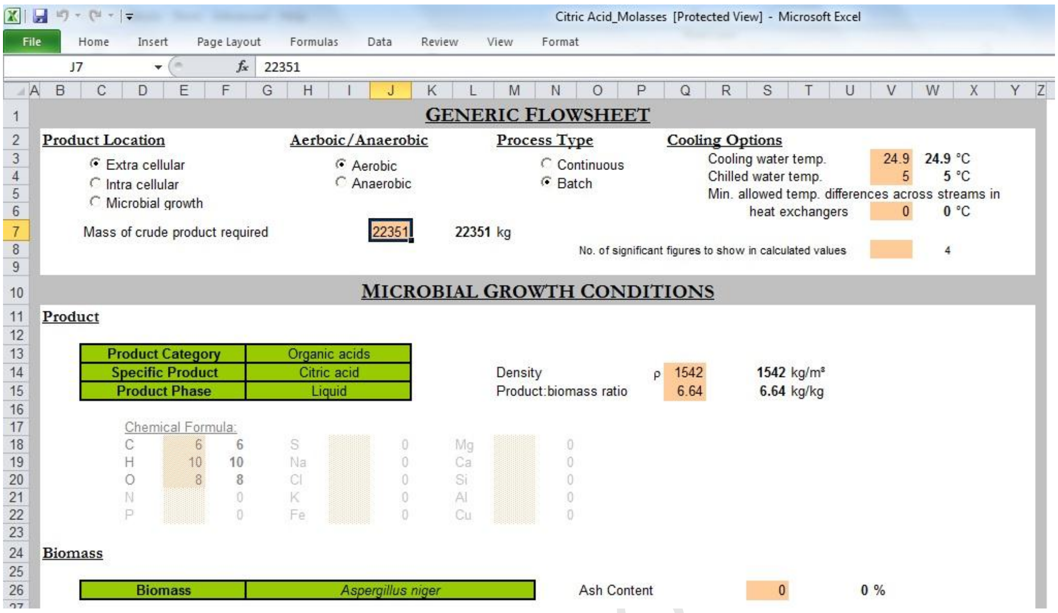

Figure 5.1 Screen-shot of the generic flowsheet model (Harding, 2008)

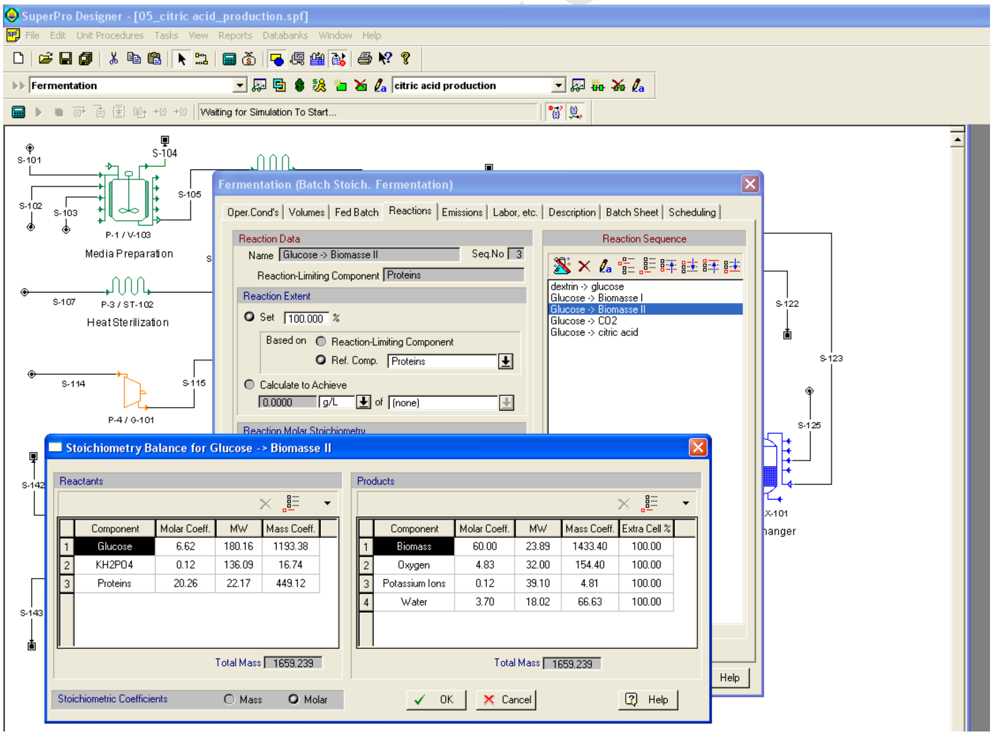

Figure 5.2 Example of a typical commercial simulation package input screen (SuperPro Designer®)

The approach to the development of the generic flowsheet graphical user interface (GUI) is shown in Figure 5.3. Implementation of this methodology is provided in Appendix C. The interface allows the user to select from a number of possible generic operations, including material and energy balance calculations, equipment costing calculations and process assessment calculations. Once the user has selected a generic operation, a number of options are available for selecting from a number of more specific categories. These include options for the process type, specific unit operations, or economic calculation categories. Within each category selection the user has the ability to select from a number of procedure or data input options. Once the necessary procedures have been selected and the process data has been inputted, the results can be generated.

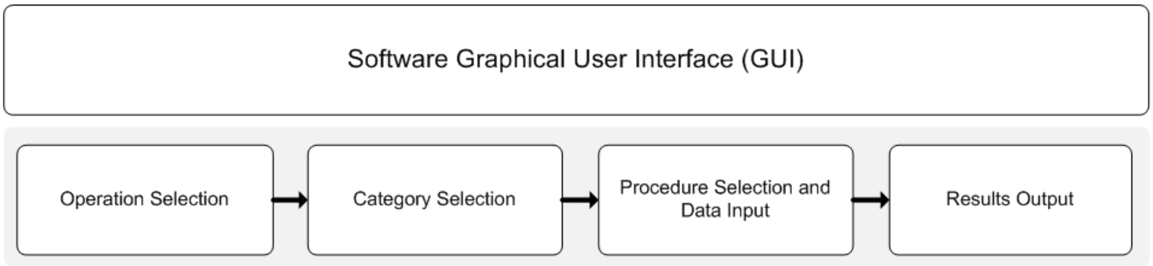

Figure 5.3 Generic flowsheet user-interface design methodology

Since the flowsheet was developed in Microsoft® Excel it is deemed justifiable to base further development within this framework. Microsoft® Visual Basic for Applications (VBA) is an obvious choice due to the ability of a first-time software developer to learn the coding language and the integrated development environment (IDE). Microsoft® specifically designed Visual Basic for Applications (VBA) to enable non-specialist software developers the ability to build user defined functions, automate processes and include low level access functionality to related software applications. Further, the VBA platform allows the developer to manipulate features of the user interface and design custom dialog boxes and user forms. Although VBA offers considerable functionality and flexibility for development it does have a number of limitations. Most importantly, the architecture has the ability to use, but not create Dynamic Link Libraries (DLLs) and is not able to function as a standalone application. The primary use of VBA is thus to add functionality and flexibility to a standard user interface.

5.2.2.2 Unit Sizing and Economic Calculations

As described in Chapter 3, unit sizing, unit costing and profitability assessment of the process design are not included in the simulation tool developed by Harding (2008). The objectives include extending the economic assessment capabilities of the generic flowsheet. Chapter 4 reveals that to provide a basis for robust economic evaluation of process options both capital and operating costs should be considered. The metrics typically used in early stages of process design and development provide the necessary methodological theory with which to develop the unit costing and economic features of the generic flowsheet model. These features are summarised in Table 5.2. This includes sizing of main equipment, capital cost estimation, operating incomes and expenses and profitability analysis. Default economic data, input requirements, calculations and outputs are included in the spreadsheet model. Using the improved user interface and software architecture, the design schematic shown in Figure 5.4 shows the design layout of the simulation flowsheet. The shaded blocks represent the flowsheet interface developed by Harding (2008), including unit operations, operating conditions and design parameters. The results of these material and energy balance calculations provide the necessary data with which to populate subsequent costing and economic calculation models.

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Table 5.2 Features and requirements of the generic flowsheet extension

Feautre	Data and Calculation Requirement	Output
Procedure Specification	Unit Throughput/Capacity Residence Time/Process Time Unit Scheduling	Design Value (Volume, Area) Unit Time
Equipment Design	Unit Parameters (e.g. Dimension Ratios) Operating Parameters (e.g. Temperature, Pressure, Efficiency)	Size/Design (Dimensions, Area) Number of Units Required
Equipment Costs	Cost Variable (Volume, Area, Height, Rating) Specified/Scaled Unit Cost Reference Reference Year Number of Units	Unit Cost
Cost Adjustments	Stagger Mode (Stand-by Units, Staggered Units) Material of Construction (e.g. SS316) Installation Factor (Cost Multiple)	Adjusted Unit Cost Total Equipment Cost
Plant Costing	Equipment Costs Plant Cost Multipliers (Microbial, Chemical)	Total Plant Cost
Material Cost/Revenue	Material/Energy Flows (e.g. kg/h, kW) Process Time (e.g. Unit Time, Production Time) Material/Energy Costs/Income (e.g. Cost/kWh)	Operating Costs Operating Incomes
Profitability	Time/Market (e.g. Depreciation Period, Discount Rate, Inflation) Project Financing (e.g. %Debt Finance, Loan Period, Loan Rate)	Gross Margin Return On Investment (ROI) Payback Period Net Present Value (NPV) Internal Rate of Return (IRR) Loan Life Coverage (LLCR)

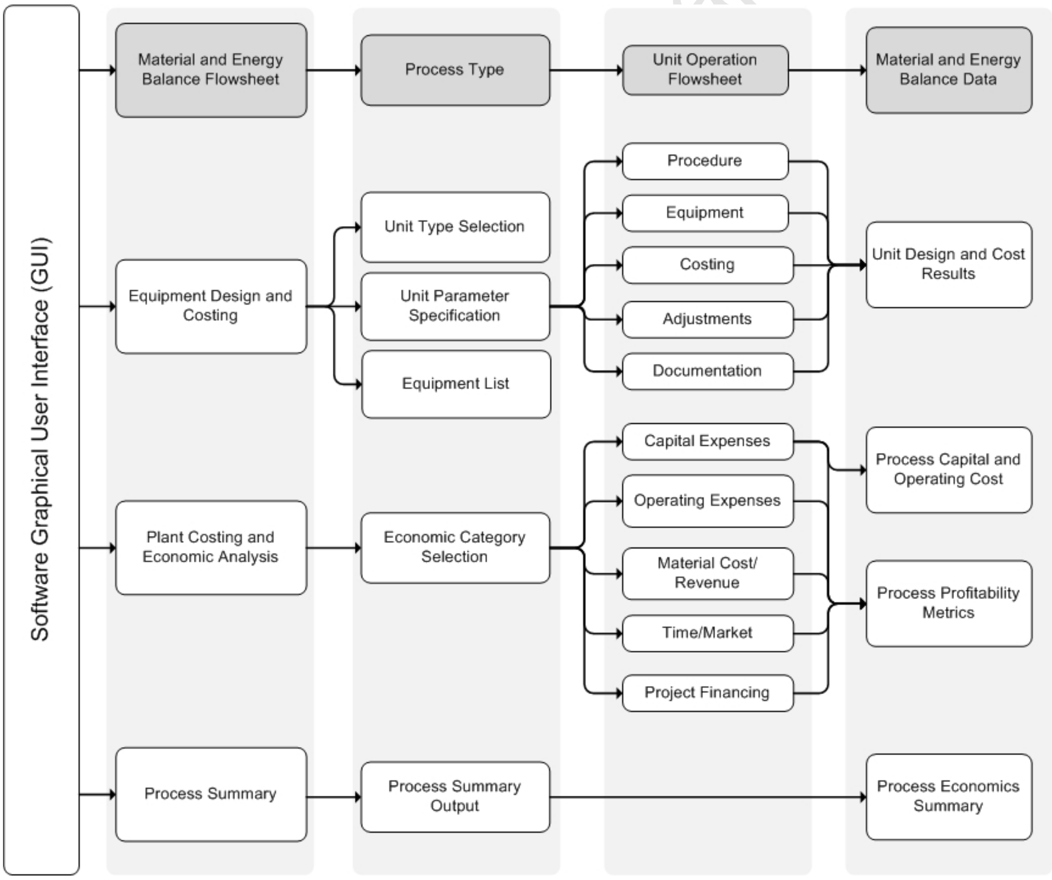

Figure 5.4: Generic flowsheet development (Grey Blocks = Harding (2008); White Blocks = this thesis)

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5.2.3 Case Study Simulation - Comparison of Process Alternatives

5.2.3.1 Selection of the Case Study

A simplified approach to bioprocess design was demonstrated using a case study for the large-scale production of citric acid. The process was selected to represent large-scale bioprocess manufacture, and demonstrate environmental and economic assessment methodologies as a basis for multi-criteria decision making in comparing process alternatives. The production process was selected on the basis of being a well-established process and consideration of process alternatives was supported by well-developed and extensive literature. Relative to other bioprocess industries (e.g. pharmaceuticals, fine chemicals), the process consumes large quantities of raw materials and energy and has a significant environmental impact by way of resource consumption, energy use and process emissions. Comparison of process modifications and alternative processing routes, using sustainability criteria developed in this thesis, was therefore deemed justifiable by the scale of the production process in terms of tonnage as well as the importance of citric acid production as a commodity chemical.

5.2.3.2 Process Simulation and Comparison

As described in Section 5.1.1 and Section 5.1.2 the need for early stage bioprocess simulation and subsequent environmental and economic assessment as a method for process screening is well justified. The basis for decision-making and process screening is generated by quantifying the environmental and economic performance of the design alternatives for the case study, using specific performance metrics. Using these results, the design practitioner may compare process alternatives, evaluate trade-offs between economic and environmental metrics and implement new and improved technologies.

The generic flowsheet model was used to simulate the process options and provide the necessary inventory data for an environmental assessment using a Life Cycle Assessment (LCA) approach. The process cost and profitability assessment was completed using the extended generic flowsheet described in Section 5.2.2. The input data to the process simulation was based primarily on detailed process data obtained from literature. The case study included two primary comparisons shown in Figure 5.5 and Figure 5.6. Firstly, results of the generic flowsheet were compared to results from a detailed simulation package for a single process option. The comparison was used to assess the ability of the simulation tool to model a production process, based on limited input data. Details of the methodology used for the comparative simulation are provided in Chapter 6 alongside the case study.

In the second comparison, alternative processing routes were compared. The comparison was used to assess the environmental and economic performance of alternative processing routes producing the same product. Details of the methodology used for the comparative simulation are provided in Chapter 7 alongside the case study. The comparison aimed to demonstrate the ability of a simplified first-estimate generic flowsheet tool to support systematic multi-criteria sustainability assessment with which to compare bioprocess design alternatives.

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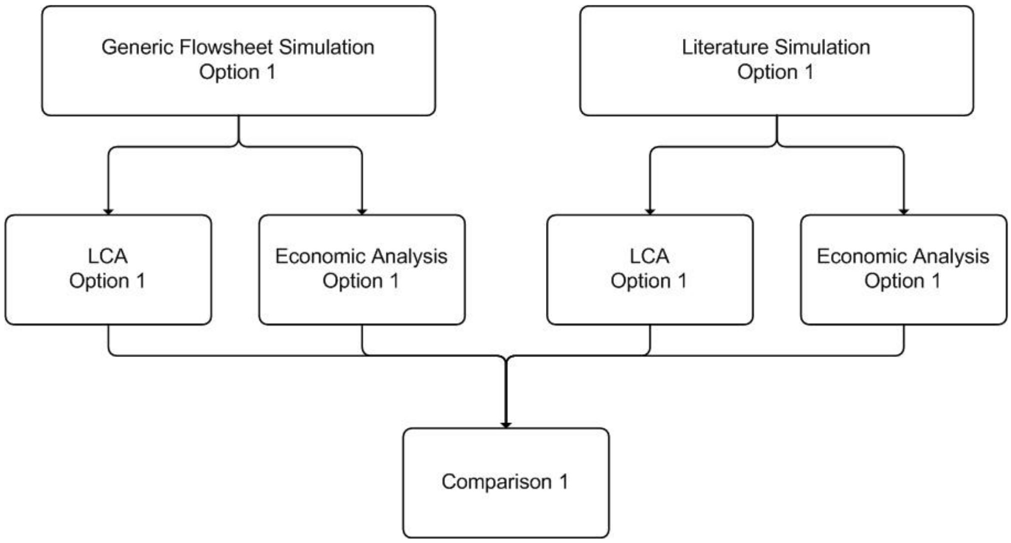

Figure 5.5 Comparison of generic flowsheet simulation tool to detailed simulation data

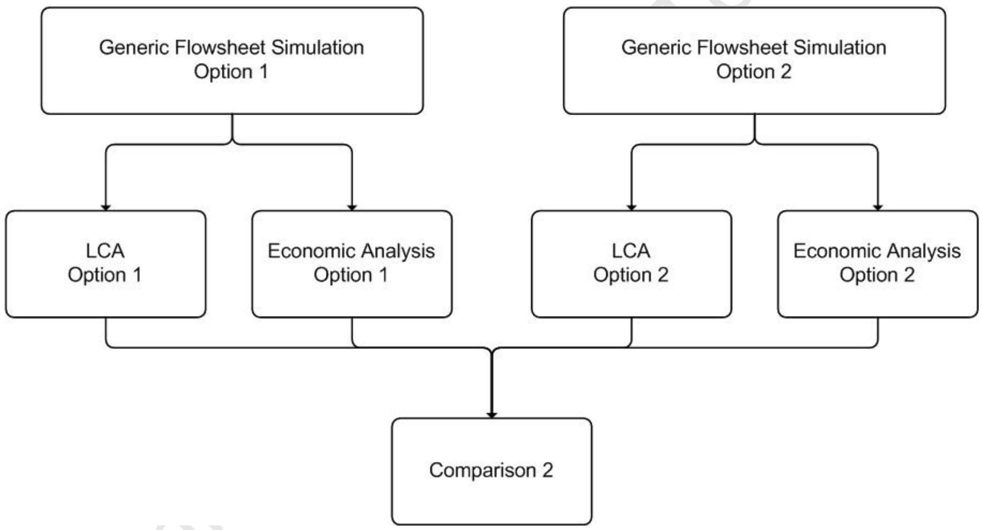

Figure 5.6 Comparison of process alternatives using the generic flowsheet

5.2.3.3 Life Cycle Assessment (LCA) as a Measure of Environmental Sustainability

The environmental assessment was performed using a cradle-to-gate Life Cycle Assessment (LCA) approach. LCA allows for an evaluation of the environmental impact of the product system as a number of discrete interdependent stages with the cumulative impacts across all stages allowing a comprehensive analysis of the environmental performance of the system. LCA is not location specific and provides results which can be compared between process options. LCA has a well-developed literature base giving the methodology a clear and systematic definition. Although a ‗cradle-to-grave' approach is typically used in LCA (Curran, 1996), the process was evaluated from cradle-to-gate, since identical products are considered. As prescribed by ISO 14040 standards, the assessment framework shown in Figure 5.7 consists of four main phases, namely *goal and scope definition*, *inventory analysis (LCI)*, *impact* *assessment (LCIA)* and *interpretation*. A detailed description of LCA methodology is provided in Appendix B.

The *goal definition* phase included details of the purpose of the LCA and the objectives to be achieved. The *scoping phase* provided details of the system boundaries and functional unit specific to the process under consideration. The *inventory analysis (LCI)* involved developing a flow diagram for the process, acquisition of the necessary data and definition of the material and energy inputs and outputs of the system in relation to the defined functional unit. The *impact assessment (LCIA)* phase was the quantitative and qualitative evaluation of the potential human health and environmental impacts of the material and energy flows identified in the *LCI* phase. Using defined mid-point impact categories such as global warming, ozone layer depletion and acidification, the *LCIA* converted the *LCI* data, using equivalency factors into these categories. *Interpretation* involved discussion of the results obtained and comparison of mid-point values for process alternatives.

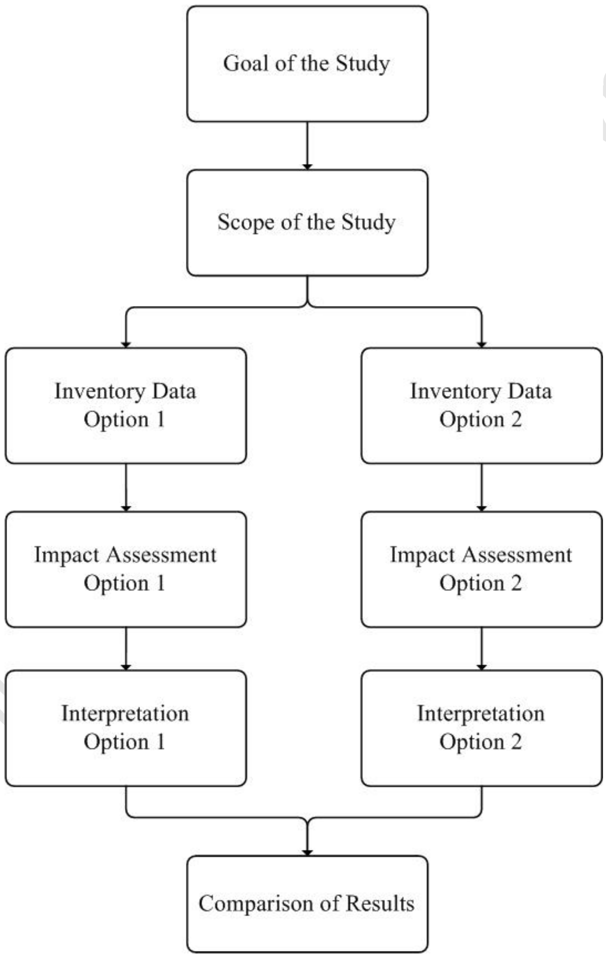

Figure 5.7 LCA phases for comparing process alternatives

The LCA was performed using the software package SimaPro v7 (PRé Consultants B.V.) and the CML 2 Baseline 2000 v2.03 assessment method. The inventory data obtained from the generic flowsheet LCA was compared to the similar inventory data from a detailed literature study using a commercial simulation package. The process options were compared on the basis of mid-point indicators, details of which are provided alongside the case studies. The use of mid-point indicators as an effective approach to LCIA and is able to reduce the complexity of the assessment. As discussed by von Blottnitz & Curran (2006), midpoint assessment minimised the amount of forecasting and effect modelling. It also results in simplifying communication of the results with fewer categories to report.

The impact categories generated by the CML 2 Baseline 2000 v2.03 assessment method include contributions to *abiotic depletion*, *global warming potential*, *ozone layer depletion, human toxicity, fresh water aquatic ecotoxicity, marine aquatic ecotoxicity, photochemical oxidation, acidification* and *eutrophication*. These are commonly used impact categories in LCA (CML, 2001; SAIC, 2006) and are consistent with the objectives of the study in providing a basis for broad environmental performance evaluation. Impacts due to land use and water consumption are not included in CML methods, but are included in the discussion of the impact categories for the defined system.

5.2.3.4 Cost and Profitability Assessment as a Measure of Economic Sustainability

An approach similar to that of the environmental assessment was used for the economic performance assessment. The assessment framework consists of four main phases, namely *goal definition, scope and design basis definition*, *unit sizing, capital cost estimation, operating cost estimation, profitability assessment*, and *interpretation.* The *goal definition* phase included details of the purpose of the economic analysis and the objectives to be achieved. The *scope and design basis* definition provided the necessary detail regarding system boundaries, production scale and process specific assumptions. The *unit sizing*, *capital cost estimation*, *operating cost estimation* and *profitability assessment* phases include specification and selection of the necessary unit parameter data and procedures as described in Section 5.2.2 above. The unit sizing results were used as the basis for capital and operating cost estimations. The capital and operating costs were used as the basis for a profitability analysis. Similarly to the environmental assessment, i*nterpretation* involved discussion of the results and comparison of economic assessment metrics.

The extended generic flowsheet model was used to size the major equipment. Details of the models and calculations used to size specific equipment units are provided in Appendix B. The material and energy balance results from the flowsheet model developed by Harding (2008) provided the basis for unit sizing. The major equipment purchase costs are estimated by scaling similar units, using Equation 5.1 for which relevant cost data is available from literature (Biwer *et al.*, 2006; Peters *et al.*, 2003; Sinnot, 1999, Atkinson & Mavituna, 1983).

$$Cost_2 = Cost_1 \left(\frac{Capacity_2}{Capacity_1} \right)^n \qquad 5.1$$

The value of the scaling factor (n) is typically taken as 0.6. The application of the 0.6 scaling factor for most purchased equipment is however an oversimplification (Peters *et al.,* 2006) and more accurate scaling factors are used where available. Specific factors for purchased equipment are mostly taken from Peters *et al.* (2003) and Perry *et al.* (1997). The data used for estimating purchased equipment cost are adjusted for inflation to current prices by a cost index as shown in Equation 5.2.

$$Cost_2 = Cost_1 \left(\frac{Inflation\ index_2}{Inflation\ index_1} \right) \qquad 5.2$$

The total purchase cost of all the major equipment is used as the basis for the total capital investment requirement of the plant. Lang factors, commonly used to obtain order-of-magnitude cost estimates

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(Peters *et al.*, 2003), are used to determine the costs for equipment erection, piping, instrumentation, insulation, electrical systems, ancillary buildings and site development. Lang factors are also used to calculate the fixed plant capital cost, including estimates for design and engineering and contractors‘ fees (Sinnott, 1999).

The costs of the raw materials and utilities required for the process are estimated using the material balance data and estimates for material and utility prices at the time of the study. Prices of raw materials are estimated from various literature sources such as *The Chemical Marketing Reporter* and previous studies using similar material inputs. Additional operating costs are estimated by various means, and details of the methodologies used are included in Chapter 7 alongside the case study and Appendix B.

The profitability metrics are determined from the results of the capital and operating cost estimates. As shown in shown Table 5.2 above, the metrics include *gross and net profit*, *payout period*, *return on investment (ROI)*, *net present worth* (NPV), *internal rate of return* (IRR) and *loan life coverage ratio* (LLCR). Details of the input variables and calculations used for the profitability analysis are provided in Appendix B.

5.3 Summary

This chapter has expanded on the literature provided in Chapters 2, 3 and 4 in justifying the need for early stage process simulation and the provided evidence for the value of early stage bioprocess simulation and subsequent environmental and economic assessment as a method for process screening. Two research hypotheses were formulated from these arguments and provided the necessary context with which to develop the overall research hypothesis. A case study approach to testing the hypothesis and answering key questions presented in Chapter 1 was justified. A research methodology was presented. The methodology included development of the generic flowsheet model, comparison of process alternatives using Life Cycle Assessment and comparison of process alternatives using order-of-magnitude cost and profitability metrics. The research methodology is applied in subsequent chapters in order to answer key questions, test the research hypothesis and provide recommendations.

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PART 2

CASE STUDY: LARGE-SCALE PRODUCTION OF CITRIC ACID

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CHAPTER 6

Overview of Citric Acid Case Study

6.1 Structure of the Case Study

In the case study, a review of the relevant literature for industrial production of citric acid is presented. Traditional microbial citric acid production, including commonly employed production techniques, culture conditions, product recovery and effluent disposal is discussed. The study demonstrates the application of a generic flow sheet simulation tool, developed and implemented in MS-Excel, to simulate the production of citric acid. The results were compared to data presented in the literature on the production of citric acid using maize starch as the main raw material (Biwer *et al.*, 2006). The results of the generic flow sheet material and energy balance calculations were used as the inventory data for a Life Cycle Assessment (LCA) of the production process. The LCA was compared to an LCA generated from inventory data obtained from the literature case study. Further, in the case study the industrial production of citric acid was compared using the alternative substrates beet molasses and maize starch as the main raw material input. The process capital and operating costs were determined by variant-based cost estimation using order-of-magnitude estimates. The overall task was aimed at bringing together various tools for sustainable bioprocess assessment in early stages of process development

6.2 Introduction to Citric Acid Production

Citric acid (2-hydroxypropane-l,2,3-tricarboxylic acid) was first isolated from lemon juice by Scheele (1784). About 100 years after citric acid had been isolated from citrus fruits, it was synthesized from glycerol by Grimocex & Adam (1890). Wehmer (1893) was the first to observe citric acid as a microbial metabolite from certain molds, providing the basis for the development of the citric acid industry. The work of Currie (1917) opened the way for industrial production of citric acid through fundamental investigations on the production capabilities of *Aspergilli sp*. The first successful operation began in 1923 in New York; later large-scale fermentation processes were developed in Czechoslovakia, the United Kingdom, Belgium and Germany. The first citric acid fermentations were performed using surface cultures using *Aspergillus niger* and a media prepared from sucrose and inorganic salts. Processes using cheaper beet molasses were soon introduced. The surface culture processes were largely replaced by submerged fermentation processes during the 1950‘s. These processes utilised *A. niger* and a media based on either purified glucose syrups, or beet or cane molasses (cited by Mislon & Meers, 1985). Currently, citric acid is produced primarily by submerged microbial techniques, using molasses as the carbon source. In recent years there has been significant interest in the use of alternative agricultural products as carbon sources for citric acid production by *A. niger*. These include maize, apple and grape pomace, pineapple, mandarin orange, brewery wastes and citrus and kiwi fruit peel (Soccol *et al.*, 2006).

Citric acid is used in a wide range of applications, of which food and beverage products are the most dominant. In 2004 approximately 64% of U.S. citric acid was used in food and beverage products, 22% for detergents and cleaning products and 10% for pharmaceutical and nutritional products. The remaining supply is used in cosmetics and toiletries and various other applications (Soccol *et al.*, 2006). The global production of citric acid in 2007 was about 1.5 million tons (Graff, 2007). The demand for citric acid is expected to grow strongly with an estimated annual increase of 2-3% (Graff, 2007) and a market value exceeding \$2 billion (Partos, 2005). The selling price has increased relatively sharply in recent years due

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to rising raw materials and energy costs, an increased demand and recent shutdowns of some production facilities. The shut-downs have occurred primarily as a result of European and North American manufacturers coming under pressure due to the fierce competition from Chinese manufacturers and high raw material costs (Heller, 2007). The increase in the cost of raw materials is mainly as a result of lack of supply, due to changes to the EU sugar regime, reduced area for production due to bad weather and increased demand for crops for the biofuel industry.

6.3 Microbial Production of Citric Acid

6.3.1 Microorganisms

Fungi, yeasts and bacteria have been used for the production of citric acid. An extensive review of microorganisms used for citric acid production can be found in literature (Rohr & Kubicek, 1987; Krishnan, 1999; Soccol *et al.*, 2006). Currie (1917) showed that certain strains of *A. niger* are able to produce large amounts of citric acid in a nutrient medium, with a high sugar and mineral salts concentration. This established the basis for commercial production of citric acid. Commercial production of citric acid using yeasts was predominant during the 1960‘s and 1970‘s, when raw materials such as hydrocarbons were relatively cheap. Although various carbon sources can be used with yeasts, a substantial quantity of unwanted isocitric acid is produced. *Aspergillus niger* thus remains the preferred organism for commercial production due to its ease of use, high yields and ability to ferment a variety of raw materials.

6.3.2 Substrates

Substrates including molasses, starchy materials and hydrocarbons have been used for commercial citric acid production (Grewal & Kalra; 1995). Molasses is commonly used due to its relatively low cost and high sugar content (40-55%) in the form of sucrose, glucose and fructose. Beet molasses is the most widely used raw material in the United States and Europe. South American and Caribbean plants mostly use sugar cane, while smaller Caribbean plants use citric wastes from citrus fruits (Krishnan, 1999). Beet molasses is preferred to cane molasses due to its lower content of trace metals and improved production yields (Soccol *et al.*, 2006). Although molasses is commonly employed for the commercial production of citric acid, starch substrates have been used as an alternative to molasses. Miles Laboratories Inc. in the USA developed a process for citric acid production from starch hydrolysates. Various publications report similar processes for the production of citric acid from a starch substrate (Marending, 1992; Rohr & Kubicek, 1987; Sarangbin & Watanapokasin, 1998; Mourya & Jauhri, 2000; Haq *et al.*, 2002).

6.3.3 Culture Conditions

The culture conditions for improved production of citric acid have been reported extensively in literature and patents. To achieve a high production rate and yield of citric acid, essential nutrients such as carbon, nitrogen, phosphorus, growth factors and trace metals need to be present. Aeration, pH and temperature need to be tightly controlled to attain optimal production conditions.

6.3.3.1 Carbon Source

The type and concentration of the carbon source strongly influences the production of citric acid (Hossain, 1992; Kristiansen, 1999; Soccol *et al.*, 2006; Papagianni, 2007). Generally, only sugars, rapidly taken up by the microorganism, are suitable for a high yield production. Polysaccharides need to first be hydrolysed before being used as a carbon source due the slow rate of uptake relative to the rate of sugar

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catabolism. The use of sucrose is preferred over glucose, fructose and lactose due to *A. niger's* ability to rapidly hydrolyze the sugar with an extracellular mycelium bound invertase. *A. niger* can also readily utilise mannose, xylose and arabinose and produce citric acid, but at lower yields than from glucose (Maddox, 1985). Careful consideration should be given in selecting the carbon source due to factors such as costs or the need for pre-treatment (Soccol *et al.*, 2006). In the case of cane molasses, the presence of certain metals (iron, calcium, magnesium, manganese, zinc), which inhibit citric acid accumulation, makes pre-treated necessary. The concentration of the carbon source influences the production rate, final product yield and the growth of the microorganism (Papagianni, 1995).

6.3.3.2 Nitrogen Source

Complex media such as molasses contain nitrogen compounds; hence a nitrogen source is rarely added to the nutrient medium. High purity media are usually supplemented with nitrogen in the form of ammonium sulphate or ammonium nitrate. It is generally preferred that ammonium salts are used, since their consumption results in a decrease in pH, a prerequisite for citric acid production (Grewal & Kalra, 1995; Papagianni, 2007). Ammonium sulphate has been shown to prolong the growth phase, while a shorter growth period is associated with the addition of ammonium nitrate (Grewal & Kalra, 1995). The nitrogen concentration required for the production is typically 0.1 to 0.4 g/L (Rohr & Kubicek, 1987, Soccol *et al.*, 2006). A high concentration of nitrogen favours fungal growth and sugar consumption, but decreases the amount of citric acid formed (Grewal & Kalra, 1995).

6.3.3.3 Phosphorus Source

Increased fungal growth and reduced citric acid production is promoted with a higher phosphorous concentration. Phosphate concentration is cited as an important factor in morphological development and process productivity (Ali, 2004), however contradiction exists in the literature (Papagianni, 2007). Shu and Johnson (1948) concluded that phosphate limitation is not required for acid accumulation, while Kubicek and Rohr (1987) showed that citric acid accumulation is achieved by phosphate limitation. Conversely, excess phosphorous results in decreased carbon dioxide fixation and hence the stimulation of mycelium growth (Soccol *et al.*, 2006). Potassium dihydrogen phosphate has been reported as the most suitable phosphorous source. A phosphorous concentration of between 0.5 and 5 g/L is required for maximum citric acid production (Grewal & Kalra, 1995).

6.3.3.4 Trace Elements

The presence of trace elements in the is one of the main factors influencing the product yield (Clark & Clark & Tymchuk, 1965; Horitsu, 1966; Sanchezm *et al.*, 1970; Wold & Suzuki, 1976; Hossain & Ahmed, 1992). Metal ions including manganese, zinc, iron, copper and magnesium have been shown to affect the accumulation of citric acid (Clark & Horitsu, 1966; Grewal & Kalra 1995). Although trace metal ions are desirable for production, too high a concentration of any of the metals can reduce production. Accurate control of the metal concentrations especially Mn^{2+} is necessary to improve acid production, especially in submerged production. Manganese is important in a number of cell functions, including cell wall synthesis, sporulation and production of secondary metabolites (Papagianni, 1999).

6.3.3.5 Lower Alcohols and Other Compounds

The addition of lower alcohols, such as methanol, ethanol and iso-propanol, decreases growth but increases citric acid production (Moyer, 1953). The optimum concentration of ethanol/methanol is about 1-3% (Grewal & Kalra, 1995; Haq *et al.*, 2002). Although the mechanism by which ethanol and methanol improve citric acid production is not clear, a number of possibilities have been suggested (Chaudhary *et*

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al., 1989; Grewal & Kalra, 1995; Haq *et al.*, 2002; Yaykash *et al.*, 2005). The addition of lipids to the nutrient medium can also result in an increase in citric acid production (Mills *et al.*, 1963; Grewal & Kalra, 1995). The fats and oils are used as a carbon source. Adham (2001), showed that improved production from beet molasses is obtained with the addition of olive, sunflower, or maize oils, added at 4% (v/v) doses.

6.3.3.6 Aeration

The rate of oxygen supply to the aerobic process has a major effect on the productivity and yield of citric acid. Improved product yields and reduced process times are achieved with higher aeration rates. The oxygen concentration should be maintained above 25% of saturation (Soccol *et al.*, 2006). Critical dissolved oxygen tension (DOT) of 8-12% and 10-15% of air saturation should be maintained for the respective growth and product phases (Grewal & Kalra, 1995). Although high aeration rates are typically required for improved product yields, a study by Prado *et al.* (2004) showed that citric acid production using solid-state techniques (SSF) is favoured at low aeration rates (0.18 m^3 /kg dry carbon source) due to limited biomass growth. It was observed that strongly aerated mediums increased sporulation, in turn reducing acid accumulation. Aeration rates in industrial operations are typically between 0.1 vvm and 1 vvm, increasing as demand increases with biomass growth (Grewal & Kalra, 1995).

6.3.3.7 pH

To maximise the production of citric acid, a low pH is essential. The working pH is typically pH 2.2 to 2.6 (Rohr & Kubicek, 1987), but is dependent on the carbon source. Above pH 4, the production of oxalic acid is accelerated, reducing the yield of citric acid. The pH should be well defined and optimised according to the microorganism, substrate and production technique (Soccol *et al.*, 2006).

6.3.4 Industrial Production Techniques

Biological production of citric acid is the most economical and widely used method for production. Industrially bioprocesses account for approximately 90% of the world‘s supply of citric acid. This can be achieved by surface, submerged and solid state techniques.

6.3.4.1 Surface Process

Early industrial manufacture was dominated by the classic surface process, whereby fungal mycelium is grown as a mat on the surface of a liquid medium in shallow trays arranged in shelves. Typically, the trays have a capacity of 50 to 100 L and a surface area of 5 m^2 with a depth ranging from 5 to 20 cm (Kristiansen *et al.*, 1999). The medium consists of sucrose, most commonly beet and sugar cane molasses. The molasses is diluted to approximately 15% sucrose medium and the pH adjusted to 5-7. The required nutrients are added and the medium is sterilised and pumped into the shallow pans. The medium is inoculated with spores, as a liquid suspension or in a sterile air stream. Further, aeration is important for both oxygen supply and temperature control. During the maximal growth stage, aeration of 10 vvm is required to ensure adequate heat removal. The bioreaction is completed after 8 to 15 days with a production of about 1 kg/m^2 per day and a 75% yield based on initial sugar concentration (Kristiansen *et al.*, 1999). On completion the mycelial mat is removed from the product liquor and washed to recover residual citric acid. Although the surface process was commercially profitable for many years, it is relatively labour intensive and requires a large production area. The process is still employed on a small to medium scale due to the comparatively lower capital and energy requirements. The process was largely replaced by the submerged process in the 1940‘s.

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6.3.4.2 Submerged Process

The submerged process is used extensively and accounts for approximately 80% of world production. The process offers high productivity, reduced labour costs, reduced space requirements, and lower risk of contamination (Kristiansen *et al.*, 1999; Soccol *et al.*, 2006). The bioreaction is performed using both conventional stirred tank reactors and air-lift reactors. The air-lift reactor is increasingly preferred due to its lower cost, larger capacity, simple operation, reduced risk of contamination and improved conditions for working with suspended solids. The vessels are typically constructed from stainless steel and coated with a protective layer to avoid corrosion and presence of trace metals in the fermentation medium. Although batch, fed-batch or continuous mode can be used, batch mode is most common. Industrial stirred tanks typically have a capacity of 50-150 m^3, where air-lift capacities can range from 200 m^3 to 950 m^3.

6.3.4.3 Solid-state fermentation

Solid-state production, also known as the Koji process, is the solid-state equivalent of the surface process. It was developed in Japan, primarily as a result of readily available rice bran and fruit wastes. It is a relatively small-scale process. The carbohydrate source is sterilised using steam and moistened to about 70% water. The watery paste, at an initial pH of 5.5, is placed in shallow pans and spray-inoculated with spores. The temperature is maintained at 28-30 °C for 4 to 5 days to completion under optimal conditions (Rohr & Kubicek, 1987). Low yields result from difficulty in controlling process parameters and the presence of trace elements (Grewal & Kalra, 1995).

6.3.5 Product Recovery

The citric acid-containing spent culture is separated from the biomass using filtration or centrifugation. Recovery of citric acid by direct crystallisation is not possible due to the presence of unwanted impurities from the raw materials and autolysis of the microbial cells (Grewal & Kalra, 1995). Intermediate purification steps follow biomass removal. The *lime-sulphuric* process or a *solvent extraction* process is used, depending on the carbon source.

6.3.5.1 Lime-Sulphuric Process

The lime-sulphuric process method is commonly employed in industrial applications. A simplified process diagram is shown in Figure 6.1. Tricalcium citrate is precipitated by the addition of lime to the product-containing solution. The precipitation typically takes place at about 50 °C for 20 minutes (Pazouki & Panda, 1998), after which the slurry is filtered from the solution. The citrate containing filter cake is reslurried and treated with dilute sulphuric acid to form soluble citric acid and insoluble calcium sulphate. The calcium sulphate is removed by filtration and washed to recover entrained citric acid. Trace amounts of calcium and metal cations are removed by ion-exchange. In some processes, the solution is decoulorised by activated carbon adsorption. The citric acid is recovered from the aqueous solution by evaporative crystallisation, followed by filtration or centrifugation. Crystallisation takes place below 37 °C, above which anhydrous citric acid is formed. The product is then dried and classified.

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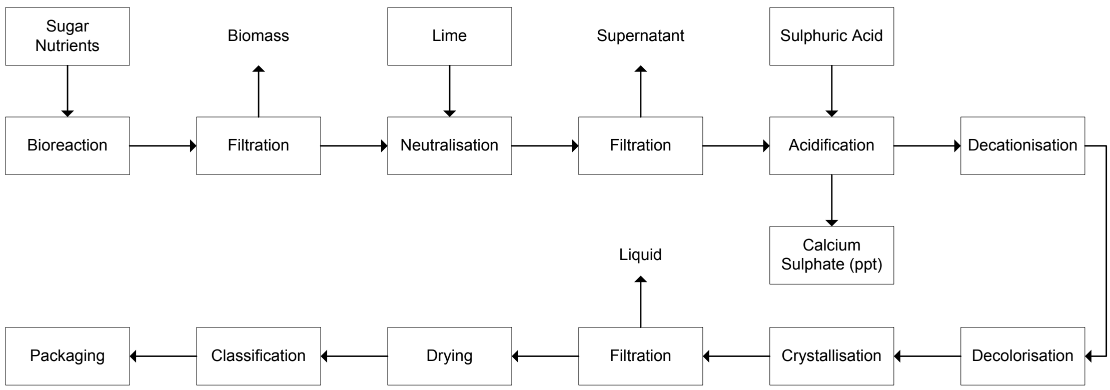

Figure 6.1 Lime sulphuric recovery process for citric acid (Adapted from Kirk-Othmer, 2004)

6.3.5.2 Liquid Extraction Process

In the liquid extraction process, citric acid is extracted into organic solvents. A simplified process diagram is shown in Figure 6.2. High selectivity is achieved with high molecular weight aliphatic amines and phosphorus-bonded oxygen-donor solvents (Pazouki & Panda, 1998). This is followed by re-extraction of the citric acid from the organic solvent phase into water. The two extraction steps are arranged as a multistage countercurrent system and differ mainly in temperature (Kirk-Othmer, 2004). The aqueous citric acid solution is further purified by activated carbon adsorption and recovered by evaporative crystallisation. Citric acid recovered by a solvent extraction process is suitable for use in food and pharmaceutical products (Grewal & Kalra, 1995).

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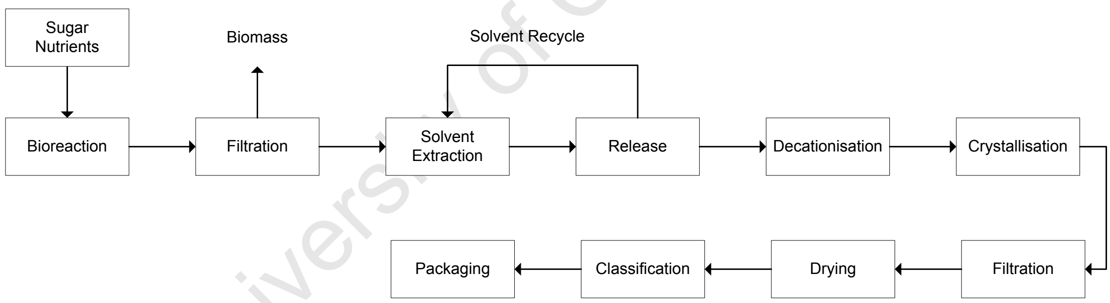

Figure 6.2 Solvent extraction recovery process for citric acid (Adapted from Kirk-Othmer, 2004)

6.3.5.3 Other Recovery Methods

More sophisticated methods such as electrodialysis, ultrafiltration and liquid membranes have been investigated to reduce cost and eliminate the large amount of calcium sulphate generated from the traditional precipitation process. Electrodialysis enables separation of salts from a solution and simultaneous conversion into corresponding acids and bases. It has been demonstrated at lab scale and shows improved economy (Grewal & Kalra, 1995; Soccol *et al.*, 2006). However, the method requires optimisation before industrial scale implementation is feasible (Kristiansen *et al.*, 1999). Recovery by ultrafiltration and nanofiltration has also been investigated (Visacky, 1996). Potential benefits include reduced energy consumption, elimination of waste materials, and the possibility of a continuous purification process. This technology requires optimisation and validation on a large scale before it can successfully displace current recovery technology (Kristiansen *et al.*, 1999).

6.3.6 Effluent Disposal

The disposal of effluent materials from industrial citric acid production process is an increasing concern from a cost and environmental viewpoint (Kristiansen *et al.*, 1999). With greater emphasis on the mitigation of potential environmental impacts, manufacturers are continuously faced with the trade-offs between cost reduction and process emissions. Calcium sulphate is most often discharged to landfill sites or the ocean. The filtrate from precipitation operations of the process using molasses typically has a high biological oxygen demand (BOD) and requires treatment before release. Traditional biological wastewater treatment practices, such as anaerobic digestion, are commonly applied.

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CHAPTER 7

Process Model and Simulation: Citric Acid Production

7.1 Introduction

In this chapter the material and energy balance data for the citric acid production process, obtained by means of computer simulation, is presented. The flowsheet model for microbial processes developed by Harding (2008) was used to generate first estimate material and energy balance data for the citric acid production process. The data provided the basis for both an environmental and economic evaluation of the process. The material and energy balance data for the citric acid process using starch as its main raw material generated in this manner was compared with process data obtained from the simulation data of Biwer *et al.* (2006). The generic flowsheet tool was then used to compare the production of citric acid using starch to that using beet molasses as the main raw material. The comparison was used to investigate the environmental and economic performance of the starch process relative to the traditional production process using molasses.

7.1.1 Application of the Generic Flowsheet to the Current Study

This case study presents a comparison of citric acid production from two feedstocks, namely maize starch and beet molasses. Input data required for the generic flowsheet simulation of the process was adapted from case studies and common citric acid process data reported in the literature. Firstly, the simulation tool was used to compare the results to values from a detailed simulation package for the production of citric acid from starch (Biwer *et al.,* 2006). The exercise aimed to determine the ability of the simulation tool to model a production process, based on limited input data. The results were compared to the results obtained from a detailed simulation package, commonly used in early stages of process development.

As presented by Harding (2008), certain critical process data is required to ensure an acceptable estimate of material and energy balances. The critical process input data required by the generic flowsheet was obtained from the simulation presented by Biwer *et al.* (2006). Similarly to the model developed for the starch process, the production of citric acid from beet molasses was simulated. The input data was gathered from various literature sources and critical input data was used where appropriate. Default input data was used for inputs that had limited effect on the process results. The detailed simulation by Biwer *et al.* (2006) provides the necessary data for simulation of the starch process, while process data reported in the literature was used for the molasses process simulation. If detailed simulation data, similar to that provided by Biwer *et al.* (2006), is not available, the data requirements may be supplied by laboratory studies or similar process studies presented in the literature.

7.2 Citric Acid Production - Starch Process

Biwer *et al.* (2006) developed a process model for citric acid production using starch based primarily on the data obtained from Marending (1992). This model was used as the basis for process simulation using the generic flowsheet developed by Harding (2008). The theoretical basis for the model developed by Biwer *et al.* (2006) is described. This was used as the basis for the input data to the generic flowsheet and subsequent simulation of citric acid production from maize starch.

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7.2.1 Model Development

7.2.1.1 Upstream Processing - Starch Hydrolysis Model

In the first step of the Biwer *et al.* (2006) process simulation, the majority of the starch was partially hydrolysed to dextrin. A number of proteins and fatty acids were found as impurities during this step. It was assumed that there was no moisture in the starch and that it contained 1% proteins and 1% fats. The ash content of the starch was not considered. The reaction scheme for the hydrolysis of the starch to dextrin, containing five glucose units, is given in Equation 7.1.

$$(C_6H_{10}O_5)_x + \frac{x}{5}H_2O \rightarrow \frac{x}{5}(C_6H_{10}O_5)_5H_2 \qquad 7.1$$

To produce 1 kg of dextrin 978.3 g of pure starch is required. The fats and proteins contained in the starch are accounted for in Equation 7.2. The detailed elementary composition of the fats and proteins is not known.

$$998.3(C_6H_{10}O_5)_5 + 27.4\,H_2O \rightarrow 1000(C_6H_{10}O_5)_5H_2O + 10\,\mathrm{Proteins} + 10\,\mathrm{Fats} \qquad 7.2$$

7.2.1.2 Bioreaction

The overall metabolic reaction scheme for citric acid production from starch using *A. niger* is shown in Figure 7.1

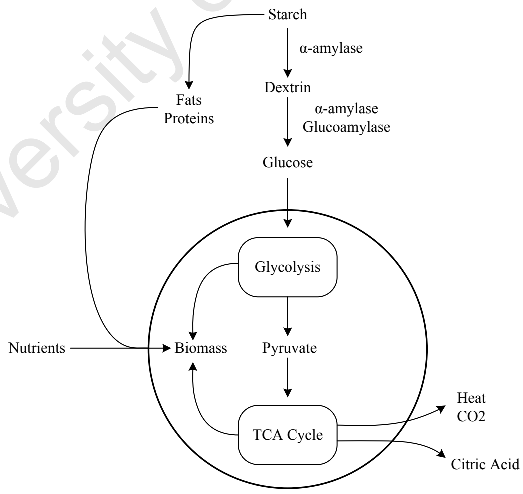

Figure 7.1 Reaction scheme for citric acid production from starch using *A. niger*

Following starch hydrolysis, the medium pH and temperature was adjusted and the inoculum added. The bioreaction took place in three distinct reaction steps. The first step was the conversion of the dextrin to glucose as shown in Equation 7.3. It is assumed that 100% of the starch was converted to glucose.

$$(C_6H_{10}O_5)_5 \cdot H_2O + 4\,H_2O \rightarrow 5\,C_6H_{12}O_6 \qquad 7.3$$

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In the second step, biomass growth occurs with concomitant consumption of the glucose, nitrogen source and mineral nutrients, under nitrogen limitation. In the final step, the glucose is transformed into citric acid. Glucose is consumed through the steps to produce citric acid, from biomass and provide energy *via* the TCA cycle.

Although the reactions were simulated as distinct steps, these reactions occur simultaneously. Biwer *et al.* (2006) calculated the ammonium nitrate added from the work of Marending (1992). The composition of protein impurities contained in the media was estimated by Biwer *et al.* (2006), using data by Nielsen & Villadsen (1994) and Creighton (1993) and the amount of nitrogen available from the protein impurities was determined. Approximately 25% of the nitrogen is obtained from the protein impurities, while the remainder is obtained from ammonium nitrate. The nitrogen content of the biomass was determined to be 5.5%. The elementary composition of the biomass was estimated as $CH_{1.72}O_{0.55}N_{0.09}P_{0.002}$ (Schlieker, 1995). Citric acid is formed via the TCA cycle and secreted into the media. The final product is expressed as citric acid monohydrate as shown in Equation 7.4. The reaction ends when the glucose concentration drops below 0.2 g/L.

$$C_6H_{12}O_6 + 1.5\,O_2 \rightarrow C_6H_8O_7 \cdot H_2O + H_2O \qquad 7.4$$

Biwer *et al*. (2006) determined the carbon dioxide formed from a carbon balance for the bioreaction and Equation 7.5, using the initial and final glucose and biomass concentrations.

$$C_6H_{12}O_6 + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O \qquad 7.5$$

7.2.1.3 Product Recovery

The process model by Biwer *et al.* (2006) used ultrafiltration for recovery. Impurities from the raw materials and autolysis of the microbial cells (Grewal & Kalra, 1995) must be removed prior to crystallisation. The ultrafiltration unit operation was used to remove cell debris and proteins that remained in the product solution following the removal of biomass from the bioreaction broth. A laboratory scale two-stage membrane was described by Vasacky (1996) to purify citric acid from spent culture following cultivation of *A. niger* on sucrose (Kristiansen, 1999). A polysulphone membrane with molecular weight (M_w) cut-off 10 000 Da was used in the study. Approximately 3% of the product, 14% of reducing sugars and 100% of the proteins was removed by the membrane.

Following removal of suspended organic materials from the product solution, citric acid can be purified using the traditional recovery steps. On ion-exchange, magnesium and potassium ions are bound to the cation resin. The solution is concentrated in an evaporative crystallisation step at about 37 °C. Citric acid monohydrate is formed at temperatures of about 20-25 °C. It is necessary to ensure that the temperature is maintained below the transition temperature of 36.5 °C, above which anhydrous product is formed. The crystals are recovered by vacuum filtration or centrifugation. The mother liquor from the filtration step is recycled back to the crystallisation step to increase yield from the solution. A portion is purged to avoid the build-up of impurities. Marending (1992) reported a typical crystallisation yield of 98%. The product crystals are dried in a fluidised bed drier below 36.5 °C and packaged.

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7.2.1.4 Effluent Disposal

The major effluent streams from the process are:

- Waste biomass
- Process water from washing and cleaning
- Retentate from filtration stages
- Gaseous emissions

The biomass removed is typically sold as a protein-rich animal feed byproduct. The aqueous effluent from the process has a relatively high biological oxygen demand (BOD) of 12 000-14 000 mg/l. It is generally treated using anaerobic digestion, producing fuel gas as a by-product.

7.2.2 Generic Flowsheet Process Simulation

The process model data presented by Biwer *et al.* (2006) formed the basis of the simulation. The process flow diagram for the model is shown in Figure 7.2. The generic flowsheet simulation was used to provide estimates for material and energy inputs and outputs per ton of citric acid produced for comparison with the more detailed simulation model presented by Biwer *et al.* (2006).

7.2.2.1 Upstream Processing

As shown in Figure 7.2, starch was supplied with α-amylase and water to the bioreactor (RX-001) for starch hydrolysis. Sterilisation of the raw materials was not required as the temperature profile of hydrolysis meets the requirements for sterilisation. The hydrolysis of starch to dextrin and finally glucose was not directly included in the generic flowsheet simulation. The flowsheet does not allow for substrate pre-treatment and requires that a pure substrate feed be specified. The material and energy input required for starch hydrolysis was obtained from the Biwer *et al.* (2006) simulation and added to the mass and energy balance results generated by the generic model. The feed to the bioreactor was specified as pure glucose. The total volume of material in the bioreactor is based on a specified final biomass concentration ($C_{x,final}$) and iteratively adjusted with the addition of water to the reactor.

The input values for upstream processing for the generic flowsheet is shown in Table 7.1. The amount of starch required was determined from Equation 7.1 and Equation 7.3 and included in the material and energy balance results. The remaining nutrient requirements (NH_4NO_3, KH_2PO_4) were prepared (TK-001), sterilised (ST-001) and supplied to the bioreactor. The inoculum preparation of *Aspergillus niger* is not shown for simplification purposes. The medium was sterilised by heat exchange with saturated steam at 140 °C for 20 minutes. The condensed steam was used to pre-heat the medium to 110 °C before sterilisation.

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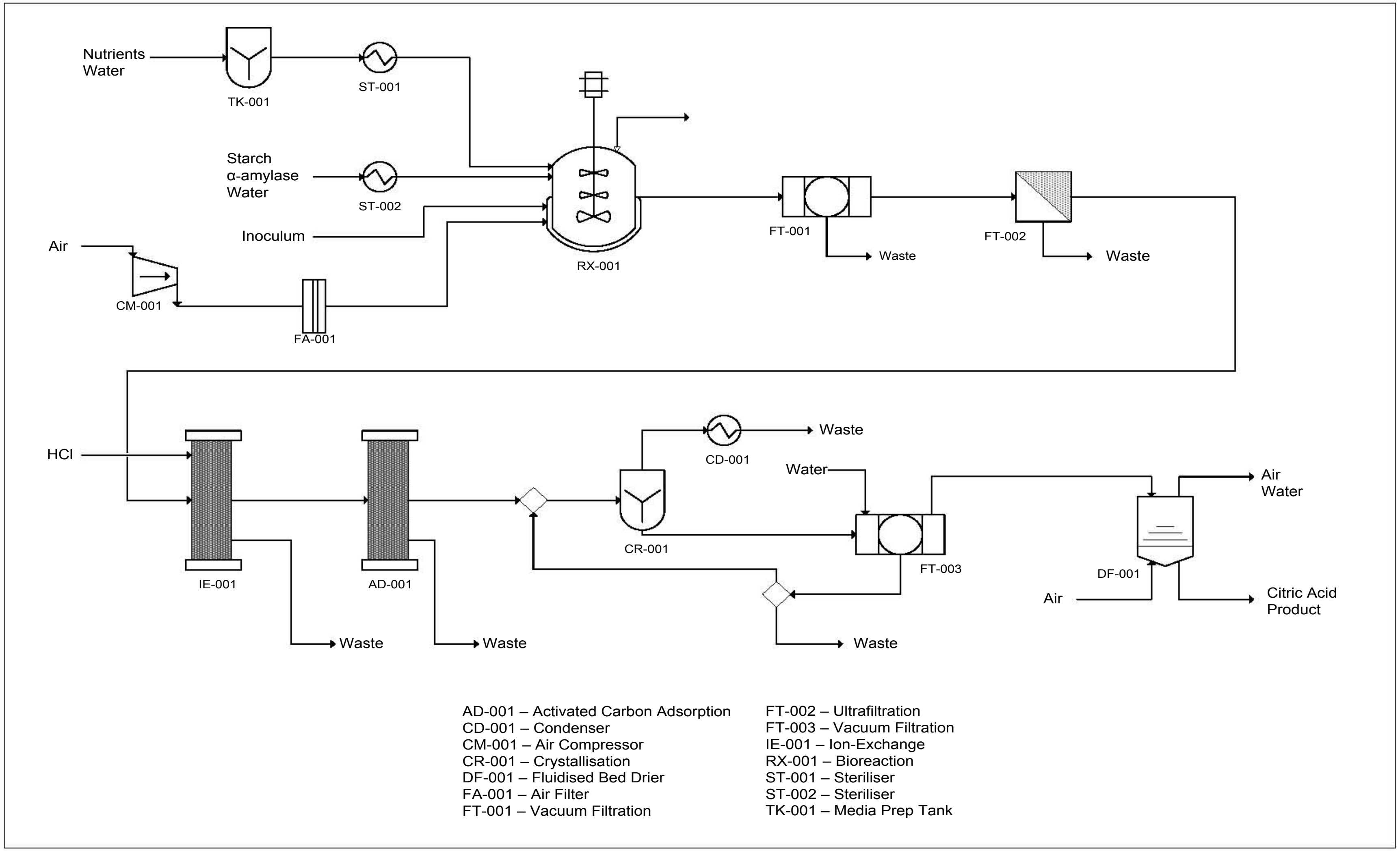

Figure 7.2 Process flow diagram for the production of citric acid from starch

Table 7.1 Upstream operating conditions for citric acid production from starch

Operating Parameter	Value	Unit
Materials		
Biomass ash content: $CH_{1.72}O_{0.55}N_{0.09}P_{0.002}$	0.0	%
Carbon source excess: $C_6H_{12}O_6$	[1.0]	%
Nitrogen source excess: NH_4NO_3	[5.0]	%
Phosphorus source excess: KH_2PO_4	[5.0]	%
Sterilisation		
Media temperature in	[110]	°C
Sterilisation temperature	[140]	°C
Steam temp	[150]	°C
Holding time	[20]	min
Oxygen supply		
Oxygen supply	Air (21% O_2:79% N_2)	-
Compression	Two-stage with intercooling	-
Cooling medium	Cooling water	-
Initial pressure	[101]	kPa
Final pressure	500	kPa
Polyentropic efficiency	0.9	-

[ ] Default values provided in simulation model

7.2.2.2 Bioreaction

The simulation inputs for the bioreaction are shown in Table 7.2. The media and inoculum were added to an aerated batch-reactor (RX-001). Product to biomass ratio, yield coefficients, initial and final biomass concentrations and aeration requirements used in the generic flowsheet model were estimated from material flows in the simulation presented by Biwer *et al.* (2006). The biomass growth and product formation were calculated using chemical balances for oxygen, nitrogen, phosphorus and the specified yield coefficients for biomass growth ($Y_{x/s}$) and product formation ($Y_{p/s}$), according to Equation 7.6.

$$\begin{aligned} &\mathrm{CarbonSource} + \mathrm{OxygenSource} + \mathrm{NitrogenSource} + \mathrm{PhosphorusSource} \\ &\rightarrow \mathrm{Biomass} + \mathrm{Product} + H_2O + CO_2 + \mathrm{NitrogenExcess} + \mathrm{PhosphorusExcess} \end{aligned} \quad 7.6$$

The growth rate input data was used to calculate the reactor residence time (t_b) and subsequently, agitation energy. Default values were used for the maximum specific growth rate (μ_{max}) and limiting nutrient concentration (K_s). Monod growth kinetics was used to predict growth rate. Although the Monod equation is an oversimplification it serves as an acceptable estimate for the simulation. Harding (2008) suggested that opportunity exits to expand the kinetic model to include other rate expressions. Air was supplied to the bioreactor at 5 bar by a two-stage centrifugal compressor (CM-001) using cooling water (25 °C) for inter-stage cooling. Sterile air supply was assumed and sterilisation by filtration (FA-001) was not specified in the generic flowsheet simulation. Heat generation during aerobic growth was determined by a degree of reduction balance (Harding, 2008). Approximately 315 kJ/mol carbon produced was released. Isothermal reactor operation was maintained by heat exchange with chilled water (5 °C). The agitation power requirement was determined using a power per unit volume (P_v) value of 0.5 kW/m^3, based on the simulation by Biwer *et al.* (2006). Once production was complete, the culture, containing mostly citric acid, *A. niger* biomass, unutilised nutrients, fatty acids and proteins, was sent for downstream processing.

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Table 7.2 Bioreaction conditions for citric acid production from starch

Operating Parameter	Symbol	Value	Unit
Reactor Operation			
Mode		Batch	
Agitation power per unit volume	Pv	[0.5]	kW/m^3
Agitation efficiency	η	[0.9]	
Aeration rate	Ar	0.25	vvm
Antifoam		[1.0]	%v/v
Reactor Temperature			
Reactor temperature	Tr	32	°C
Ambient temperature	Ta	[20]	°C
Cooling medium temperature	Tc	[18]	°C
Growth Rate			
Initial biomass concentration	Cx,in	0.04	g/l
Final biomass concentration	Cx,final	17.1	g/l
Max. specific growth rate	μ_{max}	[0.5]	hr^{-1}
Limiting nutrient conc. giving half max. growth rate	Ks	[11.9]	mg/l
Yield coefficients			
Product to Biomass Ratio	P:X	6.64	
Biomass on substrate	Yx/s	0.12	g/g
Product on substrate	Yp/s	0.76	g/g
Biomass on oxygen	Yx/o	0.26	g/g

[ ] Default values provided in simulation model (Harding, 2008)

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7.2.2.3 Downstream Processing

The downstream unit operations are summarised in Table 7.3. The majority of the biomass (99%) was removed by a rotary vacuum filter (FT-001). The separated biomass was washed to minimise the loss of citric acid product from the process. Wash water was supplied to obtain a cake porosity of 0.4 %v/v. The liquid filtrate was sent to ultrafiltration (FT-002) where 99% remaining biomass, cell debris and proteins were removed. Energy consumption for ultrafiltration was 13 MJ/m^3 of throughput. Thereafter cation exchange (IE-001) removed 99% of magnesium and potassium ions from the solution. The cations were eluted from the column with 2-molar hydrochloric acid (0.09 %v/v). Although anions were not accounted for in the generic flowsheet model, Biwer *et al.* (2006) assumed that the anions pass through the column and do not to affect the crystallisation step. The product solution was passed through an activated carbon column (AD-001) for decolourization. The citric acid solution was crystallised (CR-001) by evaporation of the majority of the water, cooling and subsequent crystallization. Biwer *et al.* (2006) assumed that the citric acid was not completely crystallized with a single pass and a recycle was included to improve product recovery. The generic flowsheet does not allow for recycle streams and it was assumed that the citric acid was recovered with a single pass. The volumetric flowrate to the crystallisation unit was increased with the addition of water to account for flowrate of the recycle stream. The solution was concentrated by vacuum evaporation at 100 °C. The product was crystallised by cooling the outlet stream to 15 °C using chilled water. The residence time was 5.4 hours and the power consumption was 0.6 kW/m^3 of throughput. Caustic soda (0.002 %v/v) was added to prevent the evaporation of hydrogen chloride during crystallisation. The evaporated water from crystallisation was condensed by heat exchange (CD-001) with cooling water and assumed to be a waste. The citric acid crystals were recovered from the mother liquor and washed in a rotary vacuum filter (FT-003). It was assumed that a crystallisation recovery of 98% was achieved, based on the work by Marending (1992), presented by Biwer *et al.* (2006). The crystals were dried by specifying 95% removal of the entrained liquid.

Table 7.3 Downstream operating conditions for citric acid production from starch

Operation	Unit	Operating Conditions
Biomass Removal (Rotary Vacuum Filtation)	FT-001	Solids removed: 99% Citric acid to retained: 99% Cake Porosity: (0.4 %v/v)
Ultrafiltration	FT-002	Solids removed: 99% Citric acid retained: 99%
Ion-Exchange	IE-001	Waste fraction removed: 99% Citric acid to retained: 99% Elution: 2N HCL (0.09 %v/v)
Activated Carbon	AD-001	Waste fraction removed: 99% Citric acid retained: 99% Elution: Water (35 %v/v)
Crystallization	CR-001	Additive: NaOH (0.002 %) Product yield: 98% Heated temp: 100 °C Outlet temp: 15 °C Residence time: 5.4 h
Crystal Recovery	FT-003	Product recovery: 99% Wash water (55 %vv) Cake Porosity: (0.4 %v/v)
Product Drying	DF-001	Solids retained: 100% Liquid removal: 99%

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7.2.2.4 Waste Treatment

The simulation did not include the material and energy balances for waste treatment. It was assumed that all waste streams are sent to an off-site waste treatment plant.

7.2.2.5 Material and Energy Balance Analysis

The results of the process yield, material requirements and energy requirements, per ton of citric acid product (P), from the simulation by Biwer *et al.* (2006) and the generic flowsheet used in this study are shown in Table 7.4, Table 7.5 and Table 7.6 respectively. A comparison of the major material and utility requirements is shown in Figure 7.3. The results of both simulations show that the major input materials were water, starch, oxygen and nitrogen source. The main nutrient requirements to produce 1 ton of citric acid, were 1270 kg of starch, 18.8 kg of ammonium nitrate and 1.88 kg of potassium phosphate. The starch requirement for generic flowsheet simulation was determined from the glucose requirement of 1330 kg, assuming complete conversion of starch to glucose. Values for biomass production (*A. niger*), nitrogen source (NH_4NO_3) and phosphorus source (KH_2PO_4) of the simulations were in relatively good agreement. The overall product yield, shown in Table 7.4, was 0.79 kg citric acid / kg starch for the generic flowsheet simulation and 0.83 kg citric acid / kg starch for the Biwer *et al.* (2006) simulation. The bioreaction and process yield values of the generic flowsheet were higher than the Biwer *et al.* (2006) model. The input yield coefficient values for the generic flowsheet of 0.77 g citric acid / g glucose and 0.16 g biomass / g glucose, were based on the bioreaction product and biomass yield values of the Biwer *et al.* (2006) simulation. Assumptions with regards to improved downstream processing operations in the generic flowsheet resulted in improved process yields.

Table 7.4 Product yields of citric acid production process from starch

	Biwer *et al.* (2006)	Generic Flowsheet
Process Yield		
C-mol citric acid/C-mol glucose	61%	66%
kg citric acid/kg starch	79%	83%
Bioreaction Yield		
C-mol citric acid/C-mol glucose	65%	71%
kg citric acid/kg starch	84%	89%

The results of the material balance for the respective simulations differ for a number of the material inputs and outputs. The larger waste flowrates of glucose, ammonium nitrate and potassium phosphate in the generic simulation was due to the assumption that excess nutrients were fed to the process. The reduced starch requirement of the generic simulation is attributed to the higher yield due to improved process recoveries in downstream operations. The batch throughput for the generic flowsheet and Biwer *et al.* (2006) model was about 10 m^3 and 9.3 m^3 per ton product respectively. The higher bioreactor volume of the generic simulation was attributed to the slightly larger volume of water (6.3 %) charged to the bioreactor relative to the Biwer *et al.* (2006) simulation. The generic simulation based the amount of water added on the initial and final concentrations ($C_{x,in}$; $C_{x,final}$), while the simulation by Biwer *et al.* (2006) specified the amount of water to the bioreactor to give an average solids concentration of 1.1 g/L. The aeration rate had a significant influence on the results and the default aeration input was not used in the generic simulation. An aeration rate of 0.25 vvm was based on the value used in the Biwer *et al.* (2006) model. The air feed of 25.1 tons per ton of product in the generic model was higher than in the Biwer *et al.* (2006) due to the larger batch volume.

Variations in simulation results may also be attributed to the calculation approaches used by the simulation models. The generic flowsheet model based the bioreaction calculations on a product to biomass ratio and yield coefficients, while the model by Biwer *et al.* (2006) used explicit chemical reactions and a limiting nutrient concentration to determine the bioreaction outputs. The inherent difference in the models is likely to result in variations in the calculation results. Default values used for non-critical input variables are also a source of variations in calculation results.

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Table 7.5 Material balance for citric acid production from starch

	Biwer *et al.* (2006)		Generic Flowsheet	
Component	In (kg/ton P)	Out (kg/ton P)	In (kg/ton P)	Out (kg/ton P)
α-Amylase	1.27	1.27	1.27	1.27
Ammonium Nitrate	18.8		23.4	
Biomass	0.367	160	0.355	156
Carbon Dioxide		410		353
Chloride		0.635		0.692
Citric Acid Monohydrate		1000		1000
Citric acid loss		62.6		30.6
COD		280		300
Fats		10		
Glucose		1.88		13.2
Hydrogen Chloride	0.577		0.712	
Magnesium Sulphate	0.984			
Magnesium (dissolved)		0.199		
Nitrogen	18920	18920	19580	19580
Oxygen	5750	5240	5950	5490
Potassium Phosphate	1.88		1.86	
Potassium (dissolved)		0.540		0.025
Sodium Hydroxide	0.346		0.402	
Sodium (dissolved)		0.113		0.231
Starch	1270		1200	
Sulfate		0.786		
Water	13880	13880	13236	13236

Energy and utility requirements, summarised in Table 7.6, include electricity, steam, cooling water and chilled water. The process simulations compared relatively well with regards to energy and utility requirements, shown in Figure 7.3. In both process simulations, most of the electricity demand was used for air compression and mechanical agitation of the bioreactor. The aeration rate and bioreaction duration determined the electricity demand on the compressor. The generic flowsheet simulation required more electricity than the Biwer *et al.* (2006) simulation for aeration and agitation due to larger reactor volume. The detailed Biwer *et al.* (2006) simulation also included electricity requirements for general process operations, including material storage and administration. Steam was required for sterilisation, media heating, evaporation in the crystallisation unit and final product drying. Cooling water was used for compressor cooling and isothermal bioreactor operation. Chilled water was used to control the bioreactor temperature and to cool the product solution during crystallisation. The Biwer *et al.* (2006) simulation required additional cooling water of 14.9 m^3 per ton of product due to the inclusion of the starch hydrolysis step, not accounted for in the generic flowsheet simulation. The simulation also required considerably more cooling water for compressor cooling. This was attributed to the compressor models used in each of the simulations. The model used in the generic flowsheet assumed a two stage centrifugal compression with inter-stage cooling, operating at 90 % polyentropic efficiency. The Biwer *et al.* (2006) model used a single stage compression model operating at an overall efficiency of 80%.

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Table 7.6 Energy and utility requirements for citric acid production from starch

Utility Input			Biwer *et al.* (2006)	Generic Flowsheet
Electricity		MJ/ton P	**9500**	**10890**
Adsorption	(AD-001)			1.83
Aeration	(CM-001)		4554	5374
Agitation	(RX-001)		2634	2897
Crystallisation	(CR-001)		48.4	39.7
Drying	(DF-001)		240	227
Filtration	(FT-001)		401	564
Filtration	(FT-002)		121	127
Filtration	(FT-003)		89	42
Ion Exchange	(IE-001)			1.83
Other			1433	
Sterilisation	(FA-001)			1620
Steam		kg/ton P	**13600**	**14100**
Bioreactor	(RX-001)		1880	1880
Crystallisation	(CR-001)		10360	11060
Drying	(DF-001)		1220	780
Sterilisation	(ST-001/2)		127	375
Cooling water		m^3/ton P	**2630**	**2230**
Aeration	(CM-001)		1507	1190
Bioreactor	(RX-001)		14.9	
Condenser	(CD-001)		1100	993
Sterilisation	(FA-001)		9.83	48.7
Chilled water		m^3/ton P	**377**	**206**
Bioreactor	(RX-001)		342	197
Crystallisation	(CR-001)		34.6	8.96

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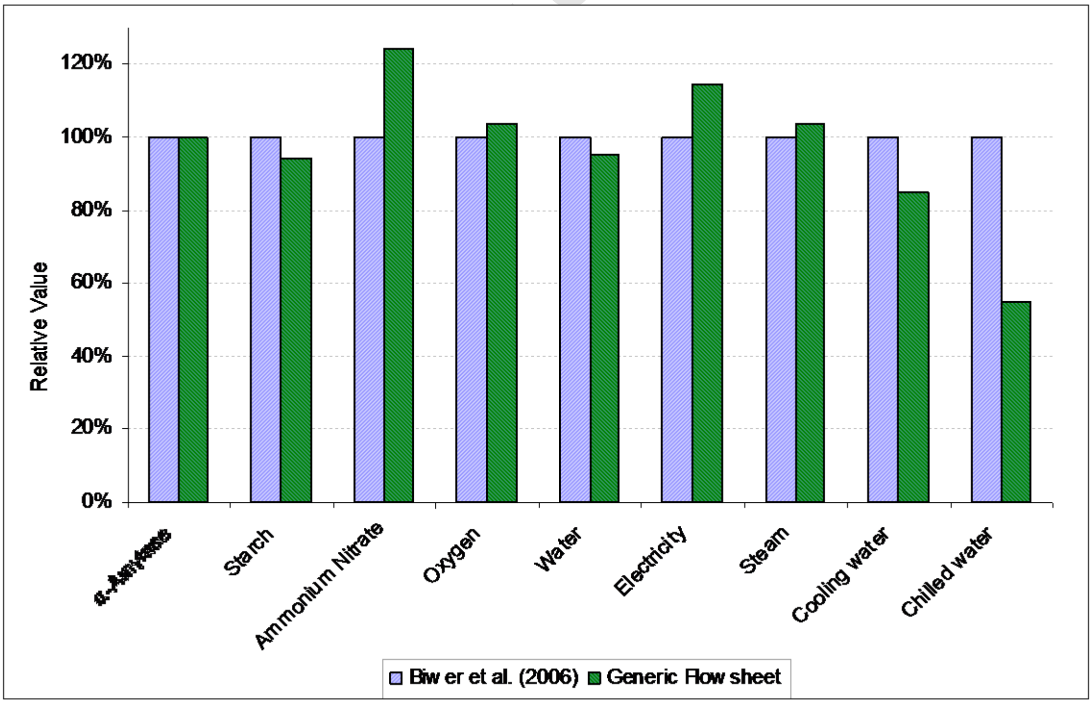

Figure 7.3 Comparison of material, energy and utility requirements for citric acid production

7.2.2.6 *Sensitivity Analysis*

To determine the sensitivity of the process model, variations in input data were investigated similarly to the approach used by Harding (2008). The process generic flowsheet model presented above, using critical inputs values from Biwer *et al.* (2006), was compared to simulations using progressively more default data from the generic flowsheet. The rationale was to demonstrate the ability of the model to model the production system with an increased reliance on default model data. The critical input data for each of the four simulations is shown in Table 7.7. Scenario 1 corresponds to the critical input data obtained from the simulation by Biwer *et al.* (2006). In scenario 2 the default aeration rate and compression pressure were used. In scenario 3 default bioreaction parameters were included. The final scenario considered the default values for biomass removal (FT-001), ultrafiltration efficiency (FT-002) and crystal recovery (FT-003).

The material and energy balance results for successive scenarios are compared in Table 7.8 and Table 7.9 respectively. The relative comparison of process inputs and outputs for each scenario is shown in Figure 7.4 and Figure 7.5 respectively. The comparisons show the relative effect of critical variables on process results. In scenario 2, a default aeration rate, 10 times the minimum stoichiometric requirement of oxygen, was used. A default pressure of 300 kPa was used for compression. There was an 80% reduction in oxygen supply using the default aeration value. The simulation flowsheet does not consider mass transfer limitations in the bioreaction model and a reduction in aeration had no influence on material requirements, other than oxygen supply. The reduction in aeration and compression pressure had a significant influence on electricity and cooling water requirements.

Table 7.7 Input parameters for citric acid production form starch

Input Parameter	Symbol	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Unit
Oxygen Supply						
Aeration rate	Ar	0.25	[10 x min. stoich.]	[10 x min. stoich.]	[10 x min. stoich.]	vvm
Compression Pressure	-	500	[300]	[300]	[300]	kPa
Bioreaction						
Initial biomass conc.	-	0.04	0.04	[1]	[1]	g/l
Final biomass conc.	$C_{x,final}$	17.1	17.1	[16.7]	[16.7]	g/l
Biomass on substrate	Yx/s	0.12	0.12	[0.445]	[0.445]	g/g
Product on substrate	Yp/s	0.76	0.76	[0.8]	[0.8]	g/g
Biomass on oxygen	Yx/o	0.26	0.26	[1.18]	[1.18]	g/g
Downstream Operations						
Biomass removal (FT-001)		99	99	99	[95]	%
Ultrafiltration efficiency		99	99	99	[95]	%
Crystal recovery (FT-003)		99	99	99	[95]	%

[ ] Default values provided in simulation model (Harding, 2008)

Electricity demand for compression was approximately 40% lower and cooling water approximately 50% lower. Scenario 3 considered the default inputs for the bioreaction model. The default final biomass concentration ($C_{x,\ final}$) and product yield coefficient ($Y_{p/s}$) compared relatively closely to the values used in scenario 1. More inoculum was required due the initial biomass concentration of 1 g/l, while slightly more biomass was produced due to the higher biomass on substrate yield coefficient ($Y_{x,s}$). Approximately 20% more starch and phosphorus source (KH_2PO_4) was required. Scenario 4 considered the effect of default recovery values for certain filtration operations. A default value of 95% was used for biomass filtration, ultrafiltration and crystal recovery. The default values had a significant influence on most of the

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major process inputs and outputs in comparison to previous scenarios. Starch, nitrogen and phosphorus requirements were increased by about 50% relative to scenario 3; owing to substantial product loss Additional water was required in the bioreactor due to increased biomass production. Similarly, the minimum oxygen requirement was increased to improve citric acid production. Citric acid loss from the process was increased by about 16 fold relative to previous scenarios.

Table 7.8 Material balance for citric acid production from starch

Component	Scenario 1 (kg/ton P)		Scenario 2 (kg/ton P)		Scenario 3 (kg/ton P)		Scenario 4 (kg/ton P)	
	In	Out	In	Out	In	Out	In	Out
Ammonium Nitrate	23.4		23.4		23.4		34.9	
Biomass	0.355	156	0.355	156	9.410	165	14.0	244
Carbon Dioxide		353		353		818		1217
Citric Acid Product		1000		1000		1000		1000
Citric acid loss		30.6		30.6		30.6		481.3
COD		300		300		319		808
Glucose		13.2		13.2		16.4		24.4
Hydrogen Chloride	0.712		0.712		0.712		0.810	0.810
Oxygen	5950	5490	910	910	1585	793	2359	793
Potassium Phosphate	1.86		1.86		1.86		2.77	
Sodium Hydroxide	0.402		0.402		0.433		0.458	
Starch	1200		1200		1488		2214	
Water	13236	13236	13236	13236	11315	11315	16388	16388

The effect of default inputs on energy and utility requirements is shown in Table 7.9. The reduction in aeration rate and compression pressure in scenario 2 resulted in a 40% reduction in electricity and a 50% reduction in cooling water. The energy requirement was further reduced in scenario 3 due to a decrease in electricity for agitation. The electricity required for agitation was 46% lower due to the shorter batch time (τ_r) as a result of increased initial biomass concentration ($C_{x,initial}$). The minimum required aeration rate was increased due to the higher biomass on substrate yield coefficient ($Y_{x/s}$). The cooling water requirement was increased by approximately 16% due to increased aeration. Similarly, scenario 4 had a significant influence on the majority of the energy and utility requirements. Electricity requirements were increased for agitation due the larger batch throughput, in comparison to scenarios 2 and 3. The electricity required for aeration was increased by 50% compared to scenario 3 due to increased biomass production. The electricity required for agitation was lower than in scenario 1 due to the shorter batch time affected by the initial biomass concentration ($C_{x,initial}$). The steam requirement for crystallisation was increased by 30% due the higher volumetric flowrate. Similarly, 30% additional cooling water was required in the condenser. Additional chilled water was required for the bioreaction and crystallisation due to the higher liquid throughput through the units. These data and comparisons illustrate that order of magnitude estimates can be obtained on a limited data set; however, data generated was refined by key process information.

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Table 7.9 Energy and utility requirements for citric acid production from starch

Utility Input			Scenario 1	Scenario 2	Scenario 3	Scenario 4
Electricity		MJ/ton P	**10890**	**6110**	**4370**	**6094**
Adsorption	(AD-001)		1.83	1.83	1.83	.
Aeration	(CM-001)		5374	590	1029	1530
Agitation	(RX-001)		2897	2897	1566	2330
Crystallisation	(CR-001)		39.7	39.7	39.7	39.7
Drying	(DF-001)		227	227	227	227
Filtration	(FT-001)		564	564	564	905
Filtration	(FT-002)		127	127	127	144
Filtration	(FT-003)		42	42	42	42
Ion Exchange	(IE-001)		1.83	1.83	1.83	1.83
Sterilisation	(FA-001)		1620	1620	777	874
Steam		kg/ton P	**14100**	**14100**	**14100**	**17900**
Bioreactor	(RX-001)		1880	1880	1880	2150
Crystallisation	(CR-001)		11060	11060	11060	14430
Drying	(DF-001)		780	780	780	780
Sterilisation	(ST-001/2)		375	375	351	522
Cooling water		m3/ton P	**2230**	**1171**	**1360**	**1680**
Aeration	(CM-001)		1190	130	317	337
Condenser	(CD-001)		993	993	993	1295
Sterilisation	(FA-001)		48.7	48.7	48.7	48.7
Chilled water		m3/ton P	**206**	**206**	**254**	**373**
Bioreactor	(RX-001)		197	197	242	361
Crystallisation	(CR-001)		8.96	8.96	11.67	12.30

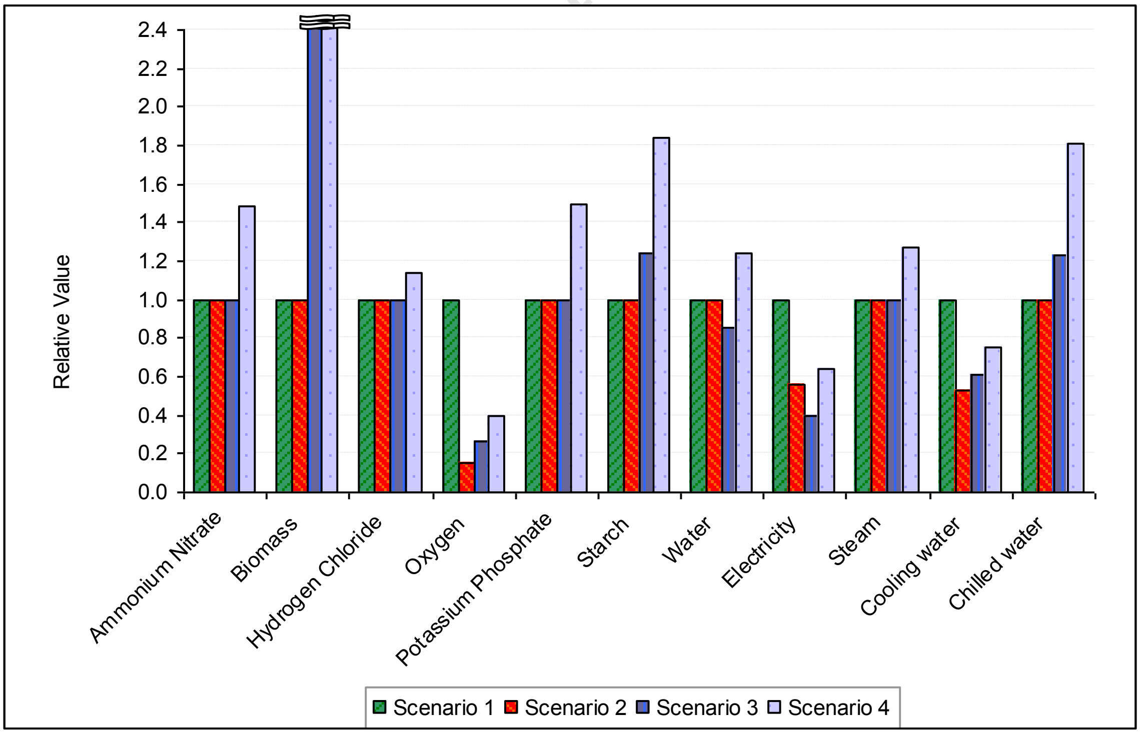

Figure 7.4 Comparison of material and energy inputs for citric acid production

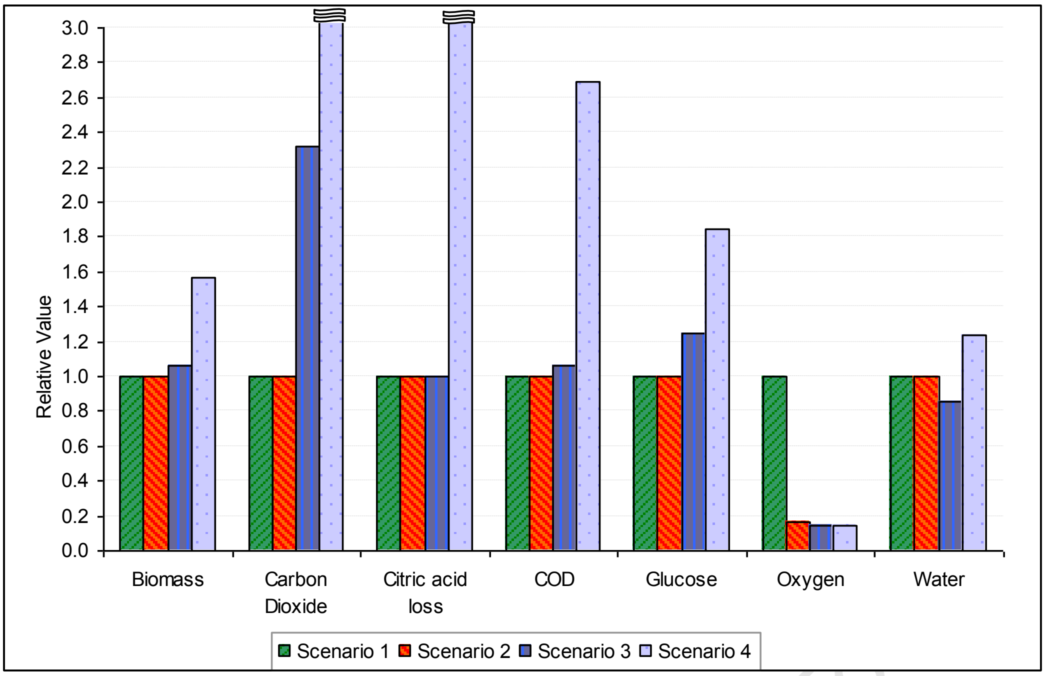

Figure 7.5 Comparison of material outputs for citric acid production

7.3 Citric Acid Production - Molasses Process

A process simulation, using the generic flowsheet model, was developed for the production of citric acid using molasses as the main raw material. The simulation input data was based on common industrial processes for the production of citric acid from molasses (Martin, 1952; Atkinson and Mavituna, 1983; Krishnan, 1999).

7.3.1 Model Development

7.3.1.1 Raw Materials and Treatment with Potassium Ferrocyanide

The composition and nutrient content of some of the most common molasses raw materials used in citric acid fermentation is shown in Table 7.10. The molasses is treated with ferrocyanide to make the carbon source suitable for the bioreaction. The ferrocyanide is added to cold molasses mash and heated so that sterilisation and ferrocyanide treatment are effected simultaneously.

In a process described by Martin (1952), beet molasses is placed in a mixing vessel and diluted to 12% (w/w sugar) with tap water. The medium is adjusted to pH 8.0 with 50% hydrochloric acid. Potassium ferrocyanide ($K_4Fe(CN)_6 3H_2 0$) is added as a 10% aqueous solution at a temperature of 80 °C. Hustede *et al.* (1972) suggested that potassium ferrocyanide be added to a minimum concentration of 0.2 g per litre with a typical concentration ranging from 0.8-1.5 g per litre. The medium is sterilised and cooled to the fermentation temperature of 20-35 °C. The medium is re-adjusted to pH 8.0 with hydrochloric acid. Potassium phosphate ($K_2HPO_4 3H_2 0$) is added as the nitrogen source to a concentration of 0.5 g/L in the form of a sterile 5% aqueous solution. A precipitate with potassium ferrocyanide is formed, but is not removed from the medium. The mash is transferred to the bioreactor and oxygenated for 2 hours at a rate of approximately 100 ml oxygen per minute. The preliminary oxygenation ensures oxygen saturation, oxidise any readily oxidisable compounds and combats severe foaming during fermentation (Martin, 1952).

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Table 7.10 Composition and nutrient content of molasses (Curtin, 1983).

Component	Cane	Beet	Citrus
Total Solids (%)	75	77	65
Specific Gravity	1.41	1.41	1.36
Total Sugars (%)	46	48	45
Crude Protein (%)	3.0	6.0	4.0
Nitrogen Free Extract (%)	63	62	55
Total Fat (%)	0.0	0.0	0.2
Total Fiber (%)	0.0	0.0	0.0
Ash (%)	8.1	8.7	6.0
Calcium, (%)	0.8	0.2	1.3
Phosphorus, (%)	0.08	0.03	0.15
Potassium, (%)	2.4	4.7	0.1
Sodium, (%)	0.2	1.0	0.3
Chlorine, (%)	1.4	0.9	0.07
Sulfur, (%)	0.5	0.5	0.17

7.3.1.2 Bioreaction

The bioreaction is similar to the process using starch as the main raw material input. Common practices for submerged production of citric acid using molasses are used as the basis for the bioreaction model in the process simulation (Atkinson and Mavituna, 1983; Martin, 1952; Krishnan, 1999, Roehr, 1981). The overall metabolic reaction scheme for citric acid production from molasses using *A. niger* is shown in Figure 7.6. Sucrose is split into glucose and fructose by invertase extracellularly and only the monosaccharides are taken up by the microorganism (Kristiansen *et al.*, 1999).

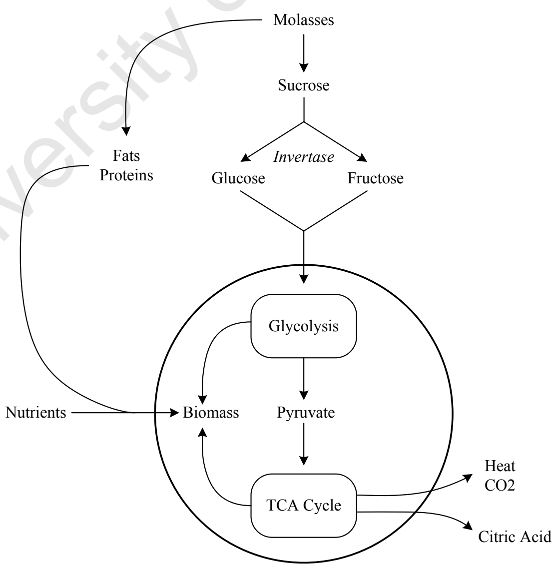

Figure 7.6 Reaction scheme for citric acid production using molasses and *A. niger*.

As described in Section 6.3, citric acid production occurs in two stages. In the first stage the inoculum is grown, followed by citric acid production. The typical medium composition used for both stages is shown in Table 7.11.

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Table 7.11 Media for the production of citric acid from *A. niger.* (Atkinson and Mavituna, 1983).

	Sporulation medium (g/l)	Production medium (g/l)
Sucrose	140	140
Bacto agar	20	0
Ammonium nitrate	2.5	2.5
Potassium dihydrogen phosphate	1	2.5
Magnesium sulphate.7H2O	0.25	0.25
Cu^{2+}	0.00048	0.00006
Zn^{2+}	0.0038	0.00025
Fe^{2+}	0.0022	0.0013
Mn^{2+}	0.001	0.001

A summary of specific media compositions, operating conditions and process yields obtained from various literature sources is given in Table 7.12. In the general process approach the inoculum preparation is added to the nutrient medium at 2-4% (v/v) (Martin, 1952; Ali, 2004) following the preliminary oxygenation in the upstream processing section. Initially, the culture is at pH 5-7, which falls to pH 1.5-2 during the growth phase. The culture is maintained at pH 2.8-4 with the addition of sulphuric acid (Krishnan, 1999; Ali, 2004). The reaction temperature is usually maintained at 28-33 °C, below which the reaction rate is considered too slow. Above 33 °C, undesired oxalic acid formation occurs.

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Table 7.12 Review of reaction conditions for the production of citric acid from molasses

Operating Parameter	Symbol	Atkinson & Mavituna, 1983	Krzystek, 1996	Ali, 2004	Roehr *et al.*, 1981	Martin, 1956	Waszczuk, 1987	Clark, 1963
Media								
Carbon source		12% Molasses Sugar	10% Sucrose	15% Cane Molasses Sugar	15-18% Sucrose	12% Beet Molasses Sugar	12-15% Sucrose	12% Beet Molasses Sugar
Nitrogen source		0.22 %NH_4NO_3	0.2%	0.2% NH_4NO_3	0.37% NH_4OH			
Phosphorus source		0.22% KH_2PO_4	0.015%	0.1% KH_2PO_4	0.01% KH_2PO_4			
Reactor								
Type		Tower	Air-lift	Stirred Tank	Stirred Tank	Tower	Air-lift	Stirred Tank
Reactor temperature	Tr	28-33 °C	32 ± 0.1 °C	30 °C	30 °C	20-35 °C	30-32 °C	31 ± 1 °C
Aeration rate	Ae	0.5-1.0 vvm	0.1-0.7 vvm	1.0 vvm	0.25 vvm	0.1-0.5 vvm		0.3 vvm
Agitation	Ag			160 rpm	Flat Blade (1650 rpm)			Flat 4-Blade (400-600
pH	-	2.5-3.5	2.5 ± 1	6	3.0		2.5	
Growth Rate								
Initial biomass conc.	$C_{x,in}$		1% v/v	4% v/v		2% v/v		
Final biomass conc.	$C_{x,final}$			14.6 g/l				
Max. growth rate	μ_{max}			0.017 hr^{-1}				
Limiting nutrient conc.	Ks							
Yield coefficients								
Biomass on substrate	$Y_{x/s}$		0.13 g/g	0.135 g/g	0.4 g/g		0.126 g/g	
Product on substrate[a]	$Y_{p/s}$	0.72 g/g	0.75 g/g	0.702 g/g	0.8 g/g	0.72 g/g	0.85 g/g	
Biomass on oxygen	$Y_{x/o}$		0.42 g/g					

[a]Based on available sugar for production of anhydrous citric acid

Mycelia are grown in a growth medium for 3 to 4 days and then placed in the production reactor. Air is sparged to the vessel at 0.25 to 1.2 vvm. Aeration rates lower than 1 vvm may be used (Atkinson and Mavituna, 1983), but higher rates are beneficial to production (Rohr & Kubicek, 1987). Production is complete after 4 to 5 days with about 65-70% of the sugar converted (Krishnan, 1999). Atkinson and Mavituna (1983) suggest that citric acid yield is highly dependent on the purity of the molasses substrate used. A yield of 68% (weight of acid produced/weight of sugar used) is typically achieved using molasses which has been treated with ferrocyanide.

The yield coefficients and growth kinetics were available from a number of literature sources (Roehr *et al.*, 1981; Waszczuk, 1987; Krzystek, 1996; Ali, 2004). Product yield coefficients ($Y_{p/s}$) of 0.7-0.85 g/g and biomass yield coefficients ($Y_{x/s}$) of 0.14-0.4 are typical. Roehr *et al.* (1981) used a comprehensive pilot plant study to investigate biomass formation and citric acid production by *A. niger*. It was shown that growth and product formation could be related to a modified Luedeking-Piret equation with the product yield coefficient ($Y_{p/s}$) of 0.8 and cell yield coefficient ($Y_{x/s}$) of 0.4 being in good agreement with the model parameters determined from the experimental data.

7.3.1.3 Product Recovery

As described in Section 6.3.5, citric acid recovery is generally achieved by precipitation, extraction, or adsorption. Precipitation by calcium hydroxide (hydrated lime) is typically employed in large-scale processes (Pazouki & Panda, 1998; Soccol *et al.*, 2006). This classical recovery process is particularly well suited for very impure liquors derived from molasses (Milson, 1985). The spent culture contains the bulk of the impurities found in the raw materials and direct crystallisation of the product is not feasible. Precipitation is the preferred method for product recovery and is thus discussed further.

The mycelial biomass is removed from the spent culture by vacuum or belt discharge filtration. Calcium hydroxide is then added to the product stream, to neutralise the broth and form an insoluble calcium citrate precipitate. Heding (1975), proposed an optimum procedure for the recovery of calcium citrate at 50 °C for 20 minutes. Although the rate of precipitation is relatively slow, the reaction, shown in Equation 7.7, goes to completion at 50 °C.

$$3\,Ca(OH)_2 + 2\,C_6H_8O_7 \cdot H_2O \rightarrow 8\,H_2O + Ca_3(C_6H_5O_7)_2 \qquad 7.7$$

Lime containing 180 to 250 kg CaO/m^3 is added to ensure that sufficiently large crystals of high purity are obtained. The lime is added at an empirically determined rate at about 90 °C at pH 7.0 (Rohr & Kubicek, 1987). The precipitated calcium citrate is filtered from the mother liquor and washed with water to remove impurities. In the washing/filtration stage about 10 m^3 of hot water (90 °C) is used per ton of citric acid produced. The filtrate from the solution contains mostly non-sugars and a small amount of sugars from the molasses. The solution has a COD of about 30 000 mg O_2/L and may be evaporated to produce a valuable by-product (―vinasse”) that can be used for animal nutrition (Rohr & Kubicek, 1987). The precipitate is treated with dilute sulphuric acid (60-70%) in slight excess (1-2 g/L) in an acidulator unit to ensure complete recovery of citric acid. The reaction scheme is shown in Equation 7.8. The product solution is filtered to remove the calcium sulphate (gypsum) precipitate.

$$3\,H_2SO_4 + Ca_3(C_6H_5O_7)_2 \rightarrow 3\,CaSO_4 + 2\,C_6H_8O_7 \qquad 7.8$$

The solution contains small amounts of organic impurities, removed by activated carbon treatment. Ion-exchange, utilising a strong cation exchange resin (e.g Dowex-50) and a medium strength anion resin (e.g. Dowex-2), removes any residual calcium sulphate and metal ions. The product solution contains approximately 200-250 g/L of citric acid following carbon treatment and ion-exchange. The solution is

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concentrated by vacuum evaporation at about 100 °C and directed to crystallisation. During crystallisation citric acid monohydrate is formed at temperatures of about 20-25 °C. The crystals are recovered by vacuum filtration or centrifugation. The mother liquor from crystallisation is recycled back to the acidulator or precipitation unit operation. The mother liquor contains about 60% citric acid (Atkinosn & Mavituna, 1983). The product crystals are dried in a fluidised bed drier and packaged. Ayers (1954) proposed a method for the precipitation of di-calcium citrate in an attempt to reduce the amount of lime used and citric acid lost. In the process, di-calcium citrate is precipitated from the product solution with the addition of calcium hydroxide, calcium oxide, or calcium carbonate, at an elevated temperature. Although di-calcium precipitation occurs above 40 °C, heating the solution to 80-95 °C is most useful in reducing the reaction time. It is claimed that one-third less lime and sulphuric acid is required for the process.

7.3.1.4 Effluent Disposal

The major effluent streams from the process are:

- Waste biomass and process water
- Gypsum from the acidulator and retentate from filtration stages
- Gaseous emissions

The biomass removed from the spent culture is typically sold as an animal feed by-product. A considerable cost factor is attached to gypsum disposal if there is limited demand for the product. Purification is often not justified by the low-value nature of the product especially if demand is low. In the case that the gypsum is not sold as a by-product it is disposed (Kristiansen *et al.* 1999). The filtrate from precipitation operations of the process has a relatively high BOD, typically 12 000-14 000 mg/l. Methods of treatment include anaerobic digestion or evaporation to produce condensed molasses solubles (CMS), which can be used for animal nutrition (Milson & Meers, 1985).

7.3.2 Generic Flowsheet Process Simulation

A process simulation was developed using a generic flowsheet developed by Harding (2008) for the production of 1000 kg of citric acid monohydrate using molasses as the main raw material. The process flow diagram is shown in Figure 7.7.

7.3.2.1 Upstream Processing

The input values for upstream processing for the generic flowsheet are given in Table 7.13. The treatment of molasses by ferrocyanide was not included in the process simulation, as the flowsheet does not allow for substrate pre-treatment. The amount of potassium ferrocyanide required for the process was determined from the approximate concentrations given by Hustede *et al.* (1972). Pure sucrose was fed to the bioreactor as the carbon source. The molasses requirement was calculated from the approximate composition of beet molasses given in Table 7.10. The remaining nutrient requirements (NH_4NO_3, KH_2PO_4) were prepared (TK-001), sterilised (ST-001) and supplied in excess to the bioreactor. The medium was pre-heated to 110 °C with condensed steam and sterilised by heat exchange with saturated steam at 140 °C for 20 minutes.

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7.3.2.2 Bioreaction

The media and inoculum were added to an aerated batch reactor (RX-001). The simulation inputs for the bioreaction are shown in Table 7.14. Product to biomass ratio, yield coefficients, initial and final biomass concentrations and aeration requirements used in the generic flowsheet model were estimated from literature sources (Roehr *et al.*, 1981; Sobotka *et al.*, 1984; Krzystek, 1996; Ali, 2004) for stirred tank systems under similar operating conditions, shown in Table 7.12. The biomass growth and product formation were calculated using chemical balances for oxygen, nitrogen, phosphorus and the specified yield coefficients for biomass growth ($Y_{x/s}$) and product formation ($Y_{p/s}$), according to Equation 7.9.

$$\begin{aligned} &\text{CarbonSource} + \text{OxygenSource} + \text{NitrogenSource} + \text{PhosphorusSource} \\ &\rightarrow \text{Biomass} + \text{Product} + H_2O + CO_2 + \text{NitrogenExcess} + \text{PhosphorusExcess} \end{aligned} \tag{7.9}$$

Similarly to the starch process simulation, growth rate input data was used to calculate the reactor residence time (t_b) and agitation energy. A default value was used for the maximum specific growth rate (μ_{max}). Monod growth kinetics was used to predict growth rate. The maintenance calculations were not considered in the simulation. Sterile air was supplied to the bioreactor at 5 bar by a two-stage centrifugal compressor (CM-001) using cooling water (25 °C) for inter-stage cooling. The bioreaction pH was not considered in the simulation, since yield coefficient data was used to predict biomass growth and product formation. The product to biomass ratio of 6.0 was based on values found in the literature (Sobotka *et al.*, 1984; Papagianni, 2007). The elementary composition of the biomass was assumed as $CH_{1.72}O_{0.55}N_{0.09}P_{0.002}$. The final biomass concentration was based on literature studies (Ali, 2004; Ho *et al.*, 1994 as cited by Papagianni, 2007).

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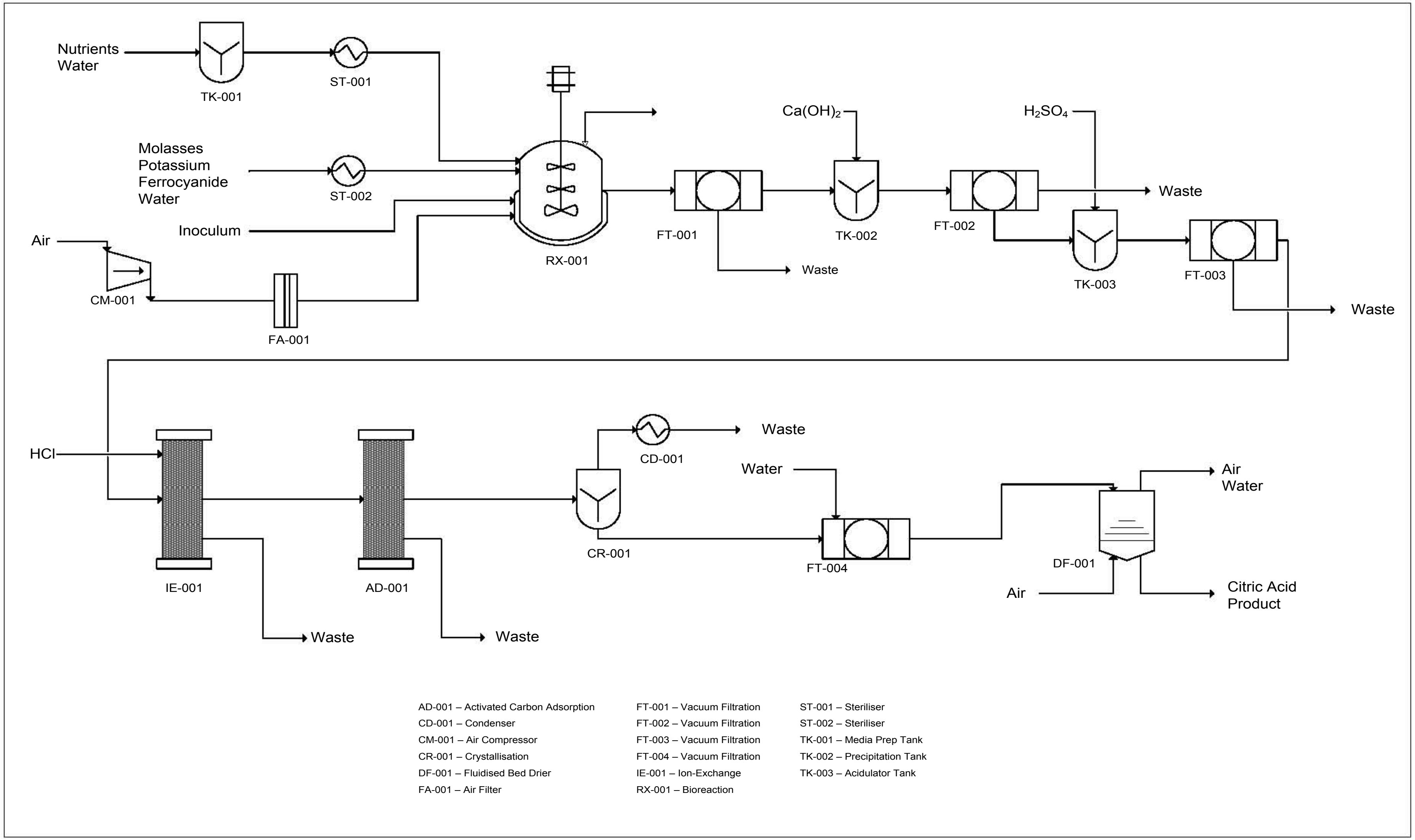

Figure 7.7 Process flow diagram for the production of citric acid from molasses

Table 7.13 Upstream operating conditions for citric acid production from molasses

Operating Parameter	Value	Unit
Materials		
Biomass ash content: $CH_{1.72}O_{0.55}N_{0.09}P_{0.002}$	0.0	%
Carbon source excess: $C_{12}H_{22}O_{11}$	[1.0]	%
Nitrogen source excess: NH_4NO_3	[5.0]	%
Phosphorus source excess: KH_2PO_4	[5.0]	%
Sterilisation		
Media temperature in	[110]	°C
Sterilisation temperature	[140]	°C
Steam temp	[150]	°C
Holding time	[20]	min
Oxygen supply		
Oxygen supply	Air (21% O_2:79% N_2)	-
Compression	Two-stage	-
Cooling medium	Cooling water	-
Initial pressure	[101]	kPa
Final pressure	500	kPa
Polyentropic efficiency	0.9	-

[ ] Default values provided in simulation model

Table 7.14 Bioreaction conditions for citric acid production from molasses

Operating Parameter	Symbol		Unit	Reference
Reactor Operation				
Mode		Batch		
Agitation power per unit volume	Pv	[0.5]	kW/m^3	
Agitation efficiency	η	[0.9]		
Aeration rate	Ar	0.25	vvm	Roehr *et al.,* 1981
Antifoam		[1.0]	%v/v	
Reactor Temperature				
Reactor temperature	Tr	30	°C	Roehr *et al.,* 1981
Ambient temperature	Ta	[20]	°C	
Cooling medium temperature	Tc	[18]	°C	
Growth Rate				
Initial biomass concentration	Cx,in	4.0	g/l	Ali, 2004
Final biomass concentration	Cx,final	16.5	g/l	Ali, 2004
Max. specific growth rate	μ_{max}	0.54	hr^{-1}	Ali, 2004
Limiting nutrient conc.	Ks	[11.9]	mg/l	
Yield coefficients				
Product to Biomass Ratio	P:X	6.0		Sobotka *et al.,* 1984
Biomass on substrate	Yx/s	0.13	g/g	Ali, 2004
Product on substrate	Yp/s	0.77	g/g	Ali, 2005
Biomass on oxygen	Yx/o	0.35	g/g	Krzystek, 1996

[ ] Default values provided in simulation model (Harding, 2008)

7.3.2.3 Downstream Processing

The downstream unit operations are summarised in Table 7.15. The majority of the biomass (99%) was removed by a rotary vacuum filter (FT-001). The separated biomass was washed

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to minimise the loss of citric acid product from the process. Wash water was supplied to obtain a cake porosity of 0.4 %v/v. Following biomass removal, tri-calcium citrate was precipitated (TK-002) from the filtrate with the addition of calcium hydroxide at 2 %v/v and filtered by rotary vacuum filtration (FT-002). It was assumed that 99% of the precipitate was recovered. The retentate was washed with 0.4 %v/v water to ensure the removal of impurities. It was assumed that 99% of all insoluble impurities were removed. The precipitate containing solution was sent to the acidulator unit (TK-003) where dilute sulphuric acid (60%) was added to ensure complete recovery of citric acid. The product solution was filtered (FT-003) to remove the calcium sulphate (gypsum) precipitate, which was sent to waste treatment.

Table 7.15 Downstream operating conditions for citric acid production from molasses

Operation	Unit	Operating Conditions
Biomass Removal (Rotary Vacuum Filtation)	FT-001	Solids removed: 99% Citric acid retained: 99% Cake Porosity: (0.4 %v/v)
Precipitation	TK-002	Additive: $Ca(OH)_2$ Heated temp: 100 °C Outlet temp: 15 °C Residence time: 2 h
Filtration	FT-002	Solids removed: 99% Citric acid to retained: 99% Cake Porosity: (0.4 %v/v)
Acidulator	TK-003	Additive: 65% H_2SO_4 Heated temp: 100 °C Outlet temp: 15 °C Residence time: 2 h
Filtration	FT-003	Solids removed: 99% Citric acid retained: 99% Cake Porosity: (0.4 %v/v)
Ion-Exchange	IE-001	Waste fraction removed: 99% Citric acid to retained: 99% Elution: HCL (0.09 %v/v)
Activated Carbon	AD-001	Waste fraction removed: 99% Citric acid to retained: 99% Elution: Water (35 %v/v)
Crystallization	CR-001	Product yield: 98% Heated temp: 100 °C Outlet temp: 25 °C Residence time: 5.4 h
Filtration	FT-004	Product recovery: 99% Wash water (55 %v/v)
Product Drying	DD-001	Solids retained: 100% Liquid removal: 99%

Cation exchange (IE-001) removed 99% of cations from the product solution. The cations were bound to the resin and eluted from the column with 2-molar hydrochloric acid (0.09 %v/v). Similarly to the starch process, it was assumed that the anions pass through the ion exchange column and did not to affect the crystallisation step. The product solution was

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passed through an activated carbon column (AD-001) for decolourization. The packing in the column was regenerated with water, added at 35 %v/v. The citric acid solution was crystallised (CR-001) by evaporation of the majority of the water, cooling and subsequent crystallization. Crystallisation was performed at 100 °C with the outlet stream cooled to 15 °C using chilled water. The residence time was 5.4 hours and the power consumption was 0.6 kW/m^3 of throughput. Caustic soda (0.002 %v/v) was added to prevent the evaporation of hydrogen chloride during crystallisation. The evaporated water from crystallisation was condensed by heat exchange (CD-001) with cooling water and assumed to be a waste. The citric acid crystals were recovered from the mother liquor by rotary vacuum filtration (FT-004) and washed with water (55 %v/v) to remove impurities. It is assumed that a crystallisation recovery of 98% is achieved. The crystals were dried in a fluid bed drier (DF-001) and packaged.

7.3.2.4 Waste Treatment

Similarly to the starch process simulation, the process flowsheet did not include the material and energy balances of the waste treatment operations. It was assumed that all waste streams were sent to an off-site waste treatment plant.

7.3.2.5 Material and Energy Balance Analysis

The results of the process yield, material requirements and energy requirements, per ton of citric acid product (P), are shown in Table 7.16 to Table 7.18. The results of the simulation showed the major input materials were water, molasses, oxygen, nitrogen source (NH_4NO_3), calcium oxide and sulphuric acid. The main nutrient requirements to produce 1 ton of citric acid, were 2760 kg of beet molasses, 25.8 kg of ammonium nitrate and 2.05 kg of potassium phosphate.

The results compared within 16-35% accuracy of values for typical commercial-scale stirred tank processes, reported by Krishnan (1999), for molasses, nutrients, lime and sulphuric acid. In the process simulation, a pure sucrose feed was assumed as the carbon source. The beet molasses requirement was calculated on this basis, using the typical sucrose content of beet molasses (Curtin, 1983). The simulated process required 1325 kg of pure sucrose to produce 1 ton of citric acid monohydrate crystals (99% purity). The process required approximately 2760 kg of beet molasses. The process yields compared well with values reported in the literature. The overall process yield, shown in Table 7.16, was 0.36 kg citric acid / kg molasses and 0.61 kg citric acid / kg sucrose. Assumptions with regards to improved downstream processing operations in the generic flowsheet resulted in slightly improved process yields relative to literature reported values.

Table 7.16 Product yields of citric acid production process from beet molasses

	Generic Flowsheet
Process Yield	
C-mol citric acid/C-mol sucrose	61%
kg citric acid/kg molasses	36%
Bioreaction Yield	
C-mol citric acid/C-mol sucrose	63%
kg citric acid/kg molasses	37%

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The process required 32% less molasses than the process reported by Krishnan (1999), attributed mainly to improved recoveries in downstream operations. The batch throughput was 11.7 m^3 per ton product. The larger bioreactor throughput of the molasses process in comparison to the starch process was attributed to higher volume of molasses containing medium, fed to the bioreactor. The initial biomass concentration ($C_{x,in}$) for the molasses process (4 g/l) was higher than the starch process (0.04 g/l). Similar final biomass concentrations, of 16.5 g/l and 17.1 g/l, were used for the molasses and starch simulations respectively and the initial biomass concentration did not have a significant influence on the bioreactor volumetric throughput. The major difference in the material flows for the respective simulations was the calcium oxide and sulphuric acid requirement for product recovery in the molasses process. This contributed to a larger water requirement in the molasses process for lime (200 g/l) and acid dilution (60 %v/v). The aeration rate of 0.25 vvm resulted in an air feed of 29.2 tons per ton of product, slightly higher than the starch process for due the larger bioreactor volume.

Table 7.17 Material balance for citric acid production from molasses

Component	In (kg/ton P)	Out (kg/ton P)
Ammonium Nitrate	25.8	
Beet Molasses	2760	
Biomass	5	174
Calcium Citrate		13.4
Calcium Oxide (Lime)	756	
Calcium Sulphate (Gypsum)		1080
Carbon Dioxide		291
Chloride		13.2
Citric Acid loss		21.5
Citric Acid Monohydrate		1000
COD		360
Hydrogen Chloride	13.5	
Nitrate (dissolved)		20.0
Nitrogen	23000	23000
Oxygen	6219	5790
Potassium Phosphate	2.05	
Potassium (dissolved)		0.589
Sodium Hydroxide	0.131	
Sodium (dissolved)		0.075
Sulphuric Acid	782	
Water	17300	17300

Energy and utility requirements, summarised in Table 7.18, include electricity, steam, cooling water and chilled water. Similarly to the starch process, most of the electricity was used for air compression (47%) and mechanical agitation of the bioreactor (25%). The aeration rate and bioreaction duration determined the electricity demand on the compressor. Approximately 9600 MJ/ton product of electricity was required for aeration and agitation in the molasses process, compared to 8300 MJ/ton product in the starch process. This was attributed to the larger bioreactor volume in the molasses process. Steam was required for sterilisation, media heating, evaporation in the crystallisation unit and final product drying. The starch process required considerably more process steam due to the higher evaporative

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crystallisation load of 263 m^3/batch. In the molasses process, liquid waste was removed during tri-calcium citrate filtration, resulting in a crystallisation load of 80.6 m^3/batch. Cooling water was used for compressor cooling and heat exchange in the condenser. Chilled water was used to control the bioreactor temperature and cool the product solution during crystallisation. The molasses process required approximately 2100 m^3 of chilled and cooling water per ton of citric acid product, compared to 3000 m^3 in the starch process simulation. This was due to the lower cooling water requirement of the condenser in the molasses process, attributed to a lower evaporative load on crystallisation.

Table 7.18 Energy and utility requirements for citric acid production from molasses

Utility Input			
Electricity		MJ/ton P	**13600**
Adsorption	(AD-001)		0.602
Acidulator	(TK-003)		68.0
Aeration	(CM-001)		6244
Agitation	(RX-001)		3362
Crystallisation	(CR-001)		20.1
Drying	(DF-001)		227
Filtration	(FT-001)		657
Filtration	(FT-002)		842
Filtration	(FT-003)		236
Filtration	(FT-004)		49.7
Ion Exchange	(IE-001)		0.731
Precipitation	(TK-002)		81.2
Sterilisation	(FA-001)		1800
Steam		kg/ton P	**8703**
Bioreactor	(RX-001)		-
Crystalstn	(CR-001)		6478
Drying	(DF-001)		1500
Sterilisation	(ST-001/2)		725
Cooling water		m^3/ton P	**1860**
Aeration	(CM-001)		1350
Bioreactor	(RX-001)		-
Condenser	(CD-001)		420
Sterilisation	(FA-001)		89.6
Chilled water		m^3/ton P	**230**
Bioreactor	(RX-001)		226
Crystallisation	(CR-001)		2.14

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7.4 Conclusions

The large-scale production of citric acid monohydrate was simulated using a generic flow sheet simulation tool, developed and implemented in MS-Excel. The production process, using two different feedstocks, namely maize starch and beet molasses was simulated. The results of the material and energy balance from the generic flowsheet were compared with the results obtained from Biwer *et al.* (2006). The results were shown to be in good agreement. Biomass production (*A. niger*), nitrogen (NH_4NO_3) requirements and phosphorus (KH_2PO_4) requirements from the process models were in relatively good agreement. The generic simulation tool was then used to simulate citric production from molasses. In the starch and molasses process, major raw material inputs included water, carbon source, and oxygen and nitrogen source. In the molasses process additional material inputs included lime (CaO) and sulphuric acid for the calcium citrate precipitation and recovery. The major material outputs common to both processes included biomass (A. *niger*), process water, carbon dioxide and suspended organics and metal ions. Significant amounts of gypsum ($CaSO_4$) were generated from the molasses process. The major utility requirements included electricity, steam, cooling water and chilled water. Electricity used for air compression and mechanical agitation of the bioreactors. The majority of the steam was used for evaporation during crystallisation. Cooling water and chilled water was required for isothermal bioreactor operation, compressor cooling and heat exchange. The electricity requirements of the processes compared relatively closely due to similar upstream and bioreaction models. The starch process required considerably more steam due to a high load on evaporative crystallisation. In the molasses process, a large amount of liquid waste was removed during the tri-calcium citrate filtration operation thus reducing the load on crystallisation

The material and energy balance results from the starch and molasses simulations provided a good first-estimate for large-scale citric acid production. The applicability and accuracy of the simulation results was largely a function of the detail included in the simulation model and the accuracy of the data used. Certain critical variables identified (e.g. yield coefficients, aeration rate, downstream efficiency) had a strong influence on the accuracy of the results. The input data for the bioreactor unit operation and the recovery in certain downstream unit operations had a strong influence on the material and energy balance results and received considerable attention when the process model was developed. The results of the material and energy balance are representative of large-scale citric acid production and can form the basis for the comparative environmental and economic assessment of process.

The first estimate approach to process simulation of a large-scale bioprocess has been demonstrated and has been shown to provide suitable material and energy data in comparison to a detailed simulation package. However, opportunity exists to include additional calculation models in the simulation tool such as substrate pre-treatment, more detailed kinetic expressions, and recycle loops. The application of a generic flowsheet tool demonstrated the ability of first estimate bioprocess simulations to serve as a tool for process management, resource utilisation, mass balance assessments, unit operation characterisation and ultimately, early planning of process development.

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CHAPTER 8

ENVIRONMENTAL ASSESSMENT

The environmental assessment was performed using Life Cycle Assessment (LCA). LCA evaluates the environmental impact of the product system as a number of discrete interdependent stages with the cumulative impacts across all stages allowing a comprehensive analysis of the environmental performance of the system. A ‗cradle-to-grave' approach is typically used in LCA (Curran, 1996); however, where identical products are considered a ‗cradle-to-gate' approach may be appropriate. The subsequent sections present a definition of the goal and scope of the LCA, the inventory data used in the assessment and finally a ‗cradle-to-gate' assessment of the process life cycle, using SimaPro assessment software.

8.1 Goal Definition

The cradle-to-gate Life Cycle Assessment of the production of citric acid used the material and energy values within the defined system to quantify the environmental implications of the system. Life cycle inventory (LCI) data for citric acid production was not available in SimaPro database library (e.g. Ecoinvent, 2007). Therefore, this work should provide the necessary LCI data to select the product and process, with the least effect on human health and the environment, for citric acid production. As discussed in the Chapter 4, the results can contribute to improved decision making in sustainable bioprocess development.

The inventory data for the LCA was generated from the flowsheet model developed by Biwer *et al.* (2006) and the generic flowsheet model presented in Chapter 7. The goals of the study were based on the main objectives of LCA, aligned with the main objectives of the overall thesis, which include:

1. Providing a basis for broad environmental assessment to evaluate the environmental performance of large-scale citric acid production.
2. Ranking the relative contribution of individual steps or processes for the production of citric acid from different feedstocks.
3. Identifying data gaps and assessing the requirements for data generation.
4. Providing information to guide product and process development.

Further the results of the LCA were compared to the material and energy balance inventories obtained from the case study presented by Biwer *et al.* (2006), utilising a detailed simulation approach, based on detailed input data. Relative accuracy of the results from the generic flowsheet inventory data could be estimated and recommendations on the applicability of the flowsheet for life cycle assessment could be formulated.

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8.2 Scope of the Study

8.2.1 System Boundaries

The study includes the comparative assessment of citric acid monohydrate production using both starch and molasses as alternative raw material inputs. A simplified flow diagram for the process system is shown in Figure 8.1 and Figure 8.2. The processes were compared, using material and energy balance data and LCA impact scores. The boundaries of the respective systems are defined as cradle-to-gate production of citric acid monohydrate. All the raw material and agricultural inputs, utility generation and supply, waste disposal outputs and products were included in the assessment.

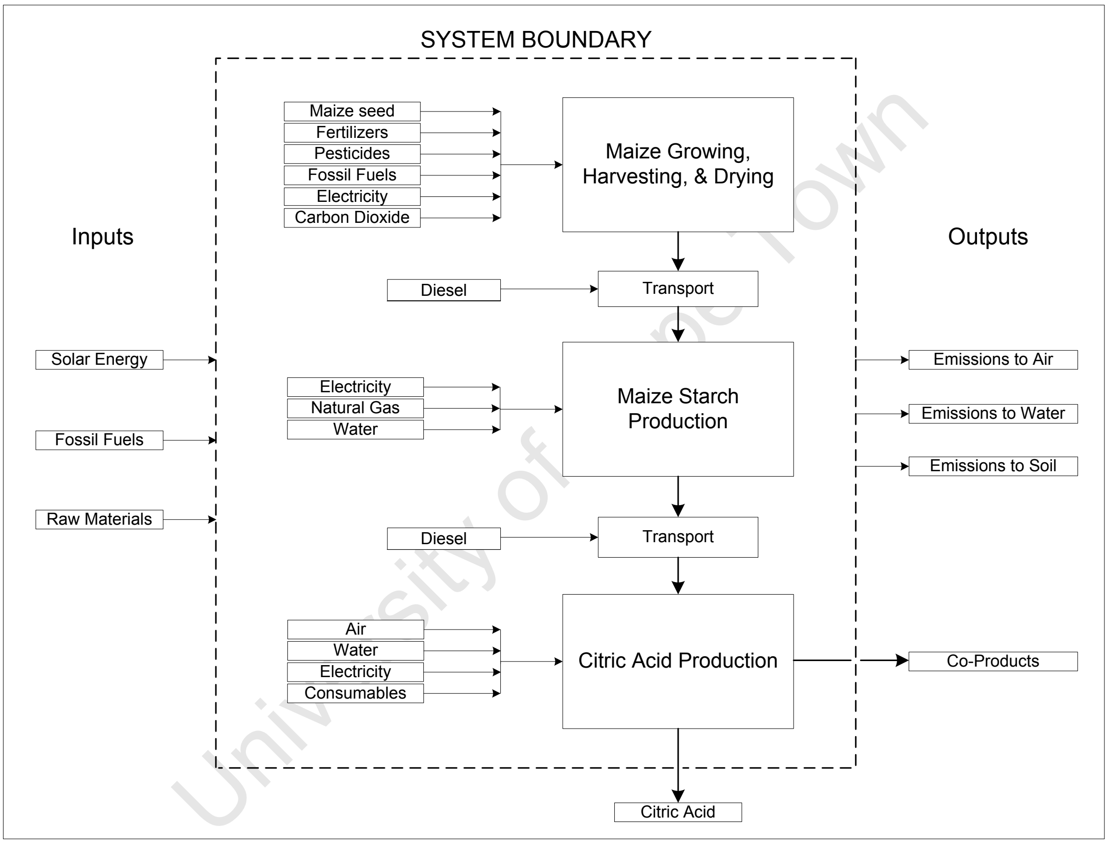

Figure 8.1 Flow diagram and system boundary for life cycle of citric acid production using starch

Raw materials, solar energy and fossil fuels were included as inputs from nature and converted to useable products within the system boundary. Biomass is assumed as a usable co-product from the citric acid process (COD: 280 g O_2/kg Product). The biomass can be further used (e.g. as animal feed), added to wastewater treatment plant as carbon source, or disposed. The citric acid product and associated biomass co-product, assumed equivalent in composition, leave the system boundary to be consumed by various processes. Emissions to air, water and soil and associated effects as a result of the conversion processes‘, were included in the Life Cycle Assessment. Wherever possible, the Ecoinvent v1.3 inventory dataset was used in the LCA. A description of data used is provided in subsequent sections.

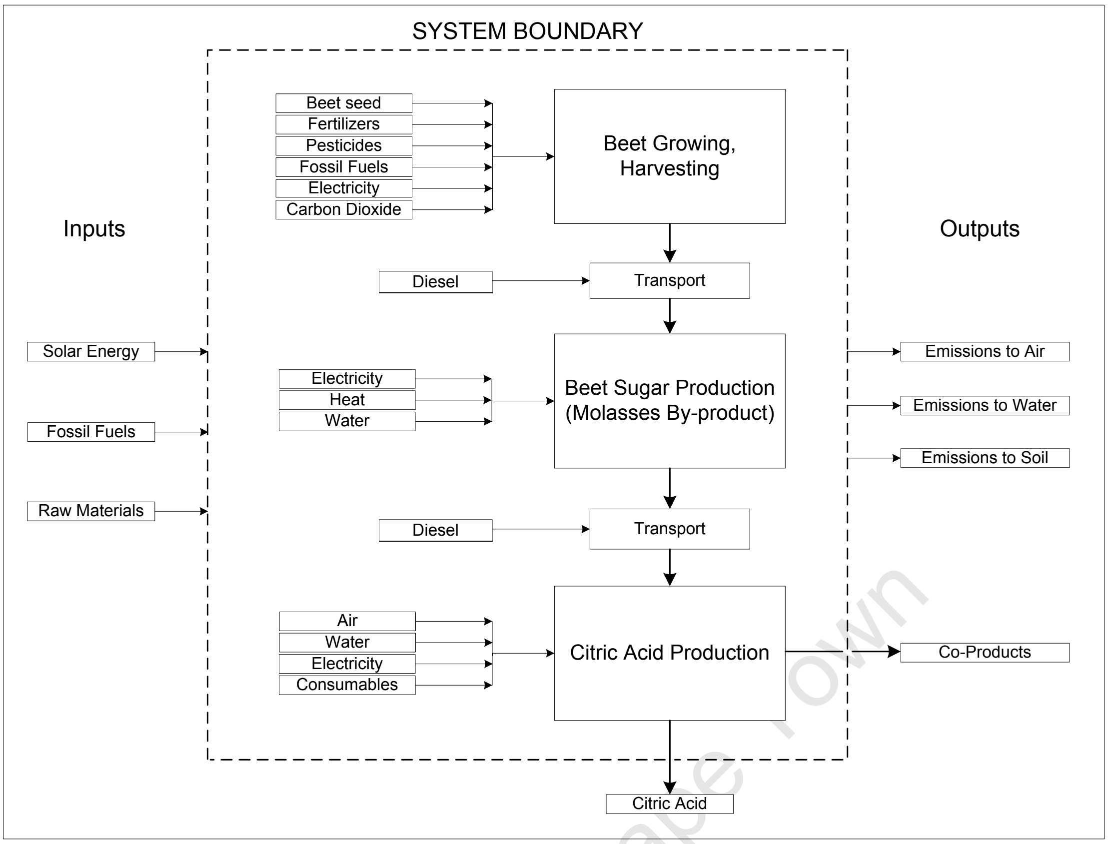

Figure 8.2 Flow diagram and system boundary for life cycle of citric acid production using molasses

i. Growing and Harvesting

The inventory for agricultural production of maize and beet included processes for soil cultivation, sowing, weed control, pest and pathogen control and harvest. Machine infrastructure and sheltering was included. The system boundary is at the farm gate.

ii. Processing of Agricultural Produce

The maize produce is transported by truck from the farm to a maize starch plant (wet mill) for processing into starch. The major unit operations included mechanical separation steps, maize steeping in process water, wet milling of steeped maize, separation of the starch and finally drying and packaging. The treatment of wastewater is also included in the system boundary. In the case of molasses production, sugar beets are transported by truck to a beet sugar factory where they are processed into sugar. Molasses and feed-pills are generated as co-products. The treatment of process wastewater, beet pulp and feed pills are included in the system boundary. Emissions associated with the major unit operations and specific inputs to the processes (e.g. energy, raw materials and supplies) are included in system boundary.

iii. Citric Acid Production

The packaged starch or beet molasses is transported to the citric acid plant, where it is used as the main raw material. The major unit operations associated with the production of citric acid were included in the system boundary. The emissions associated with each of the major unit operations and specific inputs to the process (e.g. energy, raw materials and supplies) were

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included in the system boundary. The impact as a result of plant construction and maintenance of existing equipment was not included in the scope of the assessment, according to the common, although disputed practice (Heijungs, 1992). The system boundary was the plant gate.

8.2.2 Functional Unit

The functional unit was defined as 1000 kg of citric acid monohydrate. The functional unit was used as the basis for all data tabulation. It was assumed that the citric acid produced by both the Biwer *et al.* (2006) model and the generic flowsheet model were of the same composition and sold as an identical product.

8.2.3 Data Specificity and Impact Assessment Method

The LCA work in the thesis is performed using SimaPro 7.1R (PRe Consultants B.V. 2008) with the EcoInvent v1.3 database. Where data was not available from EcoInvent v1.3, LCA Food DK (Nielsen *et al.,* 2003), UCTE, NORDEL and CENTRAL databases (Roes *et al.,* 2007) were used.

The CML 2 Baseline 2000 v2.03 assessment method was used to generate midpoint impact scores, which are commonly used in Life Cycle Assessment methods. The CML 2000 method is based on the problem-oriented (midpoint) approach. The use of mid-point indicators is justified by supported findings (Bojarski, 2008) that end-point indicators rely on damage models that feature a relatively high degree of uncertainty because of a more-complex impact model. The CML 2 method covers a core baseline range of impact categories, broadly based on the earlier CML 1992 method. It considers 10 impact categories: ozone layer depletion (mg CFC-11eq), human toxicity (kg 1,4-DBeq), photochemical oxidation (kg C_2H_4), global warming (GWP100) (kg CO_2 eq), acidification (kg SO_2 eq), abiotic depletion (kg Sbeq), eutrophication (kg $PO^{3-}{}_4$), and ecotoxicity to fresh water (kg 1,4-DBeq), marine aquatic (kg 1,4-DBeq), and terrestrial (kg 1,4-DBeq) ecosystems. A description of the impact categories is provided in the Appendix B and the life cycle impact assessment (LCIA) section in this chapter. CML is widely used in the literature, internationally recognised and the characterisation factors are largely based on work by expert groups of researchers, e.g. Center of Environmental Science of Leiden University. Water usage and biodiversity are not included as impact categories in the CML 2 Baseline 2000 v2.03 method.

8.3 Life Cycle Inventory

8.3.1 LCA Inventory Data

Firstly, inventory data obtained from the model by Biwer *et al.* (2006) was compared to the inventory data from the generic flowsheet simulation of citric acid production from starch. Thereafter, inventory data obtained from the generic model were compared for the starch and molasses process. The inventory data for inputs from the technosphere and emissions from the process is given in Table 8.1 for the starch process and Table 8.2 for the molasses process. The inventory data and differences in the input and output values is discussed in Section 7.2.2 and Section 7.3.2.

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Table 8.1 Inventory data for the production of citric acid from starch

Component	Unit	Biwer *et al.* (2006)	Generic Flowsheet	%Difference
Product				
Citric Acid Monohydrate	kg	1000	1000	0%
Inputs from Technosphere				
Ammonium Nitrate	kg	18.8	23.4	20%
Starch	kg	1270	1200	-6%
Electricity	MJ	9500	10890	13%
Hydrogen Chloride	kg	0.58	0.712	19%
Magnesium Sulphate	kg	0.984	-	
Potassium Phosphate	kg	1.88	1.86	-1%
Sodium Hydroxide	kg	0.346	0.402	14%
Steam	kg	13600	14100	4%
Inputs from Nature				
Process Water	kg	13900	13200	-5%
Cooling Water	kg	3007	2436	-23%
Oxygen	kg	510	460	-11%
Emissions to air				
Carbon Dioxide	kg	410	353	-16%
Emissions to water				
α-Amylase	kg	1.27	-	
Biomass	kg	160	156	-3%
Chloride	kg	0.635	0.692	8%
Citric acid loss	kg	62.6	30.6	-105%
COD	kg O_2	280	300	7%
Fats	kg	10	-	
Glucose	kg	1.88	13.2	86%
Magnesium (dissolved)	kg	0.199	-	
Potassium (dissolved)	kg	0.540	0.025	-2000%
Sodium (dissolved)	kg	0.113	0.231	51%
Starch	kg	-	-	
Sulfate	kg	0.786	-	
Water	kg	13880	13240	-5%

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Table 8.2 Inventory data for the production of citric acid from molasses

Component	Unit	Generic Flowsheet
Product		
Citric Acid Monohydrate	kg	1000
Inputs from Technosphere		
Ammonium Nitrate	kg	25.8
Beet Molasses	kg	2760
Calcium Oxide (Lime)		756
Electricity	MJ	13600
Hydrogen Chloride	kg	13.5
Potassium Phosphate	kg	2.05
Sodium Hydroxide	kg	0.131
Sulphuric acid	kg	782
Steam	kg	8703
Inputs from Nature		
Process Water	kg	17300
Cooling Water	kg	2090
Oxygen	kg	429
Emissions to air		
Carbon Dioxide	kg	291
Emissions to water		
Biomass	kg	174
Calcium Citrate		13.4
Calcium Sulphate (Gypsum)		1080
Chloride	kg	13.2
Citric acid loss	kg	21.5
COD	kg O_2	360
Potassium (dissolved)	kg	0.589
Sodium (dissolved)	kg	0.0753
Water	kg	17300

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8.3.2 Description of Inventory Data

The following gives a description of the inventory data used to model the citric acid production system as selected from the SimaPro database library. Due to the lack of certain data, various assumptions were taken into account when selecting data inputs. It was assumed that the inventory was representative of a typical European facility for citric acid production. Both process specific and background data, available in the SimaPro and Ecoinvent database was primarily obtained from European industrial operations. Included in the objectives of the thesis is the evaluation the environmental performance of alternative substrates and process technologies. The use of European specific data is thus deemed justifiable. However, location specific background data (e.g. electricity country mix) should be included when considering process options for different regions.

i. Citric Acid Monohydrate Product

The process allocated 100% of the environmental load to the production of 1000 kg of citric acid monohydrate. Although by-products (e.g. biomass sold as animal feed) can also be allocated a percentage of the environmental burden, these were not considered. Biomass was considered a waste material and was treated by waste treatment methods.

ii. Maize starch

The process models for maize cultivation and starch production were obtained from Ecoinvent 1.3. The model for maize growing refers to an average production in the Swiss lowlands, while the starch production model refers to typical maize starch production in Germany. The wet mill required 1.26 kg of grain maize, 0.254 kWh of electricity, 3.99 MJ of heat generated from natural gas and 1.96 kg of tap water to produce 1 kg of maize starch.

iii. Beet Molasses

The process models for beet growing and sugar production were obtained from the LCA Food DK database. The impacts associated with the production of molasses were adapted from the sugar production model available in the Ecoinvent, producing sugar, molasses and feed pills as products. The model for beet growing and sugar production was representative of average sugar production in Demark. The feed pills were returned to agriculture as animal feed. It was assumed that approximately 1 kg of feed pills displaces 0.9 kg of spring barley (Landbrugets Radgivnings Center, 2000). Approximately 7.1 kg of beet is required to produce 1 kg of beet sugar and 0.24 kg of beet molasses (LCA Food DK). Molasses accounted for 11% of the total output from sugar production which was used as the basis for the allocation of emissions.

iv. Ammonium Nitrate

The inventory data for the production of ammonium nitrate from ammonia and nitric acid, from common industrial processes, was used in the model (Zapp, 2002).

v. Hydrochloric Acid

The hydrochloric acid was assumed to be 30 %v/v hydrochloric acid in water from the combustion of chlorine with hydrogen and as a by-product of various processes. The literature used was based on plant data from Europe and North America.

vi. Potassium Phosphate

The unit process for the production of potassium phosphate was not available in the SimaPro database. The production process was substituted for the production of sodium phosphate, since potassium monophosphates are usually produced by the same process as the corresponding sodium salts, i.e., from phosphoric acid and potassium hydroxide (Schrodter *et al*., 2002).

vii. Sodium Hydroxide

The data was for a 50 wt% sodium hydroxide solution from a European production mix consisting of three different electrolysis technologies (mercury, diaphragm, membrane) (Minz, 2002). Although mercury and diaphragm systems have largely been replaced by membrane cell technologies, a production mix was assumed to ensure all potential emissions were included in the estimate.

viii. Calcium Oxide (Lime)

The input data for the lime requirement of the molasses process was representative of a production facility in Switzerland. The impacts associated with the complete product life

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cycle were included. These included the impacts associated with the mining and crushing of limestone, the calcination process and the milling and packaging operations (Oates, 2002; Ecoinvent, 2007).

ix. Transport

Transport of maize starch and beet molasses was included in the model. It was assumed that the material is transported 30 km by a 28 ton diesel truck. The production of the fuel, maintenance of the truck and maintenance of the road were included. The data used in the transport model was representative of European data. The amount of consumables used in the citric acid process was relatively small in comparison to other inputs and consumable transport was not included.

x. Electricity

A representative mix of European electricity was obtained by using a weighted average of various database values shown in Table 8.3. The models included infrastructure for distribution, system losses and direct sulphur hexafluoride (SF^{-6}) emissions. The electricity was taken as medium voltage (Roes *et al.*, 2007). Although the LCA was a comparative analysis and it was not necessary to use a European energy mix; selecting a mix provides a basis for non-comparative assessment of the results, or comparison to similar processes based on average electricity data. Energy use for the citric acid production facility is reported as final energy use. The background processes for energy generation and supply accounted partly for primary energy use, by including estimations for production and delivery losses. The models for energy generation accounted for raw material extraction, material transport and material conversion. The reported energy requirements refer to non-renewable energy use.

Table 8.3 Weighted average electricity mix

Data	%
UCTE	74.4
NORDEL	15.1
CENTRAL	10.5

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xi. Process Water, Cooling Water, Steam

There were no emissions associated with the extraction of process and cooling water from natural sources. The impacts associated with pumping (electricity) and maintenance of the water supply network were included in the model. The input data for steam included the water and energy required for production. A representative average value for European steam production from gas and heavy fuel oil was used (Ecoinvent, 2007).

xii. Process Wastewater

Impacts associated with wastewater treatment were accounted for using inputs for effluent water and chemical oxygen demand (COD). It was assumed the water is processed by a municipal wastewater treatment plant. The input data included impacts associated with infrastructure of sewage piping and water treatment plant, COD reduction and unpolluted wastewater treatment. The data was specific to the technology mix encountered in

Switzerland in 2000, but applicable to modern treatment facilities in Europe, North America and Japan (LCA Food DK; ETH-ESU, 1996).

8.4 LCA Results and Discussion

The life cycle impact assessment (LCIA) is discussed in two sections. Firstly, the impact assessment of the process data generated by the generic flowsheet and that of the Biwer *et al.* (2006) model are compared. Thereafter, the life cycle impact assessment of the respective starch and molasses based processes are presented and discussed. The individual contributions to the impact categories are presented and the overall environmental impacts are discussed.

8.4.1 Comparative Assessment of Starch Process Inventory Data

A comparative assessment of the production of citric acid from starch was based on the inventory data from the case study presented by Biwer *et al.* (2006) and the inventory data generated using the generic flowsheet model developed by Harding (2008). The impact categories are defined in the scope of the study. The approach compared the results of generic flowsheet model to the literature data to determine the relative accuracy of the generic flowsheet and draw conclusions on its appropriateness as a basis for early stage decision making. The results of the impact assessment are shown in Table 8.4 and Figure 8.3. The impact category value for each assessment is given in Table 8.4 as well as the difference in the impact category value, calculated as the percentage by which the generic flowsheet impact value is higher or lower than the category for the literature process. The individual impact categories for each assessment are compared in Figure 8.3 on a relative basis. The comparative results of the life cycle impact assessment (LCIA) from the data obtained from the simulations were in relatively close agreement. Most of the impact categories were within 2% agreement with the Biwer *et al.* (2006) model. The largest variation between the specific impact categories was observed for global warming, marine aquatic ecotoxicity, terrestrial ecotoxicity, and eutrophication. These values for the generic flowsheet varied by 1.4%, -3.2%, -1.7% and -4.7% in comparison to the Biwer *et al.* (2006) values respectively. The differences were primarily attributed to the difference in the maize starch, electricity and steam inventory data. As shown in Figure 8.3, the major contributions to the impact categories were maize starch production and electricity and steam generation. Electricity (28%) and steam (55%) generation were the major contributors to global warming. Starch production (16%) and electricity (53%) and steam generation (29%) were the largest contributors to marine aquatic ecotoxicity. Electricity (36%) and steam (46%) contributed the most to photochemical oxidation.

Although the results show LCA scores within an accuracy of 5% in comparison to the detailed simulation data, the difference in the comparative inventory data (Table 8.1) should be highlighted. Significant contributions to the all impact categories were from maize starch production and electricity and steam requirements. The carbon dioxide released from the bioreactor in the citric acid process, contributes primarily to global warming, as shown by the red bar in Figure 8.4. Significant contributions by electricity, and bioreactor CO_2 varied significantly in terms of inventory values, and were approximately 13% and 16% different, respectively. The steam and maize starch inventory values were relatively close, at 4% and 6% respectively. As shown in Figure 8.4, electricity production is a major contributor to the majority of the impact categories. The 13% difference between the inventory values for electricity generation of the generic flowsheet and the literature data is not fully represented in the results of the life cycle impact assessment (LCIA). Similarly, the 16% difference in inventory CO_2 released from the bioreactor as a result of biomass growth and citric acid accumulation is not reflected in the results of the global warming impact score when comparing the LCIA results of the two datasets.

Considering the aggregation of inventory values across the entire life cycle shows that once the life cycle impact potentials have been generated for the entire life cycle process, the difference in the inventory values of the comparative processes needs to be relatively large before differences are discernable at the life cycle impact assessment (LCIA) level. It is thus reasonable to conclude that LCA provides a fairly muted representation of the actual differences between the environmental performance of the process options. This is especially the case when LCIA results are interpreted on a full life cycle basis, as with the current case study. In the context of this thesis, where LCA is applied as a tool for screening process options, LCA is better suited to select between process options that are vastly different. The use of LCA for decision making between minor process modifications is not likely to show the environmental performance of the design modification as significantly different from the previous design. The results of the comparison above show that LCA is most valuable for process selection in early stages of process development when decision making typically considers processes designs where inventory data is significantly different. LCA is likely to show meaningful differences in impact categories for processes using different feedstocks, and considerably different processing routes. In more detailed design phases (i.e. preliminary and detailed engineering phases), as highlighted in Chapter 2, when finer distinctions are made with regard to the process design (i.e. feedstock, bioreaction catalyst, and medium components have been finalised and selection of downstream unit operations is typically of concern), LCA is unlikely to be valuable as a screening tool for selecting between design options. Further, it would suggest that the time and effort spent to generate a detailed process model is of little value considering the effects of aggregation between the process options.

The results above show that the generic flowsheet model can be used to generate inventory data of sufficient quality to be used in life cycle impact assessment of large scale bioprocesses for early stage process comparison. This is in agreement with previous case studies (Harding, 2008; Harding *et al.* 2007a, Harding *et al.* 2007b).

Table 8.4 Impact results for citric acid production using starch

Impact category	Unit	Biwer *et al.* (2006)	Generic Flowsheet	%Difference
Abiotic depl	kg Sb eq	0.046	0.046	0.21%
Global warming	kg CO_2 eq	4.8	4.8	1.4%
Ozone layer depl	kg CFC-11 eq	5.80E-07	5.81E-07	0.16%
Human tox	kg 1,4-DB eq	1.8	1.83	-0.48%
Aquatic ecotox.	kg 1,4-DB eq	0.49	0.48	-3.2%
Marine ecotox	kg 1,4-DB eq	1800	1800	0.00%
Terrestrial ecotox	kg 1,4-DB eq	0.046	0.0451	-1.7%
Photo-ox	kg C_2H4	0.00087	0.00087	0.05%
Acidification	kg SO_2 eq	0.024	0.024	-1.0%
Eutrophication	kg $PO^{3-}{}_4$ eq	0.010	0.0098	-4.7%

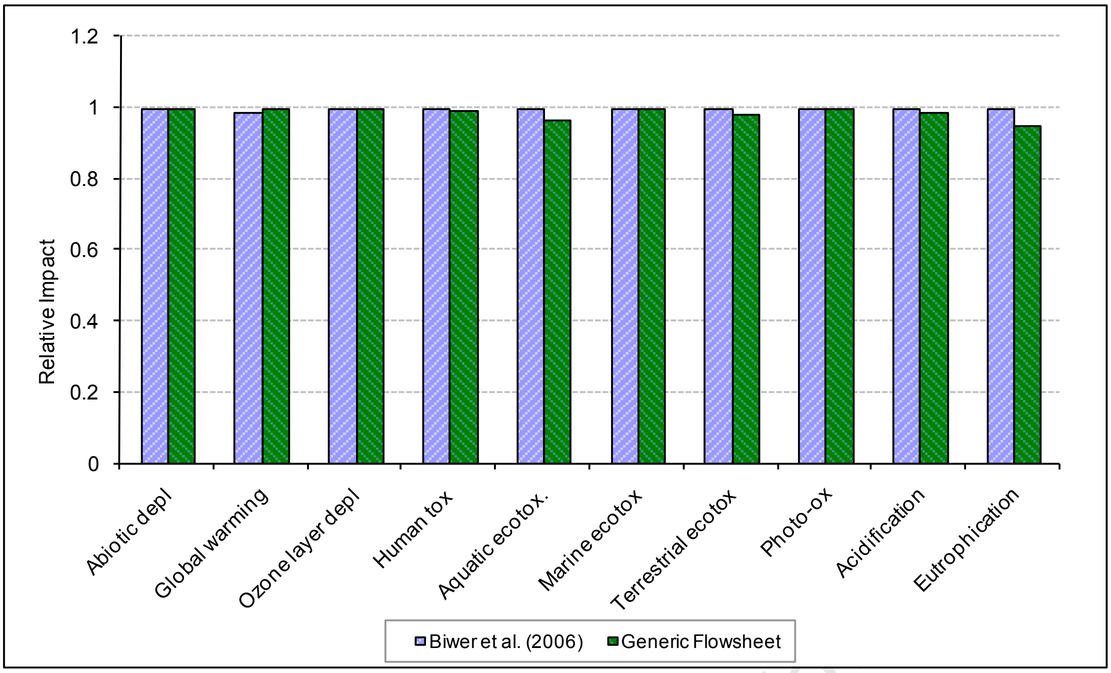

Figure 8.3 Relative results of life cycle assessment impacts of citric acid production using starch (comparing detailed simulation data with the generic flowsheet)

8.4.2 Comparative Assessment of Starch and Molasses Process

The environmental impacts of citric acid production using starch were compared to the impacts of the process using molasses as its main raw material input. The inventory data for each process was generated using the generic flowsheet model to simulate process operations, reported in Section 7.2 and 7.3. The results provided a basis for a broad environmental assessment of the production of citric acid from two different feed stocks, namely maize starch and beet molasses. Contributions by specific unit operations along the product life cycle are identified and their influence on the environmental impact categories discussed.

8.4.2.1 Overall Process Assessment

The impact assessment of the product life cycle across both process routes are shown in Table 8.5 and compared on a relative basis in Figure 8.5. The relative difference of the processes is expressed as a percentage change of the impact categories of the starch process relative to the molasses process The variation across impact categories, was most significant with regards to global warming (35%), ozone layer depletion (-47%), fresh water aquatic ecotoxicity (76%), terrestrial ecotoxicity (120%), photochemical oxidation (-22%) and eutrophication (180%). The processes compared relatively closely with the remaining categories. The variation in the impact categories can be mapped onto contributions by the individual operations in each product life cycle. The growing and processing of the maize starch and beet molasses varied with respect to input materials, energy consumption and process operations. Similarly, citric acid production varied in terms of input materials, unit operations, energy requirements and emissions.

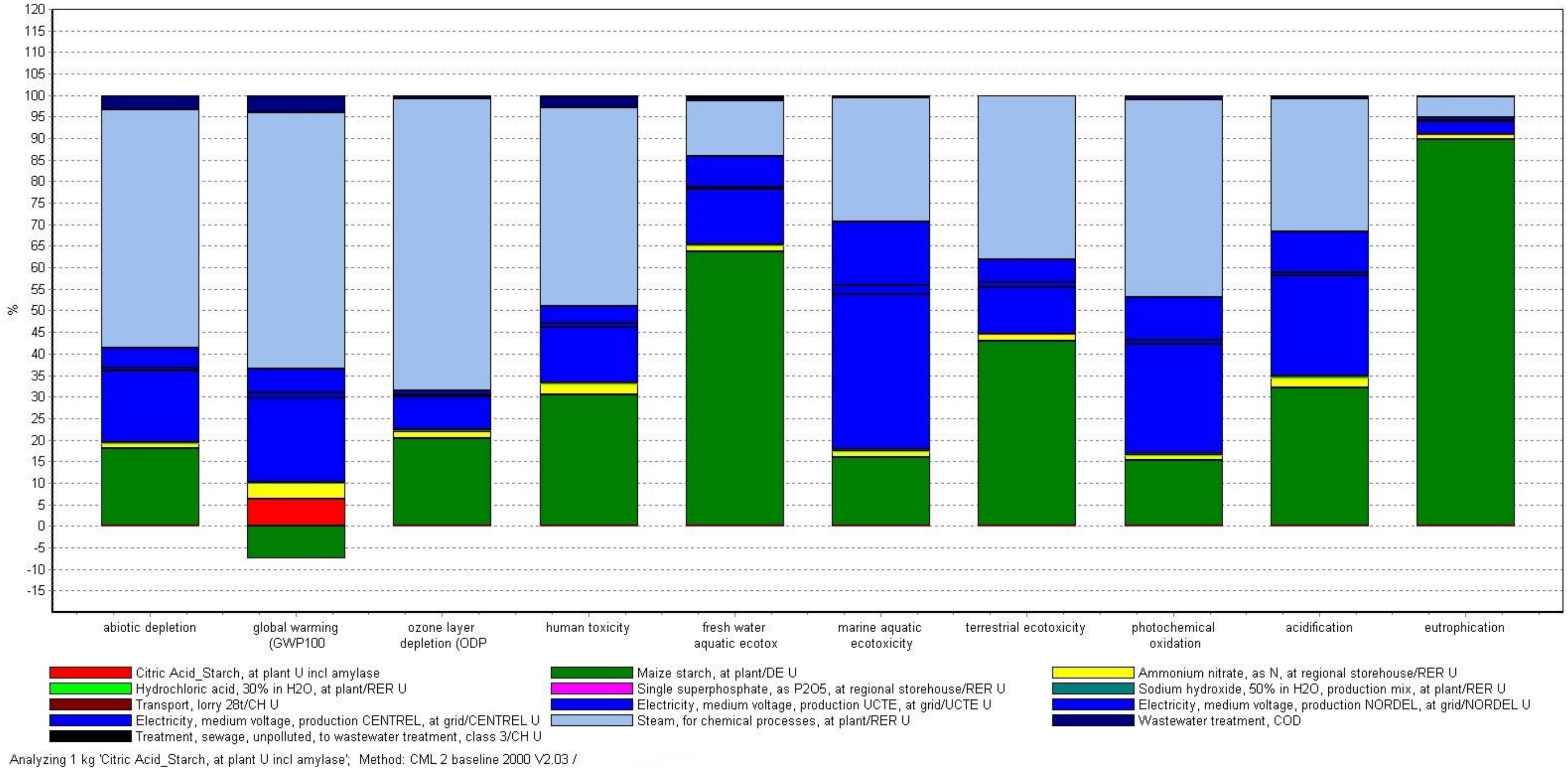

Figure 8.4 Life cycle impact characterisation of citric acid production using starch (generic flowsheet inventory)

Table 8.5 Comparison of impact results for citric acid production

Impact category	Unit	Starch Process	Molasses Process	%Difference
Abiotic depl	kg Sb eq	0.046	0.041	11%
Global warming	kg CO_2 eq	4.8	3.6	34%
Ozone layer depl	kg CFC-11 eq	5.8E-07	1.1E-06	-47%
Human tox	kg 1,4-DB eq	1.8	1.7	9%
Aquatic ecotox.	kg 1,4-DB eq	0.48	0.27	76%
Marine ecotox	kg 1,4-DB eq	1800	2300	-22%
Terrestrial ecotox	kg 1,4-DB eq	0.045	0.0208	120%
Photo-ox	kg C_2H4	0.00087	0.0011	-22%
Acidification	kg SO_2 eq	0.024	0.024	-1%
Eutrophication	kg PO^{3-}_4 eq	0.0098	0.0035	180%

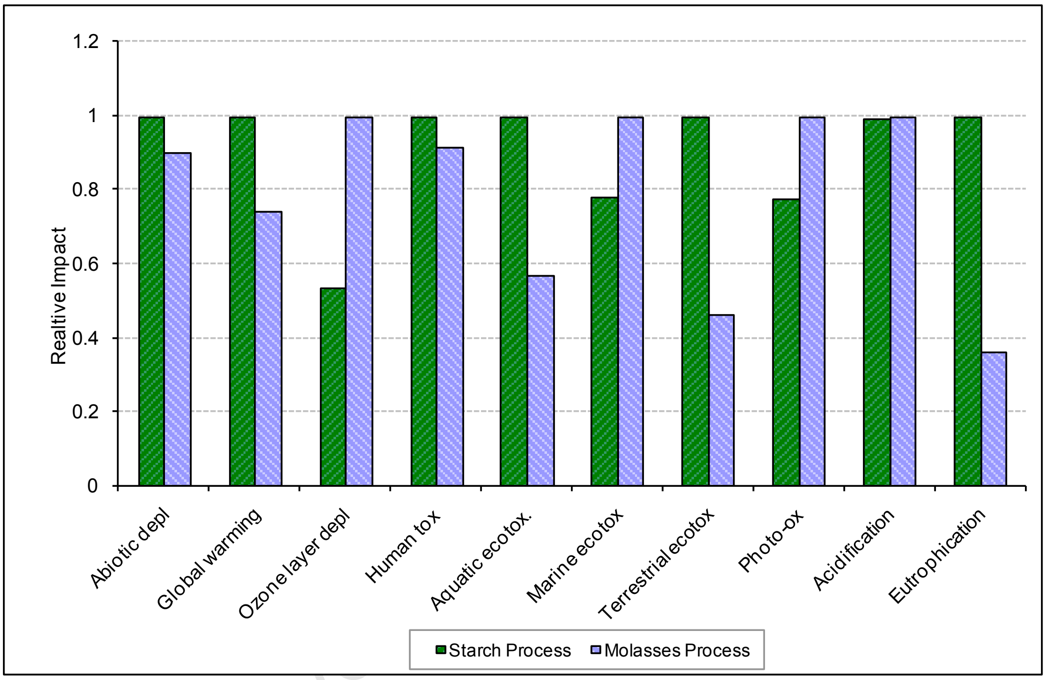

Figure 8.5 Relative results of life cycle assessment impacts of citric acid production (comparing generic flowsheet simulation for alternative process routes)

Contributions to the impact categories are presented in Figure 8.4 and Figure 8.6. The largest contributions to the environmental impact categories were the large energy requirements, namely electricity and steam; and the burdens associated with growing and processing of the maize starch and beet molasses. The generation and supply of electricity resulted in significant contributions to all the impact categories except ozone layer depletion (ODP) and eutrophication. A break-down of the relative electricity demand for individual unit operations for each process plant is shown in Figure 8.7. The majority of the electricity demand for both processes was used for air compressors (CM-001), bioreactor agitation (RX-001) and sterilisation of the media supplied to the bioreactor (ST-001, ST-002). The starch process showed a decrease in total electricity demand due to the reduced bioreactor volume and fewer filtration steps. The higher bioreactor volume and aeration rate in the molasses process resulted in a higher electricity requirement for agitation and aeration.

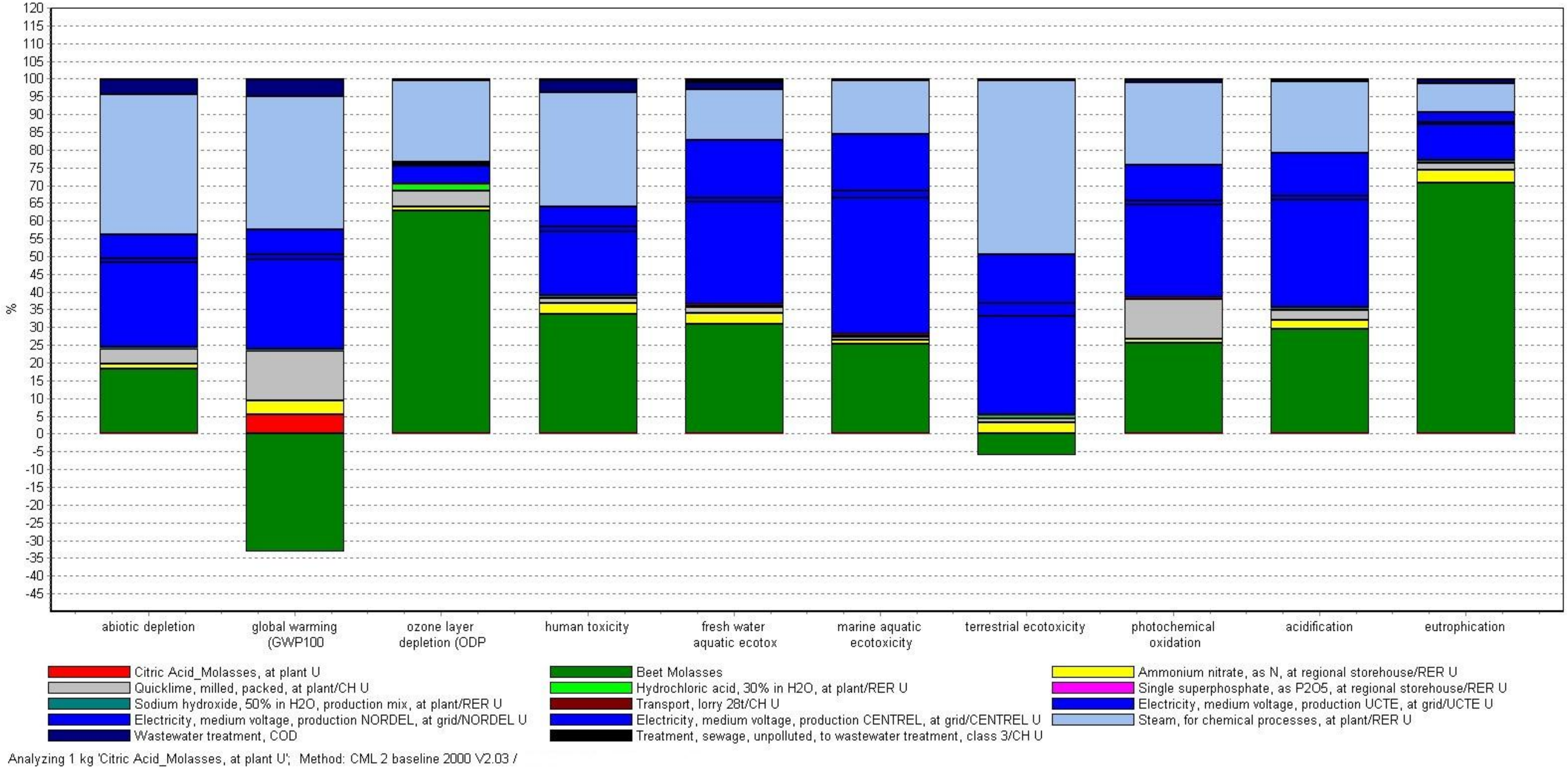

Figure 8.6 Life cycle impact characterisation of citric acid production using molasses (generic flowsheet inventory)

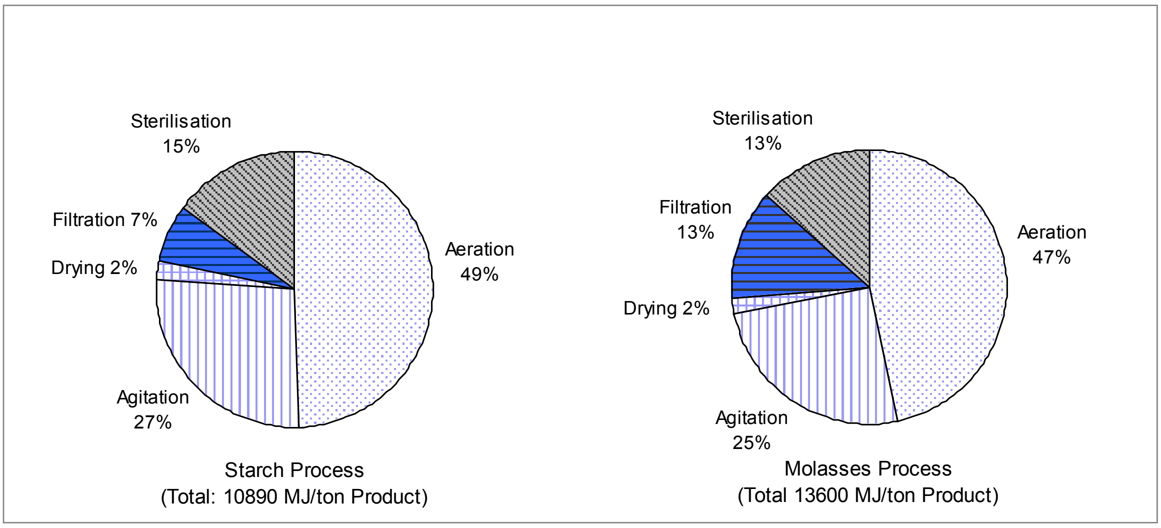

Figure 8.7 Relative electricity demand for major consumers in the production of citric acid

Similarly, steam production and supply contributed significantly to most impact categories. The contribution of individual unit operations is shown in Figure 8.8. Evaporative crystallisation (CR-001) accounted for 77% and 75% of the process steam requirement for the starch and molasses process respectively. The starch process (14,100 kg/ton product) used considerably more steam than the molasses process (8700 kg/ton product). This was due to the larger evaporative load on the unit compared to the molasses process, where a large portion of liquid filtrate was removed in the filtration operation (FT-002) preceding crystallisation. A relatively large portion of the steam requirement in the starch process was used in the bioreactor for deactivation of amylase (14%) following the hydrolysis step, contributing to the larger steam demand of the process.

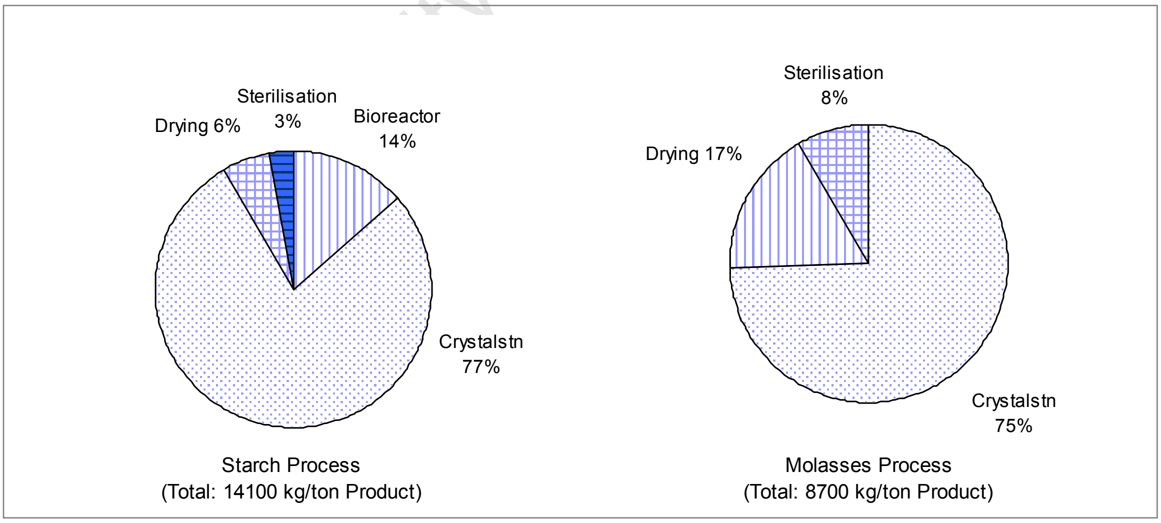

Figure 8.8 Relative steam demand for major consumers in the production of citric acid

8.4.2.2 Analysis of Impact Categories

The starch and molasses processes were compared and analysed with regards to individual impact categories. This gave insight into the individual contributions of the impacts for the respective processes. The impact categories with the most significant variation included global warming (35%), ozone layer depletion (-47%), fresh water aquatic ecotoxicity (76%), terrestrial ecotoxicity (120%) and eutrophication (180%). A detailed break-down of the impact category contributions, shown in

Figure 8.9 to Figure 8.18, by major sub-processes within the citric acid life cycle is given in Appendix D. Specific impact values expressed in the subsequent paragraphs are for the starch and molasses process respectively (e.g. starch process value /molasses process value kg CO_2 eq).

i. Abiotic Depletion

The individual contributions to abiotic depletion for the two production routes are shown in Figure 8.9. Excluding the impact of steam, the magnitudes of the contributions were similar for both processes. The larger steam requirement for crystallisation and enzymatic components of the starch process resulted in a larger abiotic depletion impact. An additional contribution for the molasses process was from lime used for tri-calcium citrate precipitation. The magnitude of abiotic depletion was mainly attributed to the extraction of natural gas (0.0248/0.0156 kg Sb eq), crude oil (0.00678/0.00482 kg Sb eq) and coal (0.00339/0.00321 kg Sb eq) for steam and electricity generation.

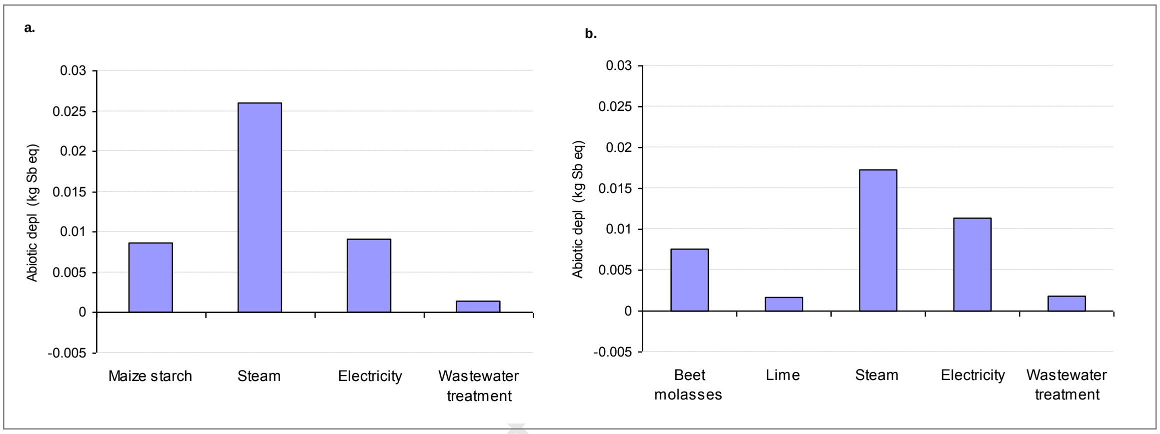

Figure 8.9 Life cycle process contributions to abiotic depletion (kg Sb eq) for citric acid production via a) starch process and b) molasses process

ii. Global Warming

The individual contributions to global warming, shown in Figure 8.10, varied considerably between the two process routes. The overall impact of the starch process on global warming was approximately 41% higher than that of the molasses process. The maize starch and beet molasses components of the life cycle system boundary both reduced the impact of global warming, attributed to carbon sequestered during agricultural growing of the maize and beet. An additional reduction in global warming was included for the beet molasses production. The model was adapted from the model based on the production of beet sugar (LCA Food DK, 2000). Co-generation of feed pills in the sugar process assumed that 1 kg used for animal feed displaced 0.9 kg of spring barley, thus reducing the contribution to associated impact categories. The major contributors to global warming included the burning of natural gas (2.31/1.35 kg CO_2 eq), heavy fuel oil (0.688/0.459 kg CO_2 eq) and lignite (0.3040/.230 kg CO_2 eq) for steam and electricity generation, required for the citric acid plant and wastewater treatment. The contribution by ammonium nitrate was primarily due to the production of nitric acid (0.209/0.189 kg CO_2 eq) required for ammonium nitrate production. The lime requirement of the molasses process contributed to the release of CO_2 (0.909 kg/kg quicklime) during the calcination of limestone. The citric acid production plant itself resulted in a small contribution to global warming by way of CO_2 emissions (0.291/0.291 kg CO_2 eq) from the bioreaction process.

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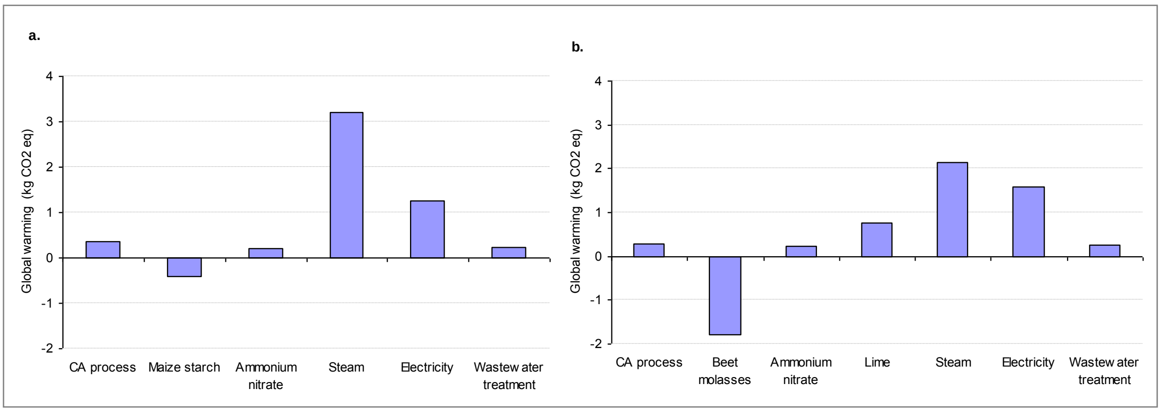

Figure 8.10 Life cycle process contributions to global warning (kg CO2 eq) for citric acid production via a) starch process and b) molasses process

iii. Ozone Layer Depletion

The effect of the process life cycles were relatively small for ozone layer depletion, shown in Figure 8.11. The majority of ozone layer depleting compounds have been phased out and are not of a major concern to a process impact assessment (Roes & Patel, 2007). The results of the impact due to ozone layer depletion impact were nonetheless compared for the two processes, but are likely to contain a high degree of uncertainty with regards to the actual impact values. The impact of the starch life cycle on ozone layer depletion was significantly smaller (-47%) relative to the molasses system. This was primarily attributed to emissions from the generation of a large quantity of heat required for the production of sugar from beet. The impact from steam generation was slightly larger for the starch process (3.96E-07/2.63E-07 kg CFC-11 eq), while the impact from electricity generation was similar for both processes.

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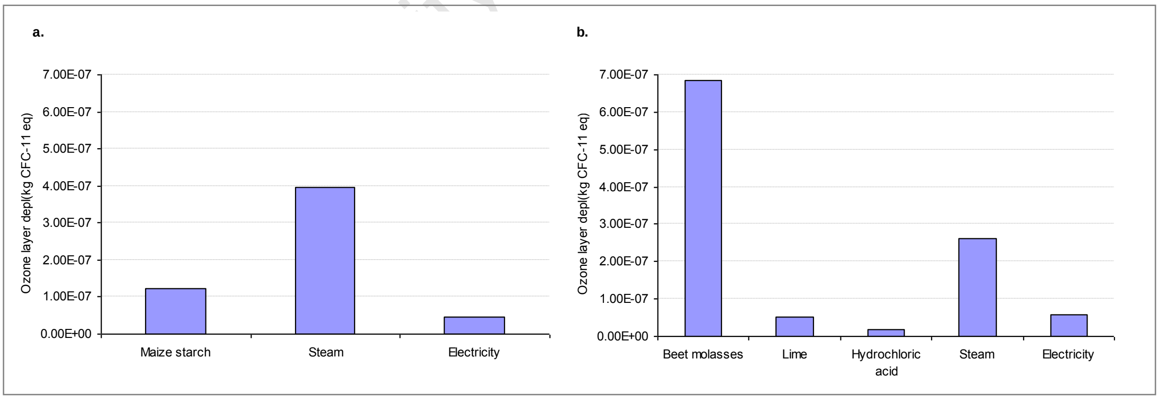

Figure 8.11 Life cycle process contributions to ozone layer depletion (kg CFC-11 eq) for citric acid production via a) starch process and b) molasses process

iv. Human Toxicity

The contributions to human toxicity, shown in Figure 8.12, were similar for both systems. The largest contributors were maize starch and beet molasses production, steam and electricity generation and wastewater treatment. The contribution by steam and electricity generation was attributed primarily to the emissions from burning of heavy fuel oil (0.449/0.299 kg 1,4-DB eq) and natural gas (0.268/0.156 kg 1,4-DB eq). Similarly, the contribution by wastewater treatment was due to emissions from natural gas burning (0.0455/0.0583 kg 1,4-DB eq) for energy requirements of treatment operations. The contribution from maize starch and beet molasses was as a result of emissions from agricultural operations, including fertilisation, weed control and pest and pathogen control.

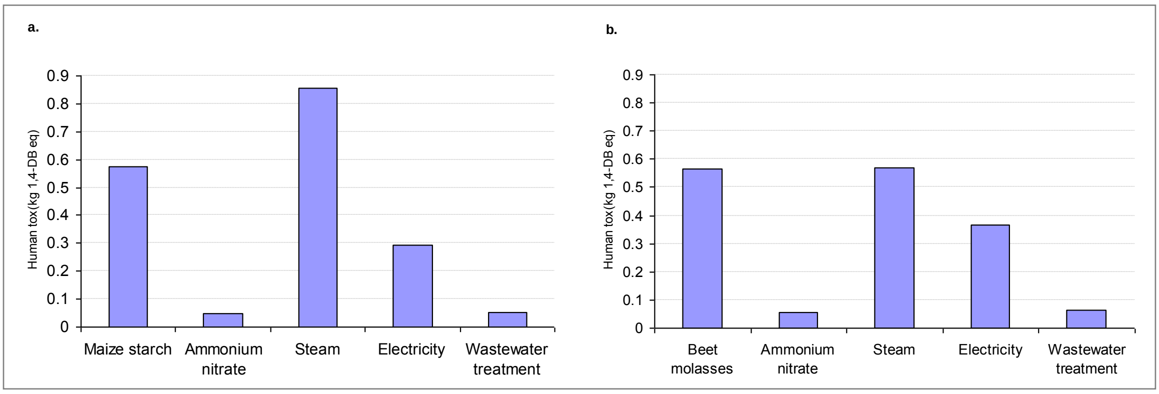

Figure 8.12 Life cycle process contributions to human toxicity (kg 1,4-DB eq) for citric acid production via a) starch process and b) molasses process

v. *Fresh Water Aquatic Ecotoxicity*

The contribution of citric acid production from maize starch to fresh water aquatic ecotoxicity was considerably higher (81%) than the contribution from the process using beet molasses, shown in Figure 8.13. This was largely attributable to the agricultural component. Maize growing operations and specifically the discharge of compounds used in herbicide (metolachlor, atrazine) and insecticide (cypermethrin) had a large effect on the impact category. Large nitrate and phosphate emissions from maize growing relative to beet growing were also significant contributors to the impact category. The contributions from steam (0.0615/0.0409 kg 1,4-DB eq) and electricity (0.088/0.11 kg 1,4-DB eq) generation were similar for both process routes. The contributions were largely as a result of the burning of heavy fuel oil (0.043/0.029 kg 1,4-DB eq) and the disposal of lignite ash (0.0432/0.0451 kg 1,4-DB eq) from lignite burning operations.

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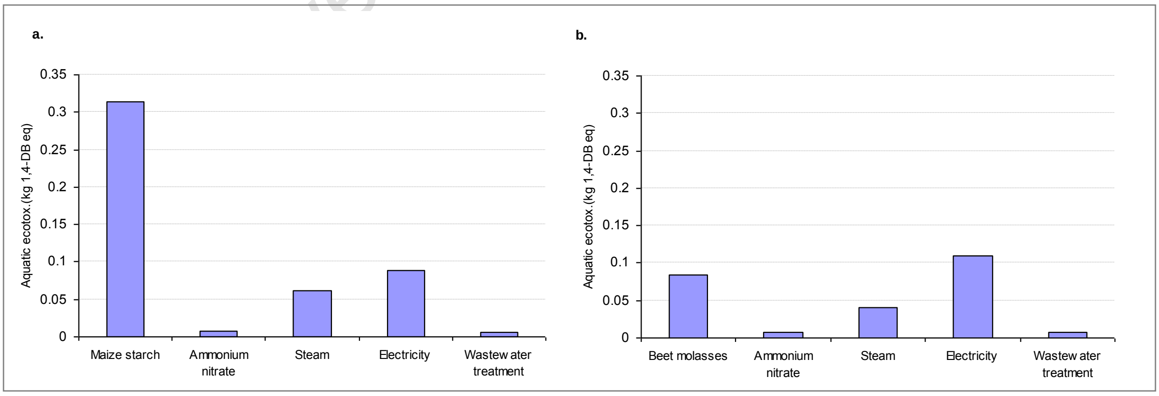

Figure 8.13 Life cycle process contributions to fresh water aquatic ecotox. (kg 1,4-DB eq) for citric acid production via a) starch process and b) molasses process

vi. *Marine Aquatic Ecotoxicity*

The results of the impact assessment for marine aquatic ecotoxicity are presented in Figure 8.14. The major contributions to the impact category were from maize starch (318 kg 1,4-DB eq) and beet molasses production (592 kg 1,4-DB eq), steam (563/374 kg 1,4-DB eq) and electricity generation (919/1150 kg 1,4-DB eq). Ammonium nitrate production (26/29 kg 1,4-DB eq) was a small contributor relative to the other inputs for both process systems. The absolute impact of marine aquatic toxicity in terms of mass equivalents of 1.4-dichlorobenzene, shown in Table 8.4, was considerably higher than comparison impact categories, namely human toxicity, fresh water aquatic ecotoxicity and terrestrial ecotoxicity. It is well known within the LCA community that characterisation factors for marine ecotoxicity and ecotoxicity to marine and fresh water ecotoxicity do not include sedimentary factors within the CML 2000/2001 baseline characterisation method. Further, there are concerns within the LCA community over the marine ecotoxicity category, with regard to the impact of hydrogen fluoride and normalisation results. This leads to particularly high estimates for the impact category in comparison to other toxicity categories. As a result it is not suggested that these three categories are compared to other impact categories on a normalised basis. The contributions from steam and electricity generation were primarily as a result of heavy fuel oil (355/237 kg 1,4-DB eq), coal (445/465 kg 1,4-DB eq) and lignite (249/258 kg 1,4-DB eq) burning.

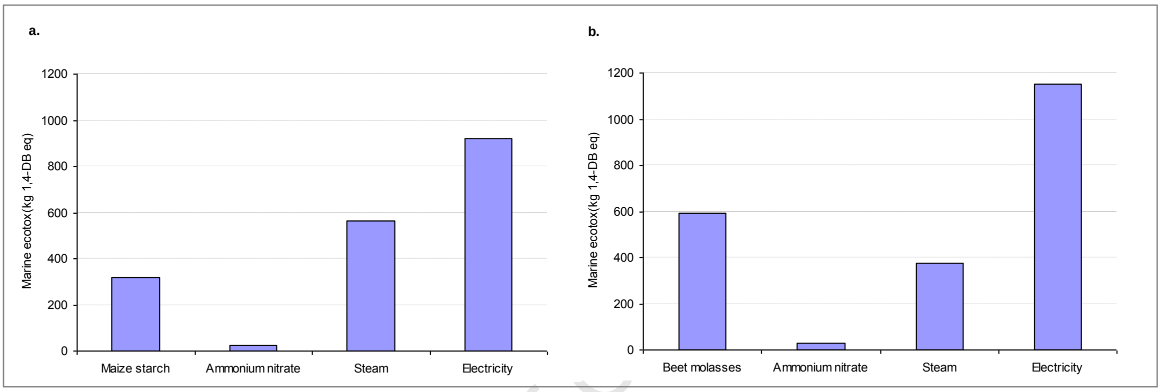

Figure 8.14 Life cycle process contributions to marine aquatic ecotoxcity (kg 1,4-DB eq) for citric acid production via a) starch process and b) molasses process

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vii. Terrestrial Ecotoxicity

The results for terrestrial ecotoxicity, presented in Figure 8.15, show the contributions that resulted in the impact of the starch process life cycle being considerably higher (120%) than that of molasses. This was attributed mainly to the comparatively larger impacts from maize starch production and steam generation for the starch process life cycle. The variation in the impact of maize starch (0.0201 kg 1,4-DB eq) and beet molasses (-0.00134 kg 1,4-DB eq) in terrestrial ecotoxicity was due to emissions of heavy metals (mercury, chromium VI) and compounds contained in agricultural chemicals (cypermethrin, atrazine) during maize growing. A net uptake of heavy metals (-0.00134 kg 1,4-DB eq) during beet growing further contributed to the large difference between the terrestrial ecotoxicity impact associated with the starch and molasses life cycles. Similarly to marine aquatic ecotoxicity contributions, steam and electricity generation were primarily as a result of heavy fuel oil (0.0161/0.0107 kg 1,4-DB eq) and lignite (0.00146/0.00183 kg 1,4-DB eq) burning.

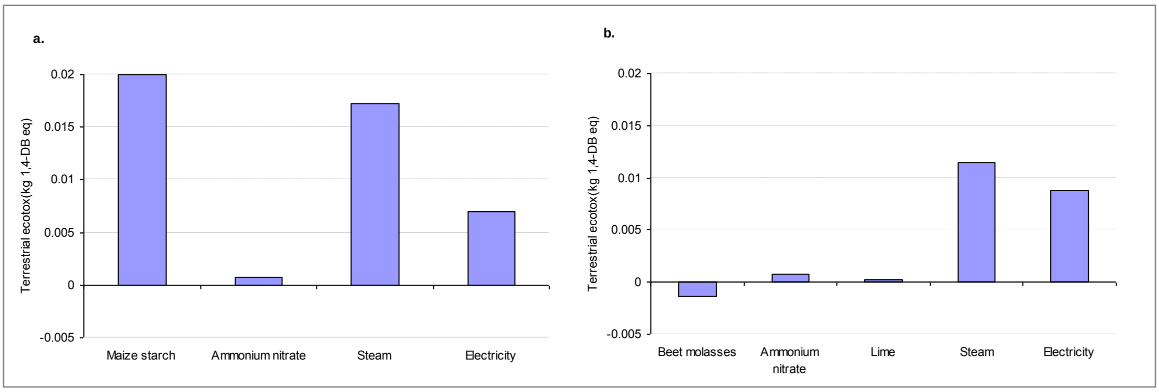

Figure 8.15 Life cycle process contributions to terrestrial ecotoxicity (kg 1,4-DB eq) for citric acid production via a) starch process and b) molasses process

viii. Photochemical Oxidation

The contributions associated with the photochemical oxidation impact category are shown in Figure 8.16. Although the absolute value of the impact category for each process life cycle was relatively small (0.00084/0.00108 kg C_2H_4 eq), there was a significant difference in total for each, as well as the specific contributions. The total impact from the starch process was lower than the molasses process life cycle (-18%), primarily as a result of the contribution by beet molasses processing and lime production. The combined impacts associated with steam and electricity generation (0.000706/0.000640 kg C_2H_4 eq) were similar for the process systems. The contribution to the impact category by steam and electricity generation was mainly from the release of SO_2 (0.000585/0.000525 kg C_2H_4 eq) during heavy fuel, natural gas and lignite burning. The contribution from maize starch and beet molasses was primarily from heat generation, required for the production of starch and molasses. A small portion of the impact was as a result of the transportation of the starch and molasses to the citric acid processing plant. Carbon monoxide emissions (0.000131 kg C_2H_4 eq) were the main component of the contribution from lime production in the molasses system.

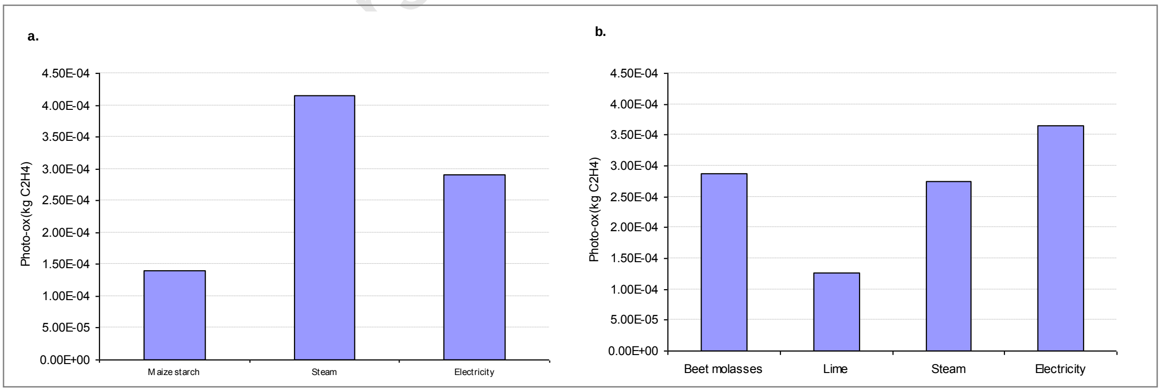

Figure 8.16 Life cycle process contributions to photochemical oxidation (kg C_2H_4 eq) for citric acid production via a) starch process and b) molasses process

ix. Acidification

The contributions to acidification, shown in Figure 8.17, were similar for both process life cycles. Emission from maize starch (0.00819 kg SO_2 e) and beet molasses (0.00723 kg SO_2 eq) production and steam (0.00773/0.00513 kg SO_2 eq) and electricity (0.00740/0.00927 kg SO_2 eq) generation were the main contributors to the impact category. The primary contributors to the impact of maize starch production on acidification were emissions (SO_2, NH_4, NO_x) from maize and beet agriculture

(0.00819/0.00723 kg SO_2 eq) and electricity generation for starch and molasses production. The impact from energy generation was mainly from emissions (SO_2, NO_x) from heavy fuel oil (0.00546/0.00405 kg SO_2 eq), coal (0.00144/0.00174 kg SO_2 eq) and lignite burning (0.00153/0.00184 kg SO_2 eq).

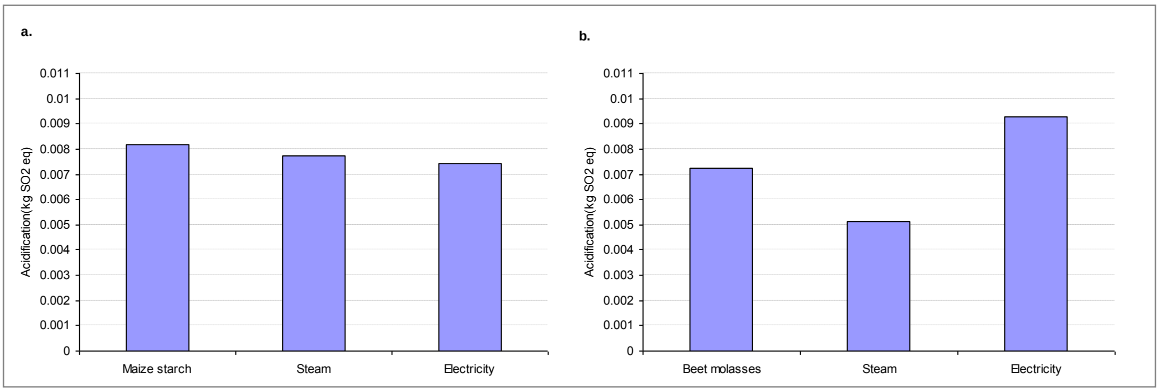

Figure 8.17 Life cycle process contributions to acidification (kg SO_2 eq) for citric acid production via a) starch process and b) molasses process

x. Eutrophication

The main contributions to eutrophication for both the starch and molasses life cycle are shown in Figure 8.18. The total eutrophication impact of the respective systems was almost entirely as a result of maize starch and beet molasses production. The impact of maize starch (0.00819 kg PO_4^{3-} eq) was considerably higher than that of beet molasses (0.00251 kg PO_4^{3-} eq). The primary contributors to the impact of maize starch production on eutrophication were emissions (NH_4, PO_4^{3-}, NO_x) from the production and use of fertilizer. Similarly, the production and use of green manure (0.000971 kg PO_4^{3-} eq) as a fertiliser in sugar beet production was the primary contributor to the eutrophication impact category.

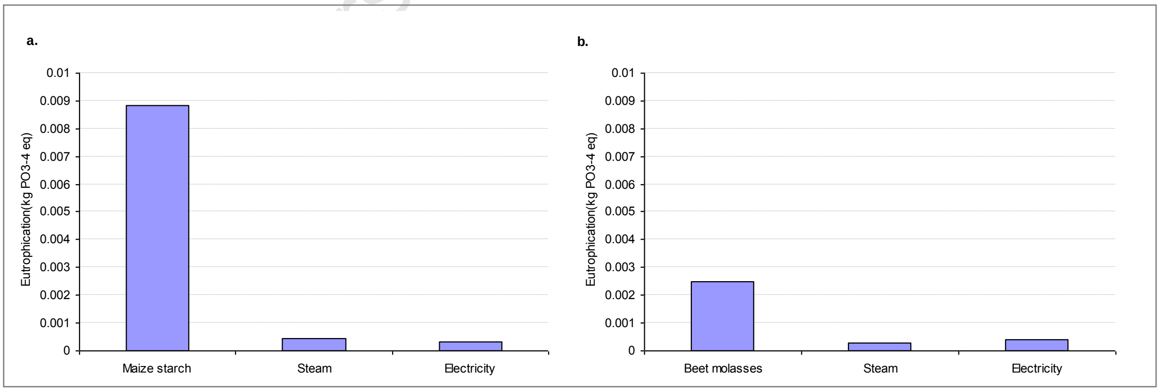

Figure 8.18 Life cycle process contributions to eutrophication (kg PO_4^{3-} eq) for citric acid production via a) starch process and b) molasses process

xi. Land Use and Water Consumption

There are no standard methodology and commonly accepted datasets currently used to analyse land use and water consumption (von Blottnitz & Curran, 2006). Land use was considered for agricultural crops producing grain maize and sugar beet. Approximately 1.23 kg of grain maize is required to produce 1 kg of sugar for the citric acid process. Taking an average crop yield for maize production in the United Sates (2004-2007), approximately 0.966 m^2 of arable land was required to produce a kilogram of maize (FAOSTAT, 2008), thus requiring 1.18 m^2 to produce 1 kg of sugar. The average crop yield for sugar beet production in Europe is approximately 5.12 kg/m^2 (i.e. 0.195 m^2/kg). Assuming that 7.1 kg of beet is required to produce 1 kg of beet sugar and 0.24 kg of molasses (LCA Food DK), approximately 5.78 m^2 of arable land is required to produce 1 kg of molasses. In comparison to sugar from maize, the production of sugar from sugar beet placed a significantly greater demand on land use. The water use of the two processes was quantified in the inventory data and showed that the molasses process consumed approximately 30% more water than the starch process. The difference was primarily due to the larger number of filtration steps in the molasses process as well as the water contained in the lime slurry.

8.4.2.3 Normalised Impact Assessment

The impact categories were dominated by impacts from energy use for the agricultural crop production and citric acid production. Normalisation is not likely to give additional insight into the relative impacts of the life cycle system. The results of human toxicity, fresh water aquatic ecotoxicity and marine aquatic toxicity showed that the maize starch and beet molasses production made significant contributions to the impact categories. The remainder of the impact score was comprised of contributions from electricity generation and steam supply, which contributed significantly to all impact categories respectively. The absolute impact of marine aquatic toxicity in terms of mass equivalents of 1.4-dichlorobenzene, was considerably higher than comparison impact categories, namely human toxicity, fresh water aquatic ecotoxicity and terrestrial ecotoxicity. A decrease in electrical and steam requirements and agricultural inputs would have the greatest impact in reducing overall LCA scores.

8.5 Analysis of Uncertainties in Early-Stage Assessment

The objective of the uncertainty analysis is to assess the reliability of the final results and conclusions by determining whether they are affected by uncertainties in the data, allocation methods or calculation of category indicator results. Although uncertainty analysis is encouraged (ISO, 2006) it is not a mandatory requirement and application of uncertainty analysis to life cycle impact assessment (LCIA) still requires development (Ross *et al.,* 2002; Huijbregts *et al.*, 2003). Uncertainties can be divided into those which affect the estimation of the potential impacts of certain activities and those that pertain to uncertainties in variables which are used for the evaluation of these impacts. Considering the latter, it is helpful to characterise uncertainty using probability distributions to determine uncertainty in the LCIA results and provide additional insight to support conclusions. Uncertainty and variability affect the outcomes of the environmental assessment presented for the citric acid case study presented in this thesis. The implementation of uncertainty analysis in the assessment can be used to test the reliability of various results produced by the generic flowsheet model as well as to identify parameters which require special attention during process development. Although parameter uncertainty was not explicitly performed in the current work, a brief discussion is provided as a primer for future investigation.

8.5.1 Model Form and Parameter Uncertainty

Uncertainty analysis of the impact categories pertains either to choice of impact categories, or LCIA methodology/types of impact indicators. Methodological choices lead to model form uncertainty and the effect of these types of uncertainties are best examined via sensitivity analysis (i.e. considering each choice and the associated effects on the results and conclusions in turn). Variances that may affect the results of the environmental impact assessment are associated with empirical parameters and their effects may be examined by Monte Carlo simulation (MCS). MCS determines how variance

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propagates through the system model to affect the results of the environmental impact assessment. The approach propagates identified parameter uncertainties into an uncertainty distribution of the output variable. In the Monte Carlo simulation each uncertain input parameter has to be specified as an uncertainty distribution. The inventory data has an associated uncertainty distribution, defined in SimaPro as *range values, triangular, normal*, or *log normal*. The majority of the input inventory data used for life cycle assessment is commonly defined as a log normal distribution (Geisler *et al.*, 2005; PRé, 2007). The analysis counts the number of times the Monte Carlo routine predicts that a specific system would be better or worse than a comparative system for each impact category. In order to assess the uncertainty in the input parameters, detailed process data or expert judgement is required on the type of distribution appropriate to each of the parameters. The results indicate the certainty with which the relative impact for each impact category was determined. As highlighted by Huijbregts *et al.* (2003), life cycle assessments involve a vast number of input parameters and it is unfeasible to characterise the uncertainty ranges for all these parameters in detail. Huijbregts *et al.* (2003) provides an iterative method of identifying the most important parameters based on measured data or expert judgement.

It is important to consider that detailed probability distributions describing the input parameters would typically not be available in early stages of process design, as in the instance of the citric acid case study presented in this thesis. The approach would most likely need to be supplemented by available process data from similar processes or implemented in later stages of the design process when more detailed process data is available. A study adopting this approach has been expertly demonstrated by Biwer *et al.* (2004), using mostly literature data and ‗internal estimates' in an early-stage case study for the simulated production of penicillin V. Provided however, that important process variables have been identified, Monte Carlo Simulation can be used to perform the uncertainty analysis, as demonstrated by Biwer *et al.* (2004). As discussed in Chapter 3, Harding (2008) investigated the sensitivity of the generic flowsheet to input data in a case study for the production of Penicillin V. The investigation was used to identify variables that have the most influence on the material and energy requirements of a process modelled using the generic flowsheet. These included the product to biomass ratio, final biomass concentration, oxygen flowrate and compression pressure, yield coefficients and downstream processing separation efficiencies. Effects from these variables, particularly in energy requirements, ultimately affect the Life Cycle Assessment (LCA) scores for the production of the product. This provides a starting point for assessing uncertainty in the process model and life cycle impact scores. The toxicity scores should however not be over-interpreted since a relatively large uncertainty is inherent in these categories, as discussed in preceding sections.

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8.6 Conclusions

The purpose of the environmental assessment was two-fold, firstly to compare the generic flowsheet to data from a detailed simulation model and then to assess the environmental impact of the production of citric acid from two different types of feedstock. The chapter presented the comparison of the environmental impact assessment of the inventory data from a literature case study and inventory data from the generic flowsheet for the production of citric acid using starch as the main raw material input. The results from the two inventory sets were in relatively good agreement with regard to all the impact categories. This was in agreement to the previous work by Harding (2008). The model can thus be used to generate inventory data of sufficient quality to be used in life cycle impact assessment of large scale bioprocesses with relative accuracy, in comparison to more detailed simulation models.

Following the initial comparison, the environmental impact as result of the production of citric acid was compared for two different process routes. The traditional process using molasses was compared to the process using starch as the main raw material. The purpose of the comparative environmental assessment of the process routes to identify the processes that had the lower environmental impact on a relative basis, ranking the relative contribution of individual steps and providing information to guide product and process development. The contributions to the environmental impact from the production of citric acid were mostly common for both the molasses and starch processing routes. The contributions were due to large electricity and steam requirements and the production of maize starch and beet molasses. Investigation of the various sub-processes used to model the citric acid production system gave deeper insight into the contributions to the impact categories. The large electricity requirements were mainly used for air compression and bioreactor agitation, while steam was mainly used for evaporation of water in the crystallisation unit operation. The emissions from electricity and steam generation were mainly from heavy fuel oil, lignite, coal and natural gas burning, which affect most impact categories. The burden associated with maize starch production and beet molasses production was largely attributed to the use of herbicides and pesticides during agricultural operations, especially for maize growing. The large heat requirement for the processing of maize and beet into starch and molasses respectively was also a significant contributor to the impact categories. A decrease in electrical energy requirements and agricultural inputs would have the greatest impact in reducing overall LCA scores.

In attempting to examine uncertainty in the results obtained from the assessment it is important to distinguish between model form and parameter uncertainty. Model form uncertainty is as a result of decision making and methodological choices in the model structure and may be investigated using a sensitivity analysis (i.e. considering each choice and the associated effects on the results and conclusions in turn). The uncertainties associated with parameter variances that may affect the results of the environmental impact assessment may be examined by parametric analysis for which Monte Carlo Simulation (MCS) is a valuable approach. MCS uses a large number of random samples from a range of potential performance scores and determines the relative preference for the alternatives. The simulation can be used to assess the reliability of the results and provide additional support for the conclusions.

Although the processes were compared on a relative basis and the process with the least environmental impact could be identified for each impact category, a conclusion with regard to the most superior process on average is a somewhat subjective decision. The limitations of the assessment did not give a single absolute measure with which to choose between the process routes, but rather improved our understanding of the process models and the associated operations. The case study presents a systematic methodology for environmental assessment when detailed process data is not

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available. Specific unit operations that contribute to specific environmental concerns can be identified and quantified and solved or mitigated in early stages of process development. Alternative process routes and new technologies can be evaluated and implemented in early stages of process design and development. The results of the study support the research hypothesis by showing that first approximation process simulation can be used as a basis for life cycle assessment, and a valuable contribution can be made to sustainable process development.

CHAPTER 9

ECONOMIC ASSESSMENT

An economic assessment of the production of citric acid was performed for the alternative process routes modelled in Chapter 7. The structure of the economic analysis was similar to the approach used for the environmental assessment. The goals of the study are defined in the context of the specific case and the overall goals of the project. Following goal and scope definition, capital and operating cost estimations for both process options are presented. The capital and operating cost estimations are used as the basis for a profitability analysis. Finally, case study specific and project specific conclusions with regards to the economic assessment are presented.

9.1 Goal Definition

The economic assessment aimed to use the material and energy values to obtain an estimate on the economic performance of the production system. Similarly to the environmental assessment, the results thereof can contribute to the integration of the various elements required for decision making in sustainable bioprocess development. The material and energy data used in the economic assessment was obtained from the generic flowsheet model developed by Harding *et al.* (2008). As detailed in Chapter 5and Appendix C, the model was extended to provide an economic assessment of the process as a first order estimate. The specific objectives of the assessment aimed to:

1. Obtain an order-of-magnitude estimate of the capital requirements for citric acid production
2. Obtain an order-of-magnitude estimate of the operating costs associated
3. Compare the profitability of the production of citric acid from starch and molasses

9.2 Scope and Process Design Basis

9.2.1 System Boundaries

The system boundaries of the economic assessment were constrained to the citric acid production facility. This included capital expenditure required for construction of the plant and operating costs associated with the production of citric acid monohydrate as the finished product. The production system is shown in Figure 7.2 and Figure 7.7 for the starch and molasses process respectively.

9.2.2 Design Basis

The annual operating capacity of the plant was based on the case study presented by Biwer *et al.* (2006) for the annual production of approximately 12,600 metric tons of citric acid monohydrate. The plant was assumed to produce 99.995% pure citric acid monohydrate, operating for 330 days a year. The bioreaction stage and associated parameters for the starch and molasses processes were sufficiently similar to use the same batch scheduling and reactor configuration for both process options. A single batch takes approximately 189 hours, with the bioreactor occupying 165 hours and the downstream processing 34 hours. The process used 12 bioreactors in a staggered configuration, which were the bottleneck of the process. Based on the bioreactor batch time, 563 batches per year were completed. Although the production capacity and unit scheduling was based on the case study by

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Biwer *et al.* (2006), the economic input data (equipment costing, material expenses, market rates etc.) were obtained from various literature and manufacturer sources.

9.2.3 Economic Assessment Assumptions

A typical, simplified schedule, of the key design, construction and commissioning stages for the citric acid plant is shown in the Figure 9.1 (Couper, 2003; Peters *et al.*, 2003). The schedule was used as the basis for the construction and start-up period in the economic calculations.

ID	Task Name	Start	Finish	Duration
1	**Citric acid plant**	**2009/01/01**	**2012/01/02**	**783d**
2	Initial design and feasibility	2009/01/01	2009/04/30	86d
3	Detailed design and verification	2009/05/01	2009/12/31	175d
4	Plant construction	2010/01/01	2010/12/31	261d
5	Staff/employee training	2010/06/01	2010/12/31	154d
6	Pre-commissioning/start-up	2011/01/03	2011/03/31	64d
7	Commissioning/ramping up production rate	2011/04/01	2011/12/30	196d
8	Full production rate	2012/01/02	2012/01/02	1d

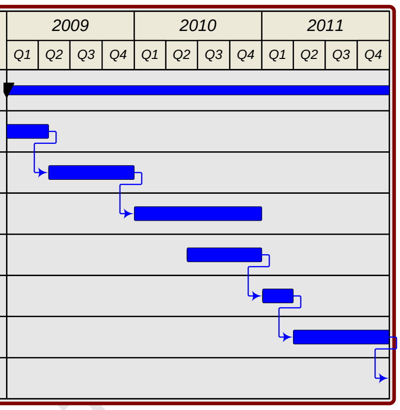

Figure 9.1 Gantt chart for the construction of a citric acid plant

Further, specific assumptions used in the economic assessment of the process plant were based on common values found in the literature (Peters *et al.,* 2003; Turton *et al.,* 2003) included:

1. All monetary values were quoted in $US
2. All purchase costs were adjusted 2005 cost index data
3. The costs incurred by the plant will increase annually at rate of 5%
4. The prime lending rate for bank finance was 12.5%
5. The plant will run at full capacity from date of start-up
6. All equipment depreciates over 10 years to a salvage value of 5% of the purchase cost (Sinnott, 1999; Turton *et al.*, 2003)

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The costs of the specific raw materials, waste disposal and utilities for the starch process model were based on the data used by Biwer *et al.* (2006). This allowed the starch process model, developed using the generic flowsheet tool, to be compared directly to the model by Biwer *et al.* (2006). The economic assessment was thus not influenced by variation in costing data, but rather based on variation in the material and energy balance results; default model parameters and process model assumptions. Similarly, the capital and operating cost estimations for comparison of the starch and molasses processes from the generic flowsheet were based on the same equipment and material cost data where applicable and the same reference year.

9.3 Unit sizing

The extended generic flowsheet model was used to size the major equipment used in the molasses and starch processes. The material and energy balance calculations (Chapter 7) were used as the basis for unit sizing. A description of the unit sizing models and input data requirements is presented in Chapter 5 and Appendix C.

i. Media Preparation Tank

The media preparation tank (TK-001) was sized on the basis of the volumetric feed of nutrients to the process, using the stirred tank reactor sizing model (Equations C.16-C.18). The contents of the unit were nitrogen source (NH_4NO_3), phosphorus source (KH_2PO_4) and water.

ii. Compressor

The individual compressor rating (kW) was calculated using the compressor model (C.6-C.7). The number of compressors required and the individual ratings were determined on the basis of the air flowrate to the bioreactor, compression time, maximum power and compression efficiency. The total throughput and compression time was based on the material and energy balance calculations for the starch and molasses process simulations. User input values to the model are shown in Table 9.1.

Table 9.1 Input variables for compressor sizing

Input	Value	Unit
Compression time	145	Hours
Max throughput for the unit	200	m^3/s
Max power for the unit	3000	kW
Compression efficiency of the unit	75	%

iii. Sterilisation

The sterilisation units (ST-001, ST-002) were sized based on the carbon source and nutrient feeds to the process and the time required for sterilisation. The input values were obtained from the material and energy balance results of the starch and molasses process simulations. The maximum heat transfer efficiency of the unit was assumed to be 90% and the maximum throughput was assumed to be 100 m^3/h. The rated throughput (m^3/h) of the units was calculated using Equations C.19 -C.20.

iv. Bioreactor

The bioreactor (RX-001) was sized using the stirred tank reactor sizing model (Equations C.16-C.18). The volume of the unit was based on the volumetric throughput of the unit (m^3/batch). User input values to the model are shown in Table 9.2. The default values for maximum capacity (80 m^3), overdesign and height to diameter ratio of the unit were provided. The maximum reactor volume was adjusted to 350 m^3 based on the model by Biwer *et al.* (2006). The large reactor volume was assumed justifiable based on the large-scale nature of the process. An overdesign factor of 10% was included (Peters *et al.*, 2003).

Table 9.2 Input variables for bioreactor sizing

Input	Value	Unit
Max volume of the reactor unit	350	m^3
Unit utilisation	90	%
Height/Diameter	3	-
Design pressure	1.5	Bar

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v. Rotary Vacuum Filtration

The rotary vacuum filters (FT-001-FT-004) used for biomass removal, precipitate recovery and crystal recovery were sized using the Equation A.7. The total area required for filtration was obtained from the material and energy balance calculations for the starch and molasses process simulations. The default maximum area (100 m^2) and efficiency (75%) was assumed.

vi. Ultrafiltration

The ultrafiltration unit (FT-002) used in the starch process was sized using the Equation C.8. The total area required for was obtained from the material and energy balance calculations. The default maximum area (80 m^2) was used to calculate the number of units required.

vii. Precipitation, Acidulation, Crystallisation

The precipitation and acidulation tanks (TK-002, TK-003) used in the molasses process and the crystallisation unit (CR-001) were sized using the stirred tank model (Equations C.16-C.18). The unit sizes were based on the volumetric throughput (m^3/batch). User input values to the model are shown in Table 9.3.

Table 9.3 Input variables for precipitation, acidulator and crystalliser sizing

Input	Value	Unit
Max volume of the reactor unit	200	m^3
Unit utilisation	90	%
Height/Diameter ratio	3	-
Design pressure	1.5	Bar

viii. Adsorption Columns

The ion exchange and activated carbon columns (IE-001, AC-001) were sized using the adsorption column model (Equations C.2-C.5). User inputs are shown in Table 9.4. The volumetric flowrate to the units was obtained from the material and energy balance calculations for the starch and molasses process simulations. Default values were used for user inputs, based on typical operating conditions (Snoeying, 1990).

Table 9.4 Input variables for adsorption column sizing

Input	Value	Unit
Unit overdesign factor	10	%
Max bed diameter	80	m^3
Bed height/diameter ratio	0.66	-
Bed/Column height ratio	0.5	-
Break time	21	min
Empty bed contact time	10	min

ix. Drying

The fluidised bed drier (DF-001) was sized using the fluid bed drier model (Equations C.9-C.12). User input values are shown in Table 9.5. Default values were used for the average particle residence time and average solids velocity, based on typical values (McCabe *et al.,* 1993).

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Table 9.5 Input variables for drier sizing

Input	Value	Unit
Unit overdesign factor	10	%
Max diameter of the unit	3	m
Height to diameter ratio	10	
Average particle residence time	30	
Average solids velocity	1.5	m/s
Evaporation rate	100	kg/h/m^3

9.4 Capital Cost Estimation

The capital cost is the total amount of funds required to purchase land, design, purchase and supply the equipment and manufacturing facilities, as well as to bring the facility into operation. The total capital investment required was comprised of the purchased equipment cost, plant direct capital cost, indirect capital costs, contractors fees and contingency, and finally working capital and start-up costs.

9.4.1 Purchased Equipment Costs

A summary of the major purchased equipment is shown in Table 9.6 for the starch based process and Table 9.7 for the molasses based process. The size and specifications of the processing units were determined from the results of the material and energy balance calculations and equipment specific parameters. The largest contributors to the purchased equipment cost for both the starch and molasses processes were the bioreactors and associated compressors and air filters. The bioreactors were the bottleneck of the process and 12 units were operated in a staggered configuration (i.e. parallel throughput) to increase process capacity. The total purchased equipment cost for the starch and molasses based processes was $18.5 million and approximately $19.8 million respectively. The difference in the purchased equipment cost of two processes was primarily due to the substitution of the precipitation equipment for an ultrafiltration unit and the variation in operating capacity of specific unit operations.

The molasses based process required additional mixing tanks (TK-002; TK-003) and associated filters (FT-002; FT-003) for precipitation of citric acid using quicklime. In the starch process these units were substituted for an ultrafiltration unit. The cost of the ultrafiltration unit ($296,000) was considerably less than the total cost of the precipitation tanks and filters ($526,400). The molasses process also required a larger bioreactor capacity to accommodate the molasses feedstock which contained less sugar than pure maize starch. This contributed to a larger volumetric flowrate from the bioreactor, and as a result, the need for a larger biomass removal capacity (FT-001). The starch process required larger adsorption and ion exchange columns and a larger crystallisation capacity in comparison to the molasses process. In the molasses process, liquid waste was removed during the tri-calcium citrate filtration operation (FT-002) thus reducing the load on the columns and crystallisation. In both processes, two activated carbon adsorption columns (AD-001) were used in a standby configuration to allow for continuous operation while the alternating column is regenerated. The processes both required approximately the same capacity for final product drying (DF-001) following crystallisation.

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Table 9.6 Summary of purchased equipment for citric acid plant using starch

Unit Code	Description	No. of Units	Staggered	Standby	Size		Material of Construction	Cost/Unit ($)	Cost ($)
AD-001	AC Column	1	0	1	29	m^3	CS	257,000	514,000
CD-001	Condenser	2	0	0	91	m^3	CS	30,000	59,000
CM-001	Compressor1	1	11	0	230	kW	CS	166,000	1,991,000
CR-001	Crystallizer	2	0	0	56	m^3	SS316	464,000	927,000
DF-001	Fluidised Drier	1	0	0	28	m^3	SS316	136,000	136,000
FA-001	Air Filter	1	11	0	0.19	m^3	CS	5,200	63,000
FT-001	Biomass Removal	1	0	0	75	m^2	CS	127,000	127,000
FT-002	Ultrafiltration	3	0	0	75	m^2	SS316	99,000	296,000
FT-003	Crystal Filter	1	0	0	37	m^2	CS	87,000	87,000
IE-001	Ion Exchange	1	0	0	0.62	m^3	CS	46,000	46,000
RX-001	Bioreactor	1	11	0	250	m^3	SS316	1,024,000	12,282,000
ST-001	SteriliserMedia	1	0	0	5.1	m^3	SS316	308,000	308,000
ST-002	Steriliser	2	0	0	58	m^3	SS316	784,000	1,569,000
TK-001	Media Prep Tank	1	0	0	2.8	m^3	SS316	54,000	54,000

Table 9.7 Summary of purchased equipment for citric acid plant using molasses

Unit Code	Description	No. of Units	Staggered	Standby	Size		Material of Construction	Cost/Unit ($)	Cost ($)
AD-001	AC Column	1	0	1	11	m^3	CS	142,200	284,500
CD-001	Condenser	1	0	0	57	m^3	CS	22,600	22,600
CM-001	Compressor	1	11	0	270	kW	CS	181,600	2,178,700
CR-001	Crystallizer	1	0	0	34	m^3	SS316	345,400	345,400
DF-001	Fluidised Drier	1	0	0	28	m^3	SS316	135,500	135,500
FA-001	Air Filter	1	11	0	0.17	m^3	CS	5,200	59,500
FT-001	Biomass Removal	2	0	0	56	m^2	CS	106,800	213,600
FT-002	Precipitation Filter	2	0	0	84	m^2	CS	135,700	271,400
FT-003	Acidulator Filter	1	0	0	47	m^2	CS	96,200	96,200
FT-004	Crystal Filter	1	0	0	11	m^2	CS	39,100	39,100
IE-001	Ion Exchange	1	0	0	0.23	m^3	CS	25,300	25,300
RX-001	Bioreactor	1	11	0	290	m^3	SS316	1,121,800	13,461,700
ST-001	Steriliser (Media)	1	0	0	5.6	m^3	SS316	326,300	326,300
ST-002	Steriliser	2	0	0	77	m^3	SS316	928,800	1,857,600
TK-001	Media Prep Tank	1	0	0	3.1	m^3	SS316	57,200	57,200
TK-002	Precipitation Tank	2	0	0	190	m^3	CS	171,900	343,900
TK-003	Acidulator Tank	1	0	0	100	m^3	CS	119,700	119,700

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9.4.2 Total Capital Investment

9.4.2.1 Plant Direct Cost

The direct plant costs (PDC) were calculated as a percentage of the purchased equipment cost (PCE). A summary of the costs and associated factors are given in Table 9.8 for each of the process simulations. The Lang factors used for the generic flowsheet simulations were taken from values presented by Biwer *et al.* (2006), based on an average of the minimum and maximum values typically encountered in microbial process designs (Holland & Wilkinson, 1997; Peters *et al.*, 2004; Biwer *et al.*, 2006). These were the default values available in the generic flowsheet. The process model by Biwer *et al.* (2006) used slightly modified factor values with a number of the direct costs. The values were closer to the minimum values typically encountered in microbial processes and was likely a more accurate estimate considering the relative simplicity and economies of scale of industrial citric acid production. The flowsheet model was intended to be a generically applicable tool to process costing and average industry values were thus used. Additionally, the Biwer *et al.* (2006) simulation specified a cost for unlisted equipment (20% of listed PCE), included in the purchased equipment cost in Table 9.8. The total direct plant costs of the generic flowsheet simulation were a slight overestimate relative to the literature simulation.

The individual cost items in Table 9.8 show that equipment erection was one of the major component costs. Some of the other major direct plant costs included process piping, instrumentation, buildings and auxiliary facilities. The purchased equipment cost and the additional costs derived from it, together, accounted for the total plant direct cost. Although the cost of land is usually calculated as 4%-8% of the purchased equipment cost (Peters *et al.*, 2003), the cost was not included in the fixed capital investment, since it is a non-depreciable component and usually included as single line item at the beginning of the project.

Table 9.8 Direct plant costs for the production of citric acid

Capital Cost	Starch[a]	f	Starch[b]	f	Molasses[b]	f
Purchased Equipment Cost	$20,935,000	*1.00*	$18,460,000	*1.0*	$19,840,000	*1.0*
Equipment erection	$9,680,000	*0.46*	$9,230,000	*0.50*	$9,920,000	*0.50*
Piping	$6,281,000	*0.30*	$12,920,000	*0.70*	$13,890,000	*0.70*
Instrumentation	$4,187,000	*0.20*	$9,230,000	*0.50*	$9,920,000	*0.50*
Insulation	$1,047,000	*0.05*	$920,000	*0.05*	$990,000	*0.05*
Electrical systems	$2,094,000	*0.10*	$2,770,000	*0.15*	$2,980,000	*0.15*
Buildings	$16,748,000	*0.80*	$9,230,000	*0.50*	$9,920,000	*0.50*
Site development	$1,227,000	*0.05*	$2,770,000	*0.15*	$2,980,000	*0.15*
Auxiliary facilities	$4,187,000	*0.20*	$12,920,000	*0.70*	$13,890,000	*0.70*
Total (PDC)	*$66,386,000*		*$78,450,000*		*$84,310,000*	

[a]Biwer et al. (2006); [b]Generic flowsheet model

9.4.2.2 *Plant Indirect Cost*

Plant indirect costs (PIC) included engineering and supervision, and construction expenses. The direct (PDC) and indirect (PIC) costs together form the total plant cost (TPC). In the generic flowsheet simulation the costs for design and engineering and construction were estimated as 25% and 35% of the plant direct cost respectively. The values were based on the values given by Biwer *et al.* (2006). The combined plant indirect costs for the starch and molasses process were estimated as $47 million and $51 million respectively.

9.4.2.3 *Contractors Fees and Contingency*

Contractor fees and contingency costs were calculated as 6% and 10% of the total plant cost (TPC) respectively based on values from Biwer *et al.* (2006). The combined contractor and contingency costs were approximately $20 million and $22 million for the starch and molasses process respectively. The total direct fixed capital (DFC) for the process was the sum of the total plant cost, contractor fees and contingency. The DFC was used as the basis for determining the working capital requirement and the plant start-up cost.

9.4.2.4 *Plant Start-up Cost*

The working capital requirement was estimated as a percentage of the direct fixed capital cost (Turton *et al.*, 2003). The working capital requirement was calculated as 15% of DFC and the start-up cost as 5% of DFC. The total cost for plant start-up and working capital was approximately $29 million and

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$31 million for the starch and molasses process respectively. The start up cost for the Biwer *et al.* (2006) study ($6.5 million), shown in Table 9.9, was considerably lower than the estimates using the generic flowsheet. The literature estimate was based on 30 days availability of labour, raw materials, utilities and waste treatment. However, start-up and working capital estimates, based on a percentage of DFC, are typically used for large scale processes (Peters *et al.*, 2003). Further, since maize starch and beet molasses are seasonal products, an overestimate of the working capital requirement for typical large-scale bioprocesses is advised (Couper, 2003).

9.4.2.5 Summary of the Total Capital Investment

The component costs were combined to give the total capital investment required for the plant. The plant start-up cost included the values for start-up and working capital. The total capital investment for the starch process from the generic flowsheet was approximately 44% larger than the starch process from the Biwer *et al.* (2006) simulation due to the larger plant direct cost (PDC) and start-up cost (PSC). The total capital investment of the molasses process was approximately 7.4% larger than the generic flowsheet starch process due to the larger plant direct cost (PDC), as a result of the larger purchased equipment cost (PCE).

Table 9.9 Summary of total capital investment for citric acid plant

	Starch[a]	Starch[b]	Molasses[b]
Plant Direct Cost (PDC)	$66,386,000	$78,450,000	$84,310,000
Plant Indirect Cost (PIC)	$33,193,000	$47,070,000	$50,590,000
Total Plant Cost (TPC = PDC + PIC)	$99,578,000	$125,520,000	$134,900,000
Contractors Fees and Contingency (CFC)	$14,937,000	$20,080,000	$21,580,000
Direct Fixed Capital Cost (DFC = TPC + CFC)	$114,514,000	$145,600,000	$156,480,000
Plant Start-up Cost (PSC)	$6,529,000	$29,120,000	$31,300,000
Total Capital Investment	$121,043,000	$174,720,000	$187,780,000

[a]Biwer *et al.* (2006); [b]Generic flowsheet model

9.5 Operating Cost Estimation

The annual operating costs of the citric acid plant were based on the material and energy balance data obtained from the process simulations. The operating costs are divided into direct and indirect operating costs.

9.5.1 Direct Operating Cost

9.5.1.1 Raw Materials

The price of specific raw materials is typically dependant on the form of the material, discounts offered for large quantities, method of delivery, and storage requirements for seasonal products. Prices quoted in literature are often inconsistent with the actual price paid by the manufacturer (Couper, 2003). The material prices for α-amylase, ammonium nitrate, potassium phosphate and sodium hydroxide, used by Biwer *et al.* (2006) were for 2005 data and represent approximate material prices paid by manufacturers at the time of the study. The USDA (2004) was used to price starch and beet molasses. Remaining raw material costs (sulphuric acid, lime, water) were obtained directly from supplier quotes.

A break-down of the specific major raw material requirements and the purchase price for the generic flowsheet simulations is shown in Table 9.10. The total raw material costs were dominated by maize starch and beet molasses, accounting for approximately 88% and 58% of the raw material cost of each process respectively. Although the purchase price of molasses ($0.09/kg) was lower than starch

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($0.15/kg) on a per mass basis, it contains approximately 48% sugars and was more expensive than maize starch on a per mass of sugar basis. In the starch process amylase was the second largest raw material cost, accounting for approximately 6% of the total raw material cost. In the molasses based process calcium oxide (19.5%) and sulphuric acid (18.5%) were also significant contributors to the total raw material cost.

Table 9.10 Major raw material inputs and costs for citric acid production

		Starch Process		Molasses Process	
Raw Material	Unit Cost ($/kg)	Consumption (kg/year)	Cost	Consumption (kg/year)	Cost
α-Amylase	10	16,000	$160,000	-	-
Ammonium Nitrate	0.15	295,000	$44,000	325,000	$49,000
Beet Molasses	0.090	-	-	34,710,000	$3,124,000
Calcium Oxide (Lime)	0.11	-	-	9,502,000	$1,045,000
Hydrogen Chloride	0.15	9,000	$1,000	170,000	$26,000
Nitrogen	0	253,610,000	$0	289,248,000	$0
Oxygen	0	67,407,000	$0	78,210,000	$0
Potassium Phosphate	0.34	23,000	$8,000	26,000	$9,000
Sodium Hydroxide	0.16	5,000	$1,000	2,000	$0
Sulphuric acid	0.10	-	-	9,834,000	$983,000
Starch	0.15	15,111,000	$2,267,000	-	-
Water	0.0005	166,670,000	$83,000	217,570,000	$109,000

9.5.1.2 Labour

The labour cost was calculated as a percentage of the operating time of individual unit processes multiplied by an hourly wage rate, shown in Table 9.11 and Table 9.12 for the starch and molasses process respectively. It was assumed that skilled labour is used at a rate of $33.7 per hour (Peters *et al.,* 2004). The total labour cost was relatively similar for the two processes, with the molasses process being slightly higher due to additional unit operations. The majority of the labour cost was used for the operation of the 12 bioreactors. The contribution from labour to the total operating cost (<1%) was significantly lower than other operating costs and should be regarded as an underestimate of the actual value.

Table 9.11 Labour demand and cost for citric acid production from starch

Unit Code	Description	Demand (hrs/year)	Labour Rate ($/hr)	Cost ($/year)
AD1 S 1	AC Column	268.17	34	9,120
CX1 S 1	Condenser	0	34	0
CM1 S 1	Compressor	0	34	0
CR1 S 1	Crystalliser	2352.72	34	79,990
DF1 S 1	Fluidised Bed Drier	1335.23	34	45,400
FA1 S 1	Air Filter	0	34	0
FT2 S 1	Biomass Removal	2535.26	34	86,200
FT1 S 2	Ultrafiltration	2028.2	34	69,000
FT3 S 3	Crystal Filter	1470.45	34	50,000
AD2 S 2	Ion Exchange	122.26	34	4,160
RX1 S 1	Bioreactor	46259.95	34	1,573,000
ST1 S 1	Steriliser	140.85	34	47,890
ST2 S 2	Steriliser	473.25	34	16,090
RX2 S 2	Media Prep Tank	281.7	34	9,580

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Table 9.12 Labour demand and cost for citric acid production from molasses

Unit Code	Description	Demand (hrs/year)	Labour Rate ($/hr)	Cost ($/year)
AD1 S 1	AC Column	270	34	9110
CX1 S 1	Condenser	0	34	0
CM1 S 1	Compressor	0	34	0
CR1 S 1	Crystalliser	2350	34	79890
DF1 S 1	Fluidised Bed Drier	1330	34	45340
FA1 S 1	Air Filter	0	34	0
FT1 S 1	Biomass Removal	2530	34	86090
FT2 S 2	Precipitation Filter	2530	34	86090
FT3 S 3	Acidulator Filter	2530	34	86090
AD2 S 2	Ion Exchange	120	34	4150
RX1 S 1	Bioreactor	46200	34	1570800
ST1 S 1	Steriliser	140	34	4780
ST2 S 2	Steriliser	470	34	16070
RX2 S 2	Media Prep Tank	0	34	0
RX3 S 3	Precipitation	560	34	19130
RX4 S 4	Acidulator	560	34	19130
FT4 S 4	Crystal Filter	1470	34	49930

9.5.1.3 Consumables

In the generic flowsheet simulation, only the packing required for the activated carbon adsorber was included in the consumable cost estimate. The cost was calculated as a function of the packing turnaround time, the number of cycles per batch and number of batches per year. The cost for GAC packing was $314,000 and $247,000 for the starch and molasses process respectively. The larger value for the starch process was due to the larger column as a result of a higher column throughput, discussed in Section 7.2.2.5. Additional consumable costs for filter media, ultrafiltration membranes and ion-exchange resin are relatively small contributors to the overall operating cost of the plant and are not included in the estimation.

9.5.1.4 Waste Treatment/Disposal

The waste treatment costs of each process, summarised in Table 9.13, were estimated using the material balance data from the generic flowsheet simulations. The disposal costs were taken from the Biwer *et al.* (2006) simulation model. The treatment costs for the starch process were dominated by costs for disposal of biomass (43%) and the treatment of wastewater (37%) from the filtration processes. The treatment costs in the molasses process were dominated by calcium sulphate disposal (75%), with a smaller contribution for biomass disposal (12%) and waste water treatment (12%). The cost associated with the disposal of gypsum from the molasses process should be especially emphasised if the plant is located in a region where there is limited demand for the material as a value added by-product (Kristiansen, 1999).

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Table 9.13 Waste treatment demand and cost for citric acid production

Waste Material	Disposal Cost ($/kg)	Starch Process Disposal (kg/year)	Starch Process Cost ($/year)	Molasses Process Disposal (kg/year)	Molasses Process Cost ($/year)
α-Amylase	0.0100	16 000	200	-	-
Biomass	0.0100	1 958 100	19 600	2 189 500	21 900
Calcium Citrate	0.0100	-	-	167 900	1 700
Calcium Sulphate	0.0100	-	-	13 582 100	135 800
Carbon Dioxide	0.0000	4 448 900	0	3 659 600	0
Chloride	0.0005	8 700	0.00	165 600	100
Citric acid loss	0.0005	385 300	200	270 400	100
Glucose	0.0100	166 200	1 700	-	-
Nitrogen	0.0000	253 609 500	0	289 248 300	0
Oxygen	0.0000	61 677 200	0	72 815 100	0
Potassium (dissolved)	0.0005	300	0	7 400	0.0
Sodium (dissolved)	0.0005	2 900	0.00	900	0
Water	0.0001	166 700 000	16 700	217 600 000	21 800

9.5.1.5 Utilities

The utility requirements, shown in Table 9.14, were determined from the material and energy balance data from the generic flowsheet simulations. The cost for utilities formed a significant portion of the total operating cost of the process, contributing about 13% in the starch process and about 11% in the molasses process. The cost of electricity and cooling water accounted for approximately 80% of utility costs in both processes. The prices of the utilities were based on average price data typically quoted in literature sources for large-scale manufacturing industries in the United States and Europe (Peters *et al.*, 2003; U.S. DOE, 2009).

Table 9.14 Utility demand and cost for citric acid production

Utility	Cost Unit	Cost ($/Unit)	Starch Process Consumption (Units/year)	Starch Process Cost ($/year)	Molasses Process Consumption (Units/year)	Molasses Process Cost ($/year)
Electricity (GJ)	GJ	13.8900	137,100	1,904,700	149,700	2,078,700
Steam (ton)	ton	4.4000	173,800	764,600	109,400	481,400
Cooling water (m3)	m3	0.0800	27,700,000	2,216,000	23,390,000	1,870,000
Chilled water (m3)	m3	0.1700	2,593,500	440,900	2,892,500	491,700

9.5.1.6 Laboratory

The laboratory cost was calculated as 60% of the total annual labour cost of the process. The laboratory costs were $1,168,000 and $1,246,000 for the starch and molasses process respectively. The costs formed a relatively small part of the operating costs and contributed about 3% to the total for both processes. Although it is prescribed that a laboratory cost estimate of 15-20% of operating labour is reasonable for preliminary purposes (Couper, 2003), an estimate of 50-100% is considered more realistic for bioprocesses where complex microbial systems need to be accurately monitored and controlled (Biwer *et al.*, 2.006).

9.5.2 Indirect Operating Costs

Indirect operating costs included amounts for depreciation, maintenance, local property taxes and insurance. These costs are a direct function of the fixed capital investment and are typically calculated as 10% to 20% of direct fixed capital for preliminary estimates (Peters *et al.*, 2003).

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9.5.2.1 Depreciation

The deprecation cost was calculated for the direct capital investment of the process for each year of operation. The depreciation value for the process simulations was determined as straight line over a usable life of 10 years with a salvage value of 5% for the equipment (Couper, 2003). The cost was included as an operating expense from the year the equipment was purchased. The depreciation cost was the largest contributor to the operating expense of both processes and accounted for about 36% to 38% of the total cost. The cost was primarily attributed to the high capital cost of the 12 bioreactors and associated compressors.

9.5.2.2 Plant Tax and Insurance

Insurance and plant tax were calculated as 1% and 2% of DFC respectively, based on values prescribed in the literature (Peters *et al.*, 2003). The cost associated with plant tax and insurance was $4,368,000 and $4,695,000 for the starch and molasses process respectively. The slightly higher cost for the molasses process was due the higher direct fixed capital investment. The costs were relatively small in comparison to other operating expenses for both processes. Although the magnitude of local property taxes depends on the location of the plant it is typically within 2%-4% of the direct fixed capital. Insurance cost depends largely on the type of process and is typically 1% of the direct fixed capital (Peters *et al.*, 2003).

9.5.2.3 Maintenance

The generic flowsheet model assumed the maintenance and repair cost as 6% of the direct fixed capital, based on typical values for average processes with normal operating conditions (Peters *et al.*, 2003). The maintenance cost was a significant contributor to the total operating expense and accounted for about 22% to 24% of the operating cost for both processes. Although maintenance costs were lower than depreciation, maintenance is likely to become the dominant contributor to the total operating cost as equipment items reach their salvage value. The estimate of 6% of DFC for maintenance is likely to increase as the plant ages.

9.5.2.4 Plant Overhead Costs

The annual plant overheads include the general costs for the actual running of the plant, such as general management, plant security, medical, canteen, general clerical staff and safety. These plant overheads are typically 50% of the total direct labour costs, including maintenance labour (Sinnot, 1999). The miscellaneous expenses associated with the running of the plant include cleaning materials, instrument charts and accessories, pipe gaskets and safety equipment and are typically 5% of the total annual maintenance costs. The annual operating costs vary considerably depending on the type of operation, location of the plant and local economic conditions. Plant overheads make a relatively small contribution to the total operating costs and were thus not included in the generic flowsheet simulation.

9.5.2.5 Summary of operating costs

A summary of the annual operating costs for the simulation models is shown in Table 9.15. A comparison of the operating costs for the starch and molasses processes from the generic flowsheet tool is shown in Figure 9.2. The largest contributions to the overall operating cost were from raw materials, utilities, maintenance and depreciation. The plant dependant operating costs, namely depreciation and maintenance, were the largest contributors to the total operating cost due to the large fixed capital investment of the plant. These costs are both calculated as a percentage of the purchased equipment cost for each year the plant is in operation.

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Table 9.15 Annual operating costs for citric acid production

Operating Cost	Starch[a]	Starch[b]	Molasses[b]
Raw Materials	$2,697,000	$2,560,000	$5,340,000
Labour	$1,347,000	$1,950,000	$2,080,000
Consumables	$478,000	$310,000	$250,000
Waste Treatment	$67,000	$44,600	$181,500
Utilities	$4,703,000	$5,330,000	$4,920,000
Insurance	$1,145,000	$1,460,000	$1,560,000
Plant Tax	$2,290,000	$2,910,000	$3,130,000
Maintenance	$7,396,000	$8,740,000	$9,390,000
Laboratory	$202,000	$1,170,000	$1,250,000
Depreciation	$10,879,000	$13,830,000	$14,870,000
Total(OC)	*$31,205,000*	*$36,360,000*	*$40,940,000*

[a]Biwer et al. (2006); [b]Generic flowsheet model

In comparing the operating costs of the starch and molasses process (Figure 9.2) it is shown that the individual operating cost contributions were relatively similar for both processes. The raw material costs of the molasses process were higher than the starch process due to additional material inputs required for tri-calcium citrate precipitation and citric acid recovery.

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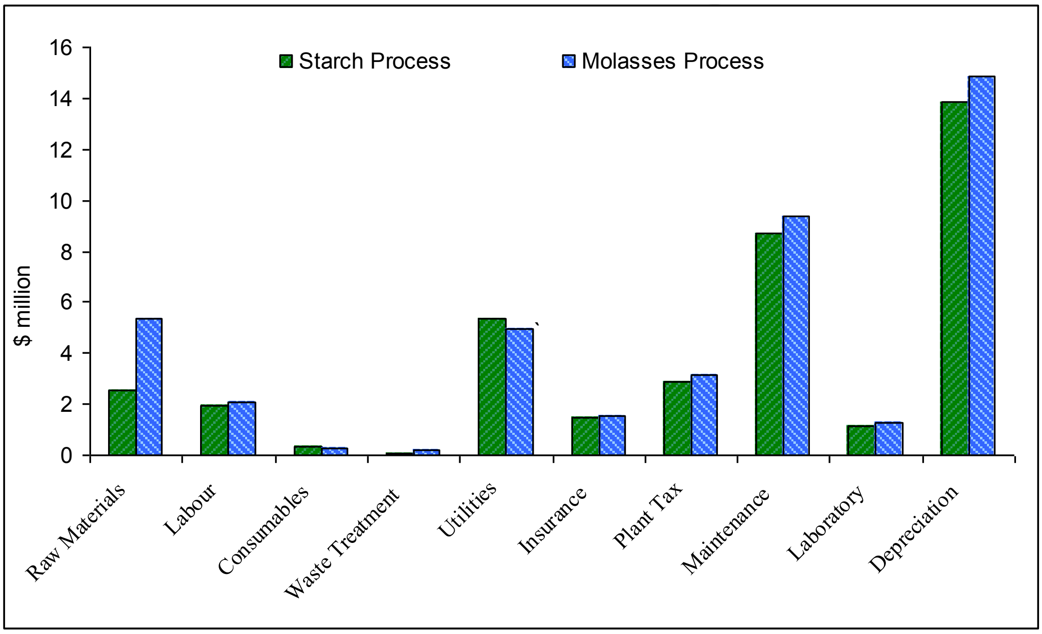

Figure 9.2 Comparison of annual operating costs for citric acid production

9.6 Profitability Assessment

The profitability assessment used the results of the capital and operating cost estimates to quantify the viability of the production process as a profit generating enterprise. The methods of evaluating profitability included *gross and net profit*, *payout period*, *return on investment*, *net present worth* and *internal rate of return*. A summary of the process profitability for each process option is shown in Table 9.16.

9.6.1 Income and Expenditure

9.6.1.1 Product Revenue and Unit Cost

Product revenue and unit production cost were dependent on the operating capacity of the production facility, the selling price of the final product and the total operating costs of the plant. Sales income was assumed approximately 36 months after plant construction was commenced. This was based on recommendations from common industry practice cited in literature (Perry, 1999). The plant was assumed to operate at full capacity from start-up. The annual production output from both the starch and molasses process was approximately 12,600 tons of citric acid monohydrate. The total process revenue was based on the selling price received and the total product output from the plant. The selling price of citric acid monohydrate was assumed as $1.8/kg (2005), based on the value used by Biwer *et al.* (2006). The unit production costs were based on the total operating costs and the total unit output from the processes. The unit production cost was approximately $2.9/kg and $3.3/kg for the starch and molasses process respectively. The case study presented by Biwer *et al.* (2006) specified a unit production cost of $2.5/kg. The difference in unit cost of the three process simulations was primarily due to the difference in the estimates for direct fixed capital of the processes. The associated depreciation and maintenance costs had a significant influence to the total operating expense and as a result the unit production cost. Although the selling price of citric acid and the cost of raw materials were estimated to increase by 5% per annum, prediction of future selling prices is unpredictable without detailed economic forecasting. It is therefore recommended that the future price of both the product and raw materials is assumed to remain constant when determining the future profitability of the process.

9.6.1.2 Gross and Net Profit

The estimated income from citric acid monohydrate sales, the cost of sales and all additional costs (e.g. tax; interest expense) were included in determining the annual gross and net profit of the operation. The simulations for both the starch and molasses process showed that the facilities operate at a gross annual loss. The loss was approximately $16.2 million and $21 million for the starch and molasses processes respectively. This was as a result of the high operating costs relative to the market selling price of citric acid monohydrate. Since no operating profit was realised, no income tax was applicable and net profit was simply equal to the gross loss. The total annual product cost did not at any point equal the total sales within the plant‘s production capacity and the processes did not break-even for the project duration.

9.6.2 Payout Period

The payout period was used to estimate the amount of time required to recover the depreciable fixed capital investment from the accrued cash flow of the project. The payout calculation used in the generic flowsheet simulation (Appendix C) has a cut-off of 50 years at which it is assumed a project is not viable. A considerably shorter payout period (3-8 years) is typical for a viable operation. The payout period for both the starch and molasses process exceeded the 50 year cut-off of the simulation model. The payout period for the starch process from the literature case study was reported as 50.3 years. The payout period should not be used in isolation as a measure of process profitability however as it does not consider cash flows after the capital is recovered.

9.6.3 Return on Investment

The return on investment (ROI) was calculated as the net profit after taxes divided by the total capital investment for the project (Appendix C.7). The net profit of the process typically varies from one

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project year to the next. To obtain a representative estimate for ROI an average value for net profit was assumed for the entire project life. The return on investment for both the starch and molasses process was calculated as a negative value, shown in Table 9.16. The estimate for ROI was similar for both processes.

9.6.4 Discounted Cash Flow and Net Present Value

The cash flow analysis for the starch and molasses process is given in Appendix E. The total cash investment required for the initial operation was determined from the total fixed capital requirement and the working capital requirement of the plant. Bank loans were used for initial plant financing. The terms of the financing was based on the Biwer *et al.* (2006) model. Fixed capital was borrowed over a 10 year loan period at an annual interest rate of 9%. Working capital was borrowed over a 6 year loan period at an annual interest rate of 12%. The process model assumed that capital expenditure was distributed over the initial years of plant construction, taken as 20% in year 1, 50% in year 2, and 30% in year 3.

A cash flow analysis of the process was used to determine the net cash flow at the end of each project year. The closing cash balance of the plant was discounted at a risk free discount rate of 7% to determine the cumulative Net Present Worth (NPW). The discount rate typically varies depending on current economic conditions but a conservative value was used to ensure process profitability was not over estimated.

The discounted closing cash balances for various discount rates, shown in Figure 9.3 and Figure 9.4, decrease sharply during the construction and commissioning phase of the plant. The discounted closing cash balance increases steadily over a 15 year period due to of a reduction in the net loss and no expected capital expenditure after the plant has been commissioned. Once the initial loan repayments are completed the rate of increase of closing cash balance increases, due to higher annual net cash flow. The discounted cumulative cash flow of the operation decreases over the plant life time. The rate of decrease slows as the annual cash flow increases.

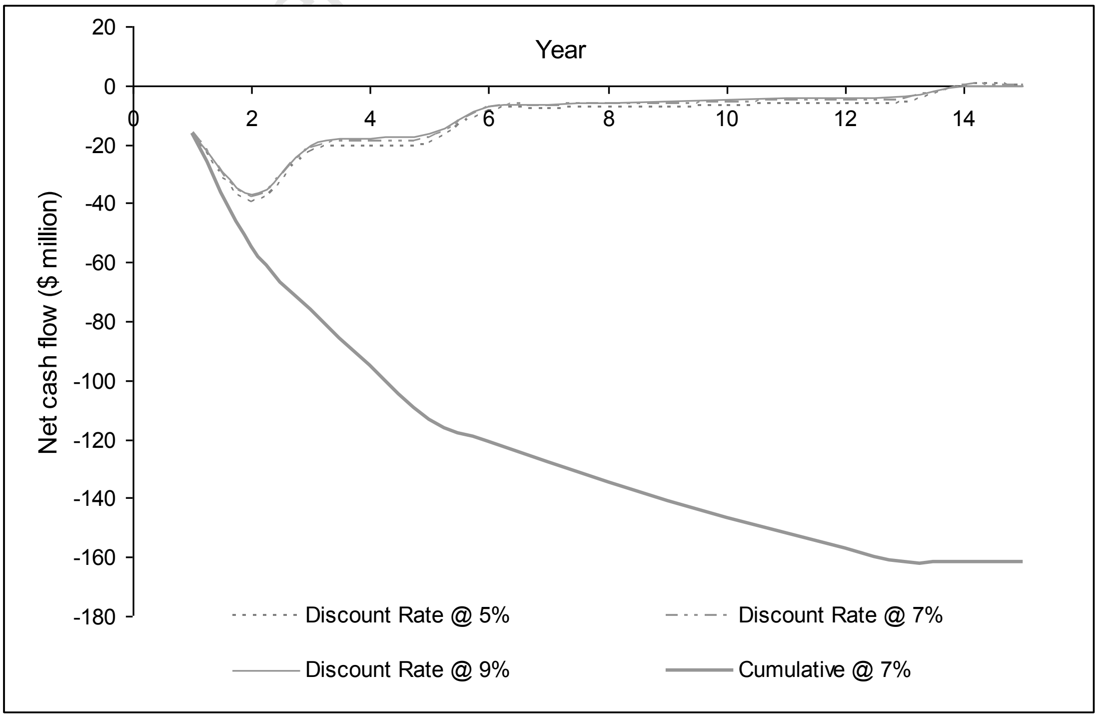

Figure 9.3 Net annual cash flow and cumulative cash flow for citric acid from starch

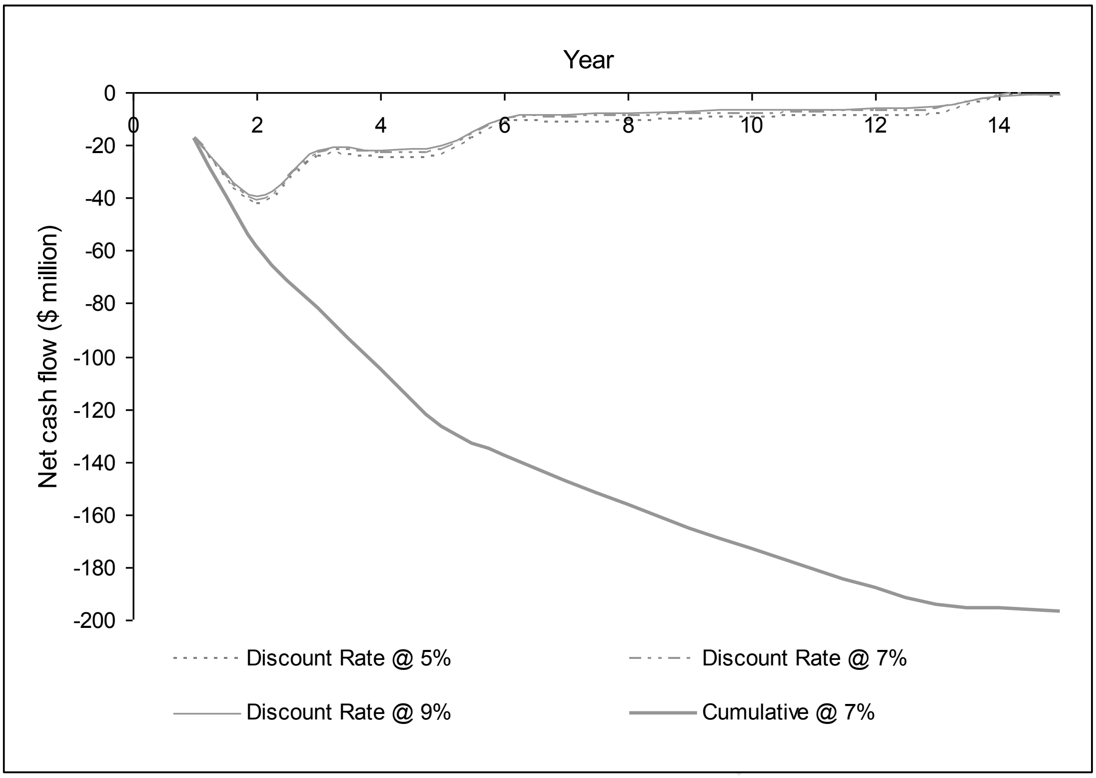

Figure 9.4 Net annual cash flow and cumulative cash flow for citric acid from molasses

9.6.5 Internal Rate of Return

The Internal Rate of Return (IRR) of operation was determined for the project duration of 15 years. The results of the IRR calculation (Table 9.16) for both generic flowsheet simulations were outside the cut-off range of 0% to 100%. The results of the process model simulation presented by Biwer *et al.* (2006) yielded similar results.

Table 9.16 Economic assessment summary of the production of citric acid

	Starch[a]	Starch[b]	Molasses[b]	
Reference Year	2005	2005	2005	
Total Capital Investment	121,043,000	174,720,400	187,781,100	$
Operating Cost	31,205,000	36,363,120	40,941,550	$
Production Rate	12,628,433	12,592,330	12,576,010	kg P/year
Unit Production Cost	2.47	2.89	3.26	$/kg P
Total Revenues	22,731,000	22,666,190	22,636,820	$
Gross Margin	-37.28	-60.43	-80.86	%
Return on Investment	1.99	-0.08	-0.1	%
Payback Time	50.33	NA (> 50 Years)	NA (> 50 Years)	years
IRR	Out of search Interval	NA (<> 0-100%)	NA (<> 0-100%)	%
NPV @ 7%	-93,973,000	-161,566,600	-197,044,800	$

[a]Biwer et al. (2006); [b]Generic flowsheet model

9.7 Comparison of Early-Stage Input Data

The default generic flowsheet data and the input data used by the Biwer *et al.* (2006) model were compared for the economic assessment of the starch production process. The comparison aimed to highlight the effect of relying on the default generic flowsheet data when detailed data is limited at early stage design.

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9.7.1 Comparison of Capital Cost Multipliers

The multipliers used by the Biwer *et al.* (2006) model differed from the generic flowsheet model for a number of capital cost estimations. The values used by Biwer *et al.* (2006) were closer to the minimum values typically encountered in microbial processes. The default values for the generic flowsheet model were based on average industry values. The values assumed by the Biwer *et al.* (2006) model were compared directly to the default values of the generic flowsheet, shown in Table 9.17. The subsequent capital cost calculations, shown in Table 9.17 and Table 9.18, were based on the same purchased equipment cost (PCE) of $18,458,000 obtained from the generic flowsheet calculations for the starch process, presented in Section 9.4.1.

Table 9.17 Comparison of capital cost multipliers for the production of citric acid

Capital Cost	Starch[a]	f[a]	Starch[b]	f[b]
Plant Direct Cost (PDC)				
Purchased Equipment Cost	$18,458,000	*1.00*	$18,458,000	*1.00*
Equipment Erection	$8,491,000	*0.46*	$9,229,000	*0.50*
Piping	$5,538,000	*0.30*	$12,921,000	*0.70*
Instrumentation	$3,692,000	*0.20*	$9,229,000	*0.50*
Insulation	$923,000	*0.05*	$923,000	*0.05*
Electrical Systems	$1,846,000	*0.10*	$2,769,000	*0.15*
Buildings	$14,767,000	*0.80*	$9,229,000	*0.50*
Site Development	$923,000	*0.05*	$2,769,000	*0.15*
Auxiliary Facilities	$3,692,000	*0.20*	$12,921,000	*0.70*
Total (PDC)	$58,329,000		$78,448,000	
Plant Indirect Cost (PIC)				
Design and Engineering	$11,666,000	*0.20*	$19,612,000	*0.25*
Construction	$17,499,000	*0.30*	$27,457,000	*0.35*
Total (PIC)	$29,164,000		$47,069,000	
Total Plant Cost (TPC = PDC + PIC)				
Plant Direct Cost (PDC)	$58,329,000		$78,448,000	
Plant Indirect Cost (PIC)	$29,164,000		$47,069,000	
Total (TPC)	$87,493,000		$125,518,000	
Contractors Fees and Contingency (CFC)				
Contractors Fee	$4,375,000	*0.05*	$7,531,000	*0.06*
Contingency	$8,749,000	*0.10*	$12,552,000	*0.10*
Total (CFC)	$13,124,000		$20,083,000	
Direct Fixed Capital Cost (DFC = TPC + CFC)				
Total Plant Cost (TPC)	$87,493,000		$125,518,000	
Contractors Fees and Contingency (CFC)	$13,124,000		$20,083,000	
Total (DFC)	$100,617,000		$145,600,000	
Plant Start-up Cost (PSC)				
Start-up and Validation	$5,031,000	*0.05*	$7,280,000	*0.05*
Working Capital	$927,000	*30 days*	$21,840,000	*0.15*
Total (PSC)	$5,958,000		$29,120,000	

[a]Generic flowsheet simulation with Biwer *et al.* (2006) input data

[b]Generic flowsheet simulation with default input data

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The larger default values used by the generic flowsheet resulted in larger estimates for all the capital cost estimations. The plant direct cost (PDC) was approximately 35% larger than the value obtained from the Biwer *et al.* (2006) multiplier values. Although similar multiplier values were used for plant indirect cost (PIC) and contractors' fees and contingency (CFC), the PDC value was used for subsequent capital cost estimations, contributing to the larger capital costs for the default multiplier values. The plant indirect cost was approximately 61% larger for the default multiplier values, due primarily to the difference in direct cost values. Similarly, the contractors' fees and contingency and direct fixed capital values were 53% and 45% larger respectively. The working capital value of the Biwer *et al.* (2006) model was based on 30 days of raw materials, labour, consumables, waste treatment and utilities; while the default value in the generic flowsheet assumed a working capital requirement of 15% of direct fixed capital (DFC). The default value in the generic flowsheet was based on average values for working capital across various chemical processes (Peters *et al.*, 2003). The generic flowsheet did not account for supervisory and administrative labour requirements and material storage costs and a conservative working capital estimate (i.e. based on DFC) is deemed justifiable. The default generic flowsheet working capital multiplier contributed to approximately 30% of the variation in the total capital investment.

Table 9.18 Comparison of total capital investment for citric acid plant

	Starch[a]	Starch[b]
Plant Direct Cost (PDC)	$58,330,000	$78,450,000
Plant Indirect Cost (PIC)	$29,160,000	$47,070,000
Total Plant Cost (TPC = PDC + PIC)	$87,490,000	$125,520,000
Contractors Fees and Contingency (CFC)	$13,120,000	$20,080,000
Direct Fixed Capital Cost (DFC = TPC + CFC)	$100,620,000	$145,600,000
Plant Start-up Cost (PSC)	$5,960,000	$29,120,000
Total Capital Investment	$106,570,000	$174,720,000

[a]Generic flowsheet simulation with Biwer *et al.* (2006) input data

[b]Generic flowsheet simulation with default input data

9.7.2 Comparison of Operating Cost Multipliers

The indirect operating costs, shown in Table 9.19, were calculated using multiplier values based on common industrial processes (Couper, 2003; Peters *et al.,* 2003). The Biwer *et al.* (2006) and the generic flowsheet assumptions were similar for insurance, plant tax and maintenance, based on the direct fixed capital. The relatively larger values for insurance, plant tax and maintenance for default generic flowsheet data was due to the larger multiplier values used for capital cost estimation. The laboratory cost estimate was based on a percentage of total labour cost for both process models. The Biwer *et al.* (2006) model assumed a laboratory cost considerably lower than the default value in the generic flowsheet. Similarly to the working capital estimation, a conservative estimate should be included for laboratory costs when basing the value on the total labour cost.

A comparison of the operating costs for the process models is shown in Table 9.20. The generic flowsheet model did not include default values for raw material costs and the material prices were based on the Biwer *et al.* (2006) model. The default labour rate ($14/hour) in the generic flowsheet was lower than the Biwer *et al.* (2006) assumed value of $34/hour, resulting in a lower annual labour cost. The labour rate is largely dependent on the location of the plant and the default value should be updated to location specific conditions. The default generic flowsheet value did however provide a reasonable estimate for operator labour. The estimates for insurance, plant tax, maintenance and depreciation were higher due to the larger estimate for direct fixed capital. The assumptions regarding estimation of direct fixed capital was the primary factor influencing the variation in the annual operating costs of the process models.

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Table 9.19 Comparison of operating cost multipliers for the production of citric acid

Operating Cost	Starch[a]	f[a]	Starch[b]	f[b]
Insurance (% of DFC)	$1,006,000	*0.01*	$1,456,000	*0.01*
Plant Tax (% of DFC)	$2,012,000	*0.02*	$2,912,000	*0.02*
Maintenance (% of DFC)	$5,031,000	*0.05*	$8,736,000	*0.06*
Laboratory (% of total labour cost)	$292,000	*0.15*	$87,360,000	*0.60*

[a]Generic flowsheet simulation with Biwer *et al.* (2006) input data

[b]Generic flowsheet simulation with default input data

Table 9.20 Comparison of annual operating costs for citric acid production

	Starch[a]	Starch[b]
Raw Materials	$2,560,000	$2,560,000
Labour	$1,950,000	$800,000
Consumables	$314,000	$314,000
Waste Treatment/Disposal	$44,600	$44,600
Utilities	$5,330,000	$5,330,000
Insurance	$1,010,000	$1,460,000
Plant Tax	$2,010,000	$2,910,000
Maintenance	$5,030,000	$8,740,000
Laboratory	$292,000	$87,360,000
Depreciation	$9,560,000	$13,830,000
Total(OC)	$26,160,000	$28,760,000

[a]Generic flowsheet simulation with Biwer *et al.* (2006) input data

[b]Generic flowsheet simulation with default input data

9.7.3 Comparison of Process Profitability

A comparison of the process profitability is shown in Table 9.21 for the process models. The annual gross profit of the process model using the default input data was considerably lower than the Biwer *et al.* (2006) model. This was due primarily to higher operating cost values for maintenance, laboratory and depreciation. Subsequently, the gross margin, return on investment (ROI) and net present value (NPV) were lower for the default input values.

Table 9.21 Comparison of profitability for citric acid production

	Units	Starch[a]	Starch[b]
Revenue	$/yr	$22,670,000	$22,670,000
Operating Cost	$/yr	$26,160,000	$36,360,000
Gross Profit	$/yr	-$3,493,000	-$13,697,000
Taxes	$/yr	0.00	0.00
Net Profit	$/yr	-$3,493,000	-$13,697,000
Gross Margin	%	-15.41	-60.43
Return on Investment	%	-0.03	-0.08
Payback Period	years	NA (> 50 Years)	NA (> 50 Years)
NPV	$	-$75,648,000	-$161,567,000
IRR	%	NA (<> 0-100%)	NA (<> 0-100%)

[a]Generic flowsheet simulation with Biwer *et al.* (2006) input data

[b]Generic flowsheet simulation with default input data

9.8 Conclusions

A study estimate of the capital and operating costs for citric acid production facilities using different feedstocks is presented. The total capital investment required for the project was $175 million and

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$188 million for the starch and molasses process respectively. The working capital requirement was $22 million and $23 million for starch molasses process respectively. The cost estimate for the production of citric acid from starch was in relatively good agreement with the study presented by Biwer *et al.* (2006).

The total purchased equipment cost was approximately $18.5 million for the starch based process and approximately $19.8 million for the molasses based process. This compared closely with the purchased equipment estimate given by Biwer *et al.* (2006), of approximately $16 million. The 12 bioreactors and the associated air compressors accounted for approximately 82% of total purchased equipment (PCE). The operating costs were dominated by contributions from equipment depreciation, plant maintenance, utilities and raw materials. Depreciation associated with the high capital cost of the bioreactors was the largest contributor to the overall operating cost of the plant. Utility costs were a significant portion of operating costs due to the large electricity and cooling water requirements. The majority of the raw material costs were attributed to the cost of maize starch and beet molasses for each process. The amylase requirement for starch hydrolysis was a significant component of the total raw material cost of the starch process. Calcium oxide and sulphuric acid were large components of raw material costs of the molasses process. The processes consumed large quantities of process water for bioreaction, filtration and adsorption, making a notable contribution to the total raw material cost. The annual unit production cost was approximately $2.8/kg and $3.3/kg for the starch and molasses process respectively. A break even analysis revealed that the processes were unable to meet the unit cost of production within the design capacity. The facilities thus operated at a net loss for the first 14 years of production after which a positive net profit is realised. The net present worth of the starch process was a loss of approximately $162 million and a loss of $197 million for the molasses based process. This was as a result of large contributions from capital depreciation and equipment maintenance.

In comparing the assumptions of the Biwer *et al.* (2006) model to the default assumptions of the generic flowsheet model it was shown that the main factors influencing profitability were the assumptions surrounding direct fixed capital estimation. It is essential that process specific multiplier values be used to obtain a reasonable estimate of process profitability. The generic flowsheet provides multiplier values, common to a range of processes, for capital cost estimation. The multiplier values were shown to result in a slightly higher estimate for direct fixed capital for the current process assessment. Further, variation in process profitability is highly correlated with the actual location of the plant, local economic conditions and specific operating conditions of the manufacturer. The profitability of the processes was mostly influenced by the selling price of citric acid monohydrate; the cost of raw materials, especially maize starch and beet molasses and the initial capital investment of the plant. The profitability of the operations can be increased by minimising the production costs of the operation and focusing on areas of the process where the largest cost reductions can be made. The economic assessments, although a general representation of the production process, give meaningful insight into the cost of producing citric acid.

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CHAPTER 10

SUMMARY AND CONCLUSIONS

The thesis provided motivation for the application of modelling and simulation to support early stage-stage process development and decision making. Techniques to assess process performance using data generated from the simulation model were discussed and the most appropriate were selected for assessing the environmental and economic performance of the process design. Citric acid production, which is representative of large-scale commercial bioprocessing, was used as a case study to apply these approaches to compare the environmental and economic performance of alternative processing routes. The aim was to demonstrate the value of the approaches to bioprocess design and decision making. A simplified flowsheet model was used to provide a first-estimate for material and energy balance data. Life cycle assessment and order-of magnitude economic analysis were used to provide a basis with which to assess the environmental and economic performance of the process designs. This chapter aims to evaluate the outcomes of the thesis so that conclusions can be formulated in light of the key questions presented in Chapter 1; insights developed in Chapter 2 to Chapter 4; the research hypothesis developed in Chapter 5, and the case study and environmental and economic results presented in Chapter 6 to Chapter 9. Finally recommendations are proposed.

10.1 Developing a Basis for the Research

10.1.1 Simulation and Modelling in Process Development

In addressing the first key question, as to the current state of the art with regards to sustainability assessment and its requisite process simulation for bioprocess development, the thesis provided a context for the application of simulation and modelling in sustainable bioprocess development. Chapter 2 reviewed stages in process development and highlighted that the freedom of development is most prevalent in the early phases of project development. This freedom decreases rapidly as the detailed design phases are initiated. Modelling and simulation may be used to fill certain data gaps, especially at early stages of process design, when lack of important data is typical. Chapter 3 explored the explicit requirements of the simulation model and how the model can be implemented in the design process. The chapter also investigated the application of modeling and simulation in the bioprocess industry. Chapter 3 affirmed the necessity for rapid process assessment to meet the typical challenges facing the biomanufacturing industry, such as rising costs, extended time periods to meet regulatory requirements and strong market competition.

In an attempt to address this difficulty, a simplified generic flowsheet for bioprocesses simulation was developed by Harding (2008). The spreadsheet model, deployed in Microsoft® Excel, is used to generate a first estimate material and energy balance for early decision making in bioprocess development. The material and energy balance data provides a basis for inventory data for life cycle assessment of the process. The tool was developed primarily in response to the need for simplified bioprocess modelling during conceptual design and development phases, when limited process data is available for environmental assessments. The simulation flowsheet was critically evaluated in Chapter 3 and the findings of Harding (2008) discussed. Although Harding (2008) demonstrated that the tool was appropriate for providing inventory data for early-stage life cycle assessment, a number of limitations of the tool were identified. These shortcomings were specific to *user interface, unit sizing and economic calculations, design calculations* and *downstream operations*. Chapter 2 and 3 showed

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that owing to the difficulty in obtaining accurate process material and energy balance data prior to completion of a detailed engineering design, the value of simplified early-stage simulation tools was recognised. Although tools have been developed specifically for early-stage bioprocess simulation, shortcomings of these tools need to be addressed to make them valuable as a basis for both environmental and economic assessment.

10.1.2 Early-Stage Process Performance Assessment

In addressing the second key question, as to the elements of sustainability that are required to form a basis for early-stage process assessment, Chapter 4 reviewed methods by which process performance may be quantified, especially in early stages of process development. The literature showed that current approaches to assessment and implementation of process sustainability are based on well-established methodologies and principles and provide a systematic basis for improvements on both a process level and a global scale. Using early stage process data as a basis for these assessments, however, accounts for major material and energy flows and does not consider data specific to minor material and energy flows, process emissions, market conditions and profitability scenarios. Bearing this in mind, an early-stage assessment approach is by no means an absolute, all-encompassing, measure of process sustainability, but rather serves as a valuable support tool to existing approaches, methodologies and tools. Life cycle assessment (LCA) was identified as a valuable approach to systematically assessing the environmental performance of process alternatives. Chapter 4 also showed that a first-estimate economic analysis of process alternatives, new technologies and process optimisation strategies can give valuable insight into the economic viability of the process. By integrating the assessment into initial process development, decision making can be greatly enhanced. A number of important performance metrics were identified for early-stage economic assessment of process alternatives. In light of challenges facing bioprocess design such as environmental regulation, competing technologies, and return on investment, environmental and economic elements of sustainability are required for early-stage process performance assessment.

10.1.3 Research Objectives

In answering the first two key questions, a basis for the thesis was established. This provided the necessary context with which to define more detailed key questions that would guide the research work and help define specific objectives. To answer the second set of key questions and achieve the research objectives, a research hypothesis was defined and a methodology was developed to guide the work. The main objectives of the research work are:

1. To refine the early-stage simulation model developed by Harding (2008), to address critical shortcomings;
2. To use Life Cycle Assessment (LCA) methodology to compare bioprocess alternatives;
3. To use order-of-magnitude economic assessment to compare bioprocess alternatives;
4. To evaluate whether an early-stage assessment tool, functioning on reduced input data, can be used to identify process ‗hot-spots‘ and compare process options;
5. To evaluate whether Life Cycle Assessment (LCA) and order-of-magnitude cost and profitability performance measures can support decision making when only first-estimate material and energy data is available, and, if so, what the benefits and limitations of these decisions are.

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Chapter 5 presented the methodology providing the necessary detail for the development of the generic flowsheet model and completion of the thesis. Development of the model included a more user-friendly and intuitive graphical user interface (GUI) and the capability for unit sizing and economic performance assessment. Further, Chapter 5 outlined the approach by which process environmental and economic performance should be evaluated using the improved flowsheet tool. This included comparison of process alternatives using Life Cycle Assessment and order-of-magnitude cost and profitability metrics. The methodology was implemented using a case study comparing the production of citric acid from alternative processing routes.

10.2 Outcomes of the Work

10.2.1 Generic Flowsheet Development

The graphical user interface of the generic flowsheet was developed to provide a more intuitive and user-friendly platform with which to select simulation procedures, input data and generate results. The user-interface allows the user to select from a number of possible generic operations, including material and energy balance calculations, equipment costing calculations and process assessment calculations. Once the user has selected a generic operation, options are available for specific categories. These include options for the process type, specific unit operations, or economic calculations. Within each category selection the user has the ability to select procedure or data input options. Once the necessary procedures have been selected and the process data entered, results can be generated. The improved interface was developed using Visual Basic for Application (VBA) and implemented in MS Excel®. The user-interface presents well designed input screens for various unit operations, process data and operating parameters, allowing for easier unit data specification. Further, the improved user interface reduces the time necessary to move between simulation procedures (i.e. material and energy balance, unit sizing, economic calculations), unit operations (e.g. bioreactor, filtration, ion-exchange), and parameter specification (batch time data, unit sizing data, unit costing data). The improvements ultimately contribute to providing an application that requires less time for specification, is easier to use, and is more functional in terms of features available for process simulation.

The generic flowsheet model was developed further to include the necessary data and calculation procedures for an order of magnitude estimate of the capital and operating costs associated with large-scale bioprocesses. The extended model uses the material and energy balance data generated using the model developed by Harding (2008). This data forms a basis for the added capabilities of unit sizing, and subsequent unit costing, capital cost estimation, operating cost estimation, and profitability assessment. The necessary default economic data, input requirements, calculations and outputs are included in the flowsheet. The extended flowsheet includes a database of default data for all the available unit operations, process costs, and profitability calculations. These data inputs may be updated by the user to reflect more representative data during specific flowsheet development. The calculation results include batch time and batch scheduling, unit residence time, throughput, configuration, size and cost; and process capital requirements, operating costs and profitability.

10.2.2 Findings of the Case Study

Chapters 6-9 presented the application of the generic flowsheet to provide a basis for the environmental and economic performance assessment of a typical large scale, high volume bioprocess. This was demonstrated using a case study for the production of citric acid. Firstly, material and energy balance results from the flowsheet tool for the production of citric acid from starch were compared to values from a detailed simulation package (Biwer *et al.,* 2006). The results of

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the inventory data from the generic flowsheet model were shown to be within 5-20% of the data from Biwer *et al.* (2006). Biomass production (*A. niger*), nitrogen (NH_4NO_3) requirements and phosphorus (KH_2PO_4) requirements from the process models were in relatively good agreement. A comparison of the material and energy inventory for citric acid production from two feedstocks, namely maize starch and beet molasses was then presented. Input data required for the generic flowsheet simulation was adapted from case studies and common citric acid process data reported in the literature. Material and energy balance results from the simulations provided a first-estimate for large-scale citric acid production. Certain critical variables identified (e.g. yield coefficients, aeration rate, downstream efficiency), had a strong influence on the results. Input data for the bioreactor unit operation and recovery in certain downstream operations had a strong influence on the material and energy balance results and received considerable attention when the process model was developed. The results of the material and energy balance are representative of large-scale citric acid production and form the basis for the comparative environmental and economic assessment of process.

The environmental assessment was performed using a ‗cradle-to-gate' life cycle assessment (LCA) methodology. The first part of the environmental assessment presented a comparative assessment of the production of citric acid from starch based on the inventory data from the case study presented by Biwer *et al.* (2006) and the inventory data generated using the generic flowsheet model. Although the results showed LCA scores within 5% in comparison to the detailed simulation data, the difference in the comparative inventory data was not within this range. This was due to the aggregation of the inventory values across the entire life cycle. The LCA provided a somewhat muted representation of the actual inventory differences between the environmental performances of the process options specific to the case study. Application of LCA over the entire life cycle is likely to be better suited to select between processes that are vastly different, since the results of the impact assessment are not likely to show the difference in environmental performance of design modifications that result in moderately different inventory data. The results of the comparison demonstrate that LCA is valuable for process selection in early stages of process development when selection between processes that have significantly different inventory data is typical.

In the second comparative life cycle assessment the traditional industrial process, based on molasses, was compared to the process using starch as the main raw material. The contributions to the environmental impact from the production of citric acid were mostly common for both the molasses and starch processing routes. Variation across impact categories was most significant with regards to global warming, ozone layer depletion, fresh water aquatic ecotoxicity, terrestrial ecotoxicity, photochemical oxidation and eutrophication. The absolute impact of marine aquatic toxicity was approximately two orders of magnitude larger than comparison impact categories, namely human toxicity, fresh water aquatic ecotoxicity and terrestrial ecotoxicity. Overall, the contributions were largely as a result of the large electricity and steam requirements and the production of maize starch and beet molasses. The electricity was mostly used for air compression and bioreactor agitation. The steam requirement was mainly for product crystallisation. The starch process required approximately 60% more process steam than the molasses process due to a high load on evaporative crystallisation. A decrease in electrical energy requirements and agricultural inputs would have the greatest impact in reducing overall LCA scores. Monte Carlo simulation should be used to determine the parameter uncertainty associated with the results of the impact assessment.

In Chapter 9, the flowsheet tool was used to provide cost estimates associated with the production of citric acid using the different feedstocks. The total capital investment required for the project was estimated at \$175 million and \$188 million for the starch and molasses processes respectively. The working capital requirement was approximately \$22 million for the starch plant and \$23 million for

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the molasses plant. The total capital required for the production of citric acid from starch was within 40% of the results of the Biwer *et al.* (2006) case study, primarily due to differences in multiplier values. The economic assessment, although a general representation of the production process, gave meaningful insight into the cost of the producing citric acid. The largest contributors to the purchased equipment cost were the 12 bioreactors and the associated air compressors, accounting for approximately 82% of PCE. The operating costs were dominated by contributions from equipment depreciation, plant maintenance, utilities and raw materials. Further, by comparing the capital and operating costs of two processes using different feedstocks and as a result, different unit operations, a basis for decision making was established. The substitution of expensive mixing vessels and raw materials in the molasses downstream process for an ultrafiltration membrane used in the starch process demonstrates how these decisions may be implemented. The high capital cost for stirred tank reactors gives further incentive to investigate the use of alternative bioreactor technology or motivate the purchase of already depreciated equipment.

10.3 Thesis Evaluation and Conclusions

The sections above have discussed the motivation for completing this thesis, the objectives that were formulated and the findings of the case study. The key findings of the simulations and assessments are presented. In concluding this thesis, these approaches and findings are further refined, in light of the initial objectives for the research, and the hypotheses developed in Chapter 5. Recommendations based on these conclusions are then presented.

10.3.1 Key Conclusions from the Research

1. *First-estimate modelling and simulation provide appropriate inventory data as a basis for early stage economic and environmental assessment of a large-scale bioprocess.*

The case study showed that first-estimate modelling and simulation could generate inventory data that is sufficiently representative to be used as the input data for process performance assessment. The data generated from the generic flowsheet tool were compared to the results from a literature study using a detailed simulation package to simulate the same process flowsheet for citric acid production from starch. Although the generic flowsheet is limited in terms of features and certain calculation procedures in comparison to the detailed package, the results from the generic tool were consistent with the accuracy typical for early-stages of process development. Using this simplified approach the most important material, energy, and unit operation data can be generated to provide a basis for environmental and economic assessment. The time required for assessment can be significantly reduced and a wider range of alternatives considered. The processes can be compared on a relative basis and the processes with the low environmental impact can be identified for each impact category. Although the choice for not including a single score in the assessment did not provide a single measure with which to choose between the process routes; the assessment approach selected, improves our understanding of the process designs and the associated operations.

2. *An early-stage assessment tool, functioning on reduced input data, can be used to identify process 'hot-spots' and compare process options*

The case study has demonstrated that an early-stage simulation tool, functioning on reduced input data, and subsequent environmental and economic assessments can be used to identify process ‗hot-spots‘ and compare process options. Assessments can enhance our insight and understanding of the process, identify potential problems and highlight areas where improvements are needed. The results from the case study showed that certain process parameters and unit operations contributed significantly to the environmental and economic performance of the process. These typically include

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aeration rates, bioreactor agitation, biomass concentration, yield coefficients, and steam heating requirements. Identifying these ‗hot-spots' and associated process parameters provides incentive and reduced scope for targeting the most important elements of the process to reduce energy requirements and improve process performance. Decisions can then be made on whether improvements should be implemented, process development should be stopped because it is not economically or environmentally sustainable, or development of the process concept into an industrial application can continue as before. The latter would be further inferred by detailed modelling and simulation of the preferred flowsheet. By using first-approximation process simulation as a basis for environmental and economic evaluation, a valuable contribution can be made to large-scale process development and optimisation and ultimately sustainable bioprocess development.

3. *Life Cycle Assessment (LCA) and order-of-magnitude cost and profitability performance measures support decision making when only first-estimate material and energy data are available.*

The case study demonstrated that results from life cycle assessment (LCA) and order-of-magnitude cost and profitability analysis can be used to identify ‗hot-spots' and provide a basis for decision making. A first-estimate flowsheeting tool was used to generate material and energy balance data on which these assessments were based. The results of LCA were used to compare alternative process options on a relative basis and the process with the least environmental impact could be identified for each impact category. It was shown that LCA provided an abstraction of environmental performance of the real world production process using a systematic methodology with which to translate inventory data into impact scores. The localised impacts are aggregated across the entire life cycle however, and identification of ‗hot-spots' may be obscured. The results of the case study in Chapter 8 show that LCA is valuable for process selection in early stages of process development when decision making typically considers processes designs where inventory data is significantly different. The case study further demonstrated, that economic assessments based on first estimate process data, give meaningful insight into the cost and profitability of the production process. The economic analysis provided insight into the variation in process profitability as a function of the actual location of the plant, local economic conditions and specific operating conditions of the manufacturer. The profitability of the processes was mostly affected by the market selling price of citric acid monohydrate; the cost of raw materials, especially maize starch, and beet molasses and the initial capital investment of the plant. This provides a basis for improvements and decision making when selecting between process options. Although the limitations of these approaches are important, the results of the case study show that first approximation process simulation can be used as a basis for life cycle assessment and order-of-magnitude economic analysis and a valuable contribution can be made to sustainable process development.

10.3.2 Research Hypothesis

The hypotheses presented in Chapter 5, provided a basis for reasoning and further investigation of the facts associated with the thesis. The validity of the hypotheses can now be evaluated:

Hypothesis 1: *A first-estimate flowsheet tool can provide holistic inventory data of sufficient reliability for process performance assessment and support decision-making in large-scale bioprocess design.*

The need for first-estimate performance assessment of bioprocess designs was justified using the discussions presented in Chapters 1, 2, 3, and 4. The justification was primarily based on the fact that the biomanufacturing faces certain problems and opportunity for addressing these problems is mostly available in early stages of the development process. Further, review of the relevant literature

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provided evidence that a simulation tool is a valuable support tool for process performance assessment and decision making. Chapter 3 showed that, typically, detailed simulation tools are used to provide a basis for these assessments, however, simplified flowsheeting tools have been successfully developed and applied to generate suitable material and energy data that is used as the basis for process assessment and decision-making. The approach was demonstrated in the second part of the thesis by applying a simplified first-estimate flowsheet simulation tool to generate process material and energy balance data for the production of citric acid. The results of the process simulation demonstrated that the flowsheeting tool was a valuable support for decision-making in large-scale process design.

Hypothesis 2: *Life Cycle Assessment and a study estimate of costs and profitability provide a method that supports systematic sustainability assessment with which to compare bioprocess design alternatives.*

Chapter 4 provides an overview of various methodologies and techniques that could be applied to assess the environmental and economic performance of the process design. Case studies presented in the literature demonstrated life cycle assessment (LCA) as a valuable method with which to assess the environmental performance of a product or service life cycle. The case studies showed that although LCA is increasingly adopted for environmental assessment there are a number of drawbacks associated with the approach. There is an immense amount of methodological choice when using LCA and the approach is often data intensive and may require extensive time and effort to generate an appropriate evaluation of the life cycle. The difficulties with LCA are mostly related to the lack of suitable inventory data, the effects of aggregation and the choices associated with allocation of burdens. Although it is conceded that LCA still requires considerable refinement, the methodology offers a systematic and well accepted approach for environmental assessment of selected activities. Further, Chapter 4 presented the application of equipment design and economic metrics to generate order-of-magnitude estimates in conceptual stages of process design. Combining these metrics provides a systematic approach to evaluate the economic performance of a process design and compare alternative process flowsheets. Although the metrics are generically applicable to chemical and biological processes and contain intrinsic inaccuracy; they are well suited for performance assessment in early stages of process design and comparison.

These approaches were demonstrated using the comparative case study for citric acid production. The first-estimate flowsheet simulation tool was used to provide the basis for the assessments. The results of the case study showed that LCA and order-of-magnitude economic assessment can be used to systematically compare bioprocess alternatives and support decision making. In the context of the three pillars of sustainability, presented in Chapter 4, life cycle assessment and order-of-magnitude estimates of process costs and profitability provide insight into the environmental and economic performance of a process design and are valuable supports for process sustainability assessment.

10.4 Value of the Thesis

The outcomes of this research project contribute to previous research work and development of the process flowsheet model within Centre for Bioprocess Engineering Research (CeBER) within the Department of Chemical Engineering, University of Cape Town. The thesis contributes to the understanding of early-stage process simulation and subsequent environmental and economic assessment of large-scale bioprocesses. This understanding supports decision making in process design and selection of process alternatives. The approach does not consider the social element of sustainability which is an important element of process sustainability. Consideration of social metrics would be valuable for improved process performance assessment, in the context of the three pillars of sustainability.

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The value of the generic flowsheet model has been improved by including unit sizing and costing as well as process profitability assessment. This enhances the practitioners‘ ability to obtain process data as a basis for both environmental and economic assessment of large-scale bioprocesses in early stages of process design. The case study demonstrates the application of the simulation tool and assessment approaches and provides a valuable example of early-stage bioprocess simulation and subsequent assessment for improved process performance. Certain critical limitations of the generic flowsheet model, such as substrate pre-treatment, single product formation, and recycle streams have not been addressed in this work. Addressing these limitations would contribute to the value of the simulation tool since the number of process designs which could be modelled would be significantly increased.

10.5 Recommendations

The following recommendations are formulated from the conclusions presented above. The recommendations provide the necessary impetus with which to apply the research theory developed in this thesis and improve future research efforts for improved sustainable bioprocess design and development.

10.5.1 Recommendations to Bioprocess Design Engineers

1. *Apply the methods for process performance assessment, presented in this thesis, to aid improved process development.*

The methods for process simulation and performance assessment, presented in this thesis, are able to highlight potential problems and opportunities earlier in the development process, avoiding costs for additional research and re-design at later stages. These methods should not be used in isolation however, but rather act as a support tool for design engineers to provide additional insight alongside other methods used during process development (e.g. literature review, laboratory research, detailed simulation, pilot-scale studies).

2. *Adopt a multi-dimensional approach*

By incorporating knowledge from systems thinking, engineering process design, environmental assessment and project finance, process development can be greatly enhanced. A multi-dimensional approach encourages holistic thinking within process development and helps the design engineer consider multiple aspects of the design process. Chemical engineers and process design practitioners form an important part of the approach to sustainable process development. The science of systems analysis, material and energy balances and process modelling form part of the fundamental competencies of chemical engineering practice. Chemical engineers function as a means to improve environmental assessment frameworks and act as a link between process industries and environmental policy makers. It is the responsibility of process engineers to drive more environmentally sustainable process development by integrating both traditional chemical engineering design approaches and quantitative environmental assessment of the designs.

10.5.2 Recommendations for Further Research

The following recommendations are proposed for further research in the context of first-estimate modelling and simulation and subsequent life cycle assessment and economic analysis for improved sustainable process development:

1. *Investigate a broader set of case studies for large-scale bioprocess design in early stages of development.*

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The thesis demonstrated the application of Life Cycle Assessment and order-of-magnitude cost and profitability assessment for a traditional industrial large-scale bioprocess based on first-estimate process material and energy data. To provide insight into the application of the methodology used in this thesis and the work of Harding (2008), to newly developed technologies and potential processing routes, a broader range of case studies should be considered.

2. *Investigate methodologies for simplified environmental and social performance assessment that are suitable to be included in the generic flowsheet.*

The generic flowsheet was extended to include capability for unit sizing and economic analysis of a large-scale bioprocess. In providing a tool that integrates all three elements of sustainability i.e. economic, environmental and social aspects it would be beneficial to include features for assessing the environmental burden and social impacts of the process design within the generic flowsheet.

3. *Improve the features of the generic flowsheet model.*

The flowsheet model developed by Harding (2008) does not make provision for substrate pre-treatment, only allows for a single product, and does not allow for recycle of process streams. The functionality of the model would be greatly enhanced by removing these constraints. This would allow for a wider range of bioprocesses to be investigated.

4. *Investigate the application of Monte Carlo simulation to gain improved insight into the uncertainty associated with the results in this thesis.*

Although the thesis did not explicitly apply Monte Carlo simulation to analyse uncertainty, the thesis provided a primer for application of the technique, which is a powerful method for assessing the uncertainty associated with the results of the simulation and provides a richer analysis. In a broader context, it would be of great value to investigate uncertainty analysis in Life Cycle Assessment as a whole, which is relatively underdeveloped.

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APPENDIX A

Bioprocess Theory

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Appendix A: Bioprocess Theory

A.1 Processes and Products

A.1.1 Types of Bioprocesses

The primary objective of bioprocesses is the transformation of feedstocks, typically organic material, in the presence of biological catalysts, such as live cells, attenuated cells, or their components, e.g. enzymes (Schlegel *et al.*, 2002). In classifying biocatalysts more precisely, one can distinguish between enzymatic biotransformations and metabolic biotransformations. Enzyme biotransformations typically consist of a single or a few specific reactions. In metabolic biotransformations an entire metabolic system of the living biocatalyst is needed.

A.1.1.1 Enzyme Biotransformation Processes

Enzymes catalyse biochemical reactions by bringing the reaction to its equilibrium position more quickly than would otherwise occur. This is achieved by lowering the activation energy of the reaction, thus dramatically increasing the rate at which the reaction occurs. Enzymes are highly specific and selective in the reaction they catalyse and the substrate they utilise. One enzyme can usually only catalyse one type of reaction. There are six classes of enzyme depending on the type of reaction that they catalyse. These are oxidoreductases, transferases, hydrolases, isomerases, lyases and ligases. About 100 types of enzymes are used industrially. Most of them are hydrolases (75%) used for the depolymerisation of natural substrates with high molecular mass (proteins, starch, pectins). The enzymes are used widely for biotransformation in fine chemical production and pharmaceutical industries. Enzymes are not only used as biocatalysts in industrial processes, but are also produced as final products. A common example is the use of enzymes (mostly proteases, amylases and lipases) as detergent additives. This represents the largest application of industrially produced enzymes. Proteases, used in laundry detergents, account for about 25% of the total worldwide sales of enzymes (Barredo, 2005).

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A.1.1.2 Microbial Bioconversion Processes

Microorganisms are extremely useful in carrying out biotransformation processes in which a compound is converted into a structurally related product by one or a number of enzymes contained in the cells. Metabolic biocatalysts are traditionally prokaryotic bacteria and eukaryotic fungi, while plant, animal and algal cell cultures have become more important in recent times. Microorganism selection depends on a range of criteria that are relevant to the optimization of the product or the process. Amongst others, the criteria include: nutritional characteristics, temperature characteristics, types of processes, response of organism to equipment, organism stability (phenotypic and genotypic), amenability to genetic manipulation, product yield, productivity and product recovery (Bull *et al.*, 1979).

i. Prokaryotes and Archea

Bacteria and archea are diverse unicellular prokaryotes which possess a rigid cell wall and no true cell nucleus. Prokaryotes are able to colonize a wide range of habitats and can be found naturally in environments of pH 1 to pH 11 and at temperatures from 0 °C to 105 °C at atmospheric pressure. They can be found in aerobic and anaerobic environments and in situations where water activity ranges from 1.0 to as low as 0.75. The optimum growth conditions for most bacteria range between pH 6.5 and pH 7.5 and temperatures between 20 °C and 45 °C (Millis, 1985). A relatively small number of bacteria have been very well studied and used as commercial biocatalysts. Commonly used genera include *Escherichia, Bacillus, Corynebacterium, Clostridium, Acetobacter, Pseudomonas, Lactobacillus and Zymomonas*. The bacteria can be cultivated in large volumes in relatively inexpensive media. The biotransformations are usually characterized by high yields of 90-100% and moderate reaction conditions. The fermentation products from the prokaryotes fall into a number of categories, namely single cell protein or biomass, end-products (e.g. solvents and acids), primary metabolites (e.g. amino acids, vitamins and nucleotides) and secondary metabolites (e.g. antibiotics, pigments and polysaccharides (Millis, 1985).

ii. Fungi

Fungi are aerobic eukaryotes and heterotrophic organisms whose usual habitat is the soil. In industrial processes it is convenient to divide fungi in two subgroups, namely yeasts and moulds. Yeasts grow as single cells, multiply by budding and can metabolise aerobically and anaerobically. Yeasts are used to produce alcohol in anaerobic fermentations and baker's yeast, yeast extract and single cell proteins in aerobic bioreactions. Filamentous fungi, which grow as mycelium or pellets, are mostly grown under aerobic conditions. In commercial applications *Aspergillus* and *Penicillium* are the dominantly used genera. Filamentous fungi are also used at large scale to produce antibiotics, organic acids and enzymes like amylases, cellulases and glucoamylases (Millis, 1985).

iii. Mammalian Cell Cultures

Mammalian cell cultures have an advantage over prokaryotic and lower eukaryotic systems as they produce products that most resemble the natural protein. They are used to produce high-value proteins where it is essential that the correct protein structure is obtained. (Peshwa, 1999). Industrial bioprocesses using animal cells are still relatively limited. There has however been an increase in the number of processes producing virus vaccines to monoclonal antibodies and complex structured glycoproteins. Production operations cover a range of capacities and configurations, including small multiple unit reactors to large 10,000 litre single batch operations. The slow growth rate of the cells and the use of rich media make mammalian cell cultures susceptible to contamination and sterile technology becomes an important consideration (Werner *et al.*, 1992).

iv. Plant Cell Cultures

Plant cell cultures are used in various applications ranging from studies of basic plant biochemistry to mass propagation and genetic engineering of crop species. The media conditions, culture vessels and conversion parameters vary depending on the intended application (Paiva, 1999). The techniques and media used to grow plant cells are similar to those of mammalian cells, except that light is provided and nutrient serum is replaced with plant extracts. Plant cell cultures are predominantly used for the production of secondary metabolites. Products include anticancer compounds (vincristine and vinblastine), food additives (colouring agents and flavour extracts), fragranced oils and organic pesticides.

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v. Insect Cells

Insect cell cultures have been commercially employed to produce recombinant proteins for vaccines and therapeutic applications. They can produce the proteins more quickly and at higher expression levels (30-50% of total intracellular protein) than mammalian cells (Terry 1999; Nagabhushan, 2002; Walsh, 2003). The use of insect cells in industry is however limited and considerable research is required before they become widely used.

vi. Algae and Protozoa

These relatively large eukaryotes have sophisticated and highly organised structures and nearly all algae have photosynthetic machinery. Commercial interest in algae is focused at their use as foodstuffs and food supplements and more recently, carbon sequestration and the production of biofuels (Schlegel, 2002). Protozoa are not typically employed for the manufacture of cells or products; they are of importance in biological waste treatment (Bailey & Ollis, 1977).

vii. Transgenic Cells

Transgenic cells contain genes that have been transferred from a different species. Genetically modified plants are able to produce a wide range of products and show great appeal to agricultural industries (Davies & Demain, 1999). The expression can either take place in the whole plant or a specific part of the plant (e.g. seeds). Plants that are commonly modified are tobacco, potato, rice and wheat. The plants are modified with specific genes to protect against potentially lethal agents such as herbicides, viruses and insects. Additional improvements include increased crop yield and product quality. Although transfer of single gene traits has been successfully achieved, many desirable traits such as disease resistance, stress tolerance and photosynthetic efficiency depend on the activity of several genes and additional work is required to characterize these genes (Nagabhushan, 2002). Transgenic animals may soon become an attractive alternative to genetically engineered, biologically active proteins. Severe regulatory issues would have to be resolved, but expression levels and product quality could be greatly improved compared to products from conventional methods (Nagabhushan, 2002).

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A.1.2 Raw Materials

Substrates required for industrial applications are usually included in complex media, which tend not to be well characterised. The media is supplemented with additional compounds, such as a nitrogen source, nutrient salts and certain trace elements. The carbon and energy for biosynthesis is usually supplied as a crude carbohydrate for industrial applications. Common carbon sources include glucose, sucrose, corn syrup, cane syrup, molasses, corn steep liquor, cereal grains, malt, rice and sorghum. Organic or inorganic nitrogen sources are suitable for the nutrient medium. Inorganic sources include soluble nitrates and ammonium compounds. Organic sources include soybean meal, cotton seed meal, peanut meal, corn steep liquor, yeast extract, urea and albumin. Oxygen requirements for metabolism are supplied by the carbon source and from aeration of the bioreactor vessel. Additional nutrients required for growth, are phosphorus and potassium. They are usually added in the form of inorganic compounds, such as phosphate salts and potassium chloride. Trace concentrations of certain micronutrients are also required for optimal growth. These include iron, zinc and manganese and are typically added as inorganic salts or as trace components of the complex media.

A.1.3 Product Classes and Types

The product mix from the bioprocess industry typically consists of pharmaceutical products; commodity biochemicals and fuels; enzymes, fine chemicals and specialities. The scale of production, process configuration and required product purity for each product class varies considerably. In classifying bioproducts it is important to consider both the chemical structure and the intended use of the product. Pharmaceutical products include diagnostic, prophylactic and therapeutic agents. The global sales of pharmaceuticals were valued at about $400 billion in 2002 (McGuire *et al.*, 2002). Pharmaceutical products are usually produced in very small amounts and have high selling prices. The need for a very high purity increases both the complexity and cost for product recovery and purification.

The production of commodity biochemicals and fuels is characterized by large production volumes (e.g. >1 million tpa) and medium to high product purities (97%). The products are usually produced by microorganisms in an inexpensive media with a high productivity and relatively simple recovery and purification stages. Examples of some of these products include citric acid, glutamic acid, acetic acid and ethanol.A wide range of fine chemicals and specialty applications are made available by the bioprocess industry. The annual production, price and required purity of fine chemicals typically lie between that of bulk chemicals and pharmaceuticals. Speciality and service applications include bioleaching in mineral processing, drug delivery systems, enzymes, antioxidants, metering probes, wastewater treatment technologies and nitrogen fixation.

A.2 Process Layout and Unit Operations

The bioprocess is often characterized by the bioreaction, by which a product or service of value is created through biocatalysis, biotransformation, or cell culture. Feed preparation and upstream processing as well as utilities such as gas compression are required. Additionally, to ensure the required production yield and product purity, it is essential that appropriate and efficient product recovery and purification be implemented. The main processing sections of a typical bioprocess are shown in Figure A.1. It is the task of the process design engineer to select the appropriate technologies and synthesise the unit operations in such a way as to ensure an efficient, economical and competitive process.

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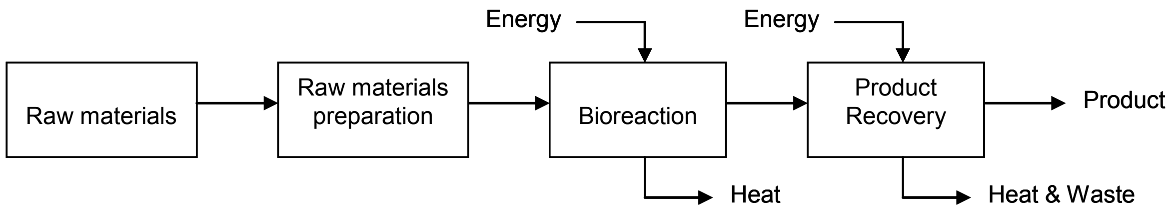

Figure A.1 Flowsheet of typical bioprocesses

A.2.1 Upstream Processing

Upstream operations include all unit operations carried out before the bioreaction stage of the process. These include typically feed preparation, sterilisation and inoculum preparation.

A.2.1.1 Handling and Preparation of Raw Materials

The role of the process design engineer is to provide the most economical means for storage of any specified quantity of gas, liquid or solid material (House, 1969). Most of the liquids encountered in bioprocesses are non-biological fluids and adhere to Newtonian fluid flow behaviour. Some liquids however, need special consideration due to sensitivity to shear or thermal factors, non-Newtonian rheology, or the need for sterility. The rheology of liquids in many bioprocesses can be complex and require special systems for handling and storage. Dough's, bioreaction broths, proteins, starch solutions and fibrous slurries all depart from typical Newtonian behaviour. The denaturation of proteins, pasting of starches, inactivation of enzymes and the destruction of microbial cells are all possible consequences of excessive shear and temperature. All of these factors need to be considered when designing the storage and handling systems to avoid material damage and redesign expenditure. An extensive analysis of fluid rheology and special properties of biological fluids is given by a number of texts (Moo-Young, 1985; Coulson & Richardson Vol 2, 1991; Perry *et al.,* 1997). Raw materials are usually stored and prepared in high concentrations to minimise storage vessel costs. The solutions are then diluted in the reactor with sterilised water. Carbon and nitrogen sources are usually prepared in separate tanks to avoid non-enzymatic browning reactions. The material used for construction of tanks is dependent on factors such as corrosion, the need for sterility and temperature.

Feedstock preparation is also an important consideration in bioprocesses. Pre-treatment of materials is often required for good assimilation by the microorganisms. Starch containing materials are prepared by milling or steam treatment for softening and swelling. In many processes, especially yeast processes, starch has to be hydrolysed by enzyme treatment (e.g. amylase). Wood feedstocks often require pre-treatment with acid or alkali. In some cases impurities, such as dissolved salts, have to be removed from feedstocks before they can be used.

A.2.1.2 Sterilisation

Sterilisation is required to prevent the growth of undesired microorganisms and is one of the most critical unit operations in bioprocesses. It can be achieved by filtration, chemical addition, heating, radiation (UV and X-rays) and sonication. Air sterilisation by filtration and media sterilisation by heat are the dominant methods used in industrial bioprocesses.

i. Air Sterilisation by Filtration

Sterilisation of gaseous streams is dominated by the use of filtration techniques. Since large volumes of air typically pass through the bioreactor in aerobic processes, it is essential that the air is adequately sterilised to avoid contamination. Membrane filters and depth filters are commonly used technologies for filtration of gaseous streams.

Membrane filters with a pore size of 0.2-0.3 μm are mostly used for microorganism removal. A gas compressor supplies the necessary pressure for flux across the membrane and a trade-off between degree of sterilisation (governed by pore size) and pressure drop is essential to reduce energy costs (Strathmann, 2002). The membranes can be sterilised by steam and functionality is not impeded by the presence of moisture. Considerable research and

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development has been directed towards the improvement of membrane filters. Their application for gas and liquid filtration has increased rapidly in recent decades.

Depth filters contain a filter-medium constructed from a synthetic porous or fibrous material usually made from glass wool, glass fibre, sintered metals, or polymers. The spacing between the fibers is large in comparison with the micro-organisms to be removed. The filters exclude micro-organisms on a probability of particle retention by interception, inertial impaction, and diffusion (Cooney, 1985). Depth filters are frequently used as pre-filters, reducing the particle load on the subsequent membrane filter and thereby pressure drop.

ii. Media Sterilisation by Heat

Heat sterilisation is most commonly used for the sterilisation of liquid media. The degree to which contaminants are removed is primarily dependent on the sterilisation temperature and the exposure time. The higher the temperature, the lower the time required for sterilisation. The sterilisation temperature-time cycle is thus determined by considering a balance between the thermal death rate of contaminants and the thermal lability of the media components. Insufficient sterilisation could lead to contamination of the process, while excessive sterilisation may lead to media destruction. The thermal death rate exhibits first order kinetics. The relationship for the death rate is shown in equation A.1.

$$k_d = \frac{1}{t} \ln \frac{N_0}{N} \qquad \text{A.1}$$

where N_0 is the initial number of microorganisms, N is the number of surviving microorganisms, t is the holding time and k_d is the thermal death rate constant (characteristic parameter for each microorganism with Arrhenius temperature dependence).

The sterilisation can be performed in a batch-wise or continuous configuration at temperatures typically above 100 °C (Cooney, 1985). Batch sterilisation is usually performed in a closed, well mixed vessel e.g., in the reaction vessel, or separate containers. Typically the medium is steam heated by means of a jacket, direct steam injection or internal heating coils, maintained at the desired sterilisation temperature and then cooled. In continuous sterilisation the cycle-time required is much shorter than batch-sterilisation and improved energy efficiency is realised. Although a higher temperature is applied (140-145 °C), heat-labile media components are less damaged due to the shorter exposure. In continuous sterilisation, tubular or plate-and-frame heat exchangers can be used to provide economical heat exchange between process streams. Direct steam injection can also be used and offers almost instantaneous heating to the required temperature. The fouling of heat exchanger surfaces is avoided with direct injection but energy is wasted since heating and cooling are not integrated. Additional disadvantages of direct injection are the dilution of the medium and difficulty in controlling pressure and temperature due to the variation in medium viscosity.

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A.2.2 Bioreactor

A.2.2.1 Types of Bioreactors

Typical types of bioreactors include stirred tank, airlift, packed-bed, fluidised bed, trickle bed, deep shaft, pulsatile, UASB and anaerobic baffled reactors. A number of factors need to be considered when deciding on the type of reactor to be used. These factors include the process type (e.g. aerobic or anaerobic), reactor configuration, geometry, mode of operation (e.g. batch or continuous), energy provision, physiochemical requirements (e.g. mixture viscosity and rheology), mass transfer requirements, liquid velocities and shear conditions. Some of the common reactor types are discussed below.

i. Stirred Tank Reactors

In most cases a stirred tank reactor (STR) is used in bioprocesses. These can range from simple small tanks to large and sophisticated aerated bioreactors. Agitation in the reactor is provided by an impeller and rising gas bubbles. The liquid mixture is assumed perfectly mixed in the ideal case. In aerobic processes, oxygen is usually supplied by air sparging at the base of the vessel. Temperature control is achieved by means of a jacket or internal cooling coils. Typically, the energy required results from agitation, aeration and temperature control.

ii. Bubble Column and Airlift Reactors

Bubble columns and airlift reactors enhance mixing and mass transfer through gas compression. In bubble columns gas is dispersed by a perforated or porous plate at the base of the column and the bubbles rise due to their buoyancy forces. There are a number of bubble column variations including simple columns, multiple columns, column with jet tube aeration and tower reactors for microorganism flocculation (Voss, 2002). Airlift reactors are essentially bubble columns that have been modified to achieve countercurrent flow of a fraction of the liquid medium in the column. The countercurrent flow is best controlled by splitting the reactor into a riser and downcomer section through which the liquid medium is circulated with and without sparging respectively (Zehner & Kraume, 2002). Although the oxygen transfer is generally lower than a stirred tank reactor the energy consumption is not as high as that of the STR. Bubble columns and airlift reactors are especially suitable for shear-sensitive, flocculating and foaming microbial systems (Voss, 2002).

iii. Packed Bed Reactors

Packed bed and plug reactors are commonly used for free and immobilised enzyme reactions. The constraints of oxygen supply and pH control are not usually applicable as with microbial growth and a greater degree of substrate conversion can be achieved in the plug flow reactor compared to the continuous STR reactor. The immobilised or particulate catalyst is placed in a tube-shaped vessel and the medium flows through the column under pressure.

iv. Fluidised Bed Reactors

In fluidised bed reactors the medium flows upwards through the catalyst bed causing the bed to expand at high flow rates. The reactor configuration promotes heat and mass transfer through good mixing of media components. The reactor is typically packed with relatively small catalyst particles to reduce pumping costs.

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A.2.2.2 Bioreactor Operational Requirements

The reactors used in bioprocessing have to meet the requirements for agitation, aeration, temperature and pH control, aseptic operation and corrosion resistance. Although optimisation of the these unit procedures is essential in improving the efficiency of the process it is important to keep in mind that the bioreactor is part of the overall process and optimising it independently from the rest of the process is often not most beneficial (Charles, 1985).

i. Agitation

Agitation is required in stirred tank reactors to maintain homogeneity, distribute nutrients and to ensure adequate energy and oxygen transfer. Some of the key parameters are agitation power, agitation time and impeller speed. The tanks are usually baffled to avoid vortex formation during mixing. The baffle and impeller size are related to the tank diameter. Depending on the aspect ratio of the vessel and the degree of mixing required, more that one impeller may be fitted to the agitation shaft to improve homogeneity. The power consumption of agitators is dependent on the speed of rotation, medium density and viscosity, impeller geometry, volume of the medium, baffle configuration and whether the vessel is gassed or ungassed. Extensive reviews of correlations for agitation power, impeller speed and baffle configurations are available (Bailey & Ollis, 1977; Moo-Young, 1985; Blanch & Clark, 1996, Ullmann, 2002).

ii. Aeration

Oxygen-transfer rate and dissolved oxygen tension are vital factors in aerobic bioreactions (Pandey *et al.,* 2000). The oxygen requirement is usually supplied by sterilised air and is sparged in at the base of the vessel. The rate of oxygen transfer to the aqueous system is dependent on the oxygen transfer coefficient (k_La) and concentration driving force in the medium. Various empirical correlations have been published for k_La for different reactor configurations and fluid properties as cited by Moo-Young (1985). In order to ensure that respiration in the fermentation system is not oxygen limited, oxygen transfer rate must equal oxygen uptake.

iii. Temperature Control

Efficient heat addition or removal from the reactor is essential to ensure the bioreaction is maintained at the optimal operating temperature. Heat transfer requirements may be determined by accounting for the reaction enthalpy, energy dissipation due to agitation and energy dissipation of the gas. A hot utility (e.g. steam) is used to supply heat, while cooling or chilled water is used for heat removal. Heat removal may be achieved by means of internal cooling coils or an external cooling jacket. Cooling coils are preferred in large reactors through allowing the transfer surface area to vary independently of external surface area. The coils can however occupy considerable volume, interfere with flow patterns and create cleaning and sterilisation problems (Moo-Young, 1985; Schlegel *et al.,* 2002).

iv. pH Control

In many biological reactions a constant pH is required. The pH has a major affect on cell growth and product formation by influencing substrate breakdown and transport through the cell membrane (Bailey & Ollis, 1977). The pH of the media will change with the metabolic

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product of the microorganisms and pH control is required during the bioreaction. The pH is controlled by buffering the medium and adding acid or base on demand. The acid or base requirements can either be determined experimentally or estimated by an ion-charge balance (Blanch & Clark, 1996; Najafpour, 2007).

v. Foam Control

Foam formation due to the combination of aeration and agitation with the presence of foam-stabilising components such as proteins, polysaccharides and fatty acids poses a major problem in many biological processes. The foam layer has the tendency to rise to the reactor headspace and leave the vessel through the air exit, causing contamination and removal of micro-organisms from their optimised environment. Foam control is achieved through the addition of antifoam agents, such as propylene glycol, silicon oil, fat, octadecanol, or plant oils. A mechanical foam breaker mounted in the reactor headspace can also be used and is usually preferred (Schlegel *et al.,* 2002).

A.2.3 Downstream Processing

The downstream unit operations for the production of bioproducts generally contribute a significant percentage to the overall production cost, especially if a high purity product is required. The type of downstream process is strongly dependent on market demand, processing cost, nature of the product, final product quality required and available technology. It is important that the purpose of each downstream unit operation is understood when developing a flowsheet model. When developing the detailed unit designs, specialised literature should be consulted (Moo-Young, 1985; Atkinson & Mavituna, 1991; Coulson & Richardson Vol 2, 1991; Perry *et al.,* 1997; McCabe *et al.*, 2001). An overview of typical purification procedures and corresponding unit operations used in bioprocesses is given. A summary of the separation principles and typical yields of common unit operations is given in Table A.1.

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Table A.1 Separation principles and yields of downstream unit operations (Moo-Young, 1985)

Unit Operation	Separation Principle	Recovery (%)	Product
Centrifugation	specific density	90-99	cells, particles
Sedimentation	specific density	80-99	cells, particles
Microfiltration	size/phase	80-99	cells, particles
Ultrafiltration	size/phase		cells debris, proteins
Chromatography		60-99	
Ion-Exchange	ionic charge		ions
Gel Filtration	size/shape		large molecules
Affinity	molecular recognition		molecules
Electrodialysis	ionic charge	70-99	ions
Extraction	solubility	70-99	hydrophilic or -phobic molecules
Distillation	volatility	80-99	volatiles
Drying/evaporation	volatility	97-99	high boiling molecules
Crystallisation	phase change	60-95	crystallised solids

A.2.3.1 Biomass Separation

In bioprocesses using cells, the first step of the product recovery process is the separation of supernatant insoluble biomass. This can be achieved by centrifugation, filtration and sedimentation. The unit operations are discussed in more detail in the subsequent sections. In the selection of the unit operation, various factors such as particle size, medium viscosity and biomass concentration need to be considered.

A.2.3.2 Cell Disruption

The isolation of intracellular products requires the disruption of the cell envelope so that the product is released into the product solution. Methods for cell disruption are generally classified as mechanical, chemical, or biological. In large-scale operations high pressure homogenizers and high speed ball mills are commonly used. In high pressure homogenization the cell suspension is passed through a narrow valve at high pressure (1500 bar) and the cells are ruptured by hydrodynamic shear due to the rate of change in pressure, impact, as well as cavitation (Moo-Young, 1985). Cell disruption using high speed ball mills involves agitating the cell suspension with steel or glass beads. Cell breakage is caused by hydrodynamic shear forces and impact during grinding of the elements. The efficiency of ball milling is primarily determined by the agitator speed, flowrate, cell density and bead diameter (Currie *et al.,* 1972; Woodrow & Quirk, 1982).

A.2.3.3 Separation of Insolubles

The separation of insoluble material is often the first step after the bioreaction and cell disruption operations. It may also be applied in later downstream operations.

i. Sedimentation and Centrifugation

Sedimentation and centrifugation are based on the density differences between insoluble particles and the surrounding fluid. Sedimentation relies on gravity, while with centrifugation a mechanically applied centrifugal force separates the insolubles from the solution. The settling velocity of the particles in the solution is dependent on particle size and density and the density and viscosity of the solution (Coulson & Richardson, 1999). Sedimentation needs a longer settling time and larger density difference than centrifugal separations. It is mostly used for biomass removal and wastewater treatment. Centrifuges are typically used for separation of biomass and cellular fragments. Disk-stack centrifuges are most commonly applied in industrial applications, with decanter centrifuges used where lower centrifugal forces suffice (Bailey & Ollis, 1977).

ii. Filtration

Filtration is a solid-liquid separation in which a hydrostatic pressure driving force is used to force a liquid medium through a filter membrane that is impermeable to a specific particle size. Filtration operations are usually characterised by pore size and the retention characteristics of the filter medium. Filter classes are classified as microporous, ultrafiltration and reverse osmosis. Conventional molecular filtration (0.1-10 µm pore size) is commonly used for biomass and cell debris removal. The rotary vacuum filter is the most common conventional filtration technique used in bioprocess operations (Shaeiwitz & Henry, 2002). It consists of a filter cloth on a rotating drum and operates under partial vacuum. As the drum rotates through a media containing bath, the liquid is drawn through the filter medium and solids are retained on the filter surface. Ultrafiltration (0.001-0.1 µm pore size) is generally applied during product purification for the separation of dissolved macromolecules such as

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proteins and peptides (Strathmann, 2002). A tangential feed flow is typically used to minimise membrane fouling. A variety of configurations such as plate and frame devices, hollow fiber cartridges and spiral wound cartridges are available for tangential flow filtration. Hollow fiber modules are the most popular configuration for ultrafiltration. Depending on the solids concentration and the viscosity of the feed solution, the flux across the filter medium is typically 20-25 L/m^2 for microfiltration and 20-100 L/m^2 for ultrafiltration (Strathmann, 2002).

A.2.3.4 Primary Product Purification

i. Liquid Extraction

Extraction is a procedure whereby components of a liquid mixture are separated by mixing the solution with a solvent in which the desired components are preferentially soluble. It is especially applied in cases where the components to be separated are heat labile, distillation cannot be applied, or when large volumes of low concentration solution have to be treated. It is commonly applied in the purification of antibiotics and separation of organic acids (Bailey & Ollis, 1977). To enlarge the contact area for mass transfer and thus improve efficiency, industrial designs use equipment to disperse droplets of one of the liquids into the other. The liquids are separated following sufficient contacting to allow migration of the extractable components to the dispersed phase. The main operating parameter for an extraction system is the partition coefficient which is strongly affected by temperature, ionic strength and pH (Seader & Henley, 2006).

ii. Precipitation

Precipitation is a typical intermediate step before final purification. Precipitation may be mediated by the addition of salts, organic solvents and polymers, as well as precipitation at the isoelectric point (Bailey & Ollis, 1977). "Salting out" is commonly used to reduce the solubility of the proteins by adding an electrolyte such as ammonium sulphate. Similarly, the addition of miscible organic solvents, such as ethanol, influences the solubility of proteins by reducing the dielectric constant of the medium. The addition of non-ionic polymers is used in protein precipitation and follows a similar mechanism to that of organic solvent addition. The existing precipitates ultimately agglomerate into larger flocs and are removed from the liquid medium by centrifugation or ultrafiltration (Blanch & Clark, 1996).

iii. Distillation

Separation by distillation is based on the relative volatilities of components in a multi-component solution. The solution is fed to a column where heat is applied, vaporising the volatile components, that leave the top of the column as distillate. The higher boiling liquid condensate leaves though the bottom of the column. Distillation is used in biological processes for the separation of high volume, low-boiling solutions, such as alcohols (Seader & Henley, 2006). It is also commonly used in the recovery of organic solvents in downstream operations (Schmid, 1996).

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iv. Chromatography

Chromatography is used to separate a mixture of components by means of selective retardation of the compounds as they move though a packing. The component solution (mobile phase) flows though a column packing of adsorbent particles (stationary phase). The solutes travel at different rates though the column depending on their affinity for the adsorbent. The solutes then exit the column at different times or the product is retained until conditions are changed for elution from the solid adsorbent matrix. Elution is typically achieved by means of gradient elution or affinity elution. The most common chromatography techniques are ion exchange, gel, affinity and hydrophobic interaction chromatography.

Ion exchange, used in purification, exploits reversible exchange of ions based on change between the solid phase and liquid phase, without any permanent change in the structure of the solid. It is the most commonly employed as a high-resolution method for the preparative separation of proteins (Bailey & Ollis, 1977).

In affinity chromatography a binding molecule is attached to an insoluble support matrix and the solute is stereoselectively bound to the immobilised molecules. Only compounds with appreciable affinity for the immobilised molecule are retained on the column, while others pass through unretarded. The technique is highly selective and provides a high yield and resolution of the purified product (Yarmush & Colton, 1985).

Hydrophobic interaction chromatography is based on the interaction of hydrophobic regions of proteins and the hydrophobic ligand matrix to separate the compounds. Adsorption occurs at high salt concentrations and bound compounds are eluted by reducing the concentration of the salt concentration of the mobile phase. The method is used primarily for the purification of proteins and is suited for purification after concentration by precipitation (Ullmann, 2002).

v. Electrodialysis

In electrodialysis an electrostatic force is applied to an electrolyte solution to transport the ions through a semi-permeable membrane. The membranes contain ion-exchange groups and have a fixed electrical charge. Electrodialysis is used for the purification of organic acids, desalination and the removal of salt from protein solutions (Greben *et al.,* 1988; Strathmann, 2002).

A.2.3.5 Final Product Purification

i. Crystallisation

Crystallisation is a solid-fluid separation operation in which crystalline particles are formed from a homogenous fluid phase. Crystallisation of the solute occurs when the concentration of the solute exceeds its solubility limit and the solution is supersaturated. The crystals are filtered from the mother solution, which is typically recycled back to the crystallizer to improve solute recovery. Solution crystallizers are generally classified according to the method by which supersaturation is achieved, e.g. cooling, evaporation, vacuum (adiabatic cooling), reaction, salting out (Mullin, 2002). Key operating parameters are yield, heat and residence time (Seader & Henley, 2006).

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ii. Drying and Stabilization

Although a number of drying techniques are available, the typical heat sensitive nature of bioproducts necessitates that water is removed with a minimal increase in temperature. Convection dryers (e.g. fluidised-bed and spray driers) are common drying techniques in industrial bioprocesses (Tsotsas *et al.,* 2002). In a fluidised-bed dryer the moist solid is fluidised by a hot gas stream. The solids are separated after sufficient contact with the hot gas (Coulson & Richardson, 1999). In spray drying, the feed solution is sprayed by a nozzle or rotating disc as small droplets into a hot dry gas (150-250 °C). Evaporation of moisture proceeds rapidly enough that the temperature of the particles does not increase significantly. Spray drying is often used for enzyme and antibiotic drying (Werner *et al.,* 1993).

A.2.4 Waste Reduction and Treatment

Waste handling, treatment and prevention are important operations in industrial bioprocessing. Treatment costs can form an important component of the overall process costs. Typical waste treatment facilities can account 10% to 20% of the total plant cost. Approaches to handling waste materials include waste avoidance and minimisation, waste reclamation, waste recycling and waste treatment (Perry *et al.,* 1997).

Waste avoidance is usually the first step in waste management, since, if feasible, treatment and handling of waste is avoided. Approaches include closed-cycle operation within plants, low-waste product design and appropriate consumption (Woodard, 2001). When waste avoidance is not possible, material reclamation is typically implemented. Material reclamation strategies include the replacement of raw materials by recovering substances from wastes as well as the use of material properties of wastes for the original and other purposes (e.g. motor oil from waste oil and composted sewage sludge in agriculture). This does not include direct energy recovery (Tome-Kozmiensky, 2002).

Recycling of materials is also important for waste reduction. The material and energy cost required for recycling should however be justified by the value of the material reclaimed. Materials that remain after avoidance, reclamation and recycling, should be treated to acceptable regulatory requirements before disposal. Treatment options are numerous and the most viable treatment method depends on the nature of the waste, environmental regulations and the geographical location of the process plant. Treatment, with energy recovery, is generally preferred over disposal (Williams, 2005).

In biological processes waste streams may contain a variety of materials, including organic and inorganic compounds, colloids and solids. Low product concentrations are generally found, requiring a large volume of material to be processed. The waste materials from a submerged microbial culture usually contain large amounts of unused nutrients that can not be recycled due to the presence of metabolites. Typical wastewater characteristics of these processes are shown in Table A.2. In processes using recombinant DNA technology, containment of waste materials is critically important. The waste streams are chemically or thermally treated before disposal, to ensure living organisms are not released to the environment (Tome-Kozmiensky, 2002).

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Table A.2 Wastewater characteristics for fermentation processes (Bailey & Ollis, 1977)

Waste Parameter	Influent Wastewater from Operations (mg/l)	Effluent in Acceptable Plant (mg/l)
BOD	4,000-40,000	5-15
COD	50,000-100,000	15-75
Phosphorus	6-10	0.2-0.6
Nitrogen	20-30	2-5
Suspended Solids	100-400	10-25

Biodegradable material from the bioreaction process is typically treated in a number of stages. Significant levels of chemical oxygen demand (COD) and biochemical oxygen demand (BOD) in bioreaction waste streams have to be reduced before the streams can be discharged from the process. The majority of the organic load is removed from the medium by screening, gravity sedimentation and chemical precipitation. Solids removed from the liquid streams may be sold as by-products e.g. animal feed. Secondary treatment of the liquid streams usually follows, where microorganisms are used to stabilize waste components (Perry *et al.,* 1997). Once sufficient organic load has been removed from the wastewater, it can be discharged to the environment or recycled back to the process. Salts and volatile organic solvents used in precipitation operations are also important wastes requiring treatment. Problems with disposal are often encountered as they are corrosive towards stainless steel and cement units used in wastewater treatment (Perham *et al.*, 2002).

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APPENDIX B

LCA and Capital Costing Methodology

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Appendix B: LCA and Capital Costing Methodology

B.1 LCA Methodology

Life Cycle Assessment (LCA) is also an extremely useful and powerful tool for rigorous environmental assessment. Applying LCA, major interrelations within process system can be accounted for and the processes that most significantly influence the environmental impact of the system as a whole can be identified. The comprehensive LCA methodology is internationally standardised and can be found in a number of literature texts and publications (SETAC, 1993; Curran, 1996; Burgess & Brennan, 2001; Ullmann, 2002; ISO, 2006; SAIC, 2006). The assessment framework typically consists of four main phases, namely *goal and scope definition*, *inventory analysis*, *impact assessment* and *interpretation*.

B.1.1 Goal Definition

Life Cycle Assessment is used to quantify the overall environmental impacts from a product, process, or service. The primary goal of the LCA is to choose the best product, process, or service with the least effect on human health and the environment. The primary goals of the LCA are thus not specific to the project under consideration. There are often also secondary goals, which vary on the intended outcomes specific project. As outlined by Curran (1996) and SAIC (2006), LCA goals typically include:

i. Support broad environmental assessments

The results from the LCA are useful in understanding the relative environmental importance of variations in the providing a product or service. It provides quantitative insight into the environmental burdens associated with an industrial process and can be used to compare alternative processes and materials used to produce a product or render a service.

ii. Establish baseline information for a process

The LCA is often used to provide a baseline of information for a given process or stages within a process, based on current or future technologies and practices. The baseline typically consists of estimates for resource and energy consumption and the environmental emissions associated with the manufacture of a specific product, or provision of a specific service. The baseline is useful in motivating improvements in current and future practices through comparative analysis and improvements of the baseline.

iii. Rank the Relative Contribution of Individual Steps or Processes

The LCA provides detailed data regarding the contributions of the individual steps of the defined product system. The data can thus be used to rank the relative importance of the individual steps in terms of energy and resource consumption and associated environmental burdens. The ranking system thus highlights areas of the product system that require the most attention in reducing resource consumption, energy intensity and waste emissions of the entire system.

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iii. Identify Data Gaps

The LCA process is very useful in identifying areas of the system where data or sufficient data quality is not available. Inventory analysis followed by impact assessment of the system helps identify areas where improved data can aid the accuracy and reliability of the LCA results.

iv. Support Public Policy

The LCA can aid the public policymaker in making decisions by providing an analysis of a broad range of environmental issues associated with a process or industry.

v. Support Product Certification

The LCA can be used as a means to provide information on the environmental effects of the individual attributes of a product or product class.

vi. Provide Information to Direct Decision Makers

The LCA can be used to provide information to industry, government and consumers on the tradeoffs of alternative processes, products and materials. The information can be used as a basis for guiding industry decisions on production materials and processes and help inform the public on environmental issues and purchasing choices.

vii. Guide Product and Process Development

LCA can form an integral part of product and process development whether in early stages of process development or during the expansion an existing process. It provides a sound basis for informed decision making towards reduction of both resource consumption and emissions.

In addition to the specific goals of the LCA, it can be used to answer a number of important questions. The questions most important to the decision makers are used to identify the study parameters of the LCA. Example questions include:

1. What part of the current process has the most significant environmental impact?
2. Which product or process alternative has the least environmental impact throughout its life cycle?
3. What are the environmental effects of proposed process or product modifications?
4. Which process technology or product route causes the least amount of global warming, acidification, or eutrophication?
5. What process modifications are necessary to reduce a specific environmental impact?

At the beginning of the study, the level of data specificity must be determined, guided by the intended use of the results. The LCA practitioner needs to decide whether the project is specific to one company or can be applied to an industry in general. In conducting a generic study, general industry data is typically used to represent the common industrial practices. In the case that the LCA is being performed for a specific process or product formulation, it is

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necessary that data from the plant used in the study. A combination of both general industry data and data from a specific process is the most likely route for data specificity, but is likely to vary from one study to the next. In defining the data specificity a distinction can be made between foreground and background data. The foreground system, defined as the set of processes directly affected by the process delivering the functional unit, is of primary concern. The background system is that which supplies energy and materials to the foreground system. The data is typically in the form of aggregated data sets in which individual plant and processes are not specified.

The data in the LCA study is organised and reported in terms of the *functional unit*, as defined in the "Goal Setting and Scoping" section of the study. The *functional unit* is used to describe the function of the product or process as well as a measure of its quantity. In a comparative study the *functional unit* is used to compare two or more products or processes on an equivalent basis. The basis of comparison is not necessarily a quantity of final material produced, but rather an equivalent use of the final product or service.

B.1.2 Scope of the Study

The elements of the LCA, to be included in the study, require scoping. The four main stages of the product or process life cycle are namely raw material acquisition, manufacturing, use/reuse/maintenance and recycle/waste disposal. To select stages for inclusion in the scope of the study, certain criteria must be assessed. These criteria include the goal of the study, the required accuracy of the results and the temporal and resource limitations. SAIC (2006) suggest that the product system is easier to define if the sequence of operations contributing to the product system is broken down into primary and secondary categories. The primary categories contribute directly to the making, using and disposing of the product. The secondary category includes auxiliary materials and processes that contribute to the primary activities. The boundaries for the systems within each of the categories can then be defined. It is important that limitations on a specific system be adequately described and justified. Certain questions can often be useful in describing the system boundaries within each category:

1. Does the analysis apply over the entire life cycle of the product?
2. In a comparative study, are additional materials or inputs required for one of the processes to achieve the same functional unit?
3. What is the basis of use for each product in the comparative study? Are they equivalent?

Logistical procedures requiring definition prior to inventory analysis of the LCA include:

i. Documenting Assumptions

All assumptions need to be documented throughout the study and reported with the final results. The assumptions are made throughout the study and an accurate record is important in keeping the final results in context of the scope and boundaries of the system.

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ii. Quality Assurance Procedure

Quality assurance is required to ensure that the specific goals of the project are met at the end of the study. This is often achieved by review of each phase of the study by interested parties, LCA practitioners and/or industry experts.

iii. Reporting Requirements

Required documentation and reports need to be defined as early as possible. This is critical in ensuring the results meet the expectations of the stakeholders involved and are consistent with the purpose of the study. The results should include the methodology used, a description of the systems and system boundaries, the basis for comparison and all assumptions made throughout the study.

B.1.3 Inventory Analysis

The life cycle inventory analysis (LCI) component of the LCA quantifies the inputs and outputs for each of the processing steps included in the defined system boundary. The phase is concerned with data collection and calculation procedures for quantifying the material and energy inputs and outputs for specific unit processes (Bauer & Maciel Filho, 2004). The key steps included in the life cycle inventory include:

1. Develop a flow diagram of the process being evaluated
2. Develop a data collection plan
3. Evaluate and document the LCI results

i. Develop a Flow Diagram

The goal definition and scoping stage defined the boundaries of the LCA. Within the defined boundaries the process unit processes can be detailed and systematically arranged using a flow diagram. The unit processes together form a complete representation of the life cycle inputs and outputs in terms of material and energy. The individual subsystems require inputs for material and energy; includes transportation of materials to and from the process; and has output of products, co-products, atmospheric emissions, waterborne wastes, solid wastes and potentially other materials (SAIC, 2006).

ii. Develop a Data Collection Plan

A number of data sources may be utilised depending on the specificity of the data required. The quality of the data required should be detailed in the goal definition and scoping phases of the study. Sources include measurements from industrial processes, industry data reports; data generated using chemical process simulation software; laboratory studies; journal publications, patents and case studies. The task of physical data collection involves a combination of research, site-visits, contact with experts, or the use of a commercially available software package. The method used is ultimately determined by the specificity of the data required.

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iv. Evaluate and Document the LCI Results

LCA studies are often stopped at the inventory stage and conclusions and recommendations are provided with regards to the ways in which inventory interventions can be minimised. This does not however consider the relative impact of the inventory components and is not recommended (Burgess & Brennan, 2001). The methodology used to generate the LCI results should be thoroughly described and reported, including specific assumptions and potential data gaps. The results within and across stages should be categorised, e.g. resource use, energy consumption and environmental releases. Data parameter groups within these categories should then the grouped, e.g. air emission, waterborne wastes and solid waste types. In addition geographical and temporal parameters should accompany the results if they are relevant to the study (SAIC, 2006).

B.1.4 Life Cycle Impact Assessment (LCIA)

The life cycle impact assessment (LCIA) phase of an LCA is the quantitative and qualitative evaluation of the potential human health and environmental impacts of the material and energy flows identified in the inventory analysis stages. Using defined impact categories such as global warming, ozone layer depletion and acidification, the LCIA converts the LCI data, using equivalency factors into these categories. These impact categories are referred to as midpoint categories and stop midway in the environmental cause-effect link. SETAC (1993) defines three distinct steps within the LCIA, namely classification, characterisation and valuation. This approach to impact assessment has been widely accepted (Burgess & Brennan, 2001).

i. Midpoint Classification

In the classification step, the resources used and waste materials generated are grouped into impact categories according to the potential environmental effects. Commonly used impact categories are shown in Table B.**1**. The impacts are described using categories such as abiotic depletion, global warming, acidification and photochemical oxidation. It is necessary to determine which impact categories are most relevant to the study based on the goal definition and scoping phase. Typically, potential impacts are grouped in three main categories, namely human health, ecological health and resource depletion (SAIC, 2006).

ii. Characterisation

The characterisation step quantifies the potential contribution to each impact category by considering the magnitude and the potency of the inventory category. The typical approach to characterisation in LCA is the use of an equivalency factor for each of the impact categories. The contribution of the individual inventory components is calculated as the amount equivalent compared to a reference material. Characterisation can put different quantities of chemicals on an equal scale to determine the amount of impact each compound has on the impact category. Characterisation thus provides a way to directly compare the LCI results within each impact category.

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iii. Normalisation, Grouping and Weighting

The normalisation, grouping and weighting step is used to weight the impact categories relative to one another. Normalisation is used to express midpoint impact indicator data to allow for direct comparison across impact categories. The indicator value is divided by a selected reference value, which may be a total resource value, a baseline resource value, or the highest value among all options (SAIC, 2006). The criteria for grouping and weighting (i.e. aggregated impact factors) may be established by means of panel discussion, either on a national, regional, or global level, with particular emphasis on the preferred environmental impact reductions (Burgess & Brennan, 2001). A number of weighting methodologies have been developed to improve the approach to valuation (SAIC, 2006).

Table B.1 Commonly used life cycle assessment impact categories (SAIC, 2006)

Impact Category	Scale	Examples of LCI Data (i.e. classification)	Common Possible Characterization Factor	Description of Characterization Factor
Global Warming	Global	Carbon Dioxide (CO_2) Nitrogen Dioxide (NO_2) Methane (CH_4) Chlorofluorocarbons (CFCs) Hydrochlorofluorocarbons (HCFCs) Methyl Bromide (CH_3Br)	Global Warming Potential	Converts LCI data to carbon dioxide (CO_2) equivalents Note: global warming potentials can be 50, 100, or 500 year potentials.
Ozone Layer Depletion	Global	Chlorofluorocarbons (CFCs) Hydrochlorofluorocarbons (HCFCs) Halons Methyl Bromide (CH_3Br)	Ozone Depleting Potential	Converts LCI data to trichlorofluoromethane (CFC^{-11}) equivalents.
Acidification	Regional Local	Sulfur Oxides (SOx) Nitrogen Oxides (NOx) Hydrochloric Acid (HCL) Hydroflouric Acid (HF) Ammonia (NH_4)	Acidification Potential	Converts LCI data to hydrogen (H^+) ion equivalents.
Eutrophication	Local	Phosphate (PO_4) Nitrogen Oxide (NO) Nitrogen Dioxide (NO_2) Nitrates Ammonia (NH_4)	Eutrophication Potential	Converts LCI data to phosphate (PO_4) equivalents.
Photochemical Smog	Local	Non-methane hydrocarbon (NMHC)	Photochemical Oxident Creation Potential	Converts LCI data to ethane (C_2H_6) equivalents.
Terrestrial Toxicity	Local	Toxic chemicals with a reported lethal concentration to rodents	LC_{50}	Converts LC_{50} data to equivalents; uses multi-media modeling, exposure pathways.
Aquatic Toxicity	Local	Toxic chemicals with a reported lethal concentration to fish	LC_{50}	Converts LC_{50} data to equivalents; uses multi-media modeling, exposure pathways.
Human Health	Global Regional Local	Total releases to air, water, and soil.	LC_{50}	Converts LC_{50} data to equivalents; uses multi-media modeling, exposure pathways.
Resource Depletion	Global Regional Local	Quantity of minerals used Quantity of fossil fuels used	Resource Depletion Potential	Converts LCI data to a ratio of quantity of resource used versus quantity of resource left in reserve.
Land Use	Global Regional Local	Quantity disposed of in a landfill or other land modifications	Land Availability	Converts mass of solid waste into volume using an estimated density.
Water Use	Regional Local	Water used or consumed Water Shortage Potential		Converts LCI data to a ratio of quantity of water used versus quantity of resource left in reserve.

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B.1.5 Life Cycle Interpretation

Life cycle interpretation is primarily focused at using the results from the impact assessment to draw conclusions and make recommendations with regards to impact mitigation. The interpretation phase also provides insight into how the results should be used for decision making in conjunction with other considerations such as socio-economic factors. The results of the impact assessment phase provide a basis for decision making. It is thus up to stakeholders involved to consider the relative trade-offs between environmental impacts and socio-economic factors and make decisions based on these trade-offs. It should be borne in mind that weighted factors need not be used in the interpretation phase. Mid-point indicators can be used to consider explicitly the process trade-offs.

B.2 Capital Costing Techniques

B.2.1 Total Capital Cost

B.2.1.1 Capital Cost Estimation

The capital cost is the total amount of funds required to purchase land, design, purchase and supply the equipment and manufacturing facilities, to bring the facility into operation (Couper, 2003). In capital cost estimation, different types of estimates can be made depending on the stage of process development. Variant cost estimation typically makes use of historical data while generative cost estimation is based on costs of constituent parts of process equipment. In early stages of process development, variant based cost estimation is usually applied. These are order-of-magnitude estimates and are determined by size adjustment, step counting, or factoring techniques. A limited number of commonly used techniques are discussed here. Discussion of further methods is provided by Peters & Timmerhaus (1991), Perry *et al.* (1997), Ullmann (2002), and Couper (2003)

i. Index Adjustments

Since most cost data used in preliminary equipment cost estimates is based on conditions in the past, it is necessary to update the data to the present. The data may be updated to present day value by means of a cost index. The present day value is found by multiplying the historical cost with the ratio of the present and past inflation index values.

$$Cost_2 = Cost_1 \left(\frac{Inflation\ index_2}{Inflation\ index_1} \right) \tag{B.1}$$

Ideally each cost item should be updated separately by weighted fractions of component indices, since different costs change at different rates. Various cost indexes that are used in chemical and biochemical engineering are regularly published e.g. *Marshall and Swift all-industry and process-industry equipment index* and *Chemical Engineering plant cost index*.

ii. Scale-Up factors

The cost of a piece of equipment or a plant can be determined by scaling the equipment or plant capacity from another similar piece of equipment or plant. Economy of scale dictates that the scaling is not linear as indicated in Equation B.2.

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$$Cost_2 = Cost_1 \left(\frac{Capacity_2}{Capacity_1} \right)^n \qquad \text{B.2}$$

The sixth-tenths rule is typically applied. Although the value of *n* is often taken as 0.6, specific exponents for different equipment and process types are also available (Peters & Timmerhaus, 1991; Perry *et al.*, 1997).

iii. Factoring

This method for estimating the total fixed capital investment requires the purchased equipment cost, including delivery. The remaining fixed capital items are then calculated as percentages of the delivered-equipment cost. The factors are derived from empirical data and the method is typically accurate to within 20-30% (Peters & Timmerhaus, 1991; Vogel, 2005). The majority of the uncertainty in the capital cost estimation is due the inherent uncertainty in the multiplier values. A more realistic estimate can be obtained by using a multiplier for individual units. In early process development however, average values are sufficient for the level of detail required (Vogel, 2005). A comprehensive list of factors for processes involving mammalian cell culture, microbial systems and enzymatic processes is presented by Biwer *et al.* (2006). The factors presented are mainly from literature (Peters & Timmerhaus, 1991) and estimates by the authors.

B.2.1.2 Direct Fixed Capital

The direct fixed capital cost represents all the capital necessary for the installed process equipment and auxiliary equipment needed for the process to be operational. This includes the purchasing and installation of major equipment, instruments and controls, piping, electrical systems, utility units and services and land and buildings etc.

i. Purchased Equipment

The cost of purchased equipment is the basis for many of the cost estimation methods used in early stages of process development. It is therefore imperative that accurate equipment costs are determined to ensure a reliable process cost estimate. Obtaining quotations from equipment suppliers is the most accurate method of determining process equipment costs. It is advised that this method be used if not too much effort on the part of supplier or design team is required. In preliminary development stages however, prices are generally estimated from purchases of similar pieces of equipment and updated using scaling factors and index adjustments. These estimates can be obtained from industry or from literature texts and journals. Peters & Timmerhaus (1991) and Atkinson & Mavituna (1991) are relatively good sources for this type of information.

ii. Additional Direct Costs

The remainder of the direct fixed costs are calculated as a percentage of the delivered-equipment cost. This is performed using empirical factors or multipliers. Equipment erection is one of the major costs and can often be up to 100% of the purchased equipment cost. Other major additional costs include process piping, instrumentation and control, land and buildings and auxiliary facilities. Land value cannot be depreciated in the financial statements and is usually included as a separate line item in capital estimation. The purchased equipment cost and the additional costs derived account for the total direct fixed capital.

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B.2.1.3 Indirect Fixed Capital

Indirect fixed capital costs include engineering and supervision, legal expenses, construction expenses, contractor's fees, contingencies and land and buildings. Supervision and construction costs are often determined by applying multiplication factors to the equipment costs or to the engineering hours. They can also be determined on the basis of construction and manpower schedules. The inclusion of contingencies takes into account that unexpected events are likely to occur during plant construction and also allows for in previously overlooked elements in early process development.

B.2.1.4 Working Capital

Working capital is used for process start-up and normal functioning of the business in the initial stages. Initially, cash flow is inconsistent and capital is needed for purchases of raw materials, payment of expenses and taxes and storage and distribution of finished goods. Working capital typically forms 10-20% of the fixed capital investment and can often be as much as 50% for processes producing seasonal products (Peters & Timmerhaus, 2003). The working capital requirement for large-scale bioprocesses is typically 15% of direct fixed capital cost (Turton *et al.*, 2003).

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B.2.2 Operating Expenses Estimation

The operating or manufacturing costs are incurred in the running of the plant, selling the products and recovering the capital investment. The operating costs are divided into fixed and variable manufacturing costs and overhead expenses.

B.2.2.1 Fixed Operating Costs

Fixed operating costs are incurred by the process whether it is operational or not. The costs include depreciation, local taxes, insurance and rent. Although several methods are used for determining the rate of depreciation, a straight line approach is usually applied. A *useful-life* period and a *salvage value* are assumed and the item's book value is reduced by the same amount every year, over the useful-life period. The equipment depreciation rate is usually dictated by local tax laws. The cost of insurance and taxes is determined from the total fixed capital investment. Insurance is typically 1-5% of fixed capital investment, while taxes vary depending on plant location.

B.2.2.2 Variable Operating Costs

i. Raw Materials

The cost of raw materials is determined from the process material balance and current material and chemical prices. Once the plant is operational, actual rates can be measured. In bioprocesses media components and the raw materials for recovery processes must be included in the costing. Raw materials for product recovery may include acids and bases, ion exchange resins and membranes. The most accurate source of material prices is from supplier quotations. Prices can also be obtained from historical industry data, published prices, or literature. A good source of published prices for preliminary estimates is available in journals such as *Chemical Marketing Reporter* (Peters & Timmerhaus, 1991; Blanch & Clark, 1996).

ii. Operating Labour

The labour required for the production process is the second largest contribution to the operating cost of the process (Perry *et al.,* 1997). The labour requirement is usually grouped into skilled and unskilled labour. In preliminary cost estimates the operating labour requirement can be determined from company industry experience, or from publication information on similar processes (Peters & Timmerhaus, 1991). It is however often difficult to estimate labour requirements for small scale bioprocesses, where correlations developed for large scale chemical processes are no longer valid. It is recommended that the simplest approach is to determine the labour requirements from the number of workers per shift for each major piece of equipment (Blanch & Clark, 1996). Labour costs can vary significantly depending on the location of the plant and should be determined from local statistical publications. Additionally, plant personnel associated with payroll, maintenance and administrative tasks are specified in plant overhead costs.

iii. Utilities

Utilities include the costs associated with the energy requirements for most unit operations. This includes power, heating and cooling requirements for distillation, aeration, agitation, centrifugation and waste treatment. The energy is provided mainly as electricity, steam and cooling water. The utility requirements are estimated from the plant material and energy balances. The cost of electricity supply depends heavily on geographical location and can vary significantly with local conditions and time. Although some costing guides are available, it is best to obtain prices from local suppliers. The prices of the utilities for study estimates are typically based on average price data quoted in literature sources for large-scale manufacturing industries in the United States and Europe (Peters *et al.*, 2003; U.S. DOE, 2009).

iv. Maintenance and Repair

The expenses involved in maintenance and repairs include cost of labour, materials and supervision. The costs for maintenance can be calculated as a percentage of the total capital investment or calculated for each piece of equipment. Annual repair costs are typically 3-6% of the invested capital. High repair costs are usually incurred shortly after plant start-up and after prolonged operation. It is suggested that 5-10% of the total capital investment should be allowed for the initial estimate (Vogel, 2005).

v. Other Operating Costs

Additional operating costs include waste treatment and disposal, royalty expenses and laboratory costs. Waste treatment and disposal is not usually included in the process model and a disposal price is typically allocated to individual waste streams. Depending on the nature of the process, these costs can vary significantly and should be investigated on a case by case basis.

B.2.2.3 Overhead Expenses

Overhead expenses include administrative costs, distribution and marketing and research and development. The various expenses vary considerably between different processes and products. A conservative estimate for administrative costs can be taken as 15-25% of operating labour (Peters & Timmerhaus, 1991). Marketing is mostly dependent on the type of

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product being produced, similar products on the market, plant location and company policies. Research and development costs are used to finance the development of new products and processes within the company. Marketing and research costs are not usually included in the cost analysis during process development.

B.2.3 Profitability Assessment

Profitability is considered a common denominator for all business operations. In order to make a decision on whether capital should be invested in a specific business or project, the profit generating potential needs to be determined. In comparing a number of different options, the profitability of each is compared in order to decide which shows potential for the best return on capital.

B.2.3.1 Product Revenue

The revenue is all the money generated by the business through the sale of products and co-products. It is calculated from the total sales volumes multiplied with the price per unit or quantity sold. The plant operating time is usually assumed to be 300-330 days per year (Peters & Timmerhaus, 1991). The price of the product can be determined by market conditions or by the cost to produce the product with a percentage mark-up.

B.2.3.2 Payback Period

The payback period (PBP) is the length of time necessary for the total return to equal the capital investment. The metric, shown in Equation B.3, is based on the fixed capital investment and annual cash flow.

$$PBP = \frac{\text{Fixed Capital}}{\text{Annual CashFlow}} \tag{B.3}$$

In order to determine an acceptable payback period it is necessary to calculate a maximum acceptable payback period. The payback period should be compared to the payback period obtained from the minimum acceptable rate of return as shown in Equation B.4.

$$PBP = \frac{0.85T}{m_{ar}T + 0.85T / N} \tag{B.4}$$

where T is the total capital investment, m_{ar} is the minimum acceptable rate of return, N is the number of years over which depreciation occurs.

B.2.3.3 Return on Investment

The return on investment (ROI) is a ratio of the profit to capital employed for a certain period and measures how effectively the business uses capital to generate profit. The ROI does not indicate how long the investment is held for and is usually stated as an annual rate of return. The ROI can be increased by minimising the production costs and maximising the revenue, assuming the process is running at capacity.

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i. Static Return on Investment

The simplest form of the return on investment calculation relates the annual profit to the capital invested, shown in Equation B.5. Although the static return on investment can be easily determined, it only takes a short-term view and does not account for future changes.

$$R = \frac{(U - H)}{I} \cdot 100 \qquad \text{B.5}$$

where R is the percentage return, U is annual revenue from sales, H is the annual production costs, U-H is the net annual profit, I is the capital invested.

ii. Dynamic Return on Investment

The dynamic return on investment, shown in Equation B.6, takes into account the time value of money and is regarded superior to the static method. Since money is not worth the same today as it is in the future the investment outlay and revenue earnings must be discounted to a fixed point in time to be comparable. The values are usually discounted to the start of production and discounted with an appropriate discount rate. The net present value (NPV) of the project thus takes into account all future annual net cash flow and discounts it to present value.

$$NPV = \sum_{t=0}^{n} \frac{(E - A)_t}{(1 + i)^t} \qquad \text{B.6}$$

where E is the expected earnings, A is the expected outlay cost, i is the discount rate, t is the number of years, n is the expected project lifetime.

A project is only considered profitable if its net present value is zero or positive. The internal rate of return (IRR) aims to find the interest rate which will yield the NPV equal to zero. The IRR is found by equating Equation 4.4 with zero and solving for the interest rate (i). Projects are compared using the calculated internal rate of return. The highest IRR indicates the most profitable project.

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APPENDIX C

Generic Flowsheet Extension

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Appendix C: Generic Flowsheet Extension

C.1 Overview

This appendix gives details of the development of the generic flowsheet application initially developed by Harding (2008). Chapter 5 details the critical shortcomings of the flowsheet pertaining to the user interface; and unit sizing and economic calculations which were not included in the flowsheet. These shortcomings were evaluated and addressed in the current work. Further, Chapter 5 provides an overview of the design architecture of the improved flowsheet model, describing the method by which the various aspects of process design and simulation (material and energy balances; unit sizing; economic calculations), included in the flowsheet, are integrated using and a simple, user-friendly, graphical interface.

The development work is completed using Microsoft® Visual Basic for Applications (VBA). The platform is used due to the relative ease of a first-time software developer to learn the coding language and the integrated development environment (IDE). Although VBA does have a number of limitations, discussed in Chapter 5, the platform allows the developer to manipulate features of the user interface and design custom dialog boxes and user forms.

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C.2 Graphical User Interface (GUI)

A graphical user interface of the generic flowsheet model is developed to provide a more user-friendly and intuitive means of integrating the features of the generic flowsheet model. The previous model developed by Harding (2008) did not provide a simple user interface and simply used a number MS Excel worksheets for data input and selection, calculation procedures and results output. The user-interface developed in this work provides a number of intuitive Microsoft® user form screens, commonly encountered in detailed simulation packages. The forms are used for selection and input for various process data and operating parameters. The user-interface allows the user to select from a number of possible design operations, including material and energy balance calculations, equipment costing calculations and process profitability assessment calculations. Once the user has selected a generic operation, a number of options are available for selecting from a number of more specific categories. These include options for the process type, specific unit operations, or economic calculation categories. Within each category selection the user has the ability to select from a number of procedure or data input options. Once the necessary procedures have been selected and the process data has been inputted the results can be generated.

The front-end screen, shown in Figure C.1, integrates the features of the software application. The user can select the process feature by clicking on one the blocks on the right-hand side of the form. The features available for selection include material and energy calculations (developed by Harding (2008)); major equipment design and costing; plant costing and economic evaluation; and process summary. The interface is designed as a top down application and features are selected from top to bottom when initially specifying the process data. The design features can however be selected at any stage of the process design. Since the model was developed for the Centre for Bioprocess Engineering Research (CeBER) within the University of Cape Town, the screen provides certain copyright information and the logo of the research centre.

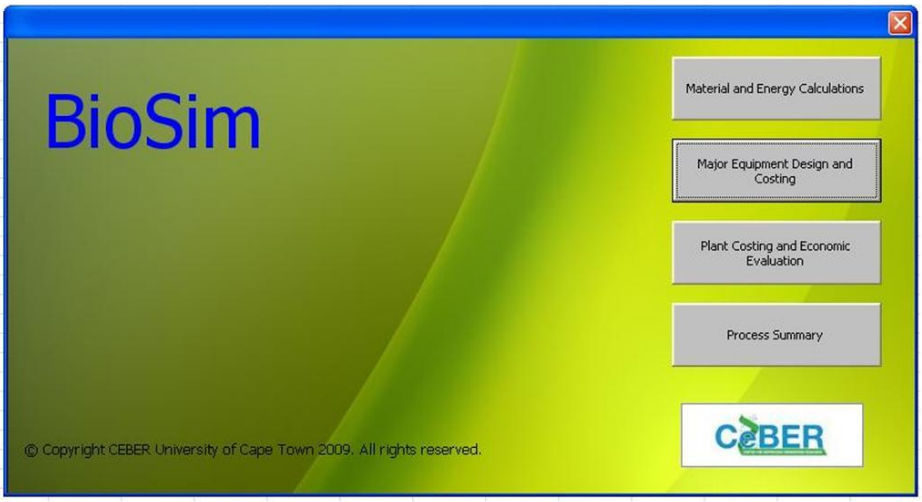

Figure C.1 Front-end user-interface for the generic flowsheet

C.3 Major Equipment Design

C.3.1 Equipment Selection

A number of unit operations typical to bioprocesses are available for selection, shown in Figure C.2. The user has the option of using predefined units („Available Units') or specifying a new unit by double-clicking on the icon representing the unit operation, which is then added to the „Available Units' list. Once the unit has been selected the unit is given a unique unit code that is used to link specific unit data in a database to the unit in the „Selected Units' list. General considerations for the individual unit operations are provided in Appendix A. Details of the design equations and input data for the unit operations used in this thesis are provided below.

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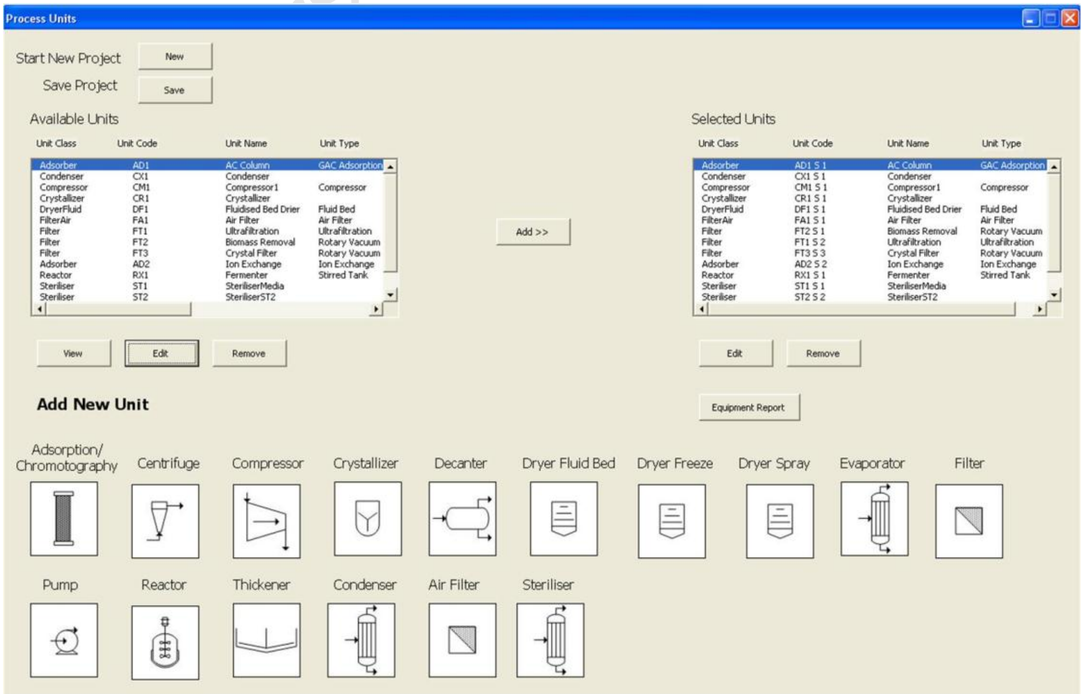

Figure C.2 Major equipment selection user form for the generic flowsheet

C.3.2 Procedure Specification and Equipment Sizing

Once a specific unit has been selected from the „Available Units' list and added to the „Selected Units' list, the unit data can be specified. The procedure tab is automatically specified with material and energy balance data (unit throughput, residence time) obtained from the generic flowsheet, as developed by Harding (2008). Batch scheduling is calculated from this data. The cycle time includes normal operating time (residence time) and the time for cleaning in place (CIP) and turnaround time of the unit. Values for CIP and turnaround are taken from SuperPro® Designer. The number of batches that the process is able to complete is a function of operating time, total batch time and the bottleneck time, shown Equation C.1. The operating time is the amount of time available per year for the process to operate. The total batch time of the process is the combined the batch time of the individual units. The „bottleneck' of the process is calculated as the unit with longest cycle time.

$$\text{BatchNumber} = \frac{\text{OperatingTime} - \text{BatchTime}}{\text{Bottleneck}} \qquad \text{C.1}$$

The equipment specification and sizing is based on the throughput data on the procedure tab. The unit name is specified and type of unit selected. These are merely to provide details in the report as to the description of the unit. Default data provided for units is specific to the unit operation icon on the equipment selection form and not the unit type. There is scope for specifying specific default data for different unit types. Although default data is provided for each unit operation, the user has the option of updating this data. Details of specific unit operations and default data used in the case study for citric acid production are provided below.

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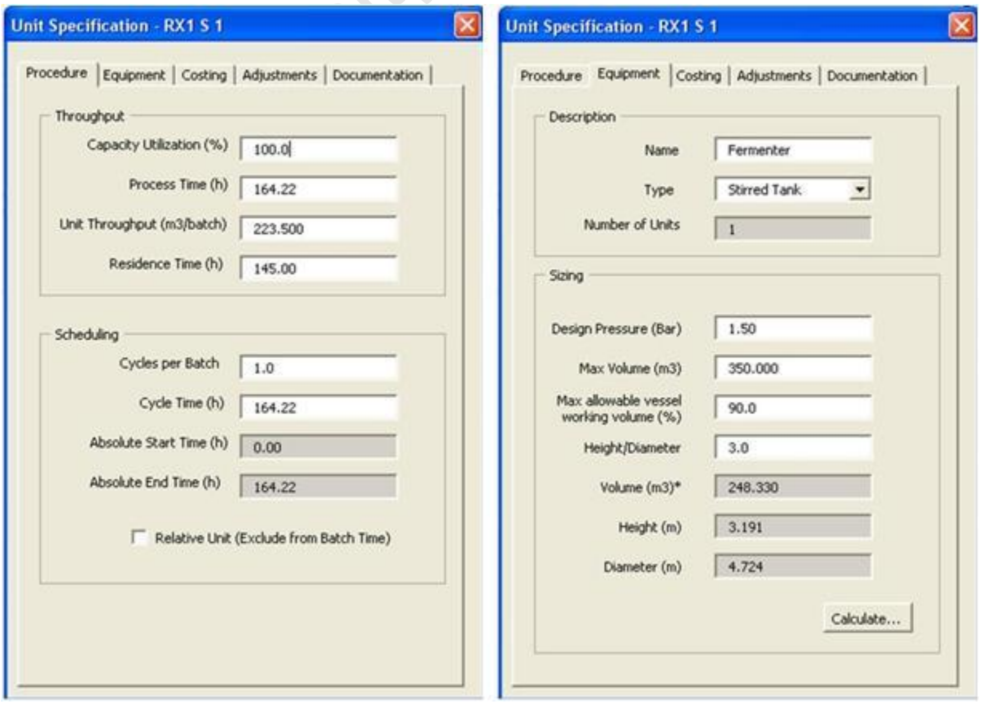

Figure C.3 Equipment specification and design user form for the generic flowsheet

C.3.2.1 Adsorption Column

The adsorption column sizing model estimates the time for loading (reaching breakthrough) and the number and dimensions of the column/s. The adsorption column model is used for activated carbon adsorption and ion-exchange. The breaktime and empty bed contact time values are based on typical values (Snoeying, 1990).

Table C.1 Design variables for adsorption column sizing

	Variable	Default Value	Units
Inputs from material and energy balance			
Unit throughput	UnitFlow	-	m^3/batch
Residence time	ResidenceTime	-	Hours
User inputs			
Unit overdesign factor	UnitOD	10	%
Max bed diameter	Dmax	80	m^3
Bed height/diameter ratio	BedHD	0.66	-
Bed/Column height ratio	BedColumn	0.5	-
Break time	Break Time	21	min
Empty bed contact time	EBCT	10	min
Calculated variables			
Number of units	UnitNumber	1	-
Bed diameter	BedDiameter	-	m
Column height	ColHeight	-	m
Bed Volume	BedVolume	-	m^3
Column volume*	ColVolume	-	m^3

*Costing variable

The volume of the adsorption bed (BedVolume), diameter of the bed (BedDiameter), height of the column (BedHeight) and volume of the column (ColVolume) is calculated using Equation C.2-C.5. The maximum bed diameter (Dmax) is used to constrain the bed diameter calculation and determine the number of units. The residence time (ResidenceTime) and unit throughput are used to calculate unit volume for continuous processes.

$$\text{BedVolume} = \frac{\text{UnitFlow} \times \text{EBCT} \times (1 \times \text{UnitOD})}{\text{BreakTime}} \qquad \text{C.2}$$

$$\text{BedDiameter} = \left(\frac{4 \times \text{BedVolume}}{\text{BedHD} \times \pi \times \text{UnitNumber}} \right)^{\frac{1}{3}} \qquad \text{C.3}$$

$$\text{BedHeight} = \text{BedHD} \times \text{BedDiameter} \qquad \text{C.4}$$

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$$\text{ColVolume} = \frac{\text{BedVolume}}{\text{BedColumn}} \quad \text{C.5}$$

C.3.2.2 Compressor

The compressor model determines the number of compressors and the individual compressor rating based on throughput and power requirement of the unit.

Table C.2 Design variables for compressor sizing and rating

	Variable	Default Value	Units
Inputs from material and energy balance			
Unit throughput	UnitFlow	-	m^3/batch
Process time	UnitTime	-	Hours
Power	Power	-	kW
User inputs			
Compression time	UnitTime	UnitTime	Hours
Max throughput for the unit	Vmax	200	m^3/s
Max power for the unit	Pmax	3000	kW
Compression efficiency of the unit	UnitEff	75	%
Calculated variables			
Number of units	UnitNumber	1	-
Individual unit rating*	UnitRating	-	kW

*Costing variable

The number of compressors (UnitNumber) and individual compressor rating (UnitRating, UnitFlowRating) is calculated using Equations C.6-C.7. The maximum volumetric throughput and maximum power rating for individual units is used to determine the number of units required.

$$\text{UnitRating} = \frac{\text{Power}}{\text{UnitTime} \times \text{UnitNumber} \times \text{UnitEff}} \quad \text{C.6}$$

$$\text{UnitFlowRating} = \frac{\text{UnitFlow}}{\text{UnitTime} \times \text{UnitNumber}} \quad \text{C.7}$$

C.3.2.3 Filter

The filter sizing model is used to size various liquid filters. The individual filter area (UnitArea) is calculated using the total filter area (TotalArea). The total filter area required is obtained from the material balance calculations (Harding, 2008). Default input values for maximum filter area and filter efficiency are dependent on the type of filter selected. Filter options include rotary vacuum filtration, ultrafiltration, micro-filtration and reverse osmosis.

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Table C.3 Design variables for filter sizing

	Variable	Default Value	Units
Inputs from material and energy balance			
Unit throughput	UnitFlow	-	m^3/batch
Process time	UnitTime	-	Hours
Total filter media area	TotalArea	-	m^2
User inputs			
Max filter area of idividual unit	Amax	80	m^2
Filtration efficiency of the unit	UnitEff	75	%
Calculated variables			
Number of units	UnitNumber	1	-
Individual unit area*	UnitArea	-	m^2

*Costing variable

The number of filters (UnitNumber) and individual filters area (UnitArea) is calculated using Equation C.8. The maximum filter area of an individual unit (Amax) is used to constrain the unit area calculation and determine the number of individual units required (UnitNumber).

$$\text{UnitArea} = \frac{\text{TotalArea}}{\text{UnitNumber} \times \text{UnitEff}} \tag{C.8}$$

C.3.2.4 Fluid-Bed Drier

The fluid bed drying model is used to determine the size of the drying unit and the rate of drying. The model uses the average particle residence time (ResidenceTime) and average solids velocity (SolidsVel) to estimate the required height. The average particle residence time is typically 30 to 120 seconds (McCabe *et al.,* 1993).

Table C.4 Design variables for fluid bed drier sizing

	Variable	Default Value	Units
Inputs from material and energy balance			
Unit throughput	UnitFlow	-	m^3/batch
Residence time	ResidenceTime	-	Hours
User inputs			
Unit overdesign factor	UnitOD	10	%
Max diameter of the unit	Dmax	3	m
Height to diameter ratio	HeightDiam	10	
Average particle residence time	ResidenceTime	30	
Average solids velocity	SolidsVel	1.5	m/s
Evaporation rate	EvapRate	100	kg/h/m^3
Calculated variables			
Number of units	UnitNumber	1	-
Unit volume*	UnitVolume	-	m^3
Unit drying capacity	Capacity	-	kg/h

*Costing variable

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The number of units (UnitNumber), volume of an individual unit (UnitVolume) and the drying capacity of the unit (Capacity) is calculated using Equations C.9-C.12. The height of the drying column is calculated using the average solids velocity and the average particle residence time, shown in Equation C.9. The default value for the average particle residence time is obtained from the material balance calculations (Harding, 2008). The value may be adjusted by the user. The maximum diameter of the unit (Dmax) is used to constrain Equations C.10 and C.11 to determine the number of units required.

$$\text{UnitHeight} = \text{SolidsVel} \times \text{ResidenceTime} \tag{C.9}$$

$$\text{UnitDiameter} = \frac{\text{UnitHeight}}{\text{HeightDiam}} \tag{C.10}$$

$$\text{UnitVolume} = \frac{1}{4}\text{UnitHeight} \times \pi \times \text{UnitDiameter}^2 \tag{C.11}$$

$$\text{Capacity} = \text{EvapRate} \times \text{UnitThroughput} \tag{C.12}$$

C.3.2.5 Heat Exchanger

The heat exchanger model is used for condenser and evaporator sizing. The total heat transfer (Q), stream flows and temperatures are obtained from the material and energy balance calculations (Harding, 2008). The area required for heat exchange is calculated using the stream flow data (energy, flowrate, temperature) and the overall heat transfer coefficient (U).

Table C.5 Design variables for heat exchanger sizing

	Variable	Default Value	Units
Inputs from material and energy balance			
Unit throughput	UnitFlow	-	m^3/batch
Process time	UnitTime	-	Hours
Total heat trasnfer	TotalHeat	-	kW
Cool stream inlet temperature	Tcin		°C
Cool stream outlet temperature	Tcout		°C
Hot stream inlet temperature	Thin		°C
Hot stream outlet temperature	Thout		°C
User inputs			
Heat transfer coefficient	U	10080.6	kJ/m^2.h.°C
Max area of idividual unit	Amax	100	m^2
Heat transfer efficiency of the unit	UnitEff	90	%
Calculated variables			
Number of units	UnitNumber	1	-
Individual unit area*	UnitArea	-	m^2

*Costing variable

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The total heat transfer (Q), log mean temperature difference (dT_{LM}) and unit area (UnitArea) is calculated using Equations C.13- C.15. The maximum area per unit (Amax) is used to constrain Equation C.15 to determine the number of heat exchange units required.

$$Q = \frac{\text{TotalHeat}}{\text{UnitEff}} \qquad \text{C.13}$$

$$dT_{LM} = \frac{(Th_{in} - Tc_{out}) - (Th_{out} - Tc_{in})}{\text{Log}\left(\dfrac{Th_{in} - Tc_{out}}{Th_{out} - Tc_{in}}\right)} \qquad \text{C.14}$$

$$\text{UnitArea} = \frac{Q}{U \times dT_{LM} \times \text{UnitNumber}} \qquad \text{C.15}$$

C.3.2.6 Reactor

The volume and dimensions of the reactor are calculated using user inputs and values from the material balance simulation. Default values are given for user input values and the number of units. The reactor model is used to size bioreactor and crystalliser units.

Table C.6 Design variables for reactor and crystalliser sizing

	Variable	Default Value	Units
Inputs from material and energy balance			
Unit throughput	UnitFlow	-	m^3/batch
Residence time	ResidenceTime	-	Hours
User inputs			
Max volume of the reactor unit	Vmax	80	m^3
Unit utilisation	UnitUtil	90	%
Height/Diameter ratio	HeightDiam	3	-
Design pressure	UnitPressure	1.5	Bar
Calculated variables			
Number of units	UnitNumber	1	-
Individual unit volume*	UnitVolume	-	m^3
Diameter	UnitDiam	-	m
Height	UnitHeight	-	m

*Costing variable

The number of reactor units (UnitNumber), individual reactor volume (UnitVolume), reactor height (UnitHeight) and reactor diameter (UnitDiam) are calculated using Equations C.16- C.18. The maximum volume of the reactor (Vmax) is used to constrain the unit volume calculation in equation C.16 and determine the number of units. The residence time (ResidenceTime) and unit throughput (UnitFlow) are used to calculate unit volume for continuous processes.

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$$\text{UnitVolume} = \frac{\text{UnitFlow}}{\text{UnitNumber} \times \text{UnitUtil}} \quad \text{C.16}$$

$$\text{UnitVolume} = \left(\frac{4 \times \text{UnitVolume}}{\text{HeightDiam} \times \pi} \right)^{\frac{1}{3}} \quad \text{C.17}$$

$$\text{UnitHeight} = \text{HeightDiam} \times \text{UnitDiam} \quad \text{C.18}$$

C.3.2.7 Steriliser

The steriliser sizing model uses the batch throughput and sterilisation time from the material and energy balances to calculate the number of units and the rated throughput of individual units.

Table C.7 Design variables for sterilizer sizing

	Variable	Default Value	Units
Inputs from material and energy balance			
Unit throughput	UnitFlow	-	m^3/batch
Residence time	UnitTime	-	Hours
User inputs			
Max throughput	MaxThrough	100	m^3/h
Heat transfer efficiency of the unit	UnitEff	90	%
Calculated variables			
Number of units	UnitNumber	1	-
Rated throughput*	UnitRating	-	m^3/h

*Costing variable

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The total flowrate (unitflow) and rated throughput (UnitRating) are calculated using Equations C.19-C.20. The maximum rated throughput (MaxThrough) is used to constrain the unit rating calculation to determine the number of individual units.

$$\text{UnitFlow} = \frac{\text{UnitThrough}}{\text{UnitTime}} \quad \text{C.19}$$

$$\text{UnitRating} = \frac{\text{UnitFlow}}{\text{UnitNumber} \times \text{UnitEff}} \quad \text{C.20}$$

C.4 Major Equipment Purchase Cost (PCE)

C.4.1 Cost Calculation Selection

The purchase cost of major equipment is based on the results of the unit sizing calculations and the cost variables of individual units. Default values are provided for total cost of most units. Default unit costs are based on estimates from Perry *et al.* (1999) and Peters *et al.* (2003) with the corresponding base capacity, scaling factor and reference year. The unit cost is calculated for a cost variable specific to the unit operation, shown in the Table C.1 to Table C.7 above. The base capacity is used as the cost variable for calculating the cost of the equipment.

The simulation allows the user to select from three costing options shown Figure C.4 and in Table C.8. In option 1, the user inputs the cost of the selected equipment and specifies a corresponding reference year. In option 2, the user selects a built-in estimation for the unit cost, based on similar unit costs provided in the database. Option 3 allows the user to specify the cost and capacity of a similar unit and the cost of the current unit is calculated.

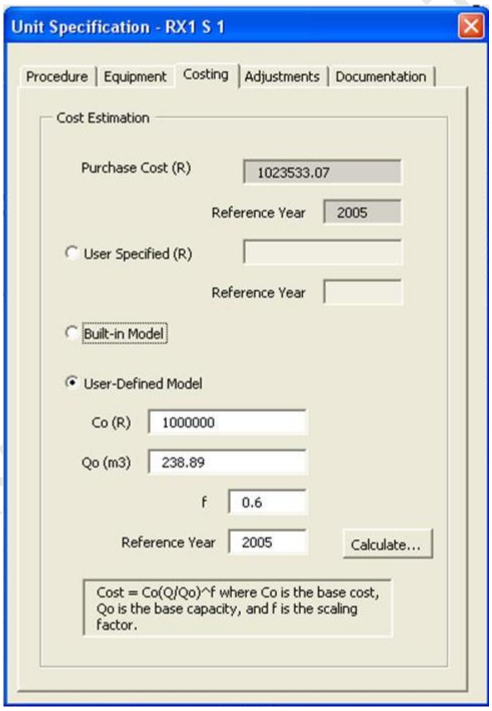

Figure C.4 Equipment cost specification user form for the generic flowsheet

The unit cost calculation in option 3 is shown by Equation C.21, where the base cost is the cost of a similar unit, the base capacity is the size of the similar unit and the scaling factor is used to scale the unit size. Improved scaling factor estimates can be entered by the user for specific unit types (Peters *et al.*, 2003).

$$\text{Cost} = \text{Base Cost} \times \left(\frac{\text{Cost variable}}{\text{Base capacity}} \right)^{\text{ScalingFactor}} \qquad \text{C.21}$$

All unit cost values are adjusted to the year of the study using Equation C.22 and the Chemical Engineering Plant Cost Index (CEPCI). Default CEPCI values are stored in the model and used to adjust the reference year cost to the current study year.

$$\text{Cost}(\text{year of study}) = \text{Cost}(\text{reference year}) \times \text{Inflation Factor} \qquad \text{C.22}$$

Table C.8 Purchase equipment cost calculation

Option 1: User defined cost	Default Value
Inputs from unit sizing	
Costing variable	-
User inputs	
Unit Cost	-
Reference Year	year of study
Calculated variables	
Unit cost (Year of study)	-
Option 2: Built-in model	**Default Value**
Inputs from unit sizing	
Costing variable	-
Unit Cost	Database value
Reference Year	Database value
Calculated variables	
Unit cost (Year of study)	-
Option 3: User defined model	**Default Value**
Inputs from unit sizing	
Costing variable	-
User inputs	
Base cost	-
Base capacity	-
Scaling factor	0.6
Reference Year	year of study
Calculated variables	
Unit cost (Year of study)	-

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C.4.2 Equipment Cost Adjustment

The capital cost of each equipment unit is adjusted according to the number of stand-by units, staggered units, material of construction and installation factor. The equipment cost is multiplied by the total number of standby and staggered units selected in the stagger mode adjustment. In selecting the material of construction and capital cost factor, the purchase cost is adjusted by a factor, higher or lower than 1, multiplied with the equipment cost. The material of construction and capital factor can be updated by a user input. The simulation model has built-in factors for various types of materials, sourced from SuperPro® Designer. The user is able to input a cost adjustment factor that is deemed more accurate.

The labour demand for each unit is specified as the number of hours of direct labour required per hour the unit is in operation. The total labour demand is based on the cycle time of the individual units, the number of cycles for each unit per batch and the number of batches per year. The specified labour demand (hrs/hr) is the combined number of hours required by all the operators for the specific unit operation.

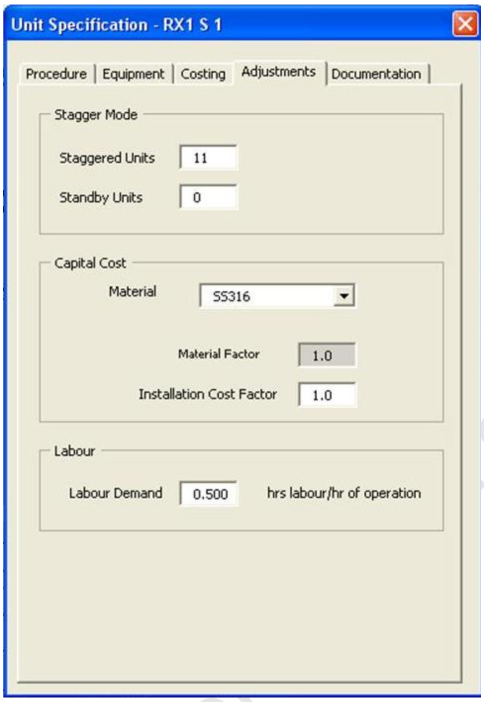

Figure C.5 Equipment cost adjustment user form for the generic flowsheet

Table C.9 Equipment cost adjustment factors

Adjustment	Default Value
Stagger Mode	
Standby Units	1
Staggered Units	0
Material of Construction Factor	
Aluminium	1
Cast Iron	1
CS	0.7
Glass-Lined	1.3
Hastelloy	3
Inconel 600	1
Monel 400	1
Nickel 200	1
Plastic	1
SS304	0.9
SS316	1
Titanium	7
Capital Cost Factor	
Installation Factor	1

C.5 Capital Expenditure

C.5.1 PDC, PIC, PSC

The direct fixed costs are calculated as a percentage of the purchased equipment cost (PCE). The capital expense tab, shown in Figure C.6, allows the user to select multiplier values for microbial, mammalian or chemical/enzymatic process options. The PCE value is multiplied with empirical factors, shown in Table C.10, to calculate the direct plant cost (PDC), plant indirect cost (PIC), total plant cost (TPC), contractors fees and contingency (CFC), direct fixed capital cost (DFC) and plant start-up cost (PSC). The working capital requirement may be calculated as a percentage of DFC or based on the number of days required for operating expenses.

The user has the option to enter multipliers that override default values by entering values that are deemed more accurate or specific to the process under consideration. The multiplier values for mammalian and microbial processes are taken from Biwer *et al.* (2006), while the values for enzymatic processes are taken from Peters *et al.* (2003).

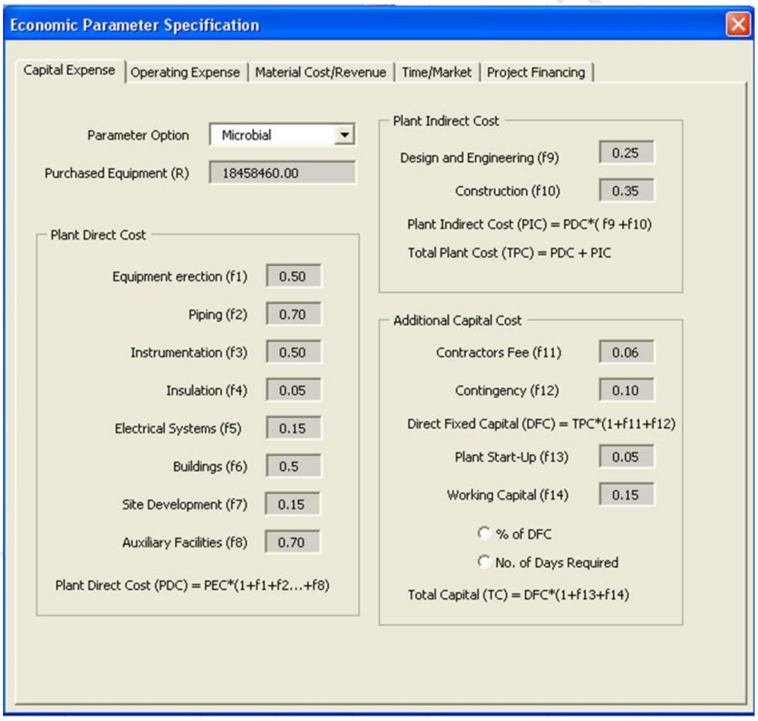

Figure C.6 Capital expenditure user form for the generic flowsheet

Table C.10 Economic multipliers for different process types

Plant direct cost, PDC		**Mammalian**[a]	**Microbial**[a]	**Enzymatic**[b]
f1	Equipment erection	0.60	0.50	0.47
f2	Piping	0.75	0.7	0.68
f3	Instrumentation	0.8	0.5	0.26
f4	Insulation	0.05	0.05	0.085
f5	Electrical systems	0.2	0.2	0.11
f6	Buildings	2.5	0.5	0.18
f7	Site development	0.2	0.2	0.10
f8	Auxiliary facilities	0.8	0.7	0.55
F1	Overall Factor, TPDC=PCE(*f* 1 +… *f8*)	**5.8**	**3.3**	**2.4**
Plant indirect cost (PIC)				
f9	Design and engineering	0.25	0.25	0.3
f10	Construction	0.35	0.35	0.35
F2	Overall Factor, TPIC=TPDC(*f9* + *f10*)	**0.6**	**0.6**	**0.65**
Total plant cost, TPC = TPDC + TPIC				
f11	Contractors fee	0.06	0.06	0.06
f12	Contingency	0.1	0.1	0.1
F3	Overall Factor, TPC(*f11* + *f12*)	**0.16**	**0.16**	**0.16**
Direct fixed capital cost, DFC = TPC(1 + f11 + f12)				
Plant start-up cost (PSC)				
f13	Start-up and validation	0.05	0.05	0.05
f14	Working capital (Percentage of DFC)	0.15	0.15	0.15
	no. of working days	30	30	
F5	Overall Factor, DFC(*f14* + *f15*)	**0.2**	**0.2**	**0.2**

[a] Biwer *et al.* (2006)

[b] Peters *et al.* (2003)

C.6 Operating Costs

Operating costs, shown in Table C.11, are calculated using the results of the process material and energy balance calculations, unit operating times, direct fixed capital (DFC) and purchased equipment costs (PCE). The material balance calculations are used as the basis for the raw material cost calculations, with input variables shown in Table C.12. The user is required to specify per hour rate for labour and the cost value per unit flow of individual material streams, shown in Figure C.7 and Figure C.8 respectively Default energy and utility costs are given in the simulation, based on data from Biwer *et al.* (2006) for steam and natural gas and internal estimates for electricity, cooling water and chilled water. The costs are updated to the year of study by a user defined inflation rate. The user has the option of specifying a per unit energy and utility cost. Insurance, plant tax, maintenance and laboratory costs are calculated as a percentage of direct fixed capital. Depreciation is calculated as a percentage of purchased equipment cost. Straight line depreciation is used for all depreciation calculations assuming a salvage value as percentage of direct fixed capital.

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Table C.11 Basis for operating cost calculations

Operating Cost	Calculation Basis
Raw Materials	Material & energy balance
Labour	Unit operating time
Consumables	Material & energy balance
Waste Treatment	Material & energy balance
Utilities	Material & energy balance
Insurance	% of direct fixed capital (DFC)
Plant Tax	% of direct fixed capital (DFC)
Maintenance	% of direct fixed capital (DFC)
Laboratory	% of direct fixed capital (DFC)
Depreciation	Purchased equipment cost (PCE)

Table C.12 Input variables operating cost calculations

Raw Materials Cost	*Inputs from material and energy balance*
	Consumption (kg/kg product)
	User inputs
	Unit Cost ($/kg)
	Calculated variables
	Demand (kg/year)
	Cost ($/year)
Labour Cost	*Inputs from equipment specification*
	Unit Code
	Demand (hrs/hr)
	Description
	User inputs
	Labour Rate ($/hr)
	Calculated variables
	Demand (hrs/year)
	Cost ($/year)
Consumables Cost	*Inputs from material and energy balance*
	Consumption (kg/kg product)
	User inputs
	Unit Cost ($/kg)
	Calculated variables
	Demand (kg/year)
	Cost ($/year)
Waste Treatment Cost	*Inputs from material and energy balance*
	Disposal (kg/kg product)
	User inputs
	Disposal Cost ($/kg)
	Calculated variables
	Disposal (kg/year)
	Cost ($/year)
Utilities Cost	*Inputs from material and energy balance*
	Consumption (kg/kg product)
	Costing Units
	User inputs
	Unit Cost ($/kg)
	Calculated variables
	Demand (kg/year)
	Cost ($/year)

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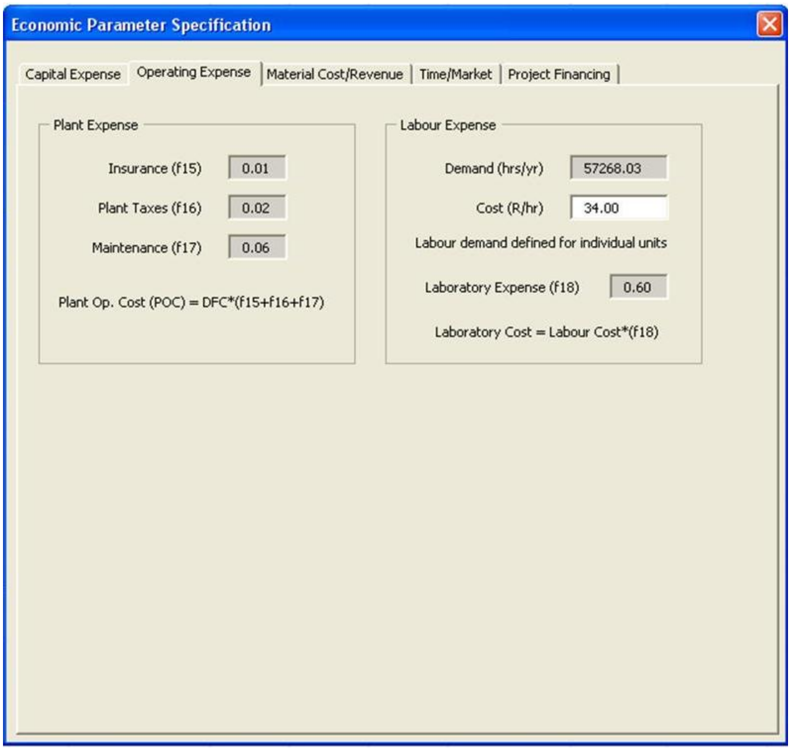

Figure C.7 Operating expenditure user form for the generic flowsheet

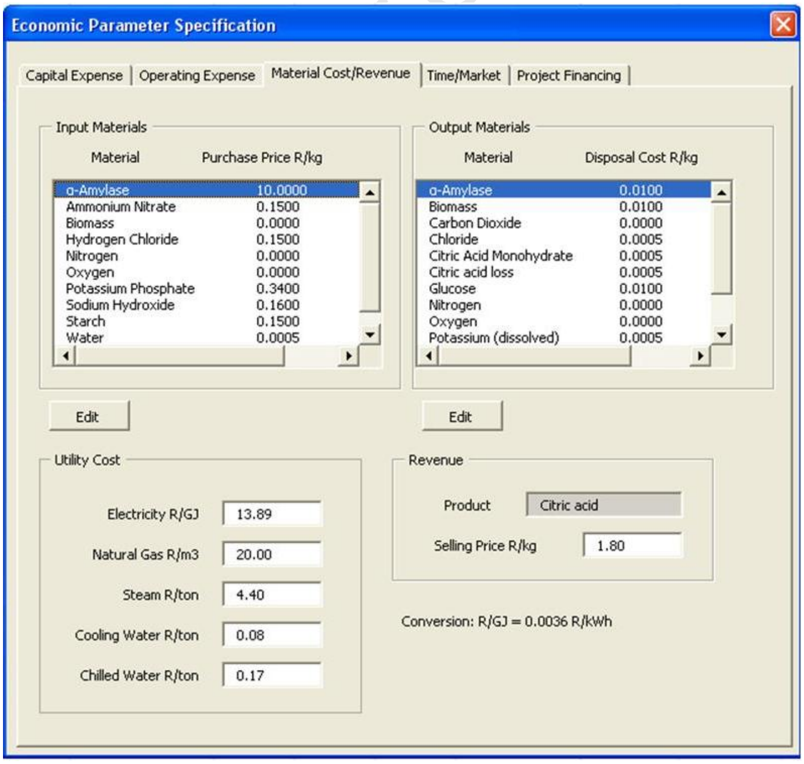

Figure C.8 Material cost/revenue user form for the generic flowsheet

C.7 Profitability assessment

The economic inputs used in the profitability assessment are shown in Table C.13. Default values are given for most input values, based on typical values (Peters *et al.*, 2003). Default values may be updated by the user. The calculation outputs for the profitability assessment are shown in Table C.14.

Table C.13 Profitability assessment inputs

Inputs	Default Value	Units
Selling Price	-	$/kg
Year of Analysis	2008	-
Year Construction Starts	2008	-
Construction Period	30	Months
Start-Up Period	4	Months
Project Lifetime	15	Yrs
Income Tax Rate	26	%
Inflation Rate	5	%
Risk-free Discount	6	%
Exchange Rate	-	-
Depreciation Period	10	Yrs
Salvage Value	5	%
Operating Capacity Y1	100	%
Operating Capacity Y2	100	%
Operating Capacity Y3	100	%
Operating Capacity Y4	100	%
Operating Capacity Y5	100	%
DFC Debt%	40	%
DFC Loan Period	10	Years
DFC Interest Rate	9	%
WCDebt%	100	%
WC Loan Period	6	Years
WC Interest Rate	13	%
Capital Expenditure Y1	30	%
Capital Expenditure Y2	40	%
Capital Expenditure Y3	30	%
Capital Expenditure Y4	0	%
Capital Expenditure Y5	0	%

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Table C.14 Profitability assessment outputs

Output	Units
Revenue	$
Operating Cost	$
Gross Profit	$
Taxes	$
Net Profit	$
Gross Margin	%
Return on Investment	%
Payback Period	Years
NPV	$
IRR	%

The revenue, gross profit and net profit calculations, shown in Equations C.23 -C.25, are based on the operating costs, product output, selling price of the product and tax rate. The user is required to specify the unit selling price of the product.

$$\text{Revenue} = \text{Output}(\text{kg}/\text{year}) \times \text{Selling Price}(\$/\text{kg}) \qquad \text{C.23}$$

$$\text{Gross Profit} = \text{Revenue} - \text{Operating Cost} \qquad \text{C.24}$$

$$\text{Net Profit} = \text{Gross Profit} \times (1 - \text{Tax Rate}) \qquad \text{C.25}$$

The gross margin and return on investment calculations, shown in Equation C.26 and C.27, are based on gross profit, revenue, net profit and the total capital investment. The total capital investment includes direct fixed capital (DFC), startup cost and working capital.

$$\text{Gross Margin}(\%) = \frac{\text{Gross Profit}}{\text{Revenue}} \times 100 \qquad \text{C.26}$$

$$\text{ROI}(\%) = \frac{\text{Net Profit}}{\text{Total Capital Invested}} \times 100 \qquad \text{C.27}$$

The payback period (PBP), net present value (NPV) and internal rate of return (IRR) are calculated using the annual cash flows, project duration (years) and a discount rate. The annual cash flow calculation is shown in Equation C.28. The payback period is calculated from plant start-up to a cumulative cash flow of zero. The NPV, shown in Equation C.29, is calculated on the basis of the project life-time and cumulative cash flows. The process internal rate of return is based on a cumulative cash flow of zero for the project duration.

$$\text{Cash Flow} = -\text{Total Capital} + \text{Financing} + \text{Net Profit} + \text{Depreciation} \qquad \text{C.28}$$

$$\text{NPV} = \sum_{t=0}^{\text{project duration}} \frac{\text{Cash Flow}_t}{(1 + \text{discount rate})^t} \qquad \text{C.29}$$

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APPENDIX D

Environmental Assessment

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Appendix D: Environmental Assessment

D.1 Life cycle assessment contributions

Table D.1 Life cycle process contributions to abiotic depletion (kg Sb eq)

Citric Acid_Starch, at plant U incl amylase	Unit	Citric Acid_Starch, at plant U incl amylase	Citric Acid_Molasses, at plant U	Citric Acid_Molasses, at plant U
Total of all processes	kg Sb eq	0.045898215	Total of all processes	0.041371623
Remaining processes	kg Sb eq	0.005226053	Remaining processes	0.009494605
Natural gas, at production onshore/RU U	kg Sb eq	0.009251887	Natural gas, at production onshore/RU U	0.005569068
Natural gas, at production offshore/NO U	kg Sb eq	0.004228616	Lignite, at mine/RER U	0.00458272
Natural gas, at production onshore/DZ U	kg Sb eq	0.004180983	Heat (coal)	0.002988007
Lignite, at mine/RER U	kg Sb eq	0.004167648	Natural gas, at production onshore/DZ U	0.002604568
Natural gas, at production onshore/NL U	kg Sb eq	0.004065187	Natural gas, at production offshore/NO U	0.002564782
Crude oil, at production onshore/RME U	kg Sb eq	0.002121534	Natural gas, at production onshore/NL U	0.002504744
Hard coal, at mine/WEU U	kg Sb eq	0.001963303	Heat (oil)	0.00235912
Crude oil, at production offshore/NO U	kg Sb eq	0.001747469	Electricity (natural gas)	0.001925309
Natural gas, at production offshore/NL U	kg Sb eq	0.001690688	Hard coal, at mine/WEU U	0.001871549
Electricity (natural gas)	kg Sb eq	0.001504032	Hard coal, at mine/EEU U	0.001743202
Crude oil, at production onshore/RU U	kg Sb eq	0.001457527	Crude oil, at production onshore/RME U	0.001583342
Crude oil, at production offshore/GB U	kg Sb eq	0.001456386	Crude oil, at production onshore/RAF U	0.001580607
Hard coal, at mine/EEU U	kg Sb eq	0.001424613		
Natural gas, at production onshore/DE U	kg Sb eq	0.001412291		

Table D.2 Life cycle process contributions to global warning (kg CO2 eq)

Process	Unit	Citric Acid_Starch, at plant U incl amylase	Citric Acid_Molasses, at plant U	Citric Acid_Molasses, at plant U
Total of all processes	kg CO2 eq	4.84642024	Total of all processes	3.608497584
Remaining processes	kg CO2 eq	1.714425367	Remaining processes	1.963526957
Natural gas, burned in industrial furnace >1(	kg CO2 eq	2.309829513	Natural gas, burned in industrial furnace >1(	1.281760603
Heavy fuel oil, burned in industrial furnace 1	kg CO2 eq	0.687614333	Quicklime, in pieces, loose, at plant/CH U	0.695908994
Citric Acid_Starch, at plant U incl amylase	kg CO2 eq	0.353	Heavy fuel oil, burned in industrial furnace 1	0.436334136
Lignite, burned in power plant/DE U	kg CO2 eq	0.304228512	Heat (coal)	0.391870708
Hard coal, burned in power plant/DE U	kg CO2 eq	0.232468962	Heat (oil)	0.37100113
Light fuel oil, burned in industrial furnace 1M	kg CO2 eq	0.232111559	Citric Acid_Molasses, at plant U	0.291
Electricity (natural gas)	kg CO2 eq	0.215780738	Electricity (natural gas)	0.276220583
Nitric acid, 50% in H2O, at plant/RER U	kg CO2 eq	0.209006603	Lignite, burned in power plant/DE U	0.260264004
Natural gas, burned in gas turbine, for comp	kg CO2 eq	0.133155541	Hard coal, burned in power plant/DE U	0.1998344
Green manure IP, until April/CH U	kg CO2 eq	0.121012518	Nitric acid, 50% in H2O, at plant/RER U	0.188748284
Transport, natural gas, pipeline, long distanc	kg CO2 eq	0.097758477	Truck 28t	0.144186487
Grain maize IP, at farm/CH U	kg CO2 eq	-1.763971883	Hard coal, burned in power plant/PL U	0.12162485

Table D.3 Life cycle process contributions to ozone layer depletion (kg CFC-11 eq)

Process	Unit	Citric Acid_Starch, at plant U incl amylase	Citric Acid_Molasses, at plant U	Citric Acid_Molasses, at plant U
Total of all processes	kg CFC-11 eq	5.80534E-07	Total of all processes	1.08965E-06
Remaining processes	kg CFC-11 eq	3.0465E-08	Remaining processes	5.93067E-08
Transport, natural gas, pipeline, long distanc	kg CFC-11 eq	2.36381E-07	Heat (oil)	5.05385E-07
Crude oil, at production onshore/RME U	kg CFC-11 eq	7.37154E-08	Truck 28t	1.97944E-07
Transport, natural gas, pipeline, long distanc	kg CFC-11 eq	5.06397E-08	Transport, natural gas, pipeline, long distanc	1.42287E-07
Crude oil, at production onshore/RU U	kg CFC-11 eq	4.9647E-08	Crude oil, at production onshore/RME U	5.50152E-08
Crude oil, at production onshore/RAF U	kg CFC-11 eq	4.2817E-08	Crude oil, at production onshore/RAF U	5.49161E-08
Transport, natural gas, onshore pipeline, lor	kg CFC-11 eq	2.82606E-08	Crude oil, at production onshore/RU U	3.44062E-08
Transport, natural gas, pipeline, long distanc	kg CFC-11 eq	2.00875E-08	Transport, natural gas, pipeline, long distanc	3.06198E-08
Crude oil, at production/NG U	kg CFC-11 eq	1.84416E-08	Crude oil, at production/NG U	2.98602E-08
Transport, natural gas, onshore pipeline, lor	kg CFC-11 eq	1.70321E-08	Heat (coal)	2.06665E-08
Transport, natural gas, offshore pipeline, lor	kg CFC-11 eq	1.30468E-08	Transport, natural gas, onshore pipeline, lor	1.76058E-08

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Table D.4 Life cycle process contributions to human toxicity (kg 1,4-DB eq)

Process	Unit	Citric Acid_Starch, at plant U incl amylase	Citric Acid_Molasses, at plant U	Citric Acid_Molasses, at plant U
Total of all processes	kg 1,4-DB eq	1.833651542	Total of all processes	1.676124416
Remaining processes	kg 1,4-DB eq	0.461265697	Remaining processes	0.535027676
Heavy fuel oil, burned in industrial furnace 1	kg 1,4-DB eq	0.449154868	Heavy fuel oil, burned in industrial furnace 1	0.285016749
Natural gas, burned in industrial furnace >1(	kg 1,4-DB eq	0.267814234	Heat (coal)	0.19625211
Ferrochromium, high-carbon, 68% Cr, at pla	kg 1,4-DB eq	0.217875515	Natural gas, burned in industrial furnace >1(	0.148614317
Well for exploration and production, offshore	kg 1,4-DB eq	0.142778634	Ferrochromium, high-carbon, 68% Cr, at pla	0.106418883
Copper, primary, at refinery/RLA U	kg 1,4-DB eq	0.087362724	Well for exploration and production, offshore	0.090181109
Grain maize IP, at farm/CH U	kg 1,4-DB eq	0.057572088	Heat (oil)	0.080631804
Electricity (natural gas)	kg 1,4-DB eq	0.045539936	Truck 28t	0.064145102
Discharge, produced water, onshore/GLO U	kg 1,4-DB eq	0.03554104	Electricity (natural gas)	0.058295601
Ammonia, steam reforming, liquid, at plant/F	kg 1,4-DB eq	0.027794673	Copper, primary, at refinery/RLA U	0.040712651
Heavy fuel oil, burned in power plant/IT U	kg 1,4-DB eq	0.022515819	Aluminium, primary, liquid, at plant/RER U	0.036852796
Coke oven gas, at plant/GLO U	kg 1,4-DB eq	0.018436313	Anode, aluminium electrolysis/RER U	0.033975618

Table D.5 Life cycle process contributions to fresh water aquatic ecotox. (kg 1,4-DB eq)

Process	Unit	Citric Acid_Starch, at plant U incl amylase	Citric Acid_Molasses, at plant U	Citric Acid_Molasses, at plant U
Total of all processes	kg 1,4-DB eq	0.478143604	Total of all processes	0.272345645
Remaining processes	kg 1,4-DB eq	0.107466998	Remaining processes	0.033154324
Grain maize IP, at farm/CH U	kg 1,4-DB eq	0.229873789	Heat (coal)	0.038911313
Heavy fuel oil, burned in industrial furnace 1	kg 1,4-DB eq	0.043494397	Heavy fuel oil, burned in industrial furnace 1MW, nc	0.027599905
Disposal, nickel smelter slag, 0% water, to r	kg 1,4-DB eq	0.042770359	Disposal, nickel smelter slag, 0% water, to residual	0.023758299
Disposal, lignite ash, 0% water, to opencast	kg 1,4-DB eq	0.016326927	Disposal, lignite ash, 0% water, to opencast refill/GI	0.01933266
Disposal, lignite ash, 0% water, to opencast	kg 1,4-DB eq	0.014444938	Heat (oil)	0.018049831
Disposal, lignite ash, 0% water, to opencast	kg 1,4-DB eq	0.012433117	Disposal, lignite ash, 0% water, to opencast refill/PL	0.017749083
Disposal, slag, unalloyed electr. steel, 0% w	kg 1,4-DB eq	0.011333079	Disposal, lignite ash, 0% water, to opencast refill/DE	0.014096745

Table D.6 Life cycle process contributions to marine aquatic ecotoxcity (kg 1,4-DB eq)

Process	Unit	Citric Acid_Starch, at plant U incl amylase	Citric Acid_Molasses, at plant U	Citric Acid_Molasses, at plant U
Total of all processes	kg 1,4-DB eq	1849.246443	Total of all processes	2340.935072
Remaining processes	kg 1,4-DB eq	451.927309	Remaining processes	527.4165399
Heavy fuel oil, burned in industrial furnace 1	kg 1,4-DB eq	355.6388544	Heat (coal)	390.6045532
Well for exploration and production, offshore	kg 1,4-DB eq	194.4126413	Heavy fuel oil, burned in industrial furnace 1MW, nc	225.6750111
Hard coal, burned in power plant/DE U	kg 1,4-DB eq	132.041872	Hard coal, burned in power plant/PL U	145.5899499
Hard coal, burned in power plant/ES U	kg 1,4-DB eq	104.3252799	Hard coal, burned in power plant/ES U	139.9952155
Hard coal, burned in power plant/PL U	kg 1,4-DB eq	103.0426575	Well for exploration and production, offshore/OCE/I	122.7939166
Lignite, burned in power plant/DE U	kg 1,4-DB eq	93.16737354	Hard coal, burned in power plant/DE U	113.5055103
Lignite, burned in power plant/GR U	kg 1,4-DB eq	69.06895851	Lignite, burned in power plant/GR U	92.43976757
Disposal, lignite ash, 0% water, to opencast	kg 1,4-DB eq	54.47659136	Lignite, burned in power plant/DE U	79.70361974
Hard coal, burned in power plant/FR U	kg 1,4-DB eq	47.63949925	Heat (oil)	70.65789684
Lignite, burned in power plant/PL U	kg 1,4-DB eq	47.31141114	Lignite, burned in power plant/PL U	67.54011503
Lignite, burned in power plant/CS U	kg 1,4-DB eq	39.82315485	Lignite, burned in power plant/CS U	53.29722932
Disposal, nickel smelter slag, 0% water, to r	kg 1,4-DB eq	35.07912292	Hard coal, burned in power plant/FR U	50.96803353
Disposal, lignite ash, 0% water, to opencast	kg 1,4-DB eq	33.75461641	Truck 28t	48.61456014
Hard coal, burned in power plant/IT U	kg 1,4-DB eq	29.71973346	Disposal, lignite ash, 0% water, to opencast refill/DE	47.03534458
Disposal, lignite ash, 0% water, to opencast	kg 1,4-DB eq	29.1625734	Disposal, lignite ash, 0% water, to opencast refill/GI	45.17613937
Hard coal, burned in power plant/NORDEL (	kg 1,4-DB eq	28.65479371	Disposal, lignite ash, 0% water, to opencast refill/PL	41.63146934

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Table D.7 Life cycle process contributions to terrestrial ecotoxicity (kg 1,4-DB eq)

Process	Unit	Citric Acid_Starch, at plant U incl amylase	Citric Acid_Molasses, at plant U	Citric Acid_Molasses, at plant U
Total of all processes	kg 1,4-DB eq	0.045118263	Total of all processes	0.020843291
Remaining processes	kg 1,4-DB eq	0.004312304	Remaining processes	0.004227993
Heavy fuel oil, burned in industrial furnace 1	kg 1,4-DB eq	0.016121661	Heavy fuel oil, burned in industrial furnace 1MW, nc	0.010230199
Grain maize IP, at farm/CH U	kg 1,4-DB eq	0.008957073	Transmission network, electricity, medium voltage/C	0.004731587
Distribution network, electricity, low voltage/	kg 1,4-DB eq	0.005222289	Lignite, burned in power plant/PL U	0.002096022
Transmission network, electricity, medium v	kg 1,4-DB eq	0.004585109	Rape seed IP, at farm/CH U	0.001973159
Rape seed IP, at farm/CH U	kg 1,4-DB eq	0.002516367	Heavy fuel oil, burned in power plant/IT U	0.000921066
Lignite, burned in power plant/PL U	kg 1,4-DB eq	0.00146825	Heat (coal)	0.000768868
Ammonia, steam reforming, liquid, at plant/F	kg 1,4-DB eq	0.000732603	Ammonia, steam reforming, liquid, at plant/RER U	0.000637123
Heavy fuel oil, burned in power plant/IT U	kg 1,4-DB eq	0.000694773	Heat (oil)	0.00045598
Steel, electric, un- and low-alloyed, at plant/	kg 1,4-DB eq	0.000507832	Truck 28t	0.00037484

Table D.8 Life cycle process contributions to photochemical oxidation (kg C2H4 eq)

Process	Unit	Citric Acid_Starch, at plant U incl amylase	Citric Acid_Molasses, at plant U	Citric Acid_Molasses, at plant U
Total of all processes	kg C2H4	0.00087127	Total of all processes	0.001123409
Remaining processes	kg C2H4	0.000412063	Remaining processes	0.000296109
Heavy fuel oil, burned in industrial furnace 1	kg C2H4	0.000175507	Heat (coal)	0.000135
Natural gas, burned in industrial furnace >1(	kg C2H4	4.67656E-05	Heavy fuel oil, burned in industrial furnace 1MW, nc	0.00011137
Sour gas, burned in gas turbine, production/	kg C2H4	4.0727E-05	Quicklime, in pieces, loose, at plant/CH U	0.000110062
Natural gas, sour, burned in production flare	kg C2H4	4.04352E-05	Truck 28t	6.44499E-05
Lignite, burned in power plant/ES U	kg C2H4	3.47761E-05	Heat (oil)	5.45513E-05
Transport, natural gas, pipeline, long distan	kg C2H4	3.39508E-05	Lignite, burned in power plant/ES U	4.6542E-05
Natural gas, at production onshore/RU U	kg C2H4	2.91097E-05	Hard coal, burned in power plant/PL U	4.0951E-05
Hard coal, burned in power plant/PL U	kg C2H4	2.89835E-05	Heavy fuel oil, burned in power plant/IT U	3.83811E-05
Heavy fuel oil, burned in power plant/IT U	kg C2H4	2.89514E-05	Lignite, burned in power plant/PL U	3.37889E-05

Table D.9 Life cycle process contributions to acidification (kg SO2 eq)

Process	Unit	Citric Acid_Starch, at plant U incl amylase	Citric Acid_Molasses, at plant U	Citric Acid_Molasses, at plant U
Total of all processes	kg SO2 eq	0.024174132	Total of all processes	0.024385361
Remaining processes	kg SO2 eq	0.0076294	Remaining processes	0.006817691
Heavy fuel oil, burned in industrial furnace 1	kg SO2 eq	0.004645395	Sugar beets IP, at farm/CH U	0.003302982
Grain maize IP, at farm/CH U	kg SO2 eq	0.004511808	Heat (coal)	0.002958289
Natural gas, sour, burned in production flare	kg SO2 eq	0.001038098	Heavy fuel oil, burned in industrial furnace 1MW, nc	0.002947792
Sour gas, burned in gas turbine, production/	kg SO2 eq	0.001018524	Lignite, burned in power plant/ES U	0.001209908
Lignite, burned in power plant/ES U	kg SO2 eq	0.000904042	Hard coal, burned in power plant/PL U	0.001126798
Heavy fuel oil, burned in power plant/IT U	kg SO2 eq	0.000811759	Heavy fuel oil, burned in power plant/IT U	0.001076156
Hard coal, burned in power plant/PL U	kg SO2 eq	0.000797502	Truck 28t	0.001035568
Hard coal, burned in power plant/ES U	kg SO2 eq	0.000639628	Lignite, burned in power plant/PL U	0.000889559
Lignite, burned in power plant/PL U	kg SO2 eq	0.00062313	Heat (oil)	0.000861342
Nitric acid, 50% in H2O, at plant/RER U	kg SO2 eq	0.000534838	Hard coal, burned in power plant/ES U	0.000858323
Lignite, burned in power plant/CS U	kg SO2 eq	0.000530331	Natural gas, sour, burned in production flare/MJ/GL	0.000713765
Green manure IP, until April/CH U	kg SO2 eq	0.000489677	Lignite, burned in power plant/CS U	0.000709767

Table D.10 Life cycle process contributions to eutrophication (kg PO3-4 eq)

Process	Unit	Citric Acid_Starch, at plant U incl amylase	Citric Acid_Molasses, at plant U	Citric Acid_Molasses, at plant U
Total of all processes	kg PO4--- eq	0.009811303	Total of all processes	0.003544692
Remaining processes	kg PO4--- eq	0.001107007	Remaining processes	0.000907134
Grain maize IP, at farm/CH U	kg PO4--- eq	0.006974055	Sugar beets IP, at farm/CH U	0.004189419
Green manure IP, until April/CH U	kg PO4--- eq	0.000971498	Green manure IP, until February/CH U	0.000745057
Treatment, maize starch production effluent,	kg PO4--- eq	0.000272668	Truck 28t	0.000173648
Phosphoric acid, fertiliser grade, 70% in H2(	kg PO4--- eq	0.000143281	Heat (coal)	0.000136946
Nitric acid, 50% in H2O, at plant/RER U	kg PO4--- eq	0.000129837	Nitric acid, 50% in H2O, at plant/RER U	0.000117252
Heavy fuel oil, burned in industrial furnace 1	kg PO4--- eq	0.000113971	Heavy fuel oil, burned in industrial furnace 1MW, nc	7.23216E-05
Crude oil, at production onshore/RU U	kg PO4--- eq	9.8986E-05	Harvesting, by complete harvester, beets/CH U	6.88709E-05

APPENDIX E

Economic Assessment

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Appendix E: Economic Assessment

E.1 Equipment Specifications

Examples of process equipment specification sheets from the simulation tool are shown below. The specification sheets of the bioreactor, ion exchange column and crystal filter for the starch process are shown.

E.1.1 Bioreactor (Starch Process: RX-001)

Form Page 1: Procedure		
Unit operaton		
Process Time	h	164.22
Batch Volume	m3/batch	223.5
Residence time	h	145
Capacity Utilisation	%	100
Cycles per Batch		1
Cycle Time	h	164.22
Absolute Start Time	h	0
Absolute End Time	h	164.22
Calc Option		2
Form Page 2: Equipment Sizing		
UnitCode		RX1
Name		Fermenter
Type		Stirred Tank
Number of Units		1
Max Volume	m3	350
Max Working Volume	%	90
Volume	m3	248.33
Height/Diameter		3
Height	m	
Diameter	m	4.724
Design Pressure	kPa	1.5
Form Page 3: Purchase Cost		
Purchase Cost	$	1023500
Reference Year		2005
Purchase Cost User		
Base Cost	$	1000000
Base Capacity	m3	239
f		0.6
Model Reference Year		2005
Cost Variable	m3	248
Calc Option		3
Form Page 4: Adjustments		
Material		SS316
Material Factor		1
Installation Cost Factor		1
Labour	hrs/hr	0.5
Staggered Units		11
Standby Units		0

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E.1.2 Adsorber (Starch Process: IE-001)

Form Page 1: Procedure		
Unit operaton		
Batch Volume	m3/batch	215.7
Process Time	h	2.17
Empty Bed Contact Time	min	0.1
Breakthrough Time	min	70.2
Washing Time	min	60
Capacity Utilisation	%	100
Cycles per Batch		1
Cycle Time	h	2.17
Absolute Start Time	h	2.17
Absolute End Time	h	4.34
Calc Option		2

Form Page 2: Equipment Sizing		
UnitCode		AD2
Name		Ion Exchange
Type		Ion Exchange
Number of Units		1
Max Bed Diameter	m3	3
Overdesign Factor (%)	%	0
Bed Height/Diameter Ratio		0.7
Bed To Column Height Ratio		0.5
Packing Density	g/L	
Bed Diameter	m	0.824
Bed Height	m	0.577
Bed Volume	m3	0.307
Column Height		3.191
Column Volume	kPa	0.615

Form Page 3: Purchase Cost		
Purchase Cost	$	45577.93
Reference Year		2005
Purchase Cost User	$	
Base Cost	$	44158
Base Capacity	m3	0.5834
f		0.6
Model Reference Year		2005
Cost Variable	m3	0.615
Calc Option		3

Form Page 4: Adjustments		
Material		CS
Material Factor		1
Installation Cost Factor		1
Labour	hrs/hr	0.1
Staggered Units		0
Standby Units		0

E.1.3 Filter (Starch Process: FT-003)

Form Page 1: Procedure		
Unit operaton		
Batch Volume	m3/batch	24.6
Residence time	h	5.22
Capacity Utilisation	%	100
Cycles per Batch		1
Cycle Time	h	5.22
Absolute Start Time	h	0
Absolute End Time	h	5.22
Calc Option		2

Form Page 2: Equipment Sizing		
UnitCode		FT3
Name		Crystal Filter
Type		Rotary Vacuum
Number of Units		1
Total Area		37.3
Max Area	m2	245
Unit Area	m2	37.3

Form Page 3: Purchase Cost		
Purchase Cost	R	87468.7
Reference Year		2005
Purchase Cost User		
Base Cost	R	84835
Base Capacity	m3	35.447
f		0.6
Model Reference Year		2005
Cost Variable	m2	37.3
Calc Option		3

Form Page 4: Adjustments		
Material		CS
Material Factor		1
Installation Cost Factor		1
Labour	hrs/hr	0.5
Staggered Units		0
Standby Units		0

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E.2 Economic Assessment Results (Starch Process)

1. EXECUTIVE SUMMARY					
Total Capital Investment				174,720,400.00	$
Operating Cost				36,363,120.00	$
Production Rate				12,592,330.00	kg P/year
Unit Production Cost				2.89	R/kg P
Total Revenues				22,666,190.00	$
Gross Margin				-60.43	%
Return on Investment				-0.08	%
Payback Time				NA (> 50 Years)	years
IRR				NA (<> 0-100%)	%
NPV @ 7%				-161,566,600.00	$

2. MAJOR EQUIPMENT PURCHASE COSTS (PCE)						
Unit Code	**Description**	**No. of Units**	**Staggered Units**	**Standby Units**	**Unit Cost ($)**	**Cost ($)**
AD1 S 1	AC Column	1	0	1	256,994.50	513,989.00
CX1 S 1	Condenser	2	0	0	29,747.38	59,494.76
CM1 S 1	Compressor1	1	11	0	165,927.20	1,991,127.00
CR1 S 1	Crystallizer	2	0	0	463,556.80	927,113.60
DF1 S 1	Fluidised Bed Drier	1	0	0	135,506.80	135,506.80
FA1 S 1	Air Filter	1	11	0	5,211.51	62,538.12
FT2 S 1	Biomass Removal	1	0	0	126,778.50	126,778.50
FT1 S 2	Ultrafiltration	3	0	0	98,605.85	295,817.60
FT3 S 3	Crystal Filter	1	0	0	87,468.70	87,468.70
AD2 S 2	Ion Exchange	1	0	0	45,577.93	45,577.93
RX1 S 1	Fermenter	1	11	0	1,023,533.00	12,282,400.00
ST1 S 1	SteriliserMedia	1	0	0	307,730.30	307,730.30
ST2 S 2	SteriliserST2	2	0	0	784,469.50	1,568,939.00
RX2 S 2	Media Prep Tank	1	0	0	53,980.20	53,980.20
					TOTAL	18,458,460.00

3. CAPITAL EXPENDITURE				Cost ($)
3.1 Plant Direct Cost (PDC)				
Purchased Equipment Cost				18,458,460.00
Equipment erection				9,229,230.00
Piping				12,920,922.00
Instrumentation				9,229,230.00
Insulation				922,923.00
Electrical systems				2,768,769.00
Buildings				9,229,230.00
Site development				2,768,769.00
Auxiliary facilities				12,920,922.00
Total (PDC)				78,448,450.00
3.2 Plant Indirect Cost (PIC)				
Design and engineering				19,612,112.00
Construction				27,456,956.80
Total (PIC)				47,069,070.00
3.3 Total Plant Cost (TPC = PDC + PIC)				
Plant Direct Cost (PDC)				78,448,450.00
Plant Indirect Cost (PIC)				47,069,070.00
Total (TPC)				125,517,500.00
3.4 Contractors Fees and Contingency (CFC)				
Contractors fee				7,531,051.20
Contingency				12,551,752.00
Total (CFC)				20,082,800.00
3.5 Direct Fixed Capital Cost (DFC = TPC + CFC)				
Total Plant Cost (TPC)				125,517,500.00
Contractors Fees and Contingency (CFC)				20,082,800.00
Total (DFC)				145,600,300.00
3.6 Plant Start-up Cost (PSC)				
Strat-up and Validation				7,280,016.00
Working Capital				21,840,048.00
Total (PSC)				29,120,060.00

4. OPERATING COSTS				Cost ($)
Raw Materials				2,564,185.00
Labour				1,947,113.00
Consumables				313,966.00
Waste Treatment/Disposal				44,564.88
Utilities				5,326,497.00
Insurance				1,456,003.20
Plant Tax				2,912,006.40
Maintenance				8,736,019.20
Laboratory				1,168,268.00
Depreciation				13,832,030.00
Total(OC)				36,363,120.00

4.1 Raw Materials Cost

Raw Material	Unit Cost ($/kg)	Consumption (kg/year)		Cost ($/year)
α-Amylase	10.0000	15,992		159,922.59
Ammonium Nitrate	0.1500	294,661		44,199.08
Biomass	0.0000	4,472		0
Hydrogen Chloride	0.1500	8,966		1,344.86
Nitrogen	0.0000	253,609,536		0
Oxygen	0.0000	67,406,744		0
Potassium Phosphate	0.3400	23,397		7,954.83
Sodium Hydroxide	0.1600	5,062		809.94
Starch	0.1500	15,110,796		2,266,619.40
Water	0.0000	166,669,024		83,334.51

4.2. Labour Cost

Unit Code	Description	Demand (hrs/year	Labour Rate ($/hr	Cost ($/year)
AD1 S 1	AC Column	268.17	34	9,117.78
CX1 S 1	Condenser	0	34	0
CM1 S 1	Compressor1	0	34	0
CR1 S 1	Crystallizer	2352.72	34	79,992.48
DF1 S 1	Fluidised Bed Drier	1335.23	34	45,397.82
FA1 S 1	Air Filter	0	34	0
FT2 S 1	Biomass Removal	2535.26	34	86,198.84
FT1 S 2	Ultrafiltration	2028.2	34	68,958.80
FT3 S 3	Crystal Filter	1470.45	34	49,995.30

AD2 S 2	Ion Exchange	122.26	34	4,156.84
RX1 S 1	Fermenter	46259.95	34	1,572,838.00
ST1 S 1	SteriliserMedia	140.85	34	4,788.90
ST2 S 2	SteriliserST2	473.25	34	16,090.50
RX2 S 2	Media Prep Tank	281.7	34	9,577.80

4.3 Consumables Cost

Consumable	Unit Cost ($/kg)	Consumption (kg/year)		Cost ($/year)
GAC Packing	4.0000	78492		313,966.00

4.4 Waste Treatment/Disposal Cost

Waste Material	Disposal Cost ($/kg)	Disposal (kg/year)		Cost ($/year)
α-Amylase	0.0100	15,992		159.92
Biomass	0.0100	1,958,107		19,581.07
Carbon Dioxide	0.0000	4,448,870		0.00
Chloride	0.0005	8,711		4.36
Citric Acid Monohydrate	0.0005	12,592,330		6,296.17
Citric acid loss	0.0005	385,325		192.66
Glucose	0.0100	166,219		1,662.19
Nitrogen	0.0000	253,609,536		0.00
Oxygen	0.0000	61,677,232		0.00
Potassium (dissolved)	0.0005	320		0.16
Sodium (dissolved)	0.0005	2,910		1.45
Water	0.0001	166,669,024		16,666.90

4.5 Utilities

Utility	Unit Cost	Cost Units	Consumption	Cost ($/year)
Electricity (GJ)	13.8900	$/GJ	137,130	1,904,742.43
Natural Gas (m3)	20.0000	$m3		0.00
Steam (ton)	4.4000	$/ton	173,774	764,606.29
Cooling water (m3)	0.0800	$/m3	27,703,126	2,216,250.08
Chilled water (m3)	0.1700	$/m3	2,593,517	440,897.81

5. PROFITABILITY ANALYSIS

5.1 Project Investment

Direct Fixed Capital				145,600,300.00	
Start-up Cost				7,280,016.00	
Working Capital				21,840,050.00	
Total Investment Cost				174,720,400.00	

5.2 Unit Output

Product Output				12,592,330.00	kg/yr
Unit Production Cost				2.89	$/kg
Product Selling Price				1.80	$/kg

5.3 Project Profitability

Revenue				22,666,190.00	$/yr
Operating Cost				36,363,120.00	$/yr
Gross Profit				-13,696,920.00	$/yr
Taxes				0.00	$/yr
Net Profit				-13,696,920.00	$/yr
Gross Margin				-60.43	%
Return on Investment				-0.08	%
Payback Period				NA (> 50 Years)	years
NPV				-161,566,600.00	$
IRR				NA (<> 0-100%)	%

6. CASH FLOW ANALYSIS

Year	Capital Investment ($)	Debt Finance ($)	Sales (kg/yr)	Selling Price ($/kg)	Sales Revenues ($)	Operating Expense ($)	Operating Income ($)
1	-29,120,060	11,648,020	0	1.799999952	0	2,766,406	-2,766,406
2	-72,800,150	29,120,060	0	1.799999952	0	9,682,420	-9,682,420
3	-43,680,090	17,472,040	0	1.799999952	0	13,832,030	-13,832,030
4	0	39,000	12,592,330	1.799999952	22,666,190	50,223,860	-27,557,670
5	0	0	12,592,330	1.799999952	22,666,190	50,223,860	-27,557,670
6	0	0	12,592,330	1.799999952	22,666,190	36,391,840	-13,725,640
7	0	0	12,592,330	1.799999952	22,666,190	36,391,840	-13,725,640
8	0	0	12,592,330	1.799999952	22,666,190	36,391,840	-13,725,640
9	0	0	12,592,330	1.799999952	22,666,190	36,391,840	-13,725,640
10	0	0	12,592,330	1.799999952	22,666,190	36,391,840	-13,725,640
11	0	0	12,592,330	1.799999952	22,666,190	33,625,430	-10,959,230
12	0	0	12,592,330	1.799999952	22,666,190	26,709,410	-4,043,220
13	0	0	12,592,330	1.799999952	22,666,190	22,559,810	106,388
14	0	0	12,592,330	1.799999952	22,666,190	22,559,810	106,388
15	0	0	12,592,330	1.799999952	22,666,190	22,559,810	106,388

Loan Payment ($)	Depreciation ($)	Taxable Income ($)	Taxes ($)	Net Profit after Taxes ($)	Net Cash Flow ($)	Discounted CashFlow @ Rate 1	Discounted CashFlow @ Rate 2	Discounted CashFlow @ Rate 3
0	2,766,406	0	0	-2,766,406	-17,472,040	-16,640,038	-16,329,009	-16,029,394
0	9,682,420	0	0	-9,682,420	-43,680,090	-39,619,131	-38,151,884	-36,764,659
0	13,832,030	0	0	-13,832,030	-26,208,050	-22,639,501	-21,393,577	-20,237,425
11,514,050	13,832,030	0	0	-39,071,720	-25,200,690	-20,732,672	-19,225,487	-17,852,805
11,514,050	13,832,030	0	0	-39,071,720	-25,239,690	-19,775,959	-17,995,552	-16,404,068
11,514,050	13,832,030	0	0	-25,239,690	-11,407,660	-8,512,574	-7,601,408	-6,802,017
11,514,050	13,832,030	0	0	-25,239,690	-11,407,660	-8,107,213	-7,104,119	-6,240,382
11,514,050	13,832,030	0	0	-25,239,690	-11,407,660	-7,721,155	-6,639,364	-5,725,121
11,514,050	13,832,030	0	0	-25,239,690	-11,407,660	-7,353,481	-6,205,013	-5,252,405
11,504,900	13,832,030	0	0	-25,230,540	-11,398,510	-6,997,698	-5,794,426	-4,814,855
11,504,900	11,065,620	0	0	-22,464,130	-11,398,510	-6,664,473	-5,415,350	-4,417,297
11,504,900	4,149,609	0	0	-15,548,120	-11,398,510	-6,347,117	-5,061,075	-4,052,566
11,504,900	0	0	0	-11,398,510	-11,398,510	-6,044,874	-4,729,977	-3,717,951
0	0	106,388	277	106,111	106,111	53,593	41,152	31,753
0	0	106,388	277	106,111	106,111	51,041	38,460	29,132

E.3 Economic Assessment Results (Molasses Process)

1. EXECUTIVE SUMMARY					
Total Capital Investment				187,781,100.00	$
Operating Cost				40,941,550.00	$
Production Rate				12,576,010.00	kg P/year
Unit Production Cost				3.26	$/kg P
Total Revenues				22,636,820.00	$
Gross Margin				-80.86	%
Return on Investment				-0.1	%
Payback Time				NA (> 50 Years)	years
IRR				NA (<> 0-100%)	%
NPV @ 7%				-197,044,800.00	$

2. MAJOR EQUIPMENT PURCHASE COSTS (PCE)						
Unit Code	**Description**	**No. of Units**	**Staggered Units**	**Standby Units**	**Unit Cost**	**Cost ($)**
AD1 S 1	AC Column	1	0	1	142,226.10	284,452.20
CX1 S 1	Condenser	1	0	0	22,601.57	22,601.57
CM1 S 1	Compressor1	1	11	0	181,558.70	2,178,705.00
CR1 S 1	Crstallizer	1	0	0	345,448.30	345,448.30
DF1 S 1	Fluidised Bed Drier	1	0	0	135,506.80	135,506.80
FA1 S 1	Air Filter	1	11	0	4,956.51	59,478.12
FT1 S 1	Biomass Removal	2	0	0	106,817.10	213,634.20
FT2 S 2	Precipitation Filter	2	0	0	135,697.00	271,394.00
FT3 S 3	AcidulatorFilter	1	0	0	96,221.10	96,221.10
AD2 S 2	Ion Exchange	1	0	0	25,261.91	25,261.91
RX1 S 1	Fermenter	1	11	0	1,121,812.00	13,461,740.00
ST1 S 1	SteriliserMedia	1	0	0	326,376.90	326,376.90
ST2 S 2	SteriliserST2	2	0	0	928,775.20	1,857,550.00
RX2 S 2	Media Prep Tank	1	0	0	57,248.25	57,248.25
RX3 S 3	Precipitation	2	0	0	171,929.00	343,858.00
RX4 S 4	Acidulator	1	0	0	119,688.30	119,688.30
FT4 S 4	Crystal Filter	1	0	0	39,110.35	39,110.35
					TOTAL	19,838,270.00

3. CAPITAL EXPENDITURE				Cost ($)
3.1 Plant Direct Cost (PDC)				
Purchased Equipment Cost				19,838,270.00
Equipment erection				9,919,135.00
Piping				13,886,789.00
Instrumentation				9,919,135.00
Insulation				991,913.50
Electrical systems				2,975,740.50
Buildings				9,919,135.00
Site development				2,975,740.50
Auxiliary facilities				13,886,789.00
Total (PDC)				84,312,650.00
3.2 Plant Indirect Cost (PIC)				
Design and engineering				21,078,162.00
Construction				29,509,426.80
Total (PIC)				50,587,590.00
3.3 Total Plant Cost (TPC = PDC + PIC)				
Plant Direct Cost (PDC)				84,312,650.00
Plant Indirect Cost (PIC)				50,587,590.00
Total (TPC)				134,900,200.00
3.4 Contractors Fees and Contingency (CFC)				
Contractors fee				8,094,014.40
Contingency				13,490,024.00
Total (CFC)				21,584,040.00
3.5 Direct Fixed Capital Cost (DFC = TPC + CFC)				
Total Plant Cost (TPC)				134,900,200.00
Contractors Fees and Contingency (CFC)				21,584,040.00
Total (DFC)				156,484,300.00
3.6 Plant Start-up Cost (PSC)				
Strat-up and Validation				7,824,213.60
Working Capital				23,472,640.80
Total (PSC)				31,296,850.00

4. OPERATING COSTS				**Cost ($)**
Raw Materials				5,344,692.00
Labour				2,076,590.00
Consumables				246,737.60
Waste Treatment/Disposal				181,499.19
Utilities				4,923,144.00
Insurance				1,564,842.72
Plant Tax				3,129,685.44
Maintenance				9,389,056.32
Laboratory				1,245,954.00
Depreciation				14,866,010.00
Total(OC)				40,941,550.00

4.1 Raw Materials Cost

Raw Material	Unit Cost ($/kg)	Consumption (kg/year)		Cost ($/year)
Ammonium Nitrate	0.1500	324,964		48,744.63
Beet Molasses	0.0900	34,709,796		3,123,881.64
Biomass	0.0000	57,975		0
Calcium Oxide (Lime)	0.1100	9,502,435		1,045,267.85
Hydrogen Chloride	0.1500	170,279		25,541.88
Nitrogen	0.0000	289,248,288		0
Oxygen	0.0000	78,210,224		0
Potassium dihydrogen phosphate	0.3400	25,781		8,765.48
Sodium Hydroxide	0.1600	1,647		263.59
Sulphuric acid	0.1000	9,834,442		983,444.20
Water	0.0000	217,565,024		108,782.51

4.2. Labour Cost

Unit Code	Description	Demand (hrs/ye	Labour Rate ($/hr)	Cost ($/year)
AD1 S 1	AC Column	267.83	34	9,106.22
CX1 S 1	Condenser	0	34	0
CM1 S 1	Compressor1	0	34	0
CR1 S 1	Crstallizer	2349.67	34	79,888.78
DF1 S 1	Fluidised Bed Drier	1333.5	34	45,339.00
FA1 S 1	Air Filter	0	34	0
FT1 S 1	Biomass Removal	2531.97	34	86,086.98
FT2 S 2	Precipitation Filter	2531.97	34	86,086.98
FT3 S 3	AcidulatorFilter	2531.97	34	86,086.98
AD2 S 2	Ion Exchange	122.1	34	4,151.40

RX1 S 1	Fermenter	46200.01	34	1,570,800.00
ST1 S 1	SteriliserMedia	140.67	34	4,782.78
ST2 S 2	SteriliserST2	472.63	34	16,069.42
RX2 S 2	Media Prep Tank	0	34	0
RX3 S 3	Precipitation	562.66	34	19,130.44
RX4 S 4	Acidulator	562.66	34	19,130.44
FT4 S 4	Crystal Filter	1468.54	34	49,930.36

4.3 Consumables Cost

Consumable	Unit Cost ($/kg)	Consumption (kg/year)		Cost ($/year)
GAC Packing	4.0000	61684		246,737.60

4.4 Waste Treatment/Disposal Cost

Waste Material	Disposal Cost ($/kg)	Disposal (kg/year)		Cost ($/year)
Biomass	0.0100	2,189,484		21,894.84
Calcium Citrate	0.0100	167,890		1,678.90
Calcium Sulphate (Gypsum)	0.0100	13,582,094		135,820.94
Carbon Dioxide	0.0000	3,659,620		0.00
Chloride	0.0005	165,562		82.78
Citric acid loss	0.0005	270,384		135.19
Nitrate (dissolved)	0.0005	251,721		125.86
Nitrogen	0.0000	289,248,288		0.00
Oxygen	0.0000	72,815,112		0.00
Potassium (dissolved)	0.0005	7,407		3.70
Sodium (dissolved)	0.0005	947		0.47
Water	0.0001	217,565,024		21,756.50

4.5 Utilities

Utility	Unit Cost	Cost Units	Consumption	Cost ($/year)
Electricity (GJ)	13.8900	$/GJ	149,655	2,078,701.66
Natural Gas (m3)	20.0000	$/m3		0.00
Steam (ton)	4.4000	$/ton	109,411	481,409.78
Cooling water (kg)	0.0800	$/m3	23,391,384	1,871,310.72
Chilled water (kg)	0.1700	$/m3	2,892,483	491,722.11

5. PROFITABILITY ANALYSIS

5.1 Project Investment

Direct Fixed Capital				156,484,300.00	
Start-up Cost				7,824,214.00	
Working Capital				23,472,640.00	
Total Investment Cost				187,781,100.00	

5.2 Unit Output

Product Output				12,576,010.00	kg/yr
Unit Production Cost				3.26	$/kg
Product Selling Price				1.80	$/kg

5.3 Project Profitability

Revenue				22,636,820.00	$/yr
Operating Cost				40,941,550.00	$/yr
Gross Profit				-18,304,730.00	$/yr
Taxes				0.00	$/yr
Net Profit				-18,304,730.00	$/yr
Gross Margin				-80.86	%
Return on Investment				-0.10	%
Payback Period				NA (> 50 Years)	years
NPV				-197,044,800.00	$
IRR				NA (<> 0-100%)	%

University of Cape Town

6. CASH FLOW ANALYSIS

Year	Capital Investment ($)	Debt Finance ($)	Sales (kg/yr)	Selling Price ($/kg)	Sales Revenues ($)	Operating Expense ($)	Operating Income ($)
1	-31,296,860	12,518,740	0	1.799999952	0	2,973,202	-2,973,202
2	-78,242,150	31,296,860	0	1.799999952	0	10,406,210	-10,406,210
3	-46,945,290	18,778,120	0	1.799999952	0	14,866,010	-14,866,010
4	0	39,000	12,576,010	1.799999952	22,636,820	55,863,800	-33,226,980
5	0	0	12,576,010	1.799999952	22,636,820	55,863,800	-33,226,980
6	0	0	12,576,010	1.799999952	22,636,820	40,997,790	-18,360,970
7	0	0	12,576,010	1.799999952	22,636,820	40,997,790	-18,360,970
8	0	0	12,576,010	1.799999952	22,636,820	40,997,790	-18,360,970
9	0	0	12,576,010	1.799999952	22,636,820	40,997,790	-18,360,970
10	0	0	12,576,010	1.799999952	22,636,820	40,997,790	-18,360,970
11	0	0	12,576,010	1.799999952	22,636,820	38,024,590	-15,387,770
12	0	0	12,576,010	1.799999952	22,636,820	30,591,590	-7,954,768
13	0	0	12,576,010	1.799999952	22,636,820	26,131,780	-3,494,964
14	0	0	12,576,010	1.799999952	22,636,820	26,131,780	-3,494,964
15	0	0	12,576,010	1.799999952	22,636,820	26,131,780	-3,494,964

Loan Payment ($)	Depreciation ($)	Taxable Income ($)	Taxes ($)	Net Profit after Taxes ($)	Net Cash Flow ($)	Discounted CashFlow @ Rate 1	Discounted CashFlow @ Rate 2	Discounted CashFlow @ Rate 3
0	2,973,202	0	0	-2,973,202	-18,778,120	-17,883,924	-17,549,645	-17,227,633
0	10,406,210	0	0	-10,406,210	-46,945,290	-42,580,764	-41,003,836	-39,512,913
0	14,866,010	0	0	-14,866,010	-28,167,170	-24,331,862	-22,992,803	-21,750,225
12,374,070	14,866,010	0	0	-45,601,050	-30,696,040	-25,253,711	-23,417,865	-21,745,851
12,374,070	14,866,010	0	0	-45,601,050	-30,735,040	-24,081,711	-21,913,662	-19,975,669
12,374,070	14,866,010	0	0	-30,735,040	-15,869,040	-11,841,718	-10,574,208	-9,462,187
12,374,070	14,866,010	0	0	-30,735,040	-15,869,040	-11,277,827	-9,882,437	-8,680,905
12,374,070	14,866,010	0	0	-30,735,040	-15,869,040	-10,740,787	-9,235,923	-7,964,133
12,374,070	14,866,010	0	0	-30,735,040	-15,869,040	-10,229,321	-8,631,704	-7,306,544
12,364,920	14,866,010	0	0	-30,725,890	-15,859,890	-9,736,594	-8,062,361	-6,699,387
12,364,920	11,892,810	0	0	-27,752,690	-15,859,880	-9,272,945	-7,534,916	-6,146,225
12,364,920	4,459,803	0	0	-20,319,690	-15,859,890	-8,831,377	-7,041,979	-5,638,740
12,364,920	0	0	0	-15,859,880	-15,859,880	-8,410,835	-6,581,288	-5,173,155
0	0	0	0	-3,494,964	-3,494,964	-1,765,194	-1,355,407	-1,045,856
0	0	0	0	-3,494,964	-3,494,964	-1,681,137	-1,266,736	-959,501