A Generic Approach to Environmental Assessment of Microbial Bioprocesses through Life Cycle Assessment (LCA)

A thesis submitted to the University of Cape Town
in fulfilment of the requirements for the degree of
Doctor of Philosophy

by

Kevin Harding

Department of Chemical Engineering
University of Cape Town
December 2008

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

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Volume 1

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Acknowledgements

I would like to thank the following groups for their assistance throughout this thesis:

- My supervisor, Prof. Sue Harrison (Centre for Bioprocess Engineering Research, Department of Chemical Engineering, University of Cape Town), for all her input and time in this project;

- The Centre for Bioprocess Engineering Research (CeBER), Department of Chemical Engineering (University of Cape Town), for being my base during my postgraduate research;

- Dr John Dennis and the Department of Chemical Engineering (Cambridge University), for time in the department and guidance on the project;

- A/Prof Harro von Blottnitz (Environmental and Process Systems Engineering, Department of Chemical Engineering, University of Cape Town) for his input on Life Cycle Assessment (LCA);

- The National Research Foundation (NRF), the KW Johnstone foundation and the Centre for Bioprocess Engineering Research (CeBER) (Department of Chemical Engineering, UCT), for financial support;

- The UNEP/SETAC LCInitiative for LCA software used; and

- My parents and brothers for all the encouragement and support throughout my studies.

Executive Summary

The intrinsic environmental advantages of industrial scale bioprocesses over chemical processes remain a discussion point owing to limited objective analysis. Studies to date are often limited to energy or global warming considerations with little regard for full Life Cycle Assessment (LCA) analyses. This, in part, may be owing to the difficulty in obtaining the material and energy balance inventory required for such assessment at an early stage in process development. However, these studies are important in designing and selecting environmentally beneficial processes for the conversion of renewable resources to commodity and energy products. The overall objective of the thesis is to obtain the data required to perform these LCA analyses.

To achieve the overall objective, the thesis presents a methodology to obtain the material and energy balance data estimates required for the LCA of industrial bioprocesses through a generic flowsheet model. The flowsheet was presented as a MS-Excel spreadsheet allowing aerobic or anaerobic production of intra- or extracellular products using batch or continuous microbial processes. A database presented in the model facilitates the use of a variety of carbon, nitrogen, sulphur and phosphorus inputs and provides relevant constants and physical data. Typically downstream processing units were taken into account and included downstream chemical inputs (reacting or inert). The model was built using a stoichiometric approach, first principles and rules of thumb.

Various validation studies were presented through case studies where adequate experimental and detailed process modelling data were available. These case studies included penicillin, cellulase and biopolymer production. Each presented three scenarios for the generic flowsheet model where the scenarios represented decreasing sets of input values, showing the sensitivity of the flowsheet models to limited input information. The material and energy balance results were then compared to material and energy results presented in the literature which gave data for specific process models. With a representative set of input data, representative material and energy results were obtained. With a more limited set of input data, increasing uncertainty arose in the material and energy results obtained.

There was a need to analyse the material and energy inventory to determine the environmental impacts of the process. Following the validation of the flowsheet model approach to generate the material and energy inventories, the model outcomes were used in Life Cycle Assessment (LCA) analyses to provide insight into the environmentally sensitive areas of an industrial bioprocess. These were compared to the LCA results from the material and energy balance

inventories obtained from the literature data to further validate the modelling approach. Thereafter, assessment of the Life Cycle Inventory data across four case studies completed was undertaken.

The first case study investigated the production of penicillin by *Penicillium chrysogenum* using a medium of glucose, Pharmamedia and phenoxyacetic acid. The key findings in the study showed that electrical and agricultural inputs were key contributors to LCA impacts. Further, it was found that poor separation efficiencies in downstream processing are the reason for high operating volumes and large recycle flows within a process. This, in turn, increased electrical and steam requirements, thereby LCA scores.

The production of cellulase was investigated using three separate flowsheets. These give a comparison of aerobic and anaerobic microbial processes, different bioreactor designs and different downstream processing units. The study showed that large water volumes associated with low biomass concentrations of a submerged fermentation system increased the energy requirements and thereby LCA scores compared to solid state cultivation. Where there was limited downstream processing, a large volume of low purity product was formed. For the cradle-to-gate approach used in the study, the LCA comparison of these systems was complex.

The third case study considered industrial production of the biopolymer, polyhydroxyalkanoate. The data predicted by the generic flowsheet was compared with the experimental data reported in literature. The comparison showed that 50 % of the material balance values predicted by the model were within 12 % of the literature results for the most comprehensive scenario modelled. Further, it was shown that electrical requirements for aeration dominated the contributions to electricity supply and thereby the LCA scores.

The generic flowsheet model was further used to inform a secondary study into biodiesel production. The material and energy balance requirements for production of the lipase, used in an enzymatic transesterification route, were modelled and the total material and energy balance obtained used in an LCA analysis. This analysis, together with the biopolymer LCA, was used to compare biological and chemical production routes for equivalent products. The LCAs for biodiesel production using a biological catalyst are compared to biodiesel using a chemical catalyst, while the biopolymer polyhydroxyalkanoate is compared to the polyolefins polypropylene, low density and high density polyethylene.

It was shown that there was no clear environmental advantage to either the chemical or biological route of production for an equivalent product. Enzyme catalysed biodiesel production had environmental advantages owing to avoided use of a chemical catalyst and neutralizing acid.

The lower pressures and temperatures required reduced LCA impacts across seven of the ten environmental impacts considered. In the production of polymers, poly-β-butyrate (PHB) production resulted in higher or similar LCA scores compared to polypropylene (PP) and polyethylene (PE) for all impact categories when using the most recent LCA database. This showed that despite the use of fossil fuels during polyolefin production, and the use of renewable resources in PHB production, PP and PE are more beneficial environmentally. The results of the LCA of polyolefins were shown to be sensitive to the database used, with substantial development occurring in these databases over the last few years. The polymer comparison was for the established industrial technology of polyolefin production versus the developing process of PHB production, which is not fully optimised. This optimisation may be expected to ultimately reduce the LCA impacts.

The thesis approaches the validation and comparison studies by interrogating the sensitivity to specified variables in the generic flowsheet model in terms of both the material and energy balance inventory generated and the life cycle impacts. These outputs are used to identify the relative contributions of particular process components and process steps to creation of environment burden, thereby allowing these to be targeted in process improvement.

A sensitivity analysis which varied all individual inputs to the generic flowsheet model for the penicillin production case study was also performed. A detailed set of inputs, based on the sensitivity of LCA results versus changes to the inputs, was used to determine the effects on the energy requirements, LCA scores, reactor volumes and product purities. The detailed set of inputs included yield coefficients, separation efficiencies and aeration rates amongst others. This sensitivity informed the process selection and decision making regarding bioprocess design. Further, the advantages of an improved bioreactor configuration were compared to optimisation options for downstream processing.

The findings from the sensitivity analysis showed that the resulting changes to LCA impacts could be larger than the relative changes to single inputs in the generic flowsheet model. For the flowsheet used, this was shown to be the case for the product to biomass ratio as well as the separation efficiencies of downstream processing units. It was also shown that LCA scores were highly sensitive to changes in aeration rates, biomass concentrations, yield coefficients and additives in downstream processing. Changes to a process that resulted in a greater volume, and in turn greater energy requirements, led to higher LCA scores.

The thesis contributes to knowledge in several ways. It provides a tool for the mass and energy balance calculation for industrial bioprocesses at an early stage of process design, facilitating the

interrogation of feedstocks and technology selection. These early stage calculations can then be used in Life Cycle Assessment (LCA) studies to allow for design considerations before detailed engineering. The interrogation of the technology by several case studies has shown the importance of volume and concentration of the biomass and product in processes. Efficient downstream processing is required to purify the product and reduce process volumes throughout. Effective aeration technologies were important to reduce overall electrical requirements.

In the case studies investigated, it was shown that energy requirements dominated the LCA impacts; hence their reduction is a key area for reducing overall the life cycle impacts. The impacts of carbon source raw materials in bioprocesses, and the agricultural feedstocks needed for these, were also shown to add significantly to LCA scores in certain case studies investigated. The thesis demonstrated that when comparing chemical and biological processes, the environmental advantages should be investigated on a case by case basis.

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List of Acronyms

BPEO	Best Practicable Environmental Option
CIP	Cleainging-in-Place
CML	Centre for Environmental Studies (University of Leiden)
COD	Chemical Oxygen Demand
DSP	Downsteam Processing
EIA	Environmental Impact Assessment
ELUs	Environmental Load Units
EPS	Environmental Priority Strategies
ESAP	Environmental Self-Assessment Program
EULA	End User License Agreement
GMAT	Green Management Assessment Tool
GWP	Global Warming Potential
HDPE	High Density Polyethylene
HPH	High Pressure Homoginisation
IChemE	Institution of Chemical Engineers
ISO	Organisation for Standardisation
LCA	Life Cycle Assessment
LCI	Life Cycle Inventory
LCIA	Life Cycle Impact Assessment
LDPE	Low Density Polyethylene
MS	Microsoft®
ODP	Ozone Depleting Potential (Ozone Layer Depletion)
PE	Polyethylene
PHA	Polyhydroxyalkanoate
PHB	Poly-β-butryic acid
PLA	Polyactides
PP	Polypropyelene
SETAC	Society of Environmental Toxicology and Chemistry
SmF	Submerged Fermentation
SSC	Solid State Cultivation
StOD	Stoichiometric Oxygen Demand
TNS	The Natural Step
TPS	Thermoplastic Starch
UNEP	United Nations Environmental Program
UML	Unified Modelling Language
US-EPA	United States Environmental Protection Agency
WWF	World Wildlife Fund, Worldwide fund for Nature
WWT	Wastewater Treatment

CHAPTER 1: INTRODUCTION

1.1. Context

Bioprocesses are becoming increasingly important for the production of chemical and energy products over conventional chemical synthesis, owing to emphasis on the use of renewable raw materials, the specificity and complexity of biologically catalysed reactions, or both (Lynd 1999, McLauglin *et al*. 2002, Dorsch and Miller 2003, Finlay 2003, Herman and Patel 2007, Herman *et al*. 2008, Lynd 2008). The use of environmental biotechnology, biocatalysis, bioremediation or similar is desired to promote biotechnology. These biological processes are frequently claimed to provide benefit over conventional chemical processes from an environmental or sustainable perspective (OECD 2001, Heller *et al*. 2003 and 2004, Sheehan *et al*. 2004, Gavrilescu and Chisti 2005, Botha and von Blottnitz 2006, von Blottnitz and Curran 2007), owing largely to their mild operating conditions, aqueous systems and the nature of the bio-system used.

The use of agricultural feedstocks could become as large an industry as the petroleum industry is now (CLS NRC 2000, Duncan 2003, Realff and Abbas 2003), motivated by the possibility of positive contributions to a sustainable resource supply (Lynd and Wang 2003). However, review of the literature shows that biological processes cannot generally be assumed to be environmentally favourable (Anex 2003, Geigrich 2003). Gerngross (1999), Gerngross and Slater (2000) and Kurdikar *et al.* (2001) reported that polyhydroxyalkanoate production did not meet expectations with respect to environmental benefits. Roes and Patel (2007) showed that the uncertainties of inputs from a range of products, including various plastics as well as ethanol, were too large to conclude that the environmental risks of biotechnologically produced chemicals were lower than those of fossil-fuel-derived chemicals. McManus *et al.* (2003) found that replacing mineral oil with rapeseed oil in hydraulic systems impacted negatively on all environmental categories, except greenhouse gas emissions. Cunningham *et al*. (2003) showed that more energy is required to make a biolubricant than for a mineral based lubricant, but that less carbon dioxide was emitted during production.

Further, many studies promoting biotechnology from a sustainability perspective, including those of Gerngross (1999), Gerngross and Slater (2000), Kurdikar *et al.* (2001), Dornburg *et al*. (2003), Pietrini *et al*. (2007) and Roes *et al*. (2007), are limited to just two considerations, namely, greenhouse gas emissions and energy consumption. Hall and Scarce (1998), Patel (2003) and Akiyama *et al.* (2003) all showed favourable environmental results in their studies encouraging biological processes, but these too were limited to one or two environmental categories only. Kim and Dale (2003) investigated the cumulative energy requirements of various energy crops to assess differences in the energy requirements compared to petroleum feedstocks. Such studies have encouraged open debate about the unquestioned environmental support for bio-based products (Miller *et al*. 2007).

It is clear that to support claims of benefit in terms of reduced environmental burden or increased process sustainability, rigorous process analysis is required, particularly at an early stage of the process design (Gasafi *et al*. 2003). Such analysis can be used both to inform process technology selection and to target improvements. In order to perform the environmental studies required, a good knowledge of the process as well as the material and energy balance data are needed. These are often not easily obtained or not freely available. Being able to get fast, accurate data to use for, amongst other things, an environmental study is desirable. Further, through use of appropriate analysis tools, environmental burden or advantage can be objectively determined and key contributors identified to assist technology selection.

1.2. Quantification of environmental damage

In order to attempt some measure of environmental damage, several organisations have looked at sustainability and the impacts of environmental change. These include the Club of Rome (Goldsmith *et al*., 1972, Meadows *et al*. 1972, Club of Rome 2008), the International Organisation for Standardisation (ISO) (ISO 14040: 2006, ISO14044: 2006, ISO 2008), the Society for Environmental Toxicology and Chemistry (SETAC) (SETAC 2008), the World Bank (World Bank 2008), WWF (World Wildlife Fund, Worldwide Fund for Nature) (WWF 2008), the United Nations Environmental Programme (UNEP 2008) and the United States Environmental Protection Agency (US-EPA 2008). Between these, academic and other role players, several methods of reporting have been proposed as shown in Table 1.1. These include Environmental Impact Assessment (EIA), Environmental Risk Assessment and Life Cycle Assessment (LCA).

Approaches to assessing environmental and health impacts may also focus on design principles. These approaches include the atom economy (or efficiency) (Sheldon 2000, Trost 2002) or the preferred use of bio-based feedstocks or catalytic reagents (Anastas and Warner 1998).

Table 1.1: Examples of tools and approaches for the quantification and management of environmental impacts

Tools	References
The Natural Step (TNS) framework	Robèrt 1991 and 2000, Robèrt 1997, Natrass and Altomare 1999, Upham 2000.
Factor X (Factor 4, Factor 10)	Schmidt-Bleek 1994 and 1997, von Weizsäcker *et al.* 1995 and 1997.
Best Practicable Environmental Option Assessment (BPEO)	Carlyle 1995.
Environmental Self-Assessment Program (ESAP)	Eagan and Joeres 1997.
Atom Efficiency/Atom economy	Sheldon 2000, Trost 2002
Bio-based feedstock use/Catalytic reagents	Anasta and Warner 1998
Green Management Assessment Tool (GMAT)	Turner *et al.* 1994.
Environmental Impact Assessment (EIA)	EIA 1982 and 1992, EU 1985 and 1997, Wilson 1998, Petts 1999, Saarikoski 2000, Cashmore *et al*. 2004, Glasson *et al*. 2005, Wang *et al*. 2006, Jay *et al*. 2007.

Tools	References
Environmental Impact Indices	Dee *et al*. 1973, Baasel 1985, Spreng 1988, Ellington and Meo 1990, Vesiland 1990, Jones 1992, Hollick 1993, Sheldon 1994, Stephan 1994, Elliott *et al*. 1996.
Environmental Risk Assessment	Kletz 1999, Slater and Jones 1999, Lees 1996, Karman 2000, Darbra *et al*. 2008.
Environmental Management Systems (ISO 14000 series)	International Institute for Sustainable Development 1996, ISO 14001: 1996, ISO 14004: 1996, Pouliot 1996, Jackson 1997, Lee 1997, Steger 2000, Hui *et al*. 2001, Morrow and Rondinelli 2002, Melnyk *et al*. 2003, Ammenberg and Sundin 2005a and 2005b.
Ecoefficiency	WBCSD 1996 and 2006, Saling *et al*. 2002, Shonnard *et al*. 2003, Saling 2005, Saling *et al*. 2005, García-Serna *et al*. 2007, Burnett and Hansen 2008.
Carbon Footprinting (ISO 14064)	IPIECA 2003, WBCSD 2004, 2007, IPCC 2006, ISO 14064: 2006, Eckel, A. 2007, Carbon Trust 2007, Defra 2007, Hammond 2007, Haven 2007, Weidemann and Minx 2007.
Life Cycle Assessment (LCA) (ISO 14040 series)	Fava *et al.* 1991, 1993 and 1994, Heijungs *et al*.1992 and 1996, Guinée *et al*. 1993a, 1993b and 2001, Consoli *et al.* 1993, Keoleian 1993, Pesso 1993, Udo de Haes 1993 and 1996, Udo de Haes *et al.* 1994 and 2002, Vigon and Harrison 1994, Aelion *et al*. 1995, Lindfors *et al*. 1995, Kniel et al. 1996, Allen *et al.* 1997, Barnthouse *et al.* 1997, Bretz and Fankhauser 1997, Christiansen 1997, Steen 1997, Jödicke *et al*. 1999, Tukker 2000, Burgess and Brennan 2001, de Beaufort-Langeveld 2003, Dubreuil 2003, Kotaji *et al.* 2003, Rebitzer and Ekvall 2004, Poulson *et al.* 2005, Russell *et al*. 2005, ISO 14040: 2006, ISO14044: 2006, Li 2006, Udo de Haes and Heijungs 2007, Niederl-Schmidinger and Narodoslawsky 2008.

1.3. Life Cycle Assessment (LCA)

In this thesis, cradle-to-factory gate LCA will be used to quantify the environmental burden associated with industrial bioprocesses. LCA is chosen as it is not location specific, gives results which can be compared readily to other processes and has a strong literature base which gives it a clear definition. In terms of the ISO 14040 standards (ISO 14040: 2006, ISO14044: 2006), the process of Life Cycle Assessment (LCA) is divided into four phases: goal and scope definition, inventory analysis (LCI), impact assessment (LCIA) and interpretation.

The goal and scope definition gives details on the LCA including reasons, audience or geographic information if applicable. A system boundary and functional unit are also defined. The inventory analysis is modelled in a flowchart, defining material and energy inputs and releases of each process in relation to the functional unit. From these inputs and releases, potential environmental impacts are determined. These results are then grouped and weighted according to different criteria. Interpretation involves discussion and conclusions of the assessment at each stage (Figure 1.1).

A more detailed explanation of life cycle assessment, and the way it is used in the thesis, is given in Appendix A.

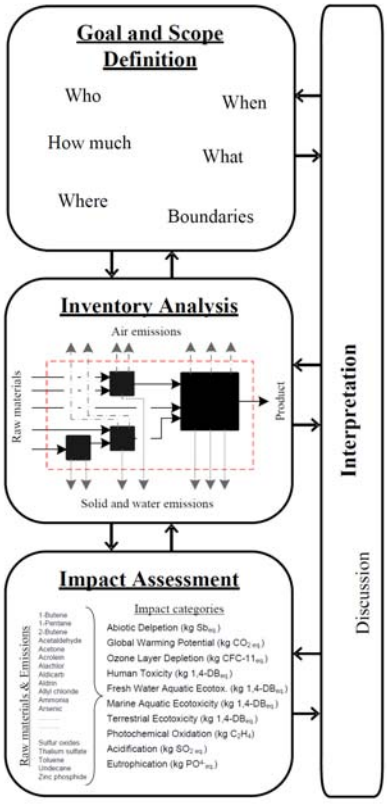

Figure 1.1: Phases of an LCA (modified from ISO 14040: 2006)

1.4. Data gathering

In order to perform a Life Cycle Assessment (LCA) study accurately, the quality of the data gathered is critical. The most important of this data is the inventory data. This is either obtained by collecting actual material and energy flows from a process or, where this is unavailable, by modelling the process using engineering software tools such as Aspen Plus® (AspenTech 2008), SuperPro Designer (Intelligen 2008) or other similar packages. Use of this software can require detailed engineering design which needs to be performed by experts with associated cost.

These software tools frequently do not adequately address the needs of bioprocess modelling. Further, this modelling, to gather information for LCA inputs or other reasons (*e.g.* costing), may require more detail than is available at early process development stages. As such, in order to determine early stage process impacts of bioprocesses, a tool for modelling these flowsheets would be useful.

1.5. Thesis objectives

The thesis covers several topics of concern. These range from comparisons of industrial scale biological and chemical process routes, the use of Life Cycle Assessment (LCA), to the investigation into questions around the sustainability of industrial biological processes. The objectives of the thesis are:

1. To develop a generic flowsheet model to provide material and energy balance data for industrial bioprocesses at an early stage in process development

   The work seeks to establish a quantitative methodology for the rigorous assessment of bioprocesses from an environmental perspective. To do this, complete material and energy balance data are required. For many systems, including bioprocess systems, the desired data are not available. To overcome this, a model describing microbial growth and product formation, sterilisation, downstream processing, purification and simple waste water treatment is developed.
2. To validate the generic flowsheet model

   Using several case studies, material and energy balance data generated from the generic flowsheet model is validated against literature data. Each case study investigates scenarios where less input data is used to generate results. This identifies the sensitivity of variables in the generic flowsheet model.

3. To use these data with Life Cycle Assessment (LCA) methodology as a means of assessing and comparing industrial bioprocess systems

   The data from the validation case studies are used to generate cradle-to-factory gate Life Cycle Assessment (LCA) profiles to determine full environmental impacts of production. These LCA results also give further validation against the LCA results calculated from material and energy balance data from literature values.

4. To use Life Cycle Assessment (LCA) methodology to compare industrial biological processes with chemical processes

   The LCA results are also used to compare LCA results for similar products obtained through chemical processes. The comparisons between biological and chemical processes allow for identification of possible areas of high environmental sensitivity in the biological processes and are investigated in the heuristics section.

5. To obtain heuristics which guide the sustainability decisions of industrial bioprocess systems

   From the comparative analysis of bioprocess systems in the case studies, variables which are most sensitive to change are identified. These variables are investigated by a more thorough sensitivity analysis to determine the effect on reactor volume, energy requirements, and cradle-to-factory gate Life Cycle Assessment results.

6. To investigate the environmental implications of bio-technology selection

   There are several process options available for industrial bioprocess systems. The environmental advantages of such things as submerged fermentation versus solid state cultivation and aerobic versus anaerobic systems are investigated. Other technology selection choices by different process routes to produce a similar product include varied recycle options and using different chemicals as raw materials. The environmental advantages of improved bioreactor production versus optimised downstream processing were also investigated.

1.6. Thesis structure

The thesis is divided into six broad sections:

- Introduction (Chapter 1);
- Generic model development (2);
- Validation of the model by case studies (3 – 5);
- Use of the model to compare biological *vs*. chemical processes (5 – 6);
- Heuristics; and
- Conclusions (7 – 8).

Chapter 1 gives the context, scope and approach to the thesis. It identifies the need for the generic flowsheet model through explaining the need for a rapid, first estimate approach to material and energy balancing in bioprocesses. The chapter also introduces the concept of Life Cycle Assessment (LCA) and its structure in the ISO standards.

The generic flowsheet model development is given in Chapter 2. The model is broken down into microbial growth and product formation, sterilisation, downstream processing and waste treatment. These are further broken down into the individual units (*e.g.* steam sterilisation, filtration, centrifugation, evaporation *etc.*). Assumptions, inputs and further details are given for each, with calculations given in Appendix B. Simplified Unified Modelling Language (UML) diagrams, flow diagrams showing the mathematical logic to the generic flowsheet model, are presented in Appendix D.

Case studies, to inform the validation of the generic flowsheet approach, were selected based on availability of comprehensive data sets to facilitate rigorous comparison. These datasets are limited in the bioprocess literature. Case studies on penicillin, cellulase and bio-polymers are presented in Chapters 3, 4 and 5 as validation studies. Material and energy balance data from detailed flowsheet models (Harrison 1990, Biwer et al. 2005, Heinzle et al. 2006a and 2006b, Zhuang et al. 2007) are compared to the material and energy output generated from the generic flowsheet model. The energy data reported in the studies were for the total renewable and non-renewable energy in the process.

Life Cycle Assessment results are also generated and compared using the software package SimaPro v7.1®. This is used as further validation of the modelled *vs.* literature flowsheet data, giving a sensitivity analysis on the material and energy balance results. The LCA burdens also help identify areas of high environmental sensitivity within the bioprocess flowsheets. These areas of possible environmental concern are investigated further in Chapter 7.

Besides acting as validation, Chapter 5 introduces the concept of comparing a biological product to a similar chemically produced one. Polyhydroxyalkanoate (biopolymer) production is compared to polyolefin production on an LCA basis. The hotspot areas identified earlier are compared and quantified against the chemical process route.

Chapter 6 provides a further case study in which biodiesel production using a chemical or biological catalyst (enzyme) is compared. The enzyme is a raw material in a process which otherwise has chemical inputs. The material and energy balance data for the enzyme are obtained from the generic flowsheet model. The relative LCA impacts of the enzyme in the total LCA score is determined, as are the overall impacts. These are compared to the LCA results from the chemical production route. Areas of high environmental sensitivity in the bioprocess route are assessed against the chemical alternative.

Chapter 7 of the thesis provides a detailed analysis of the sensitivity of the generic flowsheet model. Using Penicillin V as a model flowsheet, each variable entered is varied and LCA scores are obtained. From these results, a group of the most sensitive input variables are selected. These variables are analysed more thoroughly, with reactor volume, electrical requirements, purity and LCA scores compared. Chapter 7 also includes an assessment on the benefits of increasing the bioreactor production versus improving downstream processing.

The findings of the thesis are drawn together in Chapter 8 with conclusions.

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CHAPTER 2: GENERIC FLOWSHEET MODEL DEVELOPMENT

2.1. Introduction

2.1.1. Desirable functions of the model

There are three main desirable features of the generic flowsheet model:

1. It should act as a first estimate bioprocess simulation tool;
2. It is required to calculate all relevant information required for a comprehensive Life Cycle Assessment (LCA) study; and
3. It should require minimal inputs.

These requirements are explained in Table 2.1. It was also desirable to ensure the model was not location specific. Hence, certain aspects of the model may not use the most appropriate options for different geographic regions *e.g.* the use of natural gas in a country like South Africa where coal is found in abundance. It should be possible to modify these options as needed to introduce location specificity as required.

Table 2.1: Requirements of the generic flowsheet model

Main requirement	Secondary requirements
Act as a first estimate bioprocess simulation tool	Allow for a user defined amount of product; calculating required inputs
	Include yield coefficients, growth rates, biomass concentrations in calculations
	Allow for anaerobic or aerobic biomass growth
	Allow for microbial growth and/or product formation
	Allow for solid or liquid and intra- or extracellular product formation
	Allow for batch or continuous operation
	Allow for initial sterilisation
	Allow for downstream processing
	Include relevant cooling (bioreactor-, post bioreactor cooling)
	Calculate agitation and antifoam addition requirements
	Include maintenance calculations
	Include steam for steaming out vessels, backing steam and space heating
	Allow for preheating during sterilisation of media with recycled steam *i.e.* heat integration
	Allow for the addition of reacting and non-reacting chemicals at each process step
Calculate all relevant LCA values	Include full mass balancing (including wastewater, solid waste and air emissions)
	Track different water types (municipal, distilled, de-ionised)
	Include full energy balancing (steam, electricity and natural gas)
	Calculate product purity and recovery
	Calculate chemical oxygen demand (COD) for the wastewater
Require minimal inputs	Allow for the most minimal set of inputs
	Be quick and easy to use by non-specialists
	Call for minimal knowledge on pressure, temperature or operating conditions
	Determine the applicability at early stage of design when limited data are available

Further, the model should provide a framework to allow for future more complex modelling. These future modifications could include more complex biomass growth models, more detailed modelling of individual unit operations as well as the inclusion of complex recycle streams. It is also important that the model provides the required information for economic considerations of both capital and operating expenses.

2.1.2. Model Description

In order to determine the environmental effects of a biological process using an LCA approach, material and energy inputs and emissions are required. These data are often not easily obtained or are not available at all (*e.g.* at the early stage of process design and development). In order to obtain this data, a Microsoft® Excel (MS-Excel) (MS-Office 2008) model has been developed which allows for the use of a limited set of inputs to calculate the data needed. The outputs are the material and energy balance data needed for further LCA analysis. The approach to the generic flowsheet model is based on first principles in many instances, supplemented by data from literature and practice in certain areas.

To explain a typical industrial bioprocess, six levels have been defined. Level 1 is characterised by a bioconversion in a reactor. A microorganism is fed with nutrients (with or without oxygen) to form further biomass, a metabolic product and wastewater as shown in Figure 2.1.

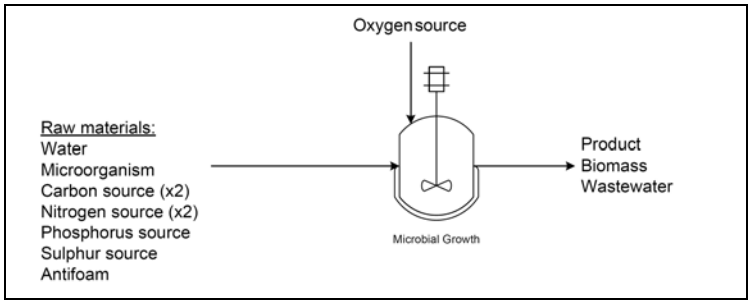

Figure 2.1: Generic bioprocess model (Level 1)

Level 2 includes sterilisation of the raw materials by heat treatment with steam or filtration, as shown in Figure 2.2. Level 3 allows for solid liquid separation after the bioreactor, followed by an optional cell disruption stage. If needed, a second solid liquid separation unit separates biomass debris from the bulk solution as seen in Figure 2.3.

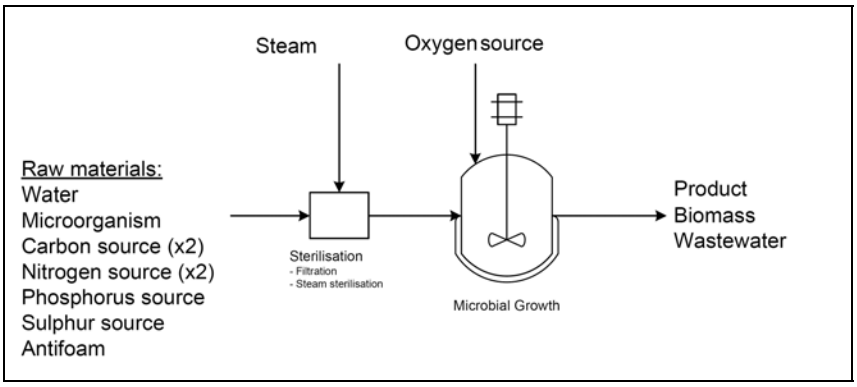

Figure 2.2: Generic bioprocess model (Level 2)

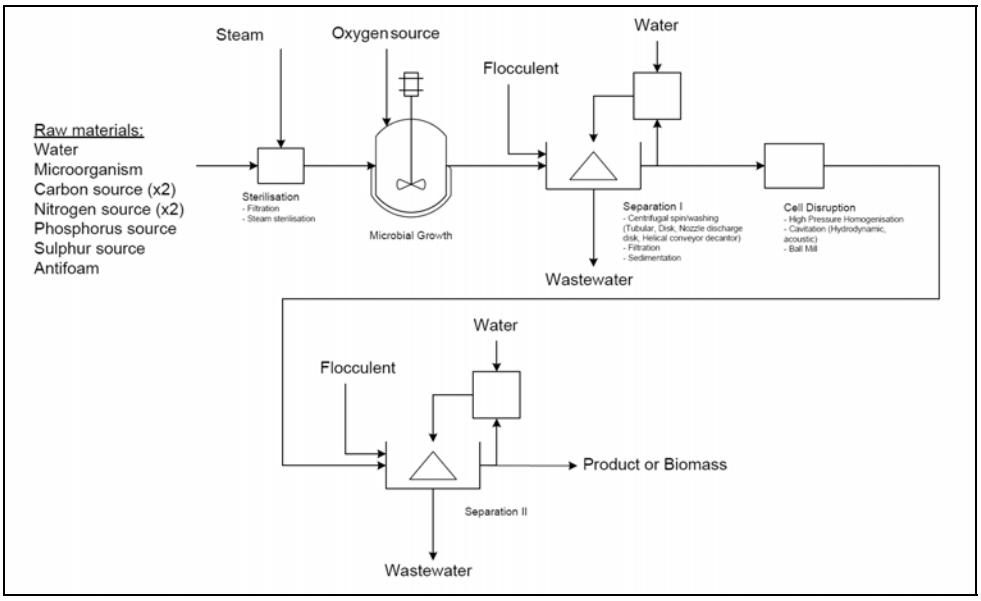

Figure 2.3: Generic bioprocess model (Level 3)

Level 4 includes the addition of a downstream purification train allowing for concentration and purification units as seen in Figure 2.4. These include such unit operations as adsorbers, chromatography units, decantors, evaporators *etc.* Level 5 includes the opportunity for formulation by spray drying, oven drying, freeze drying etc. as shown in Figure 2.5.

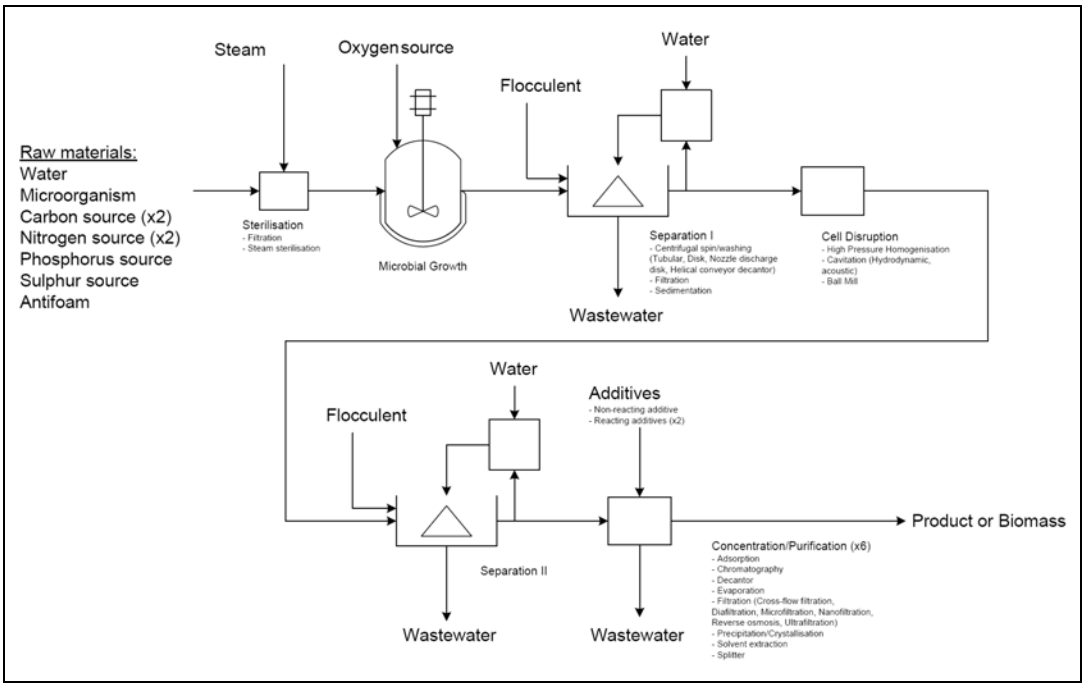

Figure 2.4: Generic bioprocess model (Level 4)

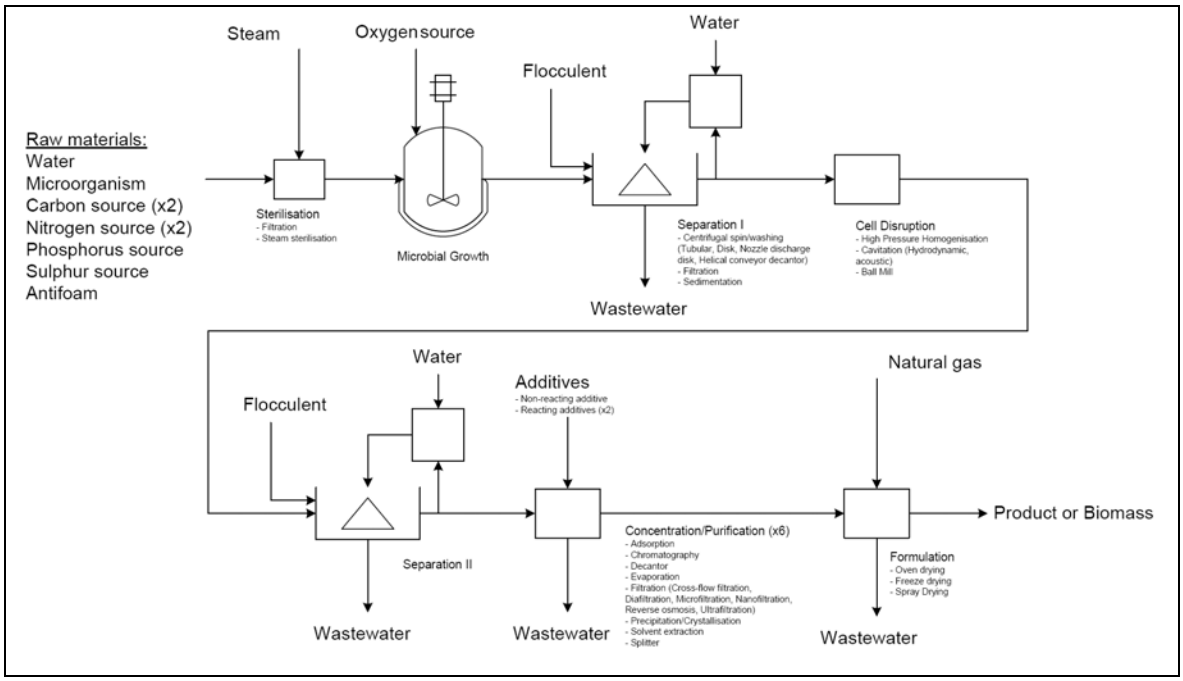

Figure 2.5: Generic bioprocess model (Level 5)

The generic flowsheet developed includes these levels as well as waste water treatment shown in Figure 2.6 (Level 6). The complete model developed allows for microbial growth with intra- or extracellular (liquid or solid) product formation in a batch or continuous set-up. Calculations are based on a pre-determined product amount. Sterilisation, inoculation, microbial growth and product formation are followed by solid liquid separation, cell disruption and further separation. Downstream processing is limited to six concentration or purification steps followed by a final formulation step. No recycle of products is taken into account and downstream reaction is only partially taken into account. The typical flowsheet is given in Figure 2.6.

The model requires a flowsheet to be specified, as well as various parameters and constants associated with the flowsheet design. A database of constants is built into the model for common values and includes yield coefficients, densities and chemical compositions based on chosen parameters. Default values, or values close to typical operating norms, are also provided for many variables (*e.g.* standard operating pressures and temperatures *etc.*). All database and default values can be altered for a specific process if desired. Each unit with its assumptions is explained below with full sample calculations given in Appendix B.

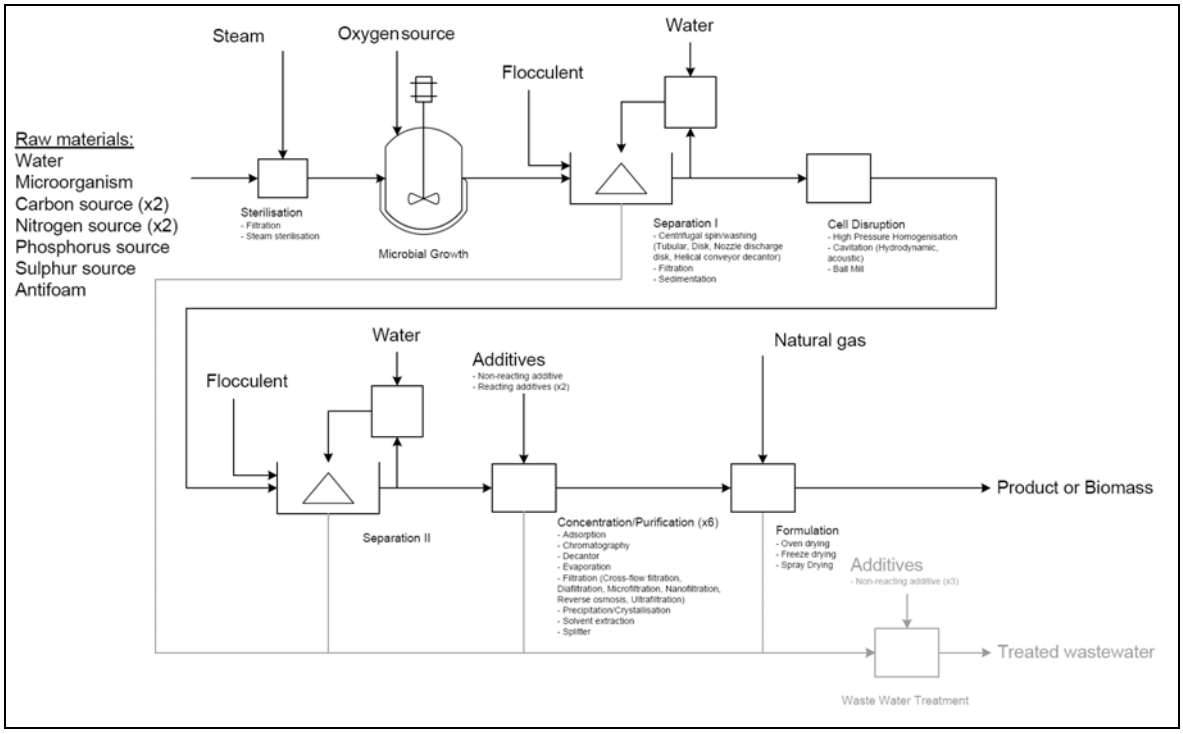

Figure 2.6: Outline of the process flowsheet used in the generic bioprocess model (Level 6)

2.1.3. Structure of the model

The generic model has been developed to give a defined amount of final product, calculating the required raw materials, impurities, energy and unit operation volumes required for this, while taking into account the losses in downstream processing. The model has been developed in Microsoft Excel to be accessible to almost all users. All functions within the MS-Excel software remain operational within the bioprocess flowsheet model.

From the levels explained in section 2.1.2, the individual unit operations and calculations of the model are explained below. The technical detail of each unit, showing the inputs required and calculations associated with each, are given. The model is presented such that the user follows selections in clearly marked cells from the top to bottom of a single MS-Excel sheet to define the desired flowsheet. Depending on choices made, default values common to these are given. These defaults are a mixture of non-numeric values (*e.g.* raw material inputs), estimates (*e.g.* ambient temperatures and pressures), average values (*e.g.* energy requirements for centrifugation), calculated values (*e.g.* heats of vaporisation) and combinations of the above (*e.g.* volume calculations). All defaults can be changed by the user.

The model is structured that advanced users could extend the model capability. Possible additions include process economics, higher level thermodynamic calculations (*e.g.* UNIQUAC) or additional kinetic data.

2.2. Sterilisation

2.2.1. Introduction

Sterilisation is performed by one of two methods: filtration or steam sterilisation. Typical industrial processes use heat sterilisation for between 1 and 90 min at temperatures between 121 and 150°C, depending on the thermal death kinetics of the microbes and containment load (Table 2.2). Sterilisation not performed by filtration or steam is given a default value of 25 MJ/m^3 of material to be sterilised (Equation B.17 in Appendix B). This, like all values in the model, can be replaced with more accurate process specific data if available.

Steam is also used between campaigns (batch operation) or during maintenance (continuous operation) for cleaning. Additional steam requirements are needed in backing steam and space heating.

2.2.2. Filtration

Constant pressure membrane filtration, with no cake build-up is assumed for sterilisation. Energy requirements, assumed to be provided by electricity, are calculated from first principles of the fluid flow through the filter as shown in Equations B.1 – B.3 of Appendix B.

2.2.3. Steam sterilisation

Typical steam requirements for sterilisation are shown in Table 2.2 and Table 2.3. Steam used to sterilise the raw materials in the model is provided continuously at 140°C. The amount of steam needed to heat the media is calculated from an energy balance assuming ambient conditions of 20°C (no heat integration). Preheated feed (from recycled steam) can also be used, increasing the inlet temperature to 60°C (partial heat integration). During the holding cycle of sterilisation, a constant rate of heat loss of 0.003 kW/m^2°C is assumed, an accepted value for insulated vessels (Woods 1995). The steam required to maintain the temperature, against this heat loss, is calculated assuming a surface area to volume ratio of 60 m^2/m^3; the median value for common piping sizes given in Welty, Wicks and Wilson (1969). After being sterilised, the raw materials are cooled by heat exchange with cooling water entering at 18°C to 37°C.

Table 2.2: Sterilisation conditions reported for different microbial growth processes

Product	Type	Temperature	Time	Amount of steam	Reference
		°C	min		
Cephalosporins	Steam	121			Smith 1985
Ethanol	Steam	135-140	1-2	0.035 kg/kg mash	Maiorella 1985
Streptomycin		121	90		Florent 1985

It is assumed that steam is pressurised isentropically, with the energy required supplied by electricity. The mass of steam, electricity and cooling water needed for steam sterilisation are determined by Equations B.4 – B.16 of Appendix B.

Table 2.3: Typical literature values for steam sterilisation

Sterilisation type	Value (kg steam/kg bioreactor medium)	Reference
Continuous sterilisation	0.2	Bartholomew and Reisman 1979, Petridies *et al.* 1989
Continuous sterilisation (143°C, 30s, with energy integration)	<0.1	Gerngross 1999
Batch sterilisation (small scale)	0.8	Bartholomew and Reisman 1979
Batch sterilisation	0.2-0.4	Kalk and Langlykke 1979

***Adapted from Patel *et al.* 2006**

2.2.4. Additional steam requirements

Additional steam requirements are needed for steaming out of the vessel, backing steam and space heating (Equations B.18 – B.28). These requirements are based on data from Dennis (2000) producing protease in a 147 m^3 vessel. For this size vessel, 40 t steam is needed to clean the reactor and 0.082 and 0.02 t steam per day per m^3 (reactor volume) are used in backing steam and space heating respectively. Space heating values from Dennis (2000) are calculated for the United Kingdom, and should be assumed as zero in a warmer South African climate.

2.3. Microbial growth and product formation

2.3.1. Introduction

Microbial biomass, product and by-products formed are related to raw material requirements and emissions produced using stoichiometric equations, yield coefficients and a mass balance approach (section 2.3.2). The default conditions used are 37°C and 1 atm, similar to common literature values for mesophilic processes (

Table 2.5). These values can be altered for a specific process. Reactor volumes and residence times are calculated from variables defined in the system *e.g.* the calculated volumes of inputs, initial and final biomass concentrations, maximum specific growth rates, *etc.*

The product can be defined as a primary product (*e.g.* ethanol), a secondary metabolite (*e.g.* Penicillin) or the microbial biomass itself (*e.g.* baker's yeast). The product can be solid or liquid, intracellular or extracellular. Raw materials needed for the system are broken into those needed for biomass growth and product formation. Stoichiometrically, the carbon, oxygen, nitrogen, sulphur and phosphorus feed, as well as the carbon dioxide and water by-products, are calculated. The addition of antifoam is included if required. The water used in the process may be municipal, distilled or de-ionised, the distinction of which is important in the Life Cycle Assessment (LCA) which follows.

2.3.2. Mass balance – biomass growth and formation

To determine the required raw materials for the specified amount of product, the calculation is split into microbial growth and product formation. From the amount of raw materials and waste calculated in each, and a specific product yield, total flow rates can be obtained.

It is possible to include one of several micro-organisms as shown in Table B.1 of Appendix B. Various experimental data are associated with these to perform the mass balance. The elemental formulae for each micro-organism, as shown in Table B.2 of Appendix B, forms part of the dataset included in the model. Where values are not available, an average value is used.

Products formed are divided into 9 categories: antibiotics, amino acids, enzymes, alcohols, vitamins, carbohydrates, organic acids, alkanes and others (Table B.3 of Appendix B). Chemical formulae for specific products are given. Those that are unknown within a specific grouping are assumed as the average of that group. These are used in the mass balance to calculate raw materials required for a specific amount of product formed. The exact chemical compositions of inputs were given as far as possible as a way to test the sensitivities of different input materials. Product densities are included as these are required for converting mass values to volumes throughout the model.

Biomass growth and product formation are calculated using chemical balances for carbon, oxygen, nitrogen, sulphur and phosphorus, as well as yield coefficients ($Y_{x/s}$ for biomass growth and $Y_{p/s}$ for product formation) (Table 2.4). The general aerobic equation for microbial growth is used for each of biomass growth and product formation (Equation 2.1). Resulting raw materials from each can be added to give the final raw material requirements. The full equations used for biomass growth (B.29 – B.50), product formation (B.51 – B.65) and material balancing (B.77 – B.90) are given in Appendix B. Typical process conditions for microbial systems with reactors greater than 0.3 m^3 are given in Table 2.5.

$$\left(\text{Carbon}_p + \text{Oxygen}_p + \text{Nitrogen}_p + \text{Sulphur}_p + \text{Phosphorus}_p\right)\text{sources}$$
$$\downarrow$$
$$\left(\text{Biomass } or \text{ Product}\right) + \left(H_2O\right)_p + \left(CO_2\right)_p + \left(\text{Nitrogen}_p + \text{Sulphur}_p + \text{Phosphorus}_p\right)\text{wastes}$$

Equation 2.1

Table 2.4: Variables in the generic bioprocess model – mass balance around the reactor

Biomass formation
Unknowns (12)
Carbon source 1, carbon source 2, oxygen source, nitrogen source 1, nitrogen source 2, sulphur source, phosphorus source, water waste, carbon dioxide waste, nitrogen waste, sulphur waste, phosphorus waste
Element Balances (6)
Carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus
Yield coefficient (1)
$Y_{x/s}$
Ratios (2)
Carbon source 1: Carbon source 2, Nitrogen source 1: Nitrogen source 2
Constraints (3)
Nitrogen-, sulphur- and phosphorus sources are only sources of N, S, P respectively in biomass
Product formation
Unknowns (12)
Carbon source 1, carbon source 2, oxygen source, nitrogen source 1, nitrogen source 2, , sulphur source, phosphorus source, water waste, carbon dioxide waste, nitrogen waste, sulphur waste, phosphorus waste
Element Balances (6)
Carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus
Yield coefficient (1)
$Y_{p/s}$
Ratios (2)
Carbon source 1: Carbon source 2, Nitrogen source 1: Nitrogen source 2
Constraints (3)
Nitrogen-, sulphur- and phosphorus sources are only sources of N, S, P respectively in product

Table 2.5: Typical process conditions for microbial systems with reactors greater than 0.3 m^3

Product	**Microorganism**	**Initial inoculum added to reactor**	**Reactor volume**	**Reactor temperature**	**Residence time**	**Aeration rate**	**Agitation rate (power/ volume)**	**Reference**
		v/v%	**m^3**	**°C**	**h**	**vvm**	**rpm (kw/m^3)**	
Amino acids	Coryneform bacteria		50-500	30-34	30-36			[5]
Anthracycline antibiotics	*Streptomyces* sp., *E. coli*	5-10	0.5-12	27-30	67-288	0.5-1.3	125 rpm	[3]
Bakers' Yeasts	*Saccharomyces* sp.			20-32.5				[2]
Cephalosporins		5-7.5	0.5-100	24-28	120-160		(4 kW/m^3)	[9]
Cephamycin		2-10	50-200	28-37	66-90	0.25-0.35		[7]

Product	Microorganism	Initial inoculum added to reactor	Reactor volume	Reactor temperature	Residence time	Aeration rate	Agitation rate (power/ volume)	Reference
		v/v%	m^3	°C	h	vvm	rpm (kw/m^3)	
Clavulanic acid	*Streptomyces* sp.	5	0.3	26		1 (air)	210 rpm	[1]
Glycerol	*Candida* sp., *Pichia* sp., *Saccharomyces* sp.		0.5-50	20-35	72-120			
Lactic acid	*Lactobacillus* sp.	5-10	2	30-60	24-144			[11]
Lincomycin	*Streptomyces* sp.	2-5	50-150	28	144-192		250 rpm (1.7 kW/ m^3)	[4]
Achromobacter delvacvate	*Achromobacter delvacvate*		3	35-36	48			[6]
Methylomonas clara	*Methylomonas clara*		0.01-4	34-40				[6]
Pseudomonas sp.	*Pseudomonas sp.*		6	36-38				[6]
Penicillin G or V	*Penicillium* sp.	10	40-200			0.5-1.0 (air)	(1- 4 kW/m^3)	[10]
Propionic Acid	*Propionibacterium* sp.			30-38	54-336			[8]
Thienamycin	*Streptomyces* sp.	8	50-57	27-28				[1]

***Further examples of bioprocesses smaller than 300 litres are given in Litchfield 1985**

References:

[1] Buckland *et al.* 1985
[2] Chen and Chiger 1985
[3] Flickinger 1985
[4] Gonzalez and Miller 1985
[5] Hermann 2003
[6] Litchfield 1985
[7] Omstead *et al.* 1985
[8] Playne 1985
[9] Smith 1985
[10] Swartz 1985
[11] Vickroy 1985
[12] Wang *et al.* 2001

2.3.3. Anaerobic growth

Both aerobic and anaerobic growth can be modelled in the flowsheeting model (Equations B.66 – B.73 in Appendix B). For anaerobic growth, oxygen is excluded from the mass balance. A secondary (anaerobic) product (Table B.4 in Appendix B) and hydrogen are formed instead of water. In addition to biomass growth and product formation equations, a stoichiometric equation is included to calculate the amount of anaerobic product (Equation 2.2).

$$\begin{gathered}\left(\text{Carbon} + \text{Nitrogen} + \text{Sulphur} + \text{Phosphorus}\right)\text{sources} \\ \downarrow \\ \text{Anaerobic product} + H_2 + CO_2 + \left(\text{Nitrogen} + \text{Sulphur} + \text{Phosphorus}\right)\text{wastes}\end{gathered} \qquad \text{Equation 2.2}$$

2.3.4. Yield coefficients

Yield coefficients, $Y_{x/s}$ (biomass yield on limiting carbon substrate), $Y_{x/o}$ (biomass yield on oxygen), sourced from the literature and summarised in Table 2.6, and $Y_{p/s}$ (yield of product per unit substrate), summarised in Table 2.7, provide information for the stoichiometric calculations used in the model and form part of a database accompanying the model. $Y_{x/s}$ and $Y_{p/s}$ provide the two additional equations required in the elemental balances used. $Y_{x/o}$ is available for validation. These data presented are also used to estimate default data. The closest approximation is chosen should the exact value not be available, according to the decision making framework of Figure 2.7. If available and desired, an alternative value can be entered.

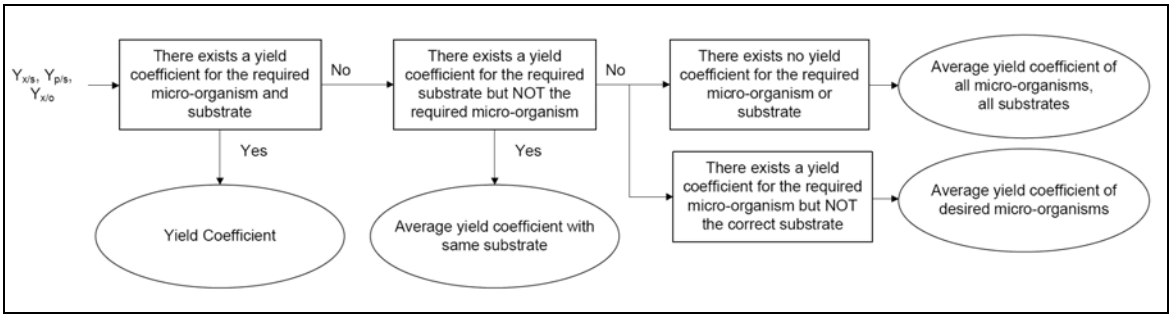

Figure 2.7: Decision method to determine yield coefficients used in generic flowsheet model

Table 2.6: Specific growth rate, concentration and yield for microbial growth

Microorganism	Carbon substrate	Max. specific growth rate, μ_{max}, (half saturation concentration, K_s)	Biomass conc., C_x	Growth yield, $Y_{x/s}$	Growth yield (O_2 consumed), $Y_{x/o}$	Reference
		h^{-1} (mg/l)	g/l	g/g substrate	g/g oxygen	
Achromobacter delvacvate	Diesel oil		10-15			[4]
Acinetobacter sp.	Acetate	0.22	6.85	0.40		[4]
	Ethanol	0.96	1.80	0.75		[4]
	Other	0.4-2.0	8-10	0.10-1.20		[4]
Aerobacter aerogenes	Acetate			0.18	0.31	[1]
	Fructose			0.42	1.46	[1]
	Glucose	1.22 (5)		0.40	1.11	[1], [2]
	Glycerol			0.45	0.97	[1]
	Lactate			0.18	0.37	[1]
	Maltose			0.46	1.50	[1]

Microorganism	Carbon substrate	Max. specific growth rate, μ_{max}, (half saturation concentration, K_s)	Biomass conc., C_x	Growth yield, $Y_{x/s}$	Growth yield (O_2 consumed), $Y_{x/o}$	Reference
		h^{-1} (mg/l)	g/l	g/g substrate	g/g oxygen	
	Mannitol			0.52	1.18	[1]
	Pyruvate			0.20	0.48	[1]
	Ribose			0.35	0.98	[1]
	Succinate			0.25	0.62	[1]
Aeromona hydrophila	Lactose-whey		1.1	0.598		[4]
Aspergillus sp.	Cassava	0.11				[5]
	Carob extract	0.16				[5]
Brevibacterium sp.	Wood			0.44		[4]
Candida sp.	Acetate			0.36	0.70	[1]
	Ethanol			0.68	0.61	[1]
	Glucose			0.51	1.32	[1]
	n-alkane		15.2	1.1		[5]
	Molasses			0.5		[5]
	Sulfite liquor		10.7	0.6		[5]
	Whey		22.5	0.54		[5]
Candida sp.	Other	0.5 (0.2)				[2]
Cellulomonas sp.	Bagasse, barley straw	0.2-0.29	10-16	0.32-0.5		[4]
Chaetomium cellulolyticum	Corn stover	0.24				[5]
Cornebacterium hydrocarboclastus	Propane	0.046	0.9	0.30		[4]
Escherichia coli	Glucose	1.1 (3)				[2]
	Glycerol	0.87 (2)				[2]
	Lactose	0.8 (20)				[2]
Fusarium sp.	Glucose	0.28				[5]
	Carob extract	0.22				[5]
	Other	0.30				[5]
Fusarium moniliforme	Carob extract		3.3	0.71		[5]
Geotrichum candidum	Whiskey distillery wash	0.385	18.0	0.57		[5]
Klebsiella sp.	Glycerol	0.85 (9)				[2]
	Glucose	0.85 (10)				[2]
	Methanol			0.38	0.56	[1]
Methalococcus capsulatus	Methane	0.14	0.4	1.00-1.03		[4]

Microorganism	Carbon substrate	Max. specific growth rate, μ_{max}, (half saturation concentration, K_s)	Biomass conc., C_x	Growth yield, $Y_{x/s}$	Growth yield (O_2 consumed), $Y_{x/o}$	Reference
		h^{-1} (mg/l)	g/l	g/g substrate	g/g oxygen	
Methophilus methylotrophus	Methanol	0.38-0.5	30	0.5		[4]
Methylomonas sp.	Methane			1.01	0.29	[1]
	Methanol	0.14-0.25	9.6-30	0.4-0.6	0.53	[1], [4], [5]
Norcadia sp.	*n*-alkanes	1.25	14.7	0.98		[4]
Norcadia sp.	*n*-propane	0.091	30	1.36		[4]
	n-butane		22			[4]
Paecilomyces variotii	Sulfite liquor	0.31	13	0.55		[5]
Penicillium chrysogenum	Glucose			0.43	1.35	[1]
Penicillium cyclopium	Whey		12.8	0.68		[5]
Penicillium sp.	Milk whey, sucrose, dextrins, starches, starch hydrolysate	0.20	30			[5], [6]
Protaminobacter ruber	Methanol		85			[4]
Pseudomonas fluorescens	Acetate			0.28	0.46	[1]
	Ethanol			0.49	0.42	[1]
	Glucose			0.38	0.85	[1]
Pseudomonas methanica	Methane			0.56	0.17	[1]
Pseudomonas sp.	Fuel oil	0.16	8-16	1.00		[4]
	Methane		0.8	0.70	0.20	[4]
	Methanol			0.41	0.44	[1]
Rhodopseudomonas gelatinosa	Bicarbonate, wheat brain	0.31	3.15-4.33			[4]
Rhizopus oligosporus	Mung bean whey	0.16				[5]
Saccharomyces cerevisiae	Glucose	0.55 (25)		0.50	0.97	[1], [2]
Saccharomyces sp.	Molasses		30-80			[3]
Thermomonospora sp.	Cellulose, pulping fines	0.48	2.3	0.35-0.44		[4]
Trichoderma sp.	Coffee wastes	0.10				[5]

References:

[1] Bailey and Ollis 1986
[2] Blanch and Clarke 1996
[3] Chen and Chiger 1985
[4] Litchfield 1985
[5] Solomons 1985
[6] Swartz 1985

2.3.5. Carbon source

All microbial growth, and associated product formation, requires a source of carbon. The database divides the carbon source into five categories: carbohydrates, hydrocarbons, volatile fatty acids or organic acids, proteins and other. Each has various examples of possible carbon sources with the associated chemical formulas for stoichiometric balancing (Table B.5 in Appendix B). The model allows two carbon sources to be chosen as raw material feed. Should the desired carbon source not be present, the chemical formula can be entered in the form $C_aH_bO_cN_dS_eP_f$.

The model assumes that the carbon source limits the microbial growth and product formation. From the mass balance calculations, 1 mol% excess is added as default. If two carbon sources are added, a mass ratio for the two carbon sources is required. This is a simplification as in biological systems, one source is often used in preference to the other through diauxic growth and catabolic repression.

2.3.6. Nitrogen source

As with the carbon source, two nitrogen sources, either organic or inorganic, can be specific in the generic flowsheet model as raw materials. Examples include ammonia gas, ammonium nitrate, ammonium sulphate and urea as shown in Table B.6 in Appendix B. Nitrogen sources required but not found in this dataset can be entered in the form $C_aH_bO_cN_dS_eP_f$. For the nitrogen source, a default of 5 mol% excess is assumed, unless specified. If two nitrogen sources are added, a mass ratio is required.

Table 2.7: Typical product concentration, yield and productivity for intra- and extracellular microbial products

Product	Microorganism	Carbon substrate	Volumetric product concentration	Product yield, $Y_{p/s}$	Product productivity	Reference
			g/l	g/g substrate	g/l/h	
2,3-butanediol	*Klebsiella oxytoca*	Molasses, pentoses, hexoses	3.4-99	0.25-0.46	0.9-2.7	[10]
Alginate	*Pseudomonas aerugenosa*	Glucose	15-30			[4]
Anthracycline antibiotics	*Streptomyces* sp.	Glucose			1.39-8	[2]
Cephalosporins	*Cephalosporium sp.*		15			[8]
Cephamycin	*Nocardia lactamdurans, Streptomyces clavuligerus*	Corn-steep liquor, distillers' solubles, yeast extract	0.5-2			[7]
Citric Acid	*Aspergillus niger*, yeasts	Glucose, *n*-alkanes, pyruvate		0.7-0.9		[5]

Product	Microorganism	Carbon substrate	Volumetric product concentration	Product yield, $Y_{p/s}$	Product productivity	Reference
			g/l	g/g substrate	g/l/h	
Clavulanic acid	*Streptomyces sp*	Dextrin	1.1			[1]
Ethanol	Yeasts	Molasses	79-86		2.2-10.8	[3]
Glycerol	*Saccharomyces cerevisiae*	Glucose	30-40	0.23-0.28	0.48-1.38	[11]
		Molasses	55-80	0.25	0.48-1.38	[11]
	Candida magnoliae	Glucose	79-170	0.43	0.71-0.83	[11]
	Candida glycerinogenes	Glucose	110-130	0.52-0.63	1.2-1.3	[11]
	Osmotolerent yeasts	Glucose, sucrose	110-130	0.60	2.75	[11]
	Pichia farinosa	Glucose	300		3.12	[11]
	Bacillus subtilis	Glucose	14.7	0.29	0.08	[11]
	Dunaliella tertiolecta	CO_2	0.12		0.0028	[11]
Gluconic acid	*Aspergillus niger*	Glucose		0.90		[5]
Itaconic acid	*Aspergillus terreus*	Glucose, anhydrous sucrose	100-180	0.55-0.65		[5]
Lactic acid	*Lactobacillus sp.*	Pentose sugars		0.90-0.95	1-3	[9]
Propionic Acid	*Propionibacterium*	Fructose, molasses	17.3-23.7	0.48-0.75		[6]
Thienamycin	*Streptomyces sp.*	Corn-steep liquor	1.1			[1]
Xanthan	*Xanthamonas campestris*	Glucose	15-30	0.31-0.60		[4]

References:

[1] Buckland *et al.* 1985
[2] Flickinger 1985
[3] Maiorella 1985
[4] Margaritis and Pace 1985
[5] Milsom and Meers 1985b
[6] Playne 1985
[7] Omstead *et al.* 1985
[8] Smith 1985
[9] Vickroy 1985
[10] Voloch *et al.* 1985
[11] Wang *et al.* 2001

2.3.7. Oxygen source

Under aerobic conditions, an oxygen source is required, either modelled as pure oxygen gas or air (Table B.7 in Appendix B). The default aeration rate is set at ten times the minimum aeration rate calculated stoichiometrically (Equations B.74 – B.76 of Appendix B), unless specified. The volume of air or oxygen enriched air passing through the reactor is often much higher than the stoichiometric amount to ensure sufficient mass transfer and to aid in mixing. Typical volumetric aeration rates are given in

Table 2.5 and Table 2.8. The energy to compress the gas used is included as an electricity value as shown in Appendix B.4. The gas compression can be performed by a one-or two-stage reciprocating, centrifugal or axial compressor with intercooling as shown in Sinnot (1983). Once

compressed, isobaric cooling with cooling water is assumed (Equations B.97 – B.105 of Appendix B). The effects of gas hold up are not included in the model.

Table 2.8: Typical literature values aeration rates

Source	Value (vvm)	Comments
Akiyama *et al.* (2003)	0.5	2500 kPa
Bartholomew and Reisman (1979)	0.2	Production of bacterial insecticide
Fong (1987)	0.5 – 1.0	Stirred tank bioreactor
Kalk and Langlykke (1986)	0.5-2	Air at 100 psia
Petrides *et al.* (1989)	1	Stirred bioreactors, limited to avoid foaming problems
	2	Airlift reactors
Queener and Swartz (1979)	0.5-1.0	Penicillin G or V production
Reisman (1988)	0.5-1.0	Citric acid production at 1.5 atm

*** Adapted from Patel et al. 2006**

2.3.8. Sulphur and phosphorus sources

Sulphur, required for proteins and other sulphur containing products is commonly provided by the sources listed in Table B.8 of Appendix B. As a simplification it is assumed that only the sulphur from the specified sulphur source is used in microbial growth or product formation. Sulphur entering in the carbon, nitrogen or phosphorus source streams is assumed to be removed as waste (SO_4). It is assumed that 5 mol% (default value) excess sulphur enters the system in the sulphur source.

Phosphorus is required to meet the phosphorus content of nucleic acids and any phosphorus in the product. Potential phosphorus sources are listed in Table B.9 of Appendix B. A 5 mol% (default value) excess is assumed.
Even where sulphur or phosphorus are not defined in the biomass or product of the system, a selection is still required to complete the material balance calculations. If this is the case, zero flows will be returned for these source values.

2.3.9. Maintenance coefficient calculations

A portion of the energy source (typically the carbon source) is metabolised to provide energy to maintain biomass functioning. This is accounted for through a maintenance coefficient. Typical values for aerobic growth lie between 0.055 and 0.25 g glucose/g cell.hr (Abbott and Clamen 1973), as illustrated in Table B.10 of Appendix B.

The maintenance coefficient is selected based on the organism. If no data exists, an average is used, based either aerobic or anaerobic values. The amount of carbon needed, defined by carbon source 1, is calculated from this maintenance coefficient and residence time. Under aerobic conditions, the carbon source required for maintenance undergoes complete oxidation to carbon dioxide, water vapour and energy. In the anaerobic process, the carbon source forms an additional anaerobic product. Calculations to determine the amount of carbon source required from maintenance are given in Equations B.91 – B.93 of Appendix B.

2.3.10. Growth rate

Growth rate data allows calculation of the reactor residence time, which is used to calculate agitation energy, maintenance energy *etc.* The required data (maximum specific growth rate, μ_{max}; half saturation constant, K_s and final biomass concentration, $C_{x,\ final}$) is obtained from literature and experimental values (Table B.11 in Appendix B or an average value is assumed (Figure 2.8). Monod kinetics have been assumed in the model developed (Equations B.94 – B.96 in Appendix B) owing to its widespread use. Opportunity exists to expand the model to incorporate other kinetic expressions.

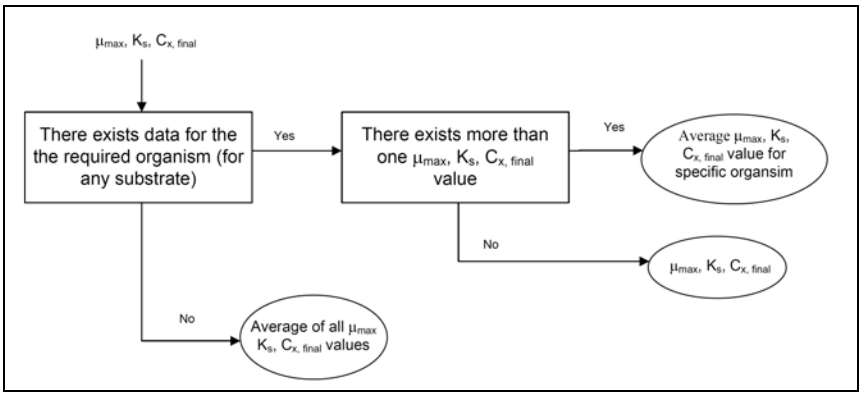

Figure 2.8: Decision method to determine μ_{max}, K_s and r_x values

2.3.11. Reactor cooling

Under aerobic conditions, microbial growth results in energy release at approximately 400 kJ/C-mol produced or 500 kJ/mol O_2 consumed. When using glucose as the main substrate 315 kJ/C-mol produced is released. Under anaerobic conditions, with a glucose substrate, approximately 225 kJ/C-mol are produced *i.e.* approximately 70 % of the aerobic value (Roels 1983).

In the model, the energy generation during aerobic growth is calculated using a degree of reduction basis using Equations B.106 – B.111 in Appendix B. (von Stochar *et al.* 2006). For anaerobic growth 70 % of the aerobic value is used, in accordance with the ratios shown above for glucose metabolism by Roels 1983 (Equation B.112 in Appendix B). Cooling or chilled water is used to maintain the reactor at the specified temperature by heat exchange.

2.3.12. Post microbial growth cooling

Once the required microbial growth and product formation is achieved, cooling of the materials can take place. A default cooled temperature of 15°C is assumed, requiring refrigeration. The refrigeration energy is calculated from compression of the refrigerant. An energy balance of the heat removal required from the microbial stream, with a 30 % compression cycle efficiency, is taken as the electrical energy requirement (Equations B.113 –B.114 in Appendix B.).

2.4. Agitation

During biomass growth and product formation, agitation may be required. the model framework provides a selection grid for agitation requirements and choice of impeller type. Impeller types include: pitch-blade turbine, paddle turbine, Rushton turbine, marine propeller, axial flow impeller, radial turbine, bar turbine, saw tooth dispenser, anchor and helical ribbon impellors. Additional requirements to be considered include the number of tanks used, tank dimensions, agitation efficiency, impeller characteristics and rotational speed.

Agitation power is calculated using a power number (N_p) for a turbulent flow regime as shown in Table B.12 of Appendix B. The power per unit volume is calculated by the method of Sinnot (1983), using power number, impeller speed and diameter. Corrections for different ratios of blade width to impeller diameter (W/D) and gassed systems are included as shown in Dickey (1984) and Atkinson and Mavituna (1983) respectively. These agitation power calculations are detailed in Equations B.115 – B.140 of Appendix B, resulting in power per unit volume. Typical literature values are shown in Table 2.9 for comparison.

Table 2.9: Typical literature values for agitation power requirements for turbulent flow

Source	Agitation power required (kW/m^3)	Comments
Akiyama *et al.* 2003, Gerngross 1999	1	-
Bartholomew and Reisman 1979	1 – 2	-
Fong 1987	0.2 – 0.7	Mild mechanical agitation
	0.2 – 2.2	Antibiotics (stirred tank bioreactor)
	3.7	Yeast (stirred tank bioreactor)
	2.2 – 6	Biomass production (stirred tank bioreactor)
Kalk and Langlykke 1979	1 – 3	-
Petrides *et al.* 1989	0.2 – 1	Mild agitation
	1.5 – 3.5	Antibiotic production
	3.5 – 5.0	Yeast production
	>7	Xanthum gum production
Seider *et al.* 1998	2	Slurry
Reisman 1988	2 – 4	
Patel *et al.* 2006	0.5 – 1	
	8 – 12	Viscous mixtures

***Adapted from Patel *et al.* 2006**

2.5. Downstream processing

Typical downstream processing operations require the recovery of the product and its purification from contaminating material. Unit operations typically used include filtration, centrifugation, solvent extraction, precipitation and freeze drying as shown in Table 2.10 with recoveries and yields as shown in Table 2.11. The downstream processing capability of the model is divided into solid-liquid separation for biomass concentration, cell disruption, a second solid-liquid separation for debris removal, six concentration and purification steps and a formulation step. The model includes the downstream units to provide for: adsorption, ball milling, cavitation, centrifugation, chromatography, decanting, evaporation, filtration, high pressure homogenisation, precipitation, crystallization, sedimentation, solvent extraction, splitter, oven drying, freeze drying and spray drying. Temperatures are tracked through the downstream process for energy calculations. Unless as a result of heat exchange, it is assumed that there is no heat loss in downstream processing.

Table 2.10: Literature review of commonly used downstream process units

Product (Microorganism)	Separation[a]	Concentration and Purification[b]	Formulation[c]	Reference
Anthracycline antibiotics (*Streptomyces* sp., *E. coli*)	Broth filtration, solvent extraction ($CHCl_3$, butanol) centrifugation	Precipitation (dissolved with *n*-butanol. Acetone added)	Freeze drying	Flickinger 1985
Cephalosporins	Filtration (rotary drum), solvent extraction (methyl isobutyl ketone)	Precipitation (acetone added), adsorption (Activated carbon, non-polar resins), enzyme treatment		Smith 1985
Cephamycin	Solvent extraction (acetone (aq.), 50 % v/v.)	Evaporation		Omstead *et al.* 1985
Citric Acid (*Aspergillus niger*, yeasts)	Filtration, solvent extraction (butan-2-ol, tributyl phosphate), centrifugation	Precipitation (calcium citrate)		Milsom and Meers 1985a
Glycerol (*Aspergillus niger*)	Broth filtration, centrifugation	Precipitation, evaporation, ion exchange	Spray drying	Milsom and Meers 1985b
Itaconic acid (*Aspergillus terreus*)	Broth filtration, centrifugation	Precipitation, evaporation		Milsom and Meers 1985b
Lactic acid (*Lactobacillus* sp.)	Filtration, solvent extraction, distillation	Precipitation, evaporation		Vickroy 1985
Lincomycin (*Streptomyces* sp.)	Filtration (4.0 % filter aid before filtration. Water washed), solvent extraction (activated carbon, *n*-butanol)	Ion exchange (cationic resins), partition chromatography (cyclohexane, methyl ethyl ketone)	Freeze drying	Gonzalez and Miller 1985
Penicillin G or V (*Penicillium* sp.)	Filtration (rotary vacuum drum), solvent extraction (amyl acetate, butyl acetate, cyclic ketones)	Precipitation (potassium or sodium added), centrifugation or filtration	Drying (pre-dried with anhydrous isopropyl alcohol, butyl alcohol. Dried with warm air, vacuum or radiant heat)	Swartz 1985
Polysaccharides (*Xanthamonas campestris*, *Pseudomonas aerugenosa*)	Centrifugation, milling	Precipitation (alcohol, salt and acid)	Forced air or vacuum drying	Margaritis and Pace 1985
Streptomycin	Broth filtration	Precipitation, adsorption (activated carbon, non-ionic resins, alcohol, acid)		Florent 1985
Thienamycin (*Streptomyces* sp.)	Pressure rotary filtration	Adsorption (Dowex 1x2 (HCO_3^-) resin)		Buckland *et al.* 1985
Yeasts (Bakers') (*Saccharomyces cerevisiae, S. uvarum, S. carlsbergensis)*			Freeze-, Roto-Louvre-, through circulation-, air-lift- and spray drying	Chen and Chiger 1985
Yeast (*Saccharomyces cerevisiae*)	Filtration (plate and frame), centrifugation			Smith 2005

a: Separation is defined as solid liquid separation.
b: Concentration and purification are meant as the same thing here. This is the increase in product purity by any means of downstream processing. Strictly speaking, this may also include the unit operations defined in separation and formulation.
c: Formulation is defined as the final stage in downstream processing. It includes processes aimed at reducing the moisture content of the product. Typical examples include oven drying, freeze drying and spray drying.

Table 2.11: Approximate product recoveries and concentrations in downstream processing units

Product	Separation	Concentration	Formulation	Overall Recovery	Purity	Reference
Cephalosporins				70 %	70-80 %	Smith 1985
Cephamycin				94 %*		Omstead *et al.* 1985
Glycerol				50-90 %	low	Wang *et al.* 2001
Gluconic acid					50 %	Milsom and Meers 1985a
Lactic acid		Evaporation: 8 % (solids concentrated to 52-82 %)				Vickroy 1985
Lincomycin				50-90 %		Gonzalez and Miller 1985
Penicillin G or V	Filtration: 90-95 % Solvent extraction: 80-90 % (single stage) 92-96 % (lead trail)		Crystallisation: 95 % Drying: 95 %	78 %		Swartz 1985
Streptomycin				75-80 %	99 %	Florent 1985
Thienamycin	Filtration: 80 %	Adsorption: 66 % Other concentration: 9-87 %		36 %	30 %	Buckland *et al.* 1985
Yeast (Bakers')	Dewatering: 18-22 % concentrated to 28-33 % solids)				Wet basis: 27-30 % Dry basis: 92-96 %	Chen and Chiger 1985
Yeast	Filtration: 90% solids removed					Smith 2005

*only includes steps AFTER filtration

2.6. Solid-liquid separation

2.6.1. Introduction

Product recoveries and waste removal are tracked through the flowsheet to determine overall recoveries and purities. In each separation step, liquid and solid removals from the desired streams are given according to the type of separation shown in Table 2.12. Depending on the phase of the product (liquid or solid), the desired flow is fed to the next unit, while the waste fraction is removed. Where the "Other" method of solid-liquid separation is used, a default electricity value of 500 MJ/m^3 is assumed (Equation B.162 of Appendix B), a representative value for all solid-liquid separation units.

Table 2.12: Product fractions recovered and waste fractions removed in separation units

	Solid or product fraction removed	Liquid or waste fraction removed
Centrifugal spin/washing	0.98	0.80
Filtration	0.95	0.70
Sedimentation	0.90	0.60
OTHER	0.94	0.70

2.6.2. Centrifugal spin and washing

Five forms of centrifugation are provided in the model: tubular, disk, nozzle-discharge disk, helical conveyor decanter or "other", each with a specific energy requirement per volume taken from Perry *et al*. (1984) (Table B.14 of Appendix B). Typical power requirements for centrifugation from the literature are given in Table 2.13. There is good agreement with the Perry *et al*. (1984) data for yeasts. However, bacterial cultures require greater energy owing to lower settling velocities, resulting from smaller cell size.

Table 2.13: Typical centrifuge power requirements

Source	Centrifuge power requirements (kJ/m^3)	Comment
Bacterial harvesting		
Bohlmann 2002	26 640 – 90 000	Axial solid ejecting centrifuge 37 kW, 1.5-5 m^3/h
Tutunjian 1985	22 320	Continuous disc-type centrifuge, 37 HP motor, water removal rate 5 m^3/h
Yeast Harvesting		
Bohlmann 2002	2 520 – 9 000	Nozzle centrifuge 149 kW 50-200 m^3/h concentrate 10-50 m^3/hr
Kalk and Langlykke 1986	8 280	Continuous desludging disk centrifuge, 4 m^3/h
Steffens *et al.* 1999, 2000	5 400	-
Tutunjian 1985	5 040	Continuous disc-type centrifuge, 37 HP motor, water removal rate 5 m^3/h

***Adapted from Patel *et al.* 2006**

The generic flowsheet model is used to calculate energy requirements from the data in Table B.14 and mass balance data assuming the solid and liquid recoveries and removals in Table 2.12 (Equations B.143 – B.148 of Appendix B). Repeat water (municipal, distilled or de-ionised) washing is allowed using mass balances to calculate further losses and accounting for the energy requirements of repeated centrifuges.

2.6.3. Filtration

The material and energy balance calculations for filtration assume constant pressure membrane filtration, with no cake build-up. Energy requirements, assumed to be provided by electricity, are calculated from first principles of the fluid flow through the filter. The mass and flux of the material entering the filter, as well as the cross sectional area of the filtration unit, are used to calculate the linear velocity. From this, the power and energy requirements are calculated (Equations B.149 – B.153). A filter media and flocculent (Table B.15 of Appendix B) can also be added (Equations B.187 – B.161).

2.6.4. Sedimentation

A flocculent (Table B.15) can also be added for a sedimentation unit operation. It is assumed that there is no energy requirement for sedimentation.

2.7. Cell disruption

2.7.1. Introduction

Cell disruption to release intracellular products can be performed chemically (using *e.g.* chloroform, toluene, EDTA or lysozyme), by freeze-thaw cycling or by mechanical stress (*e.g.* cavitation, bead milling or high pressure homogenisation) (Engler 1985, Harrison 1991, Willson 1999). The model allows for mechanical disruption by high pressure homogenisation (HPH), cavitation (hydrodynamic or acoustic) or a bead mill only, being the preferred approaches for large scale processes.

Typical disruption efficiencies (%) and energy productivities (mg/J) are summarised in Tables 2.14 to 2.16. These are used to estimate appropriate default values for the model, where necessary. It is assumed that product not released on cell disruption is lost on biomass separation in further processing. Energy requirements for high pressure homogeniser, cavitation and ball mills are calculated by Equations B.163 – B.164 of Appendix B. Where the "Other" method of cell disruption is used, a default electricity value of 25 MJ/m^3 is assumed (Equation B.165 of Appendix B), a representative value for all cell disruptions.

2.7.2. High Pressure Homogeniser

Extent of cell disruption and energy productivity (mg/J) on high pressure homogenisation are shown in Table 2.14. Data are given for *Saccharomyces cerevisiae* and *Candida utilis*. Cell disruption of bacteria requires less energy than yeasts (Harrison 1991a, 1991b). It is also

possible to reduce the energy requirement of disruption by chemical pre-treatment of the suspension (Bailey *et al*. 1995, Anand *et al*. 2007). Although other literature, including Hetherington *et al.* (1971), Follows *et al*. (1971), Doulah *et al.* (1975) and Sauer *et al*. (1989) give some data on protein release versus homogeniser pressure and number of passes, the required data for the model are not presented in these texts. A basic estimation of average values is used for other micro-organisms.

Table 2.14: Literature values for extent of disruption and energy efficiency of a high pressure homogeniser

Organism	Disruption	Biomass concentration	Energy productivity
	%	%w/v	mg/J
Saccharomyces cerevisiae	90	17	1.65
	94	20	1.25
Candida utilis	90	20	1.26
	87	20	0.83
Average	90.3	19.3	1.25

Reference:
Engler 1985

2.7.3. Cavitation

Cavitation data are split into hydrodynamic and acoustic cavitation. Energy productivity (mg/J) and energy efficiency (J/ml) values obtained from Save *et al.* (1994) and Kumar *et. al*. (2000) have been used in the model (Table 2.15). Additional literature on cavitation for the total or partial release of intracellular material have been reported by Save *et al*. (1997), Balasudaram and Pandit (2001), Gogate and Pandit (2001), Sundaram *et al*. (2003), Balasudaram and Harrison (2006), but these do not include the required data for the model.

Table 2.15: Default values for cavitation calculations

Organism	Disruption	Biomass concentration	Energy productivity	Reference
	%	%w/v	mg/J	
Hydrodynamic cavitation				
Saccharomyces cerevisiae	90	-	0.02	[1]
Acoustic cavitation				
Yeast cells	90	2.5 %w/w	1500 J/ml	[2]
	90	2.5 %w/v	0.00689 mg/J	[2]

References:
[1] Kumar *et. al.* 2000 [2] Save *et. al* 1994

2.7.4. Ball mill

Cell disruption (%) and energy productivities (mg/J) used in the model are given for a ball mill in Table 2.16. Data are given for *Saccharomyces cerevisiae*, *Saccharomyces carlsbergensis* and *Candida utilis* (Engler 1985). Additional literature on mills have been reported by Woodrow and Quirk (1982), Agerkvist and Enfors (1990) and Melendres *et al*. (1993), but these do not include the required data for the model.

Table 2.16: Default values for ball mill calculations

Organism	Disruption	Biomass concentration	Energy productivities
	%	%w/v	mg/J
Saccharomyces cerevisiae	80	6	0.51
	80	11	1.11
	80	16	1.11
	85	10-20	0.93
	95	17	1.26-1.39
	65	13.5	2.32
	42	13.5	1.25
	40	13.5	5.4
Saccharomyces carlsbergensis	95	17	1.26-1.39
Candida utilis	85-90	17	0.84-1.07
Average	75	14	1.62

Reference:
Engler 1985

2.8. Concentration and purification

2.8.1. Introduction

A maximum of six concentration and purification units can be modelled to achieve a desired product purity from solution. These include adsorption, centrifugation, chromatography, decanting, evaporation, filtration, precipitation or crystallisation, solvent extraction and splitters. Where the "Other" method of concentration and purification is used, a default electricity value of 10 MJ/m^3 is assumed (Equation B.192 of Appendix B), which is an estimated average energy based on the requirements for all other concentration and purification units.

Material balancing for centrifugation and filtration is based on solid and liquid fractional removals. For precipitation or crystallisation, it is assumed that no product is lost or waste

removed. For adsorption, chromatography, solvent extraction and decanting, it is assumed that 99 % of the product phase is retained and 95 % of the waste phase is removed as shown in Table 2.17. All default numbers are estimations based on ideal conditions or the expectations from Table 2.11 and are changeable. It is assumed that all phase changes are ideal.

Table 2.17: Product fractions recovered and waste fractions removed in concentration or purification units

	Solid or product fraction removed*	Liquid or waste fraction removed*
Adsorption	0.99	0.95
Centrifugation	0.98	0.80
Chromatography	0.99	0.95
Evaporation	1.00	0.90
Filtration	0.95	0.95
Precipitation or crystallisation	1.00	0.00
Solvent extraction and decanting	0.99	0.95
OTHER	0.99	0.80

In the separation steps, additional materials may be added to facilitate separation through, for example, phase changes, ionic modification, flocculation *etc*. It is assumed that these materials do not react, but flow through the system in the same manner as other materials (Equations B.166 – B.168 of Appendix B). Further, two reactants can also be added to facilitate separation. Following reaction, the products formed also separate in the same manner as other materials (Equations B.169 – B.170 of Appendix B). The reacting and non-reacting chemicals accommodated by the model are listed in Table B.17 of Appendix B.

2.8.2. Adsorption and chromatography

The required energy for both adsorption and chromatography is calculated as the pumping requirement to contact the liquid across a cross sectional area under a given pressure. Equations B.171 to B.174 of Appendix B used are based on first principles.

2.8.3. Centrifugation

The material and energy balance for centrifugation uses the approach presented in Section 2.6.2. Since the need for centrifugation during concentration or purification is more limited than in solid-liquid separation, the approach is simplified (Equation B.175 of Appendix B). No choice of type of centrifuge is allowed. Instead the average power per unit volume across types of centrifuges listed in Table B.14 (Appendix B) is used as default. Repeat centrifugation and wash cycles are not accommodated.

2.8.4. Decanting

In the decanter model, two phases are defined. The same approach is used as for solvent extraction as shown in Section 2.8.8, except no solvent is added. On separation, the product phase reports to the next unit operation while the waste phase is removed.

2.8.5. Evaporation

The energy to heat and evaporate water from the product is calculated from first principles, using both specific heat capacity and the energy of vaporisation as detailed in Equations B.176 – B.180 (Appendix B). Potential for energy integration within the system, as well as multi-effect evaporation, was not included. These would both reduce energy requirements.

The energy is obtained from natural gas as shown in Equations B.181 – B.182 of Appendix B. Typical energy requirements reported in the literature for different evaporators are given in Table 2.18. If natural evaporation is required, then this option of the model should not be used. Natural evaporation is accounted for by selecting the unit operation option 'other' in the model and setting energy per unit volume to zero.

Table 2.18: Typical literature values for steam and electricity requirements for different evaporation units

Source	Steam and electricity requirements		Comments
	(kg steam/kg evaporated)	(kWh/kg)	
Broklebank 1990	0.7	-	Two-stage evaporator
	0.2	-	Five-stage evaporator
	0.14	0.013	Five-stage mechanical compression
IPTS 2003	1.2 – 1.4	-	Single stage evaporator
	0.1 – 0.3	0.002	Multistage thermal vapour recompression
	-	0.01	Mechanical vapour recompression
Gerngross 1999	0.25	-	Triple-effect evaporator
Lavis 1996	1.1	-	Single-effect evaporator
	0.3 – 0.4	-	Triple-effect evaporator
	0.005	0.04	Single-effect mechanical vapour recompression
Lo 1996	0.4	-	Solvent recovery
Reisman 1988	0.2	-	-
Schweitzer 1997	0.92	0.0035	First effect, no recompression, 22 891 lb steam/h, 40 kW
	0.32	0.0049	Third effect, no recompression, 7 997 lb steam/h, 55 kW
	0.27	0.0053	Third effect, TVR recompression, 6 649 lb steam/h, 60 kW
	0.01	0.0344	Third effect, MVR recompression, 300 lb steam/h, 390 kW
Seider *et al.* 1998	1.25	-	Heuristic

***Adapted from Patel *et al.* 2006**

2.8.6. Filtration

Filtration in the concentration or purification section of the generic flowsheet model allows for five subcategories of filtration. Each has an associated energy per unit volume requirement as shown in Table 2.19. This is used to calculate the energy requirement as shown in Equation B.183 (Appendix B). This energy requirement should correspond to a pressure drop lower than the suggested limit of 3 atm (constant pressure filters) as suggested by McCabe and Smith (1976). Material balance calculations are performed as in filtration calculations of the solid-liquid separation units.

Table 2.19: Default energy per unit volumes for the different filtration options in the generic flowsheet model

Filtration method	Energy per unit volume (MJ/m^3)
Diafiltration	18
Microfiltration	7.2
Nanofiltration	252
Reverse osmosis	32.4
Ultrafiltration	18
Other	20.2[#]

#Other equals the average energy per volume for all filtration types
***Adapted from Patel *et al.* 2006**

The diafiltration option allows for the addition of a diafiltration solution (salt and water). The addition of the filter media details are included to allow for a complete Life Cycle Assessment study (Table 2.20. As in previous filtration models in solid-liquid separation, there is also allowance for a flocculent and provision is made for the filter media used (Equations B.184 – B.189 of Appendix B).

Table 2.20: Possible filtration types and filter medium

Filtration types
Diafiltration, Microfiltration, Nanofiltration, Reverse osmosis, Ultrafiltration, OTHER
Filter medium
Diatomaceous earth, Filter paper, Expanded perlite, Sintered glass, Wiremesh, OTHER
Diafiltration solutions
Water, Sodium phosphate solution, Sodium hydroxide solution, Sodium chloride solution, Other

2.8.7. Precipitation or crystallisation

Electrical energy requirements for precipitation or crystallisation are based on the energy requirement for agitation, with a default power per unit volume value of 0.8 kW/m^3 assumed (a mild to medium mixing value for precipitation as shown in Table 2.21) and an efficiency of 80 %. The unit can be heated with steam and cooled again before further processing. Both the steam and cooling water needed for this are included in material balance calculations.

In modelling the precipitation or crystallisation unit, precipitating chemicals are assumed to undergo a perfect phase change from liquid to solid. The solids formed can then be removed in further processing (*e.g.* filtration or centrifugation) as desired to further purify the product.

Table 2.21: Power requirements in baffled tanks

Agitation	Applications	Power
		kW/m^3
Mild	Blending, mixing	0.04 – 0.10
	Homogenous reactions	0.01 – 0.03
Medium	Heat transfer	0.03 – 1.0
	Liquid-liquid mixing	1.0 – 1.5
Severe	Slurry suspension	1.5 – 2.0
	Gas absorption	1.5 – 2.0
	Emulsions	1.5 – 2.0
Violent	Fine slurry suspension	> 2.0

Reference:

Sinnot 1983

2.8.8. Solvent extraction

Modelling of solvent extraction requires the addition of a solvent, selected from Table B.17 of Appendix B, and the defining of 2 phases: product and waste. The product phase reports to the next unit operation, while the chemicals in the waste phase and the remaining liquid phase are removed according to the separation efficiency specified.

2.8.9. Splitter

Defined by a split ratio of product to waste, a splitter can also be modelled in downstream processing (Equation B.191 of Appendix B).

2.9. Formulation

2.9.1. Introduction

The final step in the generic flow sheet model is formulation. This can take the form of oven-, spray- or freeze drying. Energy is supplied as natural gas for spray drying and oven drying or electricity for freeze drying. It is assumed that 99 % of the product is retained in the formulation step and that 99 % of the moisture is removed in this unit. Typical final moisture contents can be as low as 2 % in baker's yeasts, with dryer temperatures as high as 150°C (Chen and Chiger 1985). The heat lability of many bioproducts implies that careful control of the effective temperature of the product, and time of exposure, is required. Typical literature values for different drying methods and conditions are shown in Table 2.22. Where the "Other" method of formulation is used, a default electricity value of 15 MJ/m^3 is assumed (Equation B.201 of Appendix B), a representative value for all formulation units.

Table 2.22: Typical literature values for different drying methods

Drying method	Steam and electricity requirements		Temperature	Time	Drying rate (Final moisture content)	Comments	Reference
	kg steam/kg evaporated	kWh/kg	°C	min	kg water/h (%)		
Belt dryer	1.38	-				Approx. 60°C	[1]
Co-current drum dryer	1.76	-				Evap. of 46 t/h water	[1]
Fluidised bed dryer	0.24	-				25 bar steam produces 3 bar heating steam	[1]
Spray dryer	3	0.1				-	[2]
Spray dryer	1.62 – 2.33	-				1, 2 & multistage dryers	[3]
Spray dryer	2	-					[4]
Tower dryer	2.0 – 2.4	0.1				No heat recovery	[5]
(Unknown)	1.2 – 1.67	0.25 – 1				Drying of food	[1]
Air-lift dryer			100-150	10-240	160-350 (7)		[6]
Freeze drying			-35				[6]
Heated air			38		(7-8)		[6]
Roto-Louvre drying			50-60	600-1200	5400	Size: 4.85 x 2.2	[6]
Spray dryer			In: 100-120 Out: 65-67		(5-6)		[6]
Through circulation dryer			28-50				[6]

References:

[1] IPTS 2003
[2] Bartholomew and Reisman 1979
[3] Energy Centre Denmark 1992
[4] Gerngross 1999
[5] Reisman 1989
[6] Chen and Chiger 1985

2.9.2. Oven drying

The energy required to heat material and evaporate moisture by oven drying is calculated from first principles as shown in Appendix B. The energy required is obtained from natural gas. Energy calculations are similar to those for steam used elsewhere in the model.

2.9.3. Freeze drying

The energy to freeze dry the product is determined by the energy needed to cool the liquid to freezing, to freeze and subcool it, and the energy to create a vacuum in the container (Equations B.193 – B.197 of Appendix B). It is assumed that no external energy inputs are required to sublimate the liquid from the product. The energy required for vacuuming pumping is calculated as for other pumping requirements as shown in Equations B.171 – B.174 of Appendix B.

2.9.4. Spray drying

Energy data for spray drying is calculated according to the energy balance given by Baker and McKenzie (2005) for spray drier energy requirements. In the model, a simplification is made that the thermal loss factor is zero (*i.e.* adiabatic system) and the calculation reduces to a simplified form as expressed by Keey (1992) as shown in Equation B.199 (Appendix B).

2.10. Wastewater treatment (WWT)

A simplified wastewater treatment scenario is included whereby wastewater collected in the discarded streams may be neutralised by up to three chemicals (Equations B.202 – B.204 of Appendix B). A stoichiometric oxygen demand (StOD) value, as shown in IChemE (2003) (Equations B.205 – B.207 of Appendix B), has been included and can to be used as a water pollution value. Opportunity exists to expand the model to include more appropriate wastewater treatment scenarios.

2.11. Structure for the remainder of the thesis

To support Chapter 2, several appendices are presented at the end of the thesis. These include:

- Appendix B: Contains the generic flowsheet calculations, and all mathematical equations used in the model;
- Appendix C: Nomenclature used in Appendix B;

- Appendix D: UML Diagrams for the generic flowsheet model. A simplified Unified Modelling Language (UML) diagram to describe the full logic to the material balance of the generic flowsheet model; and
- Appendix E: Using the generic flowsheet model. A guide to using the MS-EXCEL model.

Subsequent chapters validate the material and energy balance results of the generic flowsheet model using several case studies. These case studies, Penicillin V, cellulase and poly-β-butryic acid (PHB) production (Chapters 3, 4, and 5 respectively), determine the accuracy of the model against rigorous material and energy balance calculations found in literature. Different process configurations are used to include, for example, aerobic versus anaerobic bioreactors and submerged fermentation versus solid state cultivation.

These case studies are also used to generate a collection Life Cycle Assessment (LCA) studies for microbial bioprocesses. These LCA studies add further validation to the material and energy balance results and highlight the key biological issues in the flowsheets. Certain LCA results are also compared to the equivalent chemical processes. This is done to determine if any common issues are raised and to calculate the relative environmental impacts of each. Polymers and biodiesel (Chapters 5 and 6 respectively) are used as case studies for this.

In Chapter 7, a sensitivity analysis is performed on the generic flowsheet model. This highlights the variables which lead to the greatest change in reactor volume, electrical requirements, purity and LCA scores. The advantages of improved production versus optimised downstream processing are also investigated. Heuristics can be drawn from these to determine reduced LCA scores during the initial design of a bioprocess as well as during operation.

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CHAPTER 3: PENICILLIN

3.1. Introduction

This chapter describes a case study for the application and validation of the generic flowsheet model, using the production of Penicillin V by the batch microbial growth of *Penicillium chrysogenum* as an example. The results obtained were used with the Life Cycle Assessment (LCA) software SimaPro v7.1® (PRé Consultants B.V. 2008) to determine the resulting environmental impacts. These were used as further validation through comparison with the LCA analysis using material and energy inventories from the literature.

The advantage of testing the model on a well-established commodity is that there are numerous published studies, not only on the experimental determination of parameters such as yield coefficients to inform the flowsheet model, but also of detailed flowsheet designs, the material and energy inventories they generate and their experimental applicability, *e.g.* Biwer *et al.* (2005) and Heinzle *et al.* (2006).

In addition to the validation of the flowsheet model, the environmental burdens of the process were calculated, and the key contributing components and feedstocks required for the production of Penicillin V were obtained. These would be used to inform the approach to bioprocess design later in the thesis.

3.2. The production of the sodium salt of Penicillin V

3.2.1. Penicillin V

Penicillin is a β-lactam antibiotic produced by various *Penicillium* fungi. It is made up of a $C_9H_{11}N_2O_4S$ framework with a variable side chain, R-, as shown in Figure 3.1. Common penicillins include Penicillin G, Oxacillin and Ampicillin (Bailey and Ollis 1986). Penicillin V, also known as Phenoxymethylpenicillin and represented as $C_{16}H_{17}N_2O_4S$, is an orally administered form of penicillin and will be considered in this case.

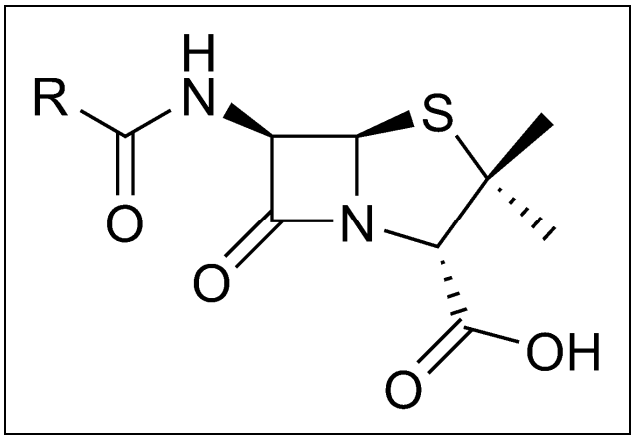


Figure 3.1: Basic penicillin structure, where R represents a side chain (R = C_7H_6 for Penicillin V)

3.2.2. Penicillin V model development

Production of Penicillin V was modelled using the fungi *Penicillium chrysogenum*, with a glucose substrate. There is substantial literature concerning penicillin production (Perry *et al.* 1997, van Nistelrooij *et al.* 1998, Falbe and Regnitz 1999, Lowe 2001, Nielsen 2001 and Ohno *et al.* 2002). In a typical process, depicted in Figure 3.2, after continuous heat sterilisation, glucose, Pharmamedia (a protein and mineral salt source (Atkinson and Mavituna 1983)), trace metals and phenoxyacetic acid entered 11 bioreactors each with a volume of 100 m^3. A primary biomass production phase of 50 h was followed by a secondary penicillin production phase of 106 h with continual glucose addition. Filter-sterilized air was added continuously, while exhaust air was filtered before being released.

After penicillin production, the fungal culture was transported to a rotary vacuum filter where the fungal biomass was removed and washed with water, as seen in Figure 3.2. Sulphuric acid was added to reduce the pH to approximately pH 3 and the temperature was lowered to minimise penicillin degradation. The penicillin was transferred to an organic phase following addition of butyl acetate. Thereafter centrifugation allowed the removal of the aqueous solution. Not shown in Figure 3.2, butyl acetate may be recycled from this stage in more complex designs.

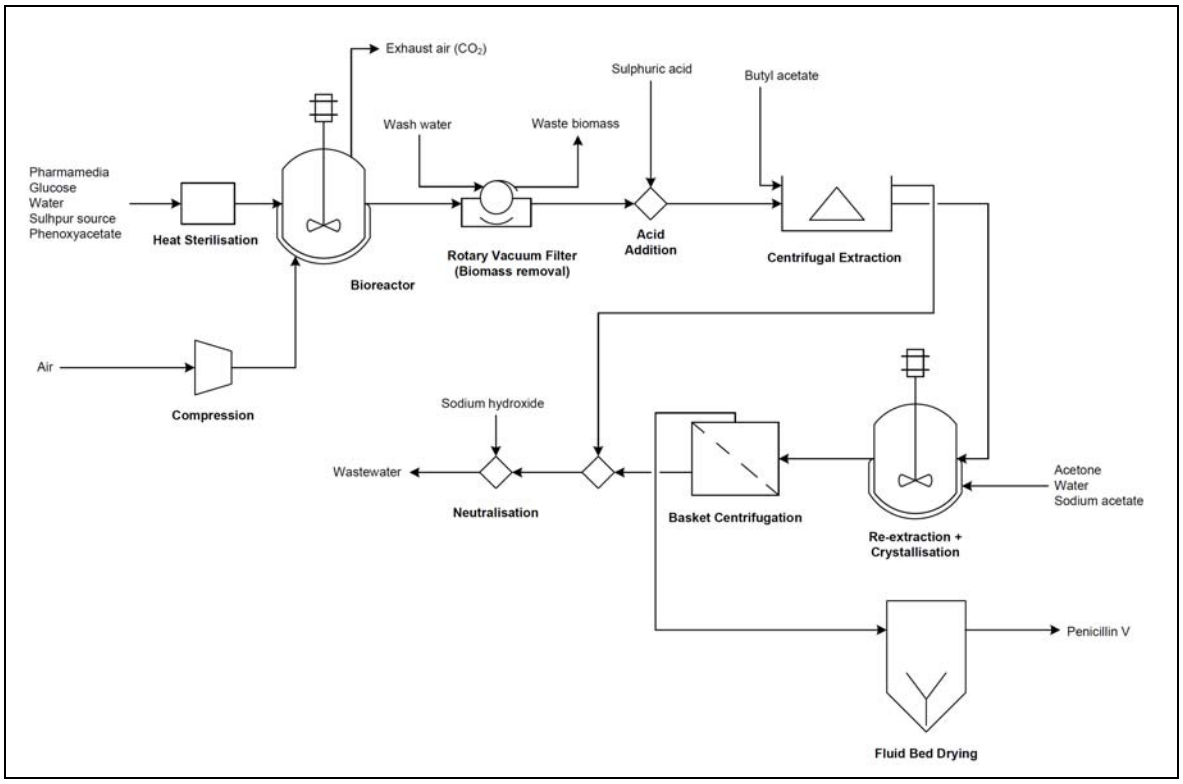


Figure 3.2: Simplified process flowsheet for penicillin V sodium salt production as modelled in the MS-Excel model (simplified from Biwer *et al.* 2005 and Heinzle *et al.* 2006)

Sodium acetate, acetone and water were added to the penicillin V solution for its re-extraction into the acetone/water phase and subsequent precipitation as crystals of the sodium salt. These were washed, separated in a basket centrifuge and air-dried in a fluidised bed dryer. The wash solution from the basket centrifuge as well as the previous centrifugal extraction wastewater was neutralised with sodium hydroxide before being discharged to effluent treatment. In a more optimum system, it may be possible to recycle sodium acetate or acetone and allow for water re-use.

Parameters used in the generic flowsheet were estimated from the literature (Table 3.1). From these, three scenarios have been presented. Each contained progressively less input from the initial full list (Scenario 1). For many processes, not all inputs are available, hence successive scenarios (Scenario 2 and Scenario 3) assumed that the less accessible and more sensitive inputs were missing and the defaults as explained in Chapter 2 were used. Scenarios 2 and 3 used approximately 45 % and 30 % of the original information respectively. The lists of inputs used for the three scenarios are shown in Table 3.2.

Table 3.1: Literature yield coefficients for the production of penicillin V

Yield coefficients		Value	Units
Biomass yield on Pharmamedia	$Y_{X/Pharmamedia}$	2.14	g/g
Biomass yield on glucose	$Y_{X/glucose}$	0.45	g/g
Penicillin yield on glucose	$Y_{penicillin/glucose}$	0.81	g/g
Penicillin yield on phenoxyacetic acid	$Y_{penicillin/phenoxyacetic\ acid}$	2.00	g/g
Biomass yield on oxygen	$Y_{X/O2}$	1.56	g/g
Maintenance coefficient	$m_{glucose}$	0.022	g glucose/g dcw h
Maintenance coefficient	m_{O2}	0.023	g O_2/g dcw h

References:

Perry *et al.* (1997), van Nistelrooij *et al.* (1998), Falbe and Regnitz (1999), Lowe (2001), Nielsen (2001) and Ohno *et al.* (2002)

Table 3.2: Sets of input values collated from Biwer *et al.* (2005) and Heinzle *et al.* (2006) for the extracellular, aerobic production of penicillin in a batch reactor

Assumptions	Scenario 1	Scenario 2	Scenario 3	Units
Cooling water temperature	5	[18]	[18]	°C
Steam Sterilisation (140°C, 3 bar)				
Reactor temperature	32	[37]	[37]	°C
Microbial growth conditions (batch production of Penicillin from *Penicillium chrysogenum*)				
Product: Biomass ratio	1.2	1.2	1.2	-
Carbon 1 source (excess): Glucose	2	[1]	[1]	%
Carbon 2 source (excess): Phenoxyacetic acid	1.7	[1]	[1]	%
Mass percentage of carbon source 2 as total carbon	10.6	10.6	10.6	%
Nitrogen source (excess): Pharmamedia ($C_{55.7}H_{6.7}O_{18.9}N_{16}S_{2.7}$ (Phyllis 2006))	15	[5]	[5]	%
Sulphur source excess	0	[5]	[5]	
Oxygen source (vvm): Air	0.021 (excluding excess)	[10x the min. stoich. requirement]	[10x the min. stoich. requirement]	
Compression: Single stage reciprocating compressor, 607.95 kPa compression pressure				
Maintenance coefficient	0.022*	0.022	0.022	h
Time for over which maintenance is considered	106	106	[10]	h
Final biomass concentration	45	45	[16.7]	g/l
Yield coefficients: $Y_{x/s}$	0.45*	[0.43]	[0.43]	g/g
$Y_{p/s}$	0.81*	[0.64]	[0.64]	g/g
$Y_{x/o}$	1.56*	[1.35]	[1.35]	g/g
Agitation (11 tanks) (Energy: Electricity)				
Power per unit volume	2.5	2.5	[0.083]	kW/m^3
Efficiency	1	[0.9]	[0.9]	

Assumptions	Scenario 1	Scenario 2	Scenario 3	Units
Post bioreactor cooling				
Outlet temperature	28	[15]	[15]	°C
Filtration (Energy: Electricity)				
Solid fraction removed	100	[95]	[95]	%
Liquid fraction retained	91	91	[70]	%
Additive: Sulphuric acid	0.028	0.028	0.028	%v/v
Centrifugation (Energy: Electricity)				
Product fraction retained	98	[99]	[99]	%
Waste fraction removed	91.8	91.8	[95]	%
Energy per unit volume	3060	[6420]	[6420]	kJ/m^3
Additive: Butyl acetate (assume no recycle)	9.1	9.1	9.1	%v/v
Precipitation and Crystallisation (Energy: Electricity and steam)				
Outlet temperature	6	[40]	[40]	°C
Residence time	12	[2]	[2]	h
Power per unit volume	0.6	[0.8]	[0.8]	kW/m^3
Additive: Acetone	12.3	12.3	12.3	%v/v
Additive: Sodium acetate	7.8	7.8	7.8	%v/v
Reaction: Sodium acetate + Penicillin → Acetic acid + Penicillin V sodium crystals (conversion: 97 % limiting reagent)				
Centrifugation (Energy: Electricity)				
Solid fraction retained	99	[98]	[98]	%
Liquid fraction removed	97.9	[80]	[80]	%
Fluid bed drying (Electricity: 72.2 MJ/m^3)				
Product fraction retained	99	[99]	[99]	%
Liquid fraction removed	90	[98]	[98]	%
Waste water treatment				
Additive: Sodium hydroxide	0.25	0.25	0.25	%v/v

*** Values available from literature and not specific to this flowsheet design**
[ ] Default values calculated or assumed in the MS-Excel model when no explicit inputs are given

3.3. Results

3.3.1. Material and energy balance outputs

From the generic flowsheet model, the material and total energy (renewable and non-reneawble) requirements calculated are shown in Table 3.3 and Table 3.4. For Scenario 1, for each kilogram of the sodium salt of Penicillin V produced, 5.18 kg of glucose, 1.30 kg of Pharmamedia and

0.36 kg of phenoxyacetic acid were required. This was a product yield of 0.19 kg penicillin/kg glucose feed. The sulphur required for the penicillin structure required 0.32 kg sulphur (as a sulphate) per kg of penicillin. Outputs of carbon, nitrogen and sulphur raw materials were all low. Despite a 55 % reduction in the number of data entries from Scenario 1, Scenario 2 gave similar material balance results as that of Scenario 1. The material required for Scenario 3 was slightly higher throughout, moreover the water requirement was over 3-fold higher than in Scenario 1. This was owing to the 3-fold lower final biomass concentration in Scenario 3. The relative amounts of material and energy inputs are shown in Figure 3.3 and the material outputs in Figure 3.4.

Table 3.3: Material balance for the production of Penicillin V for the flowsheet developed

Component	In (kg)	Out (kg)	In (kg)	Out (kg)	In (kg)	Out (kg)
	Scenario 1		Scenario 2		Scenario 3	
Acetic acid	-	0.17	-	0.17	-	0.17
Acetone	0.22	0.22	0.22	0.22	0.31	0.31
Biomass (dry cell weight)	-	0.90	-	0.90	-	1.17
Butyl acetate	0.18	0.18	0.18	0.18	0.25	0.25
Carbon dioxide	-	6.58	-	8.10	-	6.88
Glucose	5.18	0.06	6.04	0.04	5.36	0.05
Oxygen (excl. excess & N_2)	4.02	-	5.13	-	4.02	-
Penicillin V (loss)	-	0.13	-	0.13	-	0.46
Penicillin V sodium salt	-	1.00	-	1.00	-	1.00
Penicillin V sodium salt (loss)	-	0.04	-	0.04	-	0.04
Pharmamedia	1.30	0.17	1.19	0.06	1.54	0.07
Phenoxyacetic acid	0.36	0.01	0.46	0.00	0.60	0.01
Sodium acetate	0.26	0.03	0.26	0.02	0.36	0.12
Sodium hydroxide	0.11	0.11	0.11	0.11	0.37	0.37
Sulphuric acid (DSP additive)	0.01	0.01	0.01	0.01	0.04	0.04
Sulphur source	0.32	-	0.33	-	0.43	-
Water	19.1	21.3	18.86	21.4	68.24	70.36
Product yield (kg pen/kg glucose input)		0.19		0.15		0.19
Product recovery (% kg pen cr.)		96.2		96.2		96.2

Table 3.4: Energy and utility flows for the production of Penicillin V for the flowsheet developed

Energy requirements	Scenario 1	Scenario 2	Scenario 3	Units
Electricity	22.0 (79.12)	22.9 (82.37)	29.9 (107.8)	kWh/kg pen. (MJ/kg pen)
Steam (140°C, 3 bar)	3.3 (8.91)	3.4 (9.18)	10.1 (27.3)	kg/kg pen. (MJ/kg pen)
Total energy equivalent	88.03	91.55	135.1	MJ/kg penicillin
Chilled water	0.86	1.41	1.55	m^3/kg penicillin

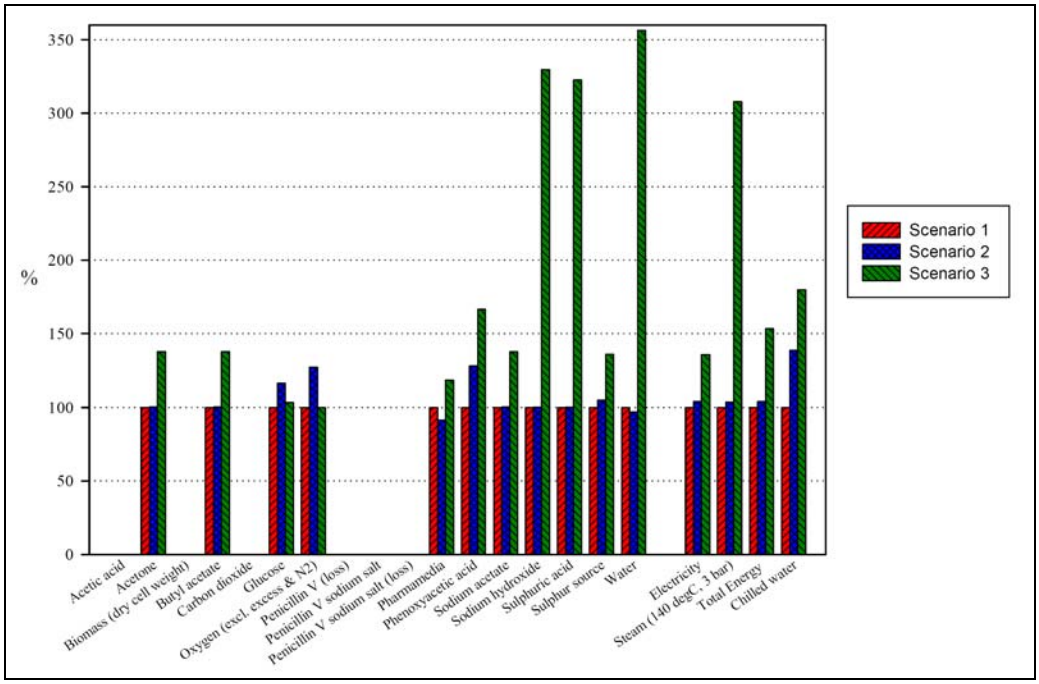


Figure 3.3: Comparison of material, energy and utility inputs from the generic model for Penicillin V sodium salt production, relative to Scenario 1

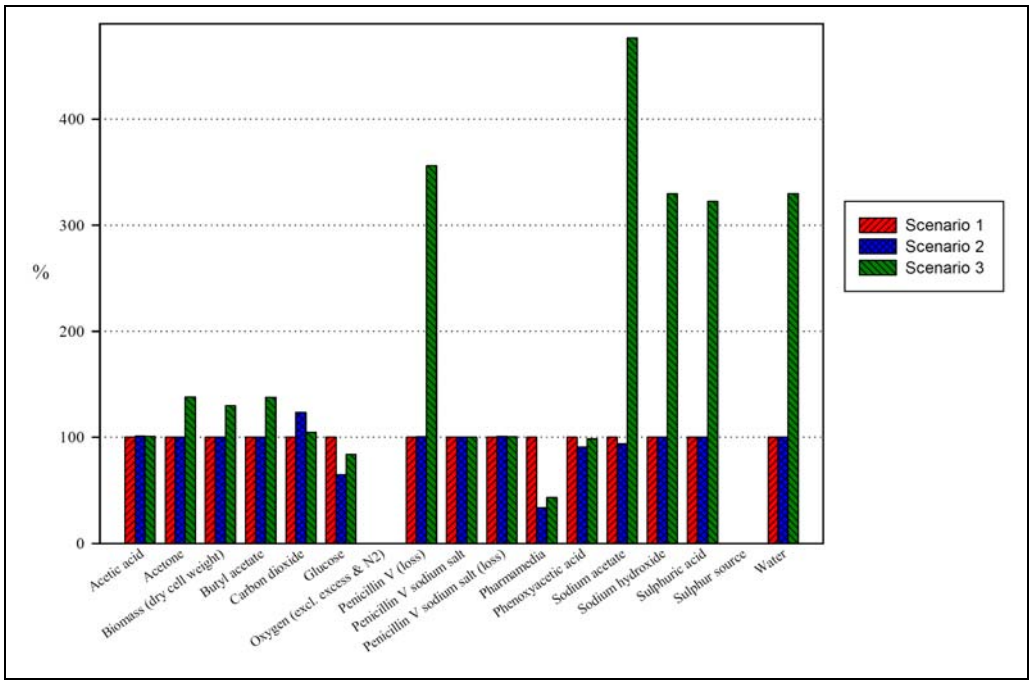


Figure 3.4: Comparison of material, energy and utility outputs from the generic model for Penicillin V sodium salt production, relative to Scenario 1

In scenario 1, for each kilogram of penicillin, 79.1 MJ of electrical energy and 3.3 kg of steam were required. The steam requirement was almost identical in Scenario 2 with the electrical energy requirement being slightly higher. In Scenario 3, both the steam and electrical energy requirements were higher than the previous scenarios. This increase was owing to the increased water requirement of Scenario 3.

From Table 3.5, it could be seen that most of the electricity was needed for the bioreactor agitation and cooling requirements. Electrical energy for downstream processing formed less than 2.1 % of the total requirement in all three scenarios. The steam and chilled water requirements in downstream processing were less than 10 % and 0.3 %. Of the total steam required, 95 % was needed for sterilisation. Of the total chilled water, 99.7 % was required for cooling between gas compression (aeration) and bioreactor cooling. Similar trends were observed in Scenarios 2 and 3.

Table 3.5: Energy contributions for the production of Penicillin V for the flowsheet developed

Energy source	Scenario 1	Scenario 2	Scenario 3
Electricity (MJ/kg pen.)	**79.12**	**75.08**	**107.8**
Sterilisation	1.23	1.27	3.78
Aeration	10.25	9.11	11.8
Agitation	32.85	32.7	26.84
Bioreactor cooling	32.51	31.25	40.54
Post bioreactor cooling	1.32	7.21	22.45
Rotary filtration	0.69	0.69	2.18
Centrifugal extraction	0.013	0.026	0.037
Re-extraction and Crystallisation	0.21	0.050	0.067
Basket Centrifugation	0.032	0.032	0.044
Fluid bed drying	0.0024	0.023	0.036
Steam (kg/kg pen.)	**3.3**	**3.4**	**10.1**
Sterilisation	3.1	3.1	9.7
Re-extraction and Crystallisation	0.2	0.3	0.3
Chilled water (m^3/kg pen.)	**0.86**	**1.19**	**1.55**
Aeration	0.11	0.02	0.03
Bioreactor cooling	0.75	1.16	1.51
Re-extraction and Crystallisation	0.0032	0.0014	0.0019

Nominal volumes for individual unit operations needed in order to give 1 kg of final penicillin V, sodium crystal product in the 3 scenarios are given in Table 3.6. As anticipated, successive downstream process volumes were progressively smaller, with exceptions being where chemical additions were made. Scenarios 1 and 2 had similar volumes owing to similar biomass concentration and separation efficiencies assumed. Scenario 3 was characterised by larger volumes as it was based on more conservative assumptions of biomass concentration and efficiency of separations. This higher volume also accounted for the higher steam and electricity requirements since energy requirements were based on a volume treated, hence energy requirement per kilogram of product were greater for less concentrated streams.

Table 3.6: Nominal volumes for the production of Penicillin V for the flowsheet model developed

Unit operation	Scenario 1	Scenario 2	Scenario 3
	dm^3/kg pen*	dm^3/kg pen*	dm^3/kg pen*
Sterilisation	23.26	23.15	72.70
Rotary vacuum filter	23.27	23.16	72.74
Centrifugal extraction	20.35	20.30	50.15
Re-extraction and Crystallisation	2.98	2.99	4.09
Basket Centrifugation	2.97	2.97	4.08
Fluid bed drying	0.76	1.16	1.38
Final volume	0.71	0.71	0.71

*** Estimated working volume for each unit for a batch process *i.e.* total volume of material passing through the unit in order to give desired product mass**

3.4. Life Cycle Assessment (LCA)

3.4.1. Goal definition and system description

The Life Cycle Assessment of the different penicillin production models was performed using the methodology explained in Section A.6. of Appendix A. The systems were defined as cradle-to-factory gate production of penicillin, including all raw material and agricultural inputs. A functional unit of 1 kg of crude sodium salt of Penicillin V, at a purity of 99 %, was used.

Wherever possible, the Ecoinvent v1.3 inventory dataset was used in the LCA. Where this was not possible, the ETH-ESU (Oxygen) or BUWAL 250 (Pharmamedia) inventories were used. A South African electricity mix, with a high coal dependence, was used in the LCA. It was assumed that the glucose requirements of penicillin production were met by sugar, modelled using the South African sugar cane data as defined in Appendix G. All air, water and solid waste emissions from penicillin production have been included in the Life Cycle Assessment. Waste water treatment (WWT) was not included in any of the LCA studies. Complete sets of life cycle inventory data are presented in Appendix I.

The Life Cycle Impact Assessment results are presented in Section 3.4.2. In the discussion that follows, the material and energy balance results are compared to the results from a full penicillin design as obtained from literature in Section 3.5.1. These material and energy flows are used in the LCA software and impacts compared to the generic flowsheet model LCA impacts in Section 3.5.2. The major process contributions to the LCA scores are presented and discussed in Section 3.5.3.

3.4.2. Life Cycle Analysis

Using the material and energy balance results from Scenario 1, and the SimaPro LCA software, for 1 kg of Penicillin V produced, life cycle impacts of 25.5 kg $CO_{2\,eq.}$, 0.227 kg $Sb_{eq.}$, 1.62 mg $CFC\text{-}11_{eq.}$ and 0.254 kg $SO_{2\,eq.}$, were obtained in the categories of global warming, abiotic depletion, ozone layer depletion and acidification respectively as shown in Table 3.7. The LCA results for the same categories for Scenario 2 were: 27.9 kg $CO_{2\,eq.}$, 0.241 kg $Sb_{eq.}$, 1.78 mg $CFC\text{-}11_{eq.}$ and 0.259 kg $SO_{2\,eq.}$. These values were all within 10 % of those calculated from Scenario 1, as were photochemical oxidation and all toxicities except terrestrial ecotoxicity, as can be seen in Figure 3.5.

Impacts from Scenario 3 were all higher than those obtained in both Scenarios 1 and 2. More raw materials were required and emissions produced were higher throughout owing to less effective downstream processing and a lower final biomass concentration. With higher volumes

in Scenario 3, the amount of water needed was much higher. To date water requirements are not reflected in life cycle impact analysis approaches explicitly. The larger volume of water increased the steam required in the sterilisation and the energy required in agitation, resulting in the highest energy need of all scenarios.

Table 3.7: LCIA of Penicillin V per kilogram of product – CML Baseline 2000 V2.03

Impact Category	Unit	Scenario 1	Scenario 2	Scenario 3
Abiotic Depletion	kg $Sb_{eq.}$	0.227	0.241	0.324
Global Warming (GWP100)	kg $CO_{2\ eq.}$	25.5	27.9	35.1
Ozone Layer Depletion (ODP)	mg $CFC\text{-}11_{eq.}$	1.62	1.78	2.31
Human Toxicity	kg $1,4\text{-}DB_{eq.}$	10.9	11.6	15.2
Fresh Water Aquatic Ecotoxicity	kg $1,4\text{-}DB_{eq.}$	2.56	2.72	3.55
Marine Aquatic Ecotoxicity	kg $1,4\text{-}DB_{eq.}$	16000	17100	22000
Terrestrial Ecotoxicity	kg $1,4\text{-}DB_{eq.}$	0.0533	0.0610	0.0861
Photochemical Oxidation	kg C_2H_4	0.00891	0.00952	0.0124
Acidification	kg $SO_{2\ eq.}$	0.254	0.272	0.348
Eutrophication	kg $PO_4^{3-}{}_{eq.}$	0.0243	0.0259	0.0306

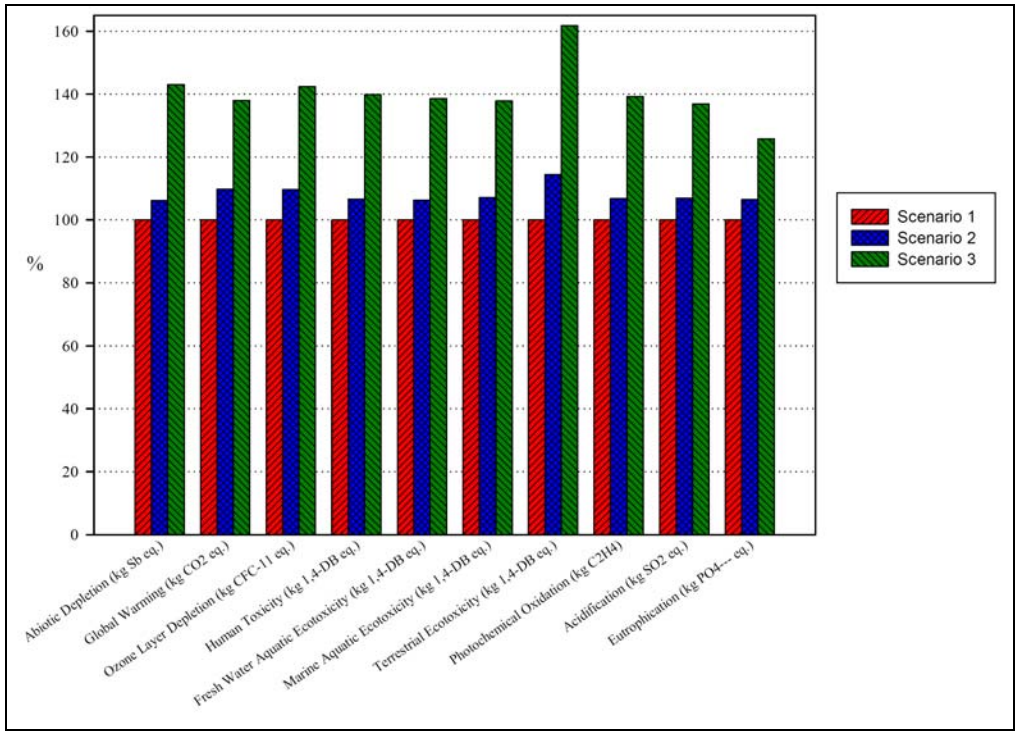


Figure 3.5: Comparison of LCA results for Penicillin V sodium salt production for Scenarios 2 and 3 relative to Scenario 1

3.5. Discussion

3.5.1. Comparison with a full penicillin design

To compare the validity of the scenarios developed, the material and energy balance results were compared to a Penicillin V model presented by Biwer *et al.* (2005) and Heinzle *et al.* (2006). This was the most complete and technologically relevant analysis available in the open literature. The model was constructed using the process flowsheeting package SuperPro Designer v5.1 (Intelligen 2008) to provide material and energy balances as well as economic costing for a base case scenario. The model included design considerations reported by Perry *et al.* (1997), van Nistelrooij *et al.* (1998), Falbe and Regnitz (1999), Lowe (2001) and Ohno *et al.* (2002).

The comparison on the basis of 1 kg penicillin V sodium salt is summarised in Table 3.8. The amount of Pharmamedia used in the generic flowsheet scenarios was higher (2.5 – 3 fold) than that for Biwer *et al.* (2005) and Heinzle *et al.* (2006); the latter was based on industrial norms and not calculation (Biwer 2006). The amount of glucose and phenoxyacetic acid was at most 18 % higher and 40 % lower respectively than the Biwer *et al.* (2005) and Heinzle *et al.* (2006) values. The amount of oxygen reported was the amount used and excludes the excess, since this was the value reported by Heinzle *et al.* (2006). The generic flowsheet model values were approximately 60 % higher than the literature.

For each kilogram of penicillin produced, approximately 19 kg of process water was required for Scenarios 1 and 2. This showed good agreement with the amount of water shown to be required in the work of Heinzle *et al*. (2006). This was less than a third of the water required for Scenario 3. This was owing to poor assumptions resulting in a large reactor volume and the additional water requirement.

The amount of steam predicted by the MS-Excel model was higher (3 – 8 fold) in all 3 scenarios, but electricity was lower (approximately 0.9 fold) as shown in Figure 3.6. This was a result of steam sterilisation in the generic flowsheet models versus heat sterilisation, by electricity, in the Heinzle *et al.* (2006) model. The total energy equivalent of Scenarios 1 and 2 were comparable (88.03- and 91.55 *vs.* 86.38 kWh/kg penicillin respectively) to the Heinzle *et al.* (2006) value, while Scenario 3 gave values approximately 60 % higher.

Butyl acetate requirements were reduced to 43 % and acetone requirements increased by 3 fold because of simplifications on a recycle stream. Other inputs and outputs in Scenarios 1 and 2 were within 10 % of the Heinzle *et al*. (2006) values. Certain of the large differences seen in the material outputs of Scenario 3, compared to the literature values as shown in Figure 3.7, were for materials of low mass (*e.g.* sodium hydroxide and Penicillin V). The small changes in these absolute values resulted in large relative differences shown on the bar graph.

Table 3.8: Material, energy and utility flows for the production of Penicillin V (Heinzle *et al.* 2006) *vs.* results from the generic flowsheet model developed

Component	In (kg)	Out (kg)	In (kg)	Out (kg)	In (kg)	Out (kg)	In (kg)	Out (kg)
	Heinzle *et al.* (2006)		Scenario 1		Scenario 2		Scenario 3	
Acetic acid	-	0.17	-	0.17	-	0.17	-	0.17
Acetone	0.12	0.12	0.22	0.22	0.22	0.22	0.31	0.31
Biomass (dry cell weight)	-	0.88	-	0.90	-	0.90	-	1.17
Butyl acetate	0.32	0.32	0.18	0.18	0.18	0.18	0.25	0.25
Carbon dioxide	-	5.47	-	6.58	-	8.10	-	6.88
Glucose	5.10	0.10	5.18	0.06	6.04	0.04	5.36	0.05
Oxygen (excl. excess & N_2)	2.56	-	4.02	-	5.13	-	4.02	-
Penicillin V (loss)	-	0.10	-	0.13	-	0.13	-	0.46
Penicillin V sodium salt	-	1.00	-	1.00	-	1.00	-	1.00
Penicillin V sodium salt (loss)	-	-	-	0.04	-	0.04	-	0.04
Pharmamedia	0.47	0.06	1.30	0.17	1.19	0.06	1.54	0.07
Phenoxyacetic acid	0.60	0.01	0.36	0.01	0.46	0.00	0.60	0.01
Sodium acetate	0.23	0.01	0.26	0.03	0.26	0.02	0.36	0.12
Sodium hydroxide	0.12	0.12	0.11	0.11	0.11	0.11	0.37	0.37
Sulphur source	-	-	0.32	-	0.33	-	0.43	-
Sulphuric acid (DSP additive)	0.01	0.01	0.01	0.01	0.01	0.01	0.04	0.04
Trace metals	0.77	0.10						
Water	19.2	21.1	19.1	21.3	18.86	21.4	68.24	70.36
Product yield (kg pen/kg glucose input)		0.20		0.19		0.15		0.19
Product recovery (% kg pen cr.)		99.0		96.2		96.2		96.2

Energy requirements	Heinzle *et al.* 2006	Scenario 1	Scenario 2	Scenario 3	Units
Electricity	23.04 (82.9)	22.0 (79.12)	22.9 (82.37)	29.9 (107.8)	kWh/kg pen. (MJ/kg pen)
Steam (140°C, 3 bar)	1.26 (3.4)	3.3 (8.91)	3.4 (9.18)	10.1 (27.3)	kg/kg pen. (MJ/kg pen)
Total energy equivalent	86.38	88.03	91.55	135.1	MJ/kg penicillin
Chilled water	3.32	0.86	1.41	1.55	m^3/kg penicillin
Cooling water	1.17	-	-	-	m^3/kg penicillin

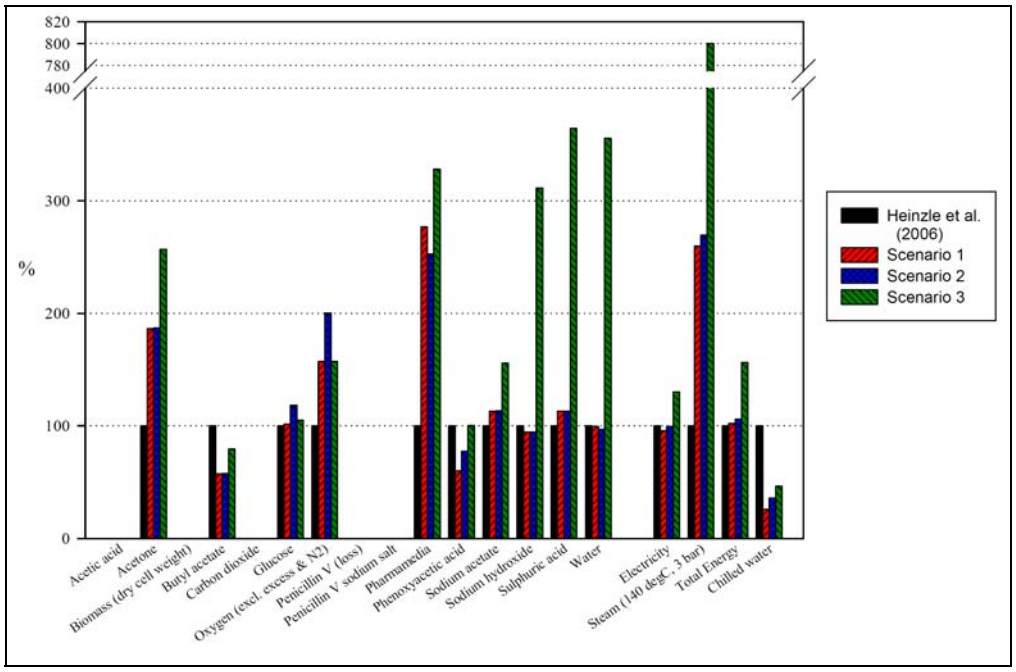


Figure 3.6: Comparison of material, energy and utility inputs calculated for Penicillin V sodium salt production, relative to literature values of Heinzle *et al.* (2006)

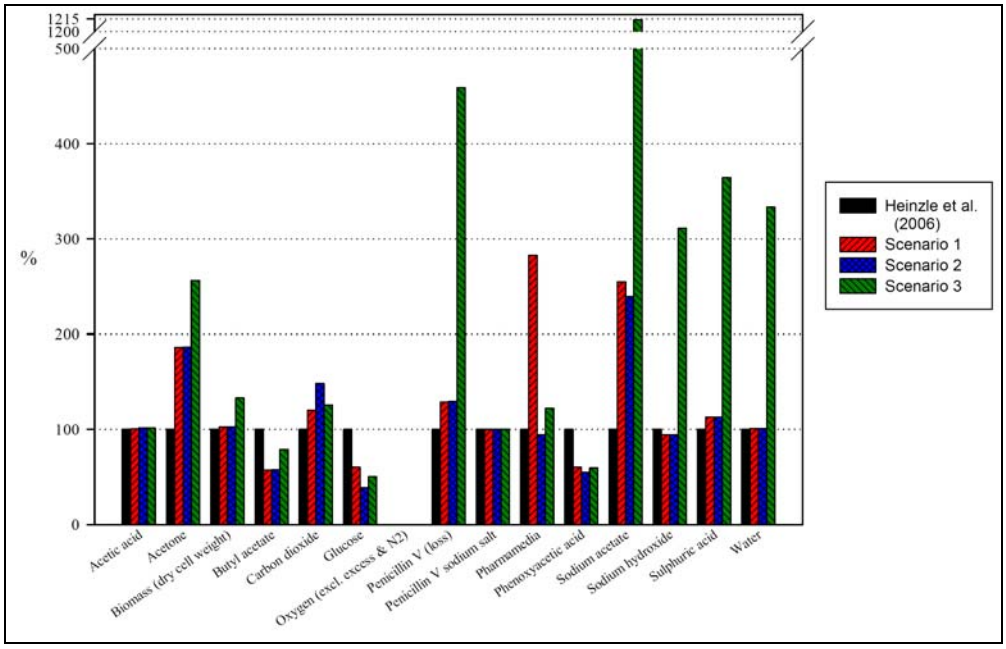


Figure 3.7: Comparison of material, energy and utility outputs calculated for Penicillin V sodium salt production, relative to literature values of Heinzle *et al.* (2006)

3.5.2. Life Cycle Impact Assessment (LCIA)

The Life Cycle Impact Assessment (LCIA) across the detailed model of Biwer *et al.* (2005) and Heinzle *et al.* (2006) and Scenarios 1 to 3 are presented in Table 3.9 and Figure 3.8. Scenarios 1 and 2 were within 10 % of the LCA values using the data of Biwer *et al.* (2005) and Heinzle *et al.* (2006) across all categories except for terrestrial ecotoxicity (16 % lower) and eutrophication (17 % higher). LCA impacts for Scenario 1 were lower than those in the Biwer *et al.* (2005) and Heinzle *et al.* (2006) model for all categories except global warming, ozone layer depletion and eutrophication as seen in Figure 3.8.

Prediction of eutrophication was higher in the MS-Excel scenarios owing to the higher usages of Pharmamedia and glucose, both produced from agricultural crops. The higher toxicity scores, particularly terrestrial ecotoxicity, in the results of Biwer *et al.* (2005) and Heinzle *et al.* (2006) were owing to greater emissions resulting from the higher phenoxyacetic acid and electricity requirements.

Table 3.9: LCIA of penicillin per kilogram of Penicillin V sodium salt product – CML 2 baseline 2000 V2.03

Impact Category	Unit	Heinzle *et al.* (2006)	Scenario 1	Scenario 2	Scenario 3
Abiotic Depletion	kg $Sb_{eq.}$	0.232	0.227	0.241	0.324
Global Warming (GWP100)	kg $CO_{2\ eq.}$	24.3	25.5	27.9	35.1
Ozone Layer Depletion (ODP)	mg $CFC\text{-}11_{eq.}$	1.64	1.62	1.78	2.31
Human Toxicity	kg 1, 4-$DB_{eq.}$	11.4	10.9	11.6	15.2
Fresh Water Aquatic Ecotoxicity	kg 1, 4-$DB_{eq.}$	2.67	2.56	2.72	3.55
Marine Aquatic Ecotoxicity	kg 1, 4-$DB_{eq.}$	16700	16000	17100	22000
Terrestrial Ecotoxicity	kg 1, 4-$DB_{eq.}$	0.0646	0.0533	0.0610	0.0861
Photochemical Oxidation	kg C_2H_4	0.00921	0.00891	0.00952	0.0124
Acidification	kg $SO_{2\ eq.}$	0.253	0.254	0.272	0.348
Eutrophication	kg $PO_4^{3-}{}_{eq.}$	0.0212	0.0243	0.0259	0.0306

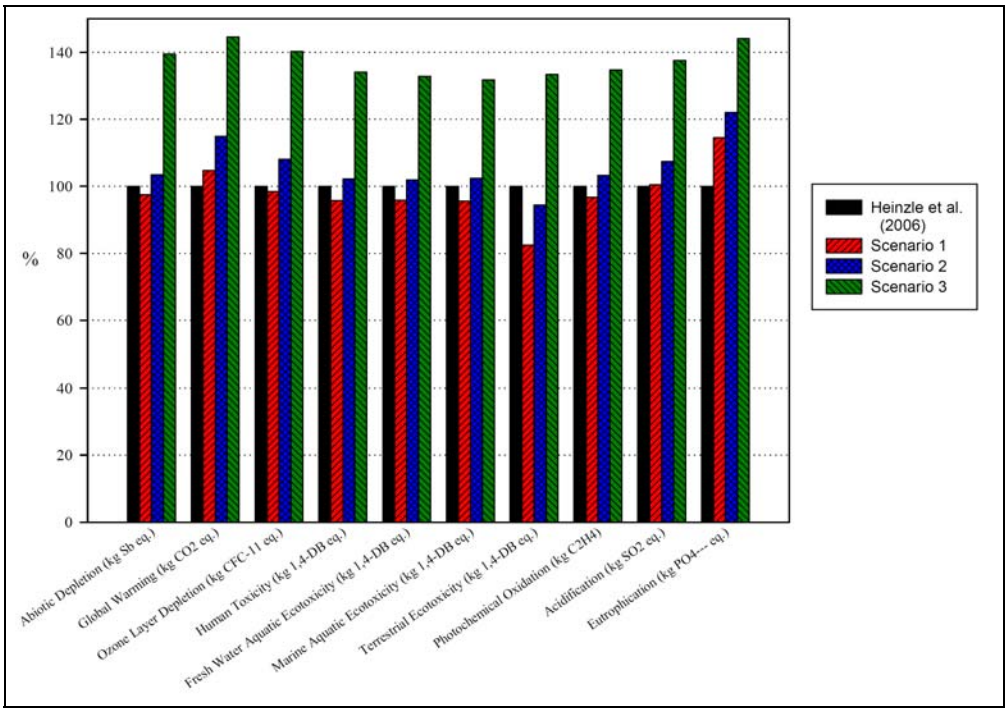


Figure 3.8: Comparison of LCA results for Penicillin V sodium salt production for the the three scenarios presented in this study relative to Heinzle *et al.* (2006) results

3.5.3. Process contributions

Six impact categories were further investigated using Scenario 1, to determine the main process contributors to total scores. Scenario 1 was chosen to undertake this analysis as its results were closest to the literature results, but included more realistic calculations for Pharmamedia inputs and not assumptions as in the Biwer *et al.* (2005) and Heinzle *et al.* (2006) reports. The LCA categories investigated include abiotic depletion, global warming, ozone layer depletion, photochemical oxidation, acidification and eutrophication shown in Figures 3.9 to 3.11. These categories do not include toxicity categories but still give a wide and varied perspective of environmental damage. The values shown in the figures represented contribution to the LCA impacts as a percentage of the total in each category. Only materials which led to a contribution greater than 3 % of the total LCA score have been included. Where there were positive impacts, *i.e.* impacts which reduced the total environmental impact, these were presented as detached wedges.

In all the impact categories shown, electricity was the largest contributor, ranging from 37 % in eutrophication to 75 % in abiotic depletion. This impact was predominantly from electricity from coal (94 % of the contribution), the major source of electricity generation in South Africa, which was the electricity source assumed (Appendix F). Other dominating contributors included

glucose, phenoxyacetic acid, Pharmamedia, acetone and sodium acetate. Steam, assumed to be raised by natural gas burning, did not play a large part in the LCA categories.

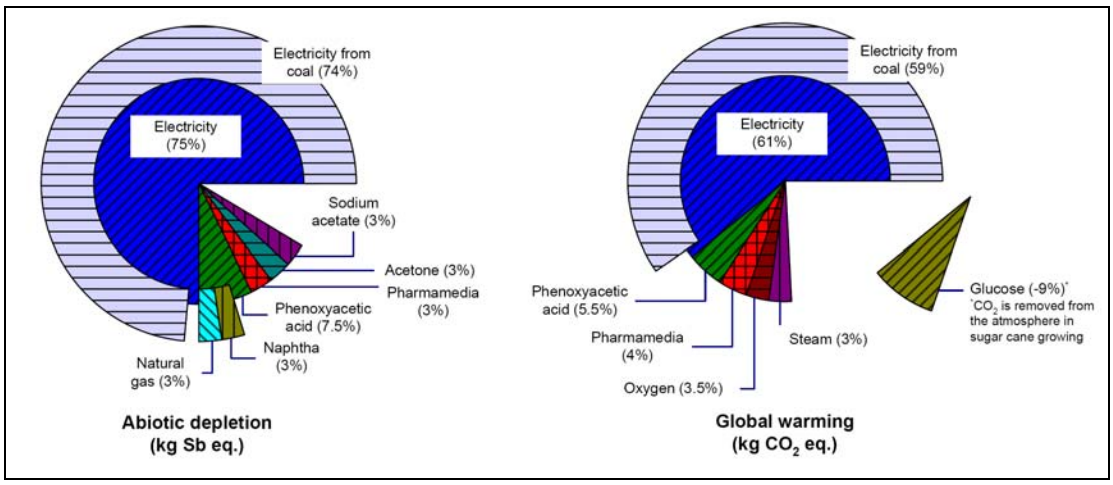
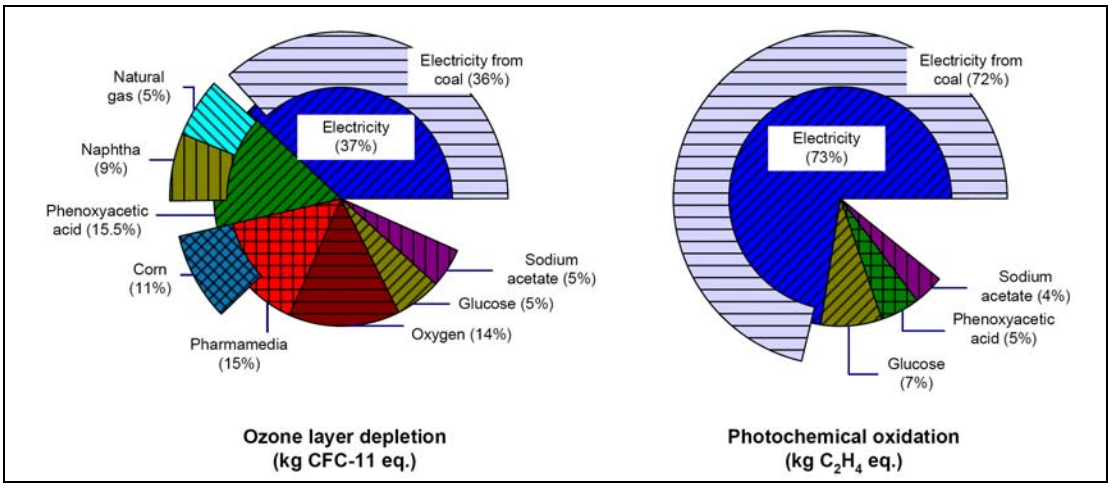


Figure 3.9: Life Cycle Assessment process contributions of penicillin production (Scenario 1) using the CML Baseline 2.03 methodology (Abiotic depletion and Global warming)

Abiotic depletion was dominated by the contribution of electricity (75 %, most from coal) and smaller portions by phenoxyacetic acid (7.5 %), Pharmamedia, acetone and sodium acetate (all 3 %). Global warming impacts were made up of similar contributors, although electricity to a lesser extent of 61 % as seen in Figure 3.9. LCA results for glucose were based on its production from sugar made from sugarcane. Data were taken from South African values as this was the most accurate LCA data available. The assumptions and calculations are presented in Appendix G. Carbon dioxide uptake was taken into account in the growth of sugarcane, resulting in a positive impact (negative value) on the global warming score. Since bio-based carbon in the final product has been deducted, it is unlikely that the ranking would change if incineration were chosen, since comparable amounts of CO_2 would be released as a consequence.



*** Note: Ozone layer depletion is of reduced importance in current processes (see Appendix A.6)**

Figure 3.10: Life Cycle Assessment process contributions of penicillin production (Scenario 1) using the CML Baseline 2.03 methodology (Ozone layer depletion and Photochemical oxidation warming)

The process contributors to ozone layer depletion score were spread more representatively owing to the contribution from intensive agriculture. Together with electricity (37 %), oxygen, Pharmamedia and phenoxyacetic acid each contributed over 10 %. In the photochemical oxidation score, electricity (73 %) dominated. Glucose (7 %), phenoxyacetic acid (5 %) and sodium acetate (4 %) had little impact as shown in Figure 3.10.

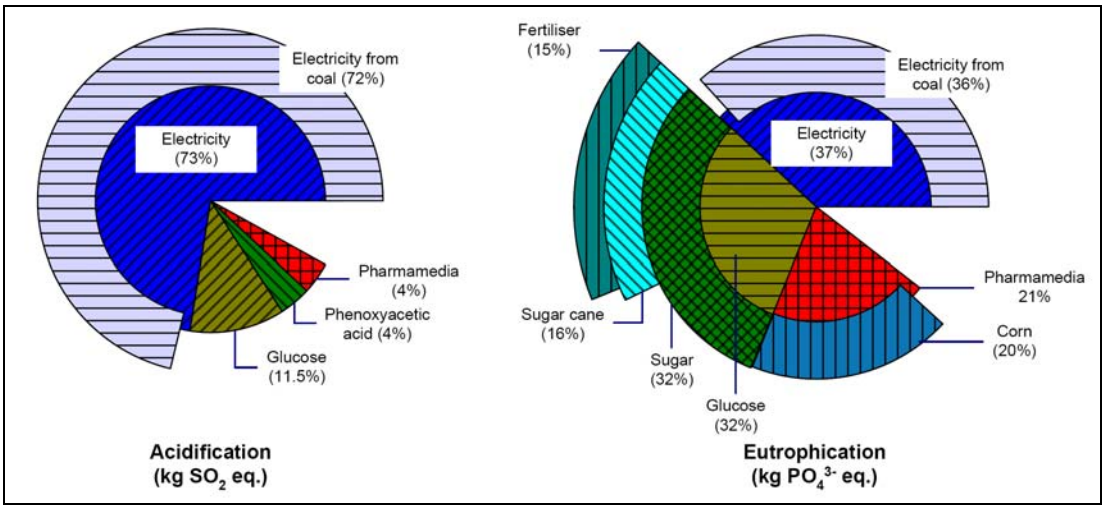


Figure 3.11: Life Cycle Assessment process contributions of penicillin production (Scenario 1) using the CML Baseline 2.03 methodology (Acidification and Eutrophication)

For acidification, the relative contributions of electricity, glucose and phenoxyacetic acid were similar to photochemical oxidation as seen in Figure 3.11. Glucose, and in turn, sugar, sugarcane and fertilisers for the agriculture, contributed 32 % of the eutrophication score. Pharmamedia, modelled as protein from corn, was also a large fraction of the score (20 %) due to fertiliser requirements.

3.6. Conclusions

The generic MS-Excel model was used to obtain material and energy balance data of a flowsheet for the production of Penicillin V sodium salt using a limited set of input data typically available at an early stage in process design. Where a maximum set of input data was used to inform the MS-Excel model, 50 % of the material and energy balance results were within 20 % of those of Biwer *et al.* (2005) and Heinzle *et al.* (2006), which was based on a detailed flowsheet design and industry values. When using a minimal set of inputs, 50 % of the material and energy balance results were within 55 % of the literature values. This illustrated that even when limited sets of inputs were available, order of magnitude results could be obtained.

Pharmamedia and phenoxyacetic acid inputs differed as the analysis of Biwer *et al.* (2005) and Heinzle *et al.* (2006) assumed industrial norms, while in the MS-Excel model presented, the required amount was calculated from stoichiometric ratios. Glucose, sodium acetate, sodium hydroxide, sulphuric acid, water and electricity requirements were all within 20 % of literature results. Pharmamedia and steam requirements were at least 2.5 times higher for the two flowsheets with the most detailed sets of inputs (i.e. Scenarios 1 and 2).

The material and energy balance data generated were used to complete a Life Cycle Assessment (LCA) study. The results of the LCA study showed good agreement between the material and energy balances in Scenarios 1 and 2 compared to the literature flowsheet provided by Biwer *et al.* (2005) and Heinzle *et al.* (2006), illustrating the appropriateness of the flowsheet model in providing early design stage data.

Using the model, with only 45 % of the input dataset (Scenario 2), provided a material and energy dataset and LCA results that represented more rigorous models (*e.g.* Scenario 1). Deviations in Scenario 3 showed a sensitivity to critical inputs, especially those which affected the system volume. An increase in the bioreactor volume impacted the system with respect to energy and losses throughout.

An analysis of the process contributions to environmental burden, targeted electricity provision and the provision for agricultural inputs as key items. Electricity requirements were over 60 % in each of abiotic depletion, global warming potential and photochemical oxidation, with the next largest process contribution in these categories represented less than 10 % of the LCA

scores. While the electrical requirements still formed the largest single contribution to LCA scores in ozone layer depletion and eutrophication (37 % in each), the combined impacts from agriculture (sugar, Pharmamedia and phenoxyacetic acid) were 35.5 and 53 % respectively. Thus, decreases in electrical energy and agricultural inputs would have the greatest impact in reducing overall LCA scores.

In summary:

- the model was appropriate if critical data was provided;
- it provided key data for a life cycle assessment analysis; and
- it provided significant insight into critical items contributing to environmental burden.

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CHAPTER 4: CELLULASE

4.1. Introduction

The aims of this chapter are multi-fold. The case study is used for additional validation of the generic model by presenting material and energy balance results and comparing these to literature flowsheets. Once the relevance of the material and energy balance inventories were determined, the values were further analysed by a Life Cycle Assessment (LCA) study. The LCA analysis was used to determine the environmental impacts of the process as well as compare LCA results obtained from the literature material and energy balance inventories with those obtained from the generic flowsheet model.

As the example in this chapter, cellulase production by three different methods is used. These flowsheets, as defined by Heinzle *et al.* (2006) and two by Zhuang *et al.* (2007), show different methods of producing the same product using:

- aerobic and anaerobic microorganisms;
- different bioreactor setups; and
- different downstream processing units.

Using three different flowsheet models allows for a greater number of validation studies to be performed. Further, it allows for a comparison of the same product using different processing technologies. The individual process contributions to the total LCA scores were calculated and compared across the different flowsheets. These key contributing components helped to inform further studies in Chapter 7 on heuristics for bioprocess technology selection.

4.2. Cellulase – Its role and production process

Bioconversion of biomass to biofuels such as bioethanol and biogas is receiving increasing attention owing to depletion of fossil fuels (Lynd 1996, Lynd *et al*. 2005, Zhang *et al.* 2007) as well as global warming issues associated with air emissions. To meet the biomass capacity for this, processing of woody biomass is desirable. This requires conversion of their cellulosic component into fermentable sugar using either hot acid hydrolysis or enzyme catalysis by cellulase (Rabinovich *et al.* 2002).

Cellulases, together with hemicellulases, accounted for approximately 20 % of the world enzyme market in 2000 (Bhat 2000). They are typically used in the food, animal feed, textile and pulp and paper industries. The aerobic fungus *Trichoderma reesei* (Himmel, *et al.* 1999, Sáez *et al.* 2002) is commonly used to produce cellulase. Anaerobic organisms such as *Clostridium papyrosolvens* and *Clostridium thermocellum* are also used. Common substrates for microbial cellulase production include cellulose and wood waste, such as bagasse, corn cobs and straw as shown in Table 4.1.

Table 4.1: Cellulase producing micro-organisms and common substrates reported in literature

Micro-organisms used	Aerobic/ Anaerobic	Substrates for micro-organism growth*	References
Aspergillus terreus	Aerobic	Bagasse, filter paper and carboxymethyl cellulose (CMC)	El-Nawwi and El-Kader 1996
Cellulomonas biazotae	(unknown)	Bagasse, cotton stalks, Kallar grass-, Dhancha-, and wheat straw, cellobiose, carboxymethyl cellulose (CMC), cotton wool, filter paper, xylan, α-cellulose, Sigmacell-100, *Atriplex lentiformis* and *Panicum maximum*	Rajoka and Malik 1997
Chaetomium erraticum	(unknown)	Carboxymethyl cellulose (CMC)	Soni *et al.* 1999
Clostridium papyrosolvens	Anaerobic	Glucose, trehalose, cellobiose and lactose	Thirumale *et al.* 2001
Clostridium thermocellum	Anaerobic	Cellulose, cellobiose, fructose, glucose, hemicellulose, laminaribiose, mannitol, sorbitol and xylose	Demain *et al.* 2005
Clostridium thermocellum	Anaerobic	Paper pulp	Zhuang *et al.* 2007
Neurospora crassa	Aerobic	Wheat straw	Romero *et al.* 1999
Phanerochaete chrysosporium (formerly *Sporotrichum pulverulentum)*	(unknown)	Glucose, wood sticks	Szabó *et al.* 1996
Phlebia gigantean	(unknown)	Glucose, microcrystalline cellulose, carboxymethyl cellulose (CMC) and cellobiose	Niranjane *et al.* 2007
Saccharomyces cerevisiae (recombinant)	Aerobic	Phosphoric acid swollen cellulose (PASC)	Den Haan *et al*. 2007
Trichoderma reesei	Aerobic	Willow	Reczey *et al.* 1996
Trichoderma reesei	Aerobic	Wood chips	Himmel, *et al.* 1999
Trichoderma reesei	Aerobic	Corn cob residue and lignocellulosic waste from xylose industry	Xia and Cen 1999
Trichoderma reesei	Aerobic	Agricultural residues (bagasse, corn stover, rice, straw, wood chips), municipal solid waste	Wooley *et al.* 1999a
Trichoderma reesei	Aerobic	Glucose, commercial cellulose (Solka-floc)	Sáez *et al.* 2002
Trichoderma reesei	Aerobic	Corn cob residues, purified cellulose, corn stover	Liming and Xueliang 2004
Trichoderma reesei	Aerobic	Dairy manure	Wen *et al.* 2005

*** Carbon sources listed are used either individually, in combination or all at once**

4.3. Case study methodology

4.3.1. Material and Energy balance

Three different cellulase production models have been defined to validate the generic flowsheet model:

- Flowsheet 1 used data from Heinzle *et al.* (2006) for aerobic submerged cultivation of *Trichoderma reesei*;
- Flowsheet 2 used data from Zhuang *et al.* 2007 for anaerobic submerged fermentation of *Clostridium thermocellumi*; and
- Flowsheet 3 used data from Zhuang *et al.* 2007 for anaerobic solid state cultivation of *Clostridium thermocellumi*.

Within each flowsheet model, three scenarios were presented, with progressively decreasing initial data. The results were validated against material and energy balance results presented in literature from detailed flowsheeting models which included experimental data. The Life Cycle Assessment (LCA) impacts were calculated and compared across all scenarios.

4.3.2. LCA goal definition and system description

The LCA was carried out using the methodology presented in Appendix A.6. The systems were defined as the cradle-to-factory gate production of cellulase, including all raw material and agricultural inputs. The functional unit was defined as the amount of product which contains 1 kg cellulase. It was assumed that equal masses of enzymes have the same activity irrespective of the method of production. Where energy is reported, this is for the total renewable and non-renewable energy in the process. Where bio-based carbon is found in the final product this was deducted in the calculations. It is unlikely that the LCA impact would change if incineration were chosen, since comparable amounts of CO_2 would be released as a consequence.

The Ecoinvent v1.3 inventory dataset was used in the LCA with a South African electricity mix characterised by a high coal dependence. It was assumed that cellulose inputs were obtained from wood chips for Flowsheet One and from wood pulp in Flowsheets Two and Three. All air, water and solid waste emissions from cellulase production have been included in the Life Cycle Assessment. Waste water treatment (WWT) was not included in any of the LCA studies for cellulase. The life cycle inventory data are presented in Appendix I.

For all three cellulase flowsheets, the material and energy balance results are presented following the description of the generic flowsheet models and literature. Thereafter Life Cycle Impact Assessment results are given, followed by a discussion. The major process contributions to the LCA scores are presented and discussed.

4.4. Cellulase Flowsheet One: Aerobic production by SmF using *Trichoderma reesei*

4.4.1. Cellulase model development

The first cellulase production model considered was an aerobic production by submerged cultivation, traditionally referred to as submerged fermentation (SmF) of the extracellular enzyme using *Trichoderma reesei*, based on the studies of Himmel *et al.* (1999), Wooley *et al.* (1999a and 1999b), Sáez *et al.* (2002) and Heinzle *et al.* (2006). Cellulose, corn liquor, ammonia and nutrients were used as raw materials.

A three stage inoculum development required three seed bioreactors. All feed streams to these reactors were individually steam sterilised. Downstream processing was limited to a rotary vacuum filtration unit to remove biomass and an ultrafiltration unit which separated waste water with unreacted raw materials from the cellulase product stream (Figure 4.1). Key parameters are shown in Table 4.2, with typical values sourced from the literature.

The submerged fermentation process is known to have a high water content. The first two flow sheets modelled in this study used a SmF approach while the third (presented in Section 4.6) used a solid state cultivation (SSC) process, in which a continuous water phase is absent.

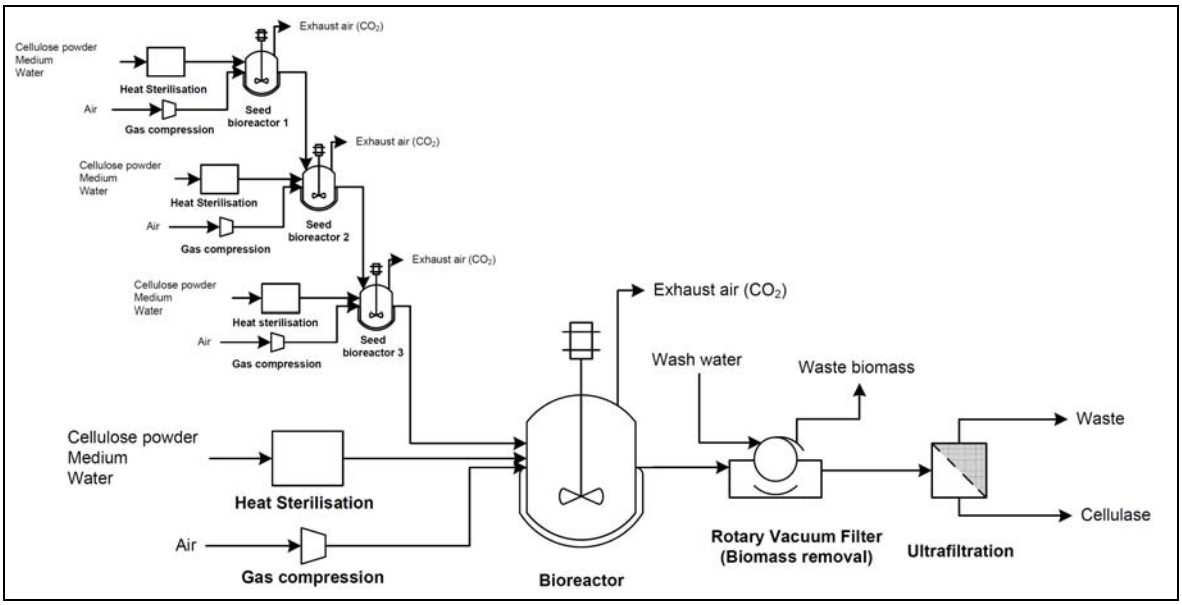


Figure 4.1: Simplified process flowsheet for cellulase production as modelled in the MS-Excel model

Table 4.2: Key parameters for the production of cellulase by *Trichoderma reesei* (from Heinzle *et al.* 2006)

Parameter		Value	Units	References
Inoculum volume	V_{inoc}	5.0	% of working volume	[2], [3]
Working volume vessel	V_{work}	80	%	[2], [3]
Aeration rate	AR	0.58	vvm	[1]
Agitator power	P_a	0.5	$kW.m^{-3}$	[1]
Bioreactor temperature	$T_{bioreactor}$	28	°C	[4]
Initial cellulose concentration	$C_{init,\ cellulose}$	45	$g.l^{-1}$	[1], [4]
Initial corn steep liquor concentration	$C_{init,\ CSL}$	7.5	$g.l^{-1}$	[2], [3]
Nutrients and trace elements concentration	$C_{nutrient}$	4.1	$g.l^{-1}$	[2], [3]
Initial ammonia concentration	$C_{init,\ ammonia}$	1.0	$g.l^{-1}$	[5]
Final cellulase concentration	$C_{final,\ cellulase}$	13.4	$g.l^{-1}$	[5]
Final biomass concentration	X_{final}	15	g dry cell weight.l^{-1}	[5]
Time for cellulase production	t_{prod}	107	h	[5]
CO_2 formation		18	$g.l^{-1}$ (bioreactor volume)	[4]
Cellulose utilisation	$U_{cellulose}$	90	%	[5]
Corn step liquor and nutrient utilisation	$U_{nutrient}$	75	%	[5]
Yield	$Y_{P/S}$	0.33	g cellulase / g cellulose	[1]
Productivity	P_{cell}	0.125	g cellulase / l.h	[1]

References:

[1] Himmel *et al.* 1999
[2] Wooley *et al.* 1999a
[3] Wooley *et al.* 1999b
[4] Sáez *et al.* 2002
[5] Heinzle *et al.* 2006

Additional values required for the model were obtained from the cellulase production model of Heinzle *et al.* (2006). Three scenarios have been presented (Scenarios H1, H2 and H3). The first scenario (Scenario H1) assumed a full set of parameters shown in Table 4.2. Scenarios H2 and H3 used a subset of these inputs, omitting less accessible inputs. Defaults within the generic flowsheet model were used in these cases. Scenarios H2 and H3 used approximately 45 % and 30 % of the original information respectively. Corn liquor was assumed as a carbon source, although it is typically a nitrogen source, since ammonia and nutrients (trace elements needed by the system) were listed as nitrogen sources. The default power per unit volume for agitation and the cross sectional area for the rotary vacuum filtration were lower in Scenarios H2 and H3 owing to the first principle calculations and estimates used compared to actual values for the specific process as used in Scenario H1. The lists of inputs used for the three scenarios are shown in Table 4.3.

Table 4.3: Sets of input values collated from Heinzle *et al.* (2006) for the extracellular, aerobic production of cellulase in a batch reactor

Assumptions	Scenario H1	Scenario H2	Scenario H3	Units
Cooling water temperature	25	[18]	[18]	°C
Chilled water temperature	5	[10]	[10]	°C
Max temp difference between exiting cooling water and hot inlet streams	10	[10]	[10]	°C
Steam Sterilisation (Cooling agent: Cooling water)				
Preheated temperature	110	110	[60]	°C
Sterilisation temperature	140	140	[121]	°C
Steam temperature	152	152	[140]	°C
Outlet temperature/Reactor temperature	35	[37]	[37]	°C
Microbial growth conditions (batch production of cellulase from *Trichoderma reesei*)				
Product: Biomass ratio	0.89	0.89	[1]	kg/kg
Carbon 1 source (excess): Cellulose	10	10	[1]	%
Carbon 2 source (excess): Corn liquor	33.3	33.3	[1]	%
Mass percentage Carbon 2 as total carbon	14.4	14.4	[50]	-
Nitrogen 1 source (excess): Ammonia	0	[5]	[5]	%
Nitrogen 2 source (excess): Nutrients*	33.3	33.3	[5]	%
Mass percentage Nitrogen 2 as total carbon	80.2	80.2	[50]	-
Oxygen source (vvm): Air	0.58	0.58	[10x the min. stoich. requirement]	
Maintain reactor temperature (Cooling agent: Chilled water)				
Yield coefficients: $Y_{x/s}$	[0.52]	[0.52]	[0.52]	g/g
$Y_{p/s}$	0.33*	[0.56]	[0.56]	g/g
$Y_{x/o}$	[0.77]	[0.77]	[0.77]	g/g

Assumptions	Scenario H1	Scenario H2	Scenario H3	Units
Final biomass concentration	15.1	[16.7]	[16.7]	g/l
Agitation (Energy: Electricity)				
No of tanks	1	[5]	[5]	
Height /Diameter (bioreactor)	3	[2]	[2]	-
Power per unit volume	0.5	0.5	[0.007]	kW/m^3
Aeration (Energy: Electricity)				
Compressed pressure	608	[300]	[300]	kPa
Compressor efficiency	0.7	[0.65]	[0.65]	-
Rotary vacuum filter (Energy: Electricity)				
Solids removed	100	100	[95]	%
Liquid retained	100	100	[70]	%
Cross sectional area	39.95	[0.037]	[0.037]	m^2
Ultrafiltration (Energy: Electricity)				
Solids (product) retained	98	98	[95]	%
Liquid (waste) removed	81	81	[70]	%

*** Micro nutrients needed were modelled as an additional nitrogen source as they cannot be accounted for in any other manner**
 # Values available from literature and not specific to this flowsheet design
[ ] Default data calculated or assumed in the MS-Excel model when no explicit inputs are given

4.4.2. Material and energy balance outputs

From the model, the material and energy inventories obtained to produce 1 kg cellulase – containing product (*i.e.* product plus impurities) are shown in Table 4.4. From the scenarios developed, cellulase purities of 6.1, 6.7 and 4.7 % were obtained for Scenarios H1, H2 and H3 respectively, with the remainder largely being water. For Scenario H1, for 1 kg of cellulase (16.4 kg of product), 3.78 kg of cellulose, 0.77 kg of corn liquor, 0.52 kg of nutrients and 0.10 kg of ammonia were required. Energy requirements for Scenario H1 included 180 MJ electricity, 2.6 kg steam and 1.6 kg chilled water per kilogram of cellulase. Scenario H2 (14.9 kg of product) had similar material requirements as shown in Figure 4.2, but electrical energy needs were approximately halved. The electricity value of Scenario H3 was 70 % lower when compared to Scenario H1. As with Scenario H3 (21.2 kg of product), the reduction in electrical needs of Scenario H2 resulted from reduction in aeration pressure, the dominant contributor to the total electrical requirements at 74 % in Scenario H1.

For Scenario H3, the material and energy inventory from the generic model showed large differences when compared to Scenarios H1 and H2. Ammonia, corn liquor and steam requirements were some 350, 450 and 250 % higher respectively. The high ammonia and corn liquor requirements resulted from the specifications for nitrogen source 2 and carbon source 2

respectively. The increase in the steam requirement (by mass) was a result of reduced heat integration with no preheating in steam sterilisation and lower steam quality. Cellulose and nutrient requirements were lower in Scenario H3 following definition of lower excesses and increased use of carbon source 2 and nitrogen source 2.

As both the material inputs and the downstream separation efficiencies were similar, the outputs of Scenarios H1 and H2 were similar. In Scenario H3, poor downstream separation efficiencies led to the cellulase lost in wastewater being 25 times greater than previous scenarios. These poor separation efficiencies also led to larger volumes, aggravating the higher steam requirements. However, cellulose, corn liquor and nutrient outputs were lower in Scenario H3. As a lower excess was used, all other outputs were higher in Scenario H3 than Scenarios H1 and H2 as can be seen in Figure 4.3.

Table 4.4: Material, energy and utility flows for the production of cellulase (Heinzle *et al.* 2006b) *vs.* results from the generic flowsheet model developed (basis: 1 kg cellulase in the product stream)

Component	In (kg)	Out (kg)	In (kg)	Out (kg)	In (kg)	Out (kg)	In (kg)	Out (kg)
	Heinzle *et al.* (2006)		Scenario H1		Scenario H2		Scenario H3	
Ammonia	0.082	0.00	0.10	0.00	0.10	0.00	0.27	0.01
Trichoderma reesei	0	1.17	0.07	1.22	0.07	1.22	0.09	1.52
Carbon dioxide	-	1.48	-	4.00	-	4.00	-	5.91
Cellulase waste*	-	0.04	-	0.02	-	0.02	-	0.50
Cellulose	3.62	0.32	3.78	0.28	3.47	0.28	2.81	0.02
Corn liquor	0.61	0.12	0.77	0.16	0.77	0.16	2.81	0.02
Product (enzyme)	-	14.9	-	16.4	-	14.9	-	21.2
Nutrients	0.33	0.065	0.52	0.10	0.52	0.10	0.27	0.01
Oxygen (reacting O_2 only)	0.81	-	3.21	-	3.21	-	5.10	-
Water	73.3	59.9	78.9	65.2	71.3	59.1	93.4	75.6
TOTAL	77.9	77.9	87.3	87.3	79.5	79.5	104.7	104.7
Product recovery (% kg cel.)		96.1		98.0		98.0		66.5
Chemical Oxygen Demand (COD)		N/A^		2.91		2.59		3.01

Energy requirements	Heinzle *et al.* 2006	Scenario H1	Scenario H2	Scenario H3	Units
Electricity	38.6 (139.0)	50.86 (183.1)	22.89 (82.4)	26.63 (95.88)	kWh/kg cel. (MJ/kg cel.)
Steam (152°C, 3 bar)	4.74 (12.8)	2.65 (7.14)	2.40 (6.47)	12.8** (34.6)	kg/kg cel. (MJ/kg cel)
Total energy equivalent	151.8	190.2	88.88	130.5	MJ/kg cellulase
Chilled water	0.84	1.60	1.60	1.38	m^3/kg cellulase
Cooling water	2.62	-	-	-	m^3/kg cellulase

*** Cellulase waste is non-recovered product lost in waste water**
****Steam at 140°C, 3 bar**
^ Not available

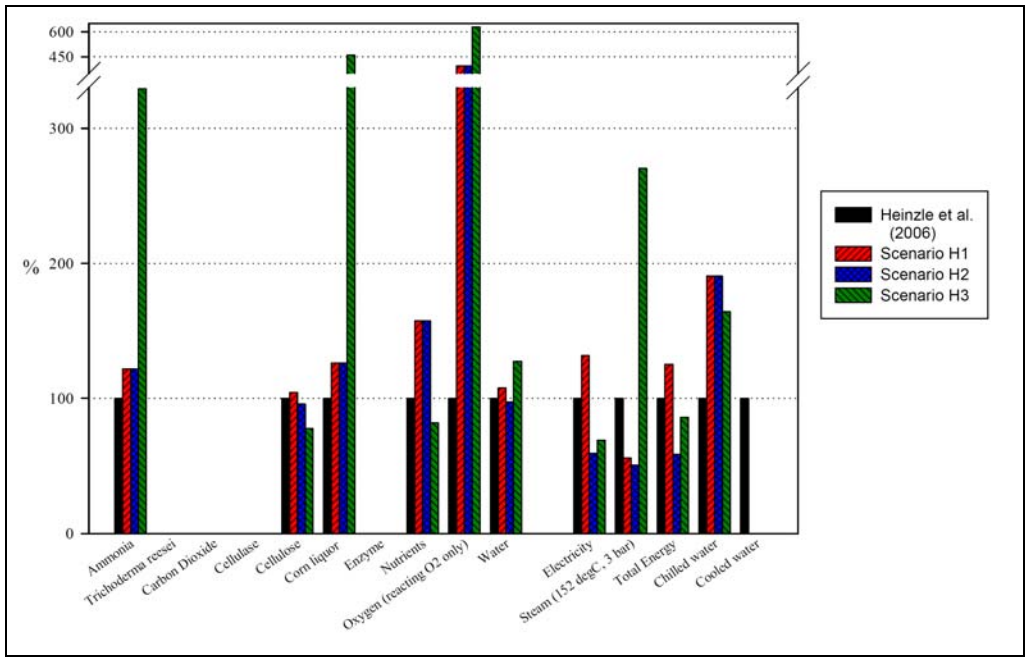


Figure 4.2: Comparison of material, energy and utility inputs calculated for cellulase production, relative to literature values of Heinzle *et al.* (2006)

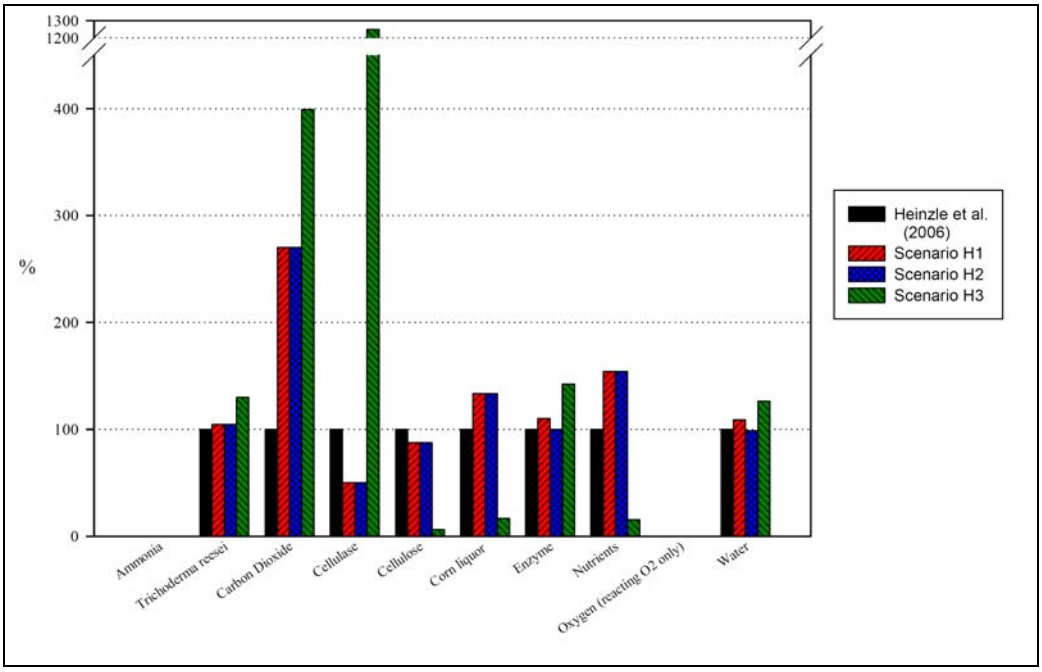


Figure 4.3: Comparison of material, energy and utility outputs calculated for cellulase production, relative to literature values of Heinzle *et al.* (2006)

Comparing the results for Scenario H1 to the published data of Heinzle *et al.* (2006), the ammonia, cellulose and water requirements were in agreement (using the equivalent input data) within 18 %. Corn liquor inputs and electricity requirements were within 30 %, while nutrient requirements were 50 % higher in Scenario H1. The oxygen requirements were 4 fold higher in the modelled scenarios. However, the Heinzle *et al.* (2006) oxygen data is questioned as the study made the assumption that all oxygen entering the system was consumed by the microorganisms. Typically in aerobic systems, 10 to 20 % of oxygen supplied on aeration is taken up into the liquid phase and consumed.

For each kilogram of cellulase produced as pure product excluding impurities, 79-, 71- and 93 kg of water was required for Scenarios H1, H2 and H3 respectively. The data of Heinzle *et al*. (2006) showed a water requirement of 73.3 kg. The amount of water removed from the systems, was shown to be under 60 kg for Scenario H2 and Heinzle *et al*. (2006) and 65- and 75 kg for Scenario H1 and H3 respectively. The large difference in the water in- and outputs of the process reported here is due to water inclusion in the product streams which are of low purities.

The amount of carbon dioxide exiting the modelled scenarios was at least double that reported by Heinzle *et al.* (2006). Since it was shown where the carbon dioxide originates in the Heinzle *et al.* (2006) study, it is uncertain as to why there is this doubling. *Trichoderma reesei*, corn liquor and nutrient outputs varied between 4 and 50 % higher in the modelled scenarios of H1 and H2. Half the amount of cellulase product was lost to waste water in Scenarios H1 and H2, while the amount of wastewater varied between 3 % lower in Scenario H2 to 15 % higher in Scenario H3 as seen in Figure 4.3.

4.4.3. Life Cycle Assessment (LCA)

For 1 kg of cellulase (16.4 kg of product) in Scenario H1, life cycle impacts of 0.50 kg $Sb_{eq.}$, 2.28 mg CFC-11 $_{eq.}$, 0.51 kg $SO_{2\,eq.}$ and 0.034 kg$PO_4^{3-}{}_{eq.}$ were obtained in the categories of abiotic depletion, ozone layer depletion, acidification and eutrophication as shown in Table 4.5. The LCA results for the same categories for Scenario H2 (14.9 kg of product) were 0.27 kg $Sb_{eq.}$, 1.52 mg CFC-11 $_{eq.}$, 0.27 kg $SO_{2\,eq.}$ and 0.022 kg$PO_4^{3-}{}_{eq.}$. In all categories, the impact of Scenario H2 was lower than Scenario H1. This reduction could be attributed chiefly to electricity needs. This reduction in electrical requirements was a result of a lower compression pressure during aeration.

Significant differences in the material and energy balance data were found in Scenario H3 (21.2 kg of product), relative to Scenario H1, owing to reduced downstream separation efficiencies and raw material input assumptions. The increased material and energy requirements in Scenario H3 resulted in higher LCA impacts compared with Scenario H2. The

total energy requirement of Scenario H3 was lower than Scenario H1. Even though the material results were greater, lower LCA scores are shown in Figure 4.4. This again showed that the energy requirements were a large contributor to LCA scores.

The global warming equivalent scores were all negative, showing a carbon dioxide reduction to the environment. This was a result of the agricultural inputs (*i.e.* cellulose, Pharmamedia and corn liquor), requiring CO_2 uptake from the atmosphere to allow for growth. This CO_2 uptake exceeded the CO_2 released during cellulase production, leading to a net reduction of greenhouse gases.

Compared to Heinzle *et al.* (2006) results, LCA impacts of Scenario H1 were at least 20 % higher in all categories except global warming as seen in Figure 4.4. The higher impacts of Scenario H1 were a result of the higher energy requirements. The largest contributor to the global warming category was cellulose.

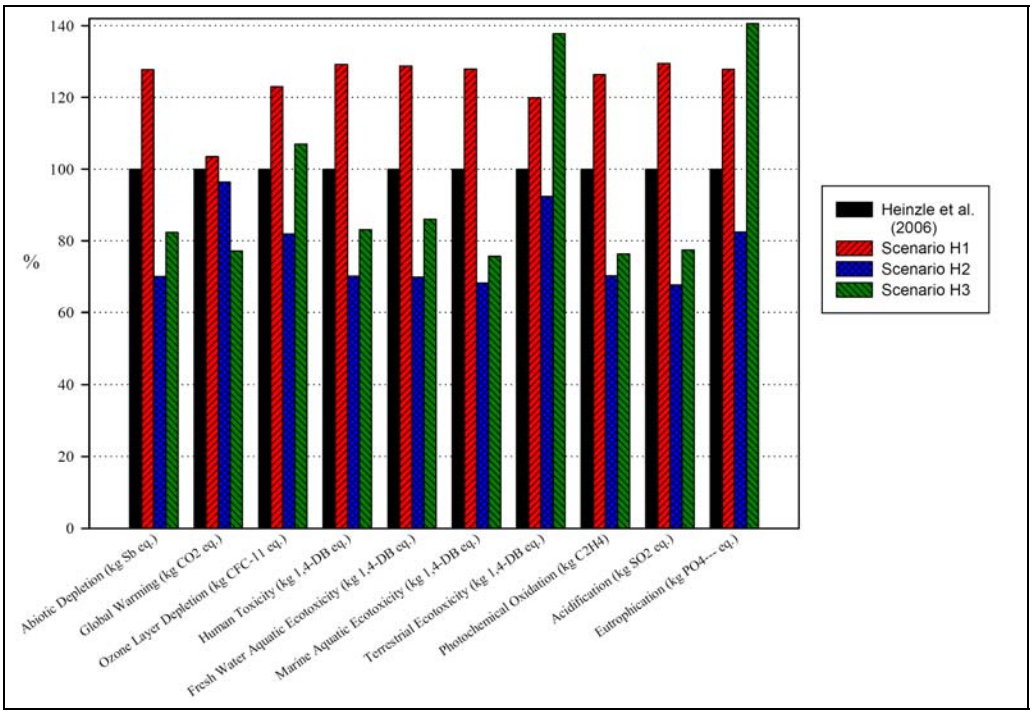
The impacts of Scenario H2 were all lower than Heinzle *et al.* (2006) owing to the lower energy requirements. Impacts from Scenario H3 were also lower owing to the lower energy requirements, except for terrestrial ecotoxicity and eutrophication which increased owing to the increased corn liquor discharge. In all LCA categories, the Heinzle *et al.* (2006) results lay within the range generated using the generic model.

LCA results which are within 5 % of each other may not be significantly different from each other owing to uncertainty in the inputs and LCA inventory datasets.

Table 4.5: LCIA of cellulase produced as described by Heinzle *et al.* (2006) – CML 2 Baseline 2000 V2.03 (basis: 1 kg cellulase in the product stream)

Impact Category	Unit	Heinzle *et al.* 2006	Scenario H1	Scenario H2	Scenario H3
Abiotic Depletion	kg $Sb_{eq.}$	0.39	0.50	0.27	0.32
Global Warming (GWP100)	kg $CO_{2\ eq.}$	-1200	-1240	-1153	-924
Ozone Layer Depletion (ODP)	mg CFC-$11_{eq.}$	1.86	2.28	1.52	1.99
Human Toxicity	kg 1,4-$DB_{eq.}$	18.50	23.89	12.98	15.40
Fresh Water Aquatic Ecotoxicity	kg 1,4-$DB_{eq.}$	4.86	6.26	3.40	4.18
Marine Aquatic Ecotoxicity	kg 1,4-$DB_{eq.}$	300000	38300	20500	22700
Terrestrial Ecotoxicity	kg 1,4-$DB_{eq.}$	0.093	0.11	0.087	0.13
Photochemical Oxidation	kg C_2H_4	0.016	0.020	0.011	0.012
Acidification	kg $SO_{2\ eq.}$	0.39	0.51	0.27	0.30
Eutrophication*	kg $PO_4^{3-}{}_{eq.}$	0.027	0.034	0.022	0.037

***Excluding the Chemical Oxygen Demand (COD) of the wastewater – which was unavailable for the Heinzle *et al.* 2006 model**



**** Since global warming values were negative, the results for Scenario H1 are most desirable and Scenario H3 the least desirable***

Figure 4.4: Comparison of LCA results for cellulase production for the three scenarios presented in the study relative to Heinzle *et al.* (2006) data

4.4.4. Process contributions

Process contributions of six LCA impact categories were investigated from the Heinzle *et al.* (2006) material and energy balance results. The Heinzle *et al.* (2006) flowsheet was chosen as it was the most detailed process flowsheeting model. The LCA categories investigated include abiotic depletion, global warming, ozone layer depletion, photochemical oxidation, acidification and eutrophication. These are categories which do not include toxicity categories but give a broad perspective of environmental damage. The values shown in the figures represented contribution to the LCA impacts as a percentage of the total in each category. Only materials which led to a contribution greater than 3 % of the total LCA score have been included.

In all six categories, electricity, cellulose and corn liquor formed the greatest portion of the LCA scores. In the abiotic depletion impact category, electricity formed 76 % of the total impact and wood chips, the assumed source of cellulose, contributed 20 % of the impact as seen in Figure 4.5. The impact of wood in the abiotic depletion category was a result of a large electrical energy requirement during processing. No other input added more than 3 % of the total abiotic score. In the global warming impact category, electricity was the main greenhouse gas emitter. The large agricultural component of cellulase production, and the associated biomass growth

(wood chips), resulted in a large carbon dioxide uptake from the atmosphere. Although corn liquor was also associated with biomass growth and carbon uptake, the ancillary energy requirements within corn liquor production resulted in an overall carbon uptake value less than 1 % of the total global warming score.

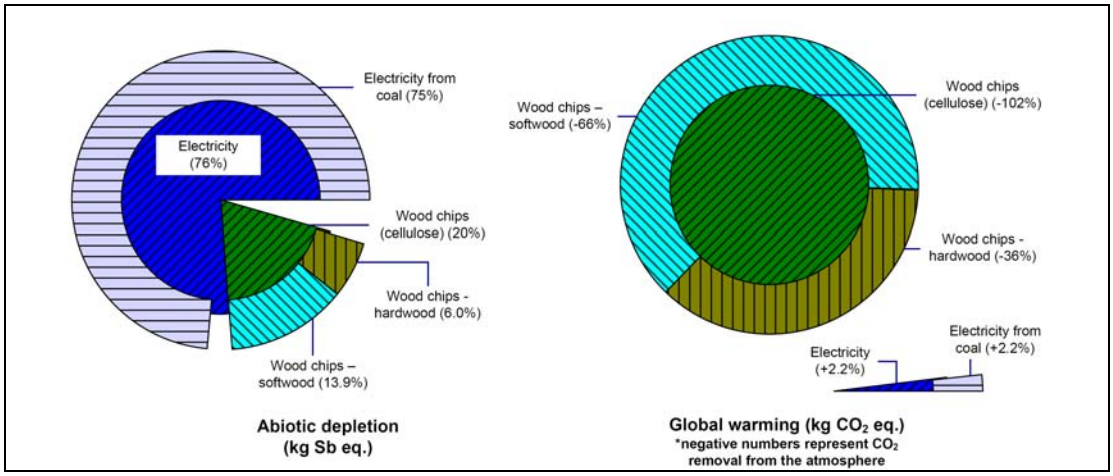
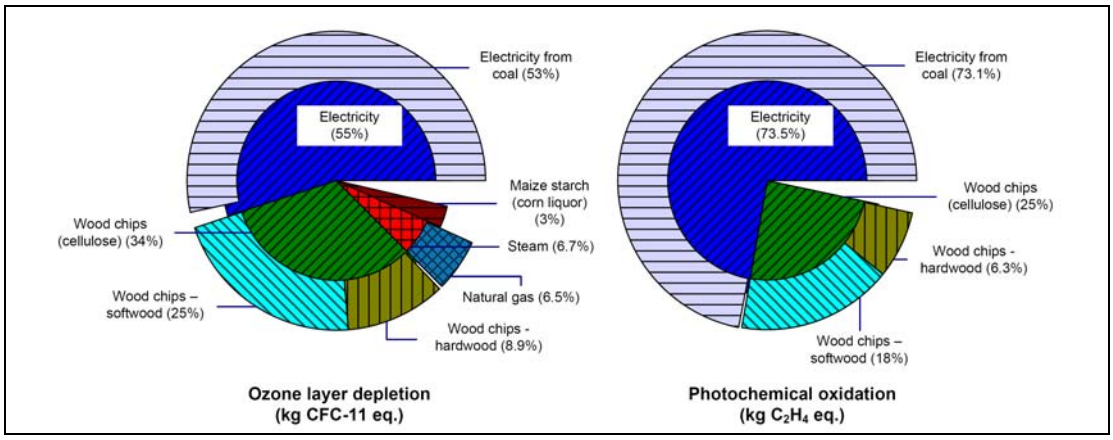


Figure 4.5: Life Cycle Assessment process contributions of cellulase production (Heinzle *et al.* 2006) using the CML baseline 2.03 methodology (Abiotic depletion and Global warming)

Electricity (73 %) and cellulose (25 %) again formed the greatest impacts in photochemical oxidation scores as shown in Figure 4.6. For ozone layer depletion impacts, steam (6.7 %) and corn liquor (3 %) were small contributors compared to the electricity (55 %) and cellulose (34 %) values.



*** Note: Ozone layer depletion is of reduced importance in current processes (see Appendix A.6)**

Figure 4.6: Life Cycle Assessment process contributions of cellulase production (Heinzle *et al.* 2006) using the CML baseline 2.03 methodology (Ozone layer depletion and Photochemical oxidation)

Electricity impacts (82.5 %) were responsible for the major impact in the acidification score, with cellulose contributing 15.6 % of the overall score as shown in Figure 4.7. Eutrophication scores were also dominated by electricity (59 %) and cellulose (23 %), with corn liquor impacts adding 17 % of the total. Agricultural products tended to increase the eutrophication scores due to fertiliser usage. The impact of dilute wastewater was not taken into account in these calculations as the chemical oxygen demand (COD) value was not given for the Heinzle *et al.* (2006) model. In Scenarios H1, H2 and H3, the impact of the COD in the wastewater contributed over 65 % of the total eutrophication score. It is expected that similar results would be achieved if the COD value had been reported in the Heinzle *et al.* (2006) model.

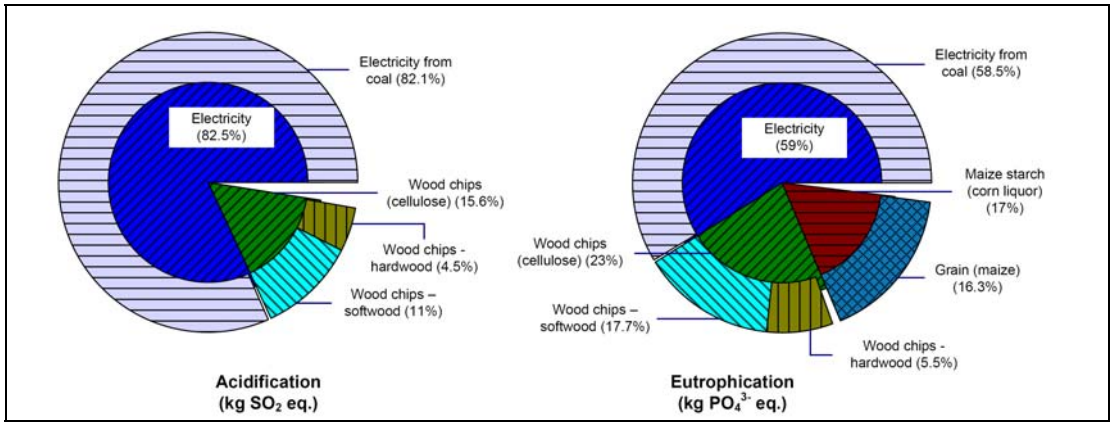


Figure 4.7: Life Cycle Assessment process contributions of cellulase production (Heinzle *et al.* 2006) using the CML baseline 2.03 methodology (Acidification and Eutrophication)

4.4.5. Discussion

It was seen in the process contributions that electricity formed more than half the LCA score for all categories investigated. The Heinzle *et al.* (2006) study made an assumption of 100 % oxygen utilisation during microbial growth and product formation. This is unrealistic and Scenarios H1, H2 and H3 assumed a typical experimental aeration rate of 0.58 vvm (Himmel *et al*. 1999). This difference in aeration rate, as well as compression pressure, was the main reason for the difference in electrical energy requirements and should be an area for further investigation.

Wood chips are a by-product in the forestry industry, and may be expected to give a relatively small contribution to overall LCA impacts. However, the large volume used, when compared to other inputs in cellulase production, resulted in a large relative impact. The Ecoinvent v1.3 inventory used for wood chips assigned burden to electricity and transportation which added to this footprint.

The importance of the volume of the operating system, through its affect on the electrical requirements of agitation, aeration and material processing was observed through material and energy balance results as well as the LCA analysis. Contributions to and the sensitivity of the volume and aeration rates were investigated further in Chapter 7.

4.5. Cellulase Flowsheet Two: Anaerobic production by SmF using *Clostridium thermocellum*

4.5.1. Cellulase model development

The second cellulase production model was based on anaerobic production using *Clostridium thermocellum*. Paper pulp, as a source of cellulose, was used with yeast extract, cellulose powder and ammonia as raw materials to the thermophilic process. Literature providing background on the process included Wooley *at al.* (1999b), Lynd *et al.* (2001), Zhang and Lynd (2003) and Zhuang *et al.* (2007).

The process used a submerged fermentation system following two seed fermenters. Cellulose powder and medium were added to the reactor, then heat sterilised before *Clostridium thermocellum* was added. Paper pulp was added and after anaerobic microbial growth and product formation, the enzyme was concentrated in a partial evaporation unit to remove moisture. It was further dried using a freeze dryer as shown in Figure 4.8. Exhaust air was filtered before being released into the atmosphere.

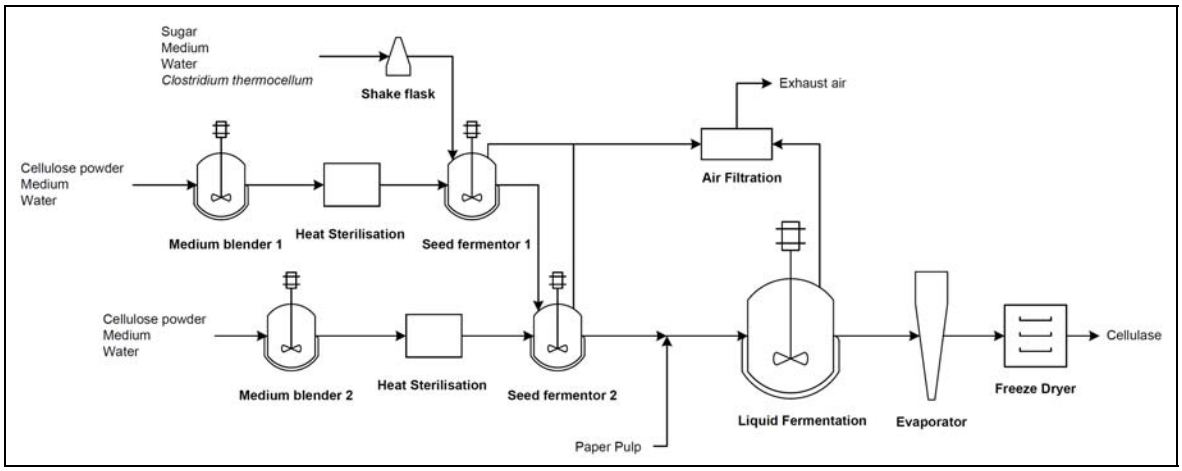


Figure 4.8: Simplified process flowsheet for cellulase production as modelled in the MS-Excel model for the SmF method

From the literature, information required for use in the generic flowsheet was extracted (Table 4.6). The cellulase production model of Zhuang *et al.* (2007) formed the major data source. Three scenarios have been presented (Scenarios SmF1, SmF2 and SmF3), representing decreasing input from the initial full list (Scenario SmF1). Defaults within the generic flowsheet model, as explained in Chapter 2, were used to estimate unspecified data. Scenarios SmF2 and SmF3 used approximately 60 % and 40 % of the original information respectively. The lists of inputs used are shown in Table 4.6.

Table 4.6: Sets of input values collated from Zhuang *et al.* (2007) for the extracellular, anaerobic production of cellulase in a batch reactor

Assumptions	Scenario SmF1	Scenario SmF2	Scenario SmF3	Units
Steam Sterilisation: (preheated with sterilised media)				
Preheated temperature	110	[60]	[60]	°C
Sterilisation temperature	121	[121]	[121]	°C
Steam temperature	152	[140]	[140]	°C
Sterilisation time	60	60	[20]	min
Microbial growth conditions (batch production of cellulase from *Clostridium thermocellum* – reactor temperature 60°C)				
Product: Biomass ratio	0.2	0.2	0.2	-
Carbon 1 source (excess): Cellulose (includes hemicellulose and paper pulp residue)	28.0^	28.0^	28.0^	%
Nitrogen source 1 (excess): Urea	0	[5]	[5]	%
Nitrogen source 2 (excess): Yeast extract	0	[5]	[5]	%
Mass percentage Nitrogen source 2 as total nitrogen	80	80	80	%
Initial biomass concentration into fermenter	0.04	[1]	[1]	g/l
Final biomass concentration	4	4	4	g/l
Agitation (Energy: Electricity)				
No of tanks	15	[5]	[5]	-
Height /Diameter (fermenter)	1	[2]	[2]	-
Concentrator: (Evaporator) (Energy: Electricity)				
Product phase retained	1	1	[0.99]	
Waste phase removed	0.5	0.5	0.5	
Freeze dryer (Energy: Electricity)				
Solid fraction retained	1	1	[0.99]	-
Liquid fraction removed	0.918	0.918	[0.99]	-

^ Cellulose excess = 0 %. Value is owing to hemicellulose and paper pulp residue included but not reacting in the cellulose stream
[ ] Default data calculated or assumed in the MS-Excel model when no explicit inputs are given

4.5.2. Material and energy balance outputs

Outputs obtained from the model for production of 1 kg cellulase – containing product (*i.e.* cellulase plus impurities) are shown in Table 4.7. From the scenarios developed, this gave cellulase mass purities of 0.83, 0.72 and 1.38 % for Scenarios SmF1, SmF2 and SmF3 respectively. For Scenario SmF1, to produce 1 kg of cellulase (120 kg of product), 90 kg of cellulose, 3.2 kg of yeast extract and 0.79 kg of urea was required. The cellulose requirement was a combination of cellulose, hemicellulose and paper pulp residue.

Scenario SmF2 (140 kg of product) required 90 kg of cellulose, 3.3 kg of yeast extract and 0.83 kg of urea, owing to the increase in excess of nitrogen source specified. Scenario SmF3 (72 kg of product) required 91 kg cellulose, 3.4 kg of yeast extract and 0.85 kg of urea, higher than requirements for both of the previous scenarios as a result of inefficient downstream separations. Water requirements, and resultant wastewater, for Scenarios SmF2 and SmF3 were 33 and 40 % higher than Scenario SmF1 owing to the changes in initial biomass concentration and downstream separation efficiencies respectively. Owing to the low purity of the enzyme stream, waste values from all scenarios were low and similar outputs were calculated.

Energy requirements for Scenario SmF1 included 1680 MJ electricity, 170 MJ steam and 6.2 kg of cooling water. The electricity requirement was only 15 % higher and 16 % lower in Scenarios SmF2 and SmF3 respectively. However, the steam requirement increased more than 3-fold in both cases owing to the assumption of no preheating of the media during sterilisation. Even though the holding time was three times lower in Scenario SmF3, steam requirements were similar to Scenario SmF2 since steam was chiefly needed to heat the media to the sterilisation temperature with the contribution to maintaining temperature being small. Cooling water requirements for Scenarios SmF1, SmF2 and SmF3 were 6.2, 6.4 and 6.0 kg per kg of cellulase respectively.

The values of the material and energy balance given by Zhuang *et al.* (2007) showed that 80 kg, 4.8 kg and 1.2 kg for cellulose, yeast extract and urea were required respectively to produce 1 kg cellulase. Additionally, the flowsheet required 30 kg potassium chloride. Inorganic salts other than those containing nitrogen, sulphur or phosphorus were not accounted for in the generic flowsheet models. The Zhuang *et al.* (2007) flowsheet also showed emissions of 1.8 kg and 0.4 kg of nitrogen and oxygen respectively which were not seen in the generic flowsheet models. The generic flowsheet model included hydrogen air emissions and phosphate and sulphate in the wastewater, none of which were shown in the Zhuang *et al.* (2007) model.

In the Zhuang *et al.* (2007) model, a simplification was made that only cellulose reacted to form biomass and cellulase. They assumed, unrealistically, that the yeast extract and urea did not form part of the reacting media for biomass growth or enzyme formation. In the generic

flowsheet models of Scenarios SmF1, SmF2 and SmF3, cellulose, yeast extract and urea all formed part of the reacting media. Accounting for these materials as reacting media resulted in differences in the raw material inputs.

Owing to the thermophilic nature of the cellulase process (60°C), large amounts of steam were modelled throughout the Zhuang *et al*. (2007) flowsheet. This was steam used to maintain temperatures, with less than 0.05 % used in sterilisation. This large steam requirement further meant a large electrical requirement for compression. The generic flowsheet models assumed that the heat was retained in the downstream processing units and the high temperatures were maintained. This explained why the steam and electrical requirements of Scenario SmF1, SmF2 and SmF3 were less than 5 % and 50 % of the Zhuang *et al.* (2007) values respectively.

Cleaning-in-place and cooling water requirements in the Zhuang *et al.* (2007) model were over 3000-fold greater than Scenarios SmF1, SmF2 and SmF3. This was because the Zhuang *et al.* (2007) value included water for cleaning-in-place (CIP), which was not included in the generic flowsheet models, which only considered cooling water. The process water required by the three scenarios modelled was between 1250 and 1700 kg of water per kilogram of cellulase (pure product excluding impurities). The amount of water required in Zhuang *et al*. (2007) study was similar to Scenario SmF1 at 1225 kg of water. These requirements were more than 17 times those of Heinzle *et al*. (2006) (Flowsheet One), owing to much reduced purity (11 times lower).

Table 4.7: Material balance for the production of cellulase by SmF (Zhuang *et al.* 2007) *vs.* results from the generic flowsheet model developed (basis: 1 kg cellulase in the product stream)

Component	In (kg)	Out (kg)	In (kg)	Out (kg)	In (kg)	Out (kg)	In (kg)	Out (kg)
	Zhuang *et al.* (2007)		Scenario SmF1		Scenario SmF2		Scenario SmF3	
Cellulose* (Cellulose, Hemicellulose, paper pulp residue)	80.4 (50.0; 11.2; 19.2)	0	89.5 (55.6, 12.5, 21.4)	0	89.5 (55.6, 12.5, 21.4)	0	91.3 (56.8, 12.7, 21.8)	0
Cellulase waste′	-	0	-	0	-	0	-	0.02
Clostridium thermocellum	0	0	0	0	1.7	0	1.7	0
Carbon dioxide	-	N/A^	-	22.0	-	24.0	-	24.5
Product (enzyme)	-	169.2	-	120.8	-	139.7	-	72.3
Hydrogen	-	-	-	-0.3	-	-0.3	-	-0.3
Phosphate ions	-	-	-	0.1	-	0.1	-	0.1
Potassium chloride	30.3	0	-	-	-	-	-	-
Urea	1.21	0	0.79	0	0.83	0	0.85	0
Water	1225	1170	1250	1199	1667	1599	1700	1699
Yeast extract	4.85	0	3.18	0	3.34	0	3.40	0
TOTAL	1341	1341	1342	1342	1762	1762	1795	1795

Component	In (kg)	Out (kg)	In (kg)	Out (kg)	In (kg)	Out (kg)	In (kg)	Out (kg)	
	Zhuang *et al.* (2007)		Scenario SmF1		Scenario SmF2		Scenario SmF3		
Product recovery (% kg cel.)		100		100		100		100	
Product purity (mass %)		0.59		0.83		0.72		1.38	
Chemical Oxygen Demand (COD)		N/A^		0		0		2.4	
Energy requirements	**Zhuang *et al.* 2007**		**Scenario SmF1**		**Scenario SmF2**		**Scenario SmF3**		**Units**
Electricity	1120** (4032)		461.4 (1678)		541.1 (1948)		389.4 (1402)		kWh/kg cel. (MJ/kg cel.)
Steam (152°C, 3 bar)	5000** (13500)		62.6 (169.0)		253.0^^ (683.1)		229.1^^ (618.6)		kg/kg cel. (MJ/kg cel.)
Total energy equivalent	17532		1847		2631		2020		MJ/kg cellulase
Chilled water	764		-		-		-		m^3/kg cellulase
CIP and cooling water#	19000		6.22		6.38		6.04		m^3/kg cellulase

*** Cellulose composition given as cellulose, hemicellulose and paper pulp residue.**

′ Cellulase waste is non-recovered product lost in waste water

^ Not available

**** Only 2.58 kg steam was reported for sterilisation. The balance maintained high temperatures throughout, which led to the high electrical requirements to compress the steam.**

^^ Steam for Scenarios SmF2 and SmF3: 140°C, 3 bar

 # Including Cleaning-in-Place (CIP) water in the Zhuang *et al.* (2007) model, but in the generic flowsheet scenarios, as well as cooling water.

4.5.3. Life Cycle Assessment (LCA)

As shown in Table 4.7, of the 5 000 kg steam used in the Zhuang *et al.* (2007) model, only 2.58 kg was used for sterilisation. The rest (4997.42 kg) was used to maintain temperatures throughout the process. The high steam requirement also added to the electrical needs for steam compression. This high steam usage was not included in the modelled Scenarios as more efficient heat retention was assumed in the downstream units. Since energy requirements often contribute significantly to the overall LCA scores, the Zhuang *et al.* (2007) model was not included in the LCA comparison so as not to obscure the comparisons between the modelled scenarios. The LCA impacts were included in Table 4.8 but not discussed.

The Life Cycle Impacts for Scenarios SmF1, SmF2 and SmF3 were calculated as shown in Table 4.8. For each kilogram of cellulase produced, values of 4.30 kg $Sb_{eq.}$, 299 kg $CO_{2\ eq.}$, 18.1mg CFC-$11_{eq.}$, 4.95 kg$SO_{2\ eq.}$ and 1.09 kg $PO_4^{3-}{}_{eq.}$ were reported for Scenario SmF1 in the categories of abiotic depletion, global warming, ozone layer depletion, acidification and eutrophication respectively. These impacts were all lower than for the impacts calculated for Scenario SmF2 as shown in Figure 4.9. This was owing to the lower material and energy inputs required and lower waste emissions.

Although the steam requirements of Scenario SmF3 were over 3-fold higher than Scenario SmF1 (by energy equivalent), the LCA impacts were lower in all categories except ozone layer depletion, terrestrial ecotoxicity and eutrophication as seen in Figure 4.9. The lower LCA scores were owing to the reduced electricity scores which dominated the LCA impacts. Ozone layer depletion, terrestrial ecotoxicity and eutrophication scores of Scenario SmF3 were higher than Scenario SmF1 owing to increased cellulose, urea and yeast extract inputs which contributed to give higher LCA scores.

Table 4.8: LCIA of cellulase produced by SmF for inputs obtained from Zhuang *et al.* (2007) and the modelled Scenarios SmF1, SmF2 and SmF3 – CML 2 Baseline 2000 V2.03 (basis: 1 kg cellulase in the product stream)

Impact Category	Unit	Zhuang et al. (2007)	Scenario SmF1	Scenario SmF2	Scenario SmF3
Abiotic Depletion	kg $Sb_{eq.}$	18.9	4.30	5.29	4.07
Global Warming (GWP100)	kg $CO_{2\ eq.}$	1940	299	403	290
Ozone Layer Depletion (ODP)	mg $CFC\text{-}11_{eq.}$	178	18.1	25.7	21.3
Human Toxicity	kg $1,4\text{-}DB_{eq.}$	775	209	252	193
Fresh Water Aquatic Ecotoxicity	kg $1,4\text{-}DB_{eq.}$	140	51.1	59.9	44.8
Marine Aquatic Ecotoxicity	kg $1,4\text{-}DB_{eq.}$	940000	328000	385000	291000
Terrestrial Ecotoxicity	kg $1,4\text{-}DB_{eq.}$	7.66	0.92	1.23	1.09
Photochemical Oxidation	kg C_2H_4	0.54	0.18	0.21	0.16
Acidification	kg $SO_{2\ eq.}$	13.6	4.95	5.76	4.48
Eutrophication	kg $PO_4^{3-}{}_{eq.}$	1.82	1.09	1.17	1.17

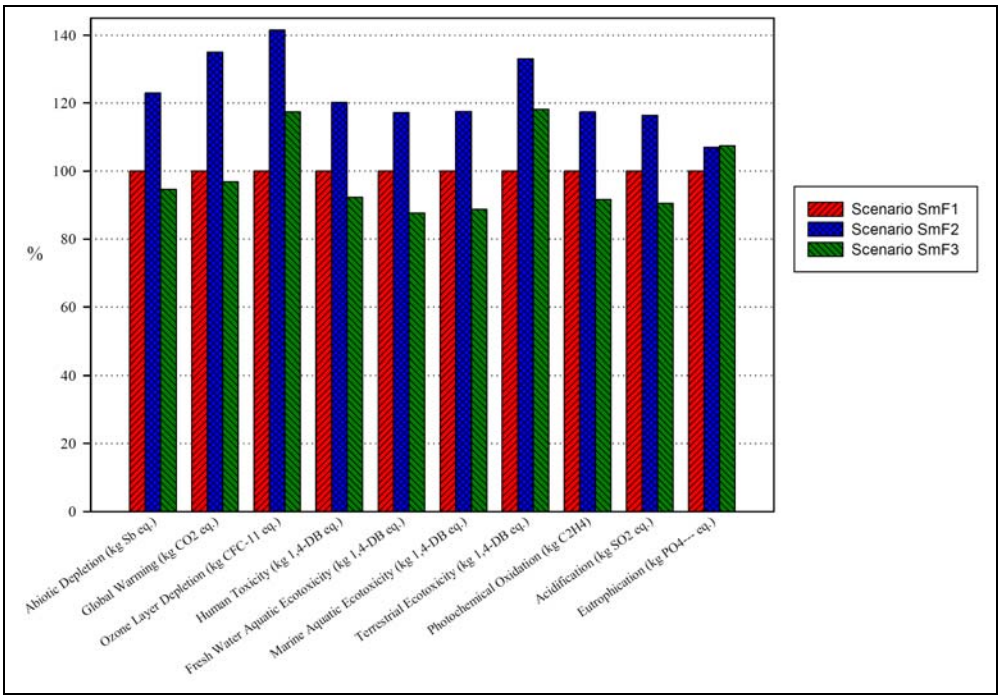


Figure 4.9: Comparison of LCA results for cellulase production by submerged fermentation for Scenarios SmF2 and SmF3 relative to Scenario SmF1

4.5.4. Process contributions

Using Scenario SmF1 as an example, process contributions for six LCA impact categories were investigated. Scenario SmF1 was chosen as it was the most detailed model of the three scenarios modelled and included yeast extract and urea as reacting media; a consideration not taken into account by Zhuang *et al.* (2007).

In all categories, electricity was the main contributor to Life cycle Assessment impacts. Cellulose (paper pulp), steam and yeast extract also formed large portions of contributions in all LCA categories. In the abiotic depletion category, electricity formed 84 % of the impact, with cellulose and steam contributing 10 % and 4.3 % respectively. The main source of the LCA impact for cellulose was electricity, resulting in a total contribution by electricity of over 80 % as shown in Figure 4.10. In the global warming impact category, the cellulose from paper pulp reduced carbon dioxide in the atmosphere. The overall emissions of global warming gases were still positive, mainly due to a large electricity impact (which was not drawn to scale in the pie chart of Figure 4.10). This large electricity impacts were a result of large energy requirements of the downstream processing units. Steam and yeast extract also added 7.8 % and 9.4 % respectively. In the previous cellulase flowsheet study (Scenario H1), the impact to global warming showed a smaller relative impact from electricity and a greater negative portion contributed by the production of cellulose. This was owing to the fact that electricity increased 33-fold in Scenario SmF1, but the cellulose requirements only increased 24-fold.

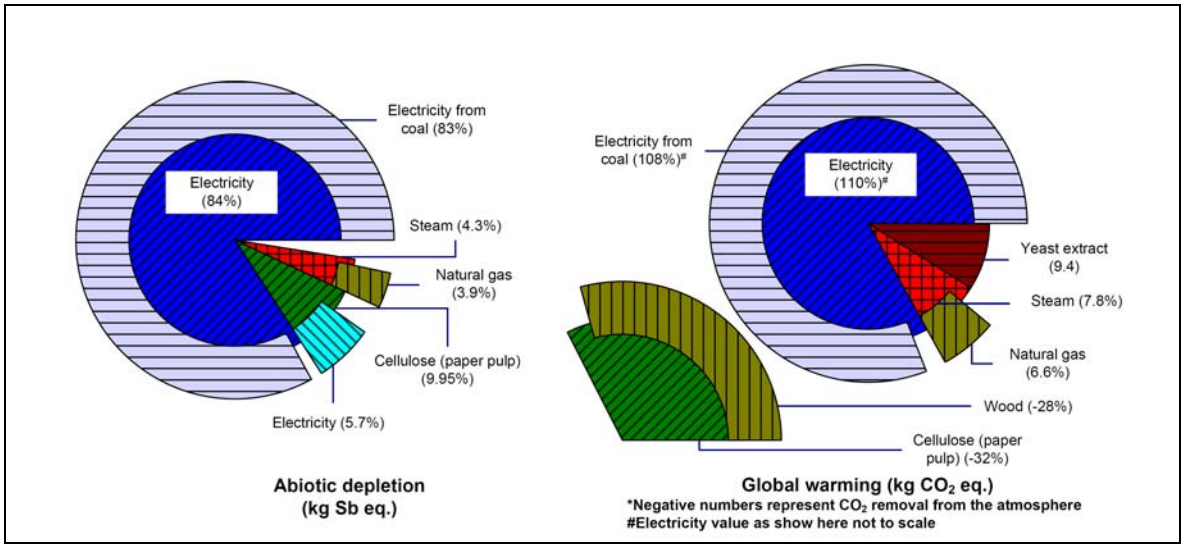
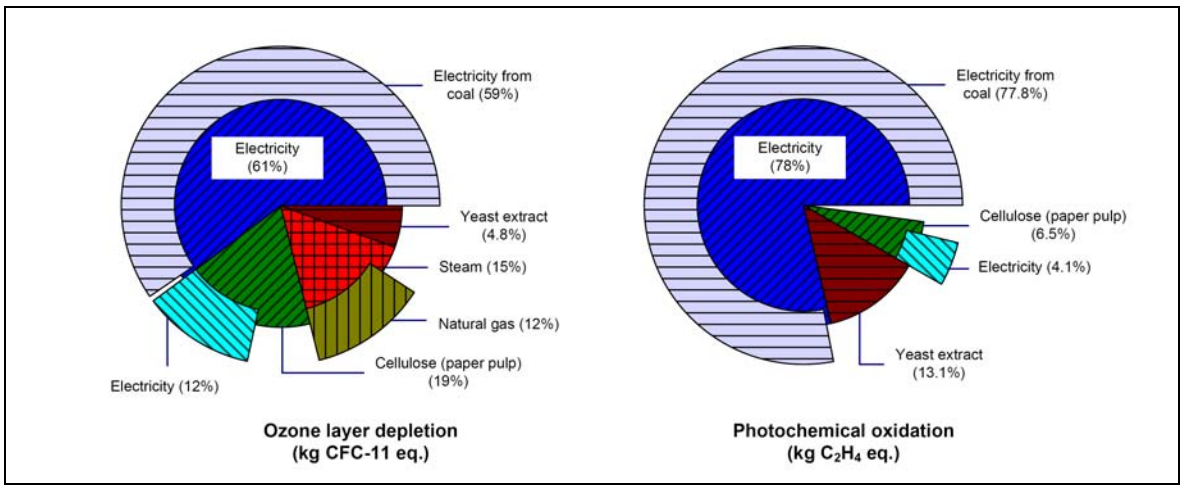


Figure 4.10: Life Cycle Assessment process contributions of cellulase production by SmF using the CML baseline 2.03 methodology (Abiotic depletion and Global warming)

Electrical impacts were calculated to be 61 % for ozone layer depletion and 78 % for photochemical oxidation as shown in Figure 4.11. The assumption of South African electricity implied the dominance of generation from coal. The remaining contributions for ozone layer depletion were made up of cellulose (19 %), steam (15 %) and yeast extract (4.8 %). Photochemical oxidation contributions were completed by yeast extract (13.1 %) and cellulose (6.5 %).



*** Note: Ozone layer depletion is of reduced importance in current processes (see Appendix A.6)**

Figure 4.11: Life Cycle Assessment process contributions of cellulase production by SmF using the CML baseline 2.03 methodology (Ozone layer depletion and Photochemical oxidation)

Electricity (79.2%) also formed the greatest contribution to acidification. Further contributors were yeast extract (11.9 %) and cellulose (7.1 %) as shown in Figure 4.12. Eutrophication scores were dominated by yeast extract (68.1 %), owing to the assumption of yeast production from glucose with an associated agricultural origin and a high eutrophication score. Electricity and cellulose contributed 17.5 % and 7.1 % respectively. Unlike the previous cellulase flowsheet, which assumed wood chips as the cellulose source, this process used paper pulp, which had a lower contribution to eutrophication. Yeast extract is typically a waste product and could have been modelled in the LCA study as a waste utilisation process. This would have meant a reduction of impacts as a result of the use of yeast extract.

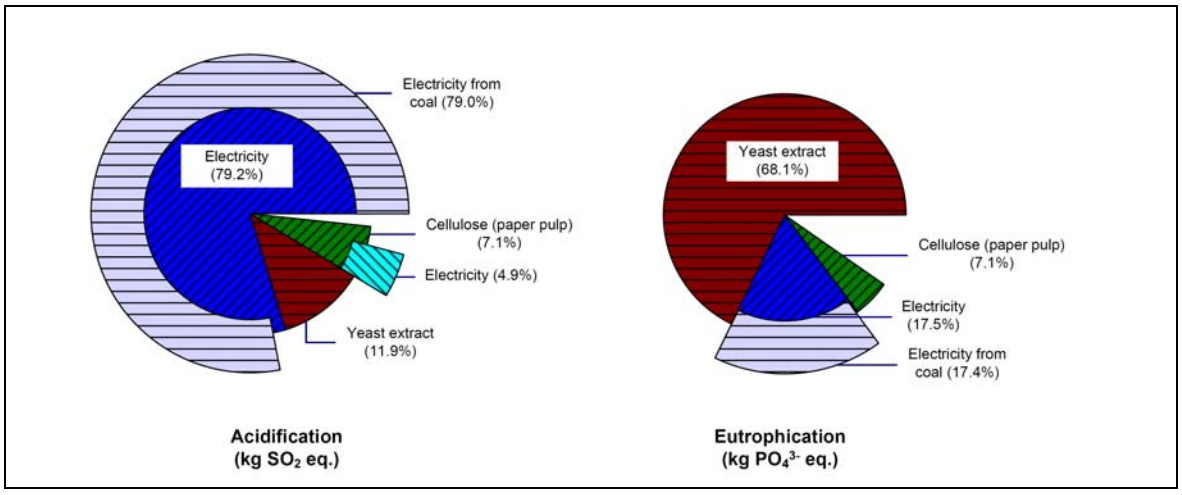


Figure 4.12: Life Cycle Assessment process contributions of cellulase production by SmF using the CML baseline 2.03 methodology (Acidification and Eutrophication)

4.5.5. Discussion

The modelled Scenarios SmF1, SmF2 and SmF3 showed good agreement for material inputs and outputs. The Zhuang *et al.* (2007) energy requirements were at least 9 fold higher than the scenario (SmF1) with the lowest energy needs. This high energy requirement was owing to high steam requirements in the Zhuang *et al.* (2007) model to maintain temperatures throughout the process. This large steam requirement was not made in the generic flowsheet models as it was assumed that heat was not lost in the downstream processing units.

Energy requirements (electricity and steam) were the main contributors in all LCA impact categories except eutrophication, which was dominated by the production of yeast extract. As such, differences in LCA results between the modelled scenarios were owing to the energy changes and had little to do with changes to the material inputs and emissions of the flowsheets. The large energy requirement was from downstream processing of the forced evaporator and freeze drying. LCA sensitivities to energy changes were investigated further in Chapter 7.

Yeast extract used in the model was assumed to add to the LCA impacts during its production. However, yeast extract is typically a waste product and it could have been modelled as a waste utilisation process by burden allocation in the LCA analysis. This would have meant a reduction of impacts and a loss of carbon credits.

4.6. Cellulase Flowsheet Three: Anaerobic production by SSC using *Clostridium thermocellum*

4.6.1. Cellulase model development

The preceding cellulase flowsheets have used submerged culture technology for enzyme formation. This resulted in large volumes of water throughout, with associated high steam and electrical requirements. An alternative method of enzyme formation, using a solid state cultivation (SSC) system, is investigated in the third production model. This system is similar to the cellulase flowsheet presented in Section 4.5, but requires no downstream processing. It is reported that the SSC method has lower water and energy consumption, results in less waste water, gives a higher concentration product (Zhuang *et al.* 2007) and has lower capital and operating costs than the SmF method (Durand *et al*., 1997, Kumar and Lonsane 1987). However, heat and mass transfer is more difficult because of limited diffusion through a solid substrate (Mitchell *et al.* 2003, Deschamps and Huet 1984). Chinn (2003) and Zhuang *et al.* (2007) provide background on the SSC process for cellulase production as used here.

The process of solid state cultivation for cellulase production required a similar front end to submerged fermentation up to the point of biomass growth. Two medium blenders were fed with cellulose powder and medium and the resultant suspension heat sterilised before filling the seed fermenters. *Clostridium thermocellum* inoculum and paper pulp were added. Following cultivation of the inoculum, the culture was transferred to an anaerobic solid state cultivation vessel as shown in Figure 4.13. The low water requirement resulted in a higher concentration of enzyme; hence downstream processing units were not included in this process.

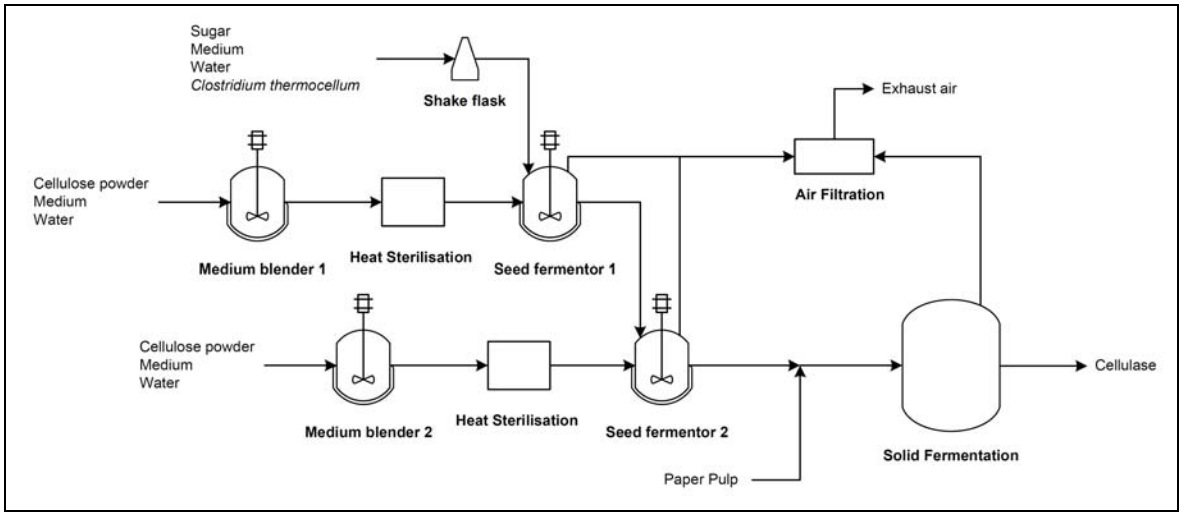


Figure 4.13: Simplified process flowsheet for cellulase production as modelled in the MS-Excel model for the SSC method

Values of the necessary parameters, summarised in Table 4.9, were extracted from the literature, predominantly from the cellulase production model of Zhuang *et al.* (2007). Three scenarios have been presented (Scenarios SSC1, SSC2 and SSC3), each less input data specified. Defaults within the generic flowsheet model, as explained in Chapter 2, were used to complete the necessary dataset. Scenarios SSC2 and SSC3 used approximately 60 % and 40 % of the original information respectively. The lists of inputs used for the three scenarios are shown in Table 4.9.

Table 4.9: Sets of input assumption from Zhuang *et al.* (2007) for the extracellular, anaerobic production of cellulase in a batch solid state cultivation reactor

Assumptions	Scenario SSC1	Scenario SSC2	Scenario SSC3	Units
Steam Sterilisation: (preheated with sterilised media)				
Preheated temperature	110	[60]	[60]	°C
Sterilisation temperature	121	[121]	[121]	°C
Steam temperature	152	[140]	[140]	°C
Sterilisation time	60	60	[20]	min
Microbial growth conditions (batch production of cellulase from *Clostridium thermocellum* – reactor temperature 60°C)				
Product: Biomass ratio	0.2	0.2	0.2	-
Carbon 1 source (excess): Cellulose (includes hemicellulose and paper pulp residue)	28.0^	28.0^	[1]	%
Nitrogen source 1 (excess): Urea	0	[5]	[5]	%
Nitrogen source 2 (excess): Yeast extract	0	[5]	[5]	%
Mass percentage Nitrogen source 2 as total nitrogen	80	80	[50]	%
Maintain rector temperature (Cooling agent: Cooling water)	60	60	60	°C
Initial biomass concentration into fermenter	0.04	[1]	[1]	g/l
Final biomass concentration	20.5	20.5	20.5	g/l

^ Cellulose excess = 0 %. Value is owing to hemicellulose and paper pulp residue included but not reacting in the cellulose stream
[ ] Default data calculated or assumed in the MS-Excel model when no explicit inputs are given

4.6.2. Material and energy balance outputs

The model outputs for the production of cellulase are shown in Table 4.10. Mass purities of 0.32, 0.31 and 0.32 % were calculated for Scenarios SSC1, SSC2 and SSC3 respectively. For Scenario SSC1, to produce 1 kg of cellulase (310 kg of product), 90 kg of cellulose, 3.2 kg of yeast extract and 0.79 kg of urea was required. The cellulose was a combination of all cellulose, hemicellulose and paper pulp residue requirements. Scenario SSC2 (330 kg of product) required 90 kg of cellulose, 3.3 kg of yeast extract and 0.83 kg of urea. Scenario SSC3 (310 kg of product) required 77 kg cellulose, 1.3 kg of yeast extract and 1.3 kg of urea. Since the microbial growth conditions of Scenarios SSC1 and SSC2 were the same as Scenarios SmF1 and SmF2

respectively, the raw material inputs of the submerged fermentation were the same as the solid state cultivation method.

Water requirements for Scenarios SSC1, SSC2 and SSC3 were 244, 257 and 257 kg respectively. The SSC models required one fifth that of the SmF models. Since there was no downstream processing in the SSC model, there was only one liquid stream. Owing to the way the scenarios were set up, gaseous emissions of carbon dioxide and hydrogen, were almost identical.

Energy requirements for Scenario SSC1 included 6.5 MJ electricity, 47 MJ steam and 5.8 kg of cooling water. In agreement with volume reduction and the absence of agitation and downstream processing in the SSC models, energy requirements were 2.9 % of the Scenario SmF1 energy requirement. The electrical energy and steam requirements of Scenario SSC2 and SSC3 were 300 % and 245 % higher than Scenario SSC1 respectively, owing to the absence of heat integration with no preheating of media before steam sterilisation. The decrease in sterilisation holding time decreased the energy requirement for Scenario SSC3. The cooling water requirements for all scenarios were within 98 % of each other.

The material input requirements for the Zhuang *et al.* (2007) model (315 kg of product) were shown to be: 80 kg, 0.87 kg and 0.22 kg for cellulose, yeast extract and urea respectively as shown in Table 4.10. Additionally, the flowsheet required 5.4 kg potassium chloride which was not accounted from in the generic flowsheet models. The Zhuang *et al.* (2007) flowsheet showed emissions of 0.26 kg and 0.097 kg of nitrogen and oxygen respectively, but did not elaborate on how these were formed since this was an anaerobic process. The generic flowsheet models included hydrogen emissions which were not shown in the Zhuang *et al.* (2007) model. Process water was higher in the SSC model of Zhuang *et al.* (2007) as cleaning-in-place (CIP) was taken into account, but not in the generic flowsheet models of Scenarios SSC1, SSC2 and SSC3.

As in the submerged fermentation model of Zhuang *et al.* (2007) there was a large difference in energy between the SSC models. Large amounts of steam were required to maintain temperatures, which were not accounted for in the generic flowsheet models. Compression of this steam meant that additional electricity was also needed. Steam and electrical requirements of Scenario SSC1 were less than 18 % and 15 % of the Zhuang *et al.* (2007) values respectively.

In the Zhuang *et al.* (2007) model for solid state cultivation, as in submerged fermentation, a simplification was made that only cellulose reacted to form biomass and cellulase. In the generic flowsheet models, cellulose, yeast extract and urea all formed part of the reacting media, resulting in the differences in the raw material inputs.

Table 4.10: Material, energy and utility flows for the production of cellulase by SSC (Zhuang *et al.* 2007) *vs.* results from the generic flowsheet model developed (basis: 1 kg cellulase in the product stream)

Component	In (kg)	Out (kg)	In (kg)	Out (kg)	In (kg)	Out (kg)	In (kg)	Out (kg)
	Zhuang *et al.* (2007)		Scenario SSC1		Scenario SSC2		Scenario SSC3	
Cellulose* (Cellulose, Hemicellulose, paper pulp residue)	80.4 (50.0, 11.2, 19.2)	0	89.5 (55.6, 12.5, 21.4)	0	89.5 (55.6, 12.5, 21.4)	0	73.3 (45.6, 10.2, 17.5)	0
Clostridium thermocellum	0.01	0	0.01	0	0.26	0	0.26	0
Carbon dioxide	-	N/A^	-	24.0	-	24.0	-	25.1
Product (enzyme)	-	315.4	-	313.9	-	327.0	-	307.1
Hydrogen	-	-	-	-0.3	-	-0.3	-	-0.3
Nitrogen	-	0.26	-	-	-	-	-	-
Oxygen	-	0.079	-	-	-	-	-	-
Potassium chloride	5.44	0	-	-	-	-	-	-
Urea	0.22	0	0.79	0	0.83	0	1.3	0
Water	228.6	0	244.3	0	256.8	0	256.8	0
Yeast extract	0.87	0	3.18	0	3.34	0	1.3	0
TOTAL	315	315	338	338	351	351	332	332
Product recovery (% kg cel.)		100		100		100		100
Product purity (mass %)		0.32		0.32		0.31		0.32
Chemical Oxygen Demand (COD)		N/A^		0		0		0

Energy requirements	Zhuang *et al.* 2007	Scenario SSC1	Scenario SSC2	Scenario SSC3	Units
Electricity	10.5 (37.8)	1.8 (6.5)	5.4 (19.5)	4.4 (16.0)	kWh/kg cel. (MJ/kg cel.)
Steam (152°C, 3 bar)	116.9 (315)	17.4 (47.0)	52.1^^ (140.7)	42.7^^ (115.3)	kg/kg cel. (MJ/kg cel.)
Total energy equivalent	352.8	53.5	160.2	131.3	MJ/kg cellulase
Chilled water	6.73	-	-	-	M^3/kg cellulase
Cooling water	304.4	5.8	5.8	5.9	m^3/kg cellulase

***Cellulose composition given as cellulose, hemicellulose and paper pulp residue.**

^ Not available

****Only 2.58 kg steam is required for sterilisation. The rest is needed for maintaining high temperatures throughout. The extra steam leads to the high electrical requirements to compress the steam.**

^Steam for Scenarios SSC2 and SSC3: 140°C, 3 bar

#Includes water for Cleaning-in-Place (CIP) for all units. This is not accounted for in the generic flowsheet models

4.6.3. Life Cycle Assessment (LCA)

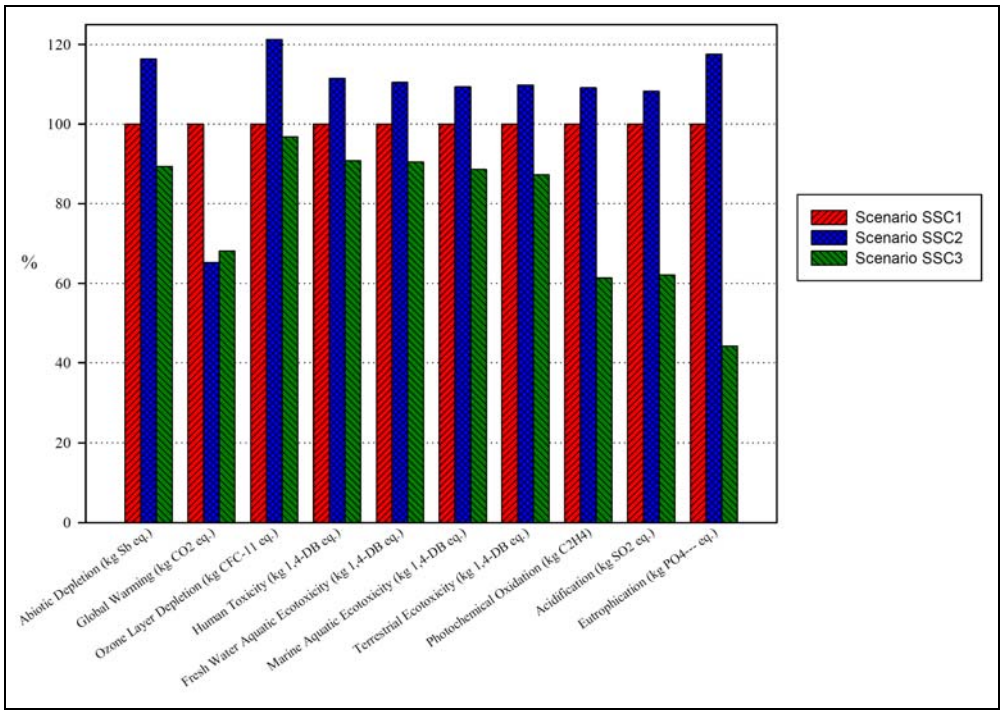
Since the total steam used in the Zhuang *et al.* (2007) model included excessive requirements to maintain temperatures, which were not shown in Scenario SSC1, SSC2 and SSC3, the Zhuang *et al.* (2007) model was not included in the LCA comparison.

For 1 kg of cellulase (314 kg of product), life cycle impacts of 0.62 kg $Sb_{eq.}$, 5.41 mg CFC-11 $_{eq.}$, 0.97 kg $SO_{2\,eq.}$ and 0.80 kg$PO_4^{3-}{}_{eq.}$ were obtained for Scenario SSC1 in the categories of abiotic depletion, ozone layer depletion, acidification and eutrophication as shown in Table 4.10. The LCA results for the same categories for Scenario SSC2 were: 0.72 kg $Sb_{eq.}$, 6.55 mg CFC-11 $_{eq.}$, 1.05 kg $SO_{2\,eq.}$ and 0.94 kg$PO_4^{3-}{}_{eq.}$. In all categories, Scenario SSC2 impacts were larger than Scenario SSC1 impacts. This was owing to the larger energy requirements for both electricity and steam.

The global warming impacts showed that there was an overall carbon dioxide equivalent reduction as a result of cellulase production. The larger negative values indicated greater carbon dioxide equivalent reductions. The higher energy requirements of Scenarios SSC2 and SSC3 resulted in higher global warming impacts than Scenario SSC1.
The burdens from Scenario SSC3 were lower than Scenario SSC1 and SSC2 in all categories except global warming (Figure 4.14). Even though the energy requirements of Scenario SSC3 were higher than for Scenario SSC1, LCA impacts were still lower by between 10 and 55 % owing to the lower cellulose and yeast extract inputs which dominated the LCA scores.

Table 4.11: LCIA of cellulase produced by SSC as modelled in Scenarios SSC1, SSC2 and SSC3 – CML 2 Baseline 2000 V2.03 (basis: 1 kg cellulase in the product stream)

Impact Category	Unit	Scenario SSC1	Scenario SSC2	Scenario SSC3
Abiotic Depletion	kg $Sb_{eq.}$	0.62	0.72	0.55
Global Warming (GWP100)	kg $CO_{2\,eq.}$	-35.0	-22.8	-23.8
Ozone Layer Depletion (ODP)	mg CFC-$11_{eq.}$	5.41	6.55	5.23
Human Toxicity	kg 1,4-$DB_{eq.}$	32.3	36.0	29.4
Fresh Water Aquatic Ecotoxicity	kg 1,4-$DB_{eq.}$	5.11	5.64	4.62
Marine Aquatic Ecotoxicity	kg 1,4-$DB_{eq.}$	40600	44400	35900
Terrestrial Ecotoxicity	kg 1,4-$DB_{eq.}$	0.51	0.56	0.45
Photochemical Oxidation	kg C_2H_4	0.036	0.039	0.022
Acidification	kg $SO_{2\,eq.}$	0.97	1.05	0.60
Eutrophication	kg $PO_4^{3-}{}_{eq.}$	0.80	0.94	0.35



**** Since global warming values are negative, the results for Scenario SSC1 were most desirable and Scenario SSC2 the least desirable***

Figure 4.14: Comparison of LCA results for cellulase production by solid state cultivation for Scenarios SSC2 and SSC3 relative to Scenario SSC1

4.6.4. Process contributions

For the production of cellulase by SSC, the individual contributions towards the overall LCA score were investigated. Using Scenario SSC1 the primary energy requirements from electricity used in cellulase production did not form a large portion of the LCA impact in any of the categories. This was expected when compared to the submerged fermentation results, since the energy requirements were up to 100 times lower in the SSC models, to produce equal product masses.

In the abiotic depletion category, cellulose from paper pulp (63 %) formed the largest contribution to Life Cycle Assessment impacts as shown in Figure 4.15. The main impacts within the cellulose impact were electricity and hydrogen peroxide. Yeast extract, steam and urea contributed 18, 15 and 3.5 % respectively. In the global warming impact category, greenhouse gases were removed from the atmosphere during biomass growth of the cellulose production cycle. Carbon dioxide was emitted during yeast extract (47 %), steam (6.8 %) and urea (4.4 %) production. The overall carbon dioxide uptake from biomass growth was larger than the emissions, resulting in a global warming reduction (Figure 4.15).

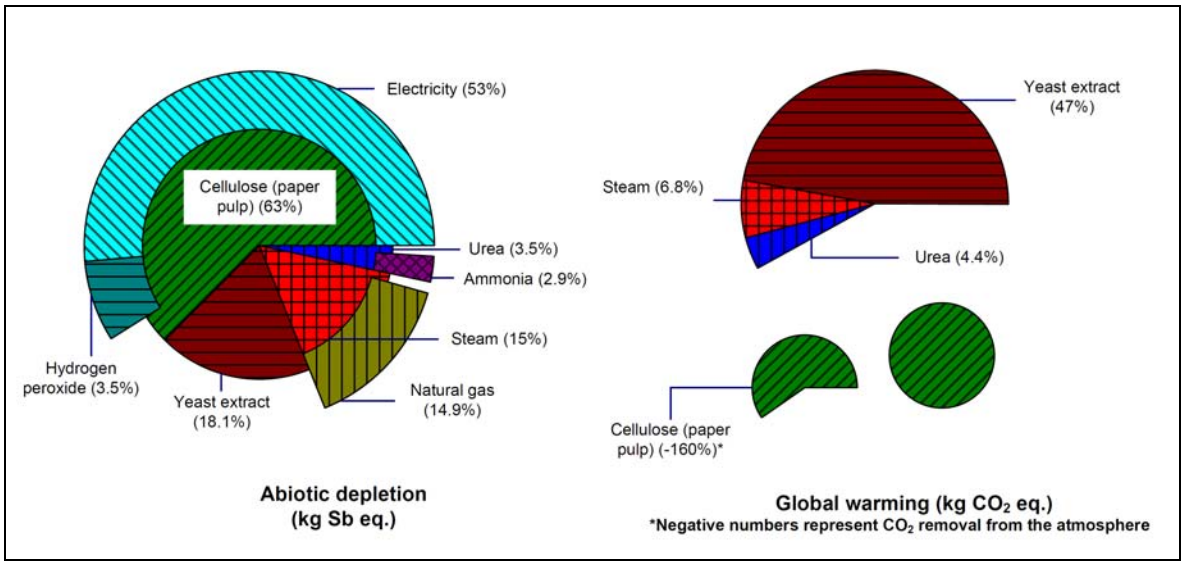
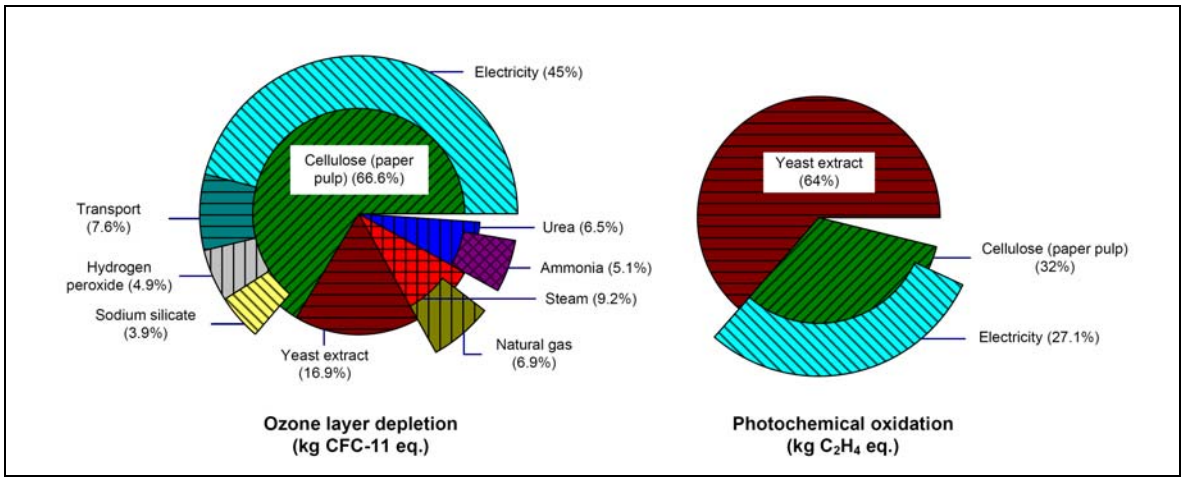


Figure 4.15: Life Cycle Assessment process contributions of cellulase production by SSC using the CML baseline 2.03 methodology (Abiotic depletion and Global warming)

In the ozone layer depletion category, cellulose production (67 %) formed two thirds of the overall impact. This impact was made up of electricity (45 %), transport (7.6 %), hydrogen peroxide (4.9%) and sodium silicate (3.9 %) as shown in Figure 4.16. Yeast extract (17 %), steam (9.2 %) and urea (6.5 %) were the remaining major contributors. In the photochemical oxidation impact category, yeast extract and cellulose contributed 64 % and 32 % respectively. The main contributor of the cellulose impact was electricity (27 %) which formed 85 % of the total cellulose score.



*** Note: Ozone layer depletion is of reduced importance in current processes (see Appendix A.6)**

Figure 4.16: Life Cycle Assessment process contributions of cellulase production by SSC using the CML baseline 2.03 methodology (Ozone layer depletion and Photochemical oxidation)

As with photochemical oxidation, the yeast extract (60.5 %) and cellulose (35.9 %) were the main process contributors in the acidification impact category (Figure 4.17). The eutrophication category was dominated by impacts from yeast extract (92.9 %), with cellulose contributing 6.7 %. The yeast extract formed a large part in eutrophication owing to the agricultural impacts of sugar assumed to have been used in the growth of yeast.

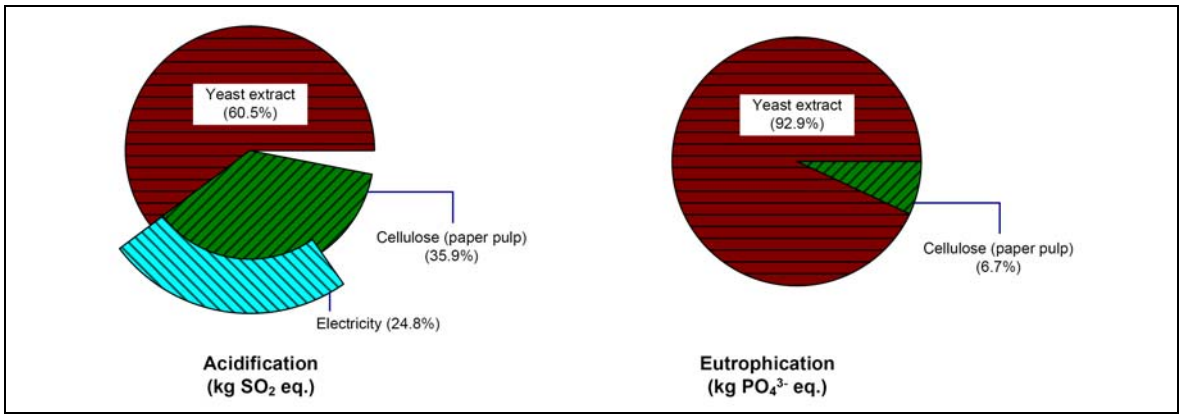


Figure 4.17: Life Cycle Assessment process contributions of cellulase production by SSC using the CML baseline 2.03 methodology (Acidification and Eutrophication)

4.6.5. Discussion

The modelled Scenarios SSC1, SSC2 and SSC3 showed good agreement with Zhuang *et al.* (2007) for material inputs, but did not show as great a steam requirement needed to maintain high temperatures. The high steam in the Zhuang *et al.* (2007) model also required a high electricity requirement and was the main reason for a greater total energy requirement compared to the modelled scenarios. The differences in the steam values were a result of limited information from the Zhuang *et al.* (2007) model, which meant a larger number of assumptions needed in the modelled flowsheets. Since there were fewer downstream processing (DSP) units for the SSC flowsheet and a smaller fraction of the total energy was attributed to DSP, the simplification to assume no DSP heating requirements, owing to perfect heat retention, was not the reason for large energy difference with respect to the SmF models.

The lower electricity resulted in the LCA impacts from cellulose, yeast extract and urea showing greater relative contributions as a result. On a Life Cycle Assessment basis, this lower energy impact meant that changes in electricity requirements had less of an effect to process contributions than in other case studies.

4.7. Comparison of flowsheet models

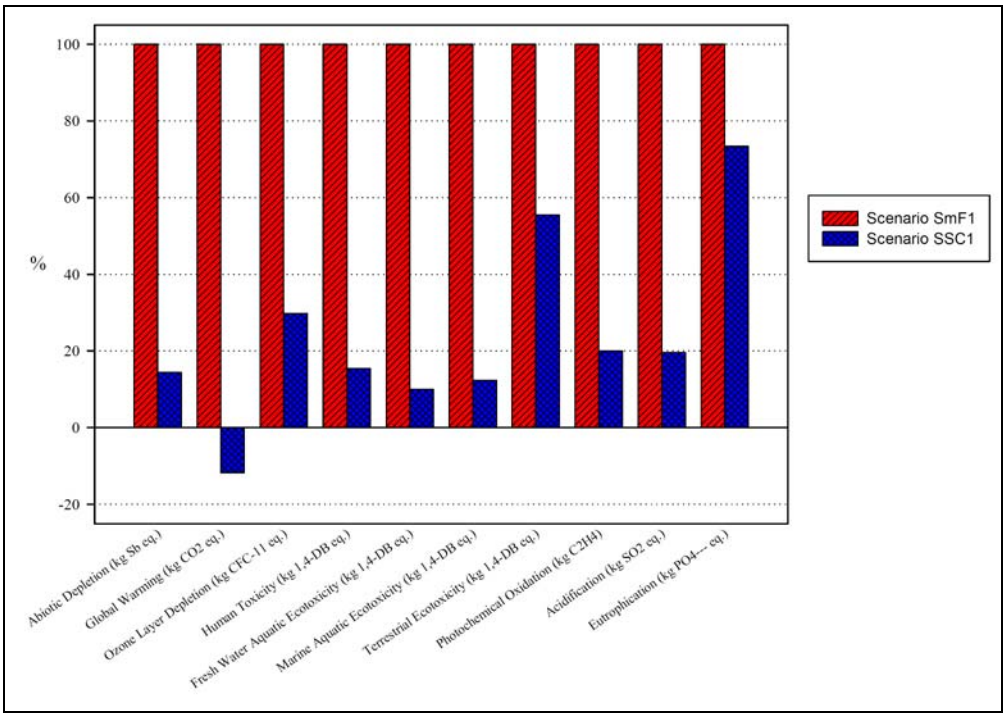
The major difference in the flowsheets investigated for the production of cellulase was the difference in volume owing to the different amounts of water in each of the flowsheets (Table 4.12). Reducing the water requirement decreased volumes, which in turn reduced the energy needed for sterilisation and the steam required for reactor temperature control. The way the submerged fermentation (SmF1) and solid state cultivation flowsheets (SSC) were set up meant that the raw material inputs were almost identical, with only the amount of water entering differing. This meant that from an LCA perspective, any differences were a result of changes in the energy requirements.

Table 4.12: Material, energy and utility flows for the production of cellulase for the three flowsheets presented – generic flowsheet model results (basis: 1 kg cellulase in the product stream)

Component	In (kg)	Out (kg)	In (kg)	Out (kg)	In (kg)	Out (kg)	
	Scenario H1		Scenario SmF1		Scenario SSC1		
Ammonia	0.10	0.00	-	-	-	-	
Trichoderma reesei	0.07	1.22	-	-	-	-	
Carbon dioxide	-	4.00	-	22.0	-	24.0	
Cellulase waste*	-	0.02	-	0	-	-	
Cellulose	3.78	0.28	89.5# (55.6, 12.5, 21.4)	0	89.5# (55.6, 12.5, 21.4)	0	
Clostridium thermocellum	-	-	0	0	0.01	0	
Corn liquor	0.77	0.16	-	-	-	-	
Hydrogen	-	-	-	-0.3	-	-0.3	
Phosphate ions	-	-	-	0.1	-	-	
Product (enzyme)	-	16.4	-	120.8	-	313.9	
Nutrients	0.52	0.10	-	-	-	-	
Oxygen (reacting O_2 only)	3.21	-	-	-	-	-	
Urea	-	-	0.79	0	0.79	0	
Water	78.9	65.2	1250	1199	244.3	0	
Yeast extract	-	-	3.18	0	3.18	0	
TOTAL	87.3	87.3	1342	1762	338	338	
Product recovery (% kg cel.)		98.0		100		100	
Chemical Oxygen Demand (COD)		2.91		0.83		0	
Energy requirements	**Scenario H1**		**Scenario SmF1**		**Scenario SSC1**		
Electricity	50.86 (183.1)		461.4 (1678)		1.8 (6.5)		kWh/kg cel. (MJ/kg cel.)
Steam (152°C, 3 bar)	2.65 (7.14)		62.6 (169.0)		17.4 (47.0)		kg/kg cel. (MJ/kg cel)
Total energy equivalent	190.2		1847		53.5		MJ/kg cellulase
Chilled water	1.60		-		-		m^3/kg cellulase
Cooling water	-		6.22		5.8		m^3/kg cellulase

*** Cellulase waste is non-recovered product lost in waste water**
****Steam at 140°C, 3 bar**
^ Not available
 # Cellulose composition given as cellulose, hemicellulose and paper pulp residue.

The reduction in energy requirements reduced LCA scores of the SSC method to between 10 and 73 % of the SmF values as shown in Figure 4.18. As a result of this lower energy requirement and the carbon dioxide uptake by agricultural processes, the global warming impact of the SSC method was negative (i.e. a net carbon dioxide equivalent reduction). Since energy was not as big an LCA contributor to the eutrophication category, the impacts were not reduced as much in the SSC flowsheet compared with other LCA categories.



**** A negative global warming value refers to net carbon dioxide uptake from the atmosphere***

Figure 4.18: Comparison of LCA results for cellulase production by solid state cultivation (Scenario SSC1) relative to submerged fermentation (Scenario SmF1) as presented in the study

In the submerged fermentation flowsheets, the process contributions from energy were the dominating contributor. It was seen in the solid state cultivation flowsheet, that the relative impacts from raw materials (cellulose and yeast extract) were larger than that of the energy requirements. This was not a result of an increased raw material requirement but owing to decreased energy requirements as shown in Table 4.13.

The total electrical energy requirement for Scenarios H1 and SmF1 was 183.1 and 1678 MJ/kg cellulase respectively compared to 6.5 MJ/kg cellulase for Scenario SSC1. Even though the steam requirement was lower in Scenario H1, the total energy requirement remained lowest in the solid state cultivation flowsheet.

Table 4.13: Energy contributions for the production of cellulase for the flowsheets developed

Energy source	Scenario H1	Scenario SmF1	Scenario SSC1
Electricity (MJ/kg cel.)	**183.1**	**1678**	**6.5**
Sterilisation	0.99	23.5	6.5
Aeration	135.2	-	-
Agitation	2.57	68.1	-
Bioreactor cooling	39.38	156.4	-
Rotary vacuum filtration	2.49	-	-
Evaporator	-	921	-
Ultrafiltration	2.46	-	-
Freeze drying	-	509	-
Steam (kg/kg cel.)	**2.65**	**62.6**	**17.4**
Sterilisation	2.65	62.6	17.4
Total Energy (MJ/kg cel.)	**190.2**	**1847**	**53.5**
Chilled water (m^3/kg cel.)	**1.60**	**-**	**-**
Aeration	1.01	-	-
Fermentation cooling	0.59	-	-
Cooling water (m^3/kg cel.)	**-**	**6.22**	**5.80**
Fermentation cooling	-	6.22	5.80

4.8. Conclusions

The generic MS-Excel model was used to obtain three sets of material and energy balances for the production of cellulase, by three separate flowsheets. For each flowsheet, three scenarios were proposed with a less detailed input set in each successive scenario. For the most detailed scenarios of each flowsheet, it was found that half of the calculated material and energy balance results of flowsheet one, two and three were within 33, 35 and 50 % of literature results respectively.

It was seen that values not within 10 % of the literature results were often a result of simplifying assumptions made in the literature models, while the generic flowsheets used more robust calculations. For example, Zhuang *et al.* (2007) assumed that the yeast extract and urea raw materials do not react in the biomass formation step. The thermophilic nature of the Zhuang *et al.* (2007) models required a large amount of steam and associated compression energy from electricity. This steam was poorly defined in the generic flowsheet model for the submerged fermentation (SmF) flowsheet. Cleaning-in-place (CIP) water was used in Zhuang *et al.* (2007) which had not been included as an option in the setup of the generic flowsheet model in Chapter 2. While water is tracked as an inventory item in the Life Cycle Impact Assessment method chosen, no water usage impact category was provided for the methodology used. This is viewed as an opening for future development owing to the important of optimising water use in bioprocesses.

Using the results generated for the material and energy balances, life cycle assessment studies were completed. The most detailed material and energy balance results for Flowsheet 1 (Scenario H1) gave LCA results which were all within 30 % of the LCA results calculated from the literature material and energy balance inventory of Heinzle *et al*. (2006). The LCA results showed high contributions to overall impacts by electricity and steam in the submerged fermentation (SmF) models. These high electrical requirements were a result of greater volumes which required greater energy for agitation and downstream separations as well as steam during sterilisation.

Cellulase production by solid state cultivation (SSC) gave an expected reduction in volumes and thus energy required. This resulted in a reduction in LCA scores to between 10 and 73 % of the SmF impacts across the various categories. With lower energy requirements in the SSC model, impacts from the raw materials (cellulose, yeast extract and urea) were more dominant.

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CHAPTER 5: POLYMERS

5.1. Introduction

Use of plastics is wide spread; however their disposal is difficult. Incinerating plastics is expensive and can release harmful chemicals. While re-use and recycling is the preferred route, implementation is difficult and results in limited applications for the recycled material (Reddy *et al.* 2003, Khanna and Srivastava 2005). Polypropylene is produced from fossil fuels hence affected by resource depletion. Polymer production from a renewable source is desirable. Such polymers include the polyhydroxyalkanoates (PHAs) group of polymers and the polylactides.

This chapter describes a case study for the application and validation of the generic flowsheet model using the microbial production of polyhydroxyalkanoate (PHA) by *Cupriavidus necator* as an example. Three scenarios were defined for the generic flowsheet, each with a more limited set of inputs. These material and energy results are compared to a full biopolymer design of Harrison (1990).

The material and energy balance results obtained from both the generic flowsheet models and the Harrison (1990) model were then used with the Life Cycle Assessment (LCA) software SimaPro v7.1® (PRé Consultants B.V. 2008) to determine the resulting environmental impacts. The LCA results were also compared to the LCA impacts for the production of polyolefins (polypropylene and polyethylene) to determine the relative impacts of an equivalent chemically produced material.

While there are several commodity biopolymers, the advantage of testing the model on polyhydroxyalkanoates is that there are numerous laboratory scale data providing parameters required for the generic flowsheet model. These are reported by Harrison (1990), Wang and Lee (1997), He *et al.* (1998), Grothe *et al.* (1999), Kim (2000), Chen *et al.* (2001), Yu (2001) and Yu *et al.* (2005).

5.2. The production of polyhydroxyalkanoates (PHAs)

5.2.1. Polyhydroxyalkanoates

A biodegradable polymer is advantageous where recycling and re-use is not possible or practicable. Biopolymers are renewable, largely biodegradable and can have very similar properties to conventional polymers. The potential environmental benefits of biopolymers currently carry an economic cost (Zinn *et al.* 2001, Godbole *et al.* 2003).

Biopolymers currently of interest include thermoplastic starch (TPS), polylactides (PLA), poly-β-hydroxybutyric acid (PHB) and its copolymers (PHAs), and polymer fills. PHBs are considered strong candidates as they have very similar properties to synthetic polymers, as seen in Table 5.1, but degrade completely to water and carbon dioxide under aerobic conditions (Lee 1996). This is advantageous in, amongst other things, medical applications (Pouton and Akhtar 1996, Shum-Tim *et al.* 1999, Williams *et al.* 1999, Sudesh *et al.* 2000, Zinn *et al.* 2001, Reddy *et al.* 2003, Volova *et al.* 2003, Wang *et al.* 2003, Chen and Wu 2005, Khanna and Srivastava 2005).

Polyhydroxyalkanoate synthesis can occur using aerobic conversion of agricultural feedstocks by wild type or recombinant micro-organisms (*e.g.* Akiyama *et al.* 2003), *in vitro* production *via* PHA-polymerase catalysed polymerisation, using genetically engineered plants (Poirer *et al.* 1995, Riesmeier *et al.* 1998, Gerngross 1999, Poirer 1999, Valentin *et al.* 1999, Gerngross and Slater 2000, Kurdikar *et al.* 2001, Moire *et al.* 2003, Snell and Peoples 2002, Capell and Christou 2004, Daae *et al.* 2004 and Mascia and Flavell 2004) or by the anaerobic digestion of biological wastes (Zinn *et al.* 2001 and Reddy *et al.* 2003). This study is confined to microbial production of poly-β-hydroxybutyric acid (PHB) under aerobic ambient growth temperatures (Lee 1996, Reddy *et al.* 2003).

For many systems, the preferred carbon source for production is cane sugar or glucose (Nonato *et al.* 2001), although starch and whey can also be used to produce PHB (Kim 2000). In addition waste effluents, including beet- or cane molasses or starchy water (Yu 2001, Sudesh *et al.* 2000), plant oils, plant-derived fatty acids and alkanes (Sudesh *et al.* 2000) are used.

Table 5.1: Properties of polypropylene and poly-β-hydroxybutyric acid (PHB)

	Units	Polypropylene	PHB
Density	kg/m^3	900-910 [4]	1250 [1]
Melting Point	°C	176 [2]	45-180 [2] P(3HB) = 180 [2]
Tensile Strength	MPa	38 [3]	13 – 40 [1]
Shrinkage	%		1 – 3 [1]
Elongation	%	400 [3]	5 – 680 [3]
Young's Modulus	MPa	17000 [3]	350 – 1000 [1]
Glass-transition Temperature	°C	-10 [2]	15 [1] P(3HB) = 4 [2]
Service Temperature	°C		-30 – 120 [1]
Specific Heat (20-80°C)	kJ/kg.K	1.9 [4]	
Thermal Conductivity (20-150°C)	kW/m.K	0.42-0.61 [4]	

References:

[1] Delft 2004
[2] Doi 1990
[3] Sudesh *et al.* 2000
[4] Ogorkiewicz 1970

Commercial data are not readily available. Chen *et al.* (2001) presented results for poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) production in a 20 m^3 reactor. Akiyama *et al.* (2003) described the computer simulated PHB production using bioreactor volumes between 300- and 700 m^3. Lee and Choi (1998) estimated the economics of production of 100 000 te/y of biopolymer but give little detail. Results from studies at the laboratory scale (Table 5.2), including process conditions and media preparation, have also been presented (Harrison 1990, Wang and Lee 1997, Grothe *et al.* 1999, Kim 2000, Chen *et al.* 2001 and Yu *et al.* 2005).

Table 5.2: Literature review of process conditions

	Organism	Process conditions	Carbon substrate	Reactor Media	Reactor type
Harrison 1990	*Cupriavidus necator* ("*Wautersia eutrophus", "Ralstonia eutrophus", "Alcaligenes eutrophus")*	6.5 dm^3, 30°C, 1500 rpm max., 2 6-blade impellors	Glucose	H_3PO_4 (1 dm^3/m^3), $(NH_4)_2SO_4$ (1.4 kg/m^3), K_2SO_4 (1.7 kg/m^3), $MgSO_4.7H_2O$ (1.9 kg/m^3), trace elements (9 dm^3/m^3).	Fed batch
Wang and Lee 1997	*Azohydromonas lata ("Alcaligenes latus")*	6.6 dm^3, 30°C, 700rpm max., 40 % DO	Sucrose	KH_2PO_4 (0.6 kg/m^3), $Na_2HPO_4.12H_2O$ (3.6 kg/m^3), $MgSO_4.7H_2O$ (1 kg/m^3), $CaCl_2.2H_2O$ (0.1 kg/m^3), citric acid (0.1 kg/m^3), trace elements (3 dm^3/m^3).	Fed batch
He *et al.* 1998	*Pseudomonas stutzeri*	0.4 dm^3, 30°C, 48 h, 200 rpm (shake flask)	Glucose, Soybean oil	$(NH_4)_2SO_4$ (0.5 kg/m^3), $MgSO_4.7H_2O$ (0.4 kg/m^3), $Na_2HPO_4.12H_2O$ (9.65 kg/m^3), KH_2PO_4(2.65 kg/m^3), microelement solution (1 ml/0.40 dm^3; $FeCl_3.6H_2O$ (20 g), $CaCl_2.H_2O$ (10 g), $CuSO_4.5H_2O$ (0.03 g), $MnCl_2.4H_2O$ (0.05 g), $ZnSO_4.7H_2O$ (0.1 g in 1 l 0.5 N HCl)	Batch
Grothe *et al.* 1999	*Azohydromonas lata ("Alcaligenes latus")*	0.2 dm^3, 25-37°C, 200 rpm (shake flask), 96 h	Sucrose	$(NH_4)_2SO_4$, KH_2PO_4, Na_2HPO_4, $MgSO_4.7H2O$, trace elements	Batch
Kim 2000	*Azotobacter chroococcum, Escherichia coli*	2.5 dm^3, 30°C	Starch	Whey powder (11.5 % proteins, 74 % lactose) (30 kg/m^3), $(NH_4)_2SO_4$ (4 kg/m^3), KH_2PO_4 (13.3 kg/m^3), $MgSO_4.7H_2O$ (1.2 kg/m^3) citric acid (1.7 kg/m^3), trace element solution (10 dm^3/m^3)	Fed batch
Chen *et al.* 2001	*Aeromonas hydrophila*	20 m^3, 46 h	Glucose	Yeast extract, lauric acid, $(NH_4)_2SO_4$ (1-2 kg/m^3), Na_2HPO_4 (3.5-5.8 kg/m^3), $MgSO_4.7H_2O$ (0.2-0.5 kg/m^3), $CaCl_2.2H_2O$ (0.05-0.1 kg/m^3), Trace elements (1-2 kg/m^3)	Fed batch
Yu 2001	*Cupriavidus necator* ("*Wautersia eutrophus", "Ralstonia eutrophus", "Alcaligenes eutrophus")*	2 dm^3, 48 h, pH = 11, 50 rpm, 30°C, 300 cm^3 air (STP) /min	Starchy wastewater	$Na_2HPO_4.2H_2O$ (4.8 kg/m^3), KH_2PO_4 (2.65 kg/m^3), $MgSO_4.7H_2O$ (0.4 kg/m^3), Trace elements (1 ml/l)	Semi-batch
Yu *et al.* 2005	*Cupriavidus necator* ("*Wautersia eutrophus", "Ralstonia eutrophus", "Alcaligenes eutrophus")*	2 dm^3, 26°C, 48 h, Aeration: 20 % of saturation	Glucose, Sodium propionate	$Na_2HPO_4.7H_2O$ (6.7 kg/m^3), KH_2PO_4 (1.5 kg/m^3), $(NH_4)_2SO_4$ (2.5 kg/m^3), $MgSO_4.7H_2O$ (0.2 kg/m^3), FeS (60 g/m^3), $CaCl_2$ (10 g/m^3), trace mineral solution (10 dm^3/m^3) 2 % (w/v) glucose, 0.2 % (w/v) yeast extract	Continuous

The data presented includes PHB production using *Azotobacter chroococcum*, *Aeromonas hydrophila*, *Azohydromonas lata*, *Cupriavidus nectar*, *Escherichia coli* and *Pseudomonas stutzeri* on carbon sources such as glucose, soybean oil, sucrose and starch in batch, fed batch and continuous processes. Partial details of microbial growth, PHB accumulation, process conditions and reviews are also described by Lee (1996), Steinbüchel and Füchtenbusch (1998), Nonato *et al.* (2001), Zinn *et al.* (2001) and Khanna and Srivastava (2005).

PHB yields and growth rates are quoted by several authors, including Ackermann and Babel (1998), Grothe *et al.* (1999), Nonato *et al.* (2001) and Khanna and Srivastava (2005). The amount of polymer, as a percentage of total biomass, ranges from 20 to above 85 %. Maximum PHB concentrations, productivities and yields are shown to be 106 kg PHB/m^3, 4.94 kg PHB/m^3/h and 0.8 kg PHB/kg substrate respectively, as seen in Table 5.3.

Table 5.3: Literature review of percentage PHB content of biomass, concentration, productivity, yield and biomass growth rates following its aerobic microbial production

	Percentage Polymer	Biomass Concentration	Polymer Concentration	Polymer Productivity	Polymer Yield	Biomass Growth Rate
	wt % PHB	kg biomass/m^3	kg PHB/m^3	kg PHB/m^3/h	kg PHB/kg substrate	/h
Harrison 1990	70	150	106	1.18*	0.36	0.11-0.33
Wang and Lee 1997, Lee and Choi 1998	87-88.3	111	98.7	4.94-5.13	0.42	0.044*
Grothe *et al.* 1999	63	1.1-3.9*	0.73-2.48	0.15 (0.38 kg sucrose/m^3/h)	0.4	0.075
Kim 2000	20-80	54-87	0.864-61	0.0149-0.9	0.04-0.33	0.017*
Chen *et al.* 2001	50	50	25	0.54	0.25*	0.029*
Nonato *et al.* 2001	65-70	120-150	78-105*	1.44	0.32	0.014-0.018*
Akiyama *et al.* 2003	75-85	100-200	75-170*	4.63	0.3-0.8	0.023-0.046*
Khanna and Srivastava 2005	76	9.3-159*	7.1-121	1.15-2.42	0.36-0.4	0.265
Yu *et al.* 2005	22-90*	3.10-7.96	1.73-2.8	0.045-0.252		0.019-0.136

*Calculated estimate values

Harrison (1990), Lee and Choi (1998), Chen *et al.* (2001), Zinn *et al.* (2001) and Akiyama *et al.* (2003) describe downstream processing for polymer recovery and purification units, including a selection of cell disruption, surfactant pre-treatment, solvent extraction, precipitation, flocculation, filter pressing, rotary vacuum drying, centrifugation and spray drying. Downstream processing can yield up to 95 % recovery of polymer (Lee and Choi 1998, Nonato *et al.* 2001) at purities greater than 98 % (Nonato *et al.* 2001, Zinn *et al.* 2001).

5.2.2. Poly-β-hydroxybutyric acid (PHB) model development

The production of poly-β-hydroxybutyric acid (PHB) was modelled using the microorganism *Cupriavidus necator* (formerly *Wautersia eutrophus*, *Ralstonia eutrophus* and *Alcaligenes eutrophus* (DSMZ 2006)), with a sucrose substrate using the approach of Harrison (1990). The flowsheet is shown in Figure 5.1. Raw materials, biomass and antifoam were sterilised in a continuous system before being fed to an aerated reactor where PHB can accumulate. After biomass growth and PHB accumulation, downstream processing was performed in batch.

Cells were disrupted in a high pressure homogeniser and solids removed by centrifugation. The PHB was re-suspended with the alkaline serine protease, Optimase L660, to digest the non-polymeric cell matter, while potassium hydroxide was added to control pH.

PHB was further processed by treatment with a non-ionic detergent (Synperonic NP8) in a stirred tank reactor. Additional product purification was achieved in repeated cycles of dilution with water and centrifuge action, followed by hydrogen peroxide treatment and a final water washing and centrifuge cycle. The purified PHB was ultimately spray dried.

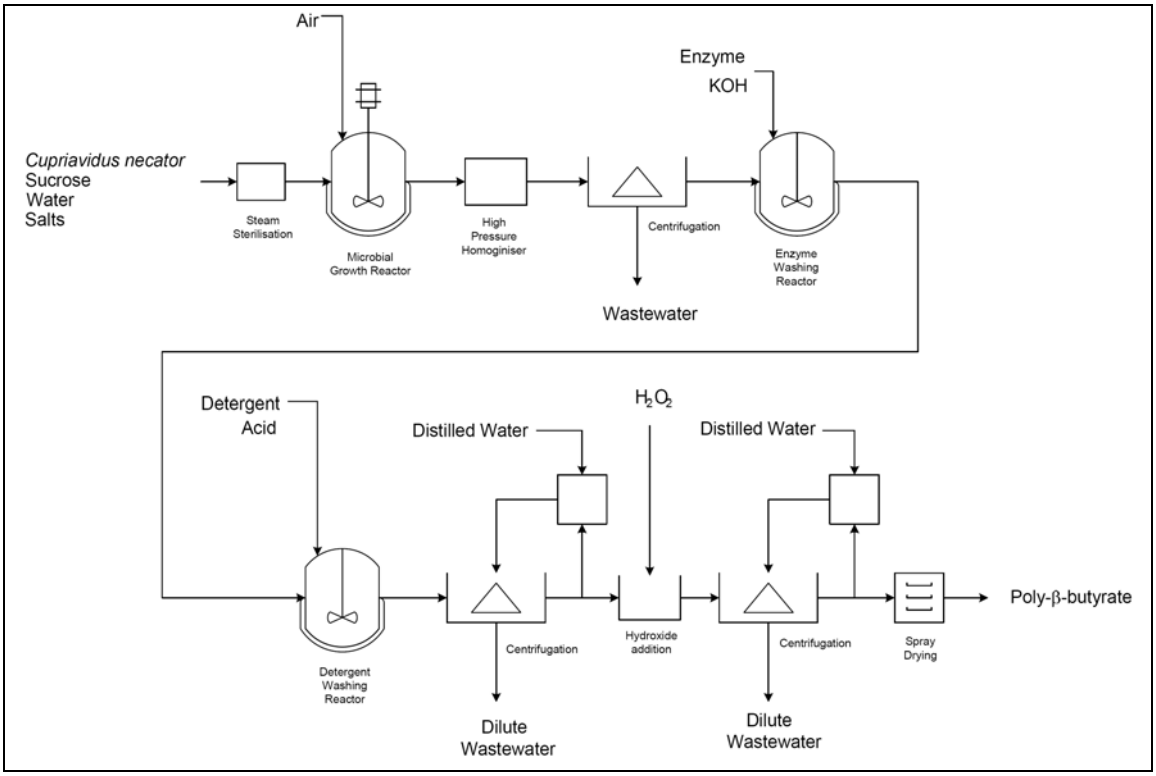


Figure 5.1: Process flowsheet for PHB production (based on Harrison 1990)

The parameters required for the model were estimated from the literature referred to in Table 5.3. Three scenarios have been presented, in which subsets of data from the initial full list (Scenario PHB1) are used. Defaults within the generic flowsheet model, as explained in Chapter 2, were used to provide data not supplied (*e.g.* yield coefficients, unit efficiencies and operating conditions). Scenarios PHB2 and PHB3 used approximately 75 % and 55 % of the original information respectively as listed in Table 5.4.

Table 5.4: Sets of input values collated from literature for the intracellular, aerobic production of PHA in a batch reactor

Assumptions	Scenario PHB1	Scenario PHB2	Scenario PHB3	Units
Steam Sterilisation: Include additional steam requirements (steam out vessel, backing steam, space heating)				
Reactor temperature (maintain reactor temperature)	30	[37]	[37]	°C
Preheat feed with sterilised media: Preheat temperature	65	[60]	[60]	°C
Heat transfer efficiency	100	[80]	[80]	%
Microbial growth conditions (batch production of poly-β-butyrate ($CH_{0.13}O_{0.67}$) from *Cupriavidus necator*)				
Product: Biomass ratio	2.4	2.4	2.4	-
Carbon source (excess): Glucose	0	0	[1]	%
Nitrogen source (excess): Ammonium sulphate	0	0	[5]	%
Oxygen source (vvm): Air	0.6	0.6	0.6	
Compression: Single stage reciprocating compressor				
Final biomass concentration	44[#]	44[#]	44[#]	g/l
Yield coefficients: $Y_{p/s}$	0.44	0.44	0.44	g/g
Antifoam addition	0.1	0.1	[1]	%v/v
Agitation				
Number of tanks	1	[5]	[5]	
Power per unit volume (Energy: Electricity)	0.5	0.5	[0.95]	kW/m^3
Efficiency	0.8	[0.9]	[0.9]	-
Cell disruption: High Pressure homogenisation				
Energy efficiency (Energy: Electricity)	0.8	[1.25]	[1.25]	mg/J
Cell disruption efficiency	100	[90.25]	[90.25]	%
Centrifugation				
Number of wash/spin cycles	1	1	1	
Energy required (Energy: Electricity)	7600	7600	7600	kJ/m^3
Efficiency	1	[0.6]	[0.6]	-
Solid fraction retained	100	100	[98]	%
Liquid fraction removed	Such that vol. equals 1/3 original vol.	Such that vol. equals 1/3 original vol.	Such that vol. equals 1/3 original vol.	-

Assumptions	Scenario PHB1	Scenario PHB2	Scenario PHB3	Units
Enzyme addition: Optimase L660 (MKC) – protease				
Protease volume added	0.08	0.08	0.08	%v/v
Potassium hydroxide added**	10	10	10	%v/v
Agitation energy (Energy: Electricity)	5.76	5.76	5.76	MJ/m^3
Detergent addition: Synperonic NP8 (ICI Ltd)				
Detergent volume added	1	1	1	%v/v
Agitation energy(Energy: Electricity)	5.76	5.76	5.76	MJ/m^3
Centrifugation				
Number of wash/spin cycles (re-suspension with H_2O)	5^	5^	5^	
Energy required (Energy: Electricity)	7600	7600	7600	kJ/m^3
Efficiency	1	[0.6]	[0.6]	-
Time	20	20	20	min
Solid fraction retained	100	100	[98]	%
Liquid fraction removed	Such that after 5 wash cycles vol. remaining equals vol. entered	Such that after 5 wash cycles vol. remaining equals vol. entered	Such that after 5 wash cycles vol. remaining equals vol. entered	-
Hydrogen peroxide addition				
H_2O_2 volume added	1.2	1.2	1.2	%v/v
Centrifugation				
Number of wash/spin cycles (re-suspension with H_2O)	2	2	2	
Energy required (Energy: Electricity)	7600	7600	7600	kJ/m^3
Efficiency	1	[0.6]	[0.6]	-
Time	20	20	20	min
Solid fraction retained	100	100	[98]	%
Liquid fraction removed	Such that after 2 wash cycles vol. remaining equals vol. entered	Such that after 2 wash cycles vol. remaining equals vol. entered	Such that after 2 wash cycles vol. remaining equals vol. entered	-
Spray drying (Energy: Natural gas)				
Solid fraction retained	100	[99]	[99]	%
Liquid fraction removed	100	100	[99]	%

*** Values available from literature and not specific to this flowsheet design**

**** The amount of potassium hydroxide added would normally be given as a mass per volume value. However, the generic flowsheet is set up to require a volume per volume value liquids are typically added.**

 # Non-PHB biomass only

^ Not a direct input to the Excel model, but used to calculate other information required for the data input here

[ ] Default values calculated or assumed in the MS-Excel model when no explicit inputs are given

5.3. Results

5.3.1. Material and energy balance outputs

Implementing the generic model as described in Chapter 2, material and energy balance data were generated and the results are shown in Table 5.5, using a basis of one kilogram poly-β-butyrate-containing product. These gave mass purities of 100, 100 and 90.5 % and recoveries of 100, 99 and 93.2 % for Scenarios PHB1, PHB2 and PHB3 respectively. Between 3.2 and 3.6 kg of glucose and between 0.23 and 0.28 kg ammonium sulphate were required as feed. These sets of values were both within 88 % of each other. At the aeration rate of 0.6 vvm, 107.6, 120.2 and 278.6 kg of air was required for Scenarios PHB1, PHB2 and PHB3 respectively. The more than doubling of air in Scenario PHB3 was owing to the increase in the reactor volume because of the assumptions made with regard to excess raw materials and the less efficient downstream processing. This increase in volume led to an increase in water, total energy (renewable and non-renewable) and downstream processing input requirements as shown in Figure 5.2. In the three scenarios, 31.8-, 34.8- and 92.2 kg of water per kilogram of plastic was required for Scenarios PHB1, PHB2 and PHB3 respectively.

It was assumed that there was no excess carbon or nitrogen source in Scenarios PHB1 and PHB2. This resulted in there being no glucose or ammonium sulphate waste in the wastewater. Mass outputs for these scenarios were within 12 % of each other (except biomass waste) as shown in Figure 5.3. The increased volumes of Scenario PHB3, which were almost three times greater than previous scenarios, also increased the additives required in downstream processing by almost three times as these were added as volume fractions. Carbon dioxide formed from the three scenarios was within 10 % for all scenarios.

For each kilogram of PHB formed in Scenario PHB1, 11.7 MJ of electrical energy, 4.96 kg steam and 503.7 kg natural gas were required. Similar, but slightly higher, requirements were found for Scenario PHB2. Compared with Scenario PHB1, the increased volumes of Scenario PHB3 required 164, 144 and 50 % more electricity, natural gas and steam respectively. Owing to the higher reactor temperatures of Scenarios PHB2 and PHB3 (37°C) versus Scenario PHB1 (30°C), less cooling water was required, since cooling water was only required for controlling reactor temperatures. A cooling water temperature of 18°C was used in all scenarios. The 7°C difference in reactor temperature resulted in a 4 fold lower cooling requirement in both Scenarios PHB2 and PHB3 compared to Scenario PHB1.

Table 5.5: Material, energy and utility flows for the production of poly-β-hydroxybutyric acid for the flowsheet developed

Component	In (kg)	Out (kg)	In (kg)	Out (kg)	In (kg)	Out (kg)	
	Scenario PHB1		Scenario PHB2		Scenario PHB3		
Glucose	3.2	0	3.6	0	3.4	0.03	
Ammonium sulphate	0.23	0	0.28	0	0.28	0.01	
Hydrogen peroxide	0.054	0.054	0.059	0.059	0.155	0.154	
Potassium hydroxide	0.36	0.36	0.39	0.39	1.00	0.06	
Optimase L660 (MKC)	0.0022	0.0022	0.0024	0.0024	0.0062	0.0062	
Synperonic NP8 (ICI Ltd)	0.028	0.028	0.030	0.030	0.078	0.078	
Air	107.6	106.4	120.2	119.0	278.6	277.4	
Carbon dioxide	-	1.9	-	2.1	-	2.0	
PHB	-	1	-	1	-	1	
PHB (lost in wastewater)	-	-	-	0.036	-	0.066	
Antifoam (PPG.EEA 142)	0.008	0.008	0.009	0.009	0.22	0.22	
Solid waste (Biomass)	-	0.42	-	0.58	-	0.55	
Water	31.8	33.1	34.8	36.2	92.2	93.5	
Total	143.3	143.3	159.4	159.4	375.9	375.9	
Chemical Oxygen Demand (COD)		0.9		1.2		1.6	
Product recovery (% PHB)		100		99		93.2	
Product purity (%)		100		100		90.5	
Energy requirements	**Scenario PHB1**		**Scenario PHB2**		**Scenario PHB3**		**Units**
Electricity	3.26 (11.7)		3.58 (12.9)		8.61 (31.0)		kWh/kg PHB (MJ/kg PHB)
Steam (140°C, 3 bar)	4.96 (13.4)		5.54 (14.9)		7.48 (20.2)		kg/kg PHB (MJ/kg PHB)
Natural gas	204.7 (5.3)		207.0 (5.4)		413.2 (10.8)		m^3/kg PHB (MJ/kg PHB)
Total energy equivalent	30.4		33.2		62.0		MJ/kg PHB
Cooling water	5.2		1.3		1.2		kg/kg PHB

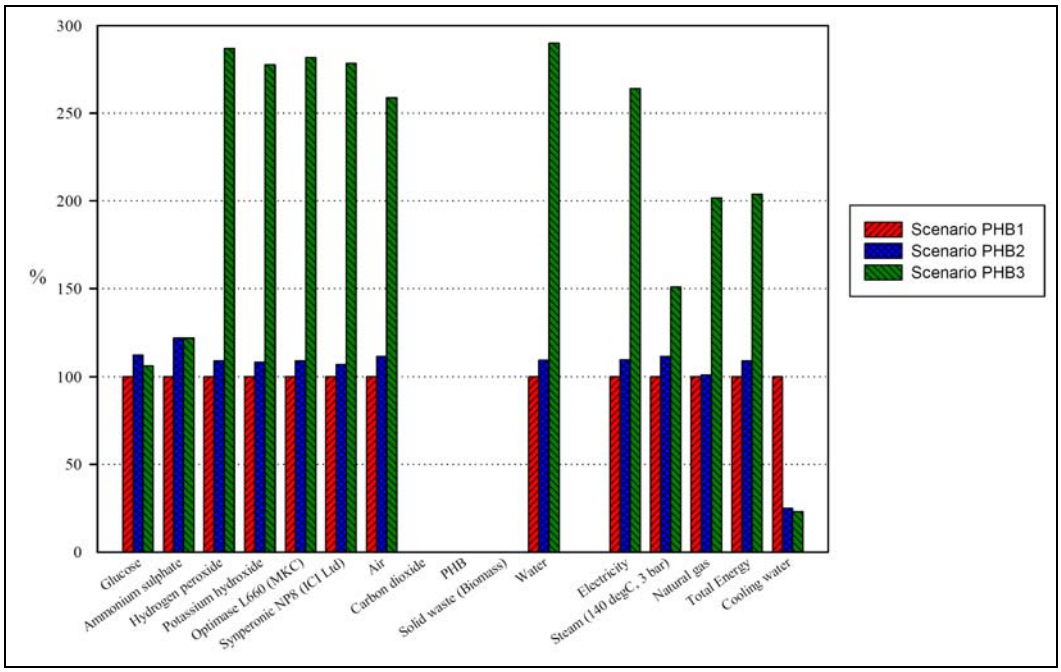


Figure 5.2: Comparison of material, energy and utility inputs from the generic model, calculated for PHB production, relative to Scenario PHB1

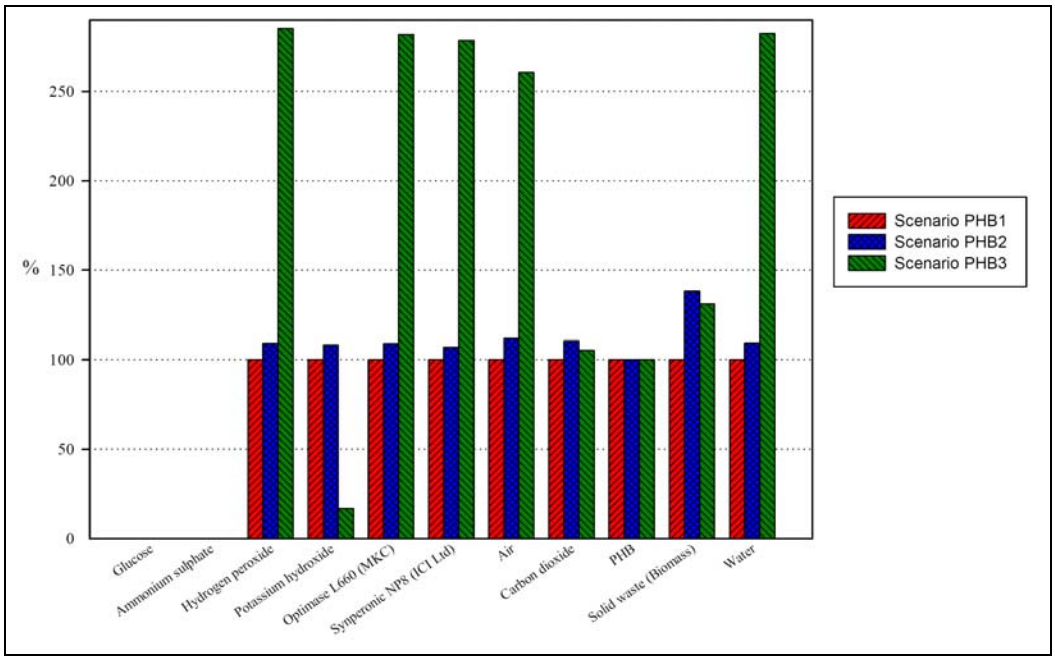


Figure 5.3: Comparison of material, energy and utility outputs from the generic model, calculated for PHB production, relative to Scenario PHB1

5.4. Life Cycle Assessment (LCA)

5.4.1. Goal definition and system description

A cradle-to-gate Life Cycle Assessment (LCA) of the poly-β-hydroxybutyrate (PHB) production was performed using the methodology presented in Section A.6 of Appendix A. A functional unit was taken as 1 kg of polymer product immediately after the spray drier in the process shown in Figure 5.1. In the case of Scenario PHB3 where the purity was not 100 %, the mass of 1 kg includes impurities. However, there was only a 9% difference in the purities. Depending on the use of the polymer, this difference may be acceptable and lead to equal functions of the polymers of different purity. As such, an equal mass functional unit was considered more appropriate than an equal purity functional unit.

The Ecoinvent v1.3 inventory dataset was used in the LCA for all inventories except sugar. It was assumed that the glucose requirements of PHB production were met by sugar, modelled using the South African sugar cane data defined in Appendix G. The LCA included a coal-based South African electricity mix,. All air, water and solid waste emissions from the polymer production have been included in the Life Cycle Assessment. Waste water from the process was assumed to be processed in a waste water treatment (WWT) facility. The life cycle inventory data sets are presented in Appendix I.

The Life Cycle Impact Assessment results are presented in Section 5.4.2, followed by a discussion. The material and energy balance results are then compared to the results from a full biopolymer process design as obtained from literature in Section 5.5.1. These material and energy flows are used in the LCA software and impacts compared to the generic flowsheet model LCA impacts in Section 5.5.2. In Section 5.5.3, the major process contributions to the LCA scores are presented and discussed. The LCA results from the poly-β-hydroxybutyrate (PHB) are then compared to the results for polyolefins (polypropylene and polyethylene).

5.4.2. Life Cycle Impact Assessment (LCIA)

Using the material and energy balance inventories shown in Table 5.5, Life Cycle Impacts for the production of 1 kg of poly-β-hydroxybutyrate (PHB) are presented in Table 5.6 using midpoint indicators. For Scenario PHB1, for each kilogram of polymer formed, global warming emissions of 5.74 kg $CO_{2\,eq.}$, abiotic depletion equal to 0.045 kg $Sb_{eq.}$, ozone layer depletion equal to 0.33 mg CFC-11 $_{eq.}$ and acidification potential of 0.047 kg $SO_{2\,eq.}$ were obtained.

The LCA results for the same categories for Scenario PHB2 were: 6.39 kg $CO_{2\ eq.}$, 0.045 kg $Sb_{eq.}$, 0.37 mg CFC-$11_{eq.}$ and 0.053 kg $SO_{2\ eq.}$. These increases were a result of the increases in electricity and steam requirements in Scenario PHB2. The increase in the eutrophication score was greater than the other LCA categories as a result of the additional impacts from increases in glucose requirements (Figure 5.4).

Although raw materials to the bioreactor were similar in all three scenarios, LCA impacts from Scenario PHB3 were between 150 % and 250 % greater than of Scenarios PHB1 and PHB2, in all categories. This was a result of a doubling of the energy requirements as well as the increased downstream chemical inputs required. These increases are owing to the larger volumes needed to achieve the desired product mass with the less efficient separations of Scenario PHB3. For each kilogram of polymer formed, life cycle impacts of 10.2 kg $CO_{2\ eq.}$, 0.093 kg $Sb_{eq.}$, 0.56 mg CFC-$11_{eq.}$ and 0.098 kg $SO_{2\ eq.}$, were obtained in the categories of global warming, abiotic depletion, ozone layer depletion and acidification respectively.

Table 5.6: LCIA of poly-β-hydroxybutyrate product per kilogram of polymer – CML Baseline 2000 V2.03

Impact Category	Unit	Scenario PHB1	Scenario PHB 2	Scenario PHB 3
Abiotic Depletion	kg $Sb_{eq.}$	0.045	0.045	0.093
Global Warming (GWP100)	kg $CO_{2\ eq.}$	5.74	6.39	10.2
Ozone Layer Depletion (ODP)	mg CFC-$11_{eq.}$	0.33	0.37	0.56
Human Toxicity	kg 1,4-$DB_{eq.}$	2.20	2.46	4.87
Fresh Water Aquatic Ecotoxicity	kg 1,4-$DB_{eq.}$	0.43	0.48	1.00
Marine Aquatic Ecotoxicity	kg 1,4-$DB_{eq.}$	2400	2700	5800
Terrestrial Ecotoxicity	kg 1,4-$DB_{eq.}$	0.012	0.014	0.020
Photochemical Oxidation	kg C_2H_4	0.0016	0.0018	0.0034
Acidification	kg $SO_{2\ eq.}$	0.047	0.053	0.098
Eutrophication	kg $PO_4^{3-}{}_{eq.}$	0.026	0.033	0.045

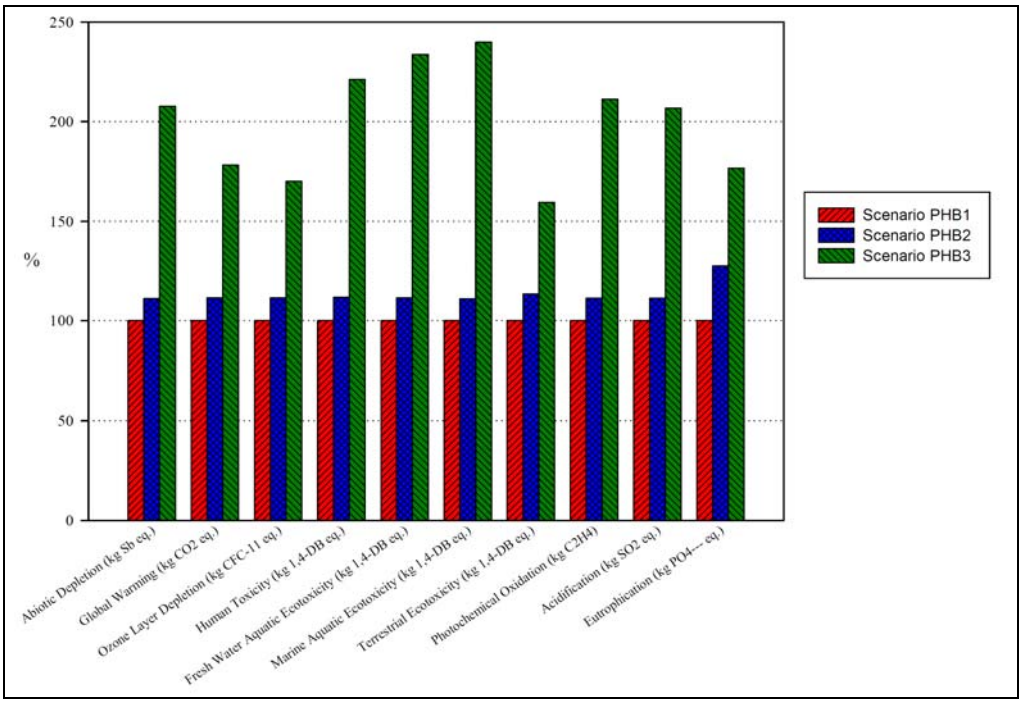


Figure 5.4: Comparison of LCA results for 1 kg poly-β-butyrate for the three scenarios developed

5.5. Comparison with a design of a biopolymer plant

5.5.1. Material and energy balance

The validity of the material and energy balance results was compared to a full flowsheet design set up specifically for the PHB process. Using MS-Excel, specific design considerations for each unit operation were included. This full design was based on the laboratory study of Harrison (1990) using a variation of the BIOPOL flowsheet developed by ICI for production of bacterial PHB from glucose (Asrar and Gruys 2002). Here it was scaled up from laboratory data to give 1 000 kg of PHB and was referred to as the Harrison (1990) model. Process conditions are given in Table 5.7. Data from Perry *et al.* (1984), Engler (1985), Woods (1995), Dennis (2000) and Baker and McKenzie (2005) were also used.

Table 5.7: Process conditions for the production of 1 000 kg of PHB according to the Harrison (1990) model

Section	Conditions
Seed media	*Cupriavidus necator*, sucrose (10 kg/m^3), $(NH_4)_2SO_4$ (1.8 kg/m^3), K_2HPO_4 (1.9 kg/m^3), $NaHPO_4$ (1.56 kg/m^3), $MgSO_4.7H_2O$ (0.8 kg/m^3), $FeSO_4.7H_2O$ (0.008 kg/m^3), trace salts solution ($CuSO_4.5H_2O$, $ZnSO_4.7H2O$, $MnSO_4.H_2O$, $CaCl_2.2H_2O$)
Reactor media	Sucrose (270 kg/m^3), H_3PO_4 (0.8 dm^3/m^3), $(NH_4)_2SO_4$ (1.1 kg/m^3), K_2SO_4 (1.4 kg/m^3), $MgSO_4.7H_2O$ (1.6 kg/m^3), trace salts (Na_2SO_4, $MnSO_4.H_2O$, $ZnSO_4.7H2O$, $CuSO_4.5H_2O$), PPG.EEA 142 antifoam (0.375 kg/m^3)
Sterilisation	139°C (continuous sterilisation) – including heat integration
Microbial growth	Temperature: 30°C; pH: 7 Reactor volume: 9.4 m^3 (working) Total reaction time: 80 h Aeration: 0.6 vol/vol/min Agitation energy: 0.5 kW/m^3 Biomass (PHB) concentration: 150 (106) g/l; Polymer concentration: 71 % PHB
Cell disruption	High pressure homogenisation 3 passes; 70 MPa; 16°C Energy efficiency of breakage: 1.25 J/mg biomass disrupted
Centrifugation	Wash volume: 1/3 of reactor volume Centrifugation: 20 min; 10 000 g Power required: 2.11 kW/m^3
Enzyme addition	Re-suspensions equivalent to 150 kg/m^3 Optimase L660 (MKC) – alkaline serine protease enzyme Agitation energy: 0.8 kW/m^3 Temperature: 70°C; pH: 8 Residence time: 2 h
(Non-ionic) detergent addition	Synperonic NP8 Agitation energy: 0.8 kW/m^3 Temperature: 70°C; pH: 7 Residence time: 2 h
Water Washing (I)	Number of washes: 4; Wash volume: 1/3 of reactor volume (3.1m^3) Centrifugation: 20 min; 10 000 g Power required: 2.11 kW/m^3 (per wash)
H_2O_2 addition	Concentration: 1.20 % v/v
Water washing (II)	Number of washes: 2; Wash volume: 1/3 of reactor volume (3.1m^3) Centrifugation: 20 min; 10 000 g Power required: 2.11 kW/m^3 (per wash)
Spray Drying	Initial moisture content: 11 %; Final moisture content: 0.1 % Drying rate: 4.8 GJ/t
Downstream processing recovery: 95 %	

References: Perry *et al.* 1984, Engler 1985, Harrison 1990, Baker and McKenzie 2005

The material and energy inventory for the scaled up Harrison (1990) data was compared on the basis of 1 kg polymer product to the three scenarios generated using the generic flowsheet in Table 5.8. The inputs for glucose, hydrogen peroxide, Optimase and steam for Scenarios PHB1 and PHB2 are within 15 % of the Harrison (1990) model. Air requirements were 35 % and 27 % lower in Scenarios PHB1 and PHB2 respectively (Figure 5.5), while the water needed was half

that of the scaled up Harrison (1990) model, which required 65.2 kg of water per kilogram of polymer.

In the Harrison (1990) model, formation of poly-β-hydroxybutyrate was achieved by nitrogen limitation. Nitrogen was supplied in Scenarios PHB1, PHB2 and PHB3 as ammonium sulphate, but since physical limitation can not be modelled, the required amount was overestimated. The Harrison (1990) model included a large number of trace elements which were not modelled in the generic flowsheets scenarios.

Since the cooling water requirement was directly affected by the amount of oxygen required for microbial growth and product formation, the lower oxygen requirements of the generic models gave a lower cooling water value. The difference in the cooling water temperature (18°C for the generic flowsheets and 20°C of the Harrison (1990) model) also contributed to the differences in the cooling water requirements. The differences in electrical requirements, from 2.55 kWh/kg polymer in the Harrison (1990) model to 3.26 kWh/kg polymer in Scenario PHB1, were a result of the difference in compression energy.

Output in the generic flowsheet model included carbon dioxide emissions which are not given for the Harrison (1990) model. Scenarios PHB1 and PHB2 both assumed no excess raw materials and thus no glucose or ammonium sulphate resulted in the waste stream. For all wastewater calculations, a chemical oxygen demand (COD) value was calculated. The Harrison (1990) value of 0.8 kg O_2 per kg polymer compared to 0.9, 1.2 and 1.6 kg O_2 per kg polymer for Scenarios PHB1, PHB2 and PHB3 respectively. Based on actual outputs, the volume of wastewater in Scenarios PHB1 and PHB2 was half of the Harrison (1990) model as shown in Figure 5.6.

Table 5.8: Material, energy and utility flows for the production of poly-β-hydroxybutyric acid *vs.* results from the generic flowsheet model developed

Component	In (kg)	Out (kg)	In (kg)	Out (kg)	In (kg)	Out (kg)	In (kg)	Out (kg)
	Harrison (1990)		Scenario PHB1		Scenario PHB2		Scenario PHB3	
Glucose	3.6	0	3.2	0	3.6	0	3.4	0.03
Ammonium sulphate	0.015	0	0.23	0	0.28	0	0.28	0.01
Sulphuric acid	0.0030	0	-	-	-	-	-	-
Phosphoric acid	0.0081	0	-	-	-	-	-	-
Hydrogen peroxide	0.052	0	0.054	0.054	0.059	0.059	0.155	0.154
Potassium hydroxide	-	-	0.36	0.36	0.39	0.39	1.00	0.06
Optimase L660 (MKC)	0.0024	0	0.0022	0.0022	0.0024	0.0024	0.0062	0.0062
Synperonic NP8 (ICI Ltd)	0.033*	0	0.028	0.028	0.030	0.030	0.078	0.078
Air	163.3	[N/D]	107.6	106.4	120.2	119.0	278.6	277.4
Carbon dioxide	-	[N/D]	-	1.9	-	2.1	-	2.0
PHB	-	1	-	1	-	1	-	1
PHB (lost in wastewater)	-	-	-	-	-	0.036	-	0.066
Antifoam (PPG.EEA 142)	0.005*	0	0.008	0.008	0.009	0.009	0.22	0.22
Trace elements:								
$MgSO_4.7H_2O$	0.021	0	-	-	-	-	-	-
K_2SO_4	0.019	0	-	-	-	-	-	-
Na_2SO_4	0.003	0	-	-	-	-	-	-
$ZnSO_4.7H_2O$	0.001	0	-	-	-	-	-	-
$MnSO_4.H_2O$	0.0009	0	-	-	-	-	-	-
$FeSO_4.7H_2O$	0.0008	0	-	-	-	-	-	-
$CuSO_4.5H_2O$	0.0001	0	-	-	-	-	-	-
$CaCl_2.2H_2O$	0.0023	0	-	-	-	-	-	-
K_2HPO_4	0.00009	0	-	-	-	-	-	-
$NaHPO_4$	0.00008	0	-	-	-	-	-	-
Solid waste (Biomass)	-	0.42	-	0.42	-	0.58	-	0.55
Water	65.2	65.2	31.8	33.1	34.8	36.2	92.2	93.5
Total**	66.6	66.6	36.9	36.9	40.4	40.4	98.5	98.5
Chemical Oxygen Demand (COD) (kg O_2)		0.8		0.9		1.2		1.6
Product recovery (% PHB)		100		100		99		93.2
Product purity (%)		100		100		100		90.5

Energy requirements	Harrison 1990	Scenario PHB1	Scenario PHB2	Scenario PHB3	Units
Electricity	2.55 (9.2)	3.26 (11.7)	3.58 (12.9)	8.61 (31.0)	kWh/kg PHB (MJ/kg PHB)
Steam (140°C, 3 bar)	4.89 (13.2)	4.96 (13.4)	5.54 (14.9)	7.48 (20.2)	kg/kg PHB (MJ/kg PHB)
Natural gas	77.4 (2.1)	204.7 (5.3)	207.0 (5.4)	413.2 (10.8)	m^3/kg PHB (MJ/kg PHB)
Total energy equivalent	24.5	30.4	33.2	62.0	MJ/kg PHB
Cooling water	13.1	5.2	1.3	1.2	kg/kg PHB

*** Volume: units = litres**
**** Excluding excess air**
[N/D] = Not Determined

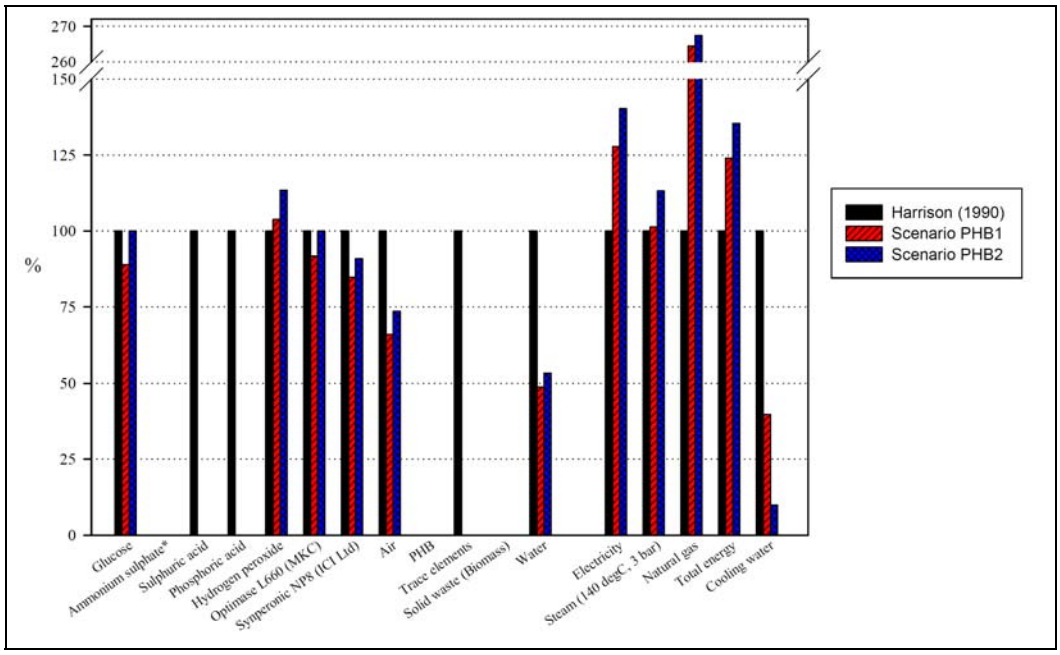
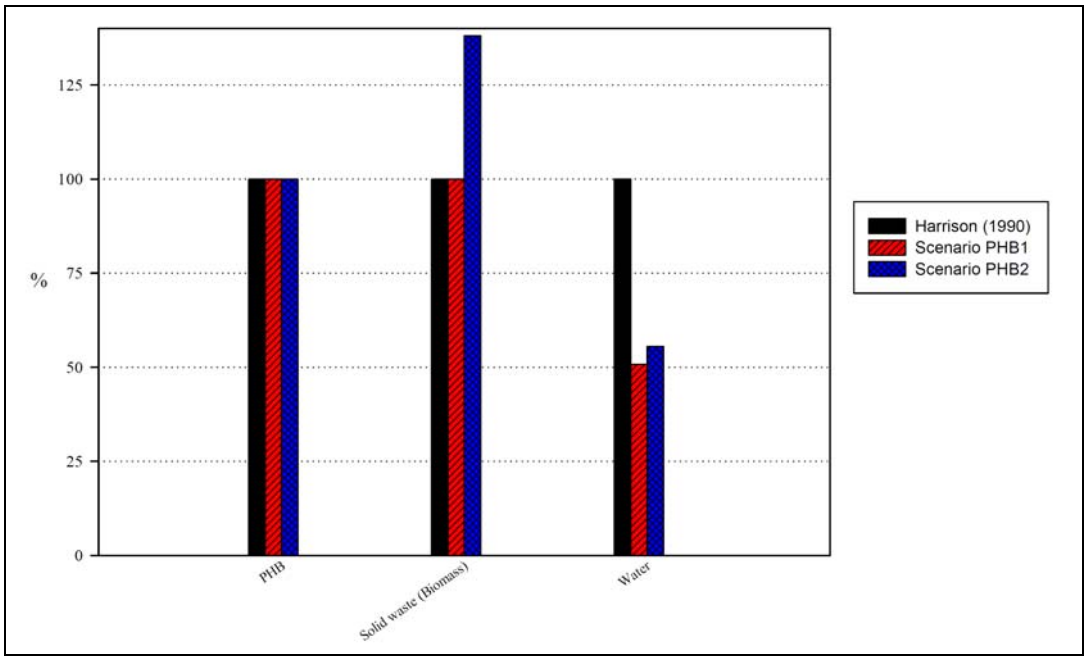


Figure 5.5: Comparison of material, energy and utility inputs (excluding ammonium sulphate) calculated for PHB production, relative to literature values of Harrison *et al.* (1990)



***Only the outputs as given in the Harrison *et al.* (1990) data are compared here**

Figure 5.6: Comparison of material, energy and utility outputs calculated for PHB production, relative to literature values of Harrison *et al.* (1990)

The individual electrical requirements for the Harrison (1990) model (aeration, agitation, cell disruption and downstream processing units) were within 80 % of the Scenario PHB1 values for all electrical requirements except steam compression, which was not taken into account in the Harrison (1990) model (Table 5.9). The total electrical energy required by the Harrison (1990) model was 78 % of the electrical energy required for Scenario PHB1 (Figure 5.7).

The amounts of natural gas needed in the spray drying process were higher in Scenarios PHB1, PHB2 and PHB3 than the Harrison (1990) model. This was owing to an assumption that the initial moisture content of material entering the spray dryers in the Harrison (1990) model was 25 % while in the generic flowsheet models, the moisture content was calculated to be 39 %.

Table 5.9: Electrical energy contributions for Scenario PHB1 versus the Harrison (1990) model for the production of 1 kg of poly-β-hydroxybutyric acid

	Scenario PHB1	Harrison (1990) model
	MJ	**MJ**
Sterilisation - Steam compression	1.86	-
Aeration	6.5	5.8
Agitation	1.31	1.36
Cell Disruption - High Pressure homogeniser	1.77	1.77
Centrifugation	0.078	0.072
Enzyme addition - agitation	0.021	0.018
Detergent addition - agitation	0.022	0.018
Water washing (I)	0.134	0.120
Water washing (II)	0.076	0.072
TOTAL	**11.7**	**9.2**

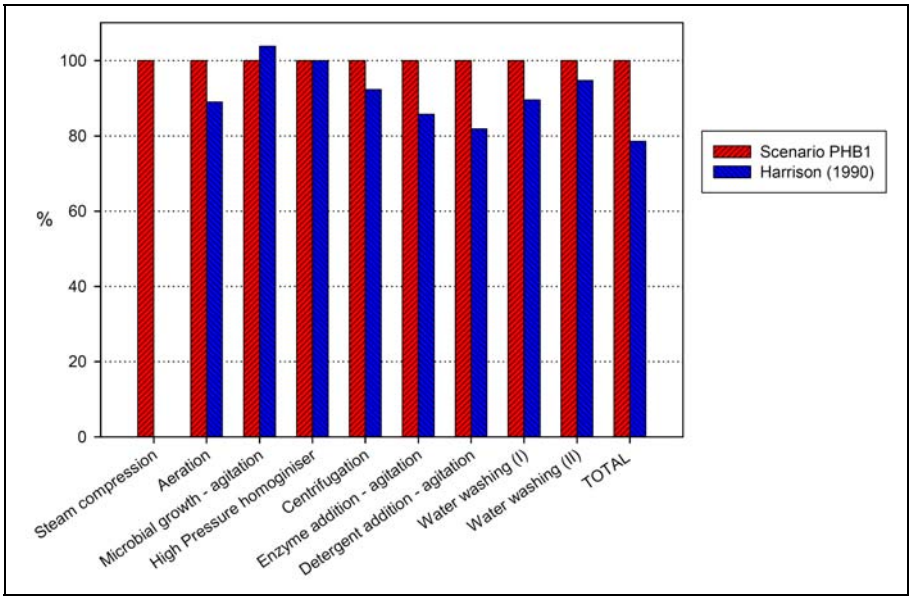


Figure 5.7: Electrical energy contributions for the production of poly-β-butyrate as determined by Harrison (1990), relative to Scenario PHB1

5.5.2. Life Cycle Impact Assessment (LCIA)

The life cycle impacts of Scenario PHB1 were less than 30 % higher than the results of the Harrison (1990) model in all categories except global warming (Figure 5.8). This was a result of the larger electricity value in the modelled scenarios as well as the over estimation in ammonium sulphate. As seen in Table 5.10, the global warming scores were 2.72, 5.74 and 6.39 kg $CO_{2\ eq.}$ for the models of Harrison (1990) and Scenarios PHB1 and PHB2 respectively. The generic flowsheet values were over twice that of the Harrison (1990) values as a result of the carbon dioxide emitted from the reactor which was included in the generic model scenarios but not the Harrison (1990) model. For the scaled up Harrison (1990) model, for each kilogram of polymer formed, life cycle impacts of 0.034 kg $Sb_{eq.}$, 0.25 mg CFC-11 $_{eq.}$, 0.042 kg $SO_{2\ eq.}$ and 0.023 kg $PO_4^{3-}{}_{eq.}$ were obtained in the categories of abiotic depletion, ozone layer depletion, acidification and eutrophication respectively. By allowing for waste water treatment, as well as possible biogas capture and use, the LCA values, particularly those for eutrophication, would decrease.

LCA results which are within 5 % of each other may not be significantly different from each other owing to uncertainty in the inputs and LCA inventory datasets.

Table 5.10: LCIA of poly-β-butyrate production per kilogram of product as described by Harrison (1990) – CML 2 Baseline 2000 V2.03

Impact Category	Unit	Harrison (1990)	Scenario PHB1	Scenario PHB 2	Scenario PHB 3
Abiotic Depletion	kg $Sb_{eq.}$	0.034	0.045	0.045	0.093
Global Warming (GWP100)	kg $CO_{2\ eq.}$	2.72	5.74	6.39	10.2
Ozone Layer Depletion (ODP)	mg CFC-$11_{eq.}$	0.25	0.33	0.37	0.56
Human Toxicity	kg 1,4-$DB_{eq.}$	1.69	2.20	2.46	4.87
Fresh Water Aquatic Ecotoxicity	kg 1,4-$DB_{eq.}$	0.34	0.43	0.48	1.00
Marine Aquatic Ecotoxicity	kg 1,4-$DB_{eq.}$	1900	2400	2700	5800
Terrestrial Ecotoxicity	kg 1,4-$DB_{eq.}$	0.0098	0.012	0.014	0.020
Photochemical Oxidation	kg C_2H_4	0.0014	0.0016	0.0018	0.0034
Acidification	kg $SO_{2\ eq.}$	0.042	0.047	0.053	0.098
Eutrophication	kg $PO_4^{3-}{}_{eq.}$	0.023	0.026	0.033	0.045

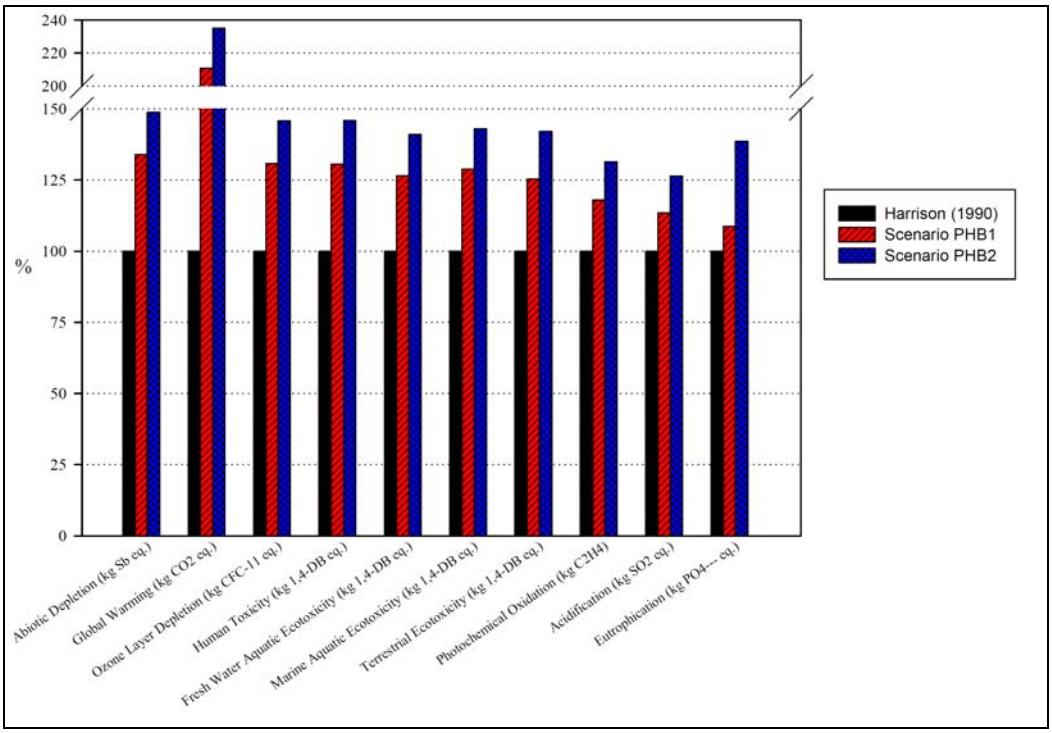


Figure 5.8: Comparison of LCA results for PHB production for Scenarios PHB1 and PHB2 relative to Harrison (1990) data

5.5.3. Process contributions

Six of the impact categories were investigated in Scenario PHB1 to determine the main contributors to total scores. The LCA categories investigated included abiotic depletion, global warming, ozone layer depletion, photochemical oxidation, acidification and eutrophication. The values shown in the figures represented contribution to the LCA impacts as a percentage of the total in each category. Only materials which led to a contribution greater than 3 % of the total LCA score have been included. Where there were positive impacts, *i.e.* impacts which reduced the total environmental impact, these were presented as detached wedges.

In the categories shown, electricity was the largest contributor in 5 of the 6 categories, while the direct impacts during PHB production process (*e.g.* CO_2 release and Chemical Oxygen Demand of wastewater) were the largest contributors in the sixth, eutrophication. Steam, natural gas, ammonium sulphate and sugar were also large contributors.

Abiotic depletion scores are dominated by electricity (56.7 %), steam (20.5 %), ammonium sulphate (10.7 %) and natural gas (7.2 %) as shown in Figure 5.9. No other source contributed more than 3 % to the overall impact. The global warming impact showed a negative value from sugar production owing to the uptake of carbon dioxide during sugar cane growth. This uptake

was not large enough to counter the overall CO_2 emissions from coal based electricity (39.3 %) and PHB production (33.1 %). There was also a global warming impact from the energy needed to raise steam (23.0 %) and ammonium sulphate (10.6 %). Since bio-based carbon in the final product has been deducted, the impact of incineration is unlikely to impact results, since comparable amounts of CO_2 would be released as a consequence.

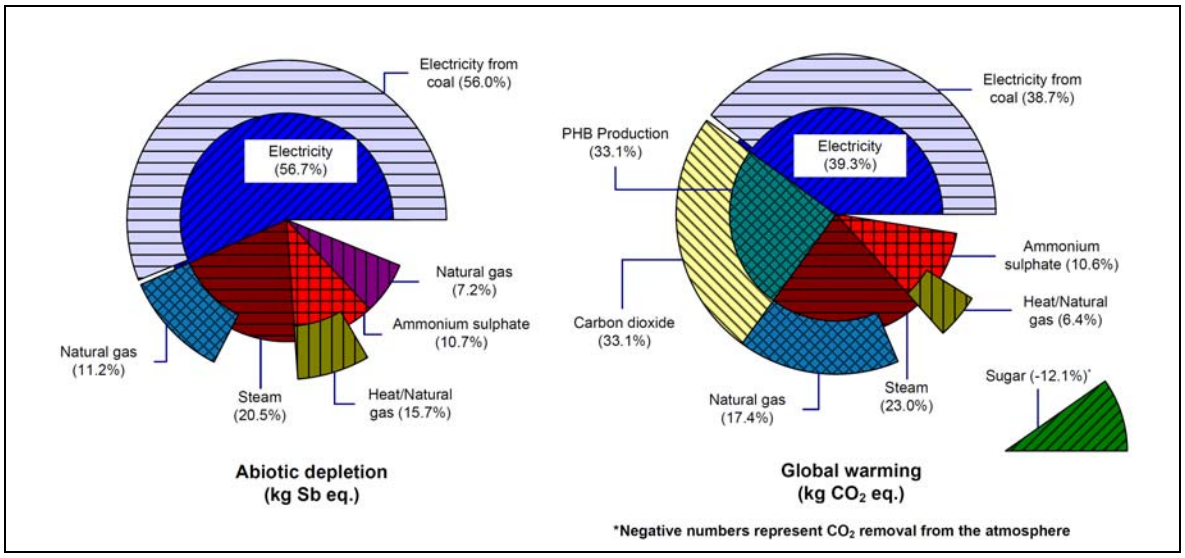
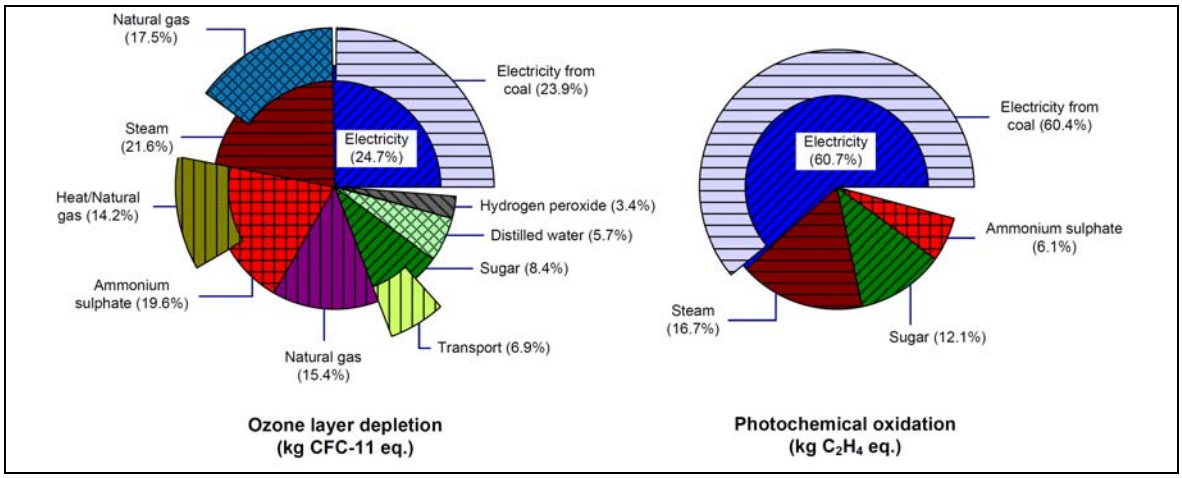


Figure 5.9: Life Cycle Assessment process contributions of PHB production (Scenario PHB1) using the CML baseline 2.03 methodology (Abiotic depletion and Global warming)

The largest contributions to the ozone layer depletion score was from electricity, steam and ammonium sulphate, which all contributed approximately 20% to the overall score as shown in Figure 5.10. Natural gas (15.4 %), sugar (8.4 %), distilled water (5.7 %) and hydrogen peroxide (3.4 %) also all contributed over 3% to the total score. Ozone layer depletion was the only LCA category where distilled water contributed more than 3 % to the overall score. These impacts were a result of chlorine compounds used during processing of the water.

Electricity contributed over 60 % to the photochemical oxidation score. Steam contributed 15 % while sugar and ammonium sulphate contributed 12 % and 6.1% respectively for Scenario PHB1.



*** Note: Ozone layer depletion is of reduced importance in current processes (see Appendix A.6)**

Figure 5.10: Life Cycle Assessment process contributions of PHB production (Scenario PHB1) using the CML baseline 2.03 methodology (Ozone layer depletion and Photochemical oxidation)

Electricity scores contributed over 58 % in the acidification impact category as shown in Figure 5.11. Sugar (19.1 %) and steam (145.7 %) and ammonium sulphate (4.1 %) also formed contributions. The eutrophication score for Scenario PHB1 was dominated by the PHB production itself. This was a result of the Chemical Oxygen Demand (COD) of the wastewater. This contribution would be greatly reduced with the addition of wastewater treatment to lower the COD value of the wastewater. Fertilisers used during sugarcane growth contributed 4.8 % of the total eutrophication score and electricity 5.22 % of the total.

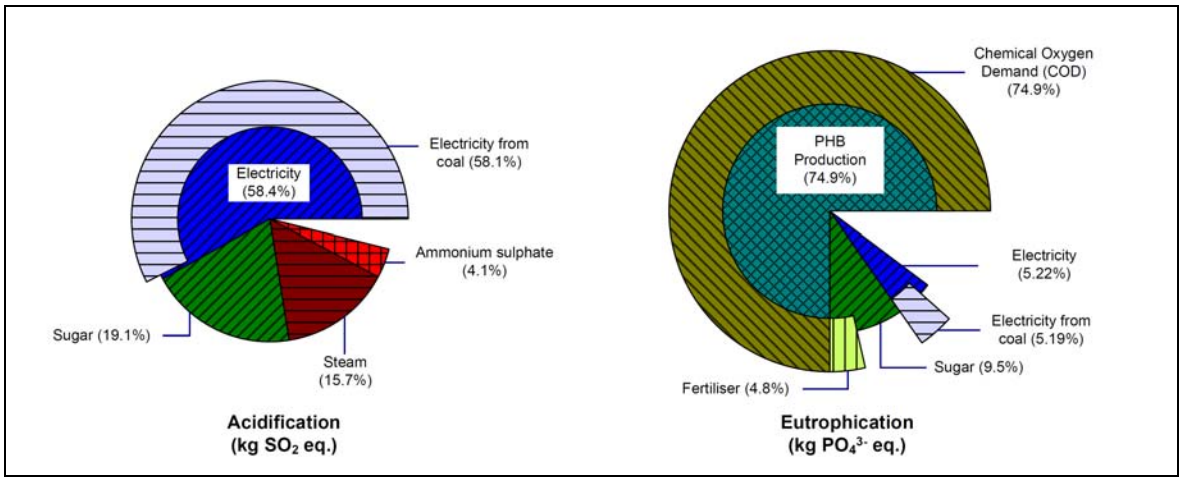


Figure 5.11: Life Cycle Assessment process contributions of PHB production (Scenario PHB1) using the CML baseline 2.03 methodology (Acidification and Eutrophication)

The lower ammonium sulphate requirement in the Harrison (1990) model resulted in process contributions which did not reflect it being a major contributor in any of the life cycle categories.

The other material and energy inputs of the Harrison (1990) model were similar to Scenario PHB1 and showed resulted in similar LCA process contributions from electricity, sugar, steam and natural gas.

5.6. Comparison of PHB LCA results to literature

Several authors have presented the carbon dioxide equivalent emissions and energy requirements for PHA/PHB production (Gerngross 1999, Kurdikar *et al*. 2001, Nonato *et al*. 2001, Akiyama *et al*. 2003). These were found to range between 0.26 and 2.4 kg $CO_{2\ eq.}$/kg-polymer and 50.4 and 113.7 MJ/kg-polymer.

When comapared to the values calculated using the generic flowsheet model (Table 5.11), it was seen that the carbon dioxide equivalent emissions estimated using the generic flowsheet were twice the literature values. As explained in Section 5.5.1, the Harrison (1990) data did not include the direct emissions of carbon dioxide from the biomass growth in the bioreactor. Similarly, it is unknown if this carbon dioxide release was fully accounted for in the literature values where bioreactors were used (Akiyama *et al*. 2003). This additional release of CO_2 could explain the higher carbon dioxide equivalent emissions of the generic flowsheet.

The energy requirements shown for the Harrison and Scenario PHB1 models in Table 5.11 were calculated using the "Cumulative Energy Demand v1.03" Life Cycle Impact Assessment method. These numbers are dependent on the energy requirements of all datasets used in the LCA calculations and have a high level of uncertainty. However, the Harrison (199) energy requirement of 54 MJ/kg of polymer is in good agreement with Gerngross (1999) and Akiyama *et al*. 2003. The higher Scenario PHB1 data (79.4 MJ/kg) remained in the range, being lower than the value reported by Nonato *et al*. (2001).

Table 5.11: Carbon dioxide equivalent emissions and total energy requirements for biopolymer production (cradle-to-gate)

Polymer	CO_2 equivalent emissions	Energy requirements
	kg $CO_{2\ eq.}$/kg-polymer	MJ/kg-polymer
PHA (Gerngross 1999)	2.4	50.4
PHA (Kurdikar *et al.* 2001)	2.0	-
PHA (Akiyama *et al.* 2003)	0.26-0.45	50-59
PHB (Nonato *et al*. 2001)	-	113.7
PHB (Harrison 1990)	2.72	54*
PHB (Scenario PHB1) (This study)	5.74	79.4*

*** Value calculated using the "Cumulative Energy Demand v1.03" Life Cycle Impact Assessment method in SimaPro**

5.7. Comparison of PHB to polyolefin production

5.7.1. Life Cycle Analysis

Environmental studies of PHB in comparison to the conventional plastics polypropylene (Akiyama *et al.* 2003, Pietrini *et al*. 2007 and Roes *et al*. 2007), polyethylene (Luck 1996, Heyde 1998, Kurdikar *et al.* 2001, Akiyama *et al.* 2003), polystyrene (Luck 1996, Heyde 1998, Gerngross 1999, Akiyama *et al.* 2003) and poly(ethylene terephthalate) (Gerngross and Slater 2000, Akiyama *et al.* 2003) have focused on carbon dioxide emissions and energy requirements and arrive at conflicting conclusions. PHB production results showed reductions in greenhouse gas emissions (Gerngross 1999, Gerngross and Slater 2000, Akiyama *et al.* 2003), but greater energy requirements (Gerngross 1999, Gerngross and Slater 2000, Kurdikar *et al.* 2001). However, Akiyama *et al.* (2003) recorded a decrease in energy requirements of PHB production. Luck (1996), Heyde (1998) and Kurdikar *et al.* (2001) reported no clear advantage or disadvantage in PHB production with respect to polyolefin production. Certain outcomes were dependent on specific process conditions. The full life cycle results from above were compared to polypropylene (PP), high density polyethylene (HDPE) and low density polyethylene (LDPE).

The life cycle inventory of granular polypropylene and polyethylene production, as given in the BUWAL 250 (Federal Office for the Environment 2007) and ETH-ESU 96 (Frischknecht *et al.* 1996) and EcoInvent v1.3 (Swiss Centre for Life Cycle Inventories 2007) databases were used. The results were for a basis of 1 kg polymer. It should be noted that while the allocation was on an equal mass basis, depending on the use of the polymer, a functional basis equating to equal tasks may be preferable i.e. owing to the slightly different properties of the polymers, different masses may be required for the same functional task. These LCA impacts were compared to the LCA results from Harrison (1990) and Scenario PHB1. While several databases were used to compare the PHB production, the EcoInvent database should be taken as the most accurate as this uses the most up to date information (Hischier 2008). Since the data was obtained from a complex array of sources, it was not easily possible to determine the process contributions in the EcoInvent database (Schivley 2008).

5.7.2. PHB vs. polypropylene

Poly-β-hydroxybutyric acid (PHB) production was less favourable or equally favourable compared to polypropylene (PP) production in all categories except ozone layer depletion, which showed high impacts using the BUWAL 250 and ETH-ESU 96 databases and terrestrial ecotoxicity which showed high impacts in the ETH-ESU 96 database. Results from the EcoInvent v1.3 database showed that production of 1 kg of PP released 2 kg $CO_{2\ eq.}$ compared to 2.72 kg $CO_{2\ eq.}$ and 5.74 kg $CO_{2\ eq.}$ for production by Harrison (1990) and Scenario PHB1

respectively as seen in Table 5.12. PP scores for photochemical oxidation and acidification were less than 50 % of the PHB values by both biopolymer production methods. The PP score for ozone layer depletion was over 99.9 % lower by the EcoInvent v1.3 database. Human-, fresh water aquatic- and marine aquatic toxicities were all less than 5 % of the PHB impacts (Figure 5.12).

For PHB results, the increased eutrophication scores were owing to fertiliser inputs in the agricultural processes, while the impacts for the other categories were a result of the energy needs and for toxicity categories, hydrogen peroxide inputs. This identified the hydrogen peroxide as a prime target for substitution and reduced LCA scores. The PP scores for abiotic depletion, global warming and terrestrial ecotoxicity were a result of disposal of hazardous and other wastes. High ozone layer depletion scores in the ETH-ESU 96 database method were a result of CFCs released to the atmosphere during the refining of crude oil which is a precursor for PP production.

Table 5.12: LCIA of polypropylene production for 1 kg of polymer – CML 2 Baseline 2000 V2.03

		Harrison (1990)	Scenario PHB1	BUWAL 250[a]	ETH-ESU 96[b]	EcoInvent v1.3[c]
Impact category	**Unit**					
Abiotic Depletion	kg Sb_{eq}	0.034	0.045	0.036	0.044	0.034
Global Warming (GWP100)	kg $CO_{2\ eq.}$	2.72	5.74	1.88	3.38	2.00
Ozone Layer Depletion (ODP)	mg $CFC\text{-}11_{eq}$	0.25	0.33	1.10	8.62	0.00013
Human Toxicity	kg $1,4\text{-}DB_{eq}$	1.69	2.20	0.187	2.47	0.051
Fresh Water Aquatic Ecotoxicity	kg $1,4\text{-}DB_{eq}$	0.34	0.43	0.025	0.45	0.014
Marine Aquatic Ecotoxicity	kg $1,4\text{-}DB_{eq}$	1900	2400	96.5	2600	70.8
Terrestrial Ecotoxicity	kg $1,4\text{-}DB_{eq}$	0.0098	0.012	0.0010	0.041	0.011
Photochemical Oxidation	kg C_2H_2	0.0014	0.0016	0.00057	0.0017	0.00068
Acidification	kg $SO_{2\ eq.}$	0.042	0.047	0.018	0.049	0.020
Eutrophication	kg $PO_4^{3-}{}_{eq.}$	0.023	0.026	0.001	0.006	0.001

a PP granulate average B250 (Average production of polypropylene in Europe according to APME. Data accounts for production from 14 companies which is half of the total Western European production.)

b PP ETH S (Polymerisation takes place with an efficiency of approximately 95 %. For this process 4 MJ electricity and 6 MJ thermal energy use is estimated. Emissions to air of VOC, NOx and dust are accounted for, water emissions (of aromatic substances, solvents, metals) can be expected during solvent and catalyst recycling.)

c Polypropylene, granulate, at plant /RER U (Data are from the Eco-profiles of the European plastics industry (APME). Not included are values reported for: recyclable wastes, amount of air/ N2/ O2 consumed, unspecified metal emission to air and to water, mercaptan emission to air, unspecified CFC/HCFC emission to air.)

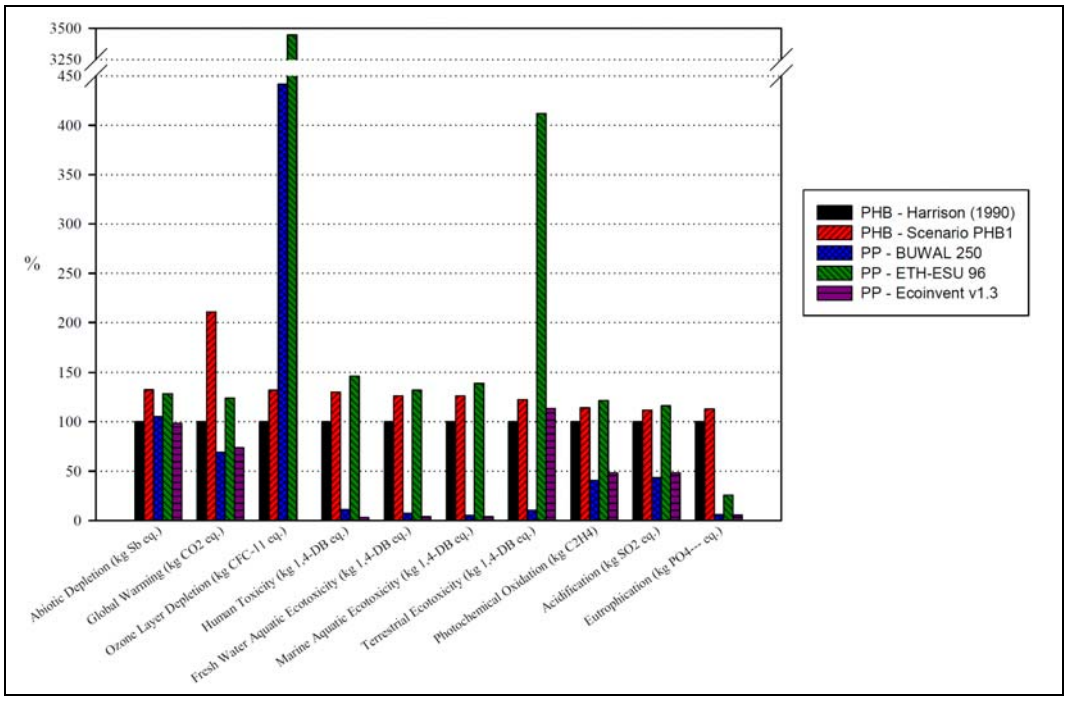


Figure 5.12: Comparison of LCA results for PHB production for polypropylene (PP) production from three different Life Cycle Inventory (LCI) sets relative to Scenario PHB1 data

5.7.3. PHB vs. high density polyethylene

Impacts for high density polyethylene (HDPE) followed similar trends to polypropylene. Poly-β-hydroxybutyric acid (PHB) production was less favourable or equally favourable compared to HDPE production in all categories except ozone layer depletion, which showed high impacts using the BUWAL 250 and ETH-ESU 96 databases and terrestrial ecotoxicity and photochemical oxidation which showed high impacts in the ETH-ESU 96 database, all as a result of refining of crude oil.

When comparing the production of PHB to that of HDPE using the EcoInvent v1.3 database, impacts were less than 50 % lower for HDPE in the categories ozone layer depletion, photochemical oxidation, acidification, eutrophication and the toxicity categories (Figure 5.13). For HDPE production, a carbon dioxide equivalent of 1.89 kg $CO_{2\ eq.}$ (Table 5.13) was released. Impacts of 0.034 kg $Sb_{eq.}$, 0.00015 mg CFC-$11_{eq.}$, 0.021 kg $SO_{2\ eq.}$, and 0.0014 kg $PO_4^{3-}{}_{eq.}$ were shown in the categories of abiotic depletion, ozone layer depletion, acidification and eutrophication respectively.

Table 5.13: LCIA of high density polyethylene production for 1 kg of polymer – CML 2 Baseline 2000 V2.03

		Harrison (1990)	Scenario PHB1	BUWAL 250[a]	ETH-ESU 96[b]	EcoInvent v1.3[c]
Impact category	**Unit**					
Abiotic Depletion	kg Sb_{eq}	0.034	0.045	0.037	0.037	0.034
Global Warming (GWP100)	kg $CO_{2\,eq.}$	2.72	5.74	2.15	2.53	1.89
Ozone Layer Depletion (ODP)	mg CFC-11_{eq}	0.25	0.33	0.80	7.66	0.00015
Human Toxicity	kg 1,4-DB_{eq}	1.69	2.20	0.14	3.12	0.067
Fresh Water Aquatic Ecotoxicity	kg 1,4-DB_{eq}	0.34	0.43	0.019	0.37	0.021
Marine Aquatic Ecotoxicity	kg 1,4-DB_{eq}	1900	2400	82.0	1900	108
Terrestrial Ecotoxicity	kg 1,4-DB_{eq}	0.0098	0.012	0.0010	0.031	0.010
Photochemical Oxidation	kg C_2H_2	0.0014	0.0016	0.00033	0.017	0.00071
Acidification	kg $SO_{2\,eq.}$	0.042	0.047	0.012	0.022	0.021
Eutrophication	kg $PO_4^{3-}{}_{eq.}$	0.023	0.026	0.0013	0.00088	0.0014

a **HDPE 250 (Average production of High Density Polyethene (HDPE) in Europe according to APME data from 10 companies, producing 1,3 Mton HDPE. Transports for imports of polymers into Switzerland are not included.)**

b **HDPE ETH S (Data from different sources. Land use is not included. Electricity use was estimated 1.5 MJ/kg, steam use 1 MJ/kg.)**

c **Polyethylene, HDPE, granulate, at plant/RER U (Data are from the Eco-profiles of the European plastics industry (APME). Not included are the values reported for: recyclable wastes, amount of air/ N2/ O2 consumed, unspecified metal emission to air and to water, mercaptan emission to air, unspecified CFC/HCFC emission to air.)**

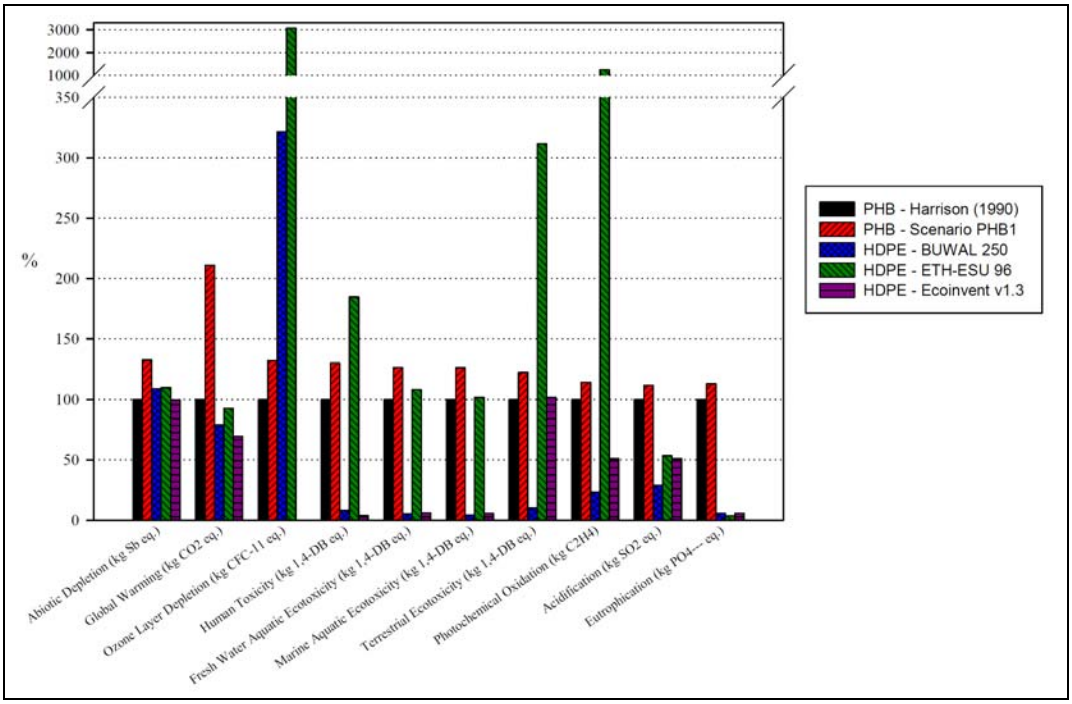


Figure 5.13: Comparison of LCA results for PHB production for high density polyethylene (HDPE) production from three different Life Cycle Inventory (LCI) sets relative to Scenario PHB1 data

5.7.4. PHB vs. low density polyethylene

The Life Cycle impacts of low density polyethylene (LDPE) production were higher than those for high density polyethylene (HDPE). From the ETH-ESU 96 database, impacts in the categories ozone layer depletion, human-, fresh water aquatic-, terrestrial ecotoxicity and photochemical oxidation were higher than for PHB production. As with HDPE, these high impacts were a result of the refining of crude oil in the LDPE process. However, using the BUWAL 250 and EcoInvent v1.3 databases, with the exception of terrestrial toxicity in the EcoInvent database, toxicity impacts were all below 5% (Figure 5.14).

When comparing PHB production to the EcoInvent v1.3 data for LDPE, as with HDPE, PHB had higher or similar impacts in all categories. The carbon dioxide equivalent released of 2.08 $kgCO_{2\ eq.}$ (Table 5.14) was 10 % higher than HDPE. Impacts of 0.034 kg $Sb_{eq.}$, 0.00014 mg $CFC\text{-}11_{eq.}$, and 0.0015 kg $PO_4^{3-}{}_{eq.}$ were shown in the categories of abiotic depletion, ozone layer depletion and eutrophication respectively. These were all within 10% of the HDPE values.

Table 5.14: LCIA of low density polyethylene production for 1 kg of polymer – CML 2 Baseline 2000 V2.03

		Harrison (1990)	Scenario PHB1	BUWAL 250[a]	ETH-ESU 96[b]	EcoInvent v1.3[c]
Impact category	**Unit**					
Abiotic Depletion	kg Sb_{eq}	0.034	0.045	0.041	0.042	0.034
Global Warming (GWP100)	kg $CO_{2\ eq.}$	2.72	5.74	2.43	3.06	2.08
Ozone Layer Depletion (ODP)	mg $CFC\text{-}11_{eq}$	0.25	0.33	0.98	8.32	0.00014
Human Toxicity	kg $1,4\text{-}DB_{eq}$	1.69	2.20	0.192	2200	0.073
Fresh Water Aquatic Ecotoxicity	kg $1,4\text{-}DB_{eq}$	0.34	0.43	0.025	220	0.019
Marine Aquatic Ecotoxicity	kg $1,4\text{-}DB_{eq}$	1900	2400	258	2400	118
Terrestrial Ecotoxicity	kg $1,4\text{-}DB_{eq}$	0.0098	0.012	0.0014	0.037	0.010
Photochemical Oxidation	kg C_2H_2	0.0014	0.0016	0.00048	0.0039	0.00046
Acidification	kg $SO_{2\ eq.}$	0.042	0.047	0.017	0.027	0.015
Eutrophication	kg $PO_4^{3-}{}_{eq.}$	0.023	0.026	0.0016	0.0010	0.0013

a LDPE B250 (Average production of Low Density Polyethene in Europe according to APME data from 22 companies, producing 2.8 Mton total. LDPE. Transports for imports of polymers into Switzerland are not included.)

b LDPE ETH S (Data from different sources. Land use is not included. Electricity use was estimated 3 MJ/kg, steam use 4 MJ/kg.)

c Polyethylene, LDPE, granulate, at plant/RER U (Data are from the Eco-profiles of the European plastics industry (APME). Not included are the values reported for: recyclable wastes, amount of air/ N2/ O2 consumed, unspecified metal emission to air and to water, mercaptan emission to air, unspecified CFC/HCFC emission to air.)

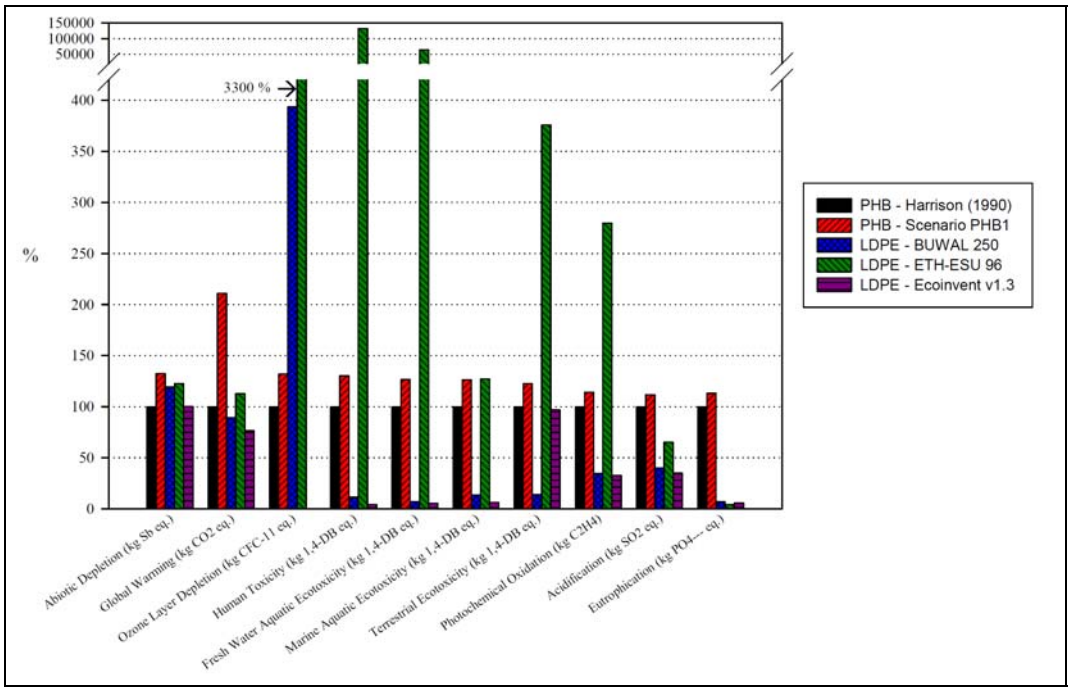


Figure 5.14: Comparison of LCA results for PHB production for low density polyethylene (LDPE) production from three different Life Cycle Inventory (LCI) sets relative to Scenario PHB1 data

5.8. Conclusions

The generic MS-Excel model was used to obtain a first approximation to a material and energy balance on a flowsheet for the production on poly-β-butyrate (PHB). When compared to a specific PHB flowsheet from literature, for the most comprehensive scenario, 50% of the material balance values modelled were within 12 % of these results. The water requirement in the generic flowsheet models was up to 50 % lower than the literature value. The electricity requirements of the generic flowsheet models were 30% higher owing to greater aeration energy required as calculated by a more rigorous model. Steam requirements were similar, while natural gas needed was more than double in the generic flowsheet models compared to the Harrison (1990) model owing to a comprehensive spray dryer model used. These differences were a result of setups in the generic flowsheet models which could not properly describe the literature model.

The largest Life Cycle Assessment (LCA) contributions were found to be from electricity and steam. The higher electricity and natural gas values in the generic model inventories led to LCA scores being approximately 25 % higher in all categories except eutrophication. In Scenario PHB3, the increased steam, hydrogen peroxide and Optimase L660 requirements, owing to

inefficient separation efficiencies, resulted in an even greater LCA impacts. It was found that the carbon dioxide uptake from the biological processes in PHB production was less than the carbon dioxide released during electricity production, giving a net carbon dioxide release. Hydrogen peroxide used in the production process of PHB was targeted as an area for possible substitution to give reduced LCA impacts.

Poly-β-butyrate (PHB) production resulted in higher or similar LCA scores compared to polypropylene (PP) and polyethylene (PE) for all impact categories when using the EcoInvent v1.3 database. This showed that despite the use of fossil fuels during polyolefin production, and the use of renewable resources in PHB production, PP and PE are more environmentally beneficial. While carbon dioxide capture during the biomass growth for sugar production of PHB helped to reduce global warming scores, this was only by 10 %. The PHB LCA assumed largely coal-based electricity production compared with the cleaner European electricity for polyolefin production. As a more direct comparison to the PHB, the production of polymers in South Africa may show less beneficial trends for biopolymers.

The LCA results were sensitive to the LCA database used. Since the EcoInvent v1.3 database was the most recent database used, it was assumed to be the most representative. Further, the polyolefin industry can be considered to be an established industry while the biopolymer industry is yet to mature. Data used in the PHB analysis can be considered dated. With research and development having moved forward, and with it likely to continue to do so, once the process is matured and optimised, the LCA scores for PHB production can be expected to be reduced significantly.

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CHAPTER 6: BIODIESEL

6.1. Introduction

As a fossil fuel, use of petroleum diesel is not sustainable indefinitely owing to limited supplies. Many nations are committed to production of a defined proportion of their transport fuels as biofuel. One such option is to produce biodiesel from oils. The purpose of this chapter was to compare different processing routes for the production of biodiesel. This was to allow for insight into flowsheet options at an early stage of design.

Various routes for biodiesel production exist, which include designs with different alcohols for esterification, different recovery options and production by an alkali or enzymatic catalyst. The environmental advantages of these different processing options were investigated by using Life Cycle Assessment (LCA) methodology. Further, the environmental impact of the enzyme needed as a catalyst was not known as there are currently no industrial-scale processes for biodiesel production based on enzymatic esterification.

The generic flowsheet model was used to provide the material and energy balance inventory for the enzyme. This inventory was used together with the material and energy balance results from five different biodiesel flowsheets to inform an LCA analysis of biodiesel production.

6.2. Biodiesel production

Biodiesel is the generic term for alkyl esters of fatty acids, produced by esterification of vegetable oils with an alcohol, methanol. Ethanol, butanol or propanol can also be used as shown in Table 6.1. It is blended with diesel fuel derived from crude oil, reducing the CO_2 attributed to the fossil-fuel on combustion. Biodiesel can also decrease other emissions characteristic of normal diesel fuel, such as particulates and SO_x. The advantages of biodiesel combustion over petroleum diesel are well documented for both private and public transport (Table 6.1).

Virgin and waste vegetable oils, as well as microalgal oils, can be converted into renewable fuel biodiesel. The most common oils include *Jatropha carcus* L., rapeseed-, sunflower- and soybean oils. Tallow or waste animal fat can also be used as a triglyceride source. Location-specific studies on biodiesel production, usage or environmental effects have been performed in many countries on both community (Van Dyne *et al.* 1996) and industrial scales (Zhang *et al.* 2003a). Typically, sodium hydroxide is used as a homogenous alkali catalyst although acids can also be used as a chemical catalyst to facilitate reaction. These include homogenous Lewis acids (acetates and synthesised stearates of various metals) as described by Di Serio *et al.* (2005). Recent studies, largely still at the laboratory stage, have explored the use of the enzyme lipase as a biological catalyst. These enzymes may be provided by immobilised enzymes (EC3.1.1.3), as

whole cell systems or crude extractions of, amongst others, *Candida antarctica* and *Geotrichum candidum* (Table 6.1).

Other methods of preparation not included in this study have also been proposed. These include using supercritical fluids (Kusdiana and Saka 2001 and 2004, Saka and Kusdiana 2001, Demibraş 2002, 2003, 2005 and 2006, Madras *et al*. 2004), supercritical fluids with co-solvents (Cao *et al*. 2005, Han *et al*. 2005), or zeolites and metal catalysts. An alkali metal salt on alumina or sulphated zirconia, tungsten zirconium or sulphated tin oxides as super acids can be used as solid-base catalysts. The use of ultrasonic energy to increase transesterification kinetics has also been studied (Stavarache *et al*. 2005) as has the use of a peroxidation step in downstream processing to improve biodiesel properties (Lin *et al*. 2006) (Table 6.1).

Table 6.1: Review of literature available on biodiesel production, including various production methods reported

Key points	Minor points and references	
Previous studies on biodiesel (showing advantages over petroleum diesel)	Krahl *et al.* 1996, Kaltschmitt *et al.* 1997, Graboski and McCormick 1998, Hall and Scarse 1998, Sheehan *et al.* 1998a, Ma and Hanna 1999, Srivastava and Prasad 2000, Fuduka *et al.* 2001, ATTRA 2002, Beer *et al.* 2002a, 2002b, Dorado *et al.* 2003, Kerschbaum and Rinke 2003, Knothe *et al.* 2003, MacLean and Lave 2003, Makareviciene and Janulis 2003, Mortimer *et al.* 2003, Turrio-Baldassarri *et al.* 2004, Nabi *et al.* 2006	
Country specific biodiesel studies	Australia, Brazil, India, Italy Nicaragua, the Philippines, the United States of America	Beer *et al.* 2002b, Abreu *et al.* 2005, Azam *et al.* 2005, Barnwell and Shamra 2005, Subramanian *et al.* 2005, Bona *et al.* 1999, Cardone *et al.* 2003 Foidl *et al.* 1996, Tan *et al.* 2004, Van Dyne *et al.* 1996,
Vegetable and plant oils used in production	*Jatropha carcus L.*, rapeseed, sunflower, soybean, *Brassica* carinata, castor bean, coconut, *Camelina sativa*, copra, cotton seed, *Cynara cardunculus L.*, groundnut (peanut), hazelnut soap stock/waste sunflower, Jojoba (*Simmondsia chinesis* Link Schneider), Karanja (*Pongamia pinnata*), linseed, niger, palm kernel, palm tree, rice bran, sesame, turnip, various Brazilian vegetable oils (*e.g.* Andiroba, Babassu, Cumara and Piqui oils), yellow nut-sedge (*Cyperus esculentus* L.), milkweed (*Asclepias*) seed, mahau (*Madhuca indica*), rubber seed, coconut and 75 different trees, shrubs and herbs in India	Foidl *et al.* 1996, Peterson *et al.* 1996, Zhang *et al.* 1996, Bona *et al.* 1999, Crabbe *et al.* 2001, Al-Widyan and Al-Shyoukh 2002, Encinar *et al.* 2002, Kalam and Masjuki 2002, Köse *et al.* 2002, Abreu *et al.* 2004, Tan *et al.* 2004, Azam *et al.* 2005, Barnwell and Sharma 2005, Bouaid *et al.* 2005, Castro *et al.* 2005, Fröhlich and Rice 2005, Ghadge and Raheman 2005, 2006, Karmee and Chadha 2005, Han *et al.* 2005, Puhan *et al.* 2005, Ramadhas *et al.* 2005, Usta *et al.* 2005, Wood 2005, Zulliakah *et al.* 2005, Canoira *et al.* 2006, Hosler and Harry-O'Kuru 2006, Jitputti *et al.* 2006, Meher *et al.* 2006
Waste animal fat as oil for biodiesel production	Ma *et al.* 1999, Alcantara *et al.* 2000, Tashtoush *et al.* 2004	
Microalgal oil for biodiesel production	Aresta *et al.* 1995, Sheehan *et al*. 1998b, Miao and Wu 2006, Sears 2006, Xu *et al*. 2006, Chisti 2007, 2008, Schmidt 2007	
Micro-organisms used in production of lipase enzyme for catalysis	Lipases from: *Candida antarctica*, *Candida lypolytica, Geotrichum candidum*, *Mucor miehei*, *Penicillium camembertii*, *Penicillium roqueforti*, *Pseudomonas cepacia*, *Pseudomonas florescens*, *Rhizomucor miehei*, *Rhizopus delemar* and *Rhizopus oryzae*	Nelson *et al.* 1996, Kaieda *et al.* 1999, 2001, Shimada *et al.* 1999, 2002, Samukawa *et al.* 2000, Watanabe *et al.* 2000, 2001, 2002, Ban *et al.* 2001, 2002, Iso *et al.* 2001, Köse *et al.* 2002, Soumanou and Bornsheuer 2003, Shieh *et al.* 2003, Pizarro and Park 2003, Hama *et al.* 2006

Key points	Minor points and references	
Alcohols used in transesterification process for biodiesel production	Methanol: Ethanol: Butanol: Propanol:	Karaosmanoğlu *et al.* 1996, Vicente *et al.* 1998, 2004, Antolin *et al.* 2001, Zhang *et al.* 2003a Peterson *et al.* 1996 Boocock *et al.* 1996 Lang *et al.* 2001
Catalyst types: Acid catalysts	Junek *et al.* 1998, Enchelmaier and Rasehorn 1994, Germani 1994, Vicente *et al.* 1998, Boocock *et al.* 1996, Karaosmanoğlu *et al.* 1996, Lang *et al.* 2001, Antolin *et al.* 2002, Zhang *et al.* 2003a, Vicente *et al.* 2004, Zulliakah *et al.* 2005, Zheng *et al.* 2006	
Catalyst types: Biological catalysts	Mittelbach 1990, Shaw *et al.* 1991, Nelson *et al.* 1996, Kaieda *et al.* 1999, 2001, Shimada *et al.* 1999, 2002, Uosukainen *et al.* 1999, Abigor *et al.* 2000, Samukawa *et al.* 2000, Watanabe *et al.* 2000, 2001, 2002, Iso *et al.* 2001, Belafi-Bako *et al.* 2002, Köse *et al.* 2002, Chen and Wu, 2003, Shieh *et al.* 2003, Du *et al.* 2004, Madras *et al.* 2004, Al-Zuhair 2005, Oda *et al.* 2005, Noureddini *et al.* 2005, Salis *et al.* 2005, Al-Zuhair *et al.* 2006	
Catalyst types: Zeolites and metal catalysts	Peterson and Scarrah 1984, Bayense *et al.* 1996, Corma *et al.* 1998, Leclercq *et al.* 2001, Suppes *et al.* 2001, 2004, Abreu *et al.* 2004	
Catalyst types: Solid base catalysts	Alkali metal salt on alumina – Ebiura *et al.* 2005, Xie and Li 2006, Xie *et al.* 2006 Sulphated zirconium, Tungsten zirconium or Sulphated tin oxides as super acids – Furuta *et al.* 2004	

6.3. Process flowsheet and mass and energy balance inventories

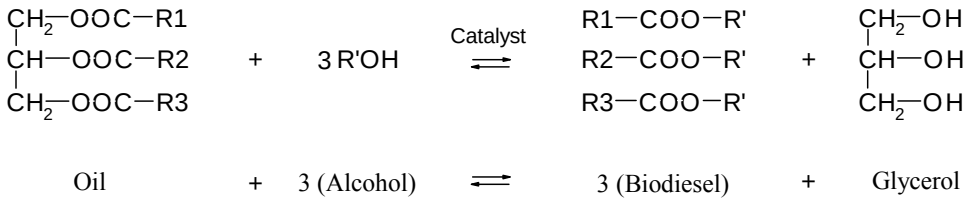
6.3.1. Introduction

In order to determine the environmental impacts of biodiesel production through life cycle assessment, process flowsheets giving material and energy inputs and outputs were required. Simulation models for five process options were developed using Aspen Plus® (AspenTech 2008). The flowsheets developed used NaOH for chemical catalysis and the enzyme lipase from *Candida antarctica* for biological catalysis (Table 6.2). The energy data reported is for the total renewable and non-renewable energy in the process.

Table 6.2: Specifications of the different biodiesel production process flowsheets investigated

Flowsheet	Case 1	Case 2	Case 3	Case 4	Case 5
Catalyst	Alkali	Lipase	Alkali	Alkali	Lipase
Alcohol Used	Methanol	Methanol	Methanol	Ethanol	Ethanol
Alcohol Recovery	94 %	-	50 %	94 %	-

The flowsheets were developed for continuous processes converting rapeseed oil (assumed to be pure triacylglycerol – $C_{57}H_{104}O_6$), via a transesterification reaction, to biodiesel as shown in Equation 6.1. The process was based on the alkali catalysed transesterification approach of Zhang *et al.* (2003a), including supportive and alternative conditions described by others as given in Table 6.3. Economic and sensitivity data were given in Zhang *et al.* (2003b).



Oil + 3 (Alcohol) ⇌ 3 (Biodiesel) + Glycerol

Equation 6.1

Table 6.3: Limited list of literature process conditions for transesterification of oils (alkali catalysts)

	Karaosmanoğlu *et al.* 1996	**Boocock *et al.* 1996**	**Vicente *et al.* 1998 & 2004**	**Lang *et al.* 2001**	**Antolin *et al.* 2002**	**Zhang *et al.* 2003a**	**This study**
Oil Used	Rapeseed	Soybean	Sunflower	Rapeseed, linseed, sunflower	Sunflower	Waste cooking oil, Triacylglycerol	Triacyalglycerol (rapeseed oil)
Alcohol Used	Methanol	Methanol, Butanol	Methanol	Methanol, Ethanol, 2-propanol, 1-butanol	Methanol	Methanol	Methanol, Ethanol
Alcohol to oil ratio	6:1	6:1-30:1	6:1	6:1	9:1	6:1	6:1
Catalyst Used	NaOH	NaOH or $NaOCH_3$ in THF*	NaOH	KOH, sodium methoxide	KOH	NaOH	NaOH
Catalyst wt. fraction	0.016	0.002-0.01	0.013	0.01-0.02	0.0028-0.0055	0.01	0.01
Reactor temperature	65 °C	20-60 °C	20-65 °C	25-110 °C	60-70 °C	60 °C	60 °C
Reactor pressure	1 atm	1 atm	1 atm	1 atm	1 atm	4 bar	4 bar
Residence Time	38-50 min	10-15 min	8 hr	40 min-3 hr			2 hr
Oil conversion	97.4-99.7 % (50 min)	70-95 %	100 %	98 % (1 hr)	96 %	95 % (2 hr)	95 % (2 hr)
Methanol recovery			~100 %			94 %	94 % 50 %
Wash water (kg/kg oil)				Includes tannic acid or brine	Includes H_3PO_4	0.01	0.26
Neutralising acid						H_3PO_4	HCl

*THF – tetrahydrofloran

6.3.2. Alkali catalysed process

As shown in Figure 6.1, in the base case (Case 1), methanol (stream 101) entered at a 6:1 molar ratio with respect to the oil (stream 105) (twice the stoichiometric requirement) allowing the equilibrium to be shifted towards biodiesel production. The catalyst (NaOH) was present at a

0.01 mass fraction with respect to oil. An assumption was made that dehydrogenated vegetable oil with less than 0.5 wt% free fatty acid, anhydrous alkali catalyst and anhydrous alcohol were used as water and free fatty acids can lead to soap formation (Liu 1994; Basu and Norris 1996). If waste cooking oils were used, a purification step would be needed before the reactor to ensure a low free fatty acid and water content.

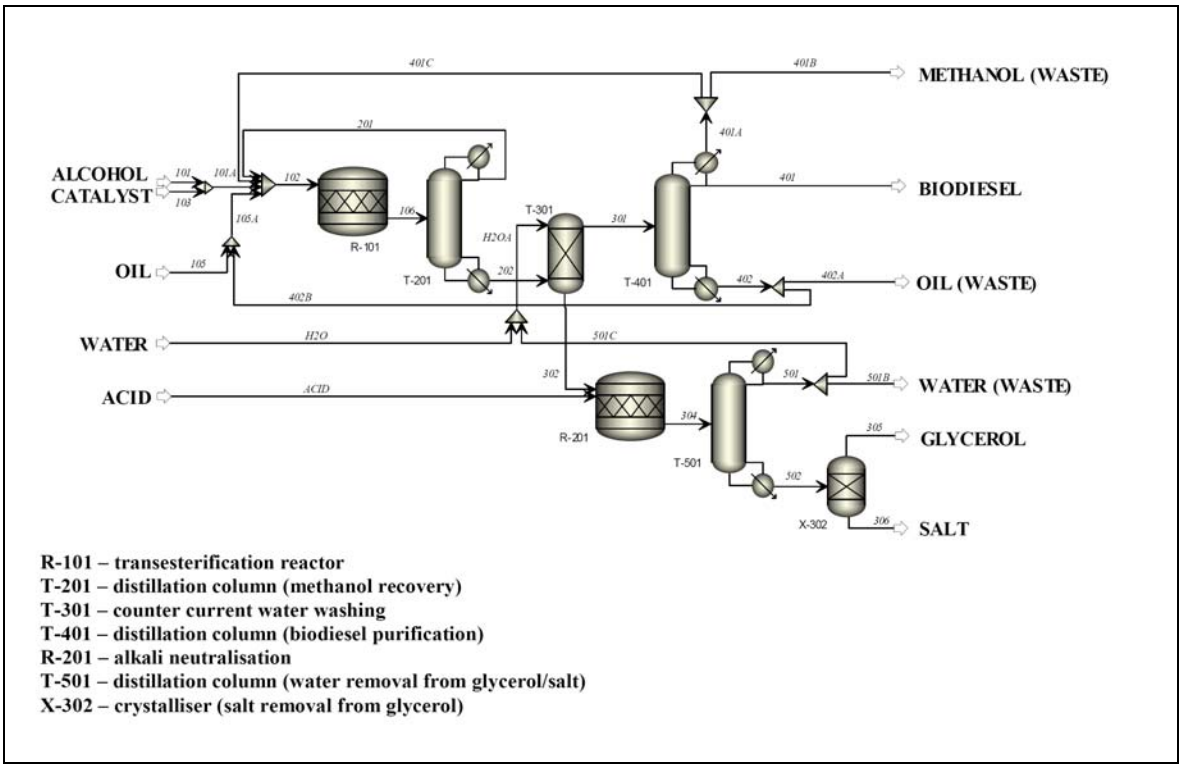


Figure 6.1: Biodiesel process flowsheet to be used in LCA – alkali catalyst; modified from Zhang *et al.* (2003a) to include recycle

The oil, catalyst and alcohol are fed (stream 102) into reactor R-101. The reactor was maintained at a temperature of 60 ºC and a pressure of 4 bar. After a 2 h residence time, a 95 % conversion of oil to biodiesel and a glycerol co-product was assumed. The reactor products (stream 106) were fed to a methanol recovery unit (distillation column, T-201) where 94 % of the methanol was separated by distillation and recycled back to R-101.

Biodiesel from the transesterification reaction (stream 202) was separated from glycerol by counter current water washing (T-301). Zhang *et al.* (2003a) recommended 11 kg of wash water per 1050 kg fresh oil feed and the use a second unspecified purification unit. The amount of water used was increased to 260 kg to achieve separation in a single step. The majority of the glycerol and sodium hydroxide was taken out with the water stream (302), while the unreacted oil and biodiesel product remained in stream 301.

Sodium hydroxide in stream 302 was neutralised in a second reactor (R-201). Zhang *et al.* (2003a) suggested phosphoric acid. As the thermodynamics of the resulting salt were unknown, hydrochloric acid (HCl) was chosen to replace it. Acid entered (acid stream) stoichiometrically so that all the alkali was treated. The products from the reactor (stream 304) were further purified in distillation column T-501 to remove water (stream 501) which was split and recycled or discarded. The salt was removed by a crystalliser (X-302), leaving 105 kg of 85 % purity glycerol (stream 305).

The biodiesel and unconverted oil from the water washing unit (T-301) proceeded to distillation column T-401 (stream 301) for separation. Oil was removed in the bottoms and recycled to reactor R-101 via stream 402B. A liquid/vapour mixture was obtained from the distillate of column containing 99.6 wt.% liquid biodiesel product (stream 401) and a vapour methanol (68 %) stream (401A) which was split and recycled to reactor R-101 or discarded.

6.3.3. Biologically catalysed process

Using the flowsheet model for biodiesel production with an alkali catalyst outlined above, relevant changes were made to accommodate an enzyme catalyst (Case 2). Where possible, features were kept unchanged to facilitate comparison through life cycle analysis. Lipase from *Candida antarctica* (immobilized on polyacrylate polymer beads as supplied by Novo Nordisk Bioindustrials – Novozym 435®) was used as the enzyme catalyst. It had been reported that Lypozyme IM-77 (also from Novo Nordisk), a commercial lipase from *Rhizomucor meihei*, could also be used (Shieh *et al.* 2003) as could any of the enzymes listed in Table 6.1. The enzyme can be used for over 25 cycles without loosing activity (Watanabe *et al.* 2002).

As with the alkali catalysed scenario, the enzyme catalysed process included the continuous feeding of methanol (stream 101) and oil (stream 105) to reactor R-101 as shown in Figure 6.2. The enzyme loading was kept at 4 wt% (biomass and support) as recommended by Shimada *et al.* (2002). Methanol was fed such that the concentration is kept low. A concentration higher than half the stoichiometric amount would denature the catalyst (Shimada *et al.* 1999, 2002). Reaction was maintained at 25°C and 1 bar with a residence time of 20 hr.

By design, and to avoid enzyme denaturation, no excess methanol was added and 90 % reacted. For this reason there was little need for methanol recovery by distillation; hence unit T-201 was omitted. Also, since no alkali was present in the system, there was no need for neutralization by acid and reactor R-201 and crystalliser X-302 were omitted.

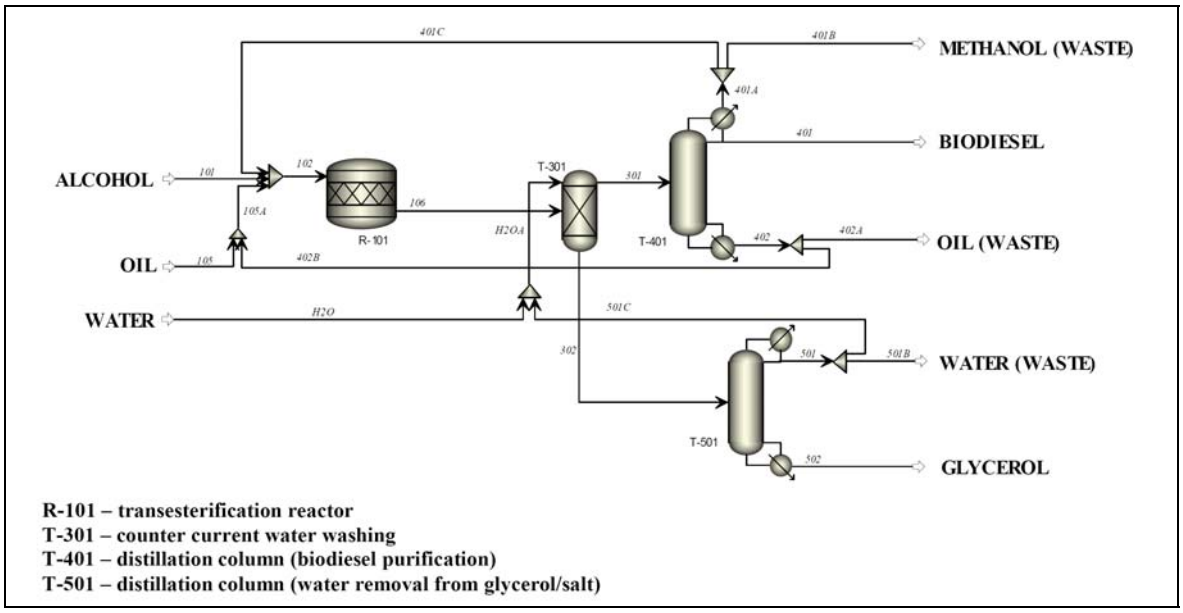


Figure 6.2: Biodiesel process flowsheet to be used in LCA – biological catalyst

6.3.4. Additional process flowsheets

Further process alternatives in this study included modification of the original alkali catalysed process (Case 1) to analyse a lower methanol recovery in T-201 (Case 3). The alkali catalysed (Case 1) and biologically catalysed (Case 2) processes have also been modelled using ethanol instead of methanol (Cases 4 and 5 respectively) (Table 6.4). The mass and energy balances obtained for 1 000 kg of biodiesel, as given in Table 6.5, were used for the Life Cycle Assessment (LCA) study.

Table 6.4: Process conditions for biodiesel production used to compare alkali catalysed and biocatalysed biodiesel production

	Case 1	Case 2	Case 3	Case 4	Case 5
Oil Used	Triacylglycerol (rapeseed oil)	Triacylglycerol (rapeseed oil)	Triacylglycerol (rapeseed oil)	Triacylglycerol (rapeseed oil)	Triacylglycerol (rapeseed oil)
Catalyst Used	NaOH	Immobilised enzyme from *Candida antarctica*	NaOH	NaOH	Immobilised enzyme from *Candida antarctica*
Catalyst fraction	0.01 (molar)	0.04 (mass)	0.01 (molar)	0.01 (molar)	0.04 (mass)
Alcohol Used	Methanol	Methanol	Methanol	Ethanol	Ethanol
Alcohol to oil ratio	6:1	3:1	6:1	6:1	3:1
Alcohol recovery	94 %	(none required)	50 %	94 %	(none required)
Oil conversion	95 %	90 %	95 %	95 %	90 %
Reactor temperature	60°C	25°C	60°C	60°C	25°C
Reactor pressure	4 bar	1 bar	4 bar	4 bar	1 bar
Residence time	1.5 hr	16 hr	1.5 hr	1.5 hr	16 hr

Table 6.5: Material, energy and utility flows obtained for each process flowsheet scenario proposed in Table 6.4

		Case 1	Case 2	Case 3	Case 4	Case 5
Products						
Biodiesel	kg	1000	1000	1000	1000	1000
Glycerol	kg	106	106	106	101	101
Feed						
Rapeseed Oil	kg	991	991	991	947	947
Methanol	kg	112	111	146	-	-
Ethanol	kg	-	-	-	149.82	0.23
NaOH	kg	10.4	-	10.4	10.0	-
Lipase (*Candida antarctica*)*	kg	-	1.6	-	-	1.6
HCl	kg	37.9	-	37.9	36.2	-
Steam	kg	1820	1540	2060	1820	1490
Electricity	kWh	8.6	34.1	12.4	9.9	35.4
Water (process)	kg	29.8	56.4	28.8	30.6	55.9
Water (cooling)	t	117	97.4	132	114	92.6
Waste						
Salt	kg	15.2	-	15.2	14.5	-
Methanol	kg	2.9	2.4	36.1	-	-
Ethanol	kg	-	-	-	0	0.23
Water	kg	57.5	50.9	57.8	57.4	51.0

***Assuming 25 cycles of biodiesel production before lipase needs replacing**

6.3.5. Production alternatives for biodiesel production

Despite slower conversion rates in the enzyme catalysed route, interest in the process is justified by the resultant simplifications and lower reaction temperatures. Advances in the technology have also resulted in a decrease in the enzyme catalyst price which had been a previous concern (Tan *et al.* 2005). The lipase catalyst does not impose restrictions on the water content or level of free fatty acids in the oil, and is able to yield similar conversions to the alkali catalysed option. There is a high ester yield and no saponification reaction occurs (Fukuda *et al.* 2001). Chemical catalysts need to be neutralised before glycerol can be removed, forming a salt by-product that contaminates the glycerol product. In contrast, the immobilized lipase remains in the reactor and does not contaminate reaction products; hence glycerol recovery and purification may be simplified.

Methanol is the most commonly used alcohol in the reaction because of its short chain length and low cost. Its use is a possible cause of environmental concern owing to high energy and crude oil requirements. Ethanol is renewable and has been reported to give advantages over methanol owing to being environmentally based and carbon dioxide neutral (Demirbaş 2003). In alkali-catalysed biodiesel production, the alcohol is added in excess to favour the transesterification reaction. Alcohol recovery is required to minimise alcohol waste. A high

recovery requires a higher energy input for distillation. The relative advantage of minimizing the distillation energy requirement requires assessment (comparison of Case 1 and Case 3). In the enzyme catalysed route, alcohol recovery is eliminated completely since a high alcohol concentration can inactivate the enzyme. Stepwise, stoichiometric addition is required (Shimada *et al.* 1999, 2002, Watanabe *et al.* 2000, 2001, 2002, Soumanou and Bornscheuer 2003). Immobilised lipases are deactivated by a high concentration of lower alcohols. When this occurs the lipase can be regenerated with 2- or *tert*-butanol (Chen and Wu 2003).

6.4. Life Cycle Assessment (LCA)

Comparison of the different routes of biodiesel production was performed using the methodology explained in Section A.6. of Appendix A. The system was defined as cradle-to-gate production of 1000 kg of biodiesel, equivalent to a fuel with a calorific value of 27.1 GJ, or 33.3MJ/l (Beer *et al.* 2002b). The LCA included all raw materials, agricultural inputs and biodiesel processing required for the final biodiesel product. The life cycle inventory data are presented in Appendix I.

The production included the useful by-product glycerol which, owing to process design and stoichiometry, is of equal purity and in equal proportion to biodiesel in each process. The assumption of equal purity may be conservative towards the enzyme process as the industrial process may give a purity slightly higher than the alkali catalysed process.

The preferred method of burden allocation is by system expansion (Azapagic and Clift 1999, Ekval and Finnveden 2001, ISO 14040:2006). However, in order for readers to compare the biodiesel results against other literature values or against petroleum diesel values, this approach was avoided. Because of varying costs of glycerol as a function of purity, burden allocation on an economic basis was also not used. Therefore, the most appropriate allocation method was by mass ratios of useful end product. Since the mass ratios of glycerol to biodiesel were similar in all studies, allocation by economics or any other method would have given similar comparative results across the scenarios. Thus these suffice for comparison of technology selection as presented here.

Before the LCA comparison of biodiesel production could be performed, the LCA impact of enzyme production needed to be calculated. Since the SimaPro v7.1® (PRé Consultants B.V. 2008) software and the EcoInvent v1.3 database (Swiss Centre for Life Cycle Inventories 2007), did not include an LCA module for immobilised enzyme production, the generic flowsheet model was used to determine material and energy balance inputs. These can then be used to determine the LCA impacts of biodiesel production.

6.5. Lipase production

6.5.1. Process flowsheet and model development

Two processes for the extracellular production of lipase using an aerobic batch reactor, with glucose and yeast extract as raw materials were considered. The first scenario (Scenario L1) assumed submerged culture (SmF) which yielded a final default biomass concentration of 16.7 g/l as shown in Table 6.6, while the second (Scenario L2) assumed solid state cultivation (SSC) giving a biomass concentration of 40 g/l. In Scenario L1, biomass is removed by a filtration unit. This was followed by a downstream train identical to Scenario L2 which did not require biomass removal. This involved a unit to precipitate the lipase product and a filtration unit to recover the lipase as shown in Figure 6.3. It was assumed that the formulation unit removed enough liquid to give a purity of 85 % in both scenarios. The assumptions around the lipase production were based first approximations. Scenario L2 (SSC) could expect to export enzyme for longer than Scenario L1 (SmF), but also had greater mass transfer problems. In these calculations it was assumed that the rates were maintained in both production scenarios.

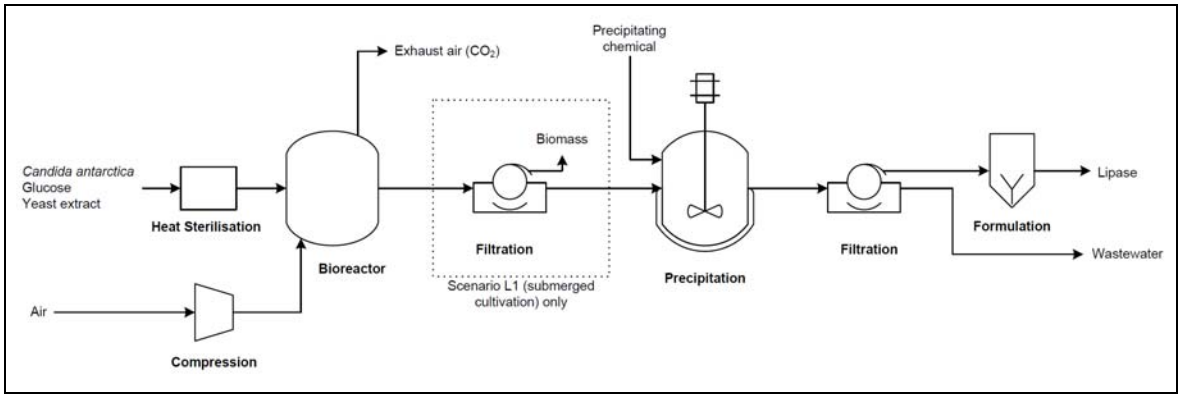


Figure 6.3: Flowsheet used for material and energy balance development for lipase production from *Candida antarctica*

6.5.2. Material and energy balance outputs

From the information used in Table 6.6, material and energy flows were calculated and are shown in Table 6.7. Although identical stoichiometry, nutrient excess and yield coefficients were used, owing to the additional filtration unit in Scenario L1 where nutrients were lost, for each kilogram of lipase product, 5.2 kg of glucose, 3.5 kg of yeast extract and 247 kg of air were required. In Scenario L2, 3.6 kg of glucose, 2.4 kg of yeast extract and 139 kg of air were required. Owing to the reduced biomass concentration with the same yield of lipase assumed per unit biomass, Scenario L1 required more water (76.5 kg) than Scenario L2 which required

28.7 kg water per kilogram of enzyme. This resulted in an increased requirement for precipitating chemical (3.7 kg in L1 *vs.* 1.5 kg in L2) since it was added on a basis of volumetric concentration. The increased volume in Scenario L1 also increased the electricity (70.7 MJ/kg) and steam (15.6 kg/kg) requirements beyond Scenario L2 which required 35.4 MJ/kg and 5.3 kg/kg respectively. The assumption of 0.5 kW/m^3 agitation energy may be slightly high for the solid state cultivation (which represents pumping energy), but it was taken as a conservative estimate.

Table 6.6: Input assumptions used for the extracellular, aerobic production of lipase in a batch reactor as required for the enzyme catalyst for biodiesel production

Assumptions*	Scenario L1 – SmF#	Scenario L2 – SSC#	Units
Steam Sterilisation: (preheated with sterilised media)			
Steam temperature	[140]	[140]	°C
Steam pressure	[300]	[300]	kPa
Microbial growth conditions: (batch production of lipase from *Candida* sp.)			
Carbon 1 source (excess): Glucose	[1]	[1]	%
Nitrogen source 1: Yeast extract	[5]	[5]	%
Maintain reactor temperature (Cooling agent: Cooling water)			
Final biomass concentration	[16.7]	40	g/l
Aeration:			
Oxygen source (vvm): Air	[10x the min. stoich. requirement]	[10x the min. stoich. requirement]	
Agitation			
Power per unit volume (Energy: Electricity)	0.5	0.5	kW/m^3
Cool before downstream processing			
Filtration			
Solid fraction removed	[0.95]	-**	%
Liquid fraction retained	[0.70]	-**	%
Precipitation			
Non-reacting additive: [Precipitating chemical]	[5]	[5]	%v/v
Filtration			
Solid fraction removed	[0.95]	[0.95]	%
Liquid fraction retained	[0.70]	[0.70]	%
Formulation (other)			
Liquid fraction removed	To give product purity of 85 %	To give product purity of 85 %	%
Product fraction retained	[0.99]	[0.99]	%

[ ] Default data calculated or assumed in the MS-Excel model when no explicit inputs are given
*** Energy assumed to be supplied by electricity throughout, with steam used for heating during precipitation**
 # Smf – submerged fermentation; SSC – solid state cultivation
**** No filtration unit required for solid state cultivation method**

Table 6.7: Material, energy and utility flows for the production of lipase from *Candida antarctica* using the generic flowsheet model

Component	In (kg)	Out (kg)	In (kg)	Out (kg)
	Scenario L1		Scenario L2	
Glucose	5.2	0.052	3.6	0.036
Yeast extract	3.5	0.16	2.4	0.12
Candida sp.	-	1.2	-	0.9
Air (reacting oxygen only)	5.2	-	3.6	-
Precipitating chemical	3.7	3.7	1.5	1.5
Lipase (lost in wastewater)	-	0.40	-	0.05
Lipase (product)	-	1	-	1
Carbon dioxide	-	7.4	-	5.2
Phosphates	-	0.13	-	0.09
Water	76.5	80.0	20.1	22.4
TOTAL	138.3	138.3	31.2	31.2
Chemical Oxygen Demand (COD)		8.1		3.7
Product recovery (% kg lip.)		65.8		94.1
Product purity (mass %)		85		85

Energy requirements	Scenario SSC1	Scenario SSC2	Units
Electricity	19.64 (70.7)	9.83 (35.4)	kWh/kg lip. (MJ/kg lip)
Steam (152°C, 3 bar)	15.6 (42.12)	5.3 (14.3)	kg/kg lip. (MJ/kg lip.)
Total energy equivalent	115.8	49.7	MJ/kg lipase
Cooling water	33.8	14.0	kg/kg lipase

6.5.3. Life Cycle Assessment (LCA) of lipase production

Using the material and energy inventory from Scenario L1, and the SimaPro software, for 1 kg of lipase produced, life cycle impacts of 53.7 kg $CO_{2\ eq.}$, 0.320 kg $Sb_{\ eq.}$, 1.810 mg CFC-11 $_{eq.}$ and 0.87 kg $SO_{2\ eq.}$ were obtained in the categories of global warming, abiotic depletion, ozone layer depletion and acidification respectively as shown in Table 6.8. The LCA results for the same categories for Scenario L2 were 33.0 kg $CO_{2\ eq.}$, 0.181 kg $Sb_{\ eq.}$, 1.07 mg CFC-11 $_{eq.}$ and 0.55 kg $SO_{2\ eq.}$. Using a solid state cultivation method and increasing the final biomass concentration as in Scenario L2, reduced the LCA burden by between 36 % and 48 % as shown in Figure 6.4. Global warming, eutrophication and abiotic depletion were reduced by 39 %, 36 % and 43 % respectively.

Table 6.8: LCIA of lipase per kilogram of product from *Candida antarctica* – CML Baseline 2000 V2.03

Impact Category	Unit	Scenario L1	Scenario L2
Abiotic Depletion	kg $Sb_{eq.}$	0.320	0.181
Global Warming (GWP100)	kg $CO_{2\ eq.}$	53.9	33.0
Ozone Layer Depletion (ODP)	mg CFC-$11_{eq.}$	1.81	1.07
Human Toxicity	kg 1,4-$DB_{eq.}$	10.8	5.86
Fresh Water Aquatic Ecotoxicity	kg 1,4-$DB_{eq.}$	2.31	1.21
Marine Aquatic Ecotoxicity	kg 1,4-$DB_{eq.}$	15 000	7 880
Terrestrial Ecotoxicity	kg 1,4-$DB_{eq.}$	0.098	0.059
Photochemical Oxidation	kg C_2H_4	0.033	0.021
Acidification	kg $SO_{2\ eq.}$	0.87	0.55
Eutrophication	kg $PO_4^{3-}{}_{eq.}$	1.01	0.65

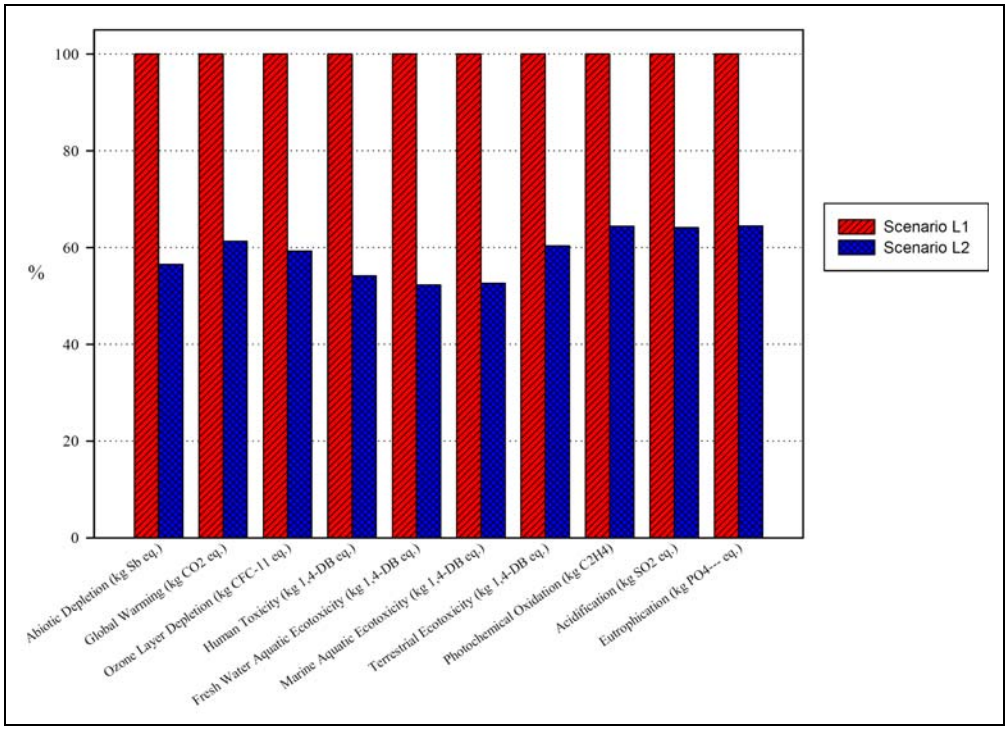


Figure 6.4: Comparison of LCA results for lipase production for Scenario L2 relative to Scenario L1

The impacts of immobilisation have been excluded from these calculations as various methods of immobilisation may be possible for biodiesel production. Watanabe *et al*. (2000, 2001, and 2002), Iso *et al*. (2001), Noureddini *et al*. (2005) and others used epoxy or resin beads in laboratory studies. Assuming an equal amount of resin to enzyme, the LCA impacts of the resin were calculated to be less than 20 % and 8 % of the lipase impacts (Scenario L2) for anionic

(anionic resin, at plant/kg/CH – Ecoinvent 1.3) and cationic resins (cationic resin, at plant/kg/CH – Ecoinvent 1.3) respectively (Figure 6.5). The exception was in the ozone layer depletion category where, owing to a large quantity of bromine compounds, the anionic score was 260 fold higher than that for the lipase. More environmentally friendly immobilisation materials such as gels, spun fibres, microcapsules can be expected to reduce this contribution still further.

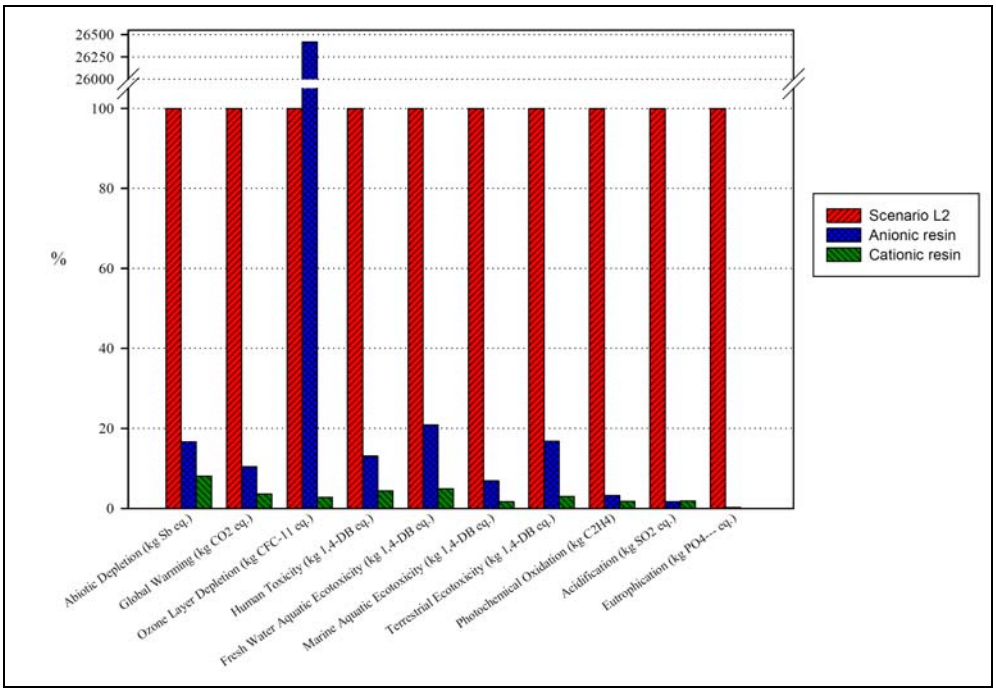


Figure 6.5: Comparison of LCA results for equal masses of anionic and cationic resins relative to lipase produced by Scenario L2

6.6. Life Cycle Assessment (LCA) of biodiesel production

The material and energy inventory for biodiesel production (Table 6.5), as calculated using the Aspen Plus® software (AspenTech 2008), was used to determine Life Cycle Assessment (LCA) indicators with the aid of SimaPro v7.1® (PRé Consultants B.V. 2008) LCA software. The impact of the lipase reported in Section 6.5, was used for Cases 2 and 5 where biological catalysts were used. Scenario L2 was used as this represented the best case. The impacts of the lipase are expected to decrease further with optimisation of the technology.

Data from LCA results which are within 5 % of each other may not be significantly different from each other owing to uncertainty in the inputs and LCA inventory datasets.

For the production of 1 kg of biodiesel, average life cycle impacts of 3.6 kg $CO_{2\ eq.}$, 0.0141 kg $Sb_{eq.}$, 0.733 mg CFC-11 $_{eq.}$ and 0.0264 kg $SO_{2\ eq.}$ were obtained in the categories of global warming, abiotic depletion, ozone layer depletion and acidification respectively as shown in Table 6.9. Differences in abiotic depletion, global warming, ozone layer depletion, photochemical oxidation, acidification and eutrophication were less than 20 % across all cases as shown in Figure 6.6. Impacts in terrestrial ecotoxicity showed as much as a 90 % variation across the different cases, with the enzyme catalysed route (Cases 2 and 5) showing the lowest impact because of the absence of hydrochloric acid.

Table 6.9: LCIA of biodiesel per kilogram of product – CML 2 Baseline 2000 V2.03

Impact category	Unit	Case 1	Case 2	Case 3	Case 4	Case 5	Average
Abiotic Depletion	kg $Sb_{eq.}$	0.0137	0.0130	0.0147	0.0149	0.0141	0.0141
Global Warming (GWP100)	kg $CO_{2\ eq.}$	3.64	3.55	3.71	3.61	3.52	3.61
Ozone Layer Depletion (ODP)	mg CFC-$11_{eq.}$	0.776	0.698	0.785	0.749	0.658	0.733
Human Toxicity	kg 1,4-$DB_{eq.}$	0.231	0.174	0.249	0.359	0.301	0.263
Fresh Water Aquatic Ecotoxicity	kg 1,4-$DB_{eq.}$	0.0412	0.0315	0.0437	0.0755	0.0660	0.052
Marine Aquatic Ecotoxicity	kg 1,4-$DB_{eq.}$	189.8	140.2	2036	293.3	242.4	580.3
Terrestrial Ecotoxicity	kg 1,4-$DB_{eq.}$	0.00216	0.000160	0.00245	0.00222	0.00023	0.00144
Photochemical Oxidation	kg C_2H_4	0.00127	0.00129	0.00128	0.00136	0.00138	0.00132
Acidification	kg $SO_{2\ eq.}$	0.0245	0.0251	0.0246	0.0288	0.0292	0.0264
Eutrophication	kg $PO_4^{3-}{}_{eq.}$	0.0374	0.0387	0.0373	0.0421	0.0433	0.0398

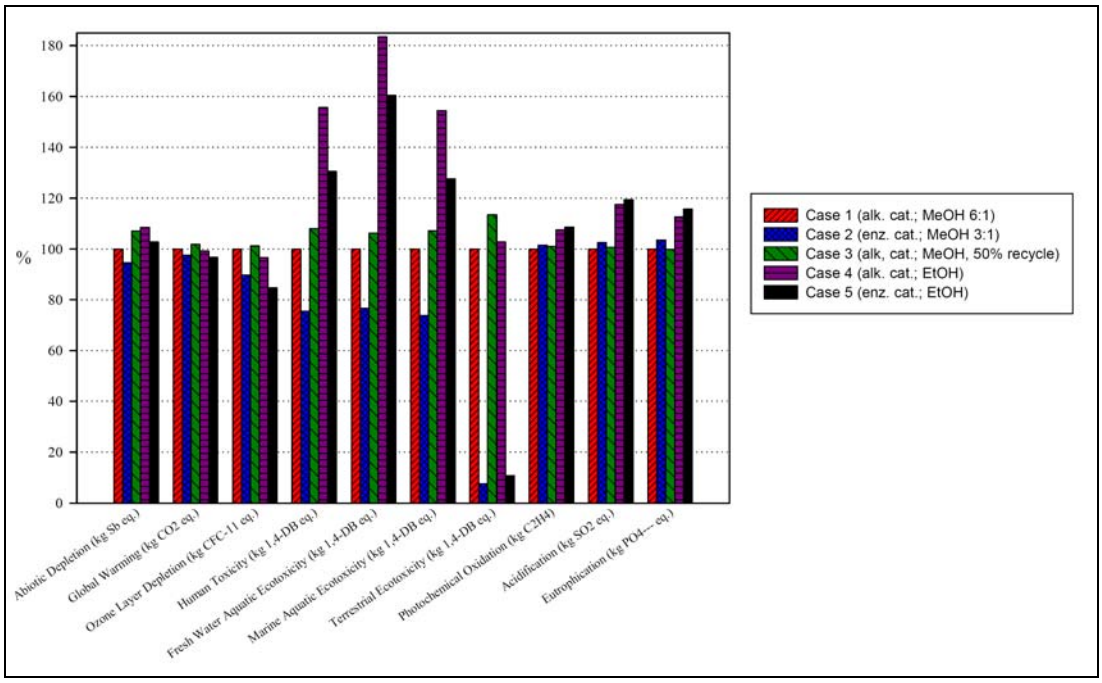


Figure 6.6: Comparison of LCA results for biodiesel for Cases 2 – 5 relative to Case 1

6.6.1. Alkali catalyst and methanol (Case 1)

Using the CML 2 Baseline 2000 v2.03 method, for 1 kg of biodiesel produced by alkali catalysis in the presence of methanol (Case 1), 3.64 kg carbon dioxide equivalent was released into the atmosphere, while 24.5 g $SO_{2\ eq.}$ and 37.4 g $PO_4^{3-}{}_{eq.}$ were emitted. The major contributing process in all LCA categories for the production of biodiesel was the farming process, including fertilizer production. Further major contributors included the energy requirements from natural gas, diesel and heat oil, as well as the impacts associated with steam production. The process contributions are further discussed in Section 6.6.5, later in this chapter.

Because of the high energy contribution, milder process conditions were expected to improve LCA results as investigated in Case 2. Reducing methanol recovery should also reduce the energy needs (Case 3). To further improve LCA results, changing the alcohol used to ethanol allowed comparison with methanol from both the original alkali and enzyme processes (Case 1 and 2 for methanol and Case 4 and 5 for ethanol).

6.6.2. Chemical vs. biological catalysts

When comparing biodiesel production (CML 2 Baseline 2000 v2.03 method) using inorganic- and biological catalysts with methanol as the transesterification alcohol (Case 1 and 2), all LCA impacts were lower for the enzyme catalysed process, except photochemical oxidation, acidification and eutrophication. Fresh water aquatic-, marine aquatic- and human toxicity were reduced by approximately 15 %. Terrestrial ecotoxicity was reduced by over 95% (mainly owing to the removal of hydrochloric acid from the biologically-catalysed process). Ozone layer depletion was reduced by 11 % and abiotic depletion by 5 %. Reductions in other categories were below 5 % as shown in Figure 6.7. Increases in photochemical oxidation, acidification and eutrophication were all less than 3 %. These reductions resulted from reduction in steam, as well as the absence of hydrochloric acid and sodium hydroxide. The increase in electricity in Case 2 balanced out the reductions from steam, hydrochloric acid and sodium hydroxide in the categories of photochemical oxidation, acidification and eutrophication.

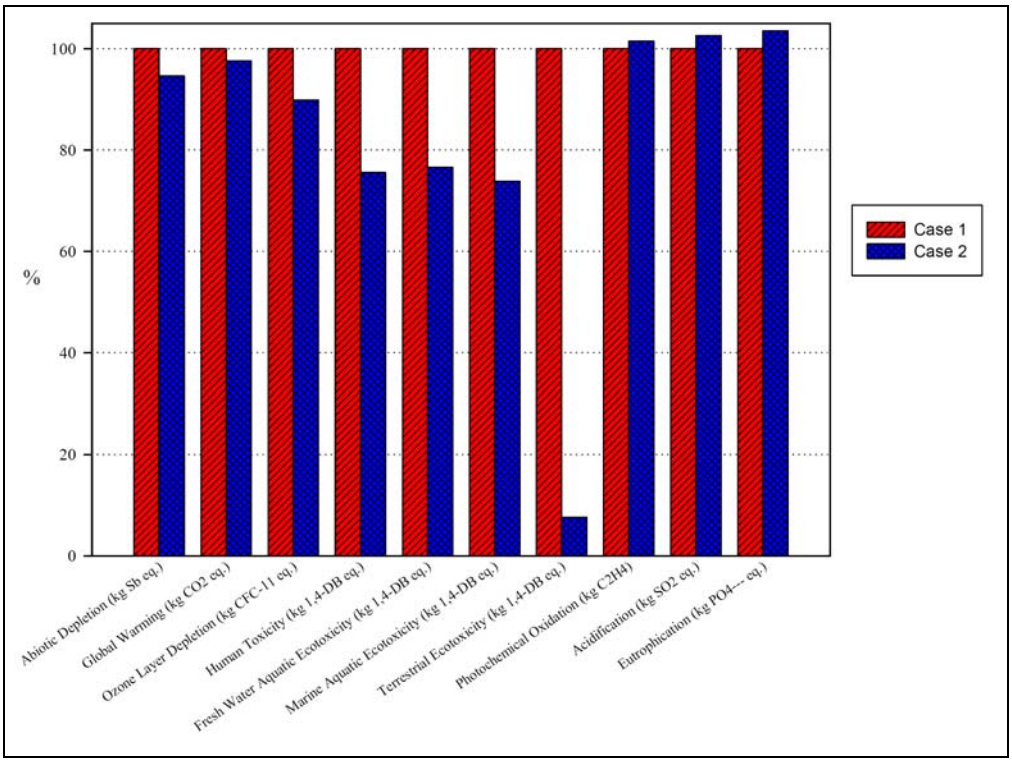


Figure 6.7: LCA results – chemical *vs.* biological catalysts (biodiesel production by alkali catalysis assuming 94 % methanol recovery (Case 1) is compared to production using lipase as a biocatalyst (Case 2))

6.6.3. Reduced methanol recovery

When methanol recovery in the alkali-catalysed process was lowered from 94 to 50 % (Case 1 *vs.* 3), impacts increased in all categories. In order to maintain an equal amount of biodiesel product, flow rates in the product recovery section increased when methanol recovery decreased. This resulted in greater pumping requirements and higher loads in distillation columns. Reducing recovery also increased waste methanol released and the methanol feed.

Reducing methanol recovery to 50 % increased all recorded toxicity levels by at least 6 %. Abiotic depletion increased by 6 %, while increases in ozone layer depletion, global warming, photochemical oxidation, acidification and eutrophication were not significant (Figure 6.8). Since flows increased, capital and operating costs were also expected to increase, which would result in further favouring of Case 1.

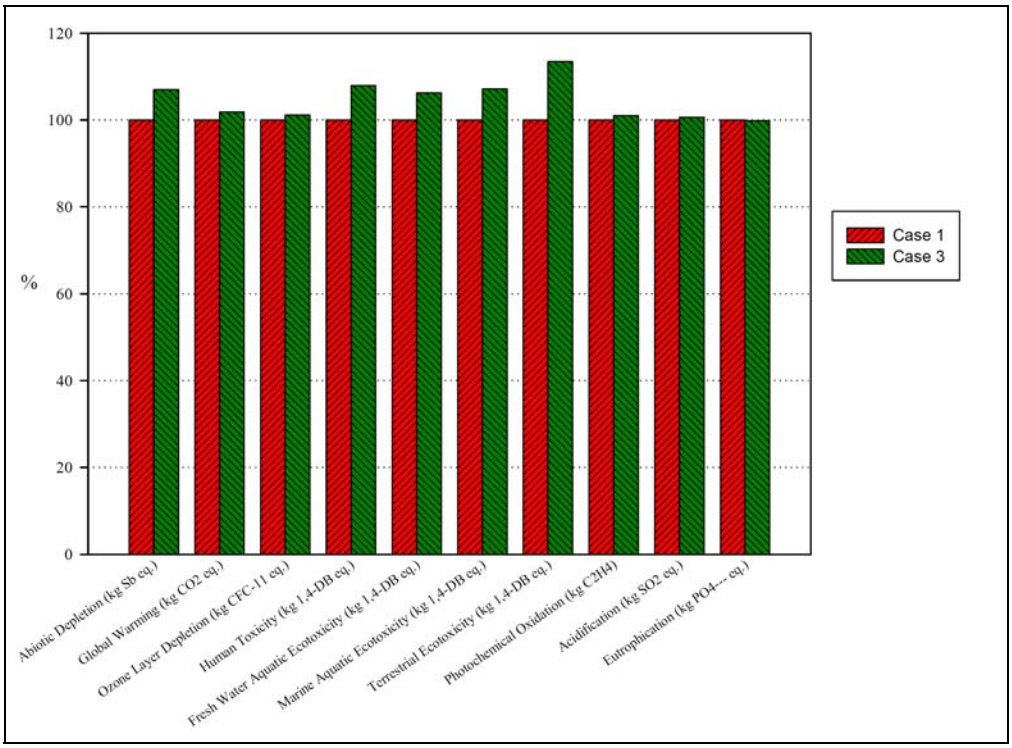


Figure 6.8: LCA results – reduced methanol recovery (biodiesel production by alkali catalysis assuming 94 % methanol recovery (Case 1) is compared to production assuming 50 % methanol recovery (Case 3))

6.6.4. Alternative alcohol (methanol vs. ethanol)

When methanol was replaced with ethanol (produced biologically from sugarcane) (Case 1 *vs.* 4 and Case 2 *vs.* 5 as shown in Figure 6.9 and Figure 6.10 respectively), LCA impacts were mainly increased, except for global warming and ozone layer depletion scores which were within 5 % of each other. The change from methanol to ethanol use showed similar trends in the alkali catalysed and enzymatic routes.

When using ethanol, increases in human-, fresh water aquatic- and marine aquatic ecotoxicity were more than 50 % and 70 % higher in the alkali and enzymatic catalysed routes respectively. These negative effects were partly owing to the sugar based-ethanol LCA module used in this study (Theka 2002) which had a high coal demand compared to other models.

Increases in abiotic depletion and photochemical oxidation were less than 10 %. The reductions in global warming and ozone layer depletion impacts were less than 1 % and 6 % respectively. The reduction in greenhouse gases was not large; even with the biologically derived ethanol taking up CO_2 during agricultural processes.

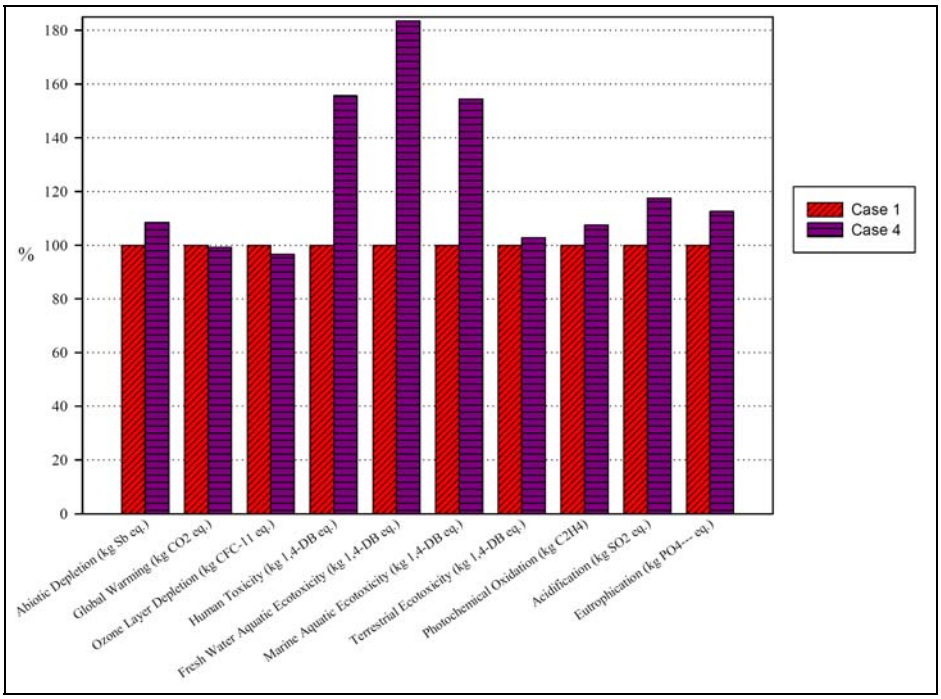


Figure 6.9: LCA results – alternative alcohol (methanol *vs.* ethanol) using alkali catalysis (biodiesel production by alkali catalysis assuming 94 % methanol recovery (Case 1) is compared to production using ethanol at 94 % recovery (Case 4))

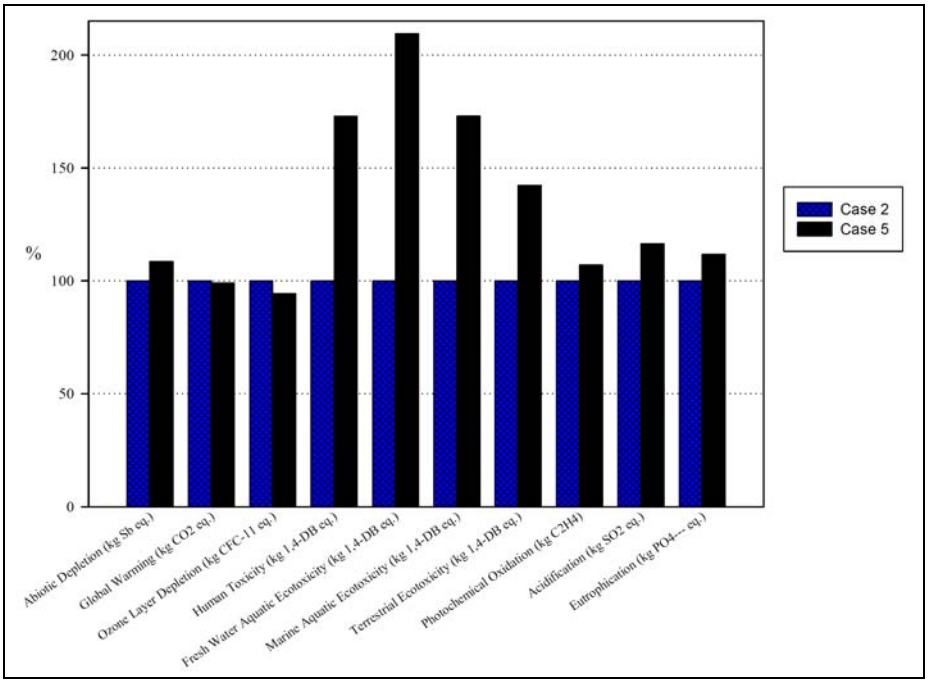


Figure 6.10: LCA results – alternative alcohol (methanol *vs.* ethanol) using lipase biocatalysis (biodiesel production by lipase biocatalysis using methanol (Case 2) is compared to production by lipase biocatalysis and ethanol (Case 5))

6.6.5. Process contributions

Using the example of biodiesel production with methanol and a lipase enzyme catalyst (Case 2), Life Cycle Assessment (LCA) process contributions were calculated to determine the impact of the lipase catalyst to the overall LCA score. The enzyme production Scenario L2 was used as the more favourable production route. The amount of lipase used was calculated assuming replacement after 1000 kg of biodiesel production.

With improvements in enzyme activity and enzyme production technology, lipase impacts and their contributions to the LCA scores may be reduced. The results showed that production of rape seed oil, steam and lipase were the major contributing factors throughout. The LCA categories investigated included abiotic depletion, global warming, ozone layer depletion, photochemical oxidation, acidification and eutrophication. The values shown in the figures represented contribution to the LCA impacts as a percentage of the total in each category. Only materials which led to a contribution greater than 3 % of the total LCA score have been included.

In the abiotic depletion category, rape seed oil made up 61.2 % of the total LCA score (Figure 6.11). This score, as in all the impact categories, was predominantly made up of the rape seed from farming, which in turn was made up of fertiliser and diesel. Steam (21 %), methanol (14.5 %) and lipase (2.0 %) made up the remaining dominant categories for abiotic depletion. Natural gas needed for steam and methanol production added a combined 30.7 % to the total score.

In the category for global warming, rape seed oil (85.9 %) and steam (9.5 %) were the only 2 impacts with a contribution of over 3 % to the total score. The global warming impacts of rape seed oil production may have been expected to have been negative owing to carbon dioxide uptake during biomass growth, as well as the credit given for the production of animal feed as a by-product during the agricultural process, but electricity, fertiliser and chemical use resulted in a net carbon dioxide release of 3.4 kg $CO_{2\ eq.}$ per kilogram of rapeseed oil produced. Where bio-based carbon is found in the final product this was deducted in the calculations. It is unlikely that the LCA impact would change if incineration were chosen, since comparable amounts of CO_2 would be released as a consequence.

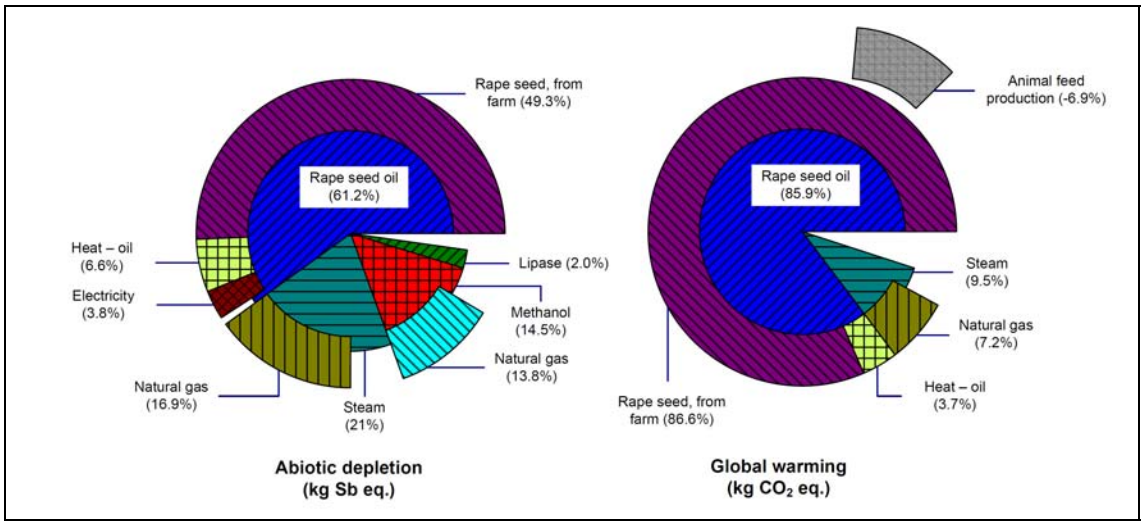
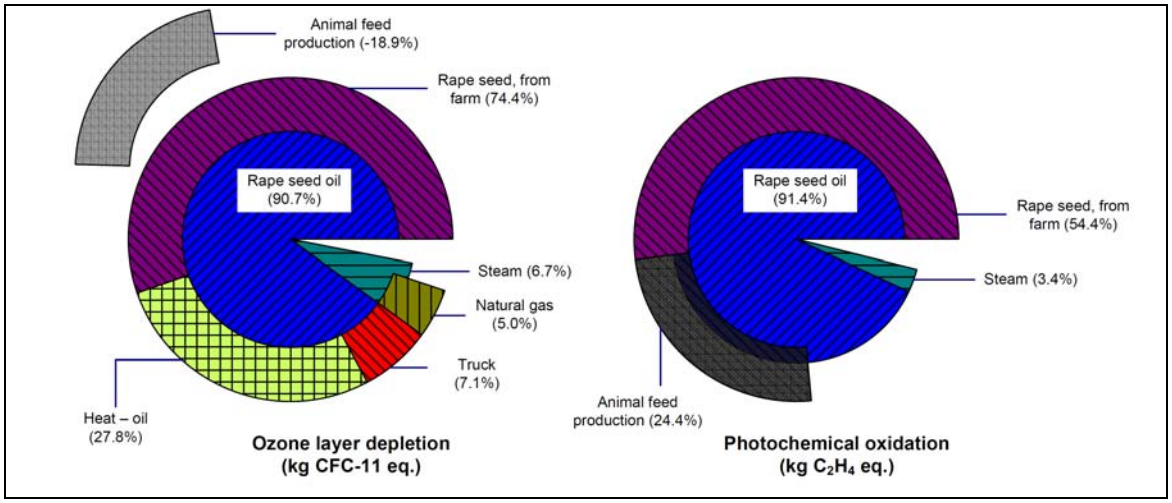


Figure 6.11: Life Cycle Assessment process contributions of biodiesel production (Case 2) using the CML baseline 2.03 methodology (Abiotic depletion and Global warming)

Rape seed oil and steam production were the two main contributors to LCA impacts in both ozone layer depletion and photochemical oxidation as shown in Figure 6.12. Rape seed oil formed more than 90 % of the impact in both. Impacts from rape seed farming, oil (for heat) and transport (truck), made up the seed oil impacts in ozone layer depletion, while rape seed farming and the co-production of animal feed appeared in the process contribution for photochemical oxidation. Emissions from the seed oil production process itself made up the remainder of the 91.4%. Steam formed 6.76 % and 3.4 % in ozone layer depletion and photochemical oxidation respectively. Natural gas, needed for steam raising, contributed 75 % of the steam scores.



*** Note: Ozone layer depletion is of reduced importance in current processes (see Appendix A.6)**

Figure 6.12: Life Cycle Assessment process contributions of biodiesel production (Case 2) using the CML baseline 2.03 methodology (Ozone layer depletion and Photochemical oxidation)

Rape seed oil, lipase and steam contributed 91.7 %, 3.3 % and 3.2 % respectively in the acidification category as shown in Figure 6.13. The rape seed oil contributed 97.3 % to the eutrophication score, a result of fertiliser used during the farming process for rape seed and the co-production of animal feed. Lipase production only formed 2.3 % of the total impact in eutrophication.

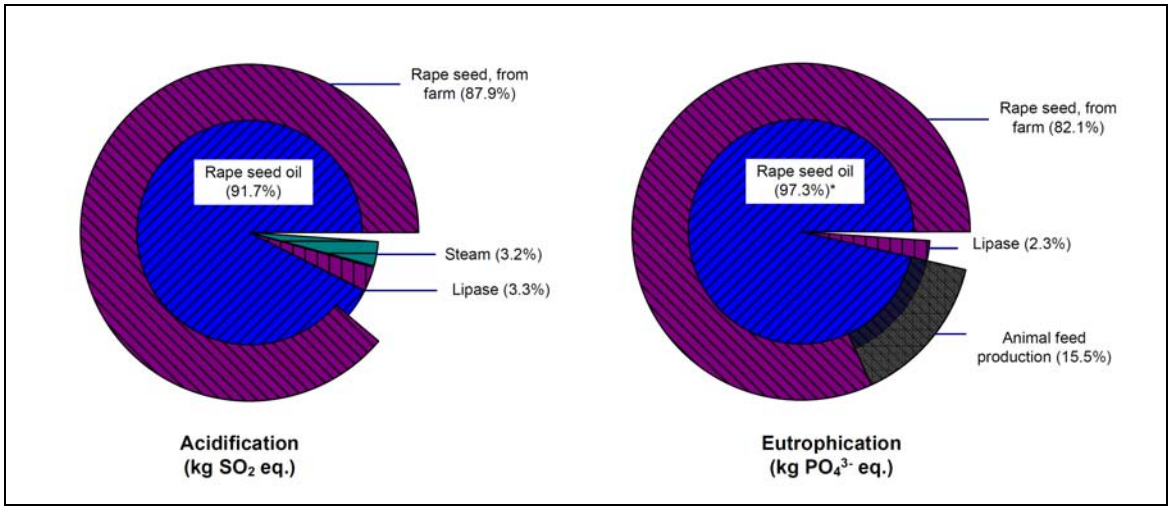


Figure 6.13: Life Cycle Assessment process contributions of biodiesel production (Case 2) using the CML baseline 2.03 methodology (Acidification and Eutrophication)

6.7. Conclusions

Biodiesel can be produced by the transesterification of oils using a chemical or biological catalyst. The environmental burdens of these production routes were investigated by a Life Cycle Assessment (LCA) methodology, where these impacts guide design considerations at an early stage of process development. In order to perform the LCA analyses, the material and energy inventories of each flowsheet design were required. These inventories were obtained by detailed Aspen Plus® (AspenTech 2008) designs. Since this data did not include the material and energy inventories for the lipase used as biological catalyst, the generic flowsheet model developed in Chapter 2 was used to generate this data. This full material and energy inventory then informed the LCA analysis for biodiesel production.

When the overall biodiesel production was studied, enzyme catalysed biodiesel production had environmental advantages owing to avoided use of chemical catalyst and neutralising acid. The lower pressures and temperatures obtained helped give more favourable LCA results across seven of the ten environmental impacts considered. The major contributing processes to LCA

scores were similar in all processes. Rapeseed oil (including diesel and fertiliser use), steam (natural gas) and methanol/ethanol were all major contributors to overall LCA scores.

Methanol production impacts were lower than the ethanol production impacts in all but two of the LCA categories. In the alkali catalysed process, lower alcohol recovery required greater flows to give similar product yield and purity. This increased the energy need, as well as pollutants, and caused a less favourable production route when analysed using life cycle assessment.

It was found that the LCA impact of lipase production was less than 4 % of the total biodiesel impact across all categories. Improvements to technology to allow for greater optimisation of lipase production would see further reduction in this contribution to total LCA scores.

As a result of recycle and distillation columns, the production of biodiesel was too complex to model using the generic flowsheet as given in Chapter 2. Therefore, Aspen Plus® was used. While Aspen Plus® gave the required material and energy data, the modelling effort was greater because of the additional engineering detail required and the complexity of the software package.

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CHAPTER 7: HEURISTICS

7.1. Introduction

The aim of this chapter was to use insight gained from the case studies presented to develop an understanding which will inform the overall approach to environmental burden minimisation in aerobic microbial bioprocesses. It was seen in these case studies that increased Life Cycle Assessment (LCA) scores were often a result of increased energy requirements caused by increased volumes. These volume increases resulted from poor downstream separation efficiencies, low biomass or product yields or a lack of downstream processing. Other factors influencing the LCA impacts were aeration and natural gas, steam and electricity usage as well as the raw materials used. A summary of the areas of high environmental sensitivity observed in these case studies is given in Table 7.1. Sensitivity to variation of these areas needs to be further understood.

In this chapter, generic areas of concern arising from the previous case studies are identified and tested more rigorously. This was done by two ways. Firstly, key variables were identified through a sensitivity analysis. Secondly, the impact of increasing production in the bioreactor was compared to optimising purification in downstream processing. The order of downstream processes was not manipulated in this investigation, although it is expected to impact the material and energy balance results.

Table 7.1: Summary of areas influencing environmental impact substantially, as identified in case studies

Case study	Hotspot identified
Penicillin V production	- Low energy efficiencies increased electrical requirements and overall LCA impacts; - Electricity and agricultural inputs are key items contribution to LCA impacts; and - Poor separation efficiencies increased operating volumes throughout, increasing electrical and steam requirements, which in turn increased LCA scores throughout.
Cellulase production	- Large water volumes associated with the low biomass concentrations of the submerged fermentation system increased the energy requirements and LCA scores compared to solid state cultivation; - Increased unit operation volumes owing to lack of downstream processing led to higher LCA scores.
Poly-β-hydroxyalkanoate production	- Aeration in the generic model was calculated differently to the literature model used, which led to different LCA scores, - Natural gas requirements in the spray drier of the generic flowsheet and literature models differed enough to affect LCA values
Lipase for biodiesel production	- Reduced reactor volumes resulting from increased biomass concentration reduced the LCA impacts.

7.2. Identifying key variables

7.2.1. Sensitivity analysis

In order to analyse the areas to which environmental burden is most sensitive, the effect of variation of each variable in the flowsheet needed to be quantified independently and systematically. From the material and energy balances obtained, LCA impacts could then be calculated and overall environmental effects determined.

The changes to variables in this section have been considered theoretically one at a time. It is recognised that in practice it may not be possible to modify one variable without modifying several others. Further, these changes may not be physically possible for the microorganism used, but will still give an indication of the possible outcomes should different organisms be used (or products formed) in similar scenarios.

7.2.2. LCA single score

When there are multiple processes to compare, a single score LCA result is useful as it will give a single definitive value over several categories. In this study, the EPS 2000 v2.02 single score method (Steen 1999a and 1999b) as presented in the SimaPro v7.1® software package (PRé Consultants B.V. 2008) was used. While the limitations of the single score method are well documented (ISO 14040: 2006, ISO14044: 2006), it provided a useful comparison for a large number of LCAs. An explanation of the single score method is described in Appendix A.

7.2.3. Summary of sensitivity results

The Penicillin V model validation provided data showing the greatest consistency with literature and was used as a representative case to study sensitivity to input variables. Using Scenario 1 of the generic model and dataset developed in Chapter 3, each input was varied by 10 and 25 % above and below the setpoint as a screening assessment in order to determine which variables should be investigated further. The resultant material and energy balance outputs, and the LCA results ensuing from these, were used to assess sensitivities (Appendix H).

The sensitivity is defined in Equation 7.1:

$$S = \frac{\Delta L}{\Delta V} \qquad \text{Equation 7.1}$$

Where:

S = Sensitivity

L = Percentage change in LCA single score [OR $(L_o - L_m)/L_o) \times 100$]

V = Percentage change in input variable [OR $(V_o - V_m)/V_o) \times 100$]

o = Original value
m = Modified value

The sensitivity results as defined above are given in Table 7.2. Low values represent LCA results which did not change when varying input variables. Sensitivities of one represented a change in the LCA value equal to the relative change in the input variable, while sensitivities greater than one represented a change in the LCA score greater than the relative change to the inputs.

Table 7.2: Sensitivity of single score LCA results for Penicillin V production (EPS 2000 v2.02)

No.^	Assumptions#	Default values	-25 %	-10 %	+10 %	+25 %
1	Cooling water temperature	0.013	0.0052	0	0	0.0052
2	Reactor temperature	0.15	0.35	0.24	0.17	0.13
3	Product to biomass ratio	**1.1**	**1.2**	**1.4**	0.35	0.19
4	Carbon 1 excess	0.003	0.0052	0.0129	0	0
5	Carbon 2 excess	0	0	0	0	0
6	Percentage Carbon 2 as total carbon	0.17	0.17	0.14	0.18	0.17
7	Nitrogen excess	0.010	0.010	0.013	0.013	0.005
8	Sulphur excess	-	-	-	-	-
9	Oxygen flowrate (vvm)	0.058	0.052	0.052	0.052	0.062
10	Compression pressure	0.041	0.021	0.039	0.026	0.021
11	Maintenance time	0.25	0.25	0.24	0.24	0.26
12	Final biomass concentration	0.97	0.49	0.44	0.04	0.18
13	Yield coefficient – $Y_{x/s}$	0.29	0.38	0.35	0.27	0.23
14	Yield coefficient – $Y_{p/s}$	0.28	0.22	0.18	0.04	0.02
15	Number of tanks	0	0	0	0	0
16	Agitation power per unit volume	0.18	0.18	0.18	0.18	0.18
17	Post microbial growth and product formation cooling	0.050	0.046	0.052	0.052	0.031
18	Solid fraction removed – Filtration	0	0	0	0	0
19	Liquid fraction retained – Filtration	**1.1**	**1.1**	0.91	0.73	0.29
20	Percentage additive – Sulphuric acid	0	0	0	0	0
21	Product fraction retained – Centrifugation 1	0.76	**1.6**	**1.1**	0.13	0.05
22	Waste fraction removed – Centrifugation 1	**7.7**	**1.3**	**1.3**	**25.1**	**10.0**
23	Energy per unit volume – Centrifugation 1	0	0	0	0	0
24	Percentage additive – Butyl acetate	0.034	0.031	0.026	0.039	0.036
25	Outlet temperature – Precipitation	0	0	0	0	0.0052
26	Power per unit volume – Precipitation	0	0	0	0	0
27	Percentage additive – Acetone	0.043	0.041	0.039	0.052	0.046

No.^	Assumptions#	Default values	-25 %	-10 %	+10 %	+25 %
28	Percentage additive – Sodium acetate	0.089	**1.1**	0.68	0.10	0.08
29	Conversion of limiting reagent	**1.2**	**1.4**	**1.1**	0.27	0.11
30	Solid fraction removed – Centrifugation 2	**1.5**	**1.36**	**1.12**	0.064	0.026
31	Liquid fraction removed – Centrifugation 2	0.19	0.21	0.18	0.026	0.010
32	Product fraction retained – Fluid bed drying	-	**1.36**	**1.12**	0.064	0.026
33	Liquid fraction removed – Fluid bed drying	0.026	0.031	0.026	0.026	0.010
34	Electricity required – Fluid bed drying	0	0	0	0	0
35	Percentage additive – Sodium hydroxide	0.007	0.010	0.013	0.013	0.005

^ Reference number for the variable changed
 # Values in bold indicate percentage changes in LCA values than the percentage changes to the variable modified

From the single score results, four areas were identified where controlled variation of the variables gave larger variations in the original LCA single scores (i.e. sensitivities greater than one). These included:

- Product to biomass ratio,
- Product fraction retained in downstream processing,
- Waste fraction removed in downstream processing, and
- Penicillin V formation: (a) Percentage additive (Sodium acetate), and (b) Percentage limiting reactant converted.

In the following sections, these areas were analysed further by extending the range through which the variables may change to determine trends in reactor volumes, electrical requirements, purities and LCA single scores. For completeness, final biomass concentration, oxygen flowrate (vvm), compression pressure and yield coefficients ($Y_{x/s}$ and $Y_{p/s}$) were also analysed as these were targeted from case studies (Table 7.1).

7.2.4. Product to biomass ratio and final biomass concentration

7.2.4.1. Product to biomass ratio

The product to biomass ratio describes the mass ratio between biomass growth and product formed. Lower ratios of product to biomass require more raw materials for biomass growth, increasing volumes and loads throughout the system, since biomass is a by-product of the product formed in the penicillin case. The product to biomass ratio was increased incrementally from zero, representing no product formation, to a minimal biomass formation representing biomass as a retained catalyst. Resultant reactor volumes, electrical requirements, recoveries, purities and single score LCA values for the production of the sodium salt of Penicillin V are shown in Table 7.3 and Figure 7.1.

Electrical requirements and reactor volumes tend to values of 47.0 MJ/kg penicillin and 2.72 m^3 respectively when increasing the product to biomass ratio. Similarly, the LCA single score tends to a constant value of 5.9 points, dominated by electricity production. At low product to biomass ratios, the electrical requirement, reactor volume and LCA single score increase sharply. This was a result of an overall increase in material and energy requirements to provide for increased biomass formation for equivalent product formation. Further, these materials led to a reduced product purity. Above a product to biomass ratio of 0.6, Penicillin V purity exceeded 99 %.

Table 7.3: Selected results when varying product to biomass ratio (all other inputs at original defined values) in the production of 1000 kg Penicillin V

Product to biomass ratio	0.1	0.5	1 (Default value)#	1.2 (Original value)^	2	5	10	100	1000
Reactor volume (m^3)	261	55.1	**28.1**	**23.6**	19.2	12.7	8.77	3.46	2.72
Electrical requirement (MJ/kg pen.)	1979	203	**95.5**	**79.1**	68.8	58.5	53.6	47.8	47.0
Purity (% kg pen.)	95.0	98.9	**99.4**	**99.5**	99.5	99.5	99.5	99.5	99.5
Single score LCA (points per kg)*	120	17.5	**9.2**	**7.8**	7.3	6.6	6.3	5.9	5.9

 # Default value: The value calculated in the generic flowsheet model if no input is defined
^ Original value: The original value used as the input in the generic flowsheet
*** EPS 2000 v2.02**

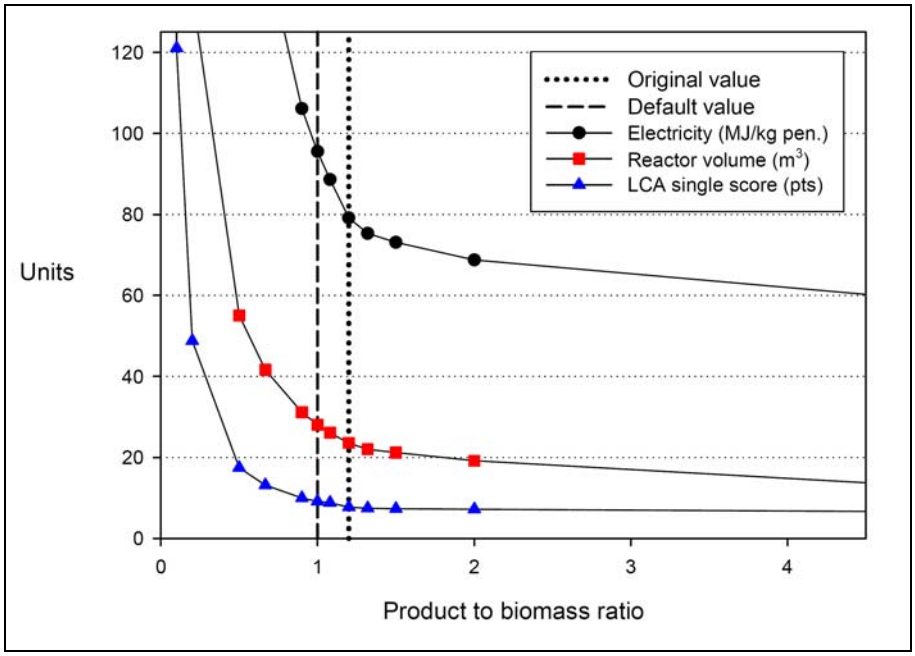

Figure 7.1: Electrical requirements, reactor volumes and LCA single scores for different product to biomass ratios (all other inputs at original defined values) in the production of 1000 kg Penicillin V

The original and default values for product to biomass ratios give LCA single scores within 16 % of each other. Over estimation of the original value showed less impact than underestimation. Doubling of the default value gives an LCA score 20 % lower, while halving it gives an LCA score 90 % higher. While increasing the product to biomass ratio benefits the process, values higher than 5 lead to diminishing returns, suggesting targeting of a product to biomass ratio of between 2 and 5 in this setup.

7.2.4.2. Final biomass concentration

Reducing the final biomass concentration in the range 5 to 45 g/l, with concomitant decrease in the product concentration owing to a constant ratio of product to biomass, sharply increased the reactor volume, electrical requirements and LCA single scores (Table 7.4). The increased volumes resulted in increased downstream processing inputs, which also resulted in increases in outputs. Unexpectedly, for Penicillin V production a biomass concentrations of 47 g/l represented a turning point for the electrical requirement and LCA single score with requirements increasing with increasing biomass concentration above 47 g/l as shown in Figure 7.2. At biomass concentrations higher than 10 g/l, the product purity was greater than 98 %. At biomass concentration lower than 10 g/l, the purity decreased sharply.

Table 7.4: Selected results when varying final biomass concentration (all other inputs at original defined values) in the production of 1000 kg Penicillin V

Final biomass concentration (g/l)	**5**	**10**	**16.7 (Default value)#**	**25**	**45 (Original value)^**	**65**	**75**
Reactor volume (m^3)	178	92.2	**56.9**	39.2	**23.3**	21.0	20.3
Electrical requirement (MJ/kg pen.)	377	213	**144.6**	110	**79.1**	82.2	84.5
Purity (% kg pen.)	96.4	98.1	**98.9**	99.2	**99.5**	99.6	99.6
Single score LCA (points per kg)*	29.5	17.5	**12.5**	10.0	**7.8**	8.5	8.9

 # Default value: The value calculated in the generic flowsheet model if no input is defined
^ Original value: The original value used as the input in the generic flowsheet
*** EPS 2000 v2.02**

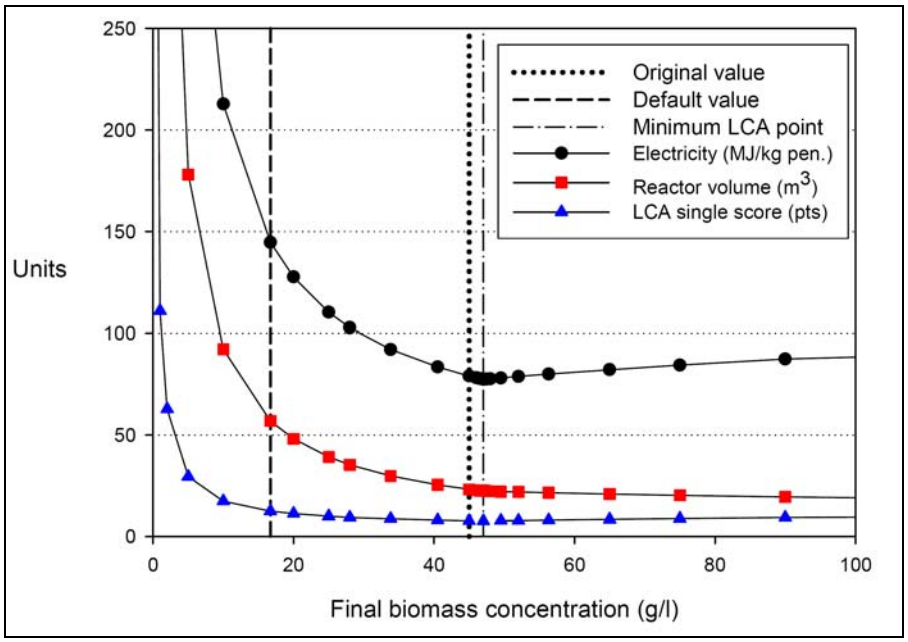

Figure 7.2: Electrical requirements, reactor volumes and LCA single scores for different final biomass concentrations (all other inputs at original defined values) in the production of 1000 kg Penicillin V

The turning point in the electrical energy requirement is analysed in Figure 7.3. While electrical contributions for agitation, aeration and sterilisation decreased with increasing biomass concentration, the electrical requirement for bioreactor cooling increased. The energy required to maintain the bioreactor temperature is directly related to the amount of biomass formed, as illustrated by the heat generation calculations shown in Section 2.3.11.

For the production of the sodium salt of Penicillin V, at concentrations above 47 g/l, the energy requirement for cooling becomes dominant. While the other electrical energy inputs show little dependence on biomass concentration at concentrations greater than 40 g/l, bioreactor cooling requirements continued to increase. This minimum electrical requirement at 47 g/l also results in a minimum LCA score.

In the model, the original value for final biomass concentration is near the minimum LCA value. However, the default value is set where the biomass concentration influences the bioreactor volume, electrical requirement and LCA single score considerably. This illustrates the importance of biomass concentration as a key variable in the design of an industrial bioprocess.

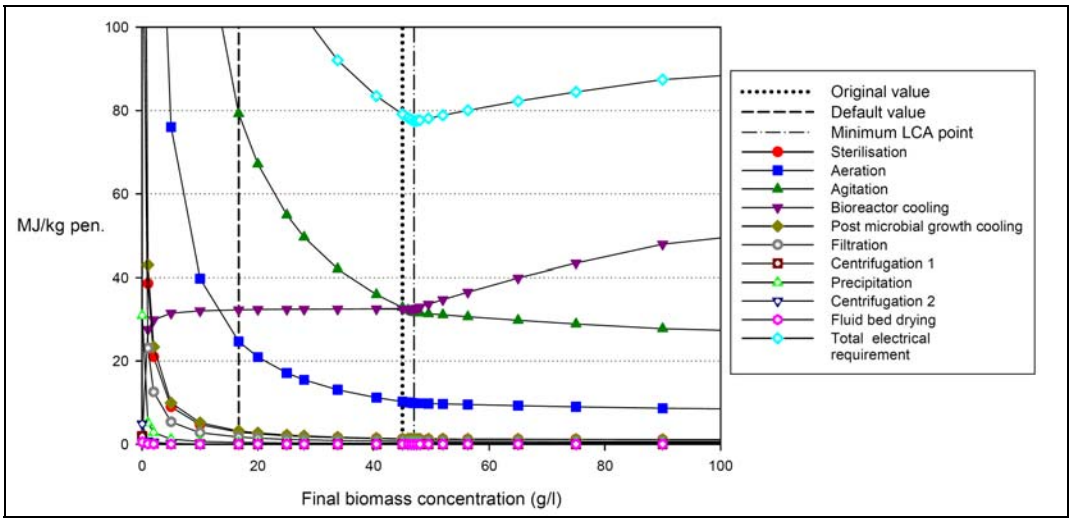

Figure 7.3: Electricity breakdown for different final biomass concentrations (all other inputs at original defined values) in the production of 1000 kg Penicillin V

The decrease in electricity, reactor volume and LCA values from a high initial value with increasing biomass concentration appeared to follow a similar trend to that with increased product to biomass ratios as shown before. However, the optimal biomass concentration was indicated by a minimum point whereas the optimum product to biomass ratio was as high as physically possible.

This trend resulted from an indirect relationship between the product to biomass ratios and biomass concentrations. Increasing the product to biomass ratio directly increased the product formed, but not the biomass concentration. Increases to the biomass concentration increased the biomass and, since the product to biomass ratio was constant, the product formed as well.

7.2.5. Oxygen flowrate (vvm) and compression pressure

7.2.5.1. Oxygen flowrate

In aerobic processes, the aeration rate is typically much higher than the stoichiometric requirement to overcome mass transfer limitations, ensure adequate oxygen provision to the micro-organisms and assist mixing. Typically air is supplied at a rate of 0.6 vvm, equating to approximately 0.12 vvm of pure oxygen. The original aeration rate of 0.021 vvm was defined for the stoichiometric oxygen requirement only for comparison to the original Biwer *et al*. (2005) and Heinzle *et al*. (2006) model.

Air itself has little impact in a Life Cycle Assessment study, but the electricity required for its compression accounts for some 10 to 15 % of the overall electricity requirements estimated, thereby contributing significantly to the LCA impacts. Selected results for varying the flowrate of oxygen (Variable 9) in the production of the sodium salt of Penicillin V are presented in Table 7.5 and discussed below.

Table 7.5: Selected results when varying oxygen flowrate (all other inputs at original defined values; compression pressure of 600 kPa) in the production of 1000 kg Penicillin V

Oxygen flowrate (vvm)	**0.021 (Original value)^**	**0.05**	**0.1**	**0.2**	**0.4**	**0.6**	**0.7**	**0.8**
Air equivalent	**0.1**	**0.25**	**0.5**	**1**	**2**	**3**	**3.5**	**4**
Reactor volume (m^3)	**23.3**	23.3	23.3	23.3	23.3	23.3	23.3	23.3
Electrical requirement (MJ/kg pen.)	**79.1**	92.7	117	164	259	355	402	450
Purity (% kg pen.)	**99.5**	99.5	99.5	99.5	99.5	99.5	99.5	99.5
Single score LCA (points per kg)*	**7.8**	8.3	9.3	11.3	15.3	19.2	21.2	23.3

^ Original value: The original value used as the input in the generic flowsheet
*** EPS 2000 v2.02**

Where the oxygen provision exceeds the stoichiometric limit, increasing the oxygen flowrate has no effect on the reactor liquid volume or product purity in the model developed. The modelling of gas holdup was not included in the model, but in reality this could play a part by increasing the volume of the reactor. Assuming a constant pressure differential on gas compression of 600 kPa, the electrical requirement was estimated to increase linearly with increasing oxygen flowrate as seen in Figure 7.4. This linear increase resulted in a linear increase in the LCA single score values.

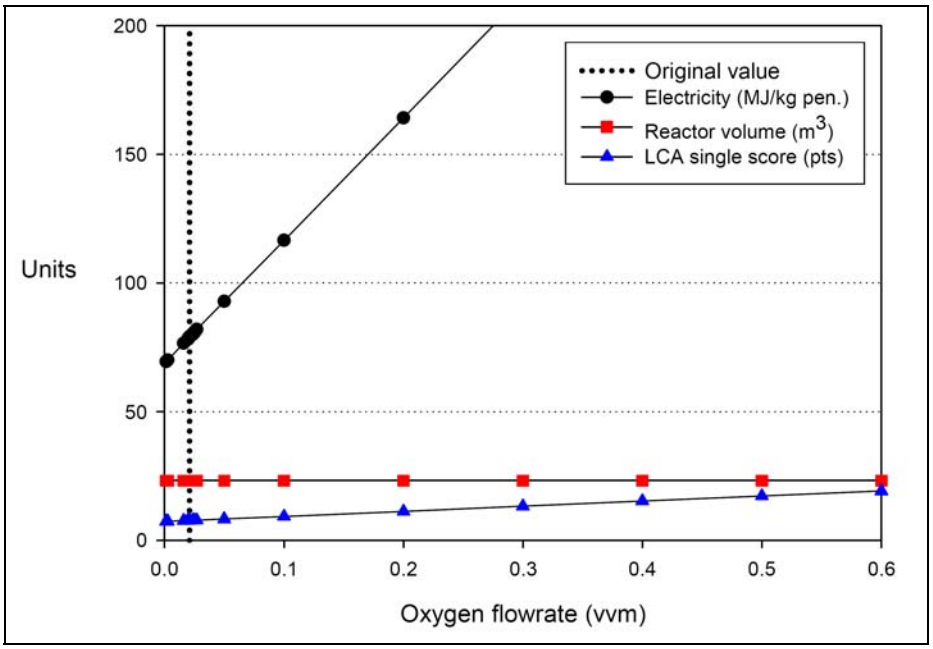

Figure 7.4: Electrical requirements, reactor volumes and LCA single scores for different oxygen flowrates (all other inputs at original defined values; compression pressure of 600 kPa) in the production of 1000 kg Penicillin V

The breakdown of electrical requirements as a function of oxygen flowrate is given in Figure 7.5. At oxygen rates less than 0.06 vvm (equivalent air rate of 0.3 vvm), electricity requirements for agitation and bioreactor cooling dominate the overall electrical requirements. Above 0.06 vvm, electrical requirements for compression became the main requirement. Electrical requirements for sterilisation, post fermentation cooling and all downstream processing form less than 2 % of the total electrical requirements. At a typical aeration of 0.6 vvm using air (oxygen rate of 0.12 vvm), 45 % of the total electrical requirement is from compression for aeration, while at approximately 1.8 vvm using air (oxygen rate of 0.36 vvm), 72 % of the electrical energy is from compression.

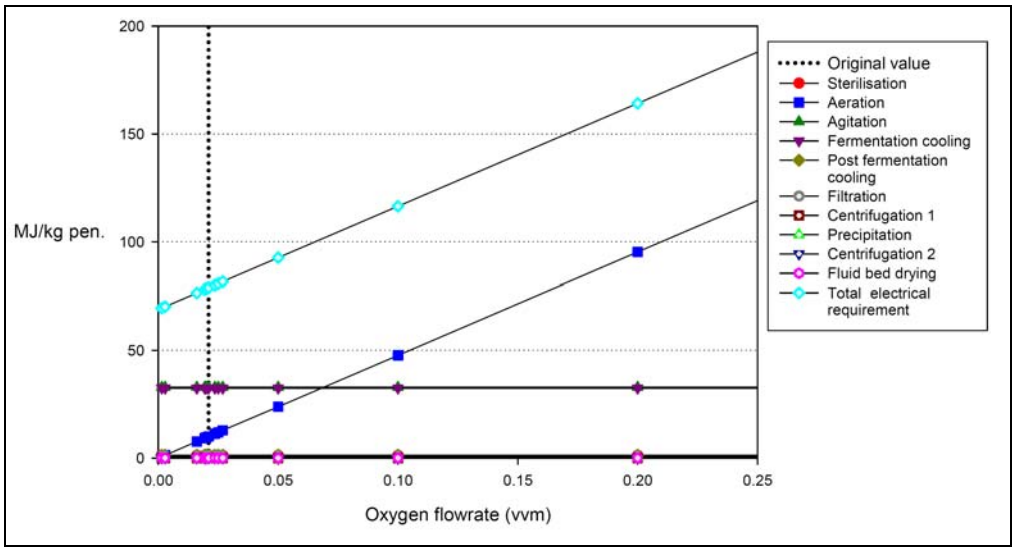

Figure 7.5: Breakdown of requirements for electricity for different oxygen flowrates (all other inputs at original defined values; compression pressure of 600 kPa) in the production of 1000 kg Penicillin V

7.2.5.2. Compression pressure

Increased aeration rate affects the compression pressure. Changes in compression affect the electrical energy requirements and cooling water needed. Increases in electrical requirements are based on the number and type of compressors (centrifugal, axial or reciprocating compressor), inlet and outlet pressures and the efficiency of compression. While the changes in cooling water requirements do not affect the LCA scores (water usage does not impact LCA), the electrical requirements to chill the water add to the overall electrical energy changes.

The compression pressure was varied from the minimum allowed pressure (atmospheric pressure) to 2000 kPa while keeping the original aeration rate of 0.021 vvm constant. Varying the compression pressure (Variable 10) does not affect reactor volume or purity obtained. Increases in the electrical requirements of compression and the Life Cycle Assessment scores with increasing compression pressure were shown in Table 7.6 and Figure 7.6. For reciprocating compressors used, the model uses three compression factors as described in Appendix B.4, depending on the compression pressure required. This led to the three distinct sections of the graph, separated by the inflection points.

Table 7.6: Selected results when varying compression pressure (all other inputs at original defined values; aeration rate of 0.021 vvm) in the production of 1000 kg Penicillin V

Compression pressure (kPa)	101.3	200	300 (Default value)#	400	600 (Original value)^	800
Reactor volume (m^3)	23.3	23.3	**23.3**	23.3	**23.3**	23.3
Electrical requirement (MJ/kg pen.)	68.9	73.6	**75.3**	77.2	**79.1**	80.5
Purity (% kg pen.)	99.5	99.5	**99.5**	99.5	**99.5**	99.5
Single score LCA (points per kg)*	7.3	7.5	**7.6**	7.7	**7.8**	7.8

 # Default value: The value calculated in the generic flowsheet model if no input is defined
^ Original value: The original value used as the input in the generic flowsheet
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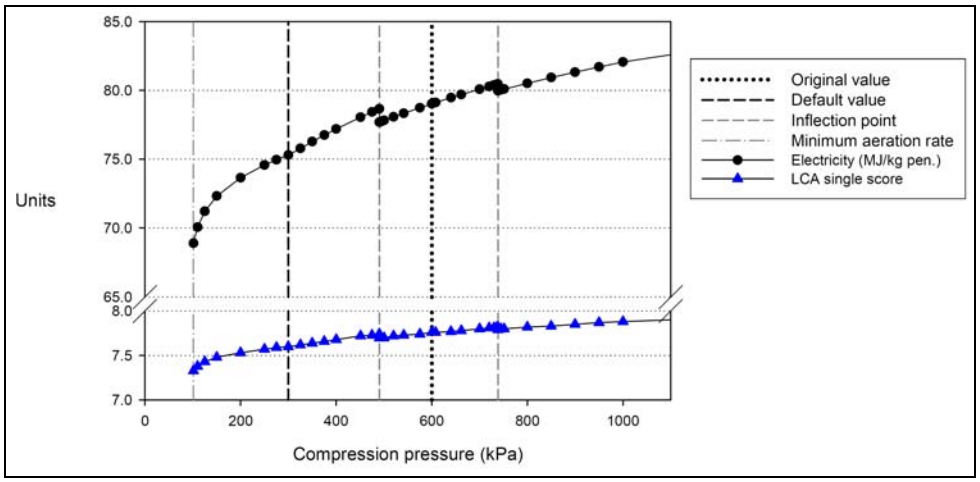

Figure 7.6: Total electrical requirements and LCA single scores for different compression pressures (all other inputs at original defined values; aeration rate of 0.021 vvm) in the production of 1000 kg Penicillin V (NOTE: Y-axis not zero)

Comparing the individual electrical energy contributions, for a pressure range of atmospheric to 2 000 kPa, the electrical energy for agitation and bioreactor cooling is always greatest as seen in Figure 7.7. As a percentage of total electrical energy needs, electricity for aeration ranges from 0 % (no compression) to approximately 16 % (at 2 000kPa).

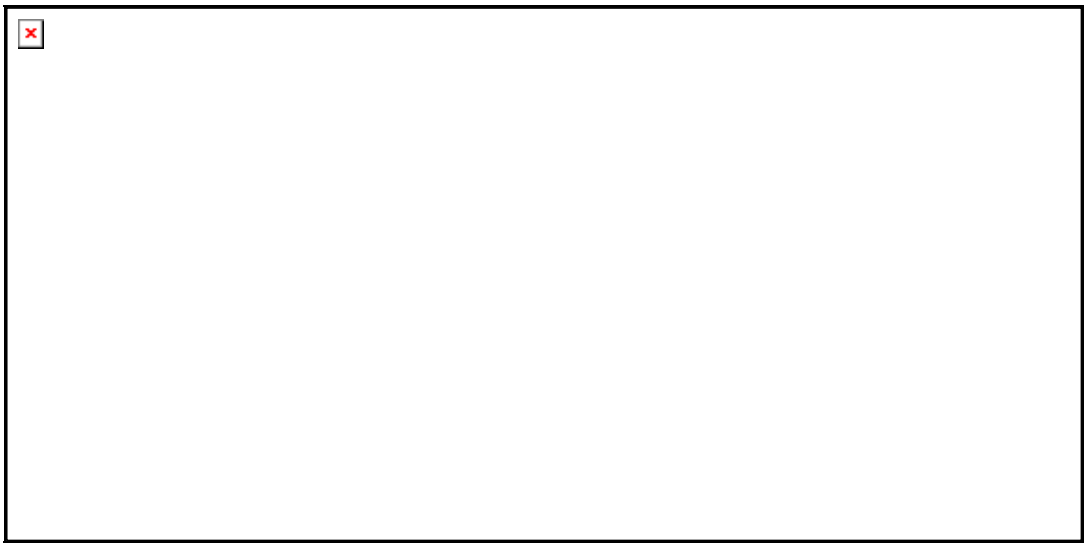

Figure 7.7: Breakdown of requirements for electricity for different compression pressures (all other inputs at original defined values; aeration rate of 0.021 vvm) in the production of Penicillin V

7.2.6. Yield coefficients

7.2.6.1. Biomass on substrate ($Y_{x/s}$)

Increases in the yield of biomass on substrate ($Y_{x/s}$), with a constant substrate feed, increased biomass production and, with a constant product to biomass ratio, resulted in more product formed. An increase in $Y_{x/s}$, with constant biomass formation, results in decreased raw material requirements to obtain the same amount of product. This can lead to a greater product purity and make for easier downstream processing separation. It should be emphasised that changing single variables may only be possible mathematically, as a way to model the effects of theoretical scenarios, but may not be possible in physical systems.

Decreasing the yield coefficient for biomass on substrate ($Y_{x/s}$) (Variable 13) results in an increasing electrical requirement, reactor volume and LCA single score. At higher $Y_{x/s}$ values, variation is less pronounced and gave minimums at a maximum yield coefficient of 0.9 g/g as shown in Figure 7.8 and presented numerically in Table 7.7. This is a maximum yield coefficient allowed by the model for Penicillin V production. Yield coefficients below 0.05 g/g loose meaning as very little biomass would be formed and the process would no longer make physical or economic sense.

Table 7.7: Selected results when varying the yield coefficient ($Y_{x/s}$) (all other inputs at original defined values) in the production of 1000 kg Penicillin V

Yield coefficient – $Y_{x/s}$ (g/g)	0.1	0.2	0.3	0.43 (Default value)#	0.45 (Original value)^	0.6	0.7
Reactor volume (m^3)	23.4	23.3	23.3	**23.3**	**23.3**	23.2	23.2
Electrical requirement (MJ/kg pen.)	163	109	91.0	**80.2**	**79.1**	73.2	70.7
Purity (% kg pen.)	99.5	99.5	99.5	**99.5**	**99.5**	99.5	99.5
Single score LCA (points per kg)*	15.8	10.6	8.9	**7.9**	**7.8**	7.2	7.0

 # Default value: The value calculated in the generic flowsheet model if no input is defined
^ Original value: The original value used as the input in the generic flowsheet
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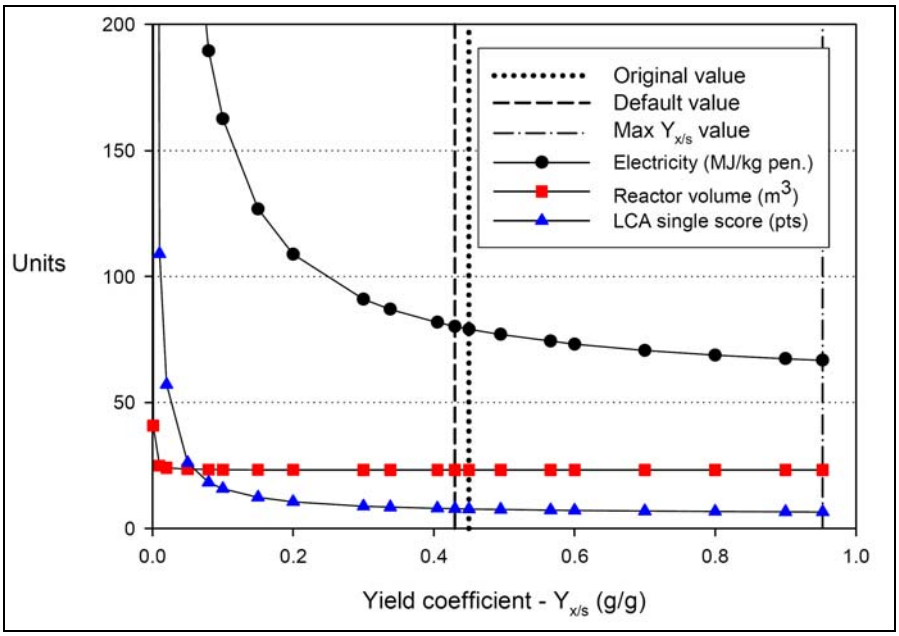

Figure 7.8: Electrical requirements, reactor volumes and LCA single scores for different yield coefficients ($Y_{x/s}$) (all other inputs at original defined values) in the production of 1000 kg Penicillin V

7.2.6.2. Product on substrate ($Y_{p/s}$)

Increases in the yield of product on substrate ($Y_{p/s}$), with a constant substrate feed, increased the amount and concentration of product formed. An increase in $Y_{p/s}$, with constant product formation, decreased raw material requirements per unit product. This led to an increased product concentration, easier downstream processing, requiring a smaller reactor volume and lower energy requirements per unit product.

Selected results when varying the yield of product on substrate ($Y_{p/s}$) (Variable 14) are presented in Table 7.8. At the highest allowed yield coefficient in the Penicillin V model (0.85 g/g), an electrical requirement, reactor volume and LCA single score of 78.9 MJ/kg, 23.2 m^3 and 7.7 Pts (per kg) were recorded respectively. These results are shown graphically in Figure 7.9. Yield coefficients below 0.05 g/g loose meaning as very little biomass would be formed and the process would no longer make physical or economic sense.

Table 7.8: Selected results when varying the yield coefficient ($Y_{p/s}$) (all other inputs at original defined values) in the production of 1000 kg Penicillin V

Yield coefficient – $Y_{p/s}$ (g/g)	0.1	0.2	0.3	0.4	0.49 (Default value)#	0.6	0.7	0.81 (Original value)^
Reactor volume (m^3)	23.4	23.3	23.3	23.3	**23.3**	23.3	23.3	**23.3**
Electrical requirement (MJ/kg pen.)	172	117	99.4	90.6	**85.9**	82.4	80.4	**79.2**
Purity (% kg pen.)	99.5	99.5	99.5	99.5	**99.5**	99.5	99.5	**99.5**
Single score LCA (points per kg)*	17.6	11.8	9.5	9.1	**8.6**	8.2	8.0	**7.8**

 # Default value: The value calculated in the generic flowsheet model if no input is defined
^ Original value: The original value used as the input in the generic flowsheet
*** EPS 2000 v2.02**

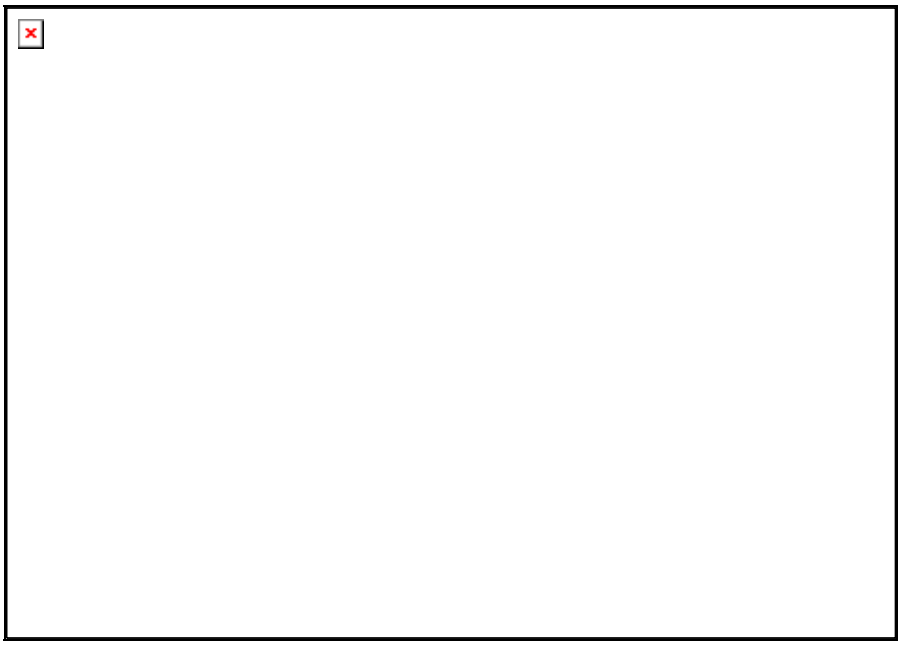

Figure 7.9: Electrical requirements, reactor volumes and LCA single scores for different yield coefficients ($Y_{p/s}$) (all other inputs at original defined values) in the production of 1000 kg Penicillin V

Over the range shown in Table 7.8, reactor volume was unchanged. The electrical requirements increased with a decreasing yield coefficient. This was a result of the stoichiometry of the calculations, predicting a greater amount of oxygen requirement, hence an increased aeration energy requirement and bioreactor cooling requirement.

The trends in reactor volume, electricity use and LCA scores seen with increased product yield coefficients ($Y_{p/s}$) were similar to those obtained for increased biomass yield coefficients ($Y_{x/s}$). This resulted from the interconnected nature of the variables through defining a fixed biomass to product ratio. Thus changing either yield coefficient affected the product and biomass values similarly.

7.2.7. Product fraction retained in downstream processing

In the downstream processing units (filtration, centrifugation and fluid bed drying), product may be lost in the waste streams. The model has been designed to give a fixed amount of final product. Losses in the downstream processing are compensated by increasing the amount of product formed through increasing biomass formation, raw material inputs, volume, electrical requirements and waste flows. For each downstream processing unit where product may be lost, the effect of varying the fraction of product retained on reactor volume, electrical requirement, purity and single score LCA value are shown in Figure 7.10. Increases in the product fractions retained for each of the filtration, centrifugation and fluid bed drying units, resulted in reduced reactor volume, decreased electricity and lower LCA scores.

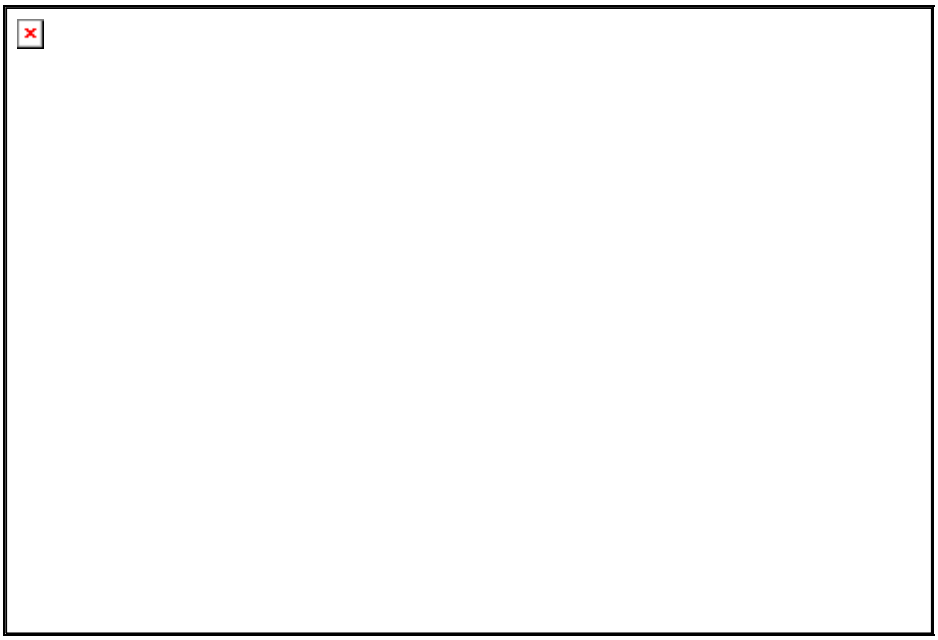

Figure 7.10: Electrical requirements, reactor volumes and LCA single scores for differing product retention in individual downstream process units (all other inputs at original defined values) in the production of 1000 kg Penicillin V

Total energy required with changing product retention was correlated to reactor volume. A decrease in product retention increased electrical requirements owing to larger reactor volumes, increasing sterilisation, aeration, agitation heat and cooling energy requirements.
A decrease in product fraction retained in any of the downstream processing units, resulted in higher LCA single score values as calculated using the EPS 2000 v2.02 method. The single score LCA values calculated from the material and energy balance data, were dominated by the patterns seen for reactor volume and electrical requirement in Figure 7.10.

7.2.8. Waste fraction removed in downstream processing

7.2.8.1. Effect of changing the waste fraction removed

Removing the waste from the product stream increases its purity and decreases the total volume entering the next downstream processing unit. This decrease in volume reduces downstream processing energy and chemical additions of subsequent operations.

However, the model is designed to give a final mass of crude product (waste plus product). An increase in the purity, owing to increased waste removal, while keeping the total mass constant,

requires more product to be formed. This increase in product requires an increase in biomass growth, which requires more raw materials, increased volumes and a higher energy requirement in the bioreactor.

While the LCA impacts of downstream processing decrease, the LCA contribution of the bioreactor increases. This trade-off of decreased downstream processing requirements may not counter the increased requirements of the bioreactor. Owing to the setup assumptions, care must be taken in interpretation of these sensitive data.

7.2.8.2. Filtration

Waste fraction removal from the filtration unit (Variable 19) was investigated and results given in Table 7.9. The filtration unit is a solid liquid separation unit with the only material removed being biomass waste (assumed the only solid at this point in the process).

Table 7.9: Selected results when varying waste fraction removed in filtration (all other inputs at original defined values) in the production of 1000 kg Penicillin V

Waste fraction removed (Filtration)	0.001	0.2	0.4	0.6	0.8	0.95 (Default value)#	1 (Original value)^
Reactor volume (m^3)	23.3	23.3	23.3	23.3	23.3	**23.3**	**23.3**
Electrical requirement (MJ/kg pen.)	79.1	79.1	79.1	79.1	79.1	**79.1**	**79.1**
Purity (% kg pen.)	99.5	99.5	99.5	99.5	99.5	**99.5**	**99.5**
Single score LCA (points per kg)*	7.8	7.8	7.8	7.8	7.8	**7.8**	**7.8**

 # Default value: The value calculated in the generic flowsheet model if no input is defined
^ Original value: The original value used as the input in the generic flowsheet
*** EPS 2000 v2.02**

There was no significant change to reactor volumes, electrical energy requirements or LCA single scores when changing the waste fraction removed in the filtration unit. This was also true for recovery and purity values which did not change with changing the waste fraction removed. As the amount of biomass in the process was less than 4 % of the total mass, variation in retention by the filtration unit did not influence overall results.

7.2.8.3. Centrifugation 1

Trends observed on varying the waste fraction removed in the first centrifugation unit (Variable 22) (removal of the aqueous phase after sulphuric acid addition) depended on the region investigated. For waste fraction removals below 0.93, little change was recorded in electrical

and volume requirements. Purity increased from 95 % to 99.5 %. For waste fraction removals above 0.93, a pronounced change resulted in the values investigated as seen in Figure 7.11. Results for reactor volume, electrical requirements, purity and LCA single scores are given in Table 7.10.

Table 7.10: Selected results when varying waste fraction removed in centrifugation 1 (all other inputs at original defined values) in the production of 1000 kg Penicillin V

Waste fraction removed (Centrifugation 1)	0	0.2	0.4	0.6	0.8	0.918 (Original value)^	0.95 (Default value)#	1
Reactor volume (m^3)	22.2	22.4	22.6	22.9	23.1	**23.3**	**30.9**	71.3
Electrical requirement (MJ/kg pen.)	77.8	78.0	78.3	78.6	78.9	**79.1**	**104**	241
Purity (% kg pen.)	95.0	95.9	96.9	97.9	98.9	**99.5**	**99.5**	99.4
Single score LCA (points per kg)*	17.5	15.4	13.3	11.2	9.0	**7.8**	**9.8**	27.2

 # Default value: The value calculated in the generic flowsheet model if no input is defined
^ Original value: The original value used as the input in the generic flowsheet
*** EPS 2000 v2.02**

For waste fraction removals below 0.93, in forming the sodium salt of Penicillin V, the limiting reagent is Penicillin V. On the improved waste removal with a fixed sodium acetate addition, sodium acetate becomes the limiting reagent. The unreacted Penicillin V in the system is not recovered. Should sodium acetate addition be increased proportionally with the change in waste fraction removal, such that Penicillin V remained the limiting reagent, trends would remain constant with those below 0.93. Hence the later are most relevant. The observation highlights the need to view each component of the sensitivity analysis holistically.

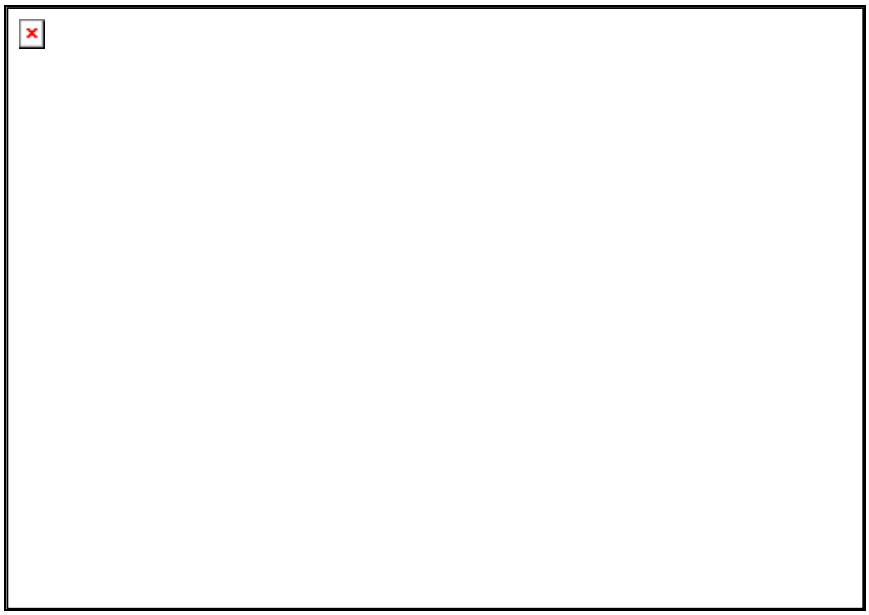

Figure 7.11: Electrical requirements, reactor volumes and LCA single scores for different waste fractions removed (all other inputs at original defined values) in centrifugation 1 in the production of 1000 kg Penicillin V

When changing the waste fraction removed in the first centrifuge, at fractions below 0.93, the reactor volume and electricity requirement decreased slightly with decreasing waste fractions removed. This was because the product purity decreased linearly and fewer raw materials were needed. However, the LCA single score increased (Figure 7.12) owing to greater downstream volumes with lower waste fraction removals. Hence, that more downstream additives were required. These additives, particularly acetone, sodium acetate and butyl acetate, influenced the total LCA score.

Waste fraction values above 0.93 can be considered irrelevant since the likelihood of the process being run with sodium acetate as the limiting reactant are low.

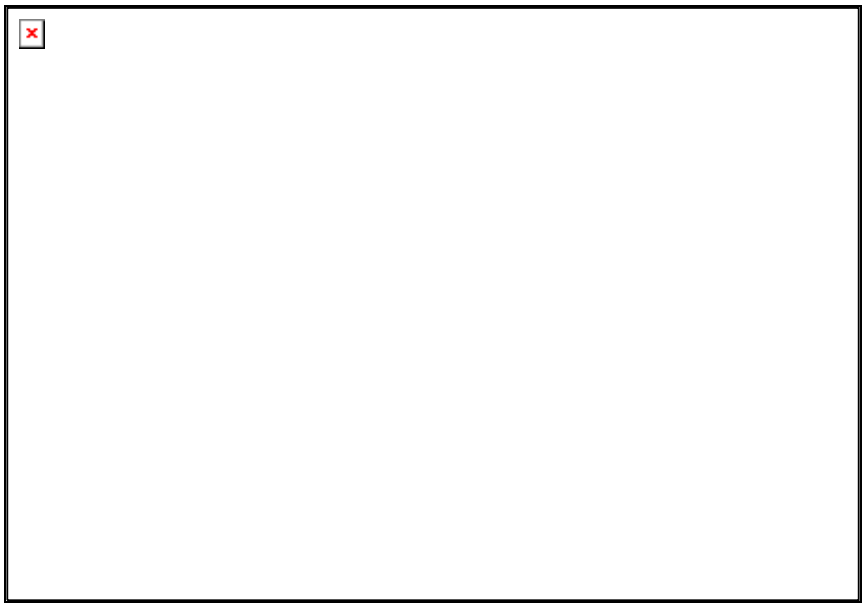

Figure 7.12: LCA single scores and purities for different waste fractions removed in centrifugation 1 (all other inputs at original defined values) in the production of 1000 kg Penicillin V

7.2.8.4. Centrifugation 2

Waste fraction removal in the second centrifugation unit used to recover the crystalline product (Variable 31) is analysed in Table 7.11. Reactor volume, electrical requirement and LCA score varied between 19.1 to 23.4 m^3, 64.9 and 79.5 MJ/kg penicillin product and 6.4 and 7.8 points respectively. The recovery remained constant at 98 % as product retained did not vary, while purity varied between 81.5 and 100 %.

Table 7.11: Selected results when varying waste fraction removed in centrifugation 2 (all other inputs at original defined values) in the production of 1000 kg Penicillin V

Waste fraction removed (Centrifugation 2)	0	0.2	0.4	0.6	0.8 (Default value)#	0.979 (Original value)^	1
Reactor volume (m^3)	19.1	19.8	20.6	21.4	**22.4**	**23.3**	23.4
Electrical requirement (MJ/kg pen.)	64.9	67.4	70.0	73.0	**76.1**	**79.1**	79.5
Purity (% kg pen.)	81.5	84.7	88.0	91.7	**95.7**	**99.5**	100
Single score LCA (points per kg)*	6.4	6.6	6.9	7.2	**7.5**	**7.8**	7.8

 # Default value: The value calculated in the generic flowsheet model if no input is defined
^ Original value: The original value used as the input in the generic flowsheet
*** EPS 2000 v2.02**

Increased waste fraction removal in centrifugation unit 2 increased electrical requirements, reactor volumes and single score LCA values as shown in Figure 7.13. While for centrifugation 1 removal of waste decreased volumes and consequently electrical requirements and LCA scores, increasing the waste fraction removal at a late stage in the downstream processing resulted in a greater increase in the product purity. In order to produce 1 000 kg of product at this increased purity, with all other variables held constant, more sodium salt of Penicillin V is required. This increased requirement for active product resulted in more raw materials required, a greater reactor volume and associated increases in energy considerations. The extra raw materials needed for this increased purity were greater than the reduction in volume achieved by a more efficient waste removal stage. Modifications to the model to give a fixed final mass of Penicillin V (*i.e.* a fixed mass of active product), show the same trends as described above.

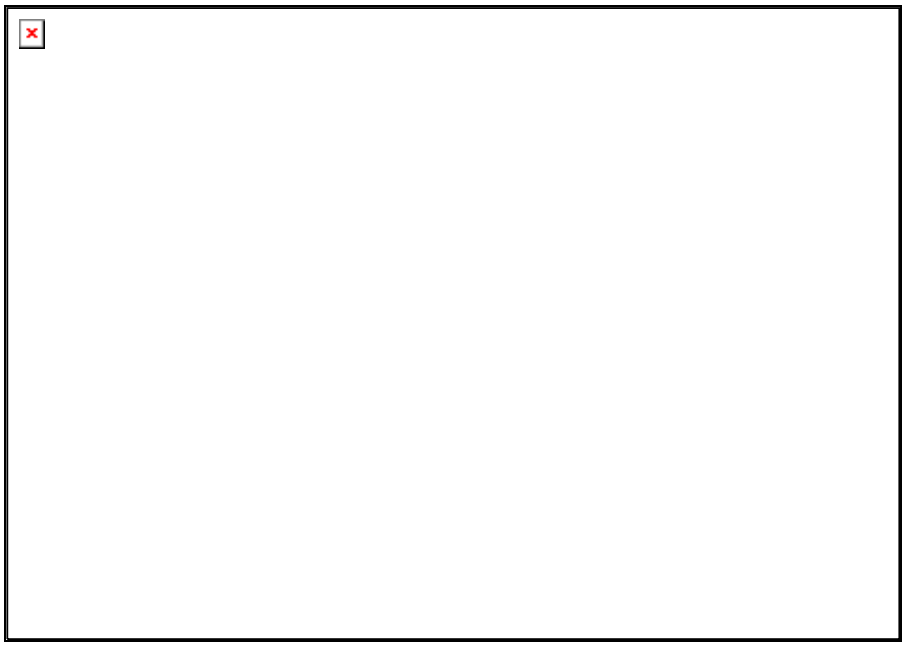

Figure 7.13: Electrical requirements, reactor volumes and LCA single scores for different waste fractions removed in centrifugation 2 (all other inputs at original defined values) in the production of 1000 kg Penicillin V

7.2.8.5. Fluid bed drying

Varying waste fraction removed from the fluid bed dryer (Variable 33) is analysed in Table 7.12. Limited impact was found. Reactor volume, electrical requirement, purity and LCA single score varied by 4.9, 4.7, 4.7 and 4% across the range of 0 to 1 respectively.

Table 7.12: Selected results when varying waste fraction removed in fluid bed drying (all other inputs at original defined values) in the production of 1000 kg Penicillin V

Waste fraction retained (Fluid bed drying)	0	0.2	0.4	0.6	0.8	0.9 (Original value)^	0.99 (Default value)#	1
Reactor volume (m^3)	22.3	22.5	22.7	22.9	23.2	**23.3**	**23.4**	23.4
Electrical requirement (MJ/kg pen.)	75.9	76.6	77.3	78.0	78.7	**79.1**	**79.4**	79.5
Purity (% kg pen.)	95.5	96.4	97.3	98.2	99.1	**99.5**	**100**	100
Single score LCA (points per kg)*	7.5	7.5	7.6	7.7	7.7	**7.8**	**7.8**	7.8

 # Default value: The value calculated in the generic flowsheet model if no input is defined
^ Original value: The original value used as the input in the generic flowsheet
*** EPS 2000 v2.02**

7.2.9. The formation of the sodium salt of Penicillin V

7.2.9.1. Percentage additive – Sodium acetate

Sodium acetate was added to react with Penicillin V to form the sodium salt of Penicillin V and acetic acid. Unreacted Penicillin V is not recovered as product.

The sodium acetate addition (Variable 28) was varied from 0.1 to 100 %v/v of stoichiometric requirement. As seen in Figure 7.14, a critical point was obtained when changing the sodium acetate addition. At values above 7.25 %v/v, electrical requirement, reactor volume and LCA single score remained constant. Below this value, these increased rapidly.

The critical point represents the change in limiting reagent in the reaction to form the sodium salt of Penicillin V from Penicillin V above 7.25 %v/v to sodium acetate below 7.25 %v/v . The latter leaves unreacted Penicillin V in the system which reports to waste. The slight increase in LCA scores at high sodium acetate additions results from the chemical itself affecting the overall LCA score.

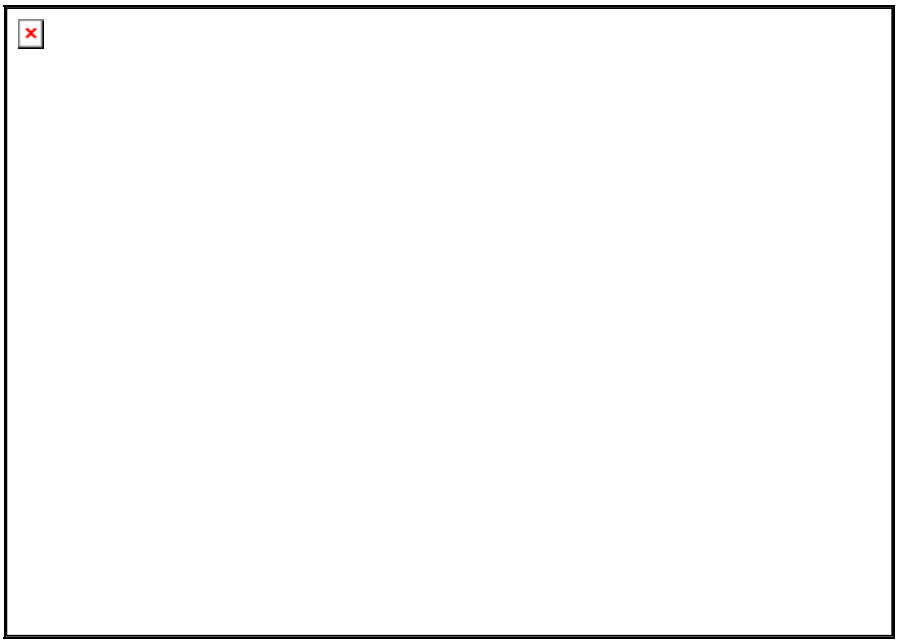

Figure 7.14: Electrical requirements, reactor volumes and LCA single scores for different percentages of sodium acetate added (all other inputs at original defined values) in the production of 1000 kg Penicillin V

7.2.9.2. Percentage of limiting reactant converted

The amount of limiting reactant converted in the reaction of sodium acetate and Penicillin V to form the sodium salt of Penicillin V and acetic acid determines the recovery of penicillin to final product. A low conversion rate results in a low product purity and higher waste flows. In turn this results in higher bioreactor volumes and raw material requirements to meet the desired product mass.

Selected results with varying the percentage limiting reactant converted are shown in Table 7.13. Halving the conversion rate resulted in a doubling of the reactor volume, electricity requirement and LCA single scores. At higher conversion rates (Figure 7.15), there are smaller changes in results, owing to smaller proportional changes.

Table 7.13: Selected results when varying conversions rates of limiting reactants (all other inputs at original defined values) in the production of 1000 kg Penicillin V

Conversion rate (%)	10	25	40	60	80 (Default value)#	97 (Original value)^	100
Reactor volume (m^3)	213	88.5	55.9	37.4	**28.2**	**23.3**	22.5
Electrical requirement (MJ/kg pen.)	725	301	190	127	**95.8**	**79.1**	76.8
Purity (% kg pen.)	94.0	97.6	98.6	99.1	**99.4**	**99.5**	99.6
Single score LCA (points per kg)*	71.3	29.6	18.7	12.5	**9.4**	**7.8**	7.6

 # Default value: The value calculated in the generic flowsheet model if no input is defined
^ Original value: The original value used as the input in the generic flowsheet
*** EPS 2000 v2.02**

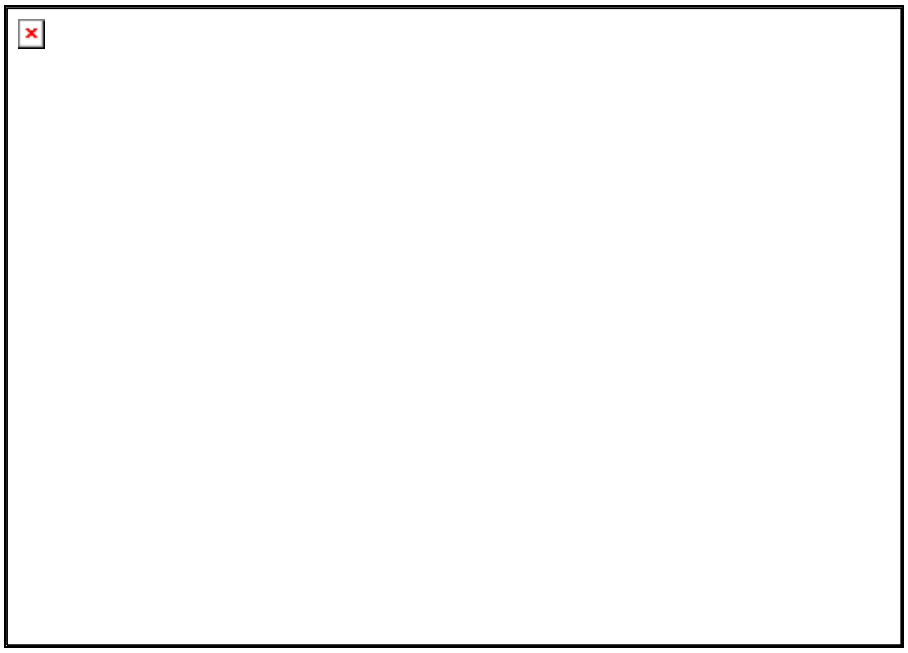

Figure 7.15: Electrical requirements, reactor volumes and LCA single scores for different conversions rates of limiting reactants (all other inputs at original defined values) in the production of 1000 kg Penicillin V

As with other scenarios where the electrical requirement, reactor volume and LCA single scores increase exponentially at lowered values, the purity also drops sharply when decreasing conversion rates. Below a conversion rate of 10 %, the purity falls below 95 % and LCA single score comparisons become less meaningful due to such large changes in concentration of Penicillin V.

7.2.10. Summary

Changing single variables in an industrial setup may have significant impacts on the performance of the process. In the example shown for the production of penicillin V, increases in the product to biomass ratio, yield coefficients, product fractions retained in downstream processing units and percentage limiting reagent converted resulted in decreasing electrical requirement, bioreactor volume and LCA single score. Increases in oxygen flowrate and compression pressure resulted in increasing electrical requirement, bioreactor volume and LCA single score values. Changes in the final biomass concentration, waste fraction removed in downstream processing units and percentage additive to a unit showed critical points for reasons such as a change in the limiting reactant. Variables showing critical points should be investigated more thoroughly to understand these critical points.

7.3. Increased production *vs.* optimised downstream processing

7.3.1. Introduction

The effect of increasing production, by increasing the final biomass concentration, increasing the product to biomass ratio or increasing yield coefficients ($Y_{p/s}$ and $Y_{x/s}$), resulted in a decreased single score LCA value. Similarly, a more effective product fraction recovery resulted in a decreased single score LCA value. To determine whether increasing the product production or optimising downstream processing has a greater effect on environmental burden, several theoretical scenarios for each of Penicillin V, cellulase and poly-β-hydroxyalkanoate production have been investigated. The reactor volume, electrical requirement, purity and single score LCA values are compared.

Four scenarios were investigated for each product. The first represents the original design. In two scenarios production was increased while keeping downstream processing unchanged. The final scenario considered perfect product retention in downstream processing while keeping production unchanged.

7.3.2. Penicillin V

7.3.2.1. Process descriptions

In the production of Penicillin V, four downstream processing units were included: filtration, centrifugation 1, centrifugation 2 and fluid bed drying. These retain 100, 98, 99 and 99 % of the product respectively (Table 7.14). To compare the original setup (Scenario C) to that with

optimised downstream processing (Scenario D), it was assumed that all units retained 100 % of the product.

Two scenarios of increased production (Scenarios B and A) assumed a theoretical increase in product to biomass ratios of 2 and 5 respectively, as well as increases in the yield coefficients and final biomass concentrations. Scenario A further assumed that no excess raw materials were fed to the bioreactor. An increase in production increased product reporting to downstream processing. It was ensured that penicillin remained the limiting reagent in the reaction to form the sodium salt of Penicillin V.

Table 7.14: Modified input values for the production of Penicillin V used in determining the impacts of improved production *vs.* optimised downstream processing

Assumptions*	Scenario A	Scenario B	Scenario C (Original scenario)	Scenario D	Units
Microbial growth conditions					
Product: Biomass ratio	5	2	1.2	1.2	-
Carbon 1 source (excess): Glucose	0	2	2	2	%
Carbon 2 source (excess): Phenoxyacetic acid	0	1.7	1.7	1.7	%
Nitrogen source (excess): Pharmamedia	0	15	15	15	%
Final biomass concentration	60	60	45	45	g/l
Yield coefficients: $Y_{x/s}$	0.60	0.50	0.45	0.45	g/g
$Y_{p/s}$	0.85#	0.85	0.81	0.81	g/g
Filtration					
Solid fraction removed	100	100	100	100	%
Liquid (product) fraction retained	91	91	91	100	%
Centrifugation 1					
Product fraction retained	98	98	98	100	%
Waste fraction removed	91.8	91.8	91.8	91.8	%
Centrifugation 2					
Solid (product) fraction retained	99	99	99	100	%
Liquid fraction removed	97.9	97.9	97.9	97.9	%
Fluid bed drying					
Product fraction retained	99	99	99	100	%
Liquid fraction removed	90	90	90	90	%

*** For the full set of original inputs see Table 3.2.**
 # Maximum allowed by the stoichiometry in the generic flowsheet model.

7.3.2.2. Material and energy balance results

Both increasing production and optimising the downstream processing resulted in decreased reactor volume and electrical energy requirement values to produce 1 000 kg of the sodium salt of Penicillin V as seen in Table 7.15. Scenario A showed the greatest improvements in reactor volume and electrical requirements. The increase in separation efficiency (Scenario D) resulted in higher electrical energy requirements and a higher reactor volume than Scenario B. The scenarios developed indicate the benefit of optimising improvement on the production side over the downstream processing side.

Table 7.15: Selected results for the five scenarios comparing increased production *vs.* optimised downstream processing in the production of 1000 kg Penicillin V

	Scenario A	Scenario B	Scenario C (Original scenario)	Scenario D	Units
Reactor volume	5.0	11.5	**23.3**	20.5	m^3
Electrical requirement	25.7	44.91	**79.1**	69.9	MJ/kg pen.
Purity	99.8	99.7	**99.5**	99.5	% kg pen.

The effect on energy when increasing production or optimising downstream processing is shown in Table 7.16. Scenario A and B resulted in decrease in both production and downstream processing energy to less than 35 % and 56 % respectively of that required in Scenario C. Improving the downstream processing efficiency (Scenario D) resulted in a 10 % decrease in production and downstream processing energy.

Table 7.16: Energy and glucose balances for the production and downstream processing of 1000 kg Penicillin V

	Scenario A	Scenario B	Scenario C (Original scenario)	Scenario D	Units
Energy					
Production	27.5	48.7	**86.5**	76.4	GJ
Downstream processing	0.5	0.8	**1.4**	1.3	GJ
Total	**28.0**	**49.5**	**87.9**	**77.7**	GJ
Glucose					
Into bioreactor	2.0	3.5	**5.2**	4.5	t
Out of bioreactor	0	0.04	**0.06**	0.05	t
Lost in wastewater	0	0.04	**0.06**	0.05	t
As impurities in product	0	0	**0**	0	t

In Table 7.16 the amount of glucose added into the bioreactor, unreacted glucose leaving the bioreactor, as well as the glucose lost in wastewater and carried through as impurities in the product is shown. This represented the raw material flow through the system and showed the relationship of unreacted raw materials in the system versus inputs. None of the scenarios have a significant amount of glucose in the product stream with all unreacted material leaving in the wastewater streams of the various purification stages of the Penicillin V production process.

For Scenario A, no excess for glucose, phenoxyacetic acid and Pharmamedia were assumed; hence no raw materials proceeded to downstream processing. In Scenario B, excess raw resulted in waste materials. This excess, and a lower yield coefficient ($Y_{x/s}$), increased raw materials required for the process. The amount of glucose required increased from 2.0 t to 3.5 t, while the amount of Pharmamedia increased from 0.6 t to 1.0 t for the production of 1 000 kg of Penicillin V. The raw materials leaving the bioreactor reported dominantly into the waste streams with less than 0.02 % of each of glucose, phenoxyacetic acid and Pharmamedia remaining in the product after formulation.

The original setup of Scenario C had the largest raw material requirements. There was a large increase in the phenoxyacetic acid required, from 0.2 t in Scenario B to 0.4 t in Scenario C. Glucose and Pharmamedia increased to 5.2 and 1.3 t respectively. Increased unreacted raw materials produced through the downstream units increased the energy requirements from 0.8 to 1.4 GJ. The energy required for the production also increased, from 48.7 to 86.5 GJ, owing to the larger volumes resulting from the greater raw material requirements.

Improved recovery in Scenario D decreased glucose, phenoxyacetic acid and Pharmamedia required compared with Scenario C. This remained more than both of Scenarios A and B which had increased production. Even with optimised downstream processing, the energy needed for product purification was higher than for Scenarios A and B. The smaller volumes that resulted from these increased production scenarios resulted in greater energy savings than the optimised downstream processing of Scenario D.

Improved product retention in downstream processing saves energy in the bioreactor since a smaller mass of raw materials was needed. This energy saving was not as large as the energy savings from the increased production scenarios.

7.3.2.3. Life Cycle Assessment (LCA) Results

The LCA results using the CML Baseline 2000 V2.03 method of assessment showed that, while similar, not all impact categories were reduced equally when improving production as seen in Figure 7.16. Scenario B impacts were approximately 40 % lower throughout, while Scenario A impacts were reduced by approximately 60 %.

When improving the downstream processing of penicillin production, all categories were reduced equally. However, these decreases of some 10 % in LCA scores were lower than the production scenarios (Table 7.17).

Table 7.17: LCIA of Penicillin V per kilogram of product for Scenarios A – D (CML Baseline 2000 V2.03)

Impact Category	Unit	Scenario A	Scenario B	Scenario C (Original scenario)	Scenario D
Abiotic Depletion	kg $Sb_{eq.}$	0.0837	0.138	0.227	0.203
Global Warming (GWP100)	kg $CO_{2\ eq.}$	8.76	15.4	25.5	22.7
Ozone Layer Depletion (ODP)	mg CFC-$11_{eq.}$	0.645	1.06	1.62	1.44
Human Toxicity	kg 1,4-$DB_{eq.}$	4.33	6.91	10.9	9.78
Fresh Water Aquatic Ecotoxicity	kg 1,4-$DB_{eq.}$	0.928	1.55	2.56	2.29
Marine Aquatic Ecotoxicity	kg 1,4-$DB_{eq.}$	5490	9450	16000	14300
Terrestrial Ecotoxicity	kg 1,4-$DB_{eq.}$	0.0228	0.0354	0.0533	0.0477
Photochemical Oxidation	kg C_2H_4	0.00328	0.00545	0.00891	0.00799
Acidification	kg $SO_{2\ eq.}$	0.0902	0.154	0.254	0.227
Eutrophication	kg $PO_4^{3-}{}_{eq.}$	0.00997	0.0162	0.0243	0.0217

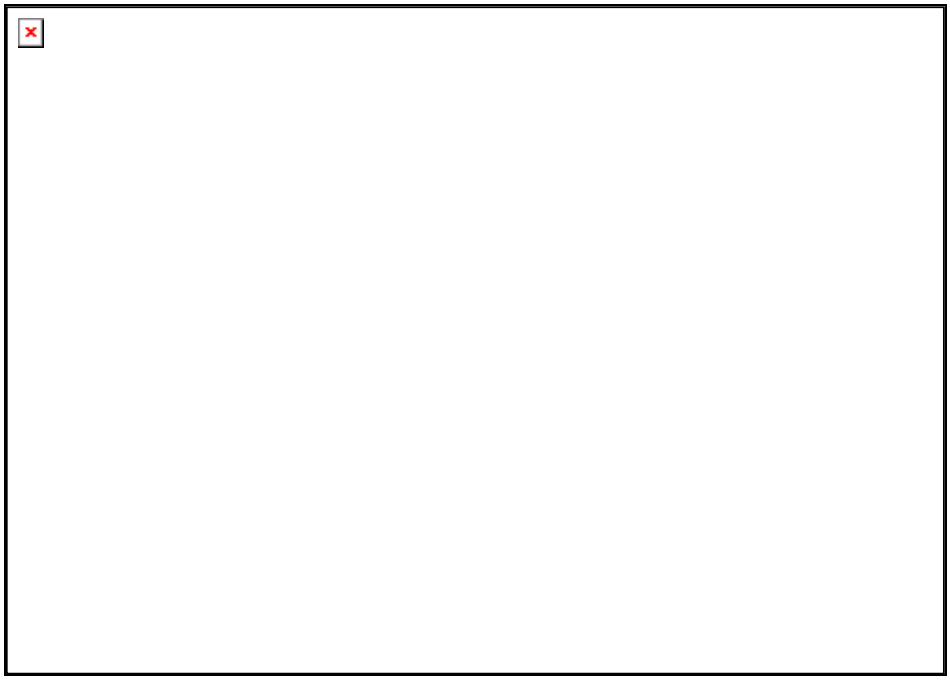

Figure 7.16: Comparison of LCA results for Penicillin V sodium salt production for Scenarios A, B and D relative to Scenario C

7.3.3. Cellulase

7.3.3.1. Process descriptions

The model for cellulase production uses a more simplified downstream process than for Penicillin V. A rotary vacuum filtration unit is used to remove biomass and an ultrafiltration unit is the only further purification stage. The original setup developed in Chapter 4 (Scenario G) was compared to one with perfect product retention (Scenario H) as shown in Table 7.18. This represents only marginally improvement from the 98 % product retention of the ultrafiltration of original scenario, with the rotary vacuum unit already having perfect product separation.

Scenarios E and F represent models with improved production setups. Scenario F assumes an increase of the product to biomass ratio to a value of 2 and a theoretical final biomass concentration of 18 g/l. Scenario E assumes an even greater increase in the product to biomass ratio to a value of 5, a theoretical final biomass concentration of 25g/l and no excess raw materials present in the bioreactor. All downstream processing for these scenarios was kept constant.

Table 7.18: Modified input values for the production of cellulase by SmF using *Trichoderma reesei* used in determining the impacts of improved production *vs.* optimised downstream processing

Assumptions[#]	Scenario E	Scenario F	Scenario G (Original scenario)	Scenario H	Units
Microbial growth conditions					
Product: Biomass ratio	5	2	0.89	0.89	kg/kg
Carbon 1 source (excess): Cellulose	0	10	10	10	%
Carbon 2 source (excess): Corn liquor	0	33.3	33.3	33.3	%
Nitrogren 2 source (excess): Nutrients	0	33.3	33.3	33.3	%
Final biomass concentration	25	18	15.12	15.12	g/l
Rotary vacuum filter					
Solids removed	100	100	100	100	%
Liquid (product) retained	100	100	100	100	%
Ultrafiltration					
Solids (product) retained	98	98	98	100	%
Liquid (waste) removed	81	81	81	81	%

*** For the full set of original inputs see Table 4.3.**
 # Since the cellulase purity is so low in the design, the model is set up (as in Chapter 4) to give a total crude product. In this example a total mass (product plus waste) of 1000 kg is assumed.

7.3.3.2. Material and energy balance results

The reactor volumes, electrical requirements and purities for the production of cellulase (Scenarios E to H), as calculated using the generic flowsheet, are shown in Table 7.19. For the production of 1000 kg cellulase (active ingredient), the optimisation of downstream processing lowered the reactor volume from 83.1 to 81.4 m^3 and the electrical requirement from 183.1 to 179.4 MJ/kg cellulase, representing a 2 % decrease.

Increasing the production of cellulase (Scenarios F and E) resulted in a much larger decrease in the reactor volume and electrical energy requirements. Improved product recovery in downstream processing presented little scope for improvement. The reactor volume and electrical requirement of Scenario E decreased to 11 % and 38 % of the original values respectively. Increasing the production also increased the purity of cellulase from 6.1 % to 38.3 %.

Table 7.19: Selected results for the five scenarios comparing increased production *vs.* optimised downstream processing in the production of product containing 1000 kg cellulase by SmF using *Trichoderma reesei*

	Scenario E	Scenario F	Scenario G (Original scenario)	Scenario H	Units
Reactor volume	9.4	31.7	**83.1**	81.4	m^3
Electrical requirement	33.8	74.6	**183.1**	179.4	MJ/kg cel.
Purity	38.34	14.75	**6.09**	6.21	% kg cel.

The main carbon entering the bioreactor for the production of cellulase was cellulose. Comparisons of cellulose required for each of Scenarios E – H is shown in Table 7.20. When no excess was added to the system and complete reaction occurred (Scenario E), no cellulose left the bioreactor. The addition of excess raw materials resulted in cellulose in the wastewater and as impurities in the product. Optimisation of the downstream processing, shown in Scenario H, resulted in the same amount of cellulose in the product as the original setup (Scenario G), but less cellulose in the wastewater stream. Although the same excess cellulose was added for Scenarios F, G and H, the improved production of Scenario F resulted in less cellulose in the product and wastewater streams.

Since the downstream processing of cellulase required less than 3 % of the total energy requirements, improvements to the downstream processing did not result in a significant energy saving in either production or downstream processing energy. However, improving the

production of cellulase reduced the volume of the bioreactor and the associated energy requirement with Scenarios F and G, showing a 60 and 80 % reduction in both the production and downstream processing energy.

Table 7.20: Energy and cellulose balances for the production and downstream processing of product containing 1000 kg cellulase by SmF using *Trichoderma reesei*

	Scenario E	Scenario F	Scenario G (Original scenario)	Scenario H	Units
Energy					
Production	34.1	75.4	**185.2**	181.6	GJ
Downstream processing	0.6	1.8	**4.9**	4.8	GJ
Total	**34.6**	**77.2**	**190.2**	**186.4**	GJ
Cellulose					
Into bioreactor	1.9	2.6	**3.8**	3.7	t
Out of bioreactor	0	0.24	**0.34**	0.33	t
Lost in wastewater	0	0.19	**0.28**	0.27	t
As impurities in product	0	0.05	**0.06**	0.06	t

Besides cellulose, corn steep liquor and ammonia were added to the bioreactor. Where no excess was added and complete reaction occurred (Scenario E), no unreacted raw materials left the bioreactor. Therefore no raw materials were present in the wastewater or as impurities in the product stream.

In Scenario F, G and H, excess raw materials were added and unreacted raw materials were present in the downstream processing. Of the 0.24 t of cellulose entering the downstream process of Scenario F, 0.19 t (80 %) was removed in the wastewater stream and 0.05 t was left as impurities in the final product. Similarly, 0.11 of the 0.13 tonnes of corn steep liquor (80 %) and 0.002 of the 0.003 t of ammonia (80 %) entering downstream processing were removed in the waste stream. The original setup (Scenario G), assumed the same excess entered the bioreactor, but owing to lower production, more cellulose, corn steep liquor and ammonia were required. With more raw materials required, a greater mass of raw materials left the bioreactor. Masses of 0.34, 0.19 and 0.005 t of cellulose, corn steep liquor and ammonia respectively entered downstream processing.

Scenario H, for optimised downstream processing, assumed a perfect product retention in the process. Since no product was lost, the mass of raw materials required was lower than in

Scenario G. However, since the original assumptions had near perfect product retention, reductions in energy usage and raw material requirements in Scenario H were small. Cellulose, corn steep liquor and ammonia input requirements of 3.7, 0.75 and 0.10 t respectively were required. This was less than 5 % lower than the original Scenario G.

7.3.3.3. Life Cycle Assessment (LCA) results

Using the CML Baseline 2000 impact assessment method, the Life Cycle scores for Scenario H are reduced by only 2 % for all categories as seen in Figure 7.17. Improving the cellulase production decreased all categories. The impacts of Scenario F were at least 40 % lower in all categories except global warming. In the theoretical Scenario E, LCA impacts are reduced to between 20 to 50 % of those in Scenario G. This was owing to the reduction in electrical energy requirements due to volume reductions as well as reduced raw material requirements due to enhanced yields. As can be seen in Table 7.21, impacts for the categories abiotic depletion, photochemical oxidation, acidification and eutrophication were reduced to values as low as 0.12 kg $Sb_{eq.}$, 0.0050 kg C_2H_4, 0.041 kg $SO_{2\ eq.}$ and 0.0098 kg $PO_4^{3-}{}_{eq.}$ respectively.

Table 7.21: LCIA of product containing 1 kg of cellulase produced by SmF using *Trichoderma reesei* for Scenarios E – F (CML Baseline 2000 V2.03)

Impact Category	Unit	Scenario E	Scenario F	Scenario G (Original scenario)	Scenario H
Abiotic Depletion	kg $Sb_{eq.}$	0.12	0.23	0.50	0.49
Global Warming (GWP100)	kg $CO_{2\ eq.}$	-632	-875	-1240	-1210
Ozone Layer Depletion (ODP)	mg CFC-$11_{eq.}$	0.695	1.23	2.28	2.24
Human Toxicity	kg 1,4-$DB_{eq.}$	5.72	11.11	23.89	23.40
Fresh Water Aquatic Ecotoxicity	kg 1,4-$DB_{eq.}$	1.50	2.90	6.26	6.13
Marine Aquatic Ecotoxicity	kg 1,4-$DB_{eq.}$	9120	17600	38300	37600
Terrestrial Ecotoxicity	kg 1,4-$DB_{eq.}$	0.041	0.068	0.11	0.11
Photochemical Oxidation	kg C_2H_4	0.0050	0.0093	0.020	0.019
Acidification	kg $SO_{2\ eq.}$	0.12	0.23	0.51	0.50
Eutrophication	kg $PO_4^{3-}{}_{eq.}$	0.0098	0.018	0.034	0.033

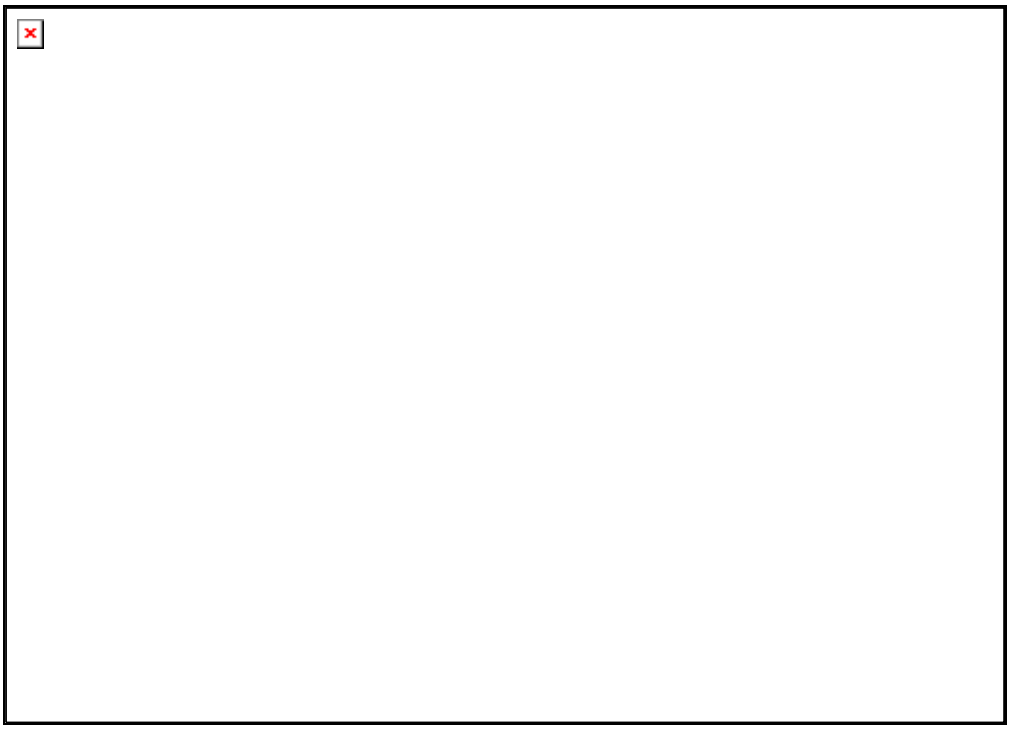

Figure 7.17: Comparison of LCA results for cellulase production by SmF using *Trichoderma reesei* for Scenarios E, F and H relative to Scenario G

7.3.4. Poly-β-hydroxybutyric acid

7.3.4.1. Process descriptions

The production of the biopolymer, poly-β-hydroxybutyric acid (PHB) as modelled in Chapter 5, assumed perfect product retention throughout. This meant that no improvement could be made to the setup. The original scenario (Scenario L) was modified to allow for product losses (Scenario K). It was assumed that only 95 % of the polymer product was retained at each downstream processing step as shown in Table 7.22.

The two scenarios for improved production (Scenarios J and I) assumed a theoretical increase in the product to biomass ratio of 4 and 6 respectively. The final biomass concentration was assumed to increase to 50 and 60 g/l respectively, while the yield coefficient of product on substrate increased to 0.50 and 0.60 g/g respectively. These are optimistic assumptions and may only be attainable with different strains of microorganism.

Table 7.22: Modified input values for the production of poly-β-hydroxybutyric acid used in determining the impacts of improved production *vs.* optimised downstream processing

Assumptions	Scenario I	Scenario J	Scenario K	Scenario L (Original scenario)	Units
Microbial growth conditions					
Product: Biomass ratio	6	4	2.4	2.4	-
Final biomass concentration	60	50	44	44	g/l
Yield coefficients: $Y_{p/s}$	0.60	0.50	0.44	0.44	g/g
Centrifugation					
Solid fraction (product) retained	100	100	95	100	%
Centrifugation					
Solid fraction (product) retained	100	100	95	100	%
Centrifugation					
Solid fraction (product) retained	100	100	95	100	%
Spray drying					
Solid fraction (product) retained	100	100	95	100	%
Liquid fraction removed	100	100	100	100	%

*** For the full set of original inputs see Table 5.4**

7.3.4.2. Material and energy balance results

As expected, an inferior downstream processing system resulted in an increase in reactor volume and electrical energy requirements as shown in Table 7.23. The loss of the product required a greater amount to be formed in the bioreactor to achieve the same mass of final polymer. The improvements of Scenario J reduced the reactor volume to 6.07 m^3 for 1000 kg of product and reduced the associated electrical requirements to less than half.

Table 7.23: Selected results for the five scenarios comparing increased production *vs.* optimised downstream processing in the production of 1000 kg poly-β-hydroxybutyric acid

	Scenario I	Scenario J	Scenario K	Scenario L (Original scenario)	Units
Reactor volume	3.77	6.07	13.12	**10.69**	m^3
Electrical requirement	5.07	9.91	24.6	**20.31**	MJ/kg PHB
Purity	100	100	100	**100**	% kg PHB

All four scenarios developed assumed that there was no excess glucose or ammonium sulphate entering the bioreactor according to the original model developed in Chapter 5. Hence, no glucose or ammonium sulphate entered downstream processing as shown in Table 7.24.

The energy required in downstream processing during production of poly-β-hydroxybutyric acid was proportionally higher than for both Penicillin V and cellulase as a result of the homogenisation and centrifugation units. In Scenarios I, J, K and L, of the total energy, 28, 24, 19 and 19 % was required for downstream processing respectively. As with other examples, both increasing production and optimising downstream production led to a reduction in both production and downstream processing energy requirements.

Table 7.24: Energy and glucose balances for the production and downstream processing of 1000 kg poly-β-hydroxybutyric acid

	Scenario I	Scenario J	Scenario K	Scenario L (Original scenario)	Units
Energy					
Production	15.1	20.4	36.2	**31.6**	GJ
Downstream processing	5.9	6.4	8.4	**7.4**	GJ
Total	**21.0**	**26.8**	**44.6**	**39.1**	GJ
Glucose					
Into bioreactor	2.0	2.6	3.9	**3.2**	t
Out of bioreactor	0	0	0	**0**	t
Lost in wastewater	0	0	0	**0**	t
As impurities in product	0	0	0	**0**	t

Scenario I, where the greatest theoretical production was modelled, gave an energy requirement for production of 15.1 GJ per 1000 kg of polymer product, 48 % of the original Scenario L. This was the lowest of all four scenarios. A production slightly lower than Scenario I, as modelled in Scenario J, resulted in a higher glucose, but similar ammonium sulphate, requirement. The production and downstream processing energy increased to 20.4 and 6.4 GJ per 1000 kg of polymer respectively. This was a 35 and 8 % increase in production energy and downstream processing energy respectively compared to Scenario I.

The original scenario (Scenario L) assumed perfect product retention. Scenario K was developed with a less than perfect efficiency but an identical production. This resulted in a material balance requiring more glucose and ammonium sulphate than any other scenario and an

energy requirement of 36.2 and 8.4 GJ per 1000 kg polymer for production and downstream processing respectively. Assuming perfect product retention as in Scenario L the production and downstream processing energy required was still higher than the increased production scenarios developed in Scenarios I and J.

7.3.4.3. Life Cycle Assessment (LCA) results

Comparing the LCA results using the CML Baseline method, Scenario K was at least 20 % higher in all categories as a result of increased raw materials requirements (Figure 7.18). Improvements to the production reduced the impacts of Scenarios J and I to an average of 48 and 65 % of the original Scenario L values respectively. The best case scenario (Scenario I) gave impacts of 0.034 kg $Sb_{eq.}$, 4.01 kg $CO_{2\ eq.}$, 0.031 kg $SO_{2\ eq.}$ and 0.014 kg $PO_4^{3-}{}_{eq.}$ in the categories abiotic depletion, global warming, acidification and eutrophication respectively (Table 7.25).

Table 7.25: LCIA of poly-β-hydroxybutyric acid per kilogram of product for Scenarios I – L (CML Baseline 2000 V2.03)

Impact Category	Unit	Scenario I	Scenario J	Scenario K	Scenario L (Original scenario)
Abiotic Depletion	kg $Sb_{eq.}$	0.034	0.047	0.088	0.072
Global Warming (GWP100)	kg $CO_{2\ eq.}$	4.01	5.74	10.7	8.46
Ozone Layer Depletion (ODP)	mg CFC-11$_{eq.}$	0.31	0.38	0.60	0.50
Human Toxicity	kg 1,4-$DB_{eq.}$	1.69	2.36	4.55	3.64
Fresh Water Aquatic Ecotoxicity	kg 1,4-$DB_{eq.}$	0.29	0.44	0.93	0.75
Marine Aquatic Ecotoxicity	kg 1,4-$DB_{eq.}$	1450	2360	5180	4224
Terrestrial Ecotoxicity	kg 1,4-$DB_{eq.}$	0.014	0.017	0.027	0.019
Photochemical Oxidation	kg C_2H_4	0.0011	0.0016	0.0032	0.0025
Acidification	kg $SO_{2\ eq.}$	0.031	0.046	0.090	0.071
Eutrophication	kg $PO_4^{3-}{}_{eq.}$	0.014	0.020	0.049	0.027

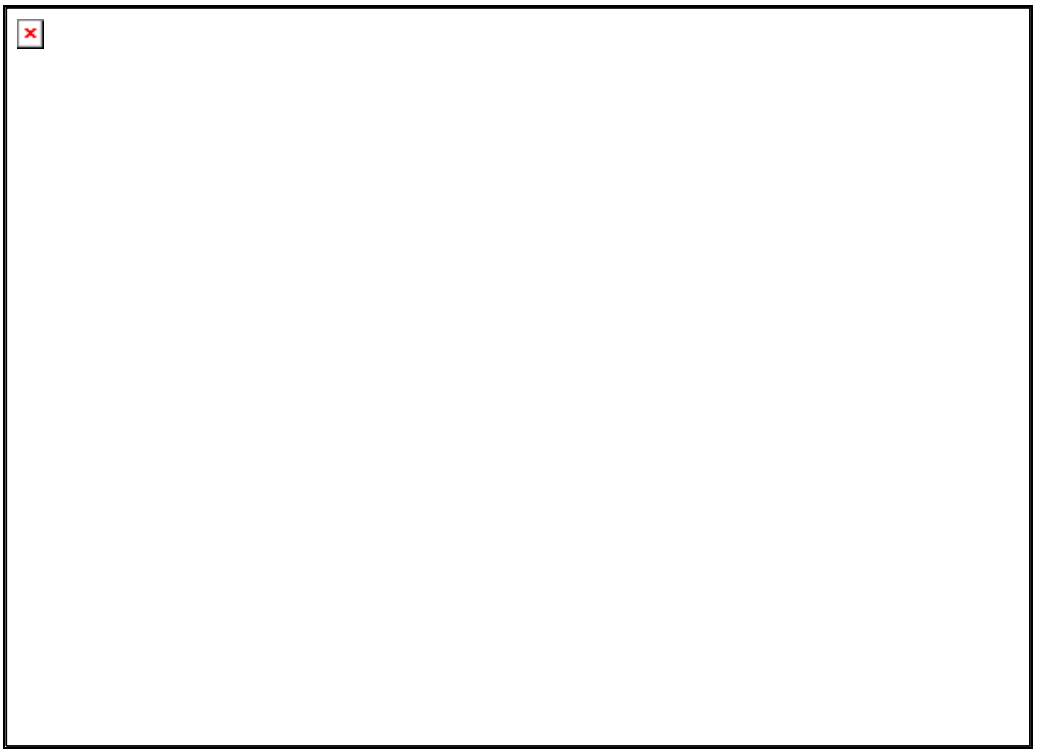

Figure 7.18: Comparison of LCA results for poly-β-hydroxybutyric acid production for Scenarios I, J and K relative to Scenario L

7.3.5. Summary

Improved production and downstream processing were analysed in the penicillin, cellulase and poly-β-hydroxybutyrate models, to determine changes to energy and raw material requirements and life cycle assessment scores. It was shown that improvements to either production or downstream processing decreased the LCA impacts, but that these were more pronounced for an improved production setup.

7.4. Conclusions

Certain variables were identified as key variables in the generic flowsheet model developed on Chapter 2. These included the product to biomass ratio, final biomass concentration, oxygen flowrate and compression pressure, yield coefficients and downstream processing separation efficiencies. Small changes to these variables may escalate the material and energy requirements of the process. These escalations, particularly in energy requirements, ultimately affect the Life Cycle Assessment (LCA) scores for the production of the product. It was seen that some variables were interconnected to others such that changing either resulted in similar trends *e.g.*

product to biomass ratio versus final biomass concentration and the yield coefficients for product on substrate and biomass on substrate. The smart breeding of strains or genetic modification may allow increased concentrations at higher yields, parallel to lower biomass production, which may result in more attractive processing conditions..

Default values in the generic flowsheet are chosen for specific reasons throughout *e.g.* literature values, common operating parameters *etc*. However, certain defaults, including product to biomass ratio and final biomass concentration, are defined in critical areas of influence. When using these defaults careful consideration needs to be taken as they are more sensitive than other defaults. Small changes near these default values influence all results including the material and energy balances as well as LCA scores.

In all case studies analysed, the improvement of a process, by increasing production, gives more favourable results than optimising downstream processing. This may be a result of various factors. These include:-

- Most downstream processing units should already be optimised and have little scope for improvement;
- There may not be a large downstream processing (DSP) setup. Improvements to a single unit DSP system will not influence an entire process as much as multiple improvements over a more complex DSP setup;
- There may be a greater scope for improvement within the bioreactor (*e.g.* improving product to biomass ratio, yield coefficients, biomass concentrations, the amount of excess raw materials fed to a system etc.) versus improvements to product retention in a few units; and
- Improvements in production impacts the material and energy values of downstream processing.

However, there is a limit to the physical production increases that can be obtained using the microorganism of choice. While it is recommended to improve the production of product by modifying the bioreactor setup there will be a point where the increase in production no longer becomes physically or economically viable. At this point it would be more beneficial to optimise the downstream processing.

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CHAPTER 8: CONCLUSIONS

8.1 Introduction

When comparing the environmental advantages of industrial scale chemical processes over biological processes, a full Life Cycle Assessment (LCA) analysis should be performed. Owing to the difficulty in obtaining the material and energy balance inventory required for an LCA analysis prior to completion of a full engineering design, a tool to obtain estimates of these was developed. Validation studies were then carried out to show the accuracy of the tool in obtaining the required material and energy values and also used compare literature and modelled LCA scores of each process. Using a combination of the modelling tool and literature values, four case studies were carried out to identify critical environmental burdens of commodity bioprocesses using LCA. The thesis also investigated the environmental advantages of industrial scale bioprocesses over chemical processes. The findings then guided a study to determine the areas of a bioprocess system which were most sensitive to change in individual input parameters. This showed which inputs resulted in high LCA scores were they not defined appropriately.

The thesis addresses several main objectives:

1. To develop a generic flowsheet model to provide material and energy balance data for industrial bioprocesses at an early stage in process development;
2. To validate the generic flowsheet model;
3. To use these data with Life Cycle Assessment (LCA) methodology as a means of assessing and comparing industrial bioprocess systems;
4. To use Life Cycle Assessment (LCA) methodology to compare industrial biological processes with chemical processes;
5. To obtain heuristics which guide the sustainability decisions of industrial bioprocess systems; and
6. To investigate the environmental implications of bio-technology selection.

8.2 Model development, validation and Life Cycle Assessment (LCA)

For many systems, including bioprocess systems, the desired material and energy balance input data are not available for Life Cycle Assessment (LCA) studies, especially at an early stage in technology selection and process design. A model was developed to provide these material and energy balance data for biological processes. The model allows for aerobic or anaerobic, intra- or extracellular biomass growth in a continuous or batch process. The model allows for various bioproducts produced from a selection of microorganisms using a range of raw materials. A database of yield coefficients, material properties and other constants required in the model,

collected from literature sources, is included. These values can be modified to reflect more representative data during specific flowsheet development.
The model, shown in an MS-Excel framework in the thesis, allows for sterilisation (steam or filtration) before the bioreactor and is followed by a downstream processing train as specified by the user. The bioreactor takes into account aeration, agitation, reactor cooling, biomass maintenance, yield coefficients, post microbial growth cooling and growth rate calculations.

The downstream processing includes solid-liquid separation, cell disruption, concentration and formulation units as required. These downstream processing units include, amongst others, centrifugation, filtration, sedimentation, cavitation, milling, evaporation, precipitation, oven drying and freeze drying units as appropriate. At each downstream processing stage, non-reacting and reacting chemicals can be added. Waste water removed at each stage can also be modelled waste water treatment step.

Several case studies were used to validate the generic flowsheet model developed in the thesis. For each validation study, three scenarios were presented. Each scenario assumed a smaller set of input data to represent the possibility of unavailable data and to determine the sensitivity of the model. Using the data from the generic flowsheets, the material and energy balance results obtained were compared to material and energy balance values of the equivalent process presented in literature. The case studies used for validation purposes included penicillin, cellulase and poly-β-hydroxybutyrate production.

Using the maximum input data sets to inform the MS-Excel model, 50 % of the material and energy balance results were within 20 % and 12% of literature values for the penicillin and poly-β-hydroxybutyrate flowsheets. For the cellulase case study, three different flowsheets were used as validation studies. For these, the maximum input data set gave results that showed 50 % of the material and energy balance results were within 33, 35 and 50 % of literature values respectively. The cellulase flowsheets had greater uncertainty, resulting in greater error. This uncertainty arose from poor flowsheet definition in the literature, resulting in an increased number of assumptions in the modelled flowsheet. This meant that a different process setup may have been modelled.

8.3 Findings from case studies

From the case study for the production of the sodium salt of Penicillin V, it was shown that the model was appropriate when critical data were provided. The material inputs for glucose, sodium acetate, sodium hydroxide, sulphuric acid, water and electricity requirements were all within 20 % of the literature results for the most representative scenario modelled. When these material and energy inventories were used to inform a Life Cycle Assessment (LCA) study, and

compared against LCA results from literature material and energy balance inputs, agreement within 5 % in eight of the ten impact categories was achievable (penicillin case study). The LCA analysis showed that the provision of electrical and agricultural inputs were the largest contributors to overall scores.

With a reduced data set, the Penicillin V flowsheet was still able to reproduce the material and energy balance data to a similar level as for the most representative flowsheet. The LCA results from literature material and energy balance inputs again agreed within 5 % in eight of the ten impact categories. This showed that with reduced datasets, even though the error may be larger, a good approximation of material and energy values and LCA results may be possible.

The second case study investigated cellulase production by three different flowsheets. These flowsheets compared aerobic and anaerobic processes, different bioreactor design and different downstream processing units by submerged fermentation (SmF) and solid state cultivation (SSC). Production by solid state cultivation (SSC) gave a reduction in volume, and thereby energy. This reduction in electricity resulted in a reduction in LCA scores. The lower energy requirements in the SSC model resulted in increased dominance of the impacts from the raw materials (cellulose, yeast extract and urea).

The generic flowsheet was used in a third case study to determine the material and energy balance results for the production of the biopolymer poly-β-hydroxybutyrate. As a result of different compression energies for aeration, the electrical energies for the generic flowsheet models were at least 25 % higher than the literature value, which resulted in the LCA values being higher. The uptake of carbon dioxide during agricultural processes was less than the carbon dioxide equivalent released and resulted in a net carbon dioxide equivalent release of at least 5.74 kg $CO_{2\ eq.}$ from the polymer production process.

8.4 Chemical versus biological processes

Using two case studies, Life Cycle Assessment (LCA) methodology was used to compare similar products produced by chemical and biological means. These were for the production of a biopolymer (poly-β-hydroxybutyrate) and the production of biodiesel.

The LCA results of poly-β-hydroxyalkanoate production were compared to the production of polyolefins with similar product characteristics (polypropylene, high density polyethylene and low density polyethylene. When using the EcoInvent v1.3 LCA database, poly-β-hydroxybutyrate (PHB) production resulted in higher LCA scores than polypropylene (PP) and polyethylene (PE) for all impact categories. Despite the use of renewable resources in the PHB

production, and the carbon dioxide captured during biomass growth, the chemical route to polymer production was shown to be more environmentally beneficial on a cradle-to-gate LCA basis.

The results for the polymer case study were shown to be sensitive to the LCA database used. As updates occur, these databases will give more representative results. Further, the polymer comparison here was for an established industrial technology against the developing biopolymer technology. Since the biotechnology is not optimised, improvements to the process will ultimately reduce the LCA impacts.

Biodiesel is typically produced using an oil, a chemical catalyst and an alcohol. Process alternatives allow for production using a biological catalyst (enzyme) and different alcohols. Methanol is typically used, but ethanol, produced from renewable resources, has been proposed to be more environmentally beneficial. Lower alcohol recycle required greater flows throughout to give similar product yields and purities. Enzymic transesterification uses a simpler process conducted without acid and at lower pressures and temperatures. This resulted in the enzyme catalysed biodiesel production being more environmentally beneficial on an LCA basis. Using ethanol instead of methanol did not reduce environmental impacts owing to the high coal based electricity requirement for sugar in the ethanol production. The impact of the enzyme production on the LCA score was less than 4 % across all categories. In the alkali catalysed process, lower alcohol recovery required greater flows to give similar product yield and purity. This increased the energy needed, as well as pollutants produced, resulting in higher environmental burden when analysed using life cycle assessment.

Where bio-based carbon is found in the final product this was deducted in the calculations. It is unlikely that the LCA impact would change if incineration were chosen, since comparable amounts of CO_2 would be released as a consequence.

8.5 Technology selection

It was found that decreasing the water requirement (*e.g.* by solid state cultivation (SSC)) decreases the overall volume requirements, which in turn decreased the energy required throughout the process. Since the energy requirement was a large contributor to the overall LCA scores, a reduction in energy also decreases the LCA scores.

Certain input variables, including product to biomass ratio, final biomass concentration, oxygen flowrate and compression pressure, yield coefficients and downstream processing separation efficiencies, were identified as key variables in the generic flowsheet model developed. Small changes to these variables may escalate the material and energy requirements of the process.

These escalations, particularly those that influence the energy requirements, affect the Life Cycle Assessment (LCA) scores for the production of the product.

It was found that in the Penicillin V production flowsheet, the downstream processing units were typically optimised. This left little scope to improve the process by improvements to separation efficiencies. However, improvements in bioproduct formation in the bioreactor dominated improvements of the overall LCA scores, as this was effective in reducing impacts from both the bioreactor and the downstream processing units as a result of higher product concentrations. This may also be the case for processes with similar bioreactor and downstream processing setups, but may not be so for processes where the product leaves the bioreactor at low concentrations as was seen in the cellulase flowsheets.

8.6 Conclusions

The thesis provided a generic flowsheet model for fast, first estimate material and energy balance inventories of industrial bioprocesses. Presented in an MS-Excel format, the model used a stoichiometric approach, together with first principles and rules of thumb. The model allowed for batch or continuous production by aerobic or anaerobic and intra- or extracellular means. Downstream processing units were included to allow for accurate representation of typical process setups. Typical information from bioprocess systems was stored in a database which included a relevant constants and physical data.

Case studies were presented to act as validations of the generic flowsheet model against literature data. These case studies, including Penicillin V, cellulase and biopolymer production, showed good agreement with the literature inventories. The material and energy balance inventories obtained were then used to inform Life Cycle Assessment (LCA) studies. These LCA studies acted as further comparisons to the generic flowsheet model. Large differences in the modelled material and energy inventories compared with the literature values, for example between steam and electricity, could be converted to LCA scores and compared on this basis.

The LCA results helped to develop an understanding of the environmental burdens and process contributions in the bioprocess flowsheets. It was possible to obtain LCA results for the case studies which were within 5 % of literature results; even when limited inputs sets were used in the generic flowsheet model. It was shown that electricity and agricultural inputs were large contributors to overall LCA scores. It was found that the carbon dioxide (CO_2) uptake from agricultural processes was not always large enough to overcome the CO_2 equivalent release from other processes, which may have otherwise resulted in a net cradle-to-gate reduction.

With energy use dominating the LCA results in case studies, and trends in the global warming category often repeated in the other LCA categories, the ratio of effort to benefit in performing a Life Cycle Assessment across a full range of impact categories may appear unnecessary. However, important contributions were noted such as the eutrophication scores of bio-based products. Further, using only energy and greenhouse gases as the measure of environmental performance allowed important burdens to be overlooked in certain categories *e.g.* hydrogen peroxide used in the biopolymer production process reported more strongly in the ozone layer depletion category. The dominance of energy throughout the LCA results may have resulted from lack of process emission data. Where more accurate flowsheet information is available, a clearer understanding of the impacts of these emissions is achieved.

The life cycle impact assessment method used did not track the water use or the biodiversity impacts, important categories in the overall understanding of the impacts of production. Water use can be followed through the inventory data, although this allows limited emphasis on the cradle-to-gate approach.

A sensitivity study was performed by changing single input variables in the generic flowsheet model. This showed that aeration rates, biomass concentrations, yield coefficients and additive concentrations in downstream processing were important variables. It was also seen that any changes to the volume of the process impacted on the energy needs, which in turn influenced the LCA scores. Further, it is shown that it was more beneficial to increase production over optimisation of downstream processing in order to reduce LCA scores.

The findings from the thesis showed that biological processes are not necessarily more environmentally friendly than chemical processes when producing similar products. While biodiesel production showed a lower LCA impact when using a biological catalyst in comparison to a chemical one, there was no advantage of a biopolymer over a conventional polyolefin. Hence, products need to be investigated on a case by case basis.

The method produced to obtain material and energy balance results, through the use of the MS-Excel generic flowsheet, showed that sufficient material and energy balance information could be abstracted for a Life Cycle Assessment to be performed. Even with a limited input set, the key features required for the LCA were not lost. The method reduced the effort required to perform an LCA compared to the detailed engineering flowsheeting often needed for a Life Cycle Assessment, which is typically not feasible owing to cost and time implications. Specifically, this methodology allows LCA to be included from the earliest decision making stages of bioprocess development.

8.7 Recommendations

It is important that a tool is available for the early estimation of material and energy balance inventories for industrial processes. The generic flowsheet presented has allowed for this, but requires a knowledgeable user. The model should be developed into a more user-friendly format which can then be widely distributed.

The analysis conducted has used a number of case studies to understand bioprocess systems and identify major contributions to Life Cycle Assessment (LCA) scores. In order to have a robust knowledge of bioprocess systems across different bioprocesses, a larger set of case studies should be investigated.

The capital costs, social costs and environmental burdens associated with the production of bioprocesses can be included in the generic flowsheet model. This will give greater input to the decision making processes of industrial bioprocesses design.

The flowsheet model was purposefully developed to only allow for a single bioproduct. The functionality of the model may be enhanced to allow for the analysis and formation of intermediate or multiple products in the bioreactor. The model also avoided recycle loops in downstream processing. The inclusion of these in future versions will allow for a greater set of bioprocesses to be investigated and help identify further ways to minimise the environmental burden of bioproducts.

It is recommended that a material and energy balance inventory and Life Cycle Assessment (LCA) analysis be routinely performed at the early stages of all bioprocess designs. This will help in the understanding of the process and aid in the reduction of environmental burden. The full environmental advantages of bioproducts should be compared to any existing chemical equivalents to ensure that there is a real benefit in the biological route.

It is further recommended to include the water inventory in LCA scores of future studies. Inclusion of an impact category primarily for water use would add to a more complete understanding of environmental impacts in LCA.

A Generic Approach to Environmental Assessment of Microbial Bioprocesses through Life Cycle Assessment (LCA)

Volume 2

Appendices

A thesis submitted to the University of Cape Town
in fulfilment of the requirements for the degree of
Doctor of Philosophy

by

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December 2008

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IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

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Appendix A

Life Cycle Assessment (LCA)

Appendix A: Life Cycle Assessment (LCA)

A. 1. Life Cycle Assessment (LCA)

Life Cycle Assessment (LCA) is an analytical tool designed to assess environmental impacts relating to the production of goods. It is defined by the Society of Environmental Toxicology and Chemistry (SETAC) as "a process to evaluate the environmental burdens associated with a product, process or activity by identifying and quantifying energy and materials uses and wastes released to the environment; to assess the impact of those energy and material uses and releases to the environment; and to identify and evaluate opportunities to effect environmental improvements. The assessment includes the entire life cycle of a product, process or activity, encompassing extracting and processing raw materials; manufacturing transportation and distribution; use, re-use, maintenance; recycling, and disposal" (Consoli *et al*. 1993). LCA methodology comprises four phases: Goal and scope definition, Inventory analysis, Impact assessment and Interpretation (Figure A.1) (ISO 14040: 2006, ISO14044: 2006).

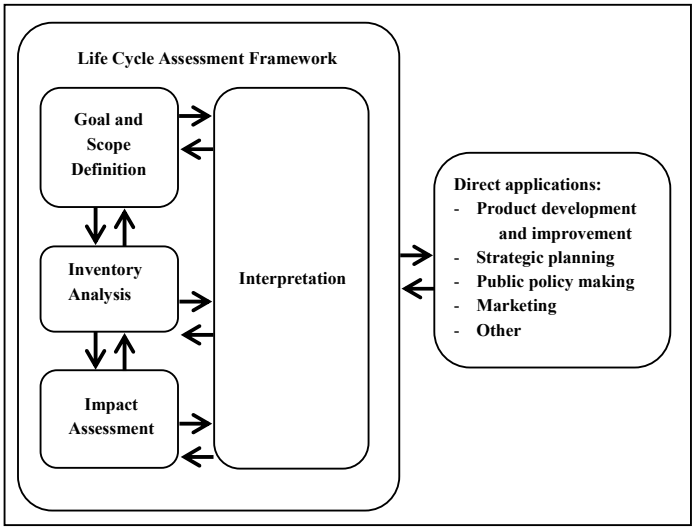

Figure A.1: Phases of an LCA as given in ISO 14040: 2006

LCA involves a certain amount of uncertainty. This arises from averaged input data, the choice of system boundary, limitations in the models used or in the variety of LCA methods used. Data errors (sometimes termed parametric uncertainty) can be described as normal or log-normal probability distributions (Steen 1997) and dealt with using Monte Carlo simulations within LCA software packages. Life Cycle assessment software packages include SimaPro (PRé Consultants B.V. 2008), GaBi (PE International 2007), Umberto (Ifu Hamburg GmbH 2007) and TEAM (Ecobilan 2007).

A.2. Goal and Scope Definition

The goal and scope of an LCA outlines the purpose and objective of the study. It sets the boundaries of the study, thereby defining the scope of a particular system. This boundary is defined as cradle-to-grave (including recycling or disposal) or as cradle-to-gate (ending as a final product). The boundary of the LCA defines where the inputs (primary resources) and outputs (final waste materials and products) are situated, drawing a system boundary around the material life cycle of interest. In theory, all processes should be included in the boundary. However, it is common practice to stop adding processes to the flowsheet where adding these does not change the outcome of the analysis.

It is at the goal and scope stage that data quality limits and categories are defined. It is also here that a target audience is typically defined. A functional unit, a mathematical quantification of products or functions being analysed, is defined, and is the reference unit from which assessment values are measured (Wentzel *et al.* 1997). This should be chosen as a unit of service or quantity of product which gives an equal function, so that different systems can be compared on an equivalent basis.

Should there be more than one product of interest; the system should be expanded to include all products or functions. Where this is not possible, burden allocation is required to share environmental burdens across products by mass, volume, economic value or other criterion.

A.3. Life Cycle Inventory Analysis (LCI)

The Life Cycle Inventory Analysis (LCI) requires the collection of data to quantify resource and energy use. Data collected include all inputs to the system and products and wastes (solid, water and air) generated. Data that are collected should be validated (*e.g.* by mass or energy balance) and reported in terms of the functional unit. Where data are not available, models need to be created for such (Vigon and Harrison 1994). The need for accurate inventory data motivates the need for an approach to obtain material and energy balance data quickly and easily. Owing to the limited availability of such bioprocess data, this need justifies the development of a generic flowsheet model in Chapter 2 of this thesis.

A.4. Life Cycle Impact Assessment (LCIA)

From the LCI results, a Life Cycle Impact Assessment (LCIA) is used to condense the large inventory data set into a manageable number of environmental impacts. Using different impact categories such as global warming, abiotic depletion and eutrophication, an LCIA converts the LCI data, using equivalency factors into, these groupings.

Where categories are left in these broad groupings, they are called midpoint categories. Midpoints are categories which stop midway in the environmental cause-effect chain. The midpoint categories can be added together to give endpoint categories. The endpoint categories attempt to model the actual damages (e.g. human health, ecosystem quality and climate change). It is also possible to group endpoints together to give a single score for all categories, but the practise of using single score values is discouraged in the ISO 14040: 2006 standard (ISO 14040: 2006).

A.5. Life Cycle Interpretation

At every stage of the LCA, an interpretation of the process is undertaken. At the end of an LCA, the interpretation phase determines the environmental significance of the findings in the LCI and LCIA phases. Since Life Cycle Assessment is seen as an iterative process, successive steps can be compared through the interpretation phase.

Interpretation can also include sensitivity or uncertainty analyses. Uncertainty data is often included in the more recent life cycle inventory datasets *e.g.* the Ecoinvent datasets. LCA software packages typically have Monte Carlo analysis options which allows for an error to be reported on each of the Life Cycle Impact Assessment results.

A.6. LCA Use in the Thesis

The LCA work in the thesis uses the software package SimaPro 7.1® (PRé Consultants B.V. 2008) with the EcoInvent v1.3 database (Swiss Centre for Life Cycle Inventories 2007). Where data is not available in the EcoInvent v1.3 database, the LCA Food (Nielsen *et al.* 2003), IDEMAT 2001 (Design for Sustainability Program 2007), BUWAL 250 (Federal Office for the Environment 2007) and ETH-ESU 96 System and Unit (Frischknecht *et al.* 1996) databases were used. The CML 2 Baseline 2000 v2.03 assessment method has been used wherever possible. This is a midpoint method presenting the life cycle impact assessment results in the categories of:

- Abiotic depletion (kg $Sb_{eq.}$);
- Global Warming (GWP100) (kg $CO_{2\ eq.}$);
- Ozone Layer Depletion (ODP) (mg $CFC\text{-}11_{eq.}$);
- Human Toxicity (kg 1,4-$DB_{eq.}$);
- Fresh Water Aquatic Ecotoxicity (kg 1,4-$DB_{eq.}$);
- Marine Aquatic Ecotoxicity (kg 1,4-$DB_{eq.}$);
- Terrestrial Ecotoxicity (kg 1,4-$DB_{eq.}$);
- Photochemical Oxidation (kg C_2H_4);
- Acidification (kg $SO_{2\ eq.}$); and
- Eutrophication (kg $PO_4^{3-}{}_{eq.}$).

The environmental categories given here are commonly included in the various Life Cycle Impact Assessment methods. However, different assessment methods define differing categories. In the CML 2 Baseline 2000 v2.03 method used, and hence this thesis, water usage and biodiversity were not included as impact categories. However, water is tracked as an inventory item.

The systems used in LCA studies were defined as cradle-to-factory gate production, including all raw material and agricultural inputs. Where possible, waste water treatment was included. The process plant and equipment impacts are excluded according to Heijungs *et al.* (1992). A typical functional unit of 1 kg of crude product was used.

Energy values reported in the material and energy inventories of the case studies are given as process inputs and represent a combination of final energy carriers and the primary energy inputs. Electricity requirements are reported as final energy, and not converted to the equivalent primary values, to enable comparison to literature. Typically, primary energy values (such as the steam and natural gas values used in this study) are used in Life Cycle Assessment studies.

As ozone depleting compounds are being phased out, ozone depletion is of reduced importance in current processes. Due to the use of data collected over an extended time period, ozone depletion has been included in this analysis. With the ongoing reduction in ozone depleting compounds, it can be assumed that ozone layer depletion as a category will have decreased significance in current and future processes.

A.7 LCA single score

The single score method used in thesis was that of the EPS 2000 v2.02 method (Steen 1999a and 1999b). The EPS 2000 v2.02 method is a robust calculation based on earlier work of Steen (1996) and Ryding *et al*. (1993). The single score is calculated by equally weighting the impacts on human health, ecosystem production capacity, abiotic stock resources and biodiversity. Each of these damage categories are quantified using characterisation indicators which include, for example, life expectancy, depletion of reserves, morbidity and severe nuisance (Figure A.2). These are typically reported in units of Environmental Load Units (ELUs) and calculated from a comprehensive substances list.

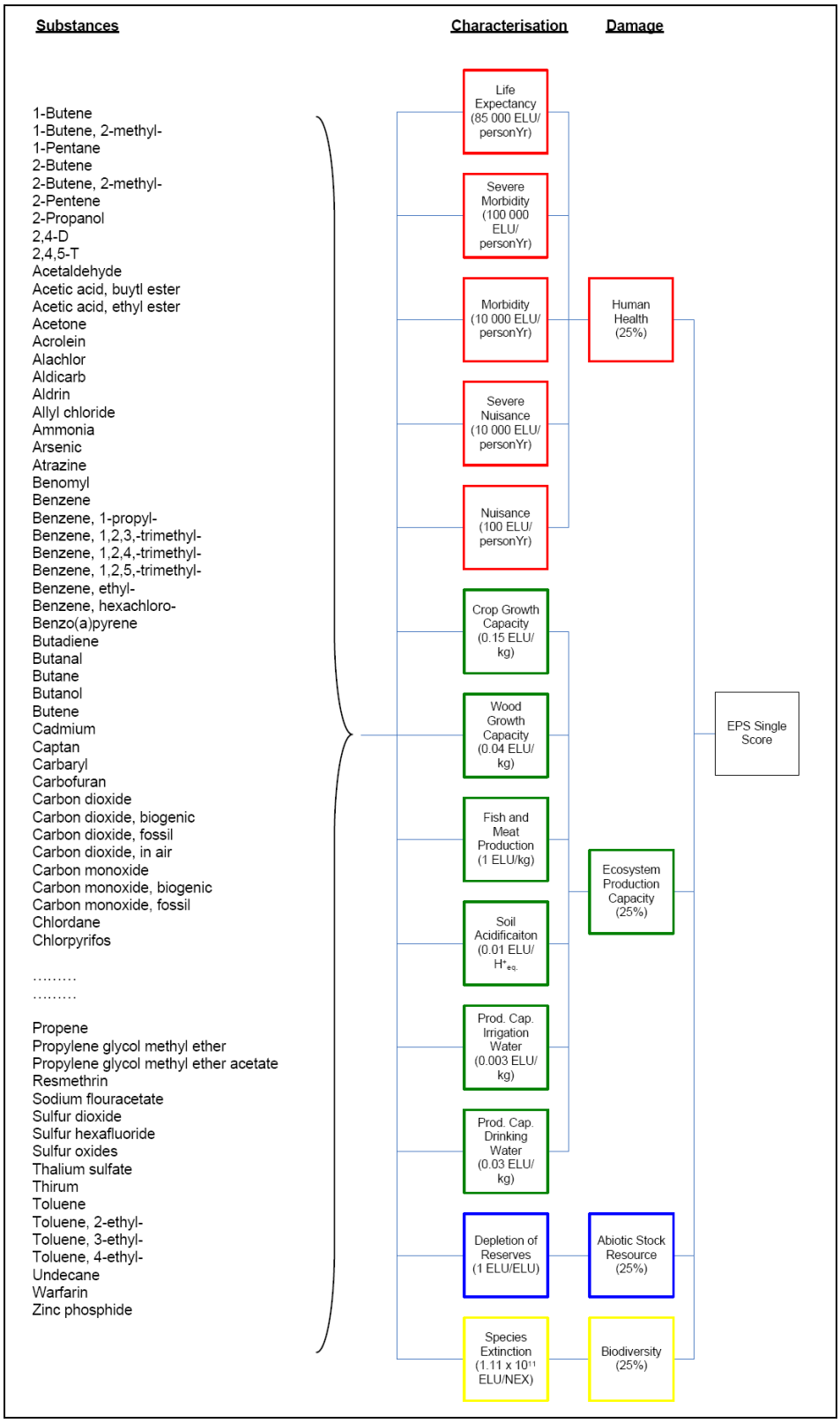

Figure A.2: EPS structure as used in the Life Cycle Assessment (LCA) calculations for single scores (Steen 1999a and 1999b)

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Appendix B

Generic Flow Sheet Calculations

Appendix B: Generic Flow Sheet Calculations

The nomenclature used in Appendix B is defined in Appendix C.

B.1. Global assumptions

- ALL initial values appearing (assumed or calculated) in the model can be changed by the user.
- Where no clear choice is made for a decision, average values or industry norms are assumed.
- Where specific heat values are unavailable, the specific heat of water is assumed ($C_{p,\,w}$ = 4.1868 kJ/kg.K).
- Complex densities (*e.g.* water, product and raw material mix) are assumed to have a density equal to water, unless specified otherwise.
- Total volumes are often assumed to equal the volume of water present in a unit. Dissolved substances and volumes changes on mixing are assumed negligible.
- Where reaction occurs, the density product density remains the same.

Global initial values

Cooling water temperature (T_{cw}) = 18°C
Chilled water temperature (T_{cw}) = 10°C
Max. temp. diff. between exiting cooling water and hot inlet streams ($T_{\Delta max,\,cw}$) = 10°C

B.2. Sterilisation

Filtration

Assumptions

No cake build-up
Constant pressure filtration

Initial values

Filtration area for sterilisation ($A_{f,\,ster}$) = 25 m^2
Pressure difference of filtration in sterilisation ($\Delta P_{f,\,ster}$) = 250 kPa
Pumping efficiency of filtration in sterilisation ($\eta_{f,\,ster}$) = 0.8
Time ($t_{f,\,ster}$) = 1 s
Note: Time basis immaterial as unit of time cancels out through calculation

Equations

$$v_{f,ster} = \frac{V_{w,in,ster}}{A_{f,ster}.t_{f,ster}} \quad \textbf{B.1}$$

where: $V_{f,ster}$ = Linear velocity through sterilisation filtration ($m.s^{-1}$)
$V_{w,in,ster}$ = Volume of water in sterilisation (m^3)

Since the volumes of the raw materials are small relative to the volume of water in the dilute systems typical of bioprocesses, only the volume of water is used as a simplification in these calculations. Volumes of raw materials are further avoided since calculating these requires the densities of each material. Although not used to calculate volumes, because the masses of raw materials are already calculated elsewhere, they are included in total mass calculations.

$$P_{f,ster} = \frac{\Delta P_{f,ster}.v_{f,ster}}{\eta_{f,ster}} \quad \textbf{B.2}$$

where: $P_{f,ster}$ = Power for filtration in sterilisation (kPa)

$$E_{f,ster} = P_{f,ster}.t_{f,ster} \quad \textbf{B.3}$$

where: $E_{f,ster}$ = Energy for filtration in sterilisation (kJ)

Steam Sterilisation

Initial values and Constants

Ambient or preheated temperature (T_a) = 20°C (60°C)
Sterilisation temperature (T_h) = 121°C
Steam temperature (T_{st})= 140°C
Reactor temperature (T_{rct}) = 37°C
Cooling or chilled water temperature (T_{cw}) = *[As for global initial values]*
Max. temp. diff. between exiting cooling water and hot inlet streams ($\Delta T_{max,\ cw}$) = 10°C
Sterilisation efficiency (η_{ster}) = 0.8 (used for all efficiencies in sterilisation)
Specific heat of water ($C_{p,w}$) = 4.1868 kJ/kg.K
Specific heat of steam ($C_{p,st}$) = $a + bT + cT^2 + dT^3$ (J/mol.K)
($a = 29.163$; $b = 14.49 \times 10^{-2}$; $c = 2.02 \times 10^{-6}$; $d = 0$)
Density (water) (ρ_w) = 1000 kg/m^3
Pressure steam (before compression) ($P_{st,in}$) = 101.325 kPa
Pressure (steam) ($P_{st,out}$) = 300 kPa

Gas constant (R) = 8.314 kJ/kg.K
Latent heat of steam at 100°C and 101.3 kPa (λ_{st}) = 2.7 MJ.kg^{-1}

Equations

$$\Delta T = T_{rct} - T_{\Delta max} \quad \textbf{B.4}$$

where: ΔT = Temperature difference (^oC)

Typically a log-mean temperature is used, but for the large ΔT values often obtained, this simplification is used.

Mass of Steam needed:

$$M_{st,h} = \left(V_{w,in}.\rho_w\right) + M_{rm} \quad \textbf{B.5}$$

where: $M_{st,h}$ = Mass of steam to heat to sterilisation temperature (T_{ster}) (kg)
M_{rm} = Mass of raw material for (kg)

$$q_h = M_{st,h} C_{p,w} \left(T_h - T_a\right) \quad \textbf{B.6}$$

where: q_h = Heat to raise temperature (kJ)

$$A_{surf,ster} = V_{ster}.SV_{ster} \quad \textbf{B.7}$$

where: A_{surf} = Surface area of sterilisation unit(m^2)
V_{ster} = Volume of sterilisation unit (m^3)
SV_{ster} = Surface area to volume ratio for sterilisation unit (m^2/m^3)

$$\Delta \underline{E} = U.A_{surf}.\Delta T \quad \textbf{B.8}$$

where: $\Delta \underline{E}$ = Rate of energy loss (kJ/s)
U = Overall heat transfer coefficient ($kW/m^2.^oC$)
ΔT = Temperature difference (^oC)

$$\Delta q = \Delta E.t_{ster} \quad \textbf{B.9}$$

where: Δq = Heat loss during constant temperature period (kJ)
t_{ster} = Time for sterilisation (hr)

$$q_T = q_h + \Delta q \quad \textbf{B.10}$$

where: q_T = Total heat lost (kJ)

$$M_{st,\,hold} = \frac{q_T}{\lambda_{st}.\eta_{St,\,hold}} \quad \textbf{B.11}$$

where: $M_{st,\,hold}$ = Mass of steam required for holding period (kg)
$\eta_{st,\,hold}$ = Efficiency for sterilisation during holding time

$$M_{st,T} = M_{st,\,h} + M_{st,\,hold} \quad \textbf{B.12}$$

where: $M_{st,\,T}$ = Total mass of steam required (kg)

Steam compression required

Assumption: Isentropic compression

$$T_{ster,\,st,\,out} = T_{st}\left(\frac{P_{ster,\,st,\,out}}{P_{ster,\,st,\,in}}\right)^{\frac{R}{C_{p,\,st}}} \quad \textbf{B.13}$$

where: $T_{ster,\,st,\,out}$ = Temperature of steam leaving steriliser (^oC)
$P_{ster,\,st,\,out}$ = Outlet pressure of steam from steriliser (kPa)
$P_{ster,\,st,\,in}$ = Inlet pressure of steam to steriliser (kPa)

$$E_{ster,\,comp} = \frac{C_{p,\,st}\left(T_{ster,\,st,\,out} - T_{st}\right).m_{st}}{\eta_{ster,\,comp}} \quad \textbf{B.14}$$

where: $E_{ster,\,comp}$ = Energy consumption for sterilisation compression (kJ)
$m_{st,\,ster}$ = Number of moles steam – sterilization (moles)
$\eta_{ster,\,comp}$ = Efficiency for sterilisation compression

Cooling water

$$M_{ster,\,cw} = \frac{M_{ster}.\left(T_h - T_{rct}\right)}{T_{ster,\,cw,\,out} - T_{cw,\,in}} \quad \textbf{B.15}$$

where: $M_{ster,\,cw}$ = Mass of cooling water following sterilisation (kg)
M_{ster} = Mass of media to be sterilised (kg)

$T_{ster, cw, out}$ = Temperature of cooling water leaving heat exchanger (^oC)

$$V_{ster, cw} = \frac{M_{ster, cw}}{\rho_w} \quad \textbf{B.16}$$

where: $V_{ster, cw}$ = Volume of cooling water for sterilisation (m^3)

Energy integration is taken into account in a simplistic assumption of preheating media with already sterilised media. If preheating occurs, no cooling water is used to reduce the temperature before the microbial growth and product formation.

Other Sterilisation

Constants

Specific energy ($\hat{E}_{ster, o}$) = 25 MJ/m^3 of material to be sterilised

Equations

$$E_{ster, o} = \hat{E}_{ster, o} . V_{w, ster, in} \quad \textbf{B.17}$$

where: $E_{ster, o}$ = Energy for sterilisation (MJ)
$V_{w, ster, in}$ = Volume of water in sterilisation (m^3)

Additional Steam

Assumptions

Steam delivered at 2.1 bar (gauge), giving a process temperature of 121°C

Continuous vs. batch processes

Either a batch or continuous process is defined:
A batch process is sterilised at the beginning of each production cycle

$$No = 1 \quad \textbf{B.18}$$

A continuous process is sterilised at the end of a campaign

Campaign time = 6 weeks

$$No = \frac{t_{camp}}{t_{cycle}} \quad \textbf{B.19}$$

where: No = Number of (equivalent) batches
t_{camp} = Time per campaign (hr)
t_{cycle} = Time per production cycle (hr)

Steam out vessel

$$\text{Steam required for } 147\ m^3 \text{ vessel} = 40\ t\ (\text{Dennis } 2000)$$

By the 2/3 rule:

$$M_{st,\,sov} = 40 * \left(\frac{V_{rct}}{147}\right)^{\frac{2}{3}} \quad \textbf{B.20}$$

where: $M_{st,\,sov}$ = Mass of steam to steam out vessel (kg)
V_{rct} = Reactor volume (m^3)

$$M_{st,\,sov,\,bat} = \frac{M_{st,sov}}{No} \quad \textbf{B.21}$$

where: $M_{st,\,sov,\,bat}$ = Mass of steam to steam out vessel per batch (kg)

Backing steam

Backing steam is the ancillary steam required in a process *e.g.* control valves

Mass of backing steam per day per unit volume ($M_{st,\,back}$) = 0.082 t steam/day/m^3 (Dennis 2000) **B.22**

$$M_{d,\,st,\,back} = M_{st,\,back}.V_{rct} \quad \textbf{B.23}$$

where: $M_{d,\,St,\,back}$ = Mass of backing steam per day (kg/day)

$$M_{T,\,St,\,back} = Md_{St,\,back}.\ t_{cycle} \quad \textbf{B.24}$$

where: $M_{T,\,st,\,back}$ = Total mass of backing steam (kg)

Space heating

Mass of space heating steam per day per unit volume ($M_{st,\ spa}$) = 0.02 t steam/day/m^3 (Dennis 2000) **B.25**

$$M_{dst,\ spa} = M_{St,\ spa.} V_{rct} \quad \textbf{B.26}$$

where: $M_{d,\ st,\ spa}$ = Mass of space heating steam per day (kg/day)

$$M_{T,\ st,\ spa} = M_{d,\ st,\ spa.}\ t_{cycle} \quad \textbf{B.27}$$

where: $M_{T,\ st,\ spa}$ = Total mass of space heating steam (kg)

Total additional steam

$$M_{T,\ st,\ ad} = M_{st,\ sov,\ bat} + M_{T,\ st,\ back} + M_{T,\ st,\ spa} \quad \textbf{B.28}$$

where: $M_{T,\ St,\ ad}$ = Total additional steam (kg)

B.3. Microbial Growth and Product Formation

Introduction

Biomass growth and product formation are calculated separately using the sets of equations outlined below. Results for each are added and proportioned to give the desired product mass.

Biomass Growth

$$\left(\text{Carbon}_x + \text{Oxygen}_x + \text{Nitrogen}_x + \text{Sulphur}_x + \text{Phosphorus}_x\right)\text{sources}$$
$$\downarrow \quad \textbf{B.29}$$
$$\text{Biomass} + \text{Product} + \left(H_2O\right)_x + \left(CO_2\right)_x + \left(\text{Nitrogen}_x + \text{Sulphur}_x + \text{Phosphorus}_x\right)\text{wastes}$$

$$\left(\alpha C_aH_bO_cN_dP_eS_f + \alpha' C_{a'}H_{b'}O_{c'}N_{d'}P_{e'}S_{f'}\right)_x + \beta\left(O_2\right)_x + \left(\chi C_gH_hO_iN_jP_kS_l + \chi' C_{g'}H_{h'}O_{i'}N_{j'}P_{k'}S_{l'}\right)_x + \delta\left(C_mH_nO_oN_pP_qS_r\right)_x + \varepsilon\left(C_sH_tO_uN_vP_wS_x\right)_x$$

$$\downarrow$$

$$\varphi C_yH_zO_{aa}N_{ab}P_{ac}S_{ad} + \varphi C_{ae}H_{af}O_{ag}N_{ah}P_{ai}S_{aj} + \gamma\left(H_2O\right)_x + \eta\left(CO_2\right)_x + \iota\left(N_2\right)_x + \kappa\left(SO_4^{-}\right)_x + \lambda\left(PO_4^{-}\right)_x \qquad \textbf{B.30}$$

where: x: Components related to biomass growth only

Table B.1: Micro-organisms that include additional experimental values and are available as part of the generic flowsheet model database

Micro-organisms
Achromobacter delvacvate, *Acinetobacter* sp., *Aerobacter aerogenes*, *Aeromona hydrophila*, *Aspergillus niger*, *Aspergillus sp.*, *Aspergillus terreus*, *Azohydromonas lata*, *Bacillus licheniformis*, *Bacillus subtilis*, *Brevibacterium* sp., *Candida glycerinogenes*, *Candida magnoliae*, *Candida* sp., *Cellulomonas* sp., *Chaetomium cellulolyticum*, *Corynebacterium glutamicum, Cornebacterium hydrocarboclastus*, *Cupriavidus necator*, *Escherichia coli*, *Fusarium moniliforme*, *Fusarium* sp., *Geotrichum candidum*, *Klebsiella oxytoca*, *Klebsiella* sp., *Lactobacillus* sp., *Methalococcus capsulatus*, *Methophilus methylotrophus*, *Methylomonas* sp., *Norcadia sp.*, *Paecilomyces variotii*, *Paracoccus denitrificans*, *Penicillium chrysogenum*, *Penicillium cyclopium*, *Penicillium* sp., *Propionibacterium* sp., *Protaminobacter rubber*, *Pseudomonas aerugenosa*, *Pseudomonas* C12B, *Pseudomonas fluorescens*, *Pseudomonas methanica*, *Pseudomonas* sp., *Rhizopus oligosporus*, *Rhodopseudomonas gelatinosa*, *Rhodopseudomonas spheroids*, *Saccharomyces cerevisiae*, *Saccharomyces* sp., *Thermomonospora* sp., *Trichoderma* sp., *Xanthamonas campestris*, OTHER.

Table B.2: Elemental formula for micro-organisms used in the model

Organism	Carbon	Hydrogen	Oxygen	Nitrogen	Sulphur	Phosphorus	Reference
	C	H	O	N	S	P	
Aerobacter aerogenes	1	1.83	0.55	0.25			[2]
Aspergillus niger	1	1.74	0.711	0.117			[1]
Azohydromonas lata	1	1.76	0.48	0.19			[2]
Candida sp.	1	1.84	0.52	0.16			[2]
Escherichia coli	1	1.77	0.49	0.24			[2]
Klebsiella sp.	1	1.75	0.43	0.23			[2]
Paracoccus denitrificans	1	1.66	0.49	0.2			[2]
Pseudomonas $C_{12}B$	1	2	0.52	0.23			[2]
Saccharomyces cerevisiae	1	1.76	0.53	0.17	0.005	0.01	[2]
OTHER	1	1.82	0.53	0.21	0	0	

References:

[1] El-Enshasy 1998 [2] Roels 1980

To solve the stoichiometric equations for biomass growth:

Initial values

Biomass: $\phi = 1$

Product: $\gamma = 0$ (assumption that no product is formed)

Unknowns (12)

Carbon source (x2):	α, α'
Oxygen source:	β
Nitrogen source (x2):	χ, χ'
Sulphur source:	δ
Phosphorus source:	ε
Water waste:	η
Carbon dioxide waste:	ι
Nitrogen waste:	φ
Sulphur waste:	κ
Phosphorus waste:	λ

Material balances (6)

Carbon:	$\alpha.a + \alpha'.a' + \chi.g + \chi'.g' + \delta.m + \varepsilon.s - \iota = \phi.y$	**B.31**
Hydrogen:	$\alpha.b + \alpha'.b' + \chi.h + \chi'.h' + \delta.n + \varepsilon.t - 2.\eta = \phi.z$	**B.32**
Oxygen:	$\alpha.c + \alpha'.c' + 2.\beta + \chi.i + \chi'.i' + \delta.o + \varepsilon.u - \eta - 2.\iota - 2.\kappa - 4.\lambda = \phi.aa$	**B.33**
Nitrogen:	$\alpha.d + \alpha'.d' + \chi.j + \chi'.j' + \delta.p + \varepsilon.v - \eta - 2.\varphi = \phi.ab$	**B.34**
Phosphorus:	$\alpha.e + \alpha'.e' + \chi.k + \chi'.k' + \delta.q + \varepsilon.w - l = \phi.ac$	**B.35**
Sulphur:	$\alpha.f + \alpha'.f' + \chi.l + \chi'.l' + \delta.r + \varepsilon.x - \kappa = \phi.ad$	**B.36**

Yield Coefficient (1)

$Y_{x/s}$ As defined by literature or experimental values in database (g/g)

$$\alpha.\left(\frac{Y_{x/s}.Mm_{C1}}{Mm_x}\right) + \alpha'.\left(\frac{Y_{x/s}.Mm_{C2}}{Mm_x}\right) = \varphi \qquad \textbf{B.37}$$

where: Mm_{C1} = Molar mass carbon source 1 (kg/kmol)
Mm_{C2} = Molar mass carbon source 2 (kg/kmol)
Mm_x = Molar mass biomass (kg/kmol)

$$\frac{Y_{x/s}}{Mm_x}.\left(\alpha.Mm_{C1}+\alpha'.Mm_{C2}\right)=\phi \qquad \textbf{B.38}$$

$$\frac{Y_{x/s}}{12.01.y+1.01.z+16.00.aa+14.01.ab+30.97.ac+32.07.ad}.\left(\alpha.Mm_{C1}+\alpha'.Mm_{C2}\right)=\phi \qquad \textbf{B.39}$$

$$\left(\frac{Y_{x/s}}{12.01.y+1.01.z+16.00.aa+14.01.ab+30.97.ac+32.07.ad}\right).\left(\begin{array}{l}\alpha.\left(12.01.a+1.01.b+16.00.c+14.01.d+30.97.e+32.07.f\right)+\\ \alpha'.\left(12.01.a'+1.01.b'+16.00.c'+14.01.d'+30.97.e'+32.07.f'\right)\end{array}\right)=\phi \qquad \textbf{B.40}$$

Ratios (2)

$$r_{C1/CT}=\frac{\alpha}{\alpha+\alpha'} \qquad \textbf{B.41}$$

where: $r_{C1/CT}$ = Carbon 1 source to total carbon ratio

$$r_{N1/NT}=\frac{\chi}{\chi+\chi'} \qquad \textbf{B.42}$$

where: $r_{N1/NT}$ = Nitrogen 1 source to total nitrogen ratio

To convert $r_{C1/CT}$ and $r_{N1/NT}$ from mass to mole values:

Using $r_{C1/CT}$ as an example, with a basis of 100 kg of total carbon source:

$$M_{C1}=100 - M_{C2} \qquad \textbf{B.43}$$

$$M_{C2}=w_{C2}\ x\ 100 \qquad \textbf{B.44}$$

where: M_{C1} = Mass of carbon source 1 (kg)
M_{C2} = Mass of carbon source 2 (kg)
w_{C2} = Mass fraction of carbon source 2

For carbon source 1 and 2:

$$m = \frac{M}{Mm} \quad \textbf{B.45}$$

where: m = Number of moles (kmol)
M = Mass (kg)
Mm = Molar mass (kg/kmol)

$$m_{CT} = m_{C1} + m_{C2} \quad \textbf{B.46}$$

where: m_{CT} = Total number of moles carbon
m_{C1} = Number of moles carbon source 1
m_{C2} = Number of moles carbon source 2

$$r_{C1/CT} = \frac{m_{C1}}{m_{CT}} \text{ x } 100 \quad \textbf{B.47}$$

A similar set of equations is required to determine $r_{N1/NT}$

Default values for $r_{C1/CT}$ and $r_{N1/NT}$ (which can be changed by the user) = 0.5

If there is no 2nd carbon or nitrogen source, $\alpha' = 0$ and $\chi' = 0$, respectively

Constraints (3)

Only nitrogen from either nitrogen source ends up in biomass. All other nitrogen is waste.
Only sulphur from sulphur source ends up in biomass. All other sulphur is waste.
Only phosphorus from nitrogen source ends in biomass. All other phosphorus is waste.

Nitrogen: $\chi_{.j} + \chi'_{.j'} = \phi_{.ab}$ **B.48**

Sulphur: $\delta_{.j} = \phi_{.ad}$ **B.49**

Phosphorus: $\varepsilon_{.w} = \phi_{.ac}$ **B.50**

Product Formation

$$\left(\text{Carbon}_p + \text{Oxygen}_p + \text{Nitrogen}_p + \text{Sulphur}_p + \text{Phosphorus}_p\right)\text{sources}$$
$$\downarrow \qquad \textbf{B.51}$$
$$\text{Biomass} + \text{Product} + \left(H_2O\right)_p + \left(CO_2\right)_p + \left(\text{Nitrogen}_p + \text{Sulphur}_p + \text{Phosphorus}_p\right)\text{wastes}$$

$$\left(\alpha C_aH_bO_cN_dP_eS_f + \alpha' C_{a'}H_{b'}O_{c'}N_{d'}P_{e'}S_{f'}\right)_p + \beta\left(O_2\right)_p + \left(\chi C_gH_hO_iN_jP_kS_l + \chi' C_{g'}H_{h'}O_{i'}N_{j'}P_{k'}S_{l'}\right)_p$$
$$+ \delta\left(C_mH_nO_oN_pP_qS_r\right)_p + \varepsilon\left(C_sH_tO_uN_vP_wS_x\right)_p$$
$$\downarrow$$
$$\varphi C_yH_zO_{aa}N_{ab}P_{ac}S_{ad} + \varphi C_{ae}H_{af}O_{ag}N_{ah}P_{ai}S_{aj} + \gamma\left(H_2O\right)_p + \eta\left(CO_2\right)_p + \iota\left(N_2\right)_p + \kappa\left(SO_4^{-}\right)_p + \lambda\left(PO_4^{-}\right)_p \qquad \textbf{B.52}$$

where: p: Components related to product formation growth only

The possible chemicals and their associated formulae are given in Table B.3. Chemical products not listed in these tables can be modelled as "OTHER" with the chemical formula modified as required.

Table B.3: Chemical formulas and associated pure component densities for products obtained in the model

Product	Carbon	Hydrogen	Oxygen	Nitrogen	Sulphur	Phosphorus	Density*
Antibiotics							
Penicillin	16	17	4	2	1	0	1410
Cephalosporin	14	13	4	8	3	0	(1410)
Erythromycin	37	67	13	1	0	0	(1410)
Nocardicin	23	24	9	4	0	0	(1410)
Clavulanic acid	8	9	5	1	0	0	(1410)
Thienamycin	11	16	4	2	1	0	(1410)
OTHER	18.17	24.33	6.5	3	0.83	0	1410
Amino Acids							
Arginine	6	14	2	4	0	0	1100
Glutamine	5	10	3	2	0	0	(1393)
Phenylalanine	9	11	2	1	0	0	1290
Tyrosine	9	11	3	1	0	0	1456
Tryptophan	11	12	2	2	0	0	(1393)
Lysine	6	14	2	2	0	0	(1393)
Glysine	2	5	2	1	0	0	1607
Alanine	3	7	2	1	0	0	1401
Histidine	6	9	2	3	0	0	(1393)
Serine	3	7	3	1	0	0	1537
Proline	5	9	2	1	0	0	(1393)

Product	Carbon	Hydrogen	Oxygen	Nitrogen	Sulphur	Phosphorus	Density*
Amino Acids (cont.)							
Glutamic acid	5	9	4	1	0	0	1538
Aspartic acid	4	7	4	1	0	0	1660
Threonine	4	9	3	1	0	0	(1393)
Cysteine	3	7	2	1	1	0	(1393)
Methionine	5	11	2	1	1	0	1340
Leucine	6	13	2	1	0	0	1165
Asparagine	4	8	3	2	0	0	(1393)
Isoleucine	6	13	2	1	0	0	(1393)
Valine	5	11	2	1	0	0	1230
Enzymes							
Lipase	6	6	0	1	0	0	(1393)**
Pectinase	6	9	2	0	0	0	(1393)**
Cellulase	4	10	13	2	0	0	(1393)**
OTHER	5.33	8.33	5	1	0	0	(1393)**
Alcohols							
2,3 Butanediol	4	10	2	0	0	0	980
Butanol	4	10	1	0	0	0	810
Ethanol	2	6	1	0	0	0	789
Ethylene glycol	2	6	2	0	0	0	1113.2
Glycerol	3	8	3	0	0	0	1261
Methanol	1	4	1	0	0	0	791.8
OTHER	2.67	7.33	1.67	0	0	0	957.5
Vitamins							
Retinol	20	30	1	0	0	0	(1000)
B1, Thiamine	12	17	1	1	1	0	(1000)
B2, Riboflavin, G	17	20	6	4	0	0	(1000)
B3, Niacin	6	4	2	1	0	0	(1000)
B5, Pantothenic acid	9	17	5	1	0	0	(1000)
B6, Pyridoxine	8	11	3	1	0	0	(1000)
B7, Biotin	10	16	3	2	1	0	(1000)
B9, Folic acid	19	19	6	7	0	0	(1000)
B12, Cyanocobalamin	63	89	14	14	0	1	(1000)
Ascorbic acid	6	8	6	0	0	0	(1000)
D2, Ergocalciferol	28	44	1	0	0	0	(1000)
Vitamins (cont.)							
D3, Cholecalciferol	27	44	1	0	0	0	(1000)
Tocopherol	29	50	2	0	0	0	(1000)
Naphthoquinone	31	46	2	0	0	0	(1000)
K1	31	46	2	0	0	0	(1000)
K2	51	76	2	0	0	0	(1000)
K3, Menadione	11	8	2	0	0	0	(1000)
Carbohydrates							
Formaldehyde	1	2	1	0	0	0	1
Glucose	6	12	6	0	0	0	1540
Sucrose	12	22	11	0	0	0	1587

Product	Carbon	Hydrogen	Oxygen	Nitrogen	Sulphur	Phosphorus	Density*
Starch	6	10	5	0	0	0	1500
OTHER	6.25	11.5	5.75	0	0	0	1157
Organic acids							
Acetic acid	2	4	2	0	0	0	1049
Citric acid	6	8	7	0	0	0	1665
Formic acid	1	2	2	0	0	0	1220
Fumaric acid	4	4	2	0	0	0	1635
Gluconic acid	6	12	7	0	0	0	1230
Itaconic acid	5	6	4	0	0	0	1573
Lactic acid	3	6	3	0	0	0	1200
Malic acid	4	6	5	0	0	0	1609
Oxalic acid	2	2	4	0	0	0	1770
Propionic acid	3	6	2	0	0	0	990
OTHER	3.60	5.60	3.80	0	0	0	1394.1
Alkanes							
Butane	4	10	0	0	0	0	810
Hexadecane	16	34	0	0	0	0	773
Hexane	6	14	0	0	0	0	654.8
Methane	1	4	0	0	0	0	700
Octane	8	18	0	0	0	0	703
OTHER	7	16	0	0	0	0	728.2
Others							
OTHER	10.2	16.0	3.2	1.2	0.1	0.0	1201

*Values in brackets are average values of the group or estimates based on the density of water (1 000 kg/m^3)

**Average protein density

To solve the stoichiometric equations for product formation:

Initial values

Biomass: $\phi = 0$ (assumption that no biomass is formed)

Product: $\gamma = 1$

Unknowns (12)

Carbon source (x2): α, α'

Oxygen source: β

Nitrogen source (x2): χ, χ'

Sulphur source: δ

Phosphorus source: ε

Water waste: η

Carbon dioxide waste: ι

Nitrogen waste: φ

Sulphur waste: κ

Phosphorus waste: λ

Material balances (6)

Carbon:	$\alpha.a + \alpha'.a' + \chi.g + \chi'.g' + \delta.m + \varepsilon.s - \iota = \gamma.ae$	**B.53**
Hydrogen:	$\alpha.b + \alpha'.b' + \chi.h + \chi'.h' + \delta.n + \varepsilon.t - 2.\eta = \gamma.af$	**B.54**
Oxygen:	$\alpha.c + \alpha'.c' + 2.\beta + \chi.i + \chi'.i' + \delta.o + \varepsilon.u - \eta - 2.\iota - 2.\kappa - 4.\lambda = \gamma.ag$	**B.55**
Nitrogen:	$\alpha.d + \alpha'.d' + \chi.j + \chi'.j' + \delta.p + \varepsilon.v - \eta - 2.\varphi = \gamma.ah$	**B.56**
Phosphorus:	$\alpha.e + \alpha'.e' + \chi.k + \chi'.k' + \delta.q + \varepsilon.w - \lambda = \gamma.ai$	**B.57**
Sulphur:	$\alpha.f + \alpha'.f' + \chi.l + \chi'.l' + \delta.r + \varepsilon.x - \kappa = \gamma.aj$	**B.58**

Yield Coefficient (1)

$Y_{p/s}$ As defined by literature or experimental values in database (g/g)

$$\alpha.\left(\frac{Y_{p/s}.Mm_{C1}}{Mm_p}\right) + \alpha'.\left(\frac{Y_{p/s}.Mm_{C2}}{Mm_p}\right) = \varphi \qquad \textbf{B.59}$$

where: Mm_p = Molar mass product

$$\frac{Y_{p/s}}{Mm_p}.\left(\alpha.Mm_{C1} + \alpha'.Mm_{C2}\right) = \varphi \qquad \textbf{B.60}$$

$$\frac{Y_{p/s}}{12.01.ae + 1.01.af + 16.00.ag + 14.01.ah + 30.97.ai + 32.07.aj}.\left(\alpha.Mm_{C1} + \alpha'.Mm_{C2}\right) = \varphi \qquad \textbf{B.61}$$

$$\left(\frac{Y_{p/s}}{12.01.ae + 1.01.af + 16.00.ag + 14.01.ah + 30.97.ai + 32.07.aj}\right). \left(\begin{matrix}\alpha.\left(12.01.a + 1.01.b + 16.00.c + 14.01.d + 30.97.e + 32.07.f\right) + \\ \alpha'.\left(12.01.a' + 1.01.b' + 16.00.c' + 14.01.d' + 30.97.e' + 32.07.f'\right)\end{matrix}\right) = \varphi \qquad \textbf{B.62}$$

Ratios (2)

[As for Equations B.41 – B.47 (biomass growth).]

Constraints (3)

Only nitrogen from either nitrogen source ends up in product. All other nitrogen is waste.
Only sulphur from sulphur source ends up in product. All other sulphur is waste.
Only phosphorus from nitrogen source ends in product. All other phosphorus is waste.

Nitrogen: $\chi.j + \chi'.j' = \gamma.ab$ **B.63**

Sulphur: $\delta.j = \gamma.ad$ **B.64**

Phosphorus: $\varepsilon.w = \gamma.ac$ **B.65**

Under certain conditions, due to over-specification of the system *e.g.* incorrect yield coefficients, results give negative mass balance values. For example, if insufficient carbon is added (a yield coefficient restriction), negative carbon dioxide emission and oxygen consumption may result.

To overcome this, the yield coefficient is assumed inaccurate. To avoid the need for iterative solution by varying Y, the unknowns present are reduced by assuming that no oxygen enters as an oxygen source. This increases the amount of oxygen-bearing carbon input required to balance the oxygen needs, with excess carbon reporting to the waste streams. It is recognized that this approach is limiting in highly aerobic processes where a high carbon waste would signal the need for improved data or further correction on an individual case basis.

An alternative approach of assuming zero carbon dioxide emissions to correct for negative values is not considered. This approach results in an increase in the amount of oxygen-bearing carbon source needed, but removes the outlet for both carbon and oxygen waste and results in negative oxygen input values.

Anaerobic Product Formation (anaerobic processes only)

$$\left(\text{Carbon}_{ap} + \text{Nitrogen}_{ap} + \text{Sulphur}_{ap} + \text{Phosphorus}_{ap}\right)\text{sources}$$

$$\downarrow$$

$$\text{Biomass} + \text{Anaerobic Product} + \left(H_2\right)_{ap} + \left(CO_2\right)_{ap} + \left(\text{Nitrogen}_{ap} + \text{Sulphur}_{ap} + \text{Phosphorus}_{ap}\right)\text{wastes} \quad \textbf{B.66}$$

$$\left(\alpha C_a H_b O_c N_d P_e S_f + \alpha' C_{a'} H_{b'} O_{c'} N_{d'} P_{e'} S_{f'}\right)_{ap} + \left(\chi C_g H_h O_i N_j P_k S_l + \chi' C_{g'} H_{h'} O_{i'} N_{j'} P_{k'} S_{l'}\right)_{ap}$$
$$+ \delta\left(C_m H_n O_o N_p P_q S_r\right)_{ap} + \varepsilon\left(C_s H_t O_u N_v P_w S_x\right)_{ap}$$

$$\downarrow$$

$$\varphi C_y H_z O_{aa} N_{ab} P_{ac} S_{ad} + \varphi C_{ae} H_{af} O_{ag} N_{ah} P_{ai} S_{aj} + \gamma\left(H_2\right)_{ap} + \eta\left(CO_2\right)_{ap} + \iota\left(N_2\right)_{ap} + \kappa\left(SO_4^-\right)_{ap} + \lambda\left(PO_4^-\right)_{ap} \quad \textbf{B.67}$$

Table B.4: Chemical formula for anaerobic products in the model

Anaerobic Product	Carbon	Hydrogen	Oxygen	Nitrogen	Sulphur	Phosphorus
2, 3-butanediol	4	10	2	0	0	0
Butanol	4	10	0	0	0	0
Butyric acid	4	8	2	0	0	0
Ethanol	2	6	0	0	0	0
Lactate	3	6	3	0	0	0
Methane	1	4	0	0	0	0
Propanol	3	8	1	0	0	0
Propionic acid	3	6	2	0	0	0
Succinic acid	4	6	4	0	0	0
OTHER	3.11	7.11	1.56	0	0	0

To solve the stoichiometric equations for anaerobic product formation:

Initial values

Biomass: $\phi = 0$

Anaerobic Product: $\gamma = 1$

Unknowns (11)

Carbon source (x2): α, α'

Nitrogen source (x2): χ, χ'

Sulphur source: δ

Phosphorus source: ε

Water waste: η

Carbon dioxide waste: ι

Nitrogen waste: φ

Sulphur waste: κ

Phosphorus waste: λ

Material balances (6)

Carbon: $\alpha.a + \alpha'.a' + \chi.g + \chi'.g' + \delta.m + \varepsilon.s - \iota = \gamma.ae$ **B.68**

Hydrogen: $\alpha.b + \alpha'.b' + \chi.h + \chi'.h' + \delta.n + \varepsilon.t - 2.\eta = \gamma.af$ **B.69**

Oxygen: $\alpha.c + \alpha'.c' + \chi.i + \chi'.i' + \delta.o + \varepsilon.u - \eta - 2.\iota - 2.\kappa - 4.\lambda = \gamma.ag$ **B.70**

Nitrogen: $\alpha.d + \alpha'.d' + \chi.j + \chi'.j' + \delta.p + \varepsilon.v - \eta - 2.\varphi = \gamma.ah$ **B.71**

Phosphorus: $\alpha.e + \alpha'.e' + \chi.k + \chi'.k' + \delta.q + \varepsilon.w - \lambda = \gamma.ai$ **B.72**

Sulphur: $\alpha.f + \alpha'.f' + \chi.l + \chi'.l' + \delta.r + \varepsilon.x - \kappa = \gamma.aj$ **B.73**

Ratios (2)

[As for Equations B.41 – B.47 (biomass growth).]

Constraints (3)

[As for Equations B.63 – B.65 (product formation).]

Raw materials

Carbon source

Table B.5: Chemical formula for carbon sources used in the generic model

Carbon source	Carbon	Hydrogen	Oxygen	Nitrogen	Sulphur	Phosphorus
Carbohydrates						
Bagasse, Lactose, Maltose, Sucrose	12	22	11	0	0	0
Carob extract[1]	6	11.35	5.68	0	0	0
Cellulose	6	10	5	0	0	0
Glucose, Fructose, Galactose and Mannose	6	12	6	0	0	0
Hemicellulose	6	11	5	0	0	0
Ribose	5	10	5	0	0	0
Starch	6	10	5	0	0	0
Whey[2]	1	1.84	0.46	0.27	0.02	0
OTHER	7.6	14.15	7.01	0	0	0
Hydrocarbons						
n-alkanes	15	32	0	0	0	0
Chemical industry wastes	8	16	1	0	0	0
Ethane	2	6	0	0	0	0
Ethanol	2	6	1	0	0	0
Fuel Oil	15	26.5	0.3	0	0.2	0
Methanol	1	4	1	0	0	0
Natural gas/methane	1	4	0	0	0	0
Propane	3	8	0	0	0	0
OTHER	5.88	12.81	0.41	0	0	0

Carbon source	Carbon	Hydrogen	Oxygen	Nitrogen	Sulphur	Phosphorus
Volatile fatty acids/Organic acids						
Acetic acid	2	4	2	0	0	0
Butyric acid	4	8	2	0	0	0
Citric acid	6	8	7	0	0	0
Formic acid	1	2	2	0	0	0
Fumaric acid	4	4	4	0	0	0
Volatile fatty acids/Organic acids (cont.)						
Gluconic acid	6	12	7	0	0	0
Itaconic acid	5	6	4	0	0	0
Lactic acid	3	6	3	0	0	0
Malic acid	4	6	5	0	0	0
Oxalic acid	2	2	4	0	0	0
Propionic acid	3	6	2	0	0	0
OTHER	3.64	5.82	3.82	0	0	0
Proteins						
Actin, Collagen, Corn steep liquor, Peptione, Tubulin[3]	5.13	9.16	2.33	1.34	0	0
Alanine	3	7	2	1	0	0
Arginine	6	14	2	4	0	0
Asparginine	4	8	3	2	0	0
Aspartic acid	4	7	4	1	0	0
Cysteine	3	7	2	1	1	0
Glutamic acid	5	9	4	1	0	0
Glutamine	5	10	3	2	0	0
Glysine	2	5	2	1	0	0
Histidine	6	9	2	3	0	0
Isoluecine, Isoluecine	6	13	2	1	0	0
Lysine	6	14	2	2	0	0
Methionine	5	11	2	1	1	0
Phenylalanine	9	11	2	1	0	0
Proline	5	9	2	1	0	0
Serine	3	7	3	1	0	0
Threonine	4	9	3	1	0	0
Tryptophan	11	12	2	2	0	0

Carbon source	Carbon	Hydrogen	Oxygen	Nitrogen	Sulphur	Phosphorus
Tyrosine	9	11	3	1	0	0
Valine	5	11	2	1	0	0
Yeast extract[4]	1	1.76	0.53	0.17	0.005	0.01
OTHER	5.13	9.16	2.33	1.34	0	0
OTHER						
Corn stover[5]	21	30.7	14	0.25	0.02	0
Glycerol	3	8	3	0	0	0
Mannitol	6	14	6	0	0	0
Pyruvate	3	4	3	0	0	0
Succinate	4	6	4	0	0	0
Sulphite liquor	7	8	2	0	0	0
Whiskey distillery wash[6]	2	6	1	0	0	0
Wood[7]	20	29	13.8	0.01	0.003	0
OTHER	8.25	13.21	5.85	0.03	0	0

Notes:

1 – From average sugar composition (sucrose, 65 % fructose and glucose, 35 %)
2 – Average of amino acid values – protein
3 - Average composition of group used
4 – Assumed same composition as yeast
5- Reference: Phyllis (2006)
6 – Pure ethanol assumed
7 – Reference: Phyllis (2006)

Nitrogen source

Table B.6: Chemical formula for nitrogen sources used in the generic model

Nitrogen source	Carbon	Hydrogen	Oxygen	Nitrogen	Sulphur	Phosphorus
Organic						
Actin, Collagen, Peptone, Tubulin[1]	5.13	9.16	2.33	1.34	0	0
Alanine	3	7	2	1	0	0
Arginine	6	14	2	4	0	0
Asparginine	4	8	3	2	0	0
Aspartic acid	4	7	4	1	0	0
Corn stover[2]	21	30.7	14	0.25	0.02	0
Cysteine	3	7	2	1	0	0
Glutamic acid	5	9	4	1	0	0
Glutamine	5	10	2	3	0	0
Glysine	2	5	2	1	0	0
Histidine	6	9	2	3	0	0

Nitrogen source	Carbon	Hydrogen	Oxygen	Nitrogen	Sulphur	Phosphorus
Isoleusine, Leusine	6	13	2	1	0	0
Lysine	6	14	2	2	0	0
Methionine	5	11	2	1	1	0
Phenylalanine	9	11	2	1	0	0
Proline	5	9	2	1	0	0
Serine	3	7	3	1	0	0
Threonine	4	9	3	1	0	0
Tryptophan	11	12	2	2	0	0
Tyrosine	9	11	3	1	0	0
Urea	1	4	1	2	0	0
Valine	5	11	2	1	0	0
Yeast extract[3]	1	1.76	0.53	0.17	0.005	0.01
OTHER	5.57	10	2.74	1.36	0	0
Inorganic						
Ammonia	0	3	0	1	0	0
Ammonium nitrate	0	4	3	2	0	0
Ammonium sulphate	0	8	4	2	1	0
Nitric acid	0	1	3	1	0	0
Nitrogen (gas)	0	0	0	2	0	0
OTHER	0	3.2	2	1.6	0	0

Notes:

1 – Chemical composition as defined in carbon source above
2- Reference: Phyllis (2006)
3 – Assumed same composition as yeast

Oxygen source

Table B.7: Chemical formula for oxygen sources used in the generic model

	Oxygen	Nitrogen
Oxygen	2	0
Air	2	2

Initial values and Constants

Number of moles of air into reactor ($m_{air,\ in,\ rct}$) = *[As calculated in biomass growth and product formation – excluding excess]*

Gas Constant (R) = 8.314 kJ/kg.K

$$V_{air,\,in,\,rct} = \frac{m_{air,\,in,\,rct}.R.T_{air,\,in,\,rct}}{P_{air,\,in,\,rct}} \qquad \textbf{B.74}$$

where: $V_{air,\,in,\,rct}$ = Volume of air into reactor (m^3)
$T_{air,\,in,\,rct}$ = Temperature of air into reactor (K)
$P_{air,\,in,\,rct}$ = Pressure of air into reactor (kPa)

$$a_{rct,\,min} = \frac{V_{air,\,in,\,rct}}{V_{rct}.\tau_{rct}} \qquad \textbf{B.75}$$

where: $a_{rct,\,min}$ = Minimum aeration rate for reactor (vvm) ($m^3/m^3/min$)

$$a_{rct} = 10.a_{rct,\,min} \qquad \textbf{B.76}$$

where: a_{rct} = Aeration rate for reactor (vvm) ($m^3/m^3/min$)

While ten times the minimum aeration rate is used as a default, aeration rate may be much higher than this to aid in mixing and ensure efficient mass transfer.

Sulphur source

Table B.8: Chemical formula for sulphur sources used in the generic model

	Carbon	Hydrogen	Oxygen	Nitrogen	Sulphur	Phosphorus	Other
Ammonia sulphate	0	8	4	2	1	0	-
Calcium sulphate	0	0	8	0	2	0	Ca
Iron sulphate	0	0	4	0	1	0	Fe
Magnesium sulphate	0	0	4	0	1	0	Mg
Potassium sulphate	0	0	4	0	1	0	K
Sodium sulphate	0	0	4	0	1	0	Na
OTHER	0	0	4	0	1	0	-

Phosphorus source

Table B.9: Chemical formula for phosphorus sources used in the generic model

	Carbon	Hydrogen	Oxygen	Nitrogen	Sulphur	Phosphorus	Other
	C	H	O	N	S	P	-
Phosphoric acid	0	3	4	0	0	1	-
K_2HPO_4	0	1	4	0	0	1	2 x K
KH_2PO_4	0	2	4	0	0	1	K
Na_2HPO_4	0	1	4	0	0	1	Na
NaH_2PO_4	0	2	4	0	0	1	2 x Na
OTHER	0	1.8	4	0	0	1	-

Mass Balance

Yield Coefficient (1)

$Y_{p/x}$ As defined by user (default value = 1)

Equations

Mass balance

Final values of the stoichiometric coefficients α to λ for biomass growth and product formation are calculated using the equations above and then scaled according to the product to biomass ratio ($Y_{p/x}$) defined.

For i = all materials required and formed during microbial growth and product formation

$$M_{i,T} = \left(\frac{M_{i,x}}{Y_{p/x}} \right) + M_{i,p} \qquad \textbf{B.77}$$

where: $M_{i,T}$ = Total mass of substance i required for formation of 1 kg product (kg)
$M_{i,x}$ = Mass of substance i required for formation of 1 kg biomass (kg)
$M_{i,p}$ = Mass of substance i required for formation of 1 kg product (kg)

Values are further scaled to give the desired amount of final product output.

$$M_{T,i}^{*} = M_{T,i} . S_c \qquad \textbf{B.78}$$

where: $M_{T,\,i}^{*}$ = Total mass of substance i after scaling (kg)
S_c = Scaling factor, where Sc is defined below.

If the desired product is biomass from microbial growth:

$$S_c = \frac{M_{p,\,sep1}}{\varphi} \qquad \textbf{B.79}$$

If the desired product is a metabolic product:

$$S_c = \frac{M_{p,\,sep1}}{\gamma} \qquad \textbf{B.80}$$

where: $M_{p,\,sep1}$ = Mass of product to solid-liquid separation unit 1 (kg)

The product mass ($M_{p,\,sep1}$) needed to give the final desired product output after downstream processing (including water and impurities not removed and taking losses into account), is determined by iterative solving of the mass balance problem.

$$M_{p,\,final,\,ass} = M_{p,\,final,\,req} \qquad \textbf{B.81}$$

where: $M_{p,\,final,\,ass}$ = Mass of final product assumed (kg)
$M_{p,\,final,\,req}$ = Mass of final product required (kg)

$$M_{p,\,final,\,calc} = M_{p,\,final,\,ass} + M_{w,\,ps} + M_{im} \qquad \textbf{B.82}$$

where: $M_{p,\,final,\,calc}$ = Mass of final product calculated (kg)
$M_{w,\,ps}$ = Mass of water in the final product stream (kg)
$M_{im,\,ps}$ = Mass of impurities in the final product stream (kg)

$$M_{p,\,final,\,ass}^{*} = M_{p,\,final,\,ass} - \left(\frac{M_{p,\,final,\,calc} - M_{p,\,final,\,req}}{\left(\frac{M_{p,\,final,\,calc} - M_{p,\,final,\,ass}}{M_{p,\,final,\,ass}} \right) - 1} \right) \qquad \textbf{B.83}$$

where: $M_{p,\,final,\,ass}^{*}$ = Mass of final product assumed (new value) (kg)
$M_{p,\,final,\,calc}$ = Mass of final product calculated (kg)
$M_{w,\,ps}$ = Mass of water in the final product stream (kg)
$M_{im,\,ps}$ = Mass of impurities in the final product stream (kg)

Three iterations of Equations B.81 –B.83 are performed to calculate a final product value. If the product is biomass growth, the initial concentration of biomass entering the process needs to be taken into account as part of the final product. Less biomass is needed since there is already a small portion in the system. The corrected (new) final product is given by the iteration process of Equations B.84 – B.89.

For j = 1 → 350:

$$M_{x,\,ass,\,j} = M_{x,\,req,\,j} \qquad \textbf{B.84}$$

where: $M_{x,\,ass,\,j}$ = Mass of biomass assumed (iteration j) (kg)
$M_{x,\,req,\,j}$ = Mass of biomass required (iteration j) (kg)

$$M_{x,\,calc,\,j} = M_{x,\,ass,\,j} + M_{x,\,0,\,j} \qquad \textbf{B.85}$$

where: $M_{x,\,calc,\,j}$ = Mass of biomass calculated (iteration j) (kg)
$M_{x,\,0,\,j}$ = Initial mass of biomass (iteration j) (kg)

$$M_{x,\,ass,\,j}^{*} = M_{x,\,ass,\,j} - \left(\frac{M_{x,\,calc,\,j} - M_{x,\,req,\,j}}{e_j} \right) \qquad \textbf{B.86}$$

where: $M_{x,\,ass,\,j}^{*}$ = Mass of biomass assumed (new value) (kg)
e_j = Error value for iteration j, where e is defined as below.

$e_1 = 1$ **B.87**

If $(M_{x,\,calc} - M_{x,\,req})_j > (M_{x,\,calc} - M_{x,\,required})_{j-1}$,

$e_j = -2.e_{j-1}$ **B.88**

This is used to help convergence by ensuring that the difference between the desired and calculated values in successive steps does not get larger.

else,

$e_j = e_{j-1}$ **B.89**

Next j

Yield coefficients

Under certain conditions, negative mass balance values are obtained. This is attributed to carbon limitation (a yield coefficient restriction), which results in negative carbon dioxide emissions and negative oxygen consumption. To remedy, it is assumed that yield coefficient values are inaccurate and ignored. It is further assumed that there is no oxygen source (see Chapter 2, Section 0 of the main text). Equation B.59 is replaced with Equation B.90.

$$b = 0 \qquad \textbf{B.90}$$

Maintenance Coefficient Calculations

Initial values and constants

Maintenance coefficient (m) = *[As in Table B.10]*

Time (t_{maint}) = 10 hrs (or as entered by the user) – a warning is given if maintenance time is less than residence time.

Table B.10: Values for maintenance coefficients as used in the model

Biomass	Carbon Source	Maintenance coefficient (g carbon source/g dry cell weight biomass.h)	Reference
Aerobic microbial growth			
Aerobacter aerogenes	Citrate	0.058	[13]
	Glucose	0.094	[10], [13]
	Glycerol	0.076	[10], [13]
Aerobacter cloacae	Glucose	0.094	[10], [11]
Azotobacter vinelandii	-	0.15-1.5	[8], [9]
Escherichia coli	Glucose	0.054-0.12	[2], [13]
Escherichia coli (uninduced recombinant strain)	Glucose	0.17	[2]
Escherichia coli (IPTG induced recombinant strain)	Glucose	0.32	[2]
Lactobacillus casei	-	0.135	[5], [11]
Methane bacteria	Methane	0.02	[13]
Penicillium chrysogenum	Glucose	0.022	[3], [12], [13]
Pseudomonas putida	Acetic acid	0.00552-0.07372	[6]
Saccharomyces cereviciae	Glucose	0.018	[13]
Anaerobic microbial growth			
Bacteroides ruminicola	Glucose	0.135	[14]
Butyrivibrio fibrisolvens	Glucose	0.049	[14]
Klebsiella aerogenes	-	2.88-3.69	[11], [15]

Biomass	Carbon Source	Maintenance coefficient (g carbon source/g dry cell weight biomass.h)	Reference
Megasphaera elsdenii	Glucose	0.187	[14]
Saccharomyces cerevisiae	-	0.036-0.36	[11], [16]
Selenomonas ruminantium	Glucose	0.022	[14]
Streptococcus bovis	Glucose	0.150	[14]
Zymomonas mobilis	Glucose	0.0-5.9	[1], [4], [7], [9]

References:

[1] Beyeler *et al.* 1984
[2] Bhattacharya and Dubey 1995
[3] Biwer *et al.* 2005
[4] Cromie and Doelle 1980
[5] De Vries *et al.* 1970
[6] Fieschko and Humphrey 1984
[7] Lee *et al.* 1979
[8] Nagai and Aiba 1972
[9] Oliviera *et al.* 1992
[10] Pirt 1965
[11] Pirt 1975
[12] Righelato *et al.* 1968
[13] Roels and Kossen 1978
[14] Russell and Baldwin 1979
[15] Stouthamer and Bettenhausen 1973
[16] Watson 1970

Equations

$$M_{C1,\,maint} = m.t_{maint}.M_x \qquad \textbf{B.91}$$

where: $M_{C1,\,maint}$ = Mass of carbon 1 for maintenance (kg)
m = Maintenance coefficient (g C source/g dry cell weight.h)
t_{maint} = Time for maintenance (hr)
M_x = Mass of biomass (kg)

Oxygen requirements (aerobic) are calculated Stoichiometrically according to Equation B.92

$$M_{C1,\,maint} + O_2 \rightarrow CO_2 + O_2 + \text{Energy} \qquad \textbf{B.92}$$

For anaerobic processes, an anaerobic product needs to be specified and Equation B.93 balanced.

$$M_{C1,\,maint} \rightarrow CO_2 + O_2 + \text{Anaerobic product} + \text{Energy} \qquad \textbf{B.93}$$

Growth Rate

Initial values and constants

Initial biomass concentration ($C_{x,\,0}$) = 1 g/l
Maximum specific growth rate (μ_{max}) = *[As in Table 2.6]* (assumed constant)
Half saturation constant (K_s) = *[As in Table 2.6]*
Biomass concentration (C_x) = *[As in Table 2.6]*
Ambient temperature (T_a) = 20°C
Reactor temperature (T_{rct}) = 37°C

Table B.11: Maximum specific growth rate, limiting nutrient concentration and final microbial concentrations used in the model

Organism	Limiting nutrient	Max. specific growth rate, μ_{max} (hr^{-1})	Half saturation constant, K_s (mg/l)	Concentration, r_x (g/l)	Reference
Achromobacter delvacvate	Diesel Oil			10-15	[3]
Acinetobacter sp.	Acetate	0.22		6.85	[3]
	Ethanol	0.96		1.8	[3]
	Other	0.4-2.0		8-10	[3]
Aerobacter aerogenes	Glucose	1.22	5-8		[1], [6]
Aeromona hydrophila	Lactose			1.1	[3]
Aspergillus sp.	Carob extract	0.11-0.16			[4]
Candida sp.	n-alkanes			15.2	[4]
	Sulfite liquor			10.7	[4]
	Lactose			22.5	[4]
	Other	0.5	0.2		[1]
Cellulomonas sp.	Bagasse	0.14-0.16		13.2	[3]
Chaetomium cellulolyticum	Corn stover	0.24			[4]
Cornebacterium hydrocarboclastus	Propane	0.046		0.9	[3]
Enterobacter cloacae	Glycerol	0.77			[6]
Escherichia coli	Glucose	1.1	3		[1]
	Glycerol	0.87	2		[1]. [6]
	Lactose	0.8	20		[1]
Fusarium moniliforme	Carob extract	0.22		3.3	[4]
Fusarium sp.	Glucose	0.28			[4]
	Other	0.3			[4]
Geotrichum candidum	Whiskey distillery wash	0.385		18	[4]
Klebsiella aerogenes	Glycerol	0.85-0.95	9		[1], [6]
	Glucose	0.85	10		[1], [6]
Klebsiella pneumoniae	Glucose	0.503-1.07	31		[6]
Methalococcus capsulatus	Methane	0.14		0.4	[3]
Methophilus methylotrophus	Methanol	0.38-0.50		30	[3]
Methylomonas sp.	Methanol	0.14-0.25		9.6-30.0	[3]
Norcadia sp.	n-alkanes	1.25		14.7	[3]
	Propane	0.091		30	[3]
	Butane			22	[3]
Paecilomyces variotii	Sulfite liquor	0.31		13	[4]

Organism	Limiting nutrient	Max. specific growth rate, μ_{max} (hr^{-1})	Half saturation constant, K_s (mg/l)	Concentration, r_x (g/l)	Reference
Penicillium cyclopium	Lactose			12.8	[4]
Penicillium sp.		0.2		30	[4], [5]
Protaminobacter ruber	Methanol			85	[3]
Pseudomonas sp.	Fuel oil	0.16		8-16	[3]
	Methane			0.8	[3]
Rhodopseudomonas gelatinosa		0.31		3.15-4.33	[3]
Rhizopus oligosporus		0.16			[4]
Saccharomyces cerevisiae	Glucose	0.55	25		[1]
Saccharomyces sp.	Molasses			30-80	[2]
Thermomonospora sp.	Cellulose	0.48		2.3	[3]
Trichoderma sp.		0.28			[4]
OTHER		0.50	11.86	16.70	

References:

[1] Blanch and Clark 1996
[2] Chen and Chiger 1985
[3] Litchfield 1985
[4] Solomons 1985
[5] Swartz 1985
[6] Roels 1983

Equations

$$S = \frac{M_{C1,in} + M_{C2,in}}{V_{rct}}.1000 \qquad \textbf{B.94}$$

For batch processes:

$$\tau_{rct} = \left(\ln\frac{C_x}{C_{x,0}}\right)\left(\frac{K_s + S}{S.\mu_{max}}\right) \text{ - Monod model} \qquad \textbf{B.95}$$

For continuous processes:

$$\tau_{rct} = D^{-1} \qquad \textbf{B.96}$$

where: S = Substrate concentration (mg/l)
$M_{C1,in}$ = Mass of carbon source 1 in (kg)
$M_{C2,in}$ = Mass of carbon source 2 in (kg)
V_{rct} = Reactor volume (m^3)
τ_{rct} = Residence time in reactor (hr)
$D = \mu_{max}$ = Dilution rate (hr^{-1})

B.4. Air compression

Initial values and Constants

Inlet pressure (oxygen source) ($P_{comp, in}$) = 101.325 kPa

Compressed pressure (oxygen source) ($P_{comp, out}$) = 300 kPa

Polyentropic efficiency (η_p) (Sinnot 1983):

Centrifugal compressor: 0.75

Axial compressor: 0.82

Reciprocating compressors: 0.75 (compression factor < 0.22)

0.82 (compression factor > 0.22 and <2.7)

0.85 (compression factor >2.7)

Specific heat of air ($C_{p, air}$) = $a + bT + cT^2 + dT^3$ (J/mol.K)

($a = 28.088$; $b = 1.97 \times 10^{-3}$; $c = 4.8 \times 10^{-6}$; $d = -1.965 \times 10^{-9}$)

Specific heat of oxygen ($C_{p, O2}$) = $a + bT + cT^2 + dT^3$ (J/mol.K)

($a = 25.46$; $b = 1.519 \times 10^{-2}$; $c = -7.15 \times 10^{-6}$; $d = 1.311 \times 10^{-9}$)

For isentropic compression, efficiencies are given in a range of 65 % to 85 % (compression ratios of 1.5 to 6), since compression ratios are low here. Energy calculations are based on first principles, examples of which can be seen in Branan (1976), Walas (1990), Sandler (1999) and Branan (2002).

Assumptions

The method of calculating energies is the same as shown for polyentropic efficiencies by Sinnot (1983).

The intercooled gas is the same temperature as the final gas temperature

$$\gamma = \frac{C_p}{C_v} = \frac{C_p}{C_p - R} \qquad \textbf{B.97}$$

where: γ = Ratio of specific heats

$C_p = C_{p, air}$ or $C_{p, O2}$ (depending on oxygen source) ((J/mol.K)$^{-1}$)

R = Gas constant (kJ.(kg.K)$^{-1}$)

$$\hat{W}_{comp} = P_{comp, in} V_{comp} \frac{n^*}{n^* - 1} \left(\left(\frac{P_{comp, out}}{P_{comp, in}} \right)^{n^* - 1 / n^*} - 1 \right) \qquad \textbf{B.98}$$

where: $\hat{W}_{comp}$ = Specific compression work (J/kg)

V_{comp}= Compression volume (m^3)

and

$$n^* = \frac{1}{1 - m^*} \quad \textbf{B.99}$$

with:

$$m^* = \frac{\gamma - 1}{\gamma . \eta_p} \quad \textbf{B.100}$$

where: m^* and n^* are polyentropic compression variables

$$W_{comp} = \frac{\hat{W}_{comp}}{\eta_p} . M_{comp,g,in} \quad \textbf{B.101}$$

where: W_{comp} = Compression energy (kJ)
$M_{comp,\,g,\,in}$ = Mass of gas for compression

If 2 compressors are used:

$$W_{comp,\,final} = 2 . W_{comp} \quad \textbf{B.102}$$

where: $W_{comp,final}$ = Final compression energy (kJ)

Cooling water

$$T_{comp,\,g,\,out} = T_h \left(\frac{P_{comp,\,out}}{P_{comp,\,in}} \right)^m \quad \textbf{B.103}$$

where: $T_{comp,\,g,\,out}$ = Temperature of gas out after compression (^oC)

$$M_{comp,\,cw} = \frac{M_{comp,\,g,\,in} . \left(T_{comp,\,g,\,out} - T_{rct} \right)}{T_{comp,\,cw,\,out} - T_{cw,\,in}} \quad \textbf{B.104}$$

where: $M_{comp,\,cw}$ = Mass of cooling water for compression (kg)
$T_{comp,\,cw,\,out}$ = Temperature of cooling water out – compression (^oC)

$$V_{comp,\,cw} = \frac{M_{comp,\,cw}}{\rho_w} \qquad \textbf{B.105}$$

where: $V_{comp,\,cw}$ = Volume of cooling water – compression (m^3)

B.5. Reactor cooling

Initial values and constants

Specific heat of water ($C_{p,\,w}$) = 4.1868 kJ/kg.K

Degree of reduction values

C: 4
H: 1
O: -2
N: -3
P: 5
S: 6

Equations

Energy generated during microbial growth

The degree of reduction is defined as:

$$\gamma_s = \sum_{i=\text{all components in carbon source}} m_i \cdot \gamma_{s,i} \qquad \textbf{B.106}$$

where: γ_s = Degree of reduction
m_i = Moles of component i (mol)
$\gamma_{s,\,i}$ = Degree of reduction, component i

From von Stochar *et al.* 2006, the heat generated for aerobic growth can then be calculated as follows:

$$q_{gen} = \frac{666.2}{\gamma_s} + 243.1 \rightarrow \text{for } \gamma_s \leq 4.67 \qquad \textbf{B.107}$$

$$q_{gen} = 157\,\gamma_s - 339 \rightarrow \text{for } \gamma_s > 4.67 \qquad \textbf{B.108}$$

where: q_{gen} = Heat generation (kJ/mol C)

$$E_{rct,\,cw} = q_{gen}.\Delta m_{C1} \quad \textbf{B.109}$$

where: $E_{rct,\,cw}$ = Energy to cool reactor (kJ)
Δm_{C1} = moles of carbon source 1 consumed (mol)

Cooling water required

$$T_{cw,\,out\,rct} = T_{rct} - \Delta T_{cw} \quad \textbf{B.110}$$

where: $T_{rct,\,cw,\,out}$ = Temperature of cooling water out reactor (^oC)
T_{rct} = Reactor temperature (^oC)
ΔT_{cw} = Temperature difference between exiting cooling water and cooled medium (^oC) – as defined globally

$$M_{cw,\,rct} = \frac{E_{rct,\,cw}}{C_{p,\,w}\left(T_{rct,\,cw,\,out} - T_{rct,\,cw,\,in}\right)} \quad \textbf{B.111}$$

where: $M_{cw,\,rct}$ = Mass of cooling water for reactor cooling (kg)
$T_{rct,\,cw,\,in}$ = Temperature of cooling water into reactor (^oC)

$$M_{cw,\,rct}^{*} = 0.7\left(M_{cw,\,rct}\right) \quad \textbf{B.112}$$

where: $M_{cw,\,rct}^{*}$ = Mass of cooling water for reactor cooling in anaerobic system (kg) – See Chapter 2.3.11.

B.6. Post microbial growth cooling

Initial values and constants

Outlet biomass temperature ($T_{rct,\,out}$) = 15°C
Specific heat (water, biomass and product mixture) ($C_{p,\,w}$) = 4.1868 kJ.(kg.K)$^{-1}$
Efficiency (η) = 0.30

Assumptions

The specific heat of the water, biomass and product mixture is the C_p value for the entire stream leaving the reactor and is estimated as the specific heat of water.

Equations

$$E_{post,\,cw} = M_{T,\,rct,\,out}.C_{p,\,w}.\Delta T_{post} \quad \textbf{B.113}$$

where: $E_{post, cw}$ = Energy removal required to cool mass out reactor (kJ)
$M_{T, rct, out}$ = Total mass leaving the reactor (kg)
ΔT_{post} = Temperature difference of cooled media (^oC)

$$E_{post, comp} = \frac{E_{post, cw}}{\eta_{post}} \quad \textbf{B.114}$$

where: $E_{post, comp}$ = Energy to compress coolant for post microbial growth cooling (kJ)
η_{post} = Compression efficiency for compression of coolant in post microbial cooling

B.7. Agitation

Assumptions

Fully baffled, Newtonian fluid behaviour.

Typically greater than 95 % of the liquid volume in the reactor is water, with unreacted raw material volume being negligible.

Initial values and constants

Number of tanks (N_t): 5

Ratio of tank height to tank diameter (H_t/D_t): 2

Impeller speed (N): 500 rpm

Number of impeller blades (b):

Pitch-blade turbine, paddle turbine: 4

Rushton turbine, bar turbine, radial turbine, 'other' turbine: 6

Ratio of impeller diameter to tank diameter (D_{imp}/D_t): 3

Ratio of impeller blade width to tank height (W_{imp}/H_t): 0.025

Efficiency (η_{ag}): 0.7

Residence time (τ_{rct}): *[As calculated in Equations B.95 or B.96]*

Table B.12: Dimensionless power numbers for agitation for various impeller types

Impeller type (Pitch)	Commercial impeller name	Number of impeller blades	Impeller diameter/ Tank diameter	Width of baffle/ Tank diameter	Power number	Reference
		b	D_{imp}/D_t	B/D_t	N_p	
Pitch-blade turbine	Chemineer-P4, Lightnin A200	4			1.27	[7]
Paddle turbine	Lightnin R200	4			3.4	[5]
Rushton turbine		3	0.33	0.083	3.3	[6]

Impeller type (Pitch)	Commercial impeller name	Number of impeller blades	Impeller diameter/ Tank diameter	Width of baffle/ Tank diameter	Power number	Reference
		b	D_{imp}/D_t	B/D_t	N_p	
		3	0.33	0.1	3.4	[6]
		4	0.33	0.083	4.3	[6]
		4	0.33	0.1	4.4	[6]
		5	0.33	0.083	5.4	[6]
		5	0.33	0.1	5.4	[6]
		6			5.2	[6]
	Chemineer D-6, Lightnin R100, Hayward Gordon RT	6	0.33	0.083	6	[6]
		6	0.33	0.1	6	[6]
		8	0.33	0.083	7.8	[6]
		8	0.33	0.1	7.8	[6]
		12	0.33	0.083	9.9	[6]
		12	0.33	0.1	10	[6]
Marine propeller (1.00)	Chemineer AP-3, Lightnin A100 & A110	3	0.35	0.1	0.32	[6]
(1.00)		3	0.35	0.083	0.31	[6]
(1.00)		3	0.22	0.1	0.36	[6]
(1.00)		3	0.22	0.083	0.35	[6]
(1.50)		3	0.22	0.1	0.62	[6]
(1.50)		3	0.22	0.083	0.61	[6]
(2.00)		3	0.31	0.1	1	[6]
(2.00)		3	0.31	0.083	0.99	[6]
(2.50)		3	0.22	0.1	1.35	[6]
(2.50)		3	0.22	0.083	1.34	[6]
Axial flow impeller	Lightnin A310 & A510	3			0.3	[7]
	Chemineer HE-3	3	0.4		0.22	[3]
	Lightnin A320 & A340	3			0.64	[8]
	Lightnin A315 & A345	3			0.75	[9]
Radial turbine	Chemineer CD-6, Lightnin R130, Philadelphia Mixers Smith Turbine, Hayward Gordon RDC	6			3.2	[4]
	Chemineer BT-6	6			2.3	[1]
Bar turbine	Lightnin R510	6			0.65	[7]
Saw tooth dispenser	Lightnin R500				0.45	[7]
Anchor					0.35	[2]
Helical ribbon					0.35	[2]
OTHER		4.7	0.3	0.1	2.9	

References:

[1] Bakker 2000
[2] Doran 1997
[3] Fasano *et al.* 1999
[4] Philadelphia Mixers 2006
[5] Post Mixing 2006
[6] Rushton *et al.* 1950
[7] Weetman and Oldshue 1988
[8] Weetman and Coyle 1989
[9] Weetman 1993

Equations

Power numbers are used in the agitation calculations to determine energy requirements. Before this is done, several steps are required:

1. Initial calculations for dimensions of the tank, impellor and the gas flowrate (Equations B.115 – B.123)
2. Correcting the power number for impellor width to diameter ratio (Equations B.124 – B.125)
3. Correcting the power number for viscosity differences (Equations B.126 – B.132)
4. Correcting the power number for gassed systems (Equation B.133)
5. Converting the power number to power and energy values (Equations B.135 – B.137)

1. Initial calculations

$$V_{rct,T} = V_{rct,w,in} + \left(\frac{M_{p,out,rct}}{\rho_{p,rct}} \right) + \frac{M_{rm,out,rct}}{1000} \qquad \textbf{B.115}$$

where: $V_{rct,T}$ = Total reactor volume (m^3)
$V_{rct,w,in}$ = Volume of water out reactor (m^3)
$M_{p,out,rct}$ = Mass of product out reactor (kg)
$\rho_{p,rct}$ = Density of product out reactor (kg/m^3)
$M_{rm,out,rct}$ = Mass of unreacted raw materials and biomass out reactor (kg) (it is assumed density of raw materials and biomass equals the density of water)

$$V_{rct,t} = \frac{V_{rct,T}}{N_t} \qquad \textbf{B.116}$$

where: $V_{rct,t}$ = Volume of single tank (m^3)

$$D_t = \sqrt[3]{\frac{4.V_{rct,t}}{\pi.\frac{H_t}{D_t}}} \qquad \textbf{B.117}$$

where: D_t = Single tank diameter (m)

$$H_t = D_t.\left(\frac{H_t}{D_t} \right) \qquad \textbf{B.118}$$

where: H_t = Single tank height (m)

$$D_{imp} = D_t . \left(\frac{D_{imp}}{D_t} \right) \quad \textbf{B.119}$$

where: D_{imp} = Impeller diameter (m)

$$W_{imp} = H_t . \left(\frac{W_{imp}}{H_t} \right) \quad \textbf{B.120}$$

where: W_{imp} = Impeller blade width (m)

$$\rho_{rct,\,T} = \frac{M_{p,\,out,\,rct} + \rho_w \left(V_{w,\,out,\,rct} \right)}{\left(\frac{M_{p,\,out,\,rct}}{\rho_{p,\,out,\,rct}} \right) + V_{w,\,out,\,rct}} \quad \textbf{B.121}$$

where: $\rho_{rct,\,T}$ = Overall density of material in reactor (kg/m^3)
$M_{p,\,out,\,rct}$ = Mass of product out reactor (kg)
$V_{w,\,out,\,rct}$ = Volume of water out reactor (kg)
ρ_ω = Density of water (1000 kg/m^3)
$\rho_{p,\,out,\,rct}$ = Density of product out reactor (kg/m^3)
Assumption: Density calculation based on water and product masses and volumes only. Change on volume owing to mixing is assumed negligible.

$$V_{g,\,rct} = \frac{m_{g,\,rct} . R . T_a}{P} \quad \textbf{B.122}$$

where: $V_{g,\,rct}$ = Volume of gas for aeration in reactor (m^3)
$m_{g,\,rct}$ = Number of moles gas in reactor for aeration (mol)
R = Gas constant (kJ.(kg.K)$^{-1}$)
T_a = Ambient temperature (^{o}C)
$P_{comp,\,g,\,rct}$ = Compressed gas pressure in reactor (kPa)

$$Q_t = \frac{V_{g,\,rct}}{\tau_{rct} . N_t} \quad \textbf{B.123}$$

where: Q_t = Volumetric gas flow rate per tank (kg/s)

2. Correcting for W_{imp}/D_{imp} ratio

To correct for different width to diameter ratios of impellor blades, the following equations are used:

4 blade impellers:

$$N_p = N_{p^*}\left(\frac{\left(\frac{W_{imp}}{D_{imp}}\right)_{act}}{\left(\frac{W_{imp}}{D_{imp}}\right)_{ref}}\right)^{1.25} \qquad \textbf{B.124}$$

6 blade impellers:

$$N_p = N_{p^*}\left(\frac{\left(\frac{W_{imp}}{D_{imp}}\right)_{act}}{\left(\frac{W_{imp}}{D_{imp}}\right)_{ref}}\right) \qquad \textbf{B.125}$$

where: N_p = Power number

N_p^* = Old power number

$(W_{imp}/D_{imp})_{act}$ = Original W_{imp}/D_{imp} ratio

$(W_{imp}/D_{imp})_{ref}$ = [Reference W_{imp}/D_{imp} ratio] given by:

- Pitch-blade turbine, paddle turbine: 1/6 (Dickey 1984)
- Rushton turbine: 1/5 (Dickey 1984)
- All other turbines not corrected for W_{imp}/D_{imp} differences.

3. Correcting for viscosity differences

It is seen that viscosity does not play a part in the power number in the model. This is because the Reynolds number (N_{Re}) is greater than 3500. Dickey (1984) showed that viscosity only affects power numbers for Reynolds number values less than 1000. Therefore, since the Reynolds number of 3500 is for conservative conditions, and expected to increase under other operating parameters, viscosity effects are assumed not to play a part in agitation energy requirements. Below is the calculation to show this why N_{Re} is above 1000.

The density of microbial biomass is typically in the order of 1100 $kg.m^{-3}$ (Krijgsman 1992). This can vary from 1003 $kg.m^{-3}$ in fungi, to 1030 $kg.m^{-3}$ in bacteria, up to 1090 $kg.m^{-3}$ in yeast cells (Aiba *et al.* 1965).

$$V_{x,\,out,\,rct} = \rho_x \,.\, M_{x,\,out,\,rct} \qquad \textbf{B.126}$$

where: $V_{x,\,out,\,rct}$ = Volume of biomass out reactor (m^3)
ρ_x = Biomass density (kg/m^3)
$M_{x,\,out,\,rct}$ = Mass of biomass out reactor (kg)

$$y_{x,\,rct} = \frac{V_{x,\,rct}}{V_{rct,\,T}} \qquad \textbf{B.127}$$

where: $y_{x,\,rct}$ = Volume fraction of biomass in reactor (m^3/m^3)
$V_{x,\,rct}$ = Volume of biomass in reactor (m^3)
$V_{rct,\,T}$ = total volume in reactor (m^3)

The viscosity of the cell suspension (μ_x^*) can be calculated by the Einstein equation (B.128), a simplification of the Vand Equation (B.129) for low volume fractions up to 14 % (Blanch and Clarke 1996).

$$\mu_{x^*} = \mu_w .\left(1 + 2.5 y_{x,\,rct}\right) \qquad \textbf{B.128}$$

$$\mu_{x^*} = \mu_w .\left(1 + 2.5 y_{x,\,rct} + 7.25 y_{x,\,rct}{}^2\right) \qquad \textbf{B.129}$$

where: μ_x^* = Viscosity of cell suspension (Pa.s)
μ_w = Viscosity of water (Pa.s)

Where μ_w is the liquid viscosity and $y_{x,\,rct}$ the volume fraction cells. For a liquid viscosity (μ_w) of 0.001 Pa.s (1 cP) and assuming a high biomass volume fraction of 14 %, using the Vand Equation (B.129):

$$\mu_x^* = 0.001\ (1 + 2.5(0.14) + 7.25(0.14)^2) \qquad \textbf{B.130}$$

$$\mu_x^* = 0.0015 \text{ Pa.s} \qquad \textbf{B.131}$$

Calculating the Reynolds (N_{Re}) number from this viscosity and conservative reaction conditions ($D_{imp} = 0.05$; $N = 125$ rpm; $\rho_w = 1000\ kg.m^{-3}$)

$$N_{Re} = \frac{D_{imp}{}^2 N \rho_w}{\mu_{x^*}} \qquad \textbf{B.132}$$

$\therefore N_{Re} = 3500$

4. Correcting for gassed systems differences (Atkinson and Mavituna 1983)

Gassed systems require less power than ungassed systems because of an effective reduction in density the gas has on the liquid.

$$N_{p,g'} = 0.72\left(\frac{N_p{}^2 N.D_{imp}{}^3}{Q_t{}^{0.56}}\right)^{0.45} \quad \text{(Michel and Miller 1962)} \qquad \textbf{B.133}$$

where: $N_{p,g'}$ = Power number for a gassed system
N_p = Power number
N = Impeller speed (s^{-1})
D_{imp} = Impeller diameter (m)
Q_t = Volumetric gas flow rate per tank (kg/s)

5. Calculating power and energy requirement

$$P_{ag,t} = N_p.\rho_{rct,T}.N.D_{imp}{}^3 \qquad \textbf{B.134}$$

where: $P_{ag,t}$ = Power for agitation for single tank(W)
$N_p = N_{p,g'}$ for gassed systems and N_p for non-gassed systems

$$Pv_{ag,t} = \frac{P_{ag,t}}{V_t} \qquad \textbf{B.135}$$

where: $Pv_{ag,t}$ = Power for agitation for single tank (W)

$$P_{rct,T} = Pv_{ag,t}.V_{rct,T} \qquad \textbf{B.136}$$

where: $P_{rct,T}$ = Power for agitation for reactor (W)
$V_{rct,T}$ = Total reactor volume (m^3)

$$E_{ag,rct} = \frac{P_{rct,T}.\tau_{rct}}{\eta_{ag}} \qquad \textbf{B.137}$$

where: $E_{ag,rct}$ = Energy for agitation for reactor (kJ)
η_{ag} = Agitation efficiency

The following are alternative correlations for gassed power requirements which could replace Equation B.133. These correlations could be included in future versions of the model. Further agitation literature is reviewed in Harnby *et al.* (1992).

Table B.13: Alternative correlations for gassed power requirements (as shown in Mann 1983 and presented by Atkinson and Mavituna 1983)

Equation		Reference
$\frac{N_{p,g'}}{N_p} = 1 - 1.6Qv.D_{imp}^{0.63}$	N_p = un-gassed power number $N_{p,g'}$ = gassed power number Qv = max. total gas flow rate per unit liquid volume (vvm)	Pharamond *et al.* (1975)
$\frac{N_{p,g'}}{N_p} = \text{constant}\left(\frac{PN^2D_{imp}^3}{\sigma}\right)^{-0.25}\left(\frac{Q}{ND_{imp}^3}\right)^{-0.38}$	Constant depends on geometry and varies with ionic strength σ = interfacial tension (N.m-1)	Hassan and Robinson (1979)
$N_{p,g'} = 0.83\left(\frac{N_pND_{imp}^3}{Q^{0.56}}\right)^{0.45}$		Loiseau *et al.* (1977)
Modified correlation for foaming systems		
$\frac{N_{p,g'}}{N_p} = 0.497\left(\frac{\rho_{rct,T}N^2D_{imp}^3}{\sigma}\right)^{-0.18}\left(\frac{Q}{ND_{imp}^3}\right)^{-0.38}$		Luong and Volesky (1979)
$N_{p,g'} = 0.812\left(\frac{N_pND_{imp}^3}{Q^{0.56}}\right)^{0.45}$		Yung *et al.* (1979)
$N_{p,g'} = (\text{constant 1})0.812N_p\exp(-(\text{constant 2}).Q)$	Constants depend on the gas flowrate Q	Brown (1981)
$N_p = 1007\left(\frac{N^{3.33}D_{imp}^{6.33}}{(\eta^*Q)^{0.4}}\right)^{0.45}$	η^* = Dispersion efficiency factor dependent upon regime of mixing	Greaves and Kobaccy (1981)
$\frac{N_{p,g'}}{N_p} = 0.10\left(\frac{Q}{NV_{rct,T}^3}\right)^{-1/4}\left(\frac{N^2D_{imp}^4}{g.D_{imp}V_{rct,T}^{2/3}}\right)^{-1/5}$	$V_{rct,T}$ = total volume in reactor (m^3) g = gravitational constant	Hughmark (1980)

Additional (ungassed) power number calculations are given in Atkinson and Mavituna (1983), derived directly from Rushton *et al*. (1950).

Flat bladed open turbines: $N_P = 0.035\rho_{rct,T}N^3D_{imp}^{3.7}W_{imp}N_{imp}^{0.8}N_b^{0.4}B^{0.3}$ **B.138**

Curve-bladed open turbines: $N_P = 0.0085\rho_{rct,T}N^3D_{imp}^{3.7}W_{imp}N_{imp}^{0.8}N_b^{0.4}B^{0.3}$ **B.139**

Disc (Rushton) turbines: $N_P = 0.25\rho_{rct,T}N^3D_{imp}^{2.2}W_{imp}L_{imp}^{1.5}N_{imp}^{0.8}N_b^{0.4}B^{0.3}$ **B.140**

where: N_p = Power number
$\rho_{rct, T}$ = Overall density of material in reactor (kg/m^3)
N = Impeller speed (s^{-1})
D_{imp} = Impeller diameter (m)
W_{imp} = Impeller width (m)
L_{imp} = Impeller length (m)
N_{imp} = Number of impeller blades
N_b = Number of baffles
B = Baffle width (m)
L_{imp} = Impeller length (m)

B.8. Solid-Liquid Separation

Initial values

Solid or product (mass) fraction removed from solid-liquid separation unit x ($w_{sol, sepx}$):
- Centrifugal spin/washing = 0.98
- Filtration = 0.95
- Sedimentation = 0.90
- OTHER = 0.94

Liquid or waste (mass) fraction removed from solid-liquid separation unit x ($w_{l, sepx}$):
- Centrifugal spin/washing = 0.80
- Filtration = 0.70
- Sedimentation = 0.60
- OTHER = 0.70

Equations

The fraction of a component removed depends on the component and product phase. Water (Solid-liquid separation unit 1) is given as an example in Equations B.141 and B.142 for wastewater (liquid) removal with a solid product.

$$M_{w, out, sepx} = M_{w, in, sepx}.(1 - w_{l, sepx}) \quad \textbf{B.141}$$

where: $M_{w, out, sepx}$ = Mass of water out of solid-liquid separation unit x (kg)
$M_{w, in, sepx}$ = Mass of water into solid-liquid separation unit x (kg)
$w_{l, sepx}$ = Liquid (mass) fraction removed from solid-liquid separation unit x (kg/kg)

$$M_{w,\,in,\,sepx} = M_{w,\,out,\,rct} \qquad \textbf{B.142}$$

where: $M_{w,\,out,\,rct}$ = Mass of water out of reactor (kg)

Centrifugal spin/washing

Initial values and constants

Number of spin/wash cycles in solid-liquid separation unit x ($N_{sp,\,sepx}$) = 2

Efficiency of centrifugation in solid-liquid separation unit x ($\eta_{cen,\,sepx}$) = 0.6

Energy per unit volume for centrifugation in solid-liquid separation unit x ($\hat{E}_{cen,\,sepx}$) = *[As selected from Table B.14]*

Table B.14: Energy per unit volume values as used in model for different centrifuge types

Centrifuge type	Energy needed ($\hat{E}_{cen,\,sepx}$)					
	kWh/1000gal		kJ/m^3			
	min.	max.	min.	max.	Average	Value used
Tubular	1	10	951.0	9510.2	5230.6	5230
Disk	1	10	951.0	9510.2	5230.6	5230
Nozzle-discharge disk	2	12	1902.0	11412.2	6657.1	6660
Helical-conveyor decanter	3	15	2853.1	14265.3	8559.2	8560
Other			1664.3	11174.5	6419.4	6420

Reference: Perry *et al.* 1984

Equations

$$V_{sepx,\,T} = V_{sepx,\,w,\,in} + \left(\frac{M_{p,\,out,\,sepx}}{\rho_{p,\,sepx}}\right) + \frac{M_{rm,\,out,\,sepx}}{1000} \qquad \textbf{B.143}$$

where: $V_{sepx,\,T}$ = Total volume of solid-liquid separation unit x (m^3)

$V_{sepx,\,w,\,in}$ = Volume of water in solid-liquid separation unit x (m^3)

$M_{p,\,out,\,sepx}$ = Mass of product out solid-liquid separation unit x (kg)

$\rho_{p,\,sepx}$ = Density of product out solid-liquid separation unit x (kg/m^3)

$M_{rm,\,out,\,sepx}$ = Mass of unreacted raw materials and biomass out solid-liquid separation unit x (kg) (it is assumed density of raw materials and biomass equals the density of water → 1000 kg/m^3)

$$E_{cen,\,sepx} = V_{sepx,\,T} \cdot \frac{\hat{E}_{cen,\,sepx}}{\eta_{cen,\,sepx}} \qquad \textbf{B.144}$$

where: $E_{cen,\,sepx}$ = Energy requirement for centrifugation in solid-liquid separation unit x (kJ)

$$M_{p,\,in,\,sepx} = \frac{M_{p,\,out,\,sepx}}{1-\left(1-w_{sol,\,sepx}\right)\left(\frac{1-\left(w_{sol,\,sepx}\right)^{N_{sp,\,sepx}}}{1-w_{sol,\,sepx}}\right)} \qquad \textbf{B.145}$$

where: $M_{p,\,in,\,sepx}$ = Mass of product into solid-liquid separation unit x (kg)
$M_{p,\,out,\,sepx}$ = Mass of product out of solid-liquid separation unit x (kg)
$w_{sol,\,sepx}$ = Solid (mass) fraction removed from solid-liquid separation unit x (kg/kg)
$N_{sp,\,sepx}$ = Number of spin/wash cycles in solid-liquid separation unit x

$$\Delta M_{p,\,sepx} = M_{p,\,in,\,sepx} - M_{p,\,out,\,sepx} \qquad \textbf{B.146}$$

where: $\Delta M_{p,\,sepx}$ = Mass of product lost in solid-liquid separation unit x (kg)

$$\Delta M_{w,\,sepx} = M_{w,\,out,\,sepx} . N_{sp,\,sepx} \qquad \textbf{B.147}$$

where: $\Delta M_{p,\,sepx}$ = Mass of water lost in solid-liquid separation unit x (kg)
$M_{w,\,out,\,sepx}$ = Mass of product lost in solid-liquid separation unit x (kg)

$$M_{w,\,ad,\,sepx} = M_{w,\,out,\,sepx} . (N_{sp,\,sepx} - 1) \qquad \textbf{B.148}$$

where: $M_{w,\,ad,\,sepx}$ = Mass of water added in solid-liquid separation unit x to make up for spin/wash cycles of centrifugation (kg)

Filtration

Initial values and constants

Pressure difference of filtration in solid-liquid separation unit x ($\Delta P_{f,\,sepx}$) = 250 kPa
Flux of filtration in solid-liquid separation unit x ($J_{f,\,sepx}$) = 10 $kg.s^{-1}$
Pumping efficiency of filtration in solid-liquid separation unit x ($\eta_{f,\,sepx}$) = 0.6
Volume fraction flocculent in solid-liquid separation unit x ($y_{fa,\,sepx}$) = 0.01
Density of flocculent in solid-liquid separation unit x ($\rho_{fa,\,sepx}$) = *[As selected from Table B.15]*

Table B.15: Flocculent chemical compositions and densities as used in the model

Flocculent	C	H	O	N	P	S	Other	Density
Alum	0	24	20	0	0	2	K, Al	2690
Aluminium chlorohydrate	0	24	24	0	0	4	12 x Cl, 12 x Al	1350
Aluminium sulphate	0	32	28	0	0	3	2 x Al	2710
Calcium oxide (Lime)	0	0	1	0	0	0	Ca	3350
Iron (III) chloride	0	0	0	0	0	0	Fe, 3 x Cl	2800
Iron (II) sulphate	0	8	8	0	0	1	Fe	1200
Sodium aluminate	0	0	1.8	0	0	0	Na, K, Al	1467
Sodium silicate	0	0	3	0	0	0	2 x Na, Si	2400
Other	0	11	10.73	0	0	1.25	-	2245.88

Equations

$$t_{f,\,sepx} = \frac{M_{in,\,sepx,\,T}}{J_{f,\,sepx}} \qquad \textbf{B.149}$$

where: $t_{f,\,sepx}$ = Time for filtration in solid-liquid separation unit x (hr)
$M_{in,\,sepx,\,T}$ = Total mass in to solid-liquid separation unit x

$$A_{f,\,sepx} = \sqrt[3]{V_{sepx,\,T}} \qquad \textbf{B.150}$$

where: $A_{f,\,sepx}$ = Cross sectional area (adsorption) in solid-liquid separation unit x (m^2)
$V_{sepx,\,T}$ = Total volume in solid-liquid separation unit x (kg) – as given by Equation B.143

$$v_{f,\,sepx} = \frac{V_{w,\,in,\,sepx}}{A_{f,\,sepx}.t_{f,\,sepx}} \qquad \textbf{B.151}$$

where: $V_{f,\,sepx}$ = Linear velocity through solid-liquid separation unit x for filtration ($m.s^{-1}$)
$V_{w,\,in,\,sepx}$ = Volume of water in solid-liquid separation unit x (m^3)

Note: Only the volume of water is assumed as it forms by far the largest portion of the total volume. The volumes of the raw materials are not considered as some will dissolve and calculating the volume of mixing is beyond the level of detail for this model.

$$P_{f,\,sepx} = \frac{\Delta P_{f,\,sepx} \cdot v_{f,\,sepx}}{\eta_{f,\,sepx}} \qquad \textbf{B.152}$$

where: $P_{f,\,sepx}$ = Power for filtration in solid-liquid separation unit x (kJ/s)

$$E_{f,\,sepx} = P_{f,\,sepx} \cdot t_{f,\,sepx} \qquad \textbf{B.153}$$

where: $E_{f,\,sepx}$ = Energy for filtration in solid-liquid separation unit x (kJ)

$$V_{sepx,\,T} = V_{sep,\,w,\,in} + \left(\frac{M_{p,\,out,\,sepx}}{\rho_{p,\,sepx}} \right) + \frac{M_{rm,\,out,\,sepx}}{1000} \qquad \textbf{B.154}$$

where: $V_{sepx,\,T}$ = Total volume in solid-liquid separation unit x (m^3)
$V_{sepx,\,w,\,in}$ = Volume of water out solid-liquid separation unit x (m^3)
$M_{p,\,out,\,sepx}$ = Mass of product out solid-liquid separation unit x (kg)
$\rho_{p,\,sepx}$ = Density of product out solid-liquid separation unit x (kg/m^3)
$M_{rm,\,out,\,sepx}$ = Mass of unreacted raw materials and biomass out solid-liquid separation unit x (kg) (it is assumed density of raw materials and biomass equals the density of water)

$$V_{fa,\,sepx,\,in} = y_{fa,\,sepx} \cdot V_{sepx,\,T} \qquad \textbf{B.155}$$

where: $V_{fa,\,sepx,\,in}$ = Volume of flocculent in solid-liquid separation unit x (m^3)

$$M_{fa,\,sepx,\,in} = V_{fa,\,sepx,\,ina} \cdot \rho_{fa,\,sepx} \qquad \textbf{B.156}$$

where: $M_{fa,\,sepx,\,in}$ = Mass of flocculent in solid-liquid separation unit x (m^3)

Filter media calculations

Initial values and constants

Filter media height in solid-liquid separation unit x ($H_{fm,\,sepx}$) = 0.1 m

Density (filter media) in solid-liquid separation x ($\rho_{fm,\,sepx}$) = [As determined by filter media type in Table B.16]

Filter media voidage in solid-liquid separation unit x ($e_{fm,\,sepx}$) = [As determined by filter media type in Table B.16]

Frequency filter media is changed in solid-liquid separation unit x ($f_{fm,\,sepx}$):

Batch: 1 per batch

Continuous process: 1 per campaign

Table B.16: Filter medium and initial voidages of the model

Filter media	Density (ρ_{fm})	Voidage (e_{fm})
Diatomaceous earth	152	0
Filter paper	700	0
Expanded perlite	1100	0.6
Sintered glass	2500	0.3
Wire mesh	7700	0.2
OTHER	2430.4	0.22

Equations

$$V_{fm,\,sepx} = H_{fm,\,sepx} . A_{f,\,sepx} \qquad \textbf{B.157}$$

where: $V_{fm,\,sepx}$ = Filter media volume in solid-liquid separation unit x (m^3)
$A_{f,\,sepx}$ = Cross sectional area (filtration) in solid-liquid separation unit x (m^2)

$$\rho_{fm,\,sepx^*} = \rho_{fm,\,sepx}\left(1 - e_{fm,\,sepx}\right) \qquad \textbf{B.158}$$

where: $\rho_{fm,\,sepx}{}^*$ = Packed density of product out solid-liquid separation unit x (kg/m^3)

$$M_{fm,\,sepx} = V_{fm,\,sepx} . \rho_{fm,\,sepx^*} \qquad \textbf{B.159}$$

where: $M_{fm,\,sepx}$ = Mass of filter media out solid-liquid separation unit x (kg)

$$M_{fm,\,sepx}{}^* = \frac{M_{fm,\,sepx}}{f_{fm,\,sepx}} \rightarrow \text{Batch system} \qquad \textbf{B.160}$$

$$M_{fm,\,sepx}{}^* = \frac{M_{fm,\,sepx}}{f_{fm,\,sepx} . No} \rightarrow \text{Continuous system} \qquad \textbf{B.161}$$

where: $M_{fm,\,sepx}{}^*$ = Mass of filter media needed in solid-liquid separation unit x (kg) per equivalent batch
No = Number of equivalent batches for a continuous system

NOTE: This is the amount of filter media required for the Life Cycle Assessment study.

Sedimentation

Assumptions

No energy needed.

A flocculent can be added as in filtration for solid-liquid separation (Table B.15).

Other Separation

Initial values

Specific energy (other) for solid-liquid separation unit x ($\hat{E}_{sepx,\ o}$) = 500 MJ/m^3 (a representative value for all solid-liquid separation units)

Equations

$$E_{sepx,\ o} = \hat{E}_{sepx,\ o} . V_{w,\ sepx,\ in} \qquad \textbf{B.162}$$

where: $E_{sepx,\ o}$ = Other energy for solid-liquid separation unit x (MJ)
$V_{w,\ sepx,\ in}$ = Volume of water in solid-liquid separation unit x (m^3)

B.9. Cell disruption

High pressure homogeniser, Cavitation, Ball Mill

Initial values

Fraction product released on rupture (R/R_{max}) = *[As selected from Table 2.14 in Chapter 2]*
Biomass conc. in cell disruption ($C_{x,\ cd}$) = *[As selected from Table 2.14 in Chapter 2]*
Energy efficiency of breakage (η_{cd}) = *[As selected from Table 2.14 in Chapter 2]*

Equations

Intracellular product is released according to Equation B.163. Product from non-disrupted cells is treated as biomass waste.

$$M_{p,\ out,\ cd} = \frac{M_{p,\ in,\ cd}}{R/R_{max}} \qquad \textbf{B.163}$$

where: $M_{p,\ out,\ cd}$ = Mass of product into cell disruption unit (kg)
$M_{p,\ in,\ cd}$ = Mass of product out of cell disruption unit (kg)

$$E_{cd} = \frac{M_{p,\,out,\,cd}}{\eta_{cd}} \qquad \textbf{B.164}$$

where: E_{cd} = Energy required to disrupt biomass in cell disruption unit (J)
η_{cd} = Disruption efficiency (kg/J)

Other Cell disruption

Specific energy (other) for cell disruption ($\hat{E}_{cd,\,o}$) = 25 MJ/m^3 (a representative value for all cell disruptions)

Equations

$$E_{cd,\,o} = \hat{E}_{cd,\,o} \,.\, V_{w,\,cd,\,in} \qquad \textbf{B.165}$$

where: $E_{cd,\,o}$ = Other energy for cell disruption unit (MJ)
$V_{w,\,cd,\,in}$ = Volume of water in cell disruption unit (m^3)

B.10. Concentration and Purification

Initial values

Solid or product (mass) fract. removed from concentration and purification unit x ($w_{sol,\,cpx}$):
- Adsorption = 1.00
- Centrifugation = 0.98
- Chromatography = 1.00
- Evaporation = 1.00
- Filtration = 0.95
- Precipitation and Crystallisation= 1.00
- Solvent extraction = 0.99
- OTHER = 0.99

Liq. or waste (mass) fraction removed from concentration and purification unit x ($w_{l,\,cpx}$):
- Adsorption = 0.00
- Centrifugation = 0.80
- Chromatography = 0.00
- Evaporation = 0.90
- Filtration = 0.95
- Precipitation and Crystallisation= 0.00
- Solvent extraction = 0.95
- OTHER = 0.80

Equations

[As for Equations B.141 – B.142 (solid-liquid separation). Subscripts as for concentration and purification unit x]

Additives (non-reacting and reacting)

Non-reacting additives can be added in each concentration and purification step.

Initial values

Volume fraction non-reacting additive in concentration and purification unit x ($y_{add,\ cpx}$) = 0.05

Density of non-reacting additive in concentration and purification unit x ($\rho_{add,\ cpx}$) = *[As per selected chemical as shown in Table B.17]*

Table B.17: Possible additional reacting and non-reacting flow materials and solvents for the concentration and purification section of the model

Materials
Acetone, Acetonitrile, Alum, Amyl acetate, Anionic flocculent, Benzene, Butanol, Butyl acetate, Calcium citrate, Carbon tetrachloride, Chloroform, Cyclohexane, Cyclopentane, Dichloromethane, Diethyl ether, Ethanol, Ethyl acetate, Ferric chloride, Heptane, Hexane, Hydrochloric acid, Lime, Magnesium, Methanol, Methyl acetate, Methyl ethyl ketone (butanone), Nitromethane, Pentane, Potassium, Propanol, Sodium, Sodium acetate, Sodium carbonate, Sodium hydroxide, Sulphuric acid, Tetrachloroethylene, Tetrahydrofuran, Toluene, Tributyl phosphate, Water, Other

Equations

$$V_{cpx,\ T} = V_{cpx,\ w,\ in} + \left(\frac{M_{p,\ out,\ cpx}}{\rho_{p,\ cpx}} \right) + \frac{M_{rm,\ out,\ cpx}}{1000} \qquad \textbf{B.166}$$

where: $V_{cpx,\ T}$ = Total volume of concentration and purification unit x (m^3)

$V_{cpx,\ w,\ in}$ = Volume of water out concentration and purification unit x (m^3)

$M_{p,\ out,\ cpx}$ = Mass of product out concentration and purification unit x (kg)

$\rho_{p,\ cpx}$ = Density of product out concentration and purification unit x (kg/m^3)

$M_{rm,\ out,\ cpx}$ = Mass of unreacted raw materials and biomass out concentration and purification unit x (kg) (it is assumed density of raw materials and biomass equals the density of water)

$$V_{add,\,cpx,\,in} = y_{add,\,cpx} \cdot V_{cpx,\,T} \qquad \textbf{B.167}$$

where: $V_{add,\,cpx,\,in}$ = Volume of additive in concentration and purification unit x (m^3)

$$M_{add,\,cpx,\,in} = V_{add,\,cpx,\,in} \cdot \rho_{add,\,cpx} \qquad \textbf{B.168}$$

where: $M_{add,\,cpx,\,in}$ = Mass of flocculent in concentration and purification unit x (m^3)

Additionally, two reacting chemicals can be added per concentration and purification step. These include any of those in Table B.17 or as specified by the user. Based on the stoichiometry of reaction, the amount of product and excess chemicals is calculated.

Initial values

Volume fraction reacting additive x in concentration and purification unit x ($y_{rax,\,cpx}$) = 0.25

Conversion rate of limiting additive in concentration and purification unit x ($X_{rax,\,cpx}$) = 80 %

$$\begin{gathered} \text{Reactant a}_{cpx} + \text{Reactant b}_{cpx} + \text{Existing chemical}_{cpx} \\ \downarrow \\ \text{Reaction product a}_{cpx} + \text{Reaction product b}_{cpx} + \text{Modified chemical}_{cpx} + \\ CO_2 + O_2 + H_2O + N_2 + SO_2 + PO_4 + Cl_2 + Na + Fe + Mg + Ca + Si + Al + Cu + K \end{gathered} \qquad \textbf{B.169}$$

Solving the material balances for all chemicals in Equation B.169 yields the number of moles required for reaction. Taking the conversion ($X_{rax,\,cpx}$) rate into account, this value scaled for the number of moles of existing chemical available and converted to mass.

$$m_{rax,\,cpx,\,i} = \frac{M_{rax,\,cpx,\,i}}{Mm_{rax,\,cpx,\,i}} \qquad \textbf{B.170}$$

where: $m_{rax,\,cpx,\,i}$ = Number of moles component i from reaction in concentration and purification unit x (kmol)

$M_{rax,\,cpx,\,i}$ = Mass of component i from reaction in concentration and purification unit x (kg)

$Mm_{rax,\,cpx,\,i}$ = Molar mass of component i from reaction in concentration and purification unit x (kg/kmol)

Adsorption

Initial values

Pressure difference for adsorption in concentration and purification unit x ($\Delta P_{ads,\ cpx}$) = 250 kPa

Pumping efficiency for adsorption in concentration and purification unit x ($\eta_{ads,\ cpx}$) = 0.6

Time basis for adsorption in concentration and purification unit x ($t_{ads,\ cpx}$) = 1 s

Note: Time basis immaterial as unit of time cancels out through calculation

Equations

$$A_{ads,\ cpx} = \left(\frac{V_{cpx,\ T}}{L/D} \right)^{\frac{2}{3}} \qquad \textbf{B.171}$$

where: $A_{ads,\ cpx}$ = Cross sectional area (adsorption) in concentration and purification unit x

L/D (Length: Diameter ratio) = 3; except freeze drying; L/D = 6

$V_{cpx,\ T}$ = Total volume in concentration and purification unit x (kg) – as given by Equation B.166

$$v_{ads,\ cpx} = \frac{V_{w,\ in,\ cpx}}{A_{ads,\ cpx} . t_{ads,\ cpx}} \qquad \textbf{B.172}$$

where: $V_{ads,\ cpx}$ = Linear velocity through adsorption in conc. and purification unit x ($m.s^{-1}$)

$V_{w,\ in,\ cpx}$ = Volume of water in concentration and purification unit x (m^3)

Note: Only the volume of water is assumed as it forms by far the largest portion of the total volume. The volumes of the raw materials are not considered as some will dissolve and calculating the volume of mixing is beyond the level of detail for this model.

$$P_{ads,\ cpx} = \frac{\Delta P_{ads,\ cpx} . v_{ads,\ cpx}}{\eta_{ads,\ cpx}} \qquad \textbf{B.173}$$

where: $P_{ads,\ cpx}$ = Power for adsorption in concentration and purification unit x (kPa)

$$E_{ads,\ cpx} = P_{ads,\ cpx} . t_{ads,\ cpx} \qquad \textbf{B.174}$$

where: $E_{ads,\ cpx}$ = Energy for adsorption in concentration and purification unit x (kJ)

Centrifugation

Initial values and constants

Efficiency of centrifugation ($\eta_{cen,\,cpx}$) = 0.6

Energy per unit volume for centrifugation ($\hat{E}_{cen,\,cpx}$) = 6420 kJ/m^3

$$E_{cen,\,cpx} = V_{cpx,\,T} \cdot \frac{\hat{E}_{cen,\,cpx}}{\eta_{cen,\,cpx}} \qquad \textbf{B.175}$$

where: $E_{cen,\,sepx}$ = Energy requirement for centrifugation in conc. and purification unit x (kJ)

$V_{cpx,\,T}$ = Total volume in concentration and purification unit x (kg) – as given by Equation B.169

Chromatography

[As for Equations B.171 – B.174 (adsorption). Subscripts as for chromatography]

Decanting

[As for Equations B.171 – B.174 (adsorption). Subscripts as for decanter]

Evaporation

Initial values and constants

Inlet temperature ($T_{cpx,\,in}$) = Outlet temperature from previous unit ($T_{cpx-1,\,out}$)

Heated temperature in concentration and purification unit x ($T_{cpx,\,h}$) = 65°C

Efficiency of evaporation in concentration and purification unit x ($\eta_{ev,\,cpx}$) = 0.8

Specific heat of water ($C_{p,\,w}$) = 4.1868kJ/kg.K

Lower heating value of natural gas (LHV_{ng}) = 40 kJ/kg

Density (natural gas) (ρ_{ng}) = 0.595 kg/m^3

Critical temperature (T_c) = 374°C

Equations

$$T_{r,w} = \frac{T_{cpx,h}}{T_c} \qquad \textbf{B.176}$$

where: $T_{r,w}$ = Reduced temperature for water

$$\lambda_{v,w} = C_1.\left(1 - T_{r,w}\right)^{C_2 + C_3.T_{r,w} + C_4.T_{r,w}^2} \quad \text{(Perry } \textit{et al.} \text{ 1997)} \qquad \textbf{B.177}$$

where: $\lambda_{v,w}$ = Latent heat of vaporisation for water ($MJ.kg^{-1}$)
C_1, C_2, C_3 and C_4 = Constants

$$q_{h,ev,cpx} = \frac{M_{cpx,T}}{\rho_w} C_{p,w}\left(T_{hw,ev} - T_{cpx,in}\right) \qquad \textbf{B.178}$$

where: $q_{h,ev,cpx}$ = Heat to raise temp. (evaporation) in conc. and purification unit x (MJ)
$M_{cpx,T}$ = Total mass in concentration and purification unit x (kg)

$$q_{v,ev,cpx} = \lambda_{v,w}.M_{w,out,ev} \qquad \textbf{B.179}$$

where: $q_{v,ev,cpx}$ = Heat to vaporise (evaporation) in concentration and purification unit x (MJ)
$M_{w,out,ev}$ = Mass of water evaporated from concentration and purification unit x (kg)

$$q_{T,ev,cpx} = \frac{q_{v,ev,cpx} + q_{h,ev,cpx}}{\eta_{ev,cpx}} \qquad \textbf{B.180}$$

where: $q_{T,ev,cpx}$ = Total heat for evaporation in concentration and purification unit x (MJ)

$$M_{ng,ev,cpx} = \frac{q_{T,ev,cpx}}{LHV_{ng}} \qquad \textbf{B.181}$$

where: $M_{ng,ev,cpx}$ = Mass natural gas (evaporation) in conc. and purification unit x (kg)

$$V_{ng,ev,cpx} = \frac{M_{ng,ev,cpx}}{\rho_{ng}} \qquad \textbf{B.182}$$

where: $V_{ng,ev,cpx}$ = Volume natural gas (evaporation) in conc. and purification unit x (kg)

Filtration

Initial values and constants

Energy per unit volume for filtration in concentration and purification unit x ($\hat{E}_{f,\ cpx}$) = *[As selected in Table 2.19 of the main text]*

Pumping efficiency of filtration in concentration and purification unit x ($\eta_{f,\ cpx}$) = 0.6

Volume fraction flocculent in concentration and purification unit x ($y_{fa,\ cpx}$) = 0.01

Density of flocculent in concentration and purification unit x ($\rho_{fa,\ cpx}$) = *[As per flocculent – Table B.15]*

Equations

Energy calculations

$$E_{f,\ cpx} = \hat{E}_{f,\ cpx} . V_{cpx,\ T} \qquad \textbf{B.183}$$

where: $E_{f,\ cpx}$ = Energy for filtration in concentration and purification unit x (MJ)

Flocculent calculations

$$V_{cpx,\ T} = V_{cp,\ w,\ in} + \left(\frac{M_{p,\ out,\ cpx}}{\rho_{p,\ cpx}} \right) + \frac{M_{rm,\ out,\ cpx}}{1000} \qquad \textbf{B.184}$$

where: $V_{cpx,\ T}$ = Total volume in concentration and purification unit x (m^3)

$V_{cpx,\ w,\ in}$ = Volume of water out concentration and purification unit x (m^3)

$M_{p,\ out,\ cpx}$ = Mass of product out concentration and purification unit x (kg)

$\rho_{p,\ cpx}$ = Density of product out concentration and purification unit x (kg/m^3)

$M_{rm,\ out,\ cpx}$ = Mass of unreacted raw materials and biomass out solid-liquid separation unit x (kg) (it is assumed density of raw materials and biomass equals the density of water)

$$V_{fa,\ cpx,\ in} = y_{fa,\ cpx} . V_{cpx,\ T} \qquad \textbf{B.185}$$

where: $V_{fa,\ cpx,\ in}$ = Volume of flocculent in concentration and purification unit x (m^3)

$$M_{fa,\ cpx,\ in} = V_{fa,\ cpx,\ ina} . \rho_{fa,\ cpx} \qquad \textbf{B.186}$$

where: $M_{fa,\ cpx,\ in}$ = Mass of flocculent in concentration and purification unit x (m^3)

Filter media calculations

[As for Equations B.157 – B.161 (filtration in solid-liquid separation unit x). Subscripts as for concentration and purification unit x]

Diafiltration calculations

Initial values

Vol. frac. diafiltration soln added (conc. and purification unit x) ($y_{dfs,\ cpx}$) = 0.25 %v/v

Mole frac. diafiltration salt in soln (conc. and purification unit x) ($x_{df,\ cpx}$) = 25 mmol/l

Equations

$$V_{dfw,\ cpx} = y_{dfs,\ cpx} . V_{cpx,\ T} \qquad \textbf{B.187}$$

where: $V_{dfw,\ cpx}$ = Volume of diafiltration water in concentration and purification unit x (m^3)

$V_{cpx,\ T}$ = Total volume in concentration and purification unit x (kg) – as given by Equation B.166

$$m_{df,\ cpx} = V_{dfw,\ cpx} . x_{df,\ cpx} \qquad \textbf{B.188}$$

where: $m_{df,\ cpx}$ = Number of moles diafiltration salt in concentration and purification unit x (m^3)

$$\text{Mass diafiltration salt } (M_{df}) = m_{df} . \frac{\text{molar mass}}{1000} \qquad \textbf{B.189}$$

Precipitation and Crystallisation

Initial values and constants

Inlet temperature ($T_{cpx,\ in}$) = Outlet temperature from previous unit ($T_{cpx\text{-}1,\ in}$)

Heated temperature in concentration and purification unit x ($T_{cpx,\ h}$) = 55°C

Outlet temperature in concentration and purification unit x ($T_{cpx,\ o}$) = 40°C

Efficiency of precipitation in concentration and purification unit x ($\eta_{prec,\ cpx}$) = 0.8

Residence time for precipitation in concentration and purification unit x ($\tau_{prec,\ cpx}$) = 2 h

Power per unit vol. (precipitation) in conc. and purification unit x (Pv_{cpx}) = 0.8 kW/m^3

Cooling or chilled water temperature ($T_{cw,\ prec}$) = *[As for global initial values]*

Max. temp. diff. between exiting cooling water and hot inlet streams ($\Delta T_{max,\ cw}$) = *[As for global initial values]*
Latent heat (steam) (λ_{St}) = 2.7 MJ/kg
Specific heat of water ($C_{p,\ w}$) = 4.1868kJ/kg.K
Density of product ($\rho_{p,\ prec}$) = *[As determined by product choice in Table B.3]*

Equations

Cooling water outlet temperature (T_o) = $T_h - T_m$ **B.190**

Agitation energy

[As for Equations B.136 – B.137 (agitation). Subscripts as for precipitation and concentration and purification unit x.]

Steam requirements

[As for Equations B.5 – B.13 (steam sterilisation). Subscripts as for precipitation and concentration and purification unit x.]

Cooling water requirements

[As for Equations B.15 – B.16 (steam sterilisation). Subscripts as for precipitation and concentration and purification unit x.]

Solvent extraction

[As for Equations B.171 – B.174 (adsorption). Subscripts as for solvent extraction]

Splitter

Initial values

Mass fraction removed in concentration and purification unit x ($w_{cpx,\ rem}$) = 0.5

Equations

$$w_{cpx,\ ret} = 1 - w_{cpx,\ rem} \qquad \textbf{B.191}$$

where: $w_{f,\ cpx,\ ret}$ = Mass fraction retained in concentration and purification unit x

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Other Concentration and Purification

Constants

Specific energy (other) for concentration and purification unit x ($\hat{E}_{cpx,\ o}$) = 10 MJ/m^3 (an estimated average energy based on requirements for all concentration and purification units)

Equations

$$E_{cpx,\ o} = \hat{E}_{cpx,\ o} . V_{w,\ cpx,\ in} \qquad \textbf{B.192}$$

where: $E_{cpx,\ o}$ = Other energy for concentration and purification unit x (MJ)
$V_{w,\ cpx,\ in}$ = Volume of water in concentration and purification unit x (m^3)

B.11. Formulation

Initial values

Solid (mass) fraction retained from formulation unit ($w_{sol,\ form}$) = 0.99
Liquid (mass) fraction removed from formulation unit x ($w_{l,\ cpx}$) = 0.99

Equations

[As for Equations B.141 – B.142 *(solid-liquid separation). Subscripts as for formulation unit]*

Oven Drying

Initial values and constants

Inlet temp. ($T_{form, in}$) = Outlet temp. from final concentration and purification unit ($T_{cpx, out}$)
Heated temperature in formulation unit ($T_{form, h}$) = 65°C
Efficiency of oven drying in formulation unit ($\eta_{ov, form}$) = 0.8

Equations

[As for Equations B.176 – B.182 *(evaporation). Subscripts as for oven drying and formulation]*

Freeze Drying

Assumptions

The sublimation energy ($E_{sub, fd}$) requires no external energy and is assumed zero
The volume of the vacuum apparatus is twice that of the total volume

Initial values and constants

Inlet temp. ($T_{form, in}$) = Outlet temp. from final concentration and purification unit ($T_{cpx, out}$)
Subcooled temperature for freeze drying ($T_{sc, fd}$) = -40°C
Freeze dryer vacuum inlet area ($A_{fd, in}$) = 0.1 m^2
Efficiency (η_{fd}) = 0.8
Specific heat of water ($C_{p, w}$) = 4.1868 kJ/kg.K
Specific heat of ice ($C_{p, ice}$) = 2.1 kJ/kg.K
Latent heat of freezing (λ_{fr}) = 333.68 kJ/kg
Sublimation energy ($E_{sub, fd}$) = 2590 kJ/kg – but assumed no external energy inputs required

Equations

<u>Heat to cool and freeze liquid</u>

$$q_{c, fd} = C_{p, w} . M_{T, form, in} . T_{form, in} \qquad \textbf{B.193}$$

where: $q_{cw, fd}$ = Heat to cool (freeze dryer) in formulation (kJ)
$M_{T, form}$ = Total mass in formulation unit (kg)

$$q_{f,\,fd} = \lambda_{fr} . M_{T,\,form,\,in} . \rho_w \qquad \textbf{B.194}$$

where: $q_{fr,\,fd}$ = Heat to freeze (freeze dryer) in formulation (kJ)

$$q_{sc,\,fd} = C_{p,\,ice} . M_{T,\,form,\,in} . \left(- T_{sc,\,fd}\right) \qquad \textbf{B.195}$$

where: $q_{sc,\,fd}$ = Heat to subcool (freeze dryer) in formulation (kJ)

$$q_{T,\,fd} = q_{cw,\,fd} + q_{fr,\,fd} + q_{sc,\,fd} \qquad \textbf{B.196}$$

where: $q_{T,\,fd}$ = Total heat for freeze drying (kJ)

Vacuum pumping

[As for Equations B.171 – B.174 (adsorption). Subscripts as for freeze drying and formulation unit]

Total energy

$$E_{T,\,fd} = \frac{q_{T,\,fd} + E_{T,\,vp,\,fd}}{\eta_{fd}} \qquad \textbf{B.197}$$

where: $E_{T,\,fd}$ = Total energy for freeze drying (kJ)
$E_{T,\,vp,\,fd}$ = Total energy for vacuum pumping in freeze drying (kJ)

Spray Drying

Assumptions

Adiabatic, therefore $\eta_{sd,\,form} = 0$

Initial values and constants

Inlet temp. ($T_{form,\,in}$) = Outlet temp. from final concentration and purification unit ($T_{cpx,\,out}$)
Heated temperature for spray dryer of formulation ($T_{out,\,sd,\,form}$) = 65°C
Outlet humidity of spray dryer ($H_{out,\,sd}$) = 0.04 kg/kg
Ambient humidity (H_a) = 0.002 kg/kg
Specific heat of air ($C_{p,\,air}$) = $a + bT + cT^2 + dT^3$ (J/mol.K)
($a = 28.088$; $b = 1.97 \times 10^{-3}$; $c = 4.8 \times 10^{-6}$; $d = -1.965 \times 10^{-9}$)

Latent heat of vaporization ($\lambda_{v,\,w}$) = *[As calculated in Equation B.177 using $T_{in,\,sd,\,form}$ in Equation B.176]*
Lower heating value of natural gas (LHV_{ng}) = 40 kJ/kg.
Density (natural gas) (ρ_{ng}) = 0.595 kg/m^3

Equations

$$\hat{E}_{sd,\,form} = 0.001 C_{p,\,air}\left[\frac{T_{out,\,sd,\,form}/\left(1-\eta_{sd,\,form}\right)-T_{form,\,in}}{H_{out,\,sd}-H_a}\right]+0.001\lambda_{v,\,w}\left[\frac{H_{out,\,sd}/\left(1-\eta_{sd,\,form}\right)-H_a}{H_{out,\,sd}-H_a}\right]$$

(Baker and McKenzie 2005) **B.198**

where: $\hat{E}_{sd,\,form}$ = Specific energy of spray drying in formulation (MJ/kg)
$\eta_{sd,\,form}$ = Thermal loss factor

Since is it assumed the system is adiabatic, $\eta_{sd,\,form} = 0$, Equation B.198 reduces to:

$$\hat{E}_{sd,\,form} = 0.001 C_{p,\,air}\left[\frac{T_{out,\,sd,\,form}-T_{form,\,in}}{H_{out,\,sd}-H_a}\right]+0.001\lambda_{v,\,w} \quad \text{(Keey 1992)} \qquad \textbf{B.199}$$

$$E_{sd,\,form} = \hat{E}_{sd,\,form}.M_{T,\,form,\,in} \qquad \textbf{B.200}$$

where: $E_{sd,\,form}$ = Energy of spray drying in formulation (MJ)
$M_{T,\,form}$ = Total mass in spray dryer in formulation unit (kg)

Volume of natural gas needed: *[As given in Equations B.181 – B.182 with $E_{sd,\,form}$ as the total energy requirement for natural gas energy.]*

Other Formulation

Constants

Specific energy (other) for formulation unit ($\hat{E}_{form,\,o}$) = 15 MJ/m^3 (a representative value for all formulation units)

Equations

$$E_{form,\,o} = \hat{E}_{form,\,o} \,.\, V_{w,\,form,\,in} \qquad \textbf{B.201}$$

where: $E_{form,\,o}$ = Other energy for formulation unit (MJ)
$V_{w,\,form,\,in}$ = Volume of water in formulation unit (m^3)

B.12. Waste Water Treatment

Initial values and constants

Volume fraction of waste water chemical x (y_{wwtx}) = 0.05 (where x = 1 → 3)

Density of waste water chemical x (ρ_{wwtx}) = *[As per selected chemical as shown in Table B.17]*

Equations

$$V_{T,\,wwt} = \sum V_{wwt,\,i} \qquad \textbf{B.202}$$

where: $V_{T,\,wwt}$ = Total volume of waste water (m^3)
$V_{wwt,\,i}$ = Volume of waste water in unit i; where i are all the downstream units

$$V_{wwtx} = \frac{V_{T,\,wwt}}{y_{wwtx}} \qquad \textbf{B.203}$$

where: V_{wwtx} = Volume of waste water treatments chemical x (m^3)

$$M_{wwtx} = V_{wwtx} \,.\, \rho_{wwtx} \qquad \textbf{B.204}$$

where: M_{wwtx} = Mass of waste water treatments chemical x (m^3)

$$StOD_i = \frac{16(2c + 0.5(h - cl) + 2.5n + 3s + 2.5p + 0.5na - o)}{Mm_i} \quad \text{(IChemE 2003)}$$

B.205

where: $StOD_i^*$ = Specific stoichiometric oxygen demand (chemical i) (kg/kg)
Mm_i = Molar mass substance i (kg/kmol)
c, h, o, n, p, s, cl, na = Coefficients for chemical i given by: $C_cH_hO_oN_nP_pS_sCl_{cl}Na_{na}$

$$StOD_i = StOD_i^* . M_i \quad \textbf{B.206}$$

where: $StOD_i$ = Stoichiometric oxygen demand (chemical i) (kg)
M_i = Mass of chemical i (kg)

$$StOD_T = \sum_{i=1}^{n} StOD_i \quad \textbf{B.207}$$

where: $StOD_T$ = Total stoichiometric oxygen demand (kg)
n = All chemicals i

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Appendix C

Nomenclature used in Appendix B

Appendix C: Nomenclature used in Appendix B

A	m^3	Area
a	vvm	Aeration rate
B		Baffle width
b		Number of impeller blades
C	$g.l^{-1}$, $kg.m^{-3}$	Concentration
C		Constants
C_p	$J.(mol.K)^{-1}$, $cal.(mol.K)^{-1}$	Specific heat
D	m	Diameter
D	hr^{-1}	Dilution rate
E	J, kJ, MJ, GJ	Energy
$\underline{E}$	kJ/s	Rate of thermal energy loss
$\hat{E}$	kJ/mol, kJ/kg, MJ/m^3, GJ/t	Specific energy
e		Voidage
e		Error
f		Frequency (filter media changes)
g	$m^3.kg^{-1}.s^{-2}$	Gravitational constant
H	m	Height
H	kg/kg	Humidity
J	$kg.s^{-1}$	Flux
K_s	$mg.l^{-1}$	Half saturation constant
L	m	Length
LHV	kJ/kg.K	Lower heating value
M	kg	Mass
Mm	g/mol	Molar mass
M	$kg/m^3/day$	Mass per volume per day
M_d	kg/day	Mass per day
m	mol	Moles
m	g C source/g dry cell weight.h	Maintenance coefficient
m^*		Polyentropic compression variable
N		Number
N	rpm, s^{-1}	Impeller speed
n^*		Polyentropic compression variable
No		Number of equivalent batches
N_p		Power number
N_{Re}		Reynold's number
P	W	Power
Pv	$kW.m^{-3}$	Power per unit volume
P	kPa	Pressure

q	MJ	Heat
Q	$m^3.s^{-1}$	Volumetric gas flow rate
Qv	$m^3.m^{-3}.min^{-1}$	Volumetric gas flow rate per unit volume
R	$kJ.(kg.K)^{-1}$	Gas constant
R		Protein release on rupture
r		Mole ratio
S	$mg.l^{-1}$	Substrate concentration
Sc		Scaling factor
SV	m^2/m^3	Surface area to volume ratio
StOD	kg	Stoichiometric oxygen demand
T	^oC	Temperature
t	s	Time
U	$kW/m^2.^oC$	Overall heat transfer coefficient
V	m^3	Volume
v	$m.s^{-1}$	Linear velocity
W	J	Work
$\hat{W}$	J/kg	Specific work
W	m	Width
w	kg/kg	Mass fraction
X	%	Conversion rate
Y	$g.g^{-1}$	Yield coefficient
y	v/v	Volume fraction

Greek letters:

$\alpha, \alpha', \beta, \beta', \chi, \delta, \varepsilon, \phi, \gamma, \eta, \iota, \varphi, \kappa, \lambda$		Stoichiometric coefficients
γ		Ratio of specific heats
γ_s		Degree of reduction
Δ		Difference
λ	$MJ.kg^{-1}$	Latent heat
η	–, %	Efficiency
μ	Pa.s	Viscosity
μ	hr^{-1}	Specific growth rate
ρ	$kg.m^{-3}$	Density
σ	$N.m^{-1}$	Interfacial tension
τ	hr	Residence time

Subscripts:

0	Initial
a	Ambient
ad	Additional
add	Additive
ads	Adsorption
ag	Agitation
air	Air
ap	Anaerobic product
ass	Assumed
b	Baffles
back	Backing (steam)
bat	Batch
c	Critical
C1	Carbon 1 (nutrient source)
C2	Carbon 2 (nutrient source)
calc	Calculated
camp	Campaign
cd	Cell disruption
cen	Centrifugation
comp	Compression
cpx	Conc. and purification; x = 1 → 6
CT	Carbon Total (nutrient source)
cw	Cooling; cooling or chilled water
cycle	Production cycle
df	Diafiltration salt
dfs	Diafiltration solution
dfw	Diafiltration water
ev	Evaporation
f	Filtration
fa	Flocculent additive
fd	Freeze drying
final	Final
fm	Filter media
form	Formulation
fr	Freezing
g	Gas
g'	Gassed system
gen	Generation
h	Heated
hold	Holding
i	Substance i
ice	Ice
in	In, Inlet
im	Impurities
imp	Impeller
j	Counter (iteration calculations)
l	Liquid
maint	Maintenance
max	Maximum
N1	Nitrogen 1 (nutrient source)
N2	Nitrogen 2 (nutrient source)
ng	Natural gas
NT	Nitrogen Total (nutrient source)
o	Other
O2	Oxygen
out	Out; Outlet
ov	Oven drying
p	Product
p	Polyentropic
post	Post bioreactor cooling
prec	Precipitation and crystallisation
ps	Product stream
R	Reduced
rax	Reacting addition; x = 1 or 2
rct	Reactor
ref	Reference
rem	Removed
req	Required
ret	Retained
rm	Raw materials
s	Substrate
sc	Subcool
sd	Spray drying
sepx	Solid-Liquid separation; x = 1 or 2
sol	Solid
sov	Steaming out vessel
sp	Spin cycles
spa	Space heating (steam)

st	Steam	v	Vaporisation
ster	Sterilisation	vp	Vacuum pumping
sub	Sublimation	w	Water
surf	Surface	wwt	Waste water treatment
t	Tank	x	Biomass
T	Total		

Appendix D

UML Diagrams for the generic flowsheet model developed

Appendix D: UML Diagrams for the Generic Flowsheet Model

A simplified Unified Modelling Language (UML) diagram for solving the entire material balance of the flowsheet model is shown in Figure D.1. This is the first part of several diagrams which can be found on the accompanying CD-Rom. Where reference is made to 'values as calculated in the main calculations', calculations as shown in Appendix B are used.

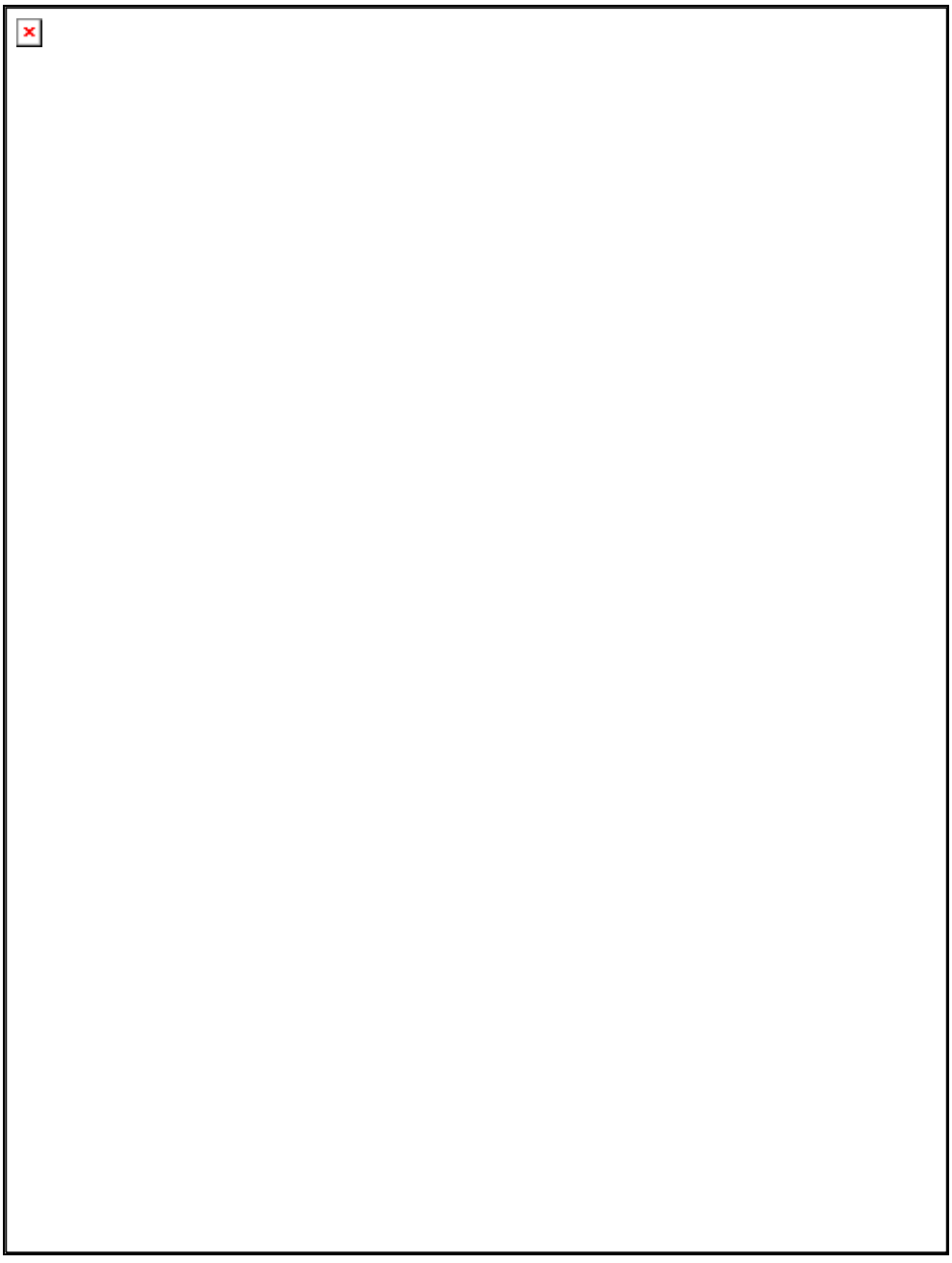

Figure D.1: Simplified UML diagram showing basic flowsheet structure to calculate product mass

Appendix E

Using the Generic Flowsheet Model

Appendix E: Using the Generic Flowsheet Model

E.1. Overview

This appendix gives details of the application of the generic flowsheet; including system requirements, installation instructions and how to use the software model.

E.2. System requirements

The software was developed on Microsoft® Office Excel 2003 and has been further tested on MS-Excel XP. Complete testing has not been done on MS-Excel 2007. The software does not work with AppleWorks, OpenOffice or other spread sheeting packages.

E.3. Getting started

The generic flowsheet model is presented in a single MS-Excel workbook: 'Generic model vx.xx.xx.xls' – where "x.xx.xx" refers to the version of the software. The workbook can be run from its current location or copied and used from the desktop.

Before opening the software ensure that Macros are enabled in MS-Excel (Tools-Macro-Security; 'Medium' security level) and ensure that macros are always enabled during use. The file will display a copyright prompt (click "OK" to continue) followed by an End user License Agreement (EULA). After agreeing to the EULA, click "I Agree" to continue. You will then be taken to the Input screen of the spreadsheet.

Note: The official name of the software, "Leap BioSoft", is used in the MS-Excel spreadsheet.

E.4. User's manual

The MS-Excel spreadsheet is designed as a top down application and should be used as such to avoid entering invalid information. Select the worksheet labelled 'Input' and from the top, select the option button and drop down menus (green) as desired. Only use the orange cells to make changes to the default values which appear to the right of these cells.

Example: Scenario 1 (sodium salt of Penicillin V) as given in Chapter 3

Below is a step-by-step guide for entering the data into the MS-Excel flowsheet model. The example applies to the generic flowsheet as provided in version v7.12.22 of the software. Modifications and enhancements which do not impact the results of this example have since been implemented in the model.

The instructions are listed as:

- 'click', where a mouse action is required;
- 'type' where inputs need to be entered into the orange input cells using the keyboard; and
- 'select' where a dropdown menu appears and a selection needs to be made.

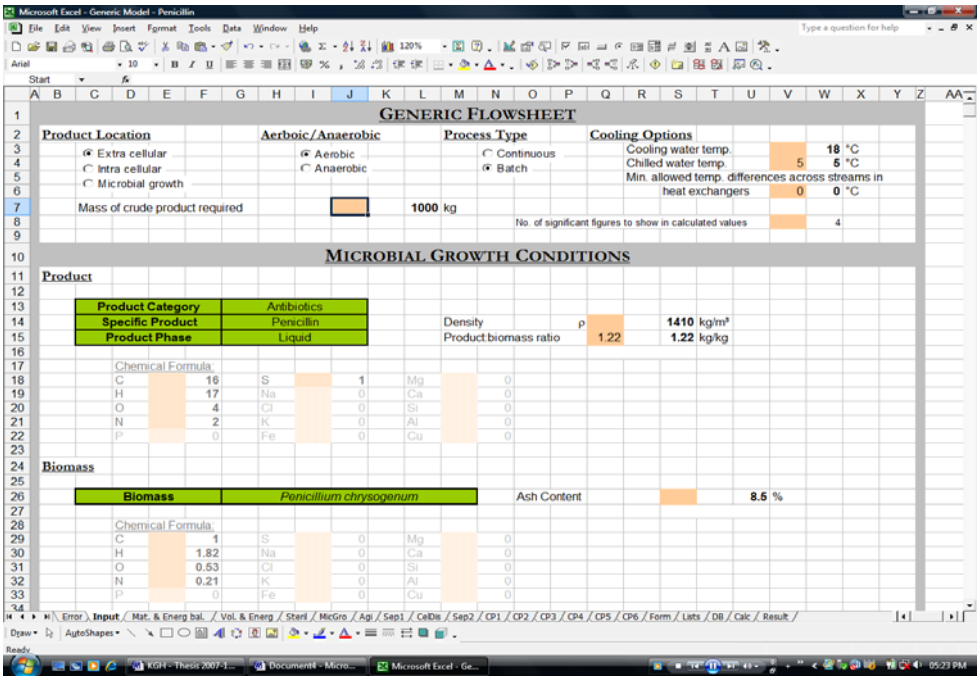

Figure E.1: Generic flowsheet model (Input) – Screenshot 1

MS-EXCEL worksheet name: Input

Worksheet (click): Input

Initial considerations
Product location (click): Extracellular
Aerobic/Anaerobic (click): Aerobic
Process type (click): Batch

Cooling options
Chilled water temp. (type): 5
Min allowed temp. differences across streams in heat exchangers (type): 0

Product description
Product category (select): Antibiotics
Specific product (select): Penicillin
Product to biomass ratio (type): 1.219

Biomass description
Biomass (select): *Penicillium chrysogenum*

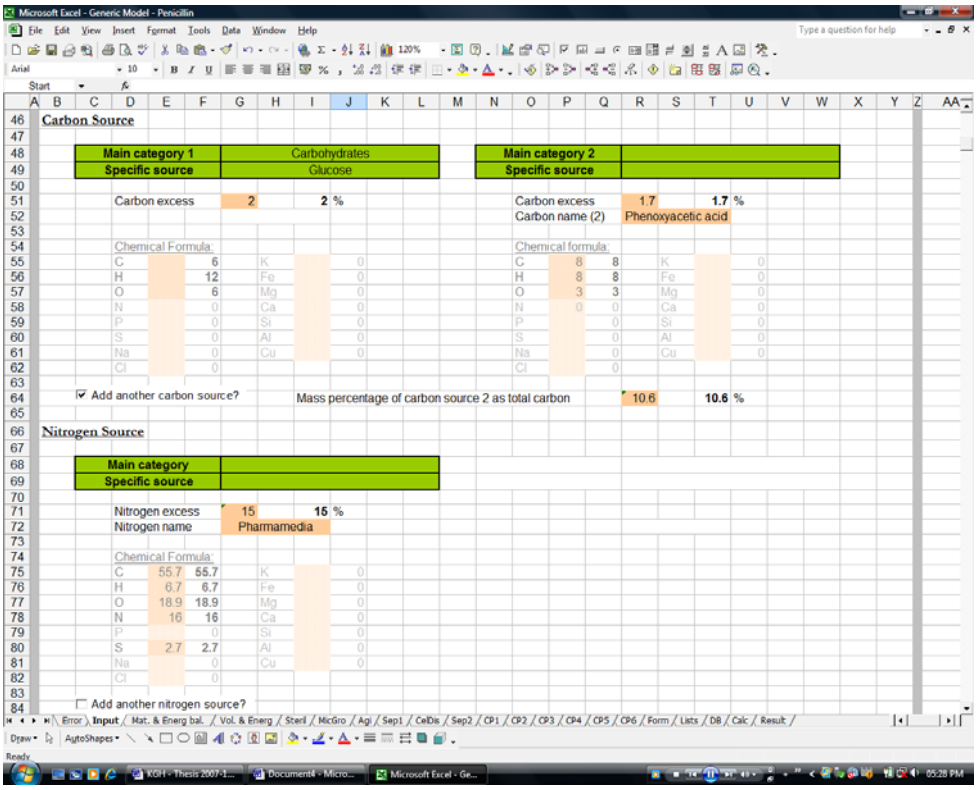

Figure E.2: Generic flowsheet model (Input) – Screenshot 2

Carbon sources
Main category (select): Carbohydrates
Specific source (select): Glucose
Carbon excess (type): 2
Add another carbon source: (Click)

Main category 2 (select) – OTHER
Carbon excess (type): 1.7
Carbon name (2) (type): Phenoxyacetic acid
Chemical formula (type): C: 8, H: 8, 0: 8, N: 0
Mass percentage carbon source 2 as total carbon (type): 10.55

Nitrogen sources
Main category (select): Organic
Specific source (select): OTHER
Nitrogen excess (type): 15
Nitrogen name (Type): Pharmamedia
Chemical formula (type): C: 55.7, H: 6.7, O: 18.9, N: 16, S: 2.7

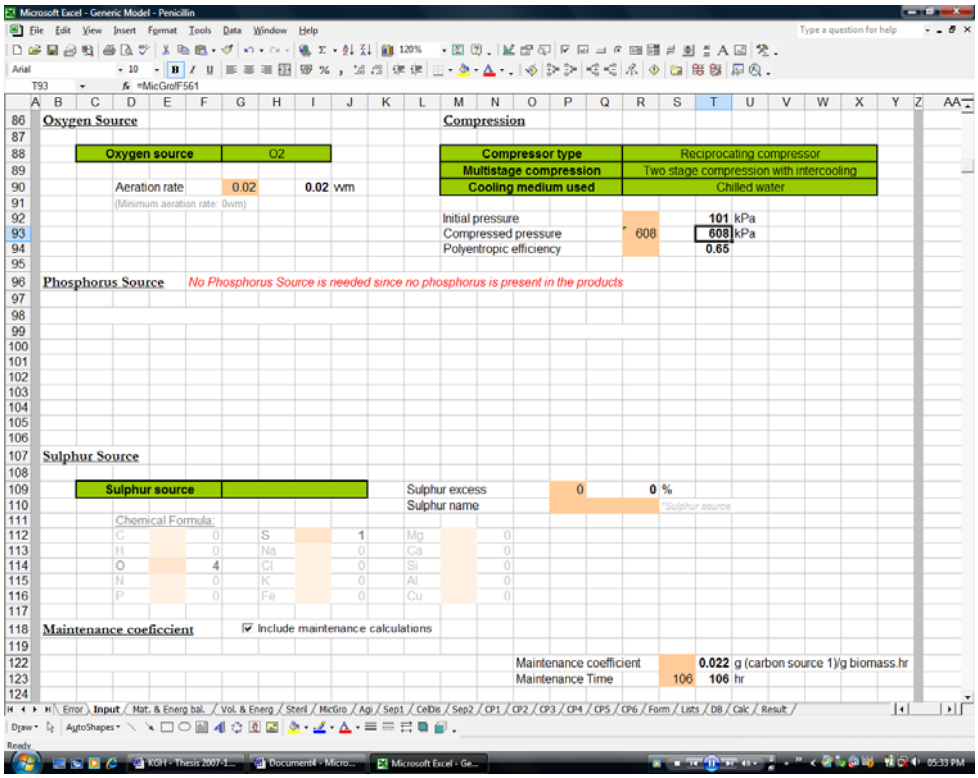

Figure E.3: Generic flowsheet model (Input) – Screenshot 3

Oxygen source
Oxygen source (select): O2
Aeration rate (type): 0.02

Compression
Compressor type (select): Reciprocating compressor
Multistage compression (select): Two stage compression with intercooling
Cooling medium used (select): Chilled water
Compressed pressure (type): 608

Sulphur source
Sulphur source (select): OTHER
Sulphur excess (type): 0

Maintenance coefficient
Include maintenance coefficient: (Click)
Maintenance time (type): 106

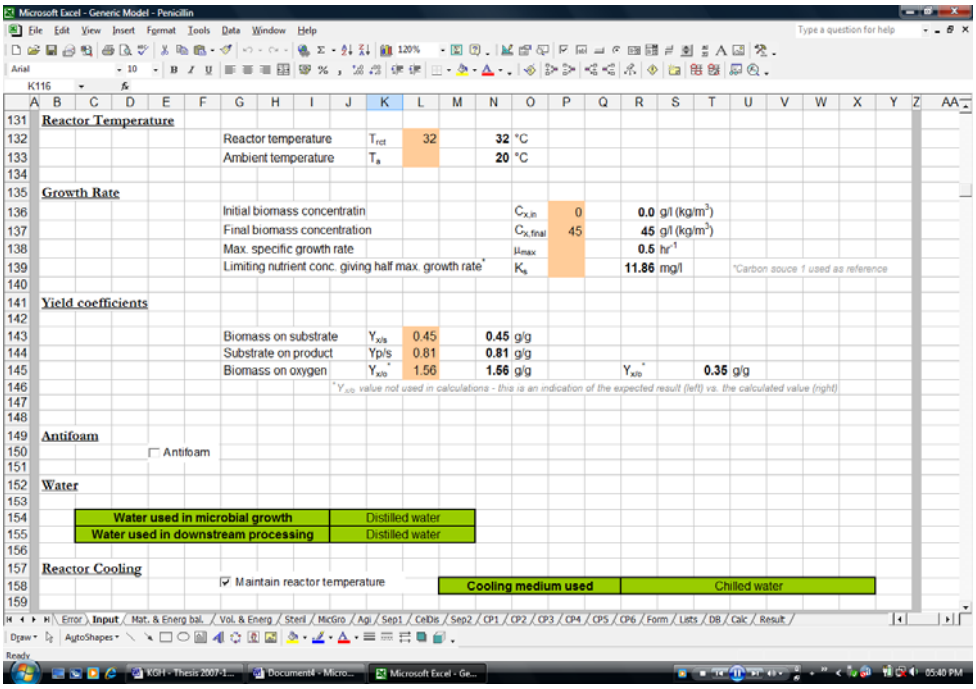

Figure E.4: Generic flowsheet model (Input) – Screenshot 4

Reactor Temperature
Reactor temperature (type): 32

Growth rate
Initial biomass concentration (type): 1e-06
Final biomass concentration (type): 45

Yield coefficients
Biomass on substrate (type): 0.45
Substrate on product (type): 0.81
Biomass on oxygen (type): 1.56

Water
Water used in microbial growth (select): Distilled water
Water used in downstream processing (select): Distilled water

Reactor Cooling
Maintain reactor temperature: (Click)
Cooling medium used (select): Chilled water

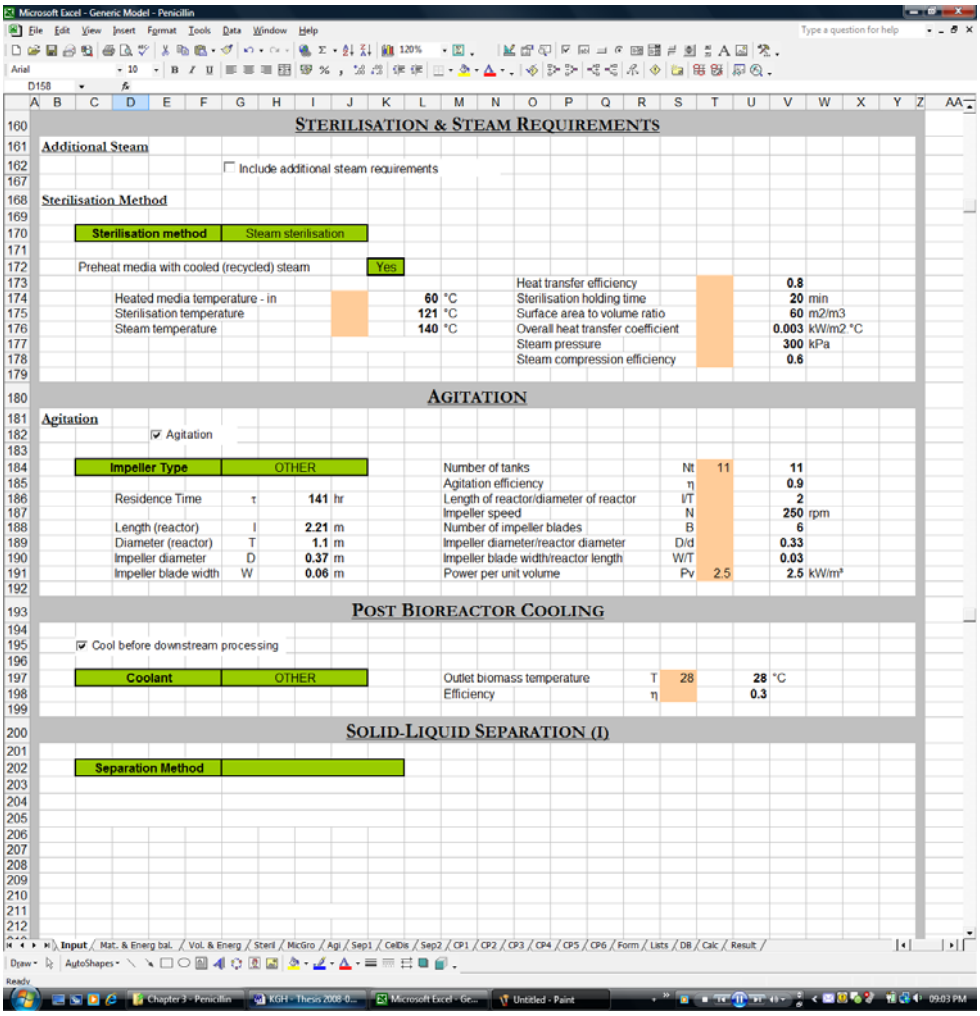

Figure E.5: Generic flowsheet model (Input) – Screenshot 5

Sterilisation Method
Sterilisation method (select): Steam
Preheat media with cooled (recycled) steam (select): Yes

Agitation
Agitation: (Click)
Impeller type (select): OTHER
Number of tanks (type): 11
Power per unit volume (type): 2.5

Post bioreactor cooling
Cool before downstream processing: (Click)
Coolant (select): OTHER
Outlet biomass temperature (type): 28

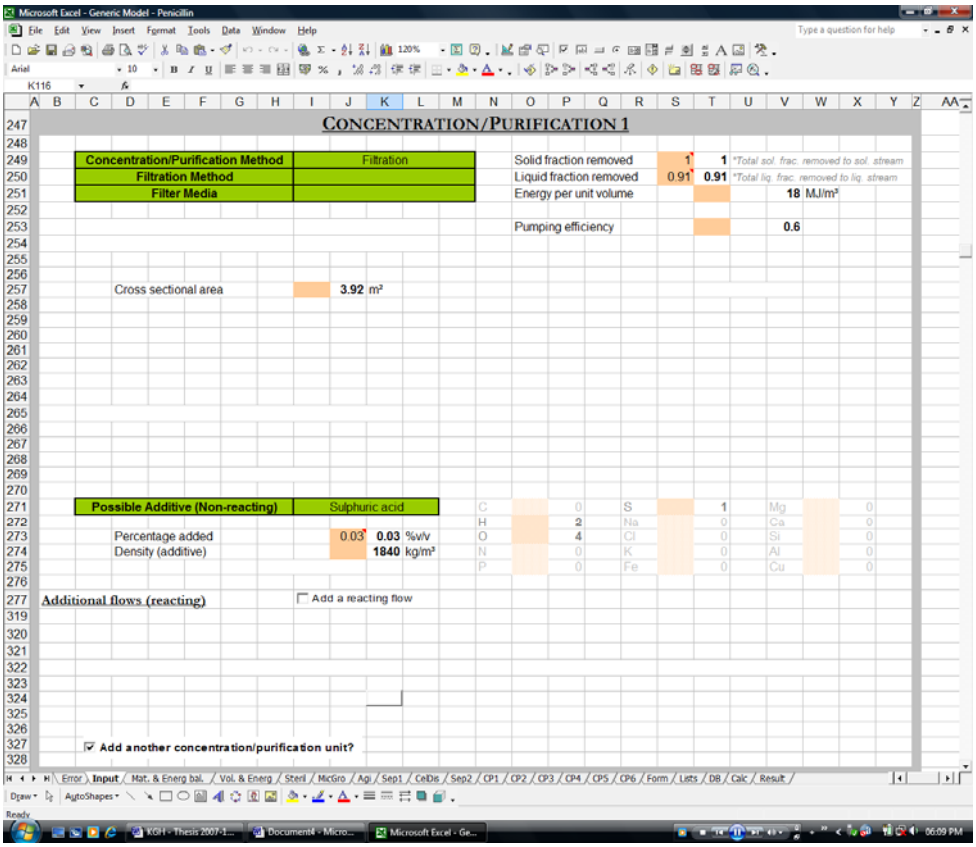

Figure E.6: Generic flowsheet model (Input) – Screenshot 6

Concentration and Purification 1

Concentration/Purification method (select): Filtration

Solid fraction removed (type): 1
Liquid fraction removed (type): 0.91

Possible additive (Non-reacting) (select): Sulphuric acid
Percentage added (type): 0.028

Add another concentration/purification unit: (Click)

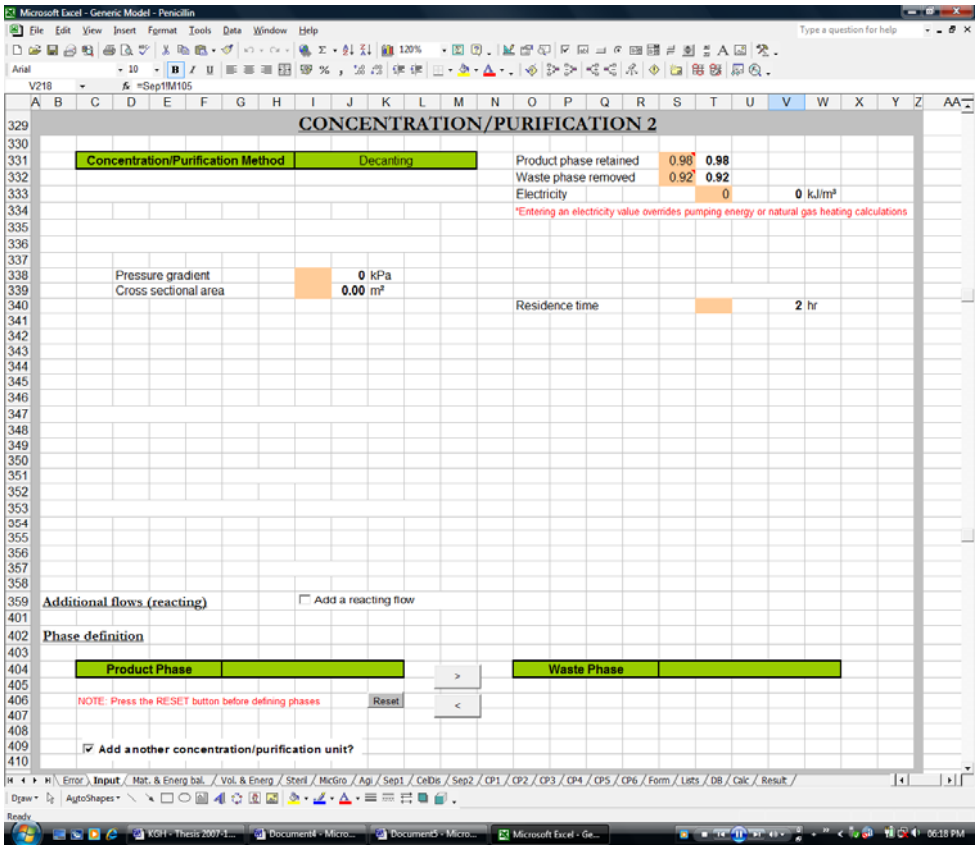

Figure E.7: Generic flowsheet model (Input) – Screenshot 7

Concentration/Purification 2

Concentration/purification method (select): Decanter

Product phase required: 0.98
Waste phase removed: 0.918

Reset: (Click)

Add another concentration/purification unit: (Click)

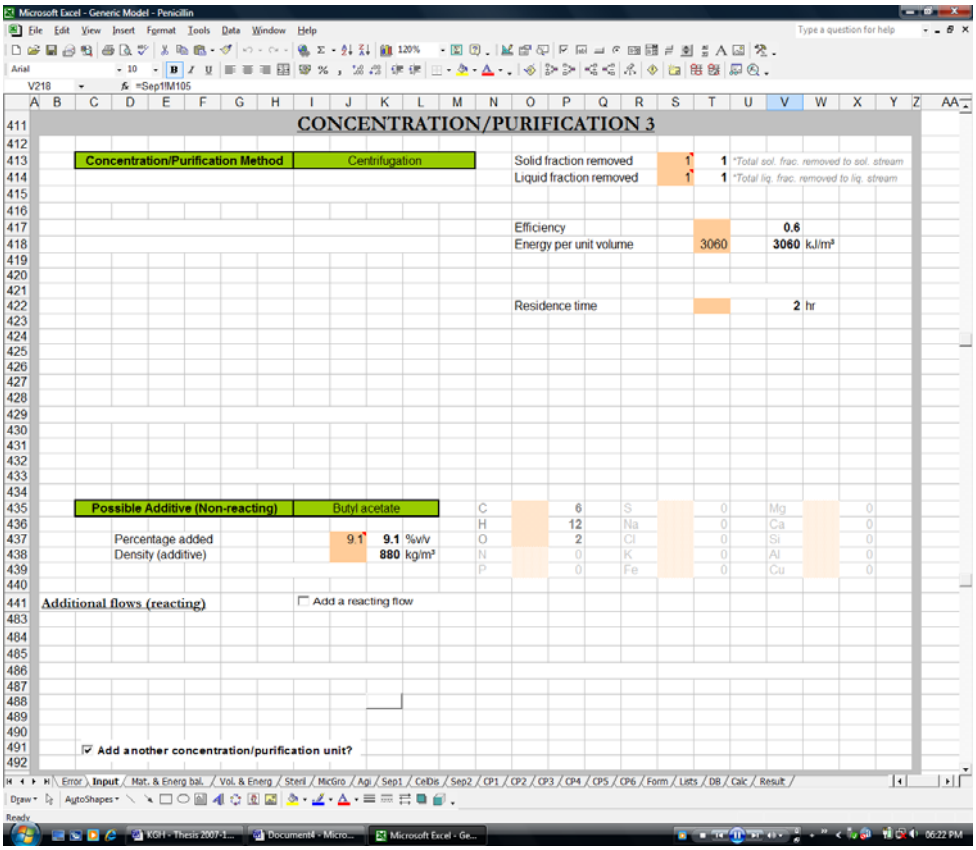

Figure E.8: Generic flowsheet model (Input) – Screenshot 8

Concentration/Purification 3

Concentration/purification method (select): Centrifugation

Solid fraction removed (type): 1
Liquid fraction removed (type): 1
Energy per unit volume (type): 3060

Possible additive (non-reacting) (select): Butyl acetate
Percentage added (type): 9.0997

Add another concentration/purification unit: (Click)

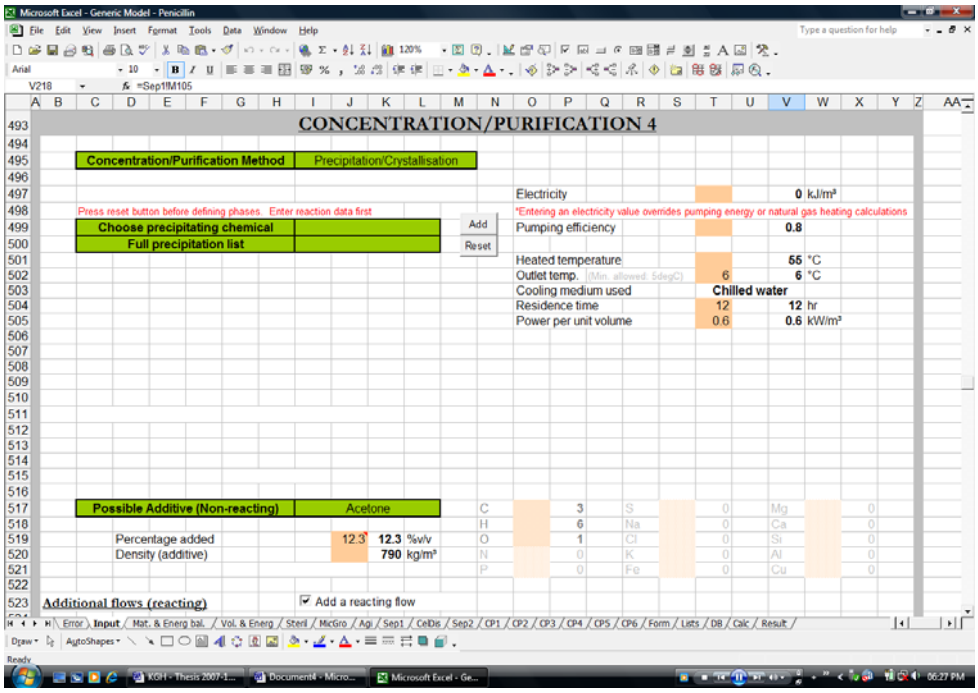

Figure E.9: Generic flowsheet model (Input) – Screenshot 9

Concentration/Purification 4

Concentration/purification method (select): Precipitation/Crystallisation

Outlet temperature (type): 6
Cooling media used (select): Chilled water
Residence time (type): 12
Power per unit volume (type): 0.6

Possible additive (non-reacting) (select): Acetone
Percentage added (type): 12.33

Add a reacting flow: (Click)

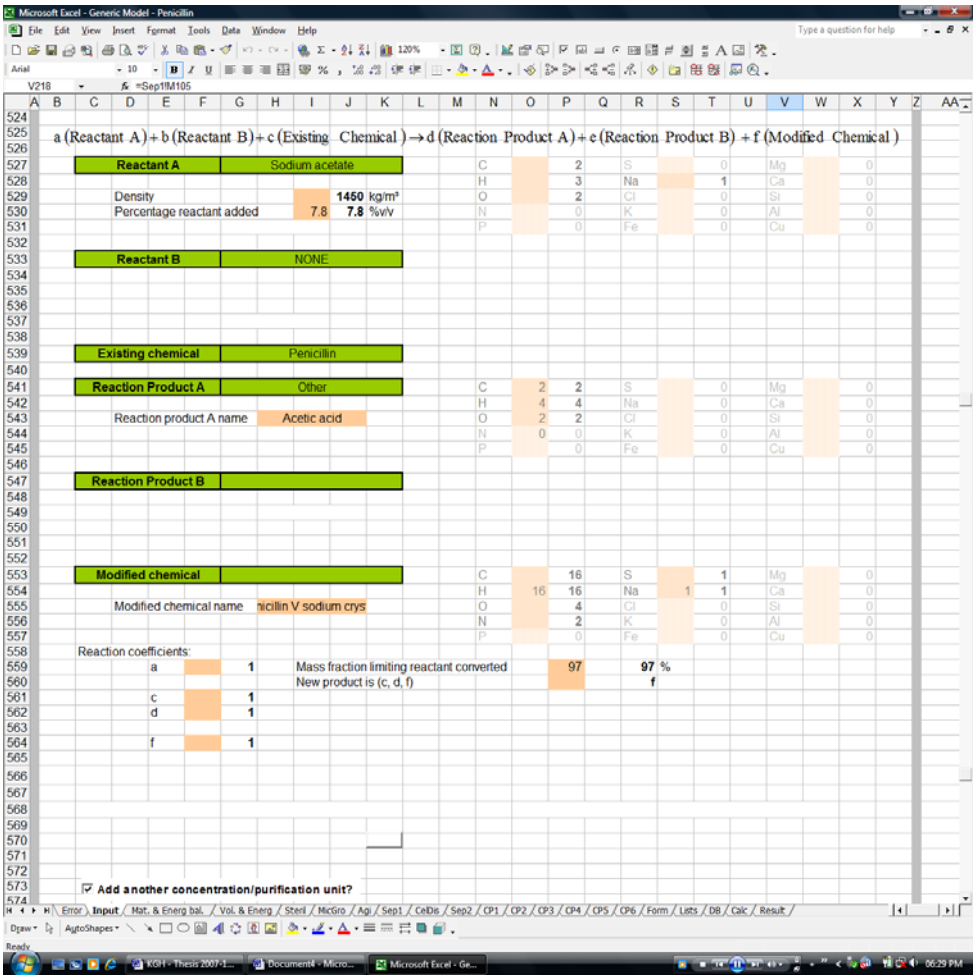

Figure E.10: Generic flowsheet model (Input) – Screenshot 10

Reactant A (select): Sodium acetate
Percentage added (type): 7.8
Existing chemical (select): Penicillin
Reaction product A (select): Other
Reaction product A name (type): Acetic acid
Chemical formula (type): C: 2, H: 4, O: 2, N: 0
Modified chemical name (type): Penicillin V sodium crystals
Chemical formula (type): H: 16, Na: 1
Mass fraction limiting reactant converted (type): 97

Choose precipitating chemical (select): Penicillin V sodium crystals
Add: (Click) [NOTE: This is an exception to the top down rule of the model]

Add another concentration/purification unit: (Click)

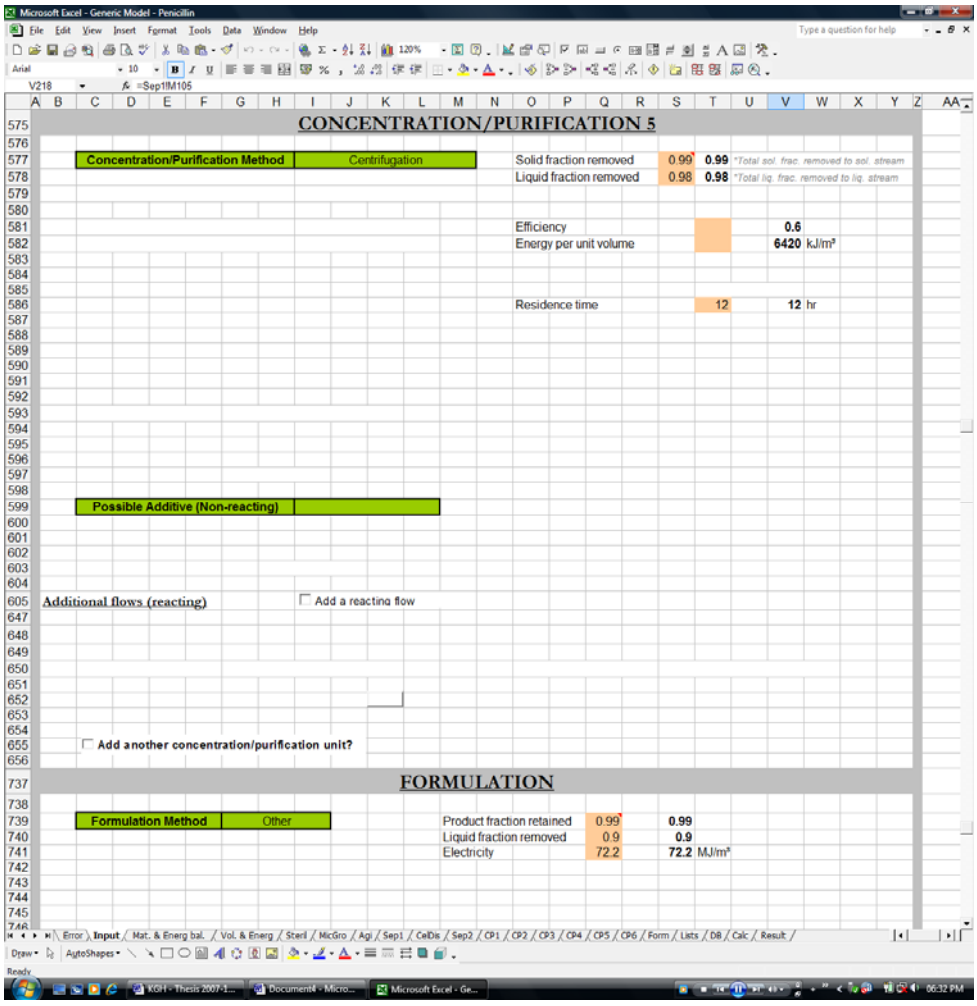

Figure E.11: Generic flowsheet model (Input) – Screenshot 11

Concentration/Purification 5

Concentration/purification method (select): Centrifugation

Solid fraction removed (type): 0.99
Liquid fraction removed (type): 0.979
Residence time (type): 12 hr

Formulation
Formulation method (select): Other
Product fraction retained (type): 0.99
Liquid fraction removed (type): 0.9
Electricity (type): 72.19

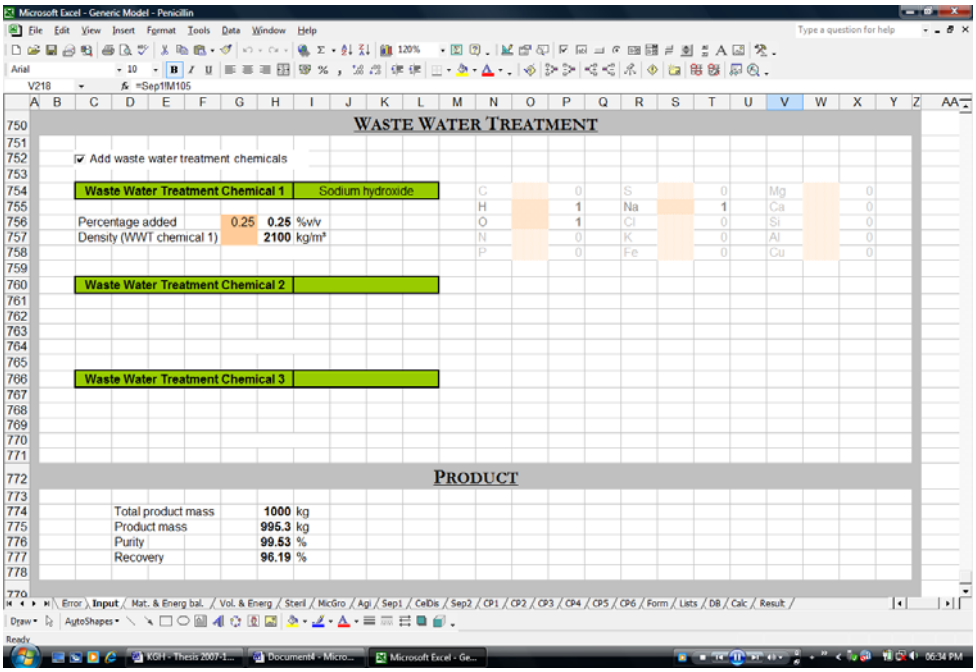

Figure E.12: Generic flowsheet model (Input) – Screenshot 12

Waste water treatment

Add waste water treatment chemicals: (Click)
Waste Water Treatment Chemical 1 (select): Sodium hydroxide

Percentage added (type): 0.25

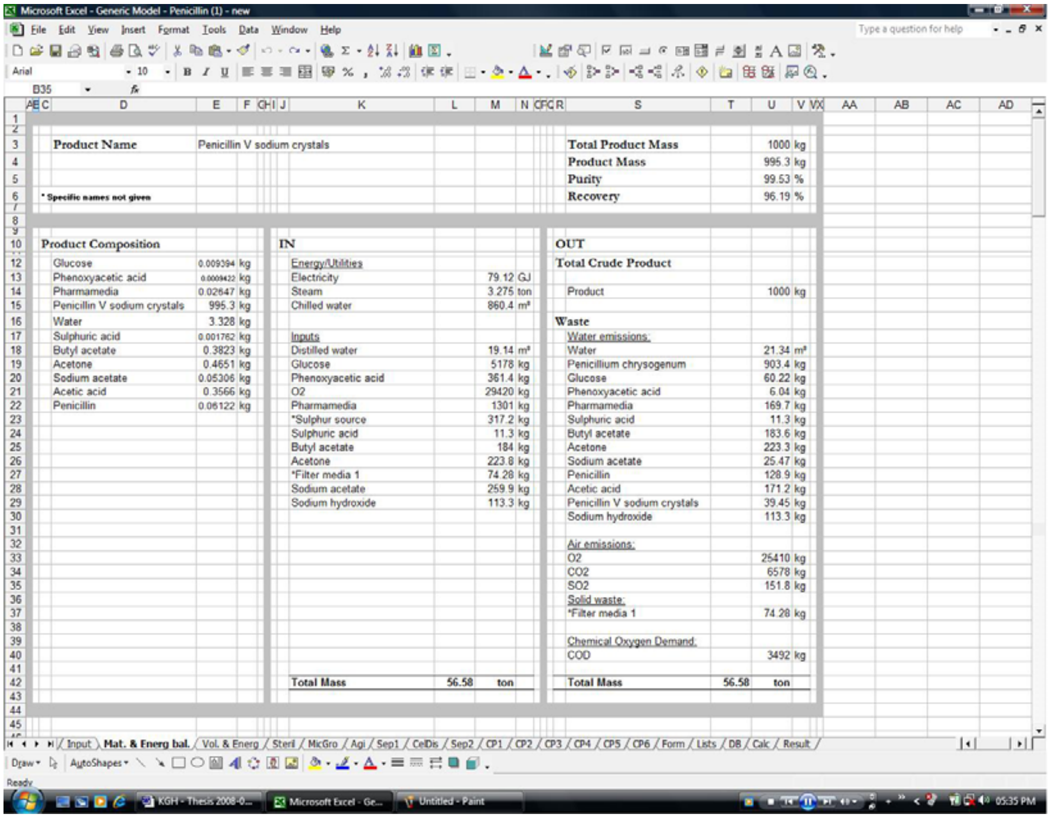

Figure E.13: Generic flowsheet model (Mat. & Energ bal.) – Screenshot 1

MS-EXCEL worksheet name: Results – Material and energy balances

Worksheet (click): Mat. & Energ bal.

This worksheet shows the results for the product produced. The product purity and recovery, product composition, material and energy requirements and waste streams are also given. Waste is given as water, air and solid emissions.

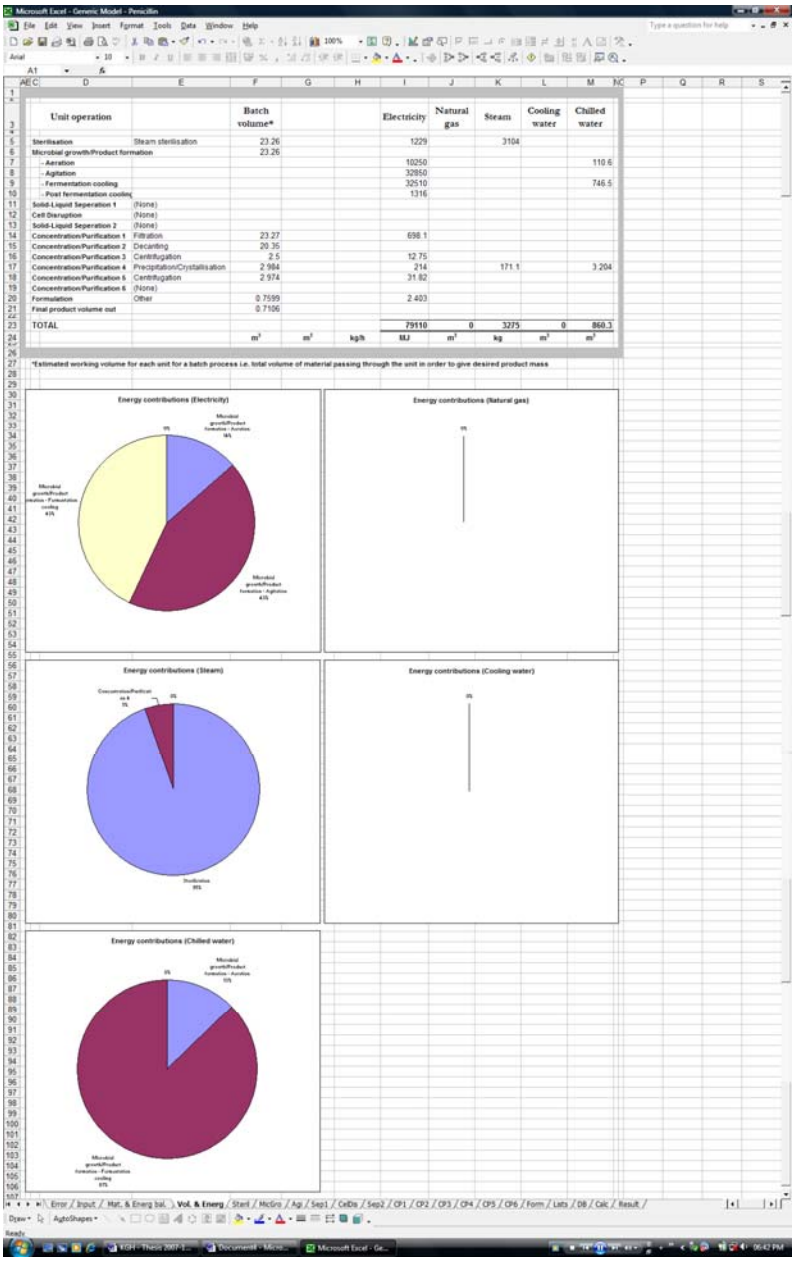

Figure E.14: Generic flowsheet model (Vol. & Energ) – Screenshot 1

MS-EXCEL worksheet name: Results – Volumes and Energy contributions

Worksheet (click): Vol. & Energ

This worksheet shows volumes calculated for each of the units used in the flowsheet. The energy requirements are broken down by unit into electricity, natural gas, steam, cooling water and chilled water. These are also presented graphically in pie charts as percentages of the total energy requirement for each unit.

Appendix F

Electricity LCA from South African Data

Appendix F: Electricity LCA from South African data

The electricity mix used in the LCA studies is for South Africa. The electricity mix is given by Eskom (2006) and shown in Table F.1. Most of South African electricity is produced by coal (93 %). The LCA impacts of electricity from coal are shown in Tables F.2 – F.7 as provided by Gediga (2006) as part of GaBi data (PE International 2007). Life Cycle inventories from the EcoInvent v1.3 (Swiss Centre for Life Cycle Inventories 2007) and ETH-ESU (Frischknecht *et al.* 1996) databases were used to complete the "underground coal mine", "coal from open coal mine", "infrastructure of underground coal mine" and "infrastructure of open coal mine" models.

LCA impacts for non-coal electricity are arbitrarily assumed equal to the impacts given for Great Britain as presented by the EcoInvent v1.3 database (Swiss Centre for Life Cycle Inventories 2007). The ways the electricity data are interlinked are shown in Figure F.1. Life Cycle Impact Assessment values are given in Table F.8.

Table F.1: Material and energy inputs and outputs for South African electricity production mix

Products and co-products	Value	Units	Allocation
Electricity production mix	221 216	GWh	100%
Inputs from technosphere (materials/fuel)	**Value**	**Units**	**Comments**
Electricity, coal	205 837	GWh	
Electricity, hydropower, at power plant	1 141	GWh	
Electricity, hydropower, at pumped storage power	2 867	GWh	
Electricity, natural gas	78	GWh	
Electricity, nuclear, water reactor power plant	11 293	GWh	
Electricity, wind power	0	GWh	No wind power taken into account

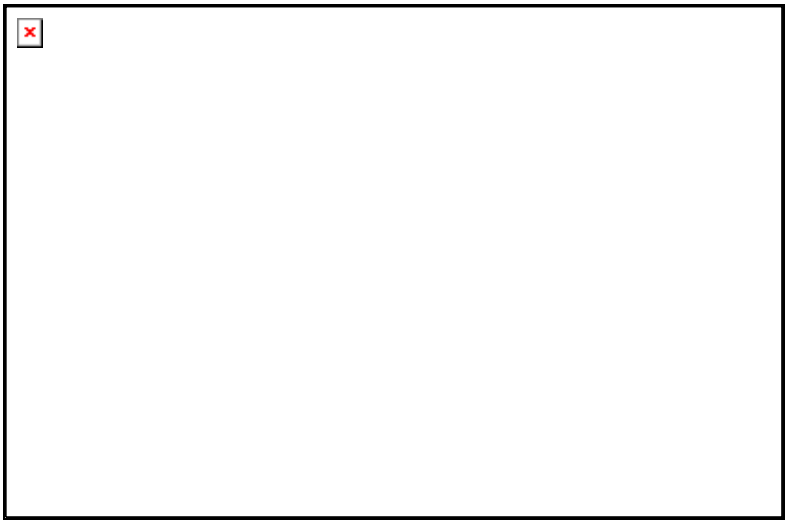

Figure F.1: Overall life cycle input/output structure for South African electricity mix

Table F.2: Life cycle inputs/outputs for electricity from coal (South Africa)

Products and co-products				**Value**	**Units**
Electricity, coal (South Africa)				1	MWh
Inputs from nature				**Value**	**Units**
Water, process and cooling, unspecified origin				0.000966	m^3
Inputs from technosphere (materials/fuel)				**Value**	**Units**
Coal, from mine (South Africa)				0.381	t
Emissions to air	**Value**	**Units**	**Emissions to air**	**Value**	**Units**
Carbon dioxide	688	kg	Arsenic	0.0000267	kg
Particulates, < 10 um	0.206	kg	Beryllium	0.00000324	kg
Sulphur dioxide	6.19	kg	Cadmium	0.000000953	kg
Nitrogen oxides	2.77	kg	Chromium	0.0000347	kg
Dinitrogen monoxide	0.0102	kg	Dioxins,	7.24E-11	kg
Ammonia	0.00000914	kg	Ethene, tetrachloro-	0.000013	kg
Benzene	0.0000103	kg	Hydrogen chloride	0.0686	kg
Methane	0.00724	kg	Hydrogen fluoride	0.00953	kg
Acrolein	0.0000137	kg	Lead	0.0000354	kg
Cobalt	0.0000103	kg	Manganese	0.0000876	kg
Formaldehyde	0.0000171	kg	Mercury	0.0000251	kg
Phenol	0.0000255	kg	Methane, dichloro-, HCC-30	0.0000533	kg
N-Nitrodimethylamine	0.0000029	kg	Methane, tetrachloro-, CFC-10	0.0000137	kg
Carbon monoxide	0.114	kg	Nickel	0.0000236	kg
Radioactive species, unspecified	0.000109	kBq	Non-methane volatile organic compounds, unspecified origin	0.0133	kg
Aldehydes, unspecified	0.0000724	kg	Selenium	0.0000953	kg
Antimony	0.00000533	kg	Ethene, trichloro-	0.000013	kg
Emissions to water	**Value**	**Units**	**Emissions to water**	**Value**	**Units**
Phosphate	0.00419	kg	Boron	0.0335	kg
Organic substances, unspecified	0.00648	kg	Chloride	0.00267	kg
Sulphuric acid	0.0838	kg	Suspended solids, unspecified	0.0495	kg
Final waste flows				**Value**	**Units**
Waste, final, inert				110	kg

Table F.3: Life cycle inputs/outputs for coal from South African coal mine

		Value	**Units**
Products and co-products	Coal, from mine (South Africa)	1	t
Inputs from technosphere (materials/fuel)	Coal from underground mine (South Africa)	0.6	t
	Coal from open mine (South Africa)	0.4	t

Table F.4: Life cycle inputs/outputs for underground coal mine (South Africa)

Products and co-products	Value	Units
Coal from underground mine (South Africa)	1	t
Inputs from nature	**Value**	**Units**
Water, unspecified natural origin	1700	kg
Gas, mine, off-gas, process, coal mining	6.7	kg
Coal, 18 MJ per kg	1670	kg
Land use II-III	4.87	m^2a
Land use II-IV	0.2	m^2a
Inputs from technosphere (materials/fuel)	**Value**	**Units**
Electricity production mix (South Africa)	0.000306	TJ
Concrete (un-reinforced)	10.6	kg
Wood	11.7	kg
Explosives	0.17	kg
Steel	2.5	kg
Diesel	1.10E-05	TJ
Industrial coal furnace (1-10MW)	0.00011	TJ
Underground coal mine infrastructure (SA)	1	t
Emissions to air	**Value**	**Units**
Methane	6.7	kg
Particulates, > 10 um	0.1	kg
Radon-222	12	kBq
Heat, waste	0.000306	TJ
Emissions to water	**Value**	**Units**
Ammonia, as N	0.001	kg
Chloride	17.05	kg
Fluoride	0.003	kg
Solved substances	1	kg
Aluminium	0.001	kg
Iron	0.002	kg
Manganese	0.001	kg
Nickel, ion	0.0001	kg
Strontium	0.005	kg
Zinc, ion	0.0001	kg
Sulphate	0.5	kg
Undissolved substances	0.015	kg
Emissions to soil	**Value**	**Units**
Coal tailings in landfill U	540	kg

Table F.5: Life cycle inputs/outputs for coal from open coal mine (South Africa)

Products and co-products	Value	Units
Coal from open mine (South Africa)	1	t
Inputs from nature	**Value**	**Units**
Water, unspecified natural origin	500	kg
Gas, mine, off-gas, process, coal mining	0.16	kg
Coal, 18 MJ per kg	1430	kg
Land use II-III	11.87	m^2a
Land use II-IV	2.7	m^2a
Land use III-IV	1.16	m^2a
Inputs from technosphere (materials/fuel)	**Value**	**Units**
Electricity production mix (SA)	0.0000540	TJ
EPDM rubber	0.14	kg
Explosives	1.7	kg
Diesel in building equipment	0.00034	TJ
Open coal mine infrastructure (SA)	1	t
Emissions to air	**Value**	**Units**
Methane	0.16	kg
Particulates, > 10 um	0.4	kg
Radon-222	12	kBq
Heat, waste	5.40E-05	TJ
Emissions to water	**Value**	**Units**
Ammonia, as N	0.001	kg
Chloride	0.35	kg
Fluoride	0.003	kg
Solved substances	1	kg
Aluminium	0.001	kg
Iron	0.002	kg
Manganese	0.001	kg
Nickel, ion	0.0001	kg
Strontium	0.005	kg
Zinc, ion	0.0001	kg
Salts, unspecified	0.1	kg
Sulphate	0.5	kg
Undissolved substances	0.015	kg
Emissions to soil	**Value**	**Units**
Coal tailings in landfill U	430	kg

Table F.6: Life cycle inputs/outputs for infrastructure of underground coal mine (South Africa)

Products and co-products	Value	Units
Underground coal mine infrastructure (South Africa)		
Inputs from nature	**Value**	**Units**
Land use II-III	0.8	m^2a
Land use II-IV	0.5	m^2a
Land use III-IV	0.5	m^2a
Inputs from technosphere (materials/fuel)	**Value**	**Units**
Electricity production mix (South Africa)	2.50E-06	TJ
Concrete (un-reinforced)	0.5	kg
Copper	0.003	kg
Steel low alloy	0.17	kg
Steel	1.5	kg
Diesel in building equipment	1.00E-05	TJ
Fuel oil lowS in boiler 1MW	1.00E-05	TJ
Emissions to air	**Value**	**Units**
Heat, waste	2.50E-06	TJ
Wastes and emissions to treatment	**Value**	**Units**
Concrete (inert) to landfill	0.5	kg

Table F.7: Life cycle inputs/outputs for infrastructure of open coal mine (South Africa)

Products and co-products	Value	Units
Open coal mine infrastructure (South Africa)	1	t
Inputs from technosphere (materials/fuel)	**Value**	**Units**
Electricity production mix (South Africa)	1.40E-06	TJ
Concrete (un-reinforced)	0.47	kg
Wood massive	0.0043	kg
Copper	0.005	kg
Steel low alloy	0.05	kg
Steel	0.1	kg
Diesel in building equipment	5.70E-06	TJ
Fuel oil lowS in boiler 1MW	5.70E-06	TJ
Emissions to air	**Value**	**Units**
Heat, waste	1.40E-06	TJ
Wastes and emissions to treatment	**Value**	**Units**
Concrete (inert) to landfill	0.47	kg

Table F.8: Life LCIA of South African electricity mix (functional unit: 1 GJ) – CML 2 Baseline 2000 V2.03

Impact category	Unit	Characterisation
Abiotic Depletion	kg Sb_{eq}	2.18
Global Warming (GWP100)	kg $CO_{2\ eq}$	196.7
Ozone Layer Depletion (ODP)	mg CFC-11_{eq}	6.92
Human Toxicity	kg 1,4-DB_{eq}	105.3
Fresh Water Aquatic Ecotoxicity	kg 1,4-DB_{eq}	27.67
Marine Aquatic Ecotoxicity	kg 1,4-DB_{eq}	172000
Terrestrial Ecotoxicity	kg 1,4-DB_{eq}	0.214
Photochemical Oxidation	kg C_2H_2	0.0833

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Appendix G

Sugar LCA from South African Sugar Cane

Appendix G: Sugar LCA from South African Sugar Cane

Sugar and bagasse are used in several parts of the thesis for raw material inputs, including the LCA equivalent for glucose. A South African model is used for this and adapted from the work by Theka (2002), Botha (2003), Botha and von Blottnitz (2006) and von Blottnitz (2006). Inputs and outputs for the production of sugar (from cane) and bagasse are given in Table G.1 and the growth of the sugar cane in Table G.2. Life Cycle Impact Assessment values are given in Table G.3.

Table G.1: Material and energy inputs and outputs for sugar (from cane) and bagasse

Products and co-products	**Value**	**Units**	**Allocation**	
Sugar (from caner)	3	t	75 %	
Molasses	1	t	25 %	
Bagasse	20.14	t	0 %	Used internally for energy
Inputs from nature	**Value**	**Units**	**Comments**	
Process water, unspecified origin	2540.64	l		
Inputs from technosphere (materials/fuel)	**Value**	**Units**	**Comments**	
Lime	25	kg		
Sugarcane	25	t		
Truck (40t)	1249	tkm	30km distance travelled	
Diesel	0.137	kg		
Inputs from technosphere (electricity/heat)	**Value**	**Units**	**Comments**	
Heat, hard coal industrial furnace	0	GJ	Assumption: Bagasse burning produces required heat (6.1 GJ)	
Electricity, production mix South Africa	0	kWh	Assumption: Bagasse burning produces required energy (23 kWh)	
Emissions to air	**Value**	**Units**	**Comments**	
Carbon dioxide	9.7863	t	Carbon uptake during cane growth shown in sugar cane data	
Nitrogen oxides	24.107	kg		
Particulates	2.3134	kg		
Sulphur oxides	7.5	kg		
Emissions to water	**Value**	**Units**	**Comments**	
Wastewater	85.479	l		
Dissolved substances	0.1258	kg		
Suspended solids	0.0014.	kg		
Chemical oxygen demand (COD)	0.0014	kg		
Final waste flows	**Value**	**Units**	**Comments**	
Solid waste	0.3	t		

Table G.2: Material and energy inputs and outputs for sugarcane

Products and co-products	Value	Units	Allocation
Sugarcane	1	t	100 %
Inputs from nature	**Value**	**Units**	**Comments**
Process water, unspecified origin	154.99	m^3	Reference: Hlatshwayo (2005)
Arable land	154.99	m^2a	Reference: SASA (2005)
Inputs from technosphere (materials/fuel)	**Value**	**Units**	**Comments**
Fertiliser	6.59	kg	
Diesel	0.95	kg	
Emissions to air	**Value**	**Units**	**Comments**
Carbon dioxide	-0.4692	t	Carbon uptake

Table G.3: LCIA of South African sugar from cane (functional unit: 1 t) – CML 2 Baseline 2000 V2.03

Impact category	Unit	Characterisation
Abiotic Depletion	kg $Sb_{eq.}$	0.579
Global Warming (GWP100)	kg $CO_{2\,eq.}$	-433.9
Ozone Layer Depletion (ODP)	mg $CFC\text{-}11_{eq.}$	17.1
Human Toxicity	kg $1,4\text{-}DB_{eq.}$	21.97
Fresh Water Aquatic Ecotoxicity	kg $1,4\text{-}DB_{eq.}$	2.30
Marine Aquatic Ecotoxicity	kg $1,4\text{-}DB_{eq.}$	12000
Terrestrial Ecotoxicity	kg $1,4\text{-}DB_{eq.}$	0.0938
Photochemical Oxidation	kg C_2H_2	0.121

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Appendix H

Sensitivity Analysis Data

Appendix H: Sensitivity Analysis Data

The data in Appendix H are the material and energy balance results for the sensitivity of changing single inputs on the Penicillin V flowsheet model as discussed in Chapter 7. Table H.1, gives the original and modified input values as used. The first of the parameters changed, giving results for a 10 % and 25 % increase and decrease in a single value is presented in Table H.2. The results for the other variables changed can be found on the accompanying CD-Rom.

Material and energy balance results which are underlined were those which changed. Results shown in bold and underlined were those which changed by more than the percentage of the changed variable (*e.g.* a change of 10 % to separation efficiency caused a change greater than 10 % in the electricity input required). The Life Cycle Assessment (LCA) results for each variable change are also given.

Table H.1: Original and modified input values for the extracellular, aerobic production of penicillin in a batch reactor

No.^	Parameter	Original value	Default value′	-25 %	-10 %	+10 %	+25 %	Units
1	Cooling water temperature	5	10	3.75	4.5	5.5	6.25	°C
	Steam Sterilisation (140°C, 3 bar)							
2	Reactor temperature	32	37	24	28.8	35.2	40	°C
	Microbial growth conditions (batch production of Penicillin from *Penicillium chrysogenum*)							
3	Product: Biomass ratio	1.2	1	0.9	1.1	1.3	1.5	-
4	Carbon 1 source (excess): Glucose	2	1	1.5	1.8	2.2	2.5	%
5	Carbon 2 source (excess): Phenoxyacetic acid	1.7	1	1.3	1.5	1.9	2.1	%
6	Mass percentage Carbon 2 as total carbon	10.6	50	7.9	9.5	11.76	13.3	%
7	Nitrogen source (excess): Pharmamedia	15	5	11.35	13.5	16.5	18.7	%
8	Sulphur source excess	0	5	-	-	-	-	
9	Oxygen flowrate (vvm)″	0.021	0.0140	0.016	0.020	0.024	0.027	
10	Single stage reciprocating compression	607.95	300	450	540	660	750	kPa
11	Time over which maintenance was considered	106	10	79.5	95.4	117	133	h
12	Final biomass concentration	45	*16.7*	33.8	40.5	49.5	56.3	g/l
13	Yield coefficients: $Y_{x/s}$	0.45	**0.43**	0.34	0.41	0.50	0.56	g/g
14	$Y_{p/s}$	0.81	**0.49**	0.61	0.73	0.85*	0.85*	g/g
	Agitation (Energy: Electricity)							
15	Number of tanks	11	5	8	10	12	14	
16	Power per unit volume	2.5	*0.88*	1.9	2.3	2.8	3.1	kW/m^3
	Efficiency#	1	-	-	-	-	-	

No.^	Parameter	Original value	Default value′	-25 %	-10 %	+10 %	+25 %	Units
	Post bioreactor cooling							
17	Outlet temperature	28	15	21	25	31	32*	°C
	Filtration (Energy: Electricity)							
18	Solid fraction removed	100	95	75	90	100*	100*	%
19	Liquid fraction retained	91	70	68	82	100*	100*	%
20	Additive: Sulphuric acid	0.028	5	0.021	0.025	0.031	0.035	%v/v
	Centrifugation (Energy: Electricity)							
21	Product fraction retained	98	99	74	88	100*	100*	%
22	Waste fraction removed	91.8	95	68.9	82.6	100*	100*	%
23	Energy per unit volume	3060	*6420*	2300	2750	3370	3820	kJ/m^3
24	Additive: Butyl acetate	9.1	5	6.8	8.2	10.0	11.4	%v/v
	Precipitation and Crystallisation (Energy: Electricity and steam)							
25	Outlet temperature	6	40	7.5	6.6	5.4	5*	°C
	Residence time**	12	-	-	-	-	-	h
26	Power per unit volume	0.6	0.8	0.45	0.54	0.66	0.75	kW/m3
27	Additive: Acetone	12.3	5	9.2	11.1	13.5	15.4	%v/v
28	Additive: Sodium acetate	7.8	25	5.8	7.0	8.6	9.8	%v/v
	Reaction: Sodium acetate + Penicillin → Acetic acid + Penicillin V sodium crystals							
29	Conversion of limiting reagent	97	80	73	87.3	100*	100*	%
	Centrifugation (Energy: Electricity)							
30	Solid fraction removed	99	98	74	89	100*	100*	%
31	Liquid fraction removed	97.9	80	73	88	100*	100*	%
	Fluid bed drying (Energy: Electricity)							
32	Product fraction retained	99	99	74	89	100*	100*	%
33	Liquid fraction removed	90	99	68	81	99	100*	%
34	Electricity required	72.7	**10**	55	65	80	91	MJ/m^3
	Waste Water Treatment							
35	Additive: Sodium hydroxide	0.25	5	0.18	0.22	0.28	0.32	%v/v

^ Reference number for the variable changed

′ Bold values indicate average values from various sources, values in italics are calculations, unaltered numbers indicate estimations based on industry norms and underlined values are those which are the authors approximation based on possibly values required

*** Maximum or minimum value allowed; compromising the 10 or 25 % increase or decrease as shown**

″ This accounts for oxygen content of the air only (excluding nitrogen)

 # Changing efficiency has the same effect as changing the power per unit volume

**** In batch units, residence time only affects power required – changing residence time has the same effect as changing power per unit volume.**

Table H.2: Sensitivity data for M&E balance for the production of Penicillin. Variable changed: Cooling water temperature

Component	Original	+10 %	+25 %	-25 %	-10 %	Default	
INPUT (kg)							
Acetone	0.22	0.22	0.22	0.22	0.22	0.22	
Butyl acetate	0.18	0.18	0.18	0.18	0.18	0.18	
Glucose	5.18	5.18	5.18	5.18	5.18	5.18	
Oxygen (excl. excess & N_2)	4.02	4.02	4.02	4.02	4.02	4.02	
Pharmamedia	1.30	1.30	1.30	1.30	1.30	1.30	
Phenoxyacetic acid	0.36	0.36	0.36	0.36	0.36	0.36	
Sodium acetate	0.26	0.26	0.26	0.26	0.26	0.26	
Sodium hydroxide	0.11	0.11	0.11	0.11	0.11	0.11	
Sulphuric acid	0.01	0.01	0.01	0.01	0.01	0.01	
Sulphur source	0.32	0.32	0.32	0.32	0.32	0.32	
Water	19.1	19.1	19.1	19.1	19.1	19.1	
Energy requirements	**Original**	**+10 %**	**+25 %**	**-25 %**	**-10 %**	**Default**	
Electricity	79.1	**79.2**	**79.3**	**79.0**	79.1	**81.5**	MJ/kg pen.
Steam (140°C, 3 bar)	3.3	3.3	3.3	3.3	3.3	3.3	kg/kg pen.
Chilled water	0.86	**0.90**	**0.95**	**0.78**	**0.83**	**1.49**	m^3/kg pen.
OUTPUT (kg)	**Original**	**+10 %**	**+25 %**	**-25 %**	**-10 %**	**Default**	
Acetic acid	0.17	0.17	0.17	0.17	0.17	0.17	
Acetone	0.22	0.22	0.22	0.22	0.22	0.22	
Biomass (dry cell weight)	0.90	0.90	0.90	0.90	0.90	0.90	
Butyl acetate	0.18	0.18	0.18	0.18	0.18	0.18	
Carbon dioxide	6.58	6.58	6.58	6.58	6.58	6.58	
Glucose	0.06	0.06	0.06	0.06	0.06	0.06	
Pen. V & Pen. V., sodium salt (loss)	0.17	0.17	0.17	0.17	0.17	0.17	
Penicillin V sodium salt	1	1	1	1	1	1	
Pharmamedia	0.17	0.17	0.17	0.17	0.17	0.17	
Phenoxyacetic acid	0.01	0.01	0.01	0.01	0.01	0.01	
Sodium acetate	0.03	0.03	0.03	0.03	0.03	0.03	
Sodium hydroxide	0.11	0.11	0.11	0.11	0.11	0.11	
Sulphuric acid	0.01	0.01	0.01	0.01	0.01	0.01	
Water	21.3	21.3	21.3	21.3	21.3	21.3	
Single Score LCA results (Pts)	**Original**	**+10 %**	**+25 %**	**-25 %**	**-10 %**	**Default**	
EPS 2000 v2.02	7.76	7.76	**7.77**	**7.75**	7.76	**7.86**	
Ecoindicator 99 (E) v2.04	2.17	2.17	2.17	**2.16**	2.17	**2.2**	
Ecopoints	31200	31200	31200	31200	31200	**31700**	

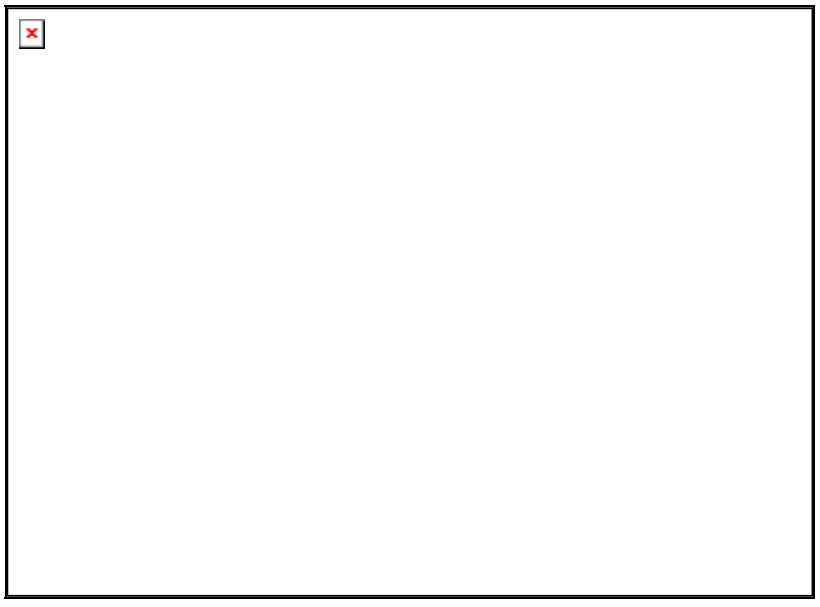

Figure H.1: LCA sensitivity results using the CML Baseline 2.03 methodology. Variable changed: Cooling water temperature

Appendix I

Life Cycle Inventory Tables

Appendix I: Life Cycle Inventory Tables

The Life Cycle Inventory data for the production of 1 kg of Penicillin V, as shown in Chapter 3, are given in Table I.1. This is a shortened version of the first of several tables which can be found on the accompanying CD-Rom for the Life Cycle Inventory data of the various LCA studies in this thesis.

Table I.1: Life Cycle Inventory for the production of 1 kg of Penicillin V, as used in Chapter 3

No	Substance	Compartment	Unit	Penicillin: Scenario 1	Penicillin: Scenario 2	Penicillin: Scenario 3	Penicillin: Biwer *et al.* (2006)
1	Air	Raw	oz	49.54	62.60	50.62	31.33
2	Aluminium, 24% in bauxite, 11% in crude ore, in ground	Raw	g	1.95	2.03	2.82	2.02
3	Anhydrite, in ground	Raw	mg	1.51	1.51	2.12	0.92
4	Atrazine	Raw	mg	264.68	242.28	313.54	95.69
5	Barite, 15% in crude ore, in ground	Raw	g	4.50	5.29	7.60	6.42
6	Baryte, in ground	Raw	mg	558.92	660.02	651.98	459.38
7	Basalt, in ground	Raw	g	1.87	2.07	2.90	2.43
8	Bauxite, in ground	Raw	g	1.36	1.61	1.47	1.18
9	Borax, in ground	Raw	µg	285.97	315.52	442.23	371.06
10	Calcite, in ground	Raw	g	121.29	127.83	179.46	128.56
11	Calcium sulfate, in ground	Raw	mg	1.30	1.30	1.93	0.70
12	Carbon dioxide, in air	Raw	g	51.14	56.20	77.79	64.48

The missing lines left out here can be found on the accompanying CD-ROM

798	Strontium	Soil	µg	246.55	291.64	407.51	356.79
799	Sulfur	Soil	mg	18.70	21.84	29.74	25.08
800	Tebutam	Soil	ng	37.18	41.23	57.50	47.74
801	Teflubenzuron	Soil	ng	8.23	8.91	12.36	9.74
802	Tin	Soil	µg	0.93	0.95	1.64	0.92
803	Titanium	Soil	µg	38.91	42.75	58.49	48.68
804	Vanadium	Soil	µg	1.11	1.22	1.67	1.39
805	Zinc	Soil	mg	0.99	1.13	1.55	1.29