Systematic investigation of potential factors that affect the production costs of the bio-based and bio-degradable plastic polyhydroxyalkanoates (PHAs) by a costing analysis based on early process simulation

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"There is no such thing as a 'self-made' man. We are made up of thousands of others. Everyone who has ever done a kind deed for us, or spoken one word of encouragement to us, has entered into the make-up of our character and of our thoughts, as well as our success" ~G. M. Adams.

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CONTENTS

Plagiarism Declaration i

Acknowledgements ii

Contents iii

List of Figures vii

List of Tables ix

Glossary xiii

Abbreviations xiv

Abstract xv

1 Introduction 1

1.1 Background to investigation 1

1.2 Problem statement 2

1.3 Project aims, objectives and impact 3

1.4 Layout of dissertation 3

2 Literature Review 5

2.1 Bio-plastics as promising alternatives to petroleum plastics 5

2.2 An overview of bio-based and bio-degradable plastics 6

2.3 Polyhydroxyalkanoates (PHAs): Bio-degradable and Bio-based plastics 8

2.3.1 Interesting features and applications of PHAs 8

2.3.2 Biosynthesis of PHAs 12

2.3.3 Microorganisms producing PHAs and the corresponding carbon sources 13

2.3.4 Cultivation methods for PHAs 16

2.3.5 Purification and recovery methods for the extraction of PHAs 16

2.4 Large scale production of PHAs 17

2.4.1 Commercial production of PHAs 17

2.4.2 Challenges with the large scale production of PHAs 19

2.4.3 PHA economics as a tool to inform reduction of costs for PHA production 23
2.4.4 Previous economic assessment on large scale production of PHAs 23
2.4.5 Reviewing simulation packages for assessing large scale production of PHAs 26
2.5 Large scale production of bio-products using the CeBER Bioprocess Modeller as simulation tool 27
2.5.1 Process design considerations for equipment sizing 29
2.5.2 Calculation of PHA production costs 31
2.6 Synthesis of Literature Review 34
2.7 Thesis Objectives 35
3 Conceptual approach to PHA production flowsheet 36
3.1 Introduction 36
3.2 Constructing the base case scenario for the large scale production of PHAs using the CeBER Bioprocess Modeller 36
3.2.1 The Harding (2008) model for the production of the PHA monomer, PHB 36
3.2.2 Adaptation of the Harding (2008) PHB model for this study 38
3.2.3 Process considerations to address for large scale production 41
3.3 Process costing for the large scale production of PHB 43
3.3.1 Approach to equipment costing 43
3.3.2 Raw materials costs 45
3.3.3 Utilities costs 45
3.3.4 Labour costs 46
3.3.5 Waste treatment costs 46
3.3.6 Computing the production costs of PHB 46
3.4 Construction of different scenarios for investigating the PHB production system 48
3.4.1 Effect of energy efficiency of centrifuges and the homogeniser efficiency 48
3.4.2 Effect of PHB purification and recovery process for downstream processing 48

3.4.3 Effect of microbial properties 53
3.4.4 Effect of carbon source 54
3.4.5 Development of an optimised scenario for the large scale production of PHB 54
3.5 Concluding remarks 55
4 Results and Discussions 56
4.1 Introduction 56
4.2 Base case scenario simulation output from the CeBER Bioprocess Modeller: material and energy inventories 56
4.2.1 Process description, flow sheet and output for the base case scenario 57
4.2.2 Approach to preliminary equipment sizing 60
4.2.3 Equipment sizing and costing results for the base case scenario 62
4.2.4 Calculation of annual PHB production costs 64
4.2.5 Analysis of breakdown of costs, electricity usage and equipment costs for the base case scenario 66
4.2.6 Process summary for the base case scenario 68
4.3 Investigating the effect of the energy efficiency of the centrifuges and the homogeniser efficiency on PHB production system 69
4.4 Investigating the effect of purification and recovery processes on PHB production 71
4.4.1 Surfactant-sodium hypochlorite purification and recovery method 71
4.4.2 Sodium hydroxide recovery method 76
4.4.3 Process comparison among the three purification and recovery processes 79
4.5 Investigating the effect of microbial properties on PHB production 81
4.6 Investigating the effect of carbon sources on PHB production 86
4.7 Optimised case scenario for PHB production 90
5 Conclusions 92
5.1 PHAs as promising alternatives to petroleum plastics 92

5.2 Importance of early stage process simulation and economics 92
5.3 Relative importance of key factors for the large scale production of PHAs 93
5.4 Key findings towards a cost effective large scale production of PHAs 95
5.5 Impact of study and recommendations for future work 96
References 98
Appendix 118
A-1: Effect of equipment efficiency additional data 118
B-1: Effect of microbial properties additional data 126
C.1: Effect of using starch as carbon source additional data 142
D-1: Effect of optimisation additional data 144

LIST OF FIGURES

Figure 2-1: Material coordinate system for bioplastics adapted from Grotto (2012) 6
Figure 2-2: Closed loop life cycle for a bio-based and bio-degradable bio-plastic (Klazinga 2009) 7
Figure 2-3: General structure of polyhydroxyalkanoates (Ebnesajjad 2013) 9
Figure 2-4: Processing technologies for different % co-monomer content in PHAs e.g. PHB/HV (Institute for Prospective Technological Studies 2005) 11
Figure 2-5: Metabolic pathways for PHA synthesis adapted from Chen *et al.* (2015) 12
Figure 2-6: Comparison of PHAs (green circle) and PLA (orange circle) to petroleum plastics (grey circle) in two environmental impacts: climate change and fossil resource depletion (Essel 2012) 22
Figure 2-7: Representation of the generic process flow diagram of the CeBER Bioprocess Modeller generic flowsheet (Harding 2008) 29
Figure 3-1: Process flow diagram for the production of 1000 kg of PHB per run using an enzymatic digestion purification recovery process developed by Harding (2008) simulated in the CeBER Bioprocess Modeller 37
Figure 3-2: Settling velocity under gravity (Stokes' Terminal Settling Velocity-TSV) and the volumetric throughput (Dunn 1998) 42
Figure 3-3: Process flowsheet for PHB production using a surfactant-sodium hypochlorite purification and recovery method (Choi & Lee 1997) 49
Figure 4-1: Process flow diagram for the simulation 20 tonnes of crude PHB per run for the base case scenario 58
Figure 4-2: Gantt chart for the simulation of 20 tonnes of crude PHB per run for the base case scenario 62
Figure 4-3: Total annual production costs breakdown for the production of 2929 tonnes of pure PHB per year for the base case scenario in which a total production cost of $ 24.4 million was estimated in 2014 66
Figure 4-4: Raw material costs breakdown for the production of 2929 tonnes of pure PHB per year for the base case scenario where raw materials costs of $ 8.22 million was estimated in 2014 66
Figure 4-5: Total annual production costs breakdown for the 2850 tonnes of pure PHB per year with a surfactant-sodium hypochlorite recovery process simulated in SuperPro in which a total production cost of $ 17.5 million was estimated in 1996 (Choi & Lee 1997) 67

Figure 4-6: Total annual production costs breakdown for the net production of 4300 tonnes of crude PHB per year using a sodium hypochlorite purification and recovery process in which a total production cost of $ 19.7 million was estimated in 1995 (Van Wegen *et al.* 1998)..... 67
Figure 4-7: Equipment costs breakdown for the net production of 2929 tonnes of pure PHB per year for the base case scenario where the total equipment costs were estimated at $ 9.6 million in 2014 67
Figure 4-8: Electricity usage breakdown for the net production of 2929 tonnes of pure PHB per year for the base case scenario where the total electricity usage was 506100 MJ 67
Figure 4-9: Process summary for the production of 2929 tonnes of pure PHB per year for the base case scenario illustrating the cost of production of PHB ($/kg), total energy usage of the process (MJ/kg PHB), total water usage (kg/kg PHB) 68
Figure 4-10: Effect of varying the energy efficiency of the centrifuges and the homogeniser efficiency on the annual large scale production of PHB using the CeBER Bioprocess Modeller 69
Figure 4-11: Process flowsheet for PHB production using a surfactant-sodium hypochlorite purification and recovery method developed in the CeBER Bioprocess Modeller simulating 20 tonnes of crude PHB per run 72
Figure 4-12: Gantt chart for the simulation of 20 tonnes of crude PHB per run with a sodium hypochlorite-surfactant purification and recovery process simulated in the CeBER Bioprocess Modeller 75
Figure 4-13: Process flowsheet for PHB production with a sodium hydroxide purification and recovery method developed in the CeBER Bioprocess Modeller simulating 20 tonnes of crude PHB per run..... 76
Figure 4-14: Gantt chart for the simulation of 20 tonnes of crude PHB per run with a sodium hydroxide purification and recovery process simulated in the CeBER Bioprocess Modeller 78
Figure 4-15: Effect of different purification and recovery processes on the annual large scale production of PHB simulated in the CeBER Bioprocess Modeller 80
Figure 4-16: Half normal probability plot displaying the absolute values of the three effects, PHB biomass ratio, final non-PHB biomass (g/L), ratio and culture time (hours) with respect to production costs 83
Figure 4-17: Effect of microbial properties for the annual large scale production of PHB simulated in the CeBER Bioprocess Modeller 85
Figure 4-18: Effect of using starch as waste carbon source for the large scale production of PHB 89
Figure 4-19: Investigation of an optimised case scenario on the large scale production of PHB with a PHB productivity of 3.8 g/L/h , a sodium hypochlorite-surfactant purification and recovery process with a cell

rupture efficiency of 95 % and an energy efficiency of 70 % for the centrifuges, and a hypothetical carbon source assumed to be free of cost.....90

LIST OF TABLES

Table 2-1: Comparison between the bio-plastics polylactic acid (PLA) and polyhydroxyalkanoates (PHAs) (Chen, 2009).....8
Table 2-2: General structure of PHAs adapted from Ebnesajjad (2013) 9
Table 2-3: Applications of PHAs in various industries adapted from Chen (2009).....11
Table 2-4: Examples of precursors for PHA production in *Cupriavidus necator* (Braunegg *et al.* 2004).....13
Table 2-5: Summary of bacterial strains producing PHAs including the type of carbon sources used and type PHAs produced (Verlinden *et al.* 2007) 14
Table 2-6: PHA production from plant oils and various wastes adapted from Chee *et al.* (2010a) 15
Table 2-7: Advantages and disadvantages of various PHA extraction methods adapted from Kunasundari & Sudesh (2011).....17
Table 2-8: Global suppliers of PHAs adapted from Chen (2009), Chanprateep (2010), Biomaterials China News, (2011), Babu *et al.* (2013), Plasteurope.com (2015) and Bio-on (2015).....18
Table 2-9: Wild type and industrial bacterial strains commonly used for pilot and large scale production of PHAs (Chen 2009) 19
Table 2-10: Comparison between the large scale production of PHAs and petroleum plastics adapted from Volova (2004) Wang *et al.* (2014): 20
Table 2-11 Tradenames and price of PHAs ($/kg) from large sale producers of PHAs adapted from Chanprateep (2010).....21
Table 2-12: Summary of feasibility studies conducted on the large scale production of PHAs 24
Table 2-13: Summary of findings from feasibility studies conducted on the large scale production of PHAs.....25
Table 2-14: Common commercial software used for process simulation adapted from Gosling (2005)...27
Table 2-15: User input requirements and corresponding simulation outputs from the CeBER Bioprocess Modeller generic flowsheet adapted from Harding (2008) and De Beer (2010).....28
Table 2-16: Factors influencing equipment scale-up and design adapted from Peters & Timmerhaus (1991).....30
Table 2-17: Categories of cost estimates and corresponding accepted accuracy (Whitesides 2012).....31

Table 2-18: Process equipment and size exponent (N) (Whitesides 2012) 31
Table 2-19: Factors affecting the cost of manufacturing a product (Turton *et al.* 2012) 32
Table 2-20: Manufacturing costs breakdown as a function of direct fixed capital (DFC) and operating labour (OL) 33
Table 2-21: Average Lang factors used to calculate the direct fixed capital (DFC) investment of a plant (Petrides 2015) 33
Table 3-1: Input data for the large scale production of PHB based on the Harding (2008) PHB model under nitrogen limitation 39
Table 3-2: Chemical Engineering Plant Cost Index (CEPCI) compiled from Business News (2014) and Chemical Engineering Essentials for the CPI Professional (2015) 43
Table 3-3: Equipment costs reported by Choi & Lee (1997) 44
Table 3-4: Raw materials costs for the large scale production of PHB using the CeBER Bioprocess Modeller 45
Table 3-5: Cost of utilities used for the large scale production of PHB using the CeBER Bioprocess Modeller from Coulson and Richardson (2005) 46
Table 3-6: Calculation of annual operating costs of a PHB production plant based on its direct fixed capital (DFC) and Lang factors adapted from Choi & Lee (1997) 47
Table 3-7: List of input data for the CeBER Bioprocess Modeller for the sodium hypochlorite-surfactant purification and recovery process 50
Table 3-8: List of input data for the CeBER Bioprocess Modeller for the sodium hydroxide purification and recovery process 51
Table 3-9: Design of factorial design investigation of microbial properties on the large scale production of PHB 53
Table 3-10: Production of PHAs from waste carbon substrates using *Cupriavidus necator* 54
Table 4-1: Original values for investigated factors for the base case scenario 57
Table 4-2: List of equipment and description for the simulation of 20 tonnes of crude PHB per run for the base case scenario 58
Table 4-3: Mass balance and utility requirements for the simulation of 20 tonnes of crude PHB for the base case scenario 59
Table 4-4: Final product stream composition exiting the spray dryer for the base case scenario 59
Table 4-5: Estimated volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the base case scenario simulating the production of 20 tonnes of crude PHB 60

Table 4-6: Equipment cost for the simulation of 20 tonnes of crude PHB per year for the base case scenario 63
Table 4-7: Comparison of calculated equipment costs with recent equipment costs calculated from an online cost estimator tool developed by Peters *et al.* (2015) and a recent techno-economic study by Koutinas *et al. (*2014) 63
Table 4-8: Equipment costs along the costs associated with raw materials, utilities, electricity, waste disposal for the simulated production of 20 tonnes of crude PHB per run for the base case scenario 64
Table 4-9: Calculation for direct production costs of 2929 tonnes of pure PHB per year for the base case scenario 65
Table 4-10: Total production cost breakdown per kg PHB as estimated for the production of 2929 tonnes of pure PHB per year for the base case scenario 69
Table 4-11: Effect of varying the energy efficiency of the centrifuges and efficiency of the homogeniser on the annual large scale production of PHB process simulated in the CeBER Bioprocess Modeller 70
Table 4-12: Effect of varying the energy efficiency of the centrifuges and efficiency of the homogeniser on the large scale production of PHB simulated in the CeBER Bioprocess Modeller 71
Table 4-13: The effect of PHB recovery process on the mass and energy inventories for the simulation of 20 tonnes of crude PHB per run for a surfactant-sodium hypochlorite process and the enzymatic digestion process used for the base case scenario 73
Table 4-14: Final product stream composition for the simulation of 20 tonnes of crude PHB per run using a surfactant-sodium hypochlorite purification and recovery process using the CeBER Bioprocess Modeller 73
Table 4-15: Estimated batch volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the simulation of 20 tonnes of crude PHB per run with a surfactant-sodium hypochlorite purification and recovery process using the CeBER Bioprocess Modeller 74
Table 4-16: Equipment costs for the simulation of 20 tonnes of crude PHB per run with a surfactant-sodium hypochlorite recovery process using the CeBER Bioprocess Modeller 75
Table 4-17: Mass balance and utility requirements for the simulation of 20 tonnes of crude PHB per run with a sodium hydroxide process and the enzymatic digestion process used for the base case 77
Table 4-18: Final product stream composition for the simulation of 20 tonnes of crude PHB per run with a sodium hydroxide purification and recovery process using the CeBER Bioprocess Modeller 77

Table 4-19: Estimated batch volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the simulation of 20 tonnes of crude PHB per run with a sodium hydroxide purification and recovery process using the CeBER Bioprocess Modeller 78
Table 4-20: Equipment costs for the simulation of 20 tonnes of crude PHB per run with a sodium hydroxide purification and recovery process using the CeBER Bioprocess Modeller 79
Table 4-21: Effect of purification and recovery process on the annual large scale production of PHB simulated in the CeBER Bioprocess Modeller 80
Table 4-22: Total annual production costs breakdown for the large scale production of PHB using either a surfactant-sodium hypochlorite or sodium hydroxide process instead of the enzymatic process used for the base case 81
Table 4-23: Coded and natural variables for 2^3 factorial design experiments to investigate the effects of the microbial properties on the production costs of PHB for the production of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller 82
Table 4-24: Effect of microbial properties on the annual large scale production of PHB simulated in the CeBER Bioprocess Modeller 86
Table 4-25: Total annual production costs breakdown for the large scale production of PHB with different microbial properties 86
Table 4-26: Effect of the carbon substrates' price on the annual production costs of PHB for the base case scenario 87
Table 4-27: Effect of the carbon substrates' price on the annual production costs of PHB with the scenarios presented in Section 4.5 87
Table 4-28: Effect of using starch as waste carbon source on the annual large scale production of PHB simulated in the CeBER Bioprocess Modeller 89
Table 4-29: Total costs breakdown for the production of PHB using starch waste as carbon source with an enzymatic digestion purification and recovery process 89
Table 4-30: Investigation of an optimised case scenario on the annual large scale production of PHB with a PHB productivity of 3.8 g/L/h , a sodium hypochlorite-surfactant purification and recovery process with a cell rupture efficiency of 95 % and an energy efficiency of 70 % for the centrifuges and, a hypothetical carbon source assumed to be free of cost 91
Table 4-31: Total costs breakdown for the production of PHB for an optimised case with a PHB productivity of 3.8 g/L/h , a sodium hypochlorite-surfactant purification and recovery process with a cell

rupture efficiency of 95 % and an energy efficiency of 70 % for the centrifuges and, a hypothetical carbon source assumed to be free of cost..... 91

GLOSSARY

Abiotic resources	resources that come from non-living, non-organic material
Bio-based	material or product derived from a biological resource
Bio-compatible	not toxic or harmful to living tissue
Bio-degradable	capable of being decomposed by bacteria or other living organisms
Bio-economy	defined as an economy where the building blocks are derived from biomass and converted to food, feed, bio-based products and bio-energy
Biomass	organic matter that has stored energy
Bio-plastic	plastic that is bio-degradable, has bio-based content or both
Bio-polymer	polymeric substance found in a biological system
Commodity product	basic resource or agricultural product
Compostable	material bio-degrading in the soil
Feedstock	any renewable and biological material that can be used as fuel
Oligomer	molecule complex consisting of a few monomer units
Polyamide	macromolecule with repeating units linked by amide bonds
Polyester	a synthetic resin in which the polymer units are linked by ester groups

ABBREVIATIONS

CDW	cell dry weight (g/L)
CeBER	Centre for Bioprocess Engineering Research
CEPCI	Chemical Engineering Plant Cost Index
D	diameter
EVOH	ethylene vinyl alcohol
H	height
mcl	medium chain length
PA	polyamide (nylon)
PBAT	poly (butylene adipate-co-terephthalate)
PBS	poly (butylene succinate)
PCL	polycaprolactone
PE	polyethylene
PEN	polyethylene naphthalate
PET	polyethylene terephthalate
PLA	polylactic acid
PHA	polyhydroxyalkanoate
PHB	polyhydroxybutyrate
PP	polypropylene
PTT	polytrimethylterephthalate
scl	short chain length
T_g	glass transition temperature

ABSTRACT

A transition is needed to shift the global economy to a more sustainable and clean economy to counteract the depletion of abiotic resources, generation of emissions and waste. Replacing petroleum plastics by bio-plastics is key to this move. This study identifies the bio-based and bio-degradable plastics, polyhydroyalkanoates (PHAs) as promising alternatives to petroleum plastics. PHAs are biologically synthesised polyesters which accumulate intracellularly in the presence of excess carbon from renewable resources and, limited nutrients in the form of nitrogen, phosphorus or sulphur. Different PHA polyesters can be synthesised by varying the growth conditions of the microbial culture. The flexible nature of PHA synthesis results in a large group of PHA polyesters with a variety of properties that span those of petroleum plastics in terms of strength, rigidity, durability and mechanical properties. Due to their versatility, PHAs have a wide range of applications in packaging and coating, health care and hygiene products, fibres, adhesives, components of toner and developer fluids and medical sutures and implant materials. Waste products of PHA production are mostly carbon dioxide and water. PHAs are biodegradable in all living systems including marine environment. PHAs are also bio-compatible and thus are not toxic to living organisms.

In spite of the numerous advantages that PHAs offer, they have not yet achieved substantial commercial success due to poor market penetration arising from their high costs of production. The typical PHA sale price ranged from $ 4 to 9/kg in 2014. Factors that have been reported to affect the production costs of PHAs are the PHA content and concentration achieved by the microorganism, the purification and recovery process of PHAs together with the type of carbon substrate used. Additionally, the efficiency of the cell disruption and energy required for the washing steps can also influence the production system and are addressed in this study along with the other factors. The overall PHA production system must also be sustainable minimising its energy, water usage and carbon dioxide emissions. When faced with limited time and resources, insight is needed to identify which of the aforementioned factors or combination of factors are more crucial to optimise in order to lower production costs and environmental burden. Economic assessment is a powerful tool to identify promising economically viable process options and discard unfavourable ones. Since PHA production is not an established technology, process conditions and pilot scale data are not easily available. This study proposes a generic large scale PHA production system that can be simulated using minimum inputs to deliver material and energy inventories which can further be used to investigate the production costs of PHAs.

The PHA monomer that was simulated is the most characterised PHA, polyhydroxybutyrate (PHB). The generic flowsheet used was originally developed by Harding (2008) using the CeBER Bioprocess Modeller, using experimental data from Harrison (1990) who worked on the refinement of the ICI – Zeneca Biopol process. In the model, PHB was produced from the microorganism *Cupriavidus necator* using glucose as the carbon source. PHB was liberated from the cells by high pressure homogenisation and recovered by centrifugation before purification by enzymatic digestion, detergent treatment and hydrogen peroxide treatment. The original model was scaled up to produce 20 tonnes of crude PHB per run to simulate the large scale production of PHB. Depending on the time for one complete run and the total operation hours of the plant, the annual production of pure PHB could be calculated.

The material and energy inventories were used for preliminary sizing and cost estimations for the equipment. From there, the direct production costs of PHB including the direct fixed capital (DFC), raw materials, utilities, labour and waste treatment costs were computed for the year 2014. This was used as the base case scenario for this study. Different scenarios were built from the base case to investigate the four factors postulated to affect PHB production. To investigate the equipment efficiency in the downstream processing, the efficiency of the cell disruption for the homogeniser and the energy efficiency of the centrifuges were varied at a lower and higher efficiency from the original values of the base case scenario. To investigate the effect of the PHB recovery and purification process, the original enzymatic digestion process used in conjunction with detergent and hydrogen peroxide treatment was replaced by either a surfactant-sodium hypochlorite process or a sodium hydroxide process. A 2^3 factorial design investigation was used to investigate the effect of microbial properties in terms of PHB content, biomass concentration and cultivation time where they were varied with respect to the base case. The effect of the carbon price was investigated by decreasing the original price incrementally. The trade-off between low carbon cost and lower microbial performance for PHB production was also addressed by using the base case scenario with a waste substrate as a carbon source and corresponding microbial performance reported.

The production costs for the base case scenario was $ 8.4/ kg with an energy usage of 31.4 MJ/kg PHB, a water usage of 33 kg/kg PHB and carbon dioxide emissions of 2.22 kg/kg PHB. The major costs were found to be the DFC dependent costs, including the plant overheads (43 %) and the raw material costs (34 %) of which 50 % was allocated to the cost of glucose. The major factors influencing the PHB production system were the efficiency of microbial production and the purification and recovery of PHB. A high PHB productivity correlated directly with lower production costs, lower use of resources and

lower carbon dioxide emissions. Efficient and simple purification and recovery processes delivering high PHB purity and recovery resulted in improved PHB production system with lower costs. Higher homogeniser efficiency in the downstream processing lowered production costs since more PHB could be released from the cells while the energy efficiency of the centrifuges had no significant effect on production costs but a higher energy efficiency led to a slight overall reduction in the energy requirement of the production system. The cost of the carbon substrate had a significant effect on production costs when production efficiency was sustained; however where low cost carbon substrates yield poor PHB productivity, the impact on PHB economics could be unfavourable. A model of the optimum process developed within the constraints of the system in this study, using a PHB productivity of 3.8 g/L/h, a sodium hypochlorite-surfactant purification and recovery process with a cell rupture efficiency of 95 % and an energy efficiency of 70% for the centrifuges, along with a hypothetical carbon source assumed to be free of costs was constructed to investigate the optimisation potential of the system. This led to a 49 % decrease for production costs of PHB from $ 8.4/kg to $ 4.2/kg, a 41 % decrease in energy usage from 31.4 MJ/kg to 18.5 MJ/kg PHB, a 77 % decrease in water usage from 33 kg/kg to 7.7 kg/kg PHB and a 14 % decrease in carbon dioxide emissions from 2.22 kg/kg to 1.90 kg/kg PHB.

This study provides an additional simple tool to investigate process options in view of achieving feasible bulk commercial production of PHAs. Insight was given on the relative importance of factors postulated to affect the PHA production system. Different microorganisms and type of PHAs can be investigated to provide support for lab scale experiments for decision making in the selection of the most viable studies that can be scaled up to industrial level. Additional process economics such as the return of investment or the break-even time of the PHA facility can be performed. Similarly, preliminary LCA studies can be performed to inform the environmental burden of the process in various impact categories.

1 INTRODUCTION

1.1 BACKGROUND TO INVESTIGATION

Limited and finite natural resources are one of the numerous global challenges. Being heavily dependent on the petroleum industry over the last decades led to a drastic increase in greenhouse gases, posing serious threat to the environment and the population. A transition is needed to shift the global economy to a more sustainable and clean one by optimising sustainable productivity from natural resources directed towards improved economic and functional outputs while minimising environmental impacts (DelftX 2014). This leads to the foundation of the bio-economy where large parts of the global economy are converted stepwise into a sustainable bio-based economy with bio-energy, bio-fuels and bio-based products as its main pillars (Kamm *et al.* 2006). The petroleum industry is a mature and established industry with numerous economic outputs namely fuels and petroleum derived products. To be analogous, the bio-economy must deliver multiple bio-based products. The attractive feature of the bio-economy is the generation of organic molecules from renewable and readily available biomass (DelftX 2014). The products can range from food, feed, pharmaceuticals, and bio-plastics to biofuels.

The production of petroleum derived plastic, produced by the polymerisation and poly-condensation of hydrocarbon monomers has been increasing steadily since the mid-20th century (British Plastics Federation 2015). Petroleum plastics have various chemical and mechanical properties such as versatility, low density, tensile strength, resistance to water, rust and natural degradation and thus have a wide variety of uses as packaging materials, computer equipment, medical applications and components in electronics (Zinn *et al.* 2001; Keshavarz & Roy 2010). Subsequently, petroleum plastics have become an ever-present commodity product in everyday life. Approximately 100 million tonnes of plastics are produced every year and the per capita consumption of plastics is 80 kg/per year in the USA, 60 kg/year in the European countries and 2 kg/year in India (Reddy *et al.* 2003). The slow process of degradation of petroleum plastics however, poses serious threat to both the environment and human population. Compounds from small undegraded plastic fragments eventually leach out in groundwater introducing toxins to the food chain (Bernard 2014). Around 40 % of the total plastics produced per year are disposed in landfills and several hundred thousand tonnes are discarded into marine environment (Reddy *et al.* 2003).

Marsalek (2011) reported three scenarios to counteract this problem. The first approach is to use higher quality and resistant plastics that can be used for a longer period of time. A second approach is to employ lasting economical and sustainable recycling technologies. The third and most recent approach is to substitute petroleum plastics by fully degradable biologically synthesised plastics, commonly known as bio-plastics. The last scenario is promising since it assists the move towards a bio-based economy where the problem of pollution caused by petroleum plastics is averted and toxic waste is not released into the environment. Bio-plastics therefore propose multiple benefits on different levels: they are eco-friendly, require less time to degrade, have low toxicity and there is a possibility to use less energy and fossil feedstock for their production than conventional plastics (Pathak *et al.* 2014).

Even though they have the potential to replace up to 85 % of petroleum plastics, bio-plastics only account for only 1.5 % of global plastic demands due to the numerous challenges that this industry faces (Rudnik 2013). Most bio-plastics manufacturing plants are small scale facilities with an annual production of around 1000 – 20 000 tonnes whereas a single polyethylene unit has an annual capacity of about 300 000 tonnes, clearly indicating that economies of scale for bio-plastics have yet to be demonstrated (Chanprateep 2010). Since the bio-plastics industry is still in its infancy, the production costs of bio-plastics are still significantly higher than petroleum plastics (Pathak *et al.* 2014).

One group of promising bio-plastics, polyhydroxyalkanoates (PHAs) are comparable to petroleum plastics in terms of strength, rigidity, durability and mechanical properties (Somleva *et al.* 2013). Additionally, PHAs are bio-based, bio-degradable and bio-compatible giving them additional benefits with respect to other bio-plastics (Reddy *et al.* 2003). This position PHAs at the cutting edge of bio-plastics technology but their production costs ($4 to 9/kg) remain too high to penetrate the market successfully (Bolck 2014). This establishes the point of this study whereby a need was identified to investigate the overall production system of PHAs systematically to provide an approach to lower their production costs.

1.2 PROBLEM STATEMENT

Economic assessment is a tool that can be used to inform approaches to decrease the cost of production of a commodity product such as PHAs. Prior to commercialisation, limited data availability results in insufficient data inputs for software packages, making an economic assessment difficult to undertake. There is a need for early process simulation to provide material and energy inventories to undertake economic and environmental assessment of new products where optimisation is required to fuel

production on an industrial scale. Adhering to the bio-economy concept, the overall production system must also be sustainable and thus minimisation of energy, water usage and carbon dioxide emissions are also desired along with a lower PHA cost. In this dissertation, this approach is tested for the bio-based and bio-degradable plastic PHAs.

1.3 PROJECT AIMS, OBJECTIVES AND IMPACT

This research aims to identify pertinent factors that influence the economics of PHA production and to investigate their relative importance with respect to one another to lower production costs. This is achieved through early design stage process simulation. Through the construction of different scenarios investigating the key factors, insight is sought towards a cost effective large scale production of PHAs. Being an early stage investigation, the outcome of this research can be used as a guide to set up and further investigate promising PHA production systems while discarding unfavourable process options. To achieve this, the study addresses the following aims and objectives:

- Using a simplified approach to simulate the large scale production of PHAs to yield material and energy inventories to determine the production costs of PHA
- Using an engineering approach and heuristics to carry out preliminary sizing of equipment in order to compute the operating costs of the PHA facility and the direct production costs of PHAs
- Investigating the large scale production of PHAs through the use of scenarios to evaluate approaches to enhance potential success on an economic and sustainability basis by identifying key variables for minimising the production costs of PHAs, energy usage, water usage and carbon dioxide emissions

1.4 LAYOUT OF DISSERTATION

In Chapter 2, a literature review on bio-plastics, their classification and market trends is presented. This is followed by a review on bio-based and bio-degradable plastics with emphasis on the bio-plastic polyhydroxyalkanoates (PHAs). The synthesis of PHAs by different microorganisms, the type of carbon substrates used, the cultivation strategies and extraction protocols for the intracellular PHAs are reviewed highlighting the various challenges that need to be addressed to overcome high production costs. An economic assessment on the large scale production of PHAs based on early process simulation has been undertaken using a generic flowsheet developed in the CeBER Bioprocess Modeller to investigate the economics of PHA production. The methodology used to achieve this is presented in Chapter 3. Potential factors that influence the production process of PHAs have been systematically

investigated in order to understand their effects on the production costs of PHAs, process energy requirement, water usage and the carbon dioxide emissions. These results are presented in Chapter 4. An integrated discussion of the findings provides insight on potential ways to decrease the production costs of PHA along with minimising its environmental footprint. This is followed by concluding remarks, the impact of the study and recommendations for further work, presented in Chapter 5.

2 LITERATURE REVIEW

2.1 BIO-PLASTICS AS PROMISING ALTERNATIVES TO PETROLEUM PLASTICS

Bio-plastics are polymers made from organic biomass sources whereas conventional plastics are made from petroleum (Ebnesajjad 2013). A bio-plastic can either be bio-based and/or biodegradable depending on their chemical composition (Klazinga 2009; Pathak *et al.* 2014). Bio-degradable plastics can be produced from both bio-based feedstocks and petrochemical feedstocks, undergoing complete degradation with potential for recycling of key elements. Bio-based plastics can be manufactured only from renewable raw materials (Shen *et al.* 2009). The three main groups of bio-plastics are listed below and are subsequently presented in a material coordinate system in Figure 2-1 (Garcia 2012):

- Non bio-based bio-polymers that are produced from non-renewable fossil resources but have bio-degradable or compostable properties such as synthetic bio-degradable aliphatic-aromatic copolyesters e.g: polybutylene terephthalate adipate (PBTA),
- Bio-based polymers that are both bio-degradable and compostable e.g.: polylatic acid (PLA) and its compounds, starch-based polymers, cellulose derived polymers and polyhydroyalkanoates (PHAs)
- Non-biodegradable polymers produced from bio-based resources e.g: bio-based PE, PP and PET

The conventional polymers group includes traditional polymers that are made from petrochemical raw materials and are non bio-based and non-biodegradable. These include PE, PP and PET. The group of non-biodegradable bio-based polymers is the bio-based counterpart of some petroleum plastics and these polymers are termed ‘drop-in’ bio-polymers (Dammer *et al.* 2013). This group is expected to be the fastest growing commodity product in the market of bio-plastics since they can make use of the distribution and supply chain of their petroleum derived counterparts (Reinshagen 2013). While these ‘drop-in’ bio-polymers do reduce the dependency on petroleum as raw materials, they do not eradicate the disposal problem associated with non bio-degradability. To adhere to the overall bio-economy concept, the group of bio-based and bio-degradable bio-plastics is expected to quadruple in production capacity over the next ten years (Reinshagen 2013).

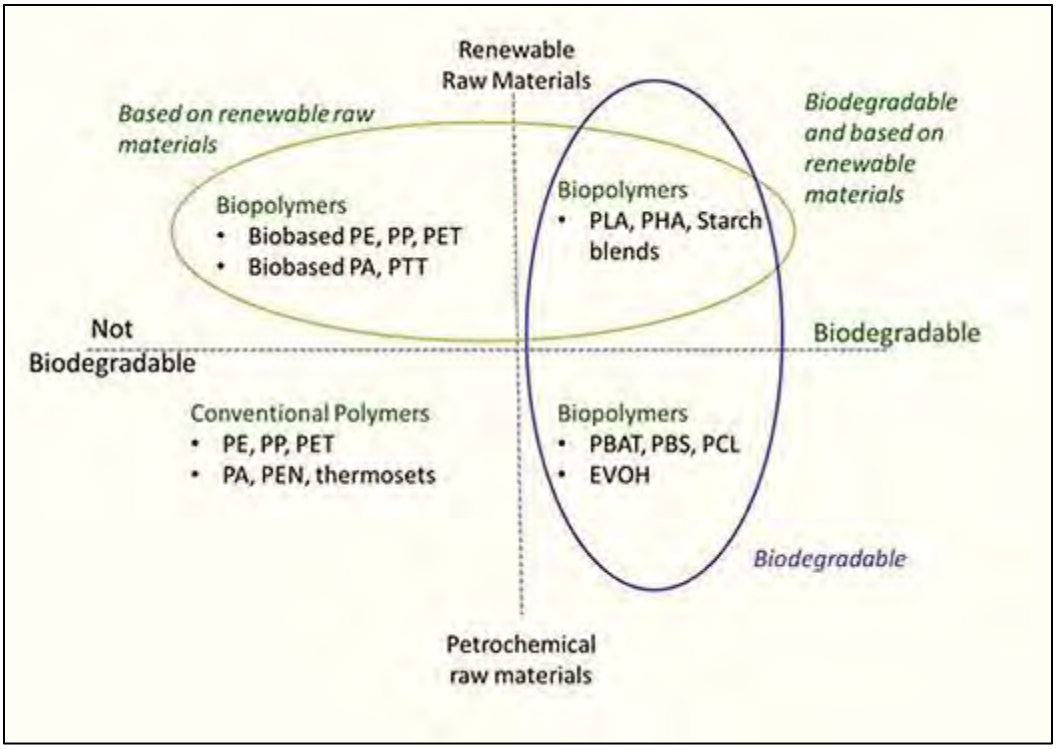


Figure 2-1: Material coordinate system for bioplastics adapted from Grotto (2012)

2.2 AN OVERVIEW OF BIO-BASED AND BIO-DEGRADABLE PLASTICS

Bio-based and bio-degradable bio-plastics such as polylactides, aliphatic polyesters, polysaccharides and polyhydroxyalkanoanates can be fully degraded by microorganisms (Reddy *et al.* 2003). To be classified as completely degradable, the bio-degradation must occur within the 180 days equivalent to the rate of degradation of natural materials in all soil, marine/ocean, and compost in aerobic and anaerobic environments (Narayan 2006). Figure 2-2 shows the ideal 'cradle-to-cradle' life cycle of a bio-based and biodegradable bio-plastic.

The major classes of bio-plastics are described in this paragraph. Starch- and cellulose- based plastics are the most common bio-based and bio-degradable plastics and have been used for decades (Shen *et al.* 2009). PLA was discovered in 1932 but was only commercialised in the early 1990's owing to its similar properties to hydrocarbon polymers such as PET (Babu *et al.* 2013). PLA is mainly used in food packaging applications but is not suitable for use in electronic devices and engineering applications (Babu *et al*. 2013). PHAs are biologically synthesised polyesters which occur naturally in a variety of microorganisms. They were first discovered as bacterial storage products in the 1920's and commercialisation started in the 1990's (DiGregorio 2009).

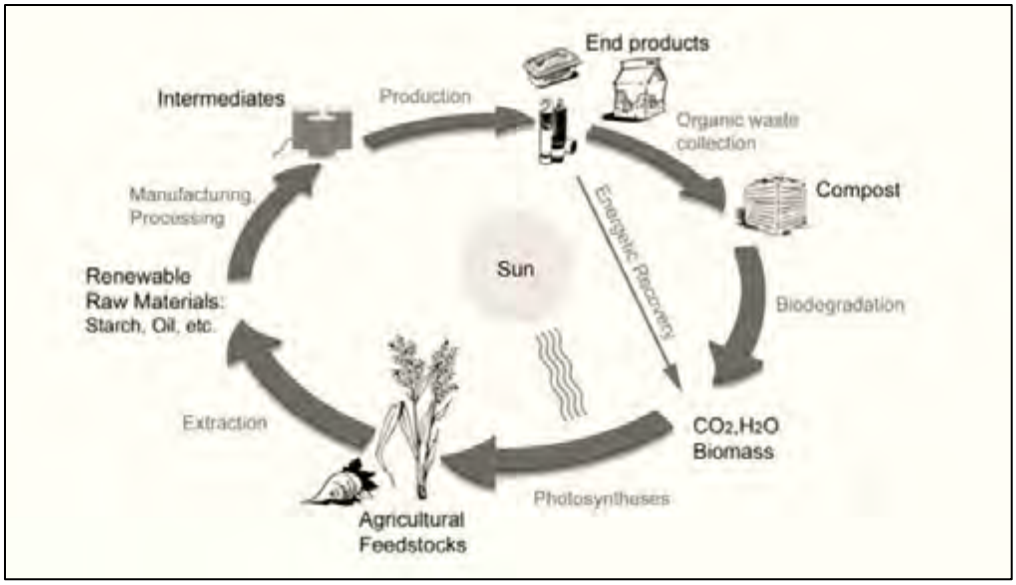


Figure 2-2: Closed loop life cycle for a bio-based and bio-degradable bio-plastic (Klazinga 2009)

PLA and PHAs are among the most promising bio-polymers due to their wide range applications (Chanprateep 2010; Mohan 2011). PLA has reached commercial scale production while PHA production is in between pilot and commercial scale (Bolck 2014). The large scale commercial success of PLA and PHAs is being hampered by their high cost of production. The PLA cost is at least 5 - 20 % more than petroleum plastics, while PHAs usually cost at least twice more (Howard 2014; The Plastics Exchange 2015). Increase in the production scale and optimisation of the industrial PLA process over the last 10 years succeeded in decreasing the cost of production of PLA from $ 6.6 /kg to $ 2 - 3/kg. The price of PHAs remains at $ 4 – 9/kg resulting in PLA dominating the bio-based plastic market (Regina & Telis 2012 ;Liew *et al.* 2014). The interesting feature of PHAs is the ability to tailor the type of polymer produced to end needs demonstrating their full potential as bio-plastics. This can be done by varying the microorganisms used and the raw materials, mostly the carbon and nitrogen sources (Babu *et al.* 2013). Thus, owing to its broad range of properties, rapid growth is predicted in the PHA market leading to reduction in production costs in the coming years. PHAs and PLA are compared in Table 2-1 (Markets and Markets 2013).

Table 2-1: Comparison between the bio-plastics polylactic acid (PLA) and polyhydroxyalkanoates (PHAs) (Chen, 2009)

	Polylactic acid (PLA)	**Polyhydroxyalkanoates (PHAs)**
Monomer structure	Only D- and L-lactic acids (LA)	At least 150 monomers
Production methods	Bio-production of LA and chemical synthesis of PLA	Totally biosynthesised as intracellular polyesters
Production cost	Comparable with conventional plastics like PET	At least twice that of PLA
Material properties	Poor, could be adjusted by regulating D- and L- LA ratios	Full range including brittle, flexible and elastic polymers. Fully controllable
Technology maturity	LA production well-established. LA polymerization to PLA is complicated. Only one company, NatureWork, produces PLA on a large scale	At least 10 companies worldwide produced or are producing PHAs up to 2000 tonnes per year scale via microbial routes
Investment	Large fixed capital investment: NatureWork has invested 1 billion US$ over several years to run a 140 000 ton PLA plant	Small investment: existing aerobic microbial bioreactors plants with process modifications can be used for PHA production
Intellectual properties	Cover almost all areas of production and applications	Limited IP in areas of production and applications
Application areas	Packaging, medical implants, printing, coating, yet limited by Tg, glass transition temperature	Almost all areas of conventional plastic industry, including bio-medical uses, limited only by current high costs.

Due to the high number of versatile monomers, PHAs can replace petroleum plastics in numerous areas of applications. Additionally, while both PHAs and PLA are biodegradable on land, recent studies have demonstrated that only PHAs are biodegradable in marine environment based on the American and European standards for biodegradation in the marine environment (Greene 2012). While the high production costs of PHAs are restricting its market penetration, opportunity exists to optimise its production to ensure its economic viability (Howard 2014).

2.3 POLYHYDROXYALKANOATES (PHAS): BIO-DEGRADABLE AND BIO-BASED PLASTICS

In Sections 2.3, 2.4 and 2.5 the literature on PHAs is reviewed with emphasis on its potential applications in industry, the various microorganisms for synthesis, the appropriate substrates, production techniques, extraction protocols and the associated challenges with large scale production.

2.3.1 Interesting features and applications of PHAs

An interesting feature of PHAs setting them apart from bio-plastics like polylactic acid (PLA), is their ability to be tailored to meet end needs through incorporation of different monomers. This gives PHAs the potential to replace petroleum plastics in various applications (Chen 2009). Polyhydroxybutyrate (PHB) was the first PHA monomer discovered by the French chemist and bacteriologist Maurice

Lemoigne as an intracellular bio-polyester in the gram-positive bacterium *Bacillus megaterium* in 1926 at the Lille branch of the Pasteur institute (DiGregorio 2009). However, it was only during the 1970's oil crisis that it was re-discovered that bacteria can produce PHAs naturally. To date there are about 150 different variations of PHAs produced by using different monomers (Braunegg *et al.* 1998; Chee *et al.* 2010a; Bernard 2014). PHAs come from a family of optically active biological polyesters which contain hydroxyalkanoic units in the R configuration because of the stereospecificity of the enzymes involved in synthesis (Garate 2014). Most PHAs are aliphatic polyesters of carbon, oxygen and hydrogen as shown in Figure 2-3 (Braunegg *et al.* 1998).

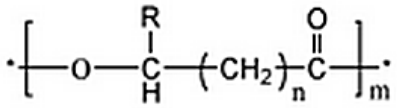


Figure 2-3: General structure of polyhydroxyalkanoates (Ebnesajjad 2013)

where, R is the side chain on the monomer, n defines the length of the monomer and m is the number of monomeric units in the polymer chain. Both n and R determine the type of HA monomer unit as depicted in Table 2-2.

Table 2-2: General structure of PHAs adapted from Ebnesajjad (2013)

n value	Alky group	Type of PHAs
n = 1	R = hydrogen, -H-	poly(3-hydroxypropionate)
	R = methyl, $-CH_3-$	poly(3-butyrate)
	R = ethyl, $-CH_2-CH_3-$	poly(3-hydroxyvalerate)
	R = propyl, $-(CH2)_2-CH_3-$	poly(3-hydroxyhexanoate)
	R = pentyl, $-(CH2)_4-CH_3-$	poly(3-hydroxyoctanoate)
	R = nonyl , $-(CH2)_8-CH_3-$	poly(3-hydroxydodecanoate)
n = 2	R = hydrogen, -H-	poly(4-hydroxybutyrate)
	R = methyl, $-CH_3-$	poly(4-hydroxyvalerate)
n = 3	R = hydrogen, -H-	poly(5-hydroxyvalerate)
	R = methyl, $(-CH_3)-$	poly(5-hydroxyhexanate)
n = 4	R = hexyl, $-(CH_2)_5-CH_3-$	poly(6-hydroxydodecanoate)

The average molecular weight of PHAs is in the range of 50 to 1000 kDa (Sudesh *et al.* 2007). PHAs can be divided into two main groups based on the number of carbon atoms in the monomer unit: short chain length (scl) PHAs consisting of 3 to 5 carbon atoms and medium chain length (mcl) PHAs consisting of 6 to 14 carbon atoms (Lee 1996; Madison & Huisman 1999). There can also be a subgroup by the combination of short chain length and medium chain length (scl-mcl) PHAs (Roy & Visakh 2015). The (mcl) PHAs tend to be more elastomeric while (scl) PHAs are more brittle and stiff (Roy & Visakh 2015).

(scl) PHAs have been studied more extensively due to their close resemblance to conventional plastics (Loo & Sudesh, 2007). PHB is the most common PHA synthesised by microorganisms and has similar properties to polypropylene (Harding *et al.* 2007). By varying the side chain and compositions of the homopolymer PHB along with addition of other monomers presented in Table 2-2, PHA copolymers with enhanced properties can be obtained. The two most studied (scl) copolymers are poly(3-hydroxybutyrate-co-4-hydroxyvalerate) [P(3HB-*co*-4HB)] and poly(3-hydroxybutyrate-co-hydroxybutyrate) [P(3HB-*co*-4HB)]. An example of scl-mcl PHAs is poly(3-hydroxybutyrate-co-3-hexanoate) [P(3HB-*co*-3HHx)] (Roy & Visakh 2015).

As shown in Figure 2-4, different co-monomers of PHAs result in different types of PHAs with each having distinct properties. The PHB copolymers poly(3-hydroxybutyrate-co-3-hydroxyvalerate [P(3HB-co-3HV)] is hard and has remarkable processability; poly(3-hydroxybutyrate-co-4-hydroxybutyrate [P(3HB-co-4HB)] has excellent mechanical properties and bio-absorbability; poly(3-hydroxybutyrate-co-3-hydroxyhexanoate [P(3HB-co-3HHx)] is comparable to polypropylene (PP) and low density polyethylene (LDPE) (Loo & Sudesh 2007). At low co-monomer content (5 %) and molecular weight (500 kDa), PHAs are suited for injection moulding and melt blowing. At medium molecular weight, they are suited for melt spun fibres (Roy & Visakh 2015). As the co-monomer content and molecular weight increase in the range of 10 % and 600 kDa respectively, PHAs are adequate as melt resin and cast films. Above 15 % co-monomer content results in softer and more elastic PHAs, applicable in adhesives and elastomeric films (Roy & Visakh 2015).

In addition to their wide range of applications, PHAs are also bio-degradable in all living systems including the marine environment, mainly breaking down to carbon dioxide and water (Snell & Peoples 2009). However, the biodegradability of PHAs limits its use in aqueous ecosystems. PHAs are also bio-compatible and thus are not toxic in living organisms. This facilitates medical application and further, ingestion by mammals has been proven to be of no harm (Forni *et al.* 1999a; Forni *et al.* 1999b). PHAs are degraded into 3-hydroxyacid by the enzyme PHA depolymerase (endogenous in many microorganisms) with no harmful effects on animals since 3-hydroxyacid is present naturally in their systems (Suriyamongkol *et al.* 2007). The promising aspects for PHAs in various industries such as packaging, medical and coating materials are shown in Table 2-3.

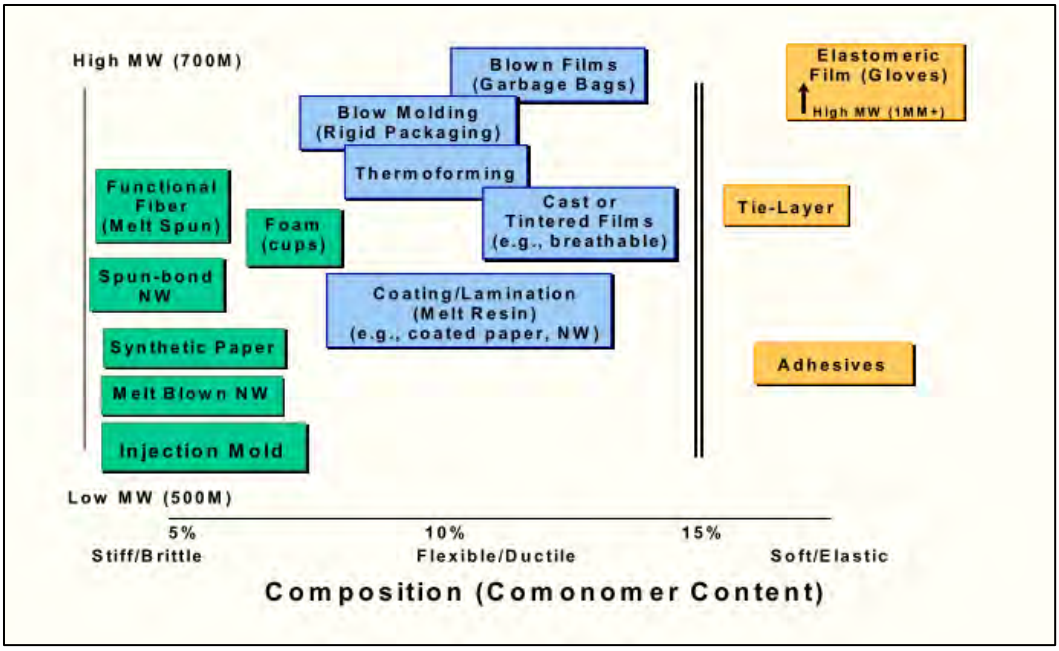


Figure 2-4: Processing technologies for different % co-monomer content in PHAs e.g. PHB/HV (Institute for Prospective Technological Studies 2005)

Table 2-3: Applications of PHAs in various industries adapted from Chen (2009)

Applications of PHAs	Examples
Packaging industry	All packaging materials that are used for a short period of time, including films, daily consumables, electronic appliances
Disposal items	Food utensils and containers
Hygiene products and healthcare	Diapers, sanitary products, hospital supplies
Printing & photographic industry	PHAs are polyesters that can be easily stained
Other bulk chemicals	Heat adhesives. Latex, smart gels. PHA non-woven matrices can be used to remove facial oils
Plastic processing	PHAs can be used as processing aids for plastic processing
Textile industry	Like nylons, PHA can be used as processing aids for plastic processing
Fine chemical industry	Different monomers can be used as chiral starting materials for the synthesis of antibiotics and other fine chemicals
Medical implant biomaterials	PHAs are biodegradable and biocompatible and can be developed into medical implant materials. PHA can also be turned into drug controlled release matrices
Healthy food additives	PHA oligomers can be used as food supplements
Industrial microbiology	The PHA synthesis operon can be used as a metabolic regulator or resistance enhancer to improve the performance of industrial microbial strains
Biofuels or fuels additives	PHAs can be hydrolysed to form hydroxyl-alkanoate methyl esters that are combustible
Protein purification	PHA granule binding proteins phasin or PhaP are used to purify recombinant proteins

2.3.2 Biosynthesis of PHAs

An imbalanced growth condition in the form of a limiting nutrient like nitrogen, phosphorus, sulfur, oxygen or magnesium in the presence of excess carbon triggers the polymerisation of soluble carbon intermediates into water-insoluble molecules like PHAs (Annuar *et al.* 2008). By accumulating PHAs, microorganisms have a natural reserve of carbon and energy. On restoring the limiting nutrient, the PHAs can be degraded by intracellular enzymes and used as carbon or energy source (Lee 1996). PHA synthesis relies on important biochemical pathways such as the tricarboxylic acid (TCA) cycle, fatty acid degradation (β-oxidation) and fatty acid biosynthesis, as shown in Figure 2-5 (Chen *et al.* 2015). Pathway 1 is specific for (C3 – C5) substrates where acetyl-CoA is converted to hydroxybutyrate-CoA before being polymerised by the PHA synthase to PHB. Pathway 2 uses intermediate products from the β-oxidation cycle and fatty acids as carbon sources to produce (mcl) PHA. Pathway 3 relies on the *in situ* fatty acid synthesis to produce (mcl) PHAs (Chen *et al.* 2015).

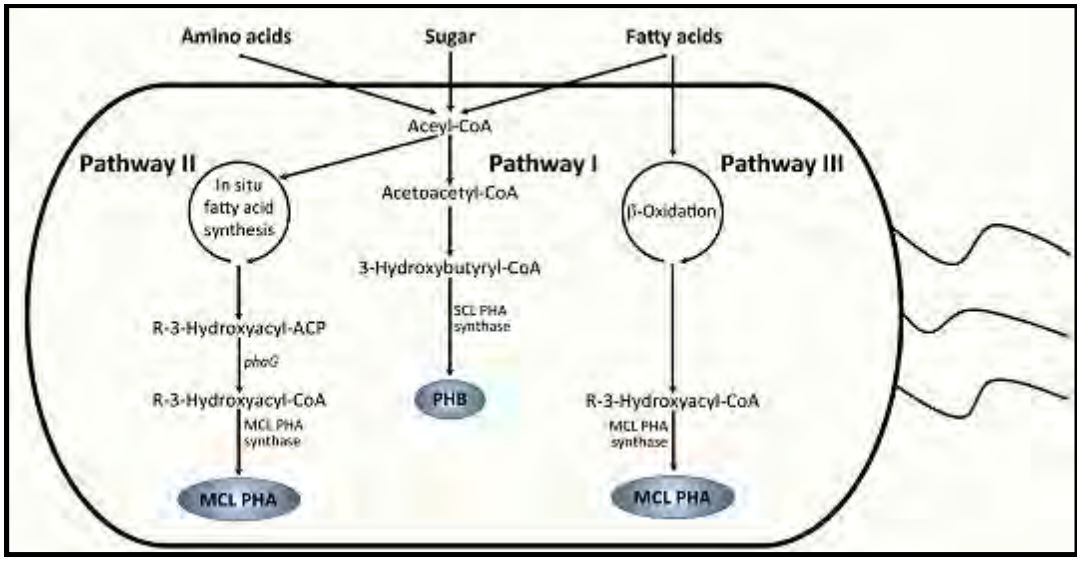


Figure 2-5: Metabolic pathways for PHA synthesis adapted from Chen *et al.* (2015)

Owing to the flexible nature of the PHA biosynthesis, PHB copolymers such as P(3HB-co-3HV) can be produced when precusors are fed during the PHA accumulation phase. The concentrations of these precursors must be regularly monitored to prevent growth inhibition in the microorganims and to maximise PHA accumulation (Garcia 2012). Table 2-4 gives some common precursors for the microorganism *Cupriavidus necator* and the corresponding polymers produced:

Table 2-4: Examples of precursors for PHA production in *Cupriavidus necator* (Braunegg *et al.* 2004)

Precursor	Polyester
Propionate	Poly-3-hydroxybutyrate-co-3-hyroxyvalerate [P(3HB-co-3HV)]
γ-Butyrolactone	Poly-3-hydroxybutyrate-co-4-hydroxybutyrate [P(3HB-co-4HV)]
1,4-Butanadiol	Poly-3-hydroxybutyrate-co-4-hydroxybutyrate [P(3HB-co-4HV)]
4-hydroxybutyrate	Poly-3-hydroxybutyrate-co-4-hydroxybutyrate[P(3HB-co-4HV)]

2.3.3 Microorganisms producing PHAs and the corresponding carbon sources

PHAs are synthesised in more than 75 genera of prokaryotes and archaea (Bernard 2014). For most microorganisms, the intracellular synthesis and accumulation of PHAs is triggered by unfavourable growth conditions. There is a smaller group of microorganisms that do not need nutrient limitation to accumulate PHAs which is further explained in Section 2.3.4. These include *Alcaligenes latus*, a mutant strain of *Azobacter vinelandii* and recombinant strains of *E. coli* amongst others (Doi 1990; Lee 1996; Akaraonye *et al.* 2010). The type of carbon source used ultimately determines the type of PHA monomer produced since it influences the metabolic pathways shown in Figure 2-5 (Domb *et al.* 2011). Table 2-5 gives an overview of various microorganisms, their carbon sources that they can metabolise and the corresponding type of PHA monomer produced.

Some desirable features of potential commercial PHA producers include the ability to be cultivated efficiently to a high cell density with a high PHA content in a short period of time resulting in a high PHA productivity, and when efficient polymer extraction does not represent a big challenge (Volova 2004). Of over 300 microorganisms that can produce PHAs, few have been identified as suitable contestants for commercial PHA production which is discussed in Section 2.4. While a wide range of carbon substrates can be metabolised by microorganisms to produce PHAs, mostly pure carbon sources have been reported to result in high enough PHA productivities suitable for commercial scale production (Volova 2004). The high cost of pure carbon sources such as glucose and sucrose contributes to some 50 % of the total production costs of PHAs giving rise to the need for cheaper carbon sources, potentially surplus or waste materials from industry (Scott 2013). Table 2-6 lists some of these cheap carbon substrates and corresponding PHA production (Chee *et al.* 2010a).

Table 2-5: Summary of bacterial strains producing PHAs including the type of carbon sources used and type PHAs produced (Verlinden *et al.* 2007)

Bacteria strain (s)	Carbon source (s)	Polymer (s) produced
Aeromonas hydrophila	Lauric acid, oleic acid	mcl-PHAs
Alcaligenes latus	Malt, soy waste, milk waste, vinegar waste, sesame oil	PHB
Bacillus cereus	Glucose, ε-caprolactone, sugarbeet molasses	PHB
Bacillus spp.	Nutrient broth, glucose, alkanoates, ε-caprolactone, soy molasses	PHB, PHBV copolymers
Burkholderia sacchari sp. nov.	Adonitol, arabinose, arabitol, cellobiose, fructose, lactose, maltose, melibiose, raffinose, rhamnose, sorbitol, sucrose, trehalose, xylitol	PHB, PHBV
Burkholderia cepacia	Palm olein, palm stearin, crude palm oil, palm kernel oil, oleic acid, xylose, levulinic acid, sugarbeet molasses	PHB, PHBV
Caulobacter crescentus	Caulobacter medium, glucose	PHB
Cupriavidus necator (Ralstonia eutropha)	Glucose, sucrose, fructose, valerate, octanoate, lactic acid, soybean oil	PHB, copolymers
Escherichia coli mutants	Glucose, glycerol, palm oil, ethanol, sucrose, molasses	PHB
Halomonas boliviensis	Starch hydolysate, maltose, maltotetraose and maltohexaose	PHB
Legionella pneumophila	Nutrient broth	PHB
Methylocystis sp.	Methane	PHB
Microlunatus phosphovorus	Glucose, acetate	PHB
Pseudomonas aeruginosa	Glucose, technical oleic acid, waste free fatty acids, waste free frying oil	mcl-PHAs
Pseudomonas oleovorans	Octanoic acid	mcl-PHAs
Pseudomonas putida	Glucose, octanoic acid, undecenoic acid	mcl-PHAs
Pseudomonas putida, P. fluorescens, P. jessenii	Glucose, aromatic monomers	Aromatic polymers
Pseudomonas stutzeri	Glucose, soybean oil, alcohols, alkanoates	mcl-PHAs
Rhizobium meliloti, R. viciae, Bradyrhizobium japonicum	Glucose, sucrose, galactose, mannitol, trehalose, xylose, raffinose, maltose, dextrose, lactose, pyruvate, sugar beet molasses, whey	PHB
Rhodopseudomonas palustris	Acetate, malate, fumarate, succinate, propionate, malonate, gluconate, butyrate, glycerol, citrate	PHB, PHBV
Spirulina platensis (cyanobacterium)	Carbon dioxide	PHB
Staphylococcus epidermidis	Malt, soy waste, milk waste, vinegar waste, sesame oil	PHB

Table 2-6: PHA production from plant oils and various wastes adapted from Chee *et al.* (2010a)

Strains	PHA type	Substrates	PHA content (%)	Cell concentration (g/L)	Reference
Alcaligenes latus DSM 1124	P(3HB)	Soya waste, malt waste	32.6 - 70	18.4 - 32.6	Yu *et al.* (1999)
Bacillus megaterium	P(3HB)	Beet molasses, date syrup	~50	3	Omar *et al*. (2001)
Burkholderia sp. USM (JCM 15050)	P(3HB)	Palm oil derivatives, fatty acids, glycerol	22 - 70	0.7 - 2	Chee *et al.* (2010b)
Comamonas testosteroni	mcl-PHA	Castor oil, coconut oil, mustard oil, cottonseed oil, groundnut oil, olive oil	79 - 88	n. m	Thankor *et al.* (2005)
Cupriavidus necator	P(3HB)	Molasses	37.5	20	Beaulieu *et al.* (1995)
Cupriavidus necator	PHB	Potato starch, saccharified waste	46	230	Haas *et al.* (2008)
Cupriavidus necator H16	P(3HB), P(3HB-co-HV)	Molasses	60 - 70	100 – 120	Harrison (personal communication)
Cupriavidus necator	PHB	Olive oil, corn oil, oleic oil	79 - 82	4.1 – 4.3	Fukui & Doi (1998)
Cupriavidus necator DSM 545	P(3HB)	Waste glycerol	62	82.5	Cavalheiro *et al.* (2009)
Cupriavidus necator (wild strain)	PHB, PHV	Soybean oil	74	122	Kahar *et al.* (2004)
Cupriavidus necator (recombinant strain)	PHB, PHV	Soybean oil	72.5	133	Kahar *et al.* (2004)
Recombinant Escherichia coli	P(3HB-co-3HHx-co-3HO)	Soybean oil	6	0.92	Gustavo & Regina (2006)
Pseudomonas aeruginosa IFO3924	mcl PHA	Palm oil	18 - 36	1.8 – 2.2	Marsudi et al. 2008
Pseudomonas aeruginosa NCIB 40045	mcl PHA	Waste frying oil	29	3.5 – 5.5	Fernandez *et al.* 2005
Pseudomonas guezennei biovar. Tikehau	mcl PHA	Coprah oil	63	n. m	Simon-Colin *et al.* 2008
Thermus thermophiles HB8	P(3HV-co-3HHp-co-3HN-co-3HU)	Whey	13 - 36	0.1 – 0.6	Pantazaki *et al.* 2009

*n.m: not mentioned

2.3.4 Cultivation methods for PHAs

PHAs are produced industrially by microbial cultivation. Mostly PHB, PHBV and (mcl) PHAs are produced on a large scale (Reddy *et al.* 2003). As mentioned in Section 2.3.3 there are two groups of bacteria that can produce PHAs; the first group requires a limiting nutrient in the presence of excess carbon to synthesise PHAs while the second group can accumulate PHAs during growth. For the first group, PHA production is comprised of two stages. In the first stage, bacterial cells are grown until a desired cell concentration without nutrient limitation. In the nutrient limited second stage, the cells do not multiply but the intracellular accumulation of PHA increases (Chee *et al.* 2010a). The cultivation step ranges from 38 to 48 hours and under these conditions *Cupriavidus necator* (formerly known as *Ralstonia eutropha* and *Alcaligenes eutrophus*) can accumulate PHAs up to 80% of its cell dry weight (Institute for Prospective Technological Studies 2005). An optimal amount of biomass needs to be accumulated in the first stage before entering into the nutrient limitation phase to maximise volumetric PHA concentration (Chee *et al.* 2010a). For the second group, fed-batch cultivation with no nutrient limitation is used since PHA accumulation is growth associated (Chee *et al.* 2010a). This approach is less widely practiced since there are only a few microorganisms belonging to the second group. Another less popular and more challenging production strategy is to use mixed cultures grown on non-sterile waste streams leading to a decrease in pure raw materials costs (Verlinden *et al*. 2007). The buildup of microbial cell numbers occurs in the feast (excess nutrients) phase while accumulation of PHAs is triggered by famine (nutrient limitation) conditions, employing the cycle normally used in biological treatment plants (Din *et al.* 2006).

2.3.5 Purification and recovery methods for the extraction of PHAs

In addition to cell cultivation and PHA production, the recovery of PHAs from biomass is also a crucial step. Intracellular PHAs require various downstream processing steps to liberate it from the cells, prior to recovery and purification. A simple and efficient method of extraction of intracellular PHAs from biomass is solvent extraction using halogenated compounds such as chloroform, dicholoromethane, dichloroethane and chloropropane (Kunasundari & Sudesh 2011). However, halogenated compounds are hazardous to health and the environment, posing a threat on large scale. Thus, alternate methods are required (Chee *et al.* 2010a). The final application of PHAs influences the type of recovery process; for example, for medical applications, high purity PHAs is desired while for use in the packaging industry, the degree of purity required depends on the end uses of the PHAs, whether they are used for food packaging or as garbage bags (Kunasundari & Sudesh 2011). Nonetheless, any loss in purity and low recovery of PHAs decrease their value, thus recovery methods which result in high purity (95-98%) and high yield (87-98%) of PHAs are preferred (Kunasundari & Sudesh 2011). It is also important to use

environmentally-friendly and non-hazardous chemicals while minimising the cost of (Kunasundari & Sudesh 2011). The advantages and disadvantages of the various PHA extraction and purification methods are summed up in Table 2-7.

Table 2-7: Advantages and disadvantages of various PHA extraction methods adapted from Kunasundari & Sudesh (2011)

Recovery method	Advantages	Disadvantages
Solvent extraction	-High purity -Negligible/limited degradation to the polymer -Higher molecular weight	-Not environmentally friendly -High capital and operation cost - Lengthy process
Chemical digestion surfactant	-Extracted PHAs retain original molecular weight -Native order of polymer chains in PHA granules is retained	-Low purity of PHAs -Treatment required to remove surfactant from wastewater
Sodium hypochlorite	- Higher purity of PHAs can be obtained	-Severe reduction in molecular weight of the extracted PHAs
Surfactant and hypochlorite treatment	-High quality of PHAs -Rapid recovery and simple process -Lower operating cost compared to solvent extraction	-Combined cost of surfactant and sodium hypochlorite -Wastewater treatment required to remove residual surfactant and sodium hypochlorite
Dispersion sodium hypochlorite and solvent extraction	-High purity of PHAs	-Not environmentally friendly -Consumption of large volume of toxic and volatile solvents -Higher recovery cost
Enzymatic digestion	-Mild operation conditions -Good recovery	-Complex process -High cost of enzymes

2.4 LARGE SCALE PRODUCTION OF PHAS

2.4.1 Commercial production of PHAs

PHAs were first produced in 1959 by W.R Grace and Company in the United States with the intent of commercialisation but lack of production efficiency and adequate recovery process caused the company to shut down (Chanprateep 2010). In 1976, PHB and a copolymer of P3HB/3HV under the tradename BIOPOL™ was developed by Imperial Chemical Industries (ICI, Ltd.,Bellingham, UK), later known as Zeneca Ltd but it was only in the 90's that these biopolymers started to be economically viable when the oil prices started to rise again (Chanprateep 2010). Thereafter, more companies started PHA production as shown in Table 2-8. Some PHA production strains used commercially are shown in Table 2-9 with corresponding carbon sources, final cell concentration and PHA content. However commercial success still remains challenging and is addressed in Section 2.4.2.

Table 2-8: Global suppliers of PHAs adapted from Chen (2009), Chanprateep (2010), Biomaterials China News, (2011), Babu *et al.* (2013), Plasteurope.com (2015) and Bio-on (2015)

Company	Types of PHA	Production scale (tonnes/year)	Period
Zeneca (formerly as ICI, UK)	PHBV	300	1980s to 1990s
Monsanto, USA (with Zeneca technology)	PHB, PHBV	Development of PHA production plant, not commericalised	1990s - 2001
BtF, Austria	PHB	20 to 100	1990s - 1993
Biomers, Germany	PHB	50	1990s to present
Biomers with Newlight technologies	scl and mcl PHAs	1000 to 22 500	2014 to present
Mitsubishi gas chemical company Inc., Japan	PHB	10 000	1990S to unknown
Kaneka, Japan (with P&G)	Several PHA	10 000	1990s to 2004
Kaneka, Japan	PHA, PHBH	1000	2011 – present
Shantou Lianyi Biotech, China	Several PHA	2000	1990s to 2010
Metabolix, USA	Several PHA	Unknown	1990s to present
Metabolix with ADM	Several PHA	50 000	2005 to 2012
Meredian, USA (with P&G)	Several PHA	Pilot (13 500) Large scale (91 000 or higher)	2012 to present
Tepha, USA	Several PHA	PHA medical implants	1990s to present
Biocycles, Brazil	PHB	50 - 100	1990s to present
Bio-on, Italy	PHB (unclear)	10 000	2008 to present
Bio-on with Moore capital, Brazil	unclear	10 000	Not yet started
Bio-on with Cristal Union	unclear	5000 to 10 000	Not yet started
Bio-on with Pizzoli, Italy	unclear	2000 - 4000	Production starts in 2017
Zhejiang Tian An, China	PHBV	2000	1990s to present
Jiangmen Biotech Ctr, China	PHBHHx	Unknown	1990s
Yikeman, Shandong, China	PHB (unclear)	3000	2008 to present
Tianjin Northern Food, China	PHB	10 000	1990s to present
Jiang Su Nan Tian, China	PHB	10	1990s to present
Shenzhen O'Bioer, China	Several PHA	Unknown	2004 to present
Tianjin Green Bio-Science (+ DSM)	P3HB-4HB	10 000	2004 to present
Shandong Lukang, China	Several PHA	5	2005 to present

Table 2-9: Wild type and industrial bacterial strains commonly used for pilot and large scale production of PHAs (Chen 2009)

Strain	DNA manipulation	PHA type and scale (tonnes/year)	Carbon source	Final CDW (g/l)	Final PHA (% CDW)	Companies
*Cupriavidus necator (*previously *Ralstonia eutropha* and *Alcaligenes eutrophus)*	No	PHB (10)	Glucose	> 200	> 80%	Tianjin North. Food, China
Alcaligenes latus	No	PHB (30 – 100)	Glucose or sucrose	> 60	> 75%	Chemie Linz, btF, Austria Biomers, Germany
Escherichia coli	Yes	PHB (10)	Glucose	> 150	> 80%	Jiang Su Nan Tian, China
*Cupriavidus necator (*previously *Ralstonia eutropha* and *Alcaligenes eutrophus)*	No	PHBV (300 – 2000)	Glucose + propionate	> 160	> 75%	ICI, UK Zhejiang Tian An, China
*Cupriavidus necator (*previously *Ralstonia eutropha* and *Alcaligenes eutrophus)/ Escherichia Coli*	No	P3HB4HB (> 10 000)	Glucose + 1,4-butanediol	> 100	> 75%	Metabolix, USA Tianjin Green Biosci. China
*Cupriavidus necator (*previously *Ralstonia eutropha* and *Alcaligenes eutrophus)*	Yes	PHBHHx (1)	Fatty acids	> 100	> 80%	P&G, Kaneka, Japan
Aeromonas hydrophilia	No	PHBHHx (1)	Lauric acid	< 50	< 50%	P&G, Jiangmen Biotech Ctr.
Aeromonas hydrophilia	Yes	PHBHHx (0.1)	Lauric acid	~ 50	> 50%	Shandong, Lukang,
P. oleovorans Bacillus spp.	No	PHB (5)	Sucrose	> 90%	> 50%	Biocycles, Brazil

2.4.2 Challenges with the large scale production of PHAs

2.4.2.1 Identifying challenges

Although there are an increasing number of companies producing PHAs, economies of scale have been difficult to achieve. A number of challenges are listed in Table 2-10 and further discussed in the following sections. Compared to the chemical process where products can attain a concentration of at least 500 g/L , the final maximum PHA content of the culture is usually in the range of 200 g/L (a high concentration with respect to other bioprocesses) resulting in high downstream processing costs. The usual substrate to product conversion in PHA production is usually around 33 % (g/g) whereas the conversion for typical plastics such as PE or PET is typically over 90% (Wang *et al.* 2014). This results in

challenging process economics for PHA production. Additionally, since the main driving force to produce PHAs on large scale is to shift the current petroleum-based economy to the bio-economy, PHAs production must be sustainable and environmental friendly, further discussed in Section 2.4.2.3.

Table 2-10: Comparison between the large scale production of PHAs and petroleum plastics adapted from Volova (2004) Wang *et al.* (2014):

Comparison parameter	Polyhydroxyalkanoates (PHAs)	Petroleum plastics
Production scale	Low	High
Raw materials	Usually high cost pure substrates	Petroleum
Reaction conditions	Ambient temperature and atmosphere pressure, aqueous medium	Mostly high temperature, high pressure and organic solvents as reaction medium
Process	Mostly batch and fed-batch processes	Mostly continuous processes
Process duration	Dependent on time from inoculum preparation to downstream processing	Mostly complete within hours/days
Energy requirement	Can be comparable or lower than petroleum plastics production	Depends on type of plastics produced but generally higher than PHAs
Greenhouse gas emissions	Low	High
Water consumption	High water consumption	Less water consumption
Final product concentration	Mostly low from mg up to 200 g/L	Mostly over 500 g/L
Cost of product recovery	Very high (influenced by cell disruption ,centrifugation is long and energy intensive)	Low
Substrate to product conversion efficiency (g/g)	Low (~ 33 %)	Mostly very high (> 90%)
Risk	Low level	High due to flammable and explosive a well as toxic or product leakages
Waste water	Mostly non-toxic and easily treated	Mostly toxic, acidic or alkali, difficult to treat

2.4.2.2 Commercial price of PHAs

The final price of PHAs depends on the cost of production determined by substrate cost, PHA yield on the substrate, productivity and the efficiency of the downstream processing (Lee 1996b). Based on an economic evaluation on the large scale production of PHB, Choi and Lee (1999) presented a wide range of PHB production costs from $ 3 to 7/kg for the year 1996. This price range still holds for current PHA prices as shown in Table 2-11, suggesting that optimisation for PHA production is still needed.

Table 2-11 Tradenames and price of PHAs ($/kg) from large sale producers of PHAs adapted from Chanprateep (2010)

Polymer	Trade names	Manufacturers	Price in $/kg in 2010
PHB	Biogreen	Mitsubishi gas chemical company Inc.	3.86 to 4.64
PHB	Mirel™	Metabolix with ADM (Telles)	2.32 (expected) but $ 5.50 when released into the market
PHBV and PHB	Biomer R	Biomer Inc. (Germany)	4.64 to 7.73
PHBH	NodaxTM	Lianyi Biotech (China)	5.71

The price of Mirel developed by Metabolix Inc and Telles was reported to be $ 5.5/kg in 2010, making it comparatively more expensive than plastics and other bio-plastics; the price of PLA was $ 1.98/kg in the last quarter of 2011 and PS and PET costs at $ 2.20/kg and $ 1.76/kg respectively. This led to a poor market penetration of Mirel (Naitove 2012; Smock 2012; Roland-Holst *et al.* 2013). PHA price is still 20 – 80 % higher than petroleum plastics (Markets and Markets 2013). According to Roland-Holst *et al.* (2013) Stanford estimates place the average price of PHAs at $ 4.70 /kg in 2013 while Bolck (2014) mentioned a PHA price range of $ 4.6 to 9.3/kg in 2014. Decreasing the price of PHAs by lowering the cost of production still presents a major challenge due to the complexity of PHA production (Koller *et al.* 2010).

2.4.2.3 Environmental impacts of PHAs

Although PHA production relies on biological and renewable resources, early LCA studies on PHAs concluded that the overall production of PHAs from raw materials to the final desired product was more energy-intensive than the production of conventional plastics (Hyde 1998; Gerngross 1999). Gerngross (1999) expressed great concerns on whether replacing petroleum plastics by PHAs will result in lower environmental burden if the consumption of non renewable resources needed for the overall PHA production is taken into account. However, a more recent study in the form of a cradle to gate comparative life cycle assessment of PHAs in all major impact categories by Harding *et al.* (2007) suggested that PHB is superior to polypropylene (PP).

To address conflicting environmental asessment for bio-plastics, Essel (2012) conducted a meta-analysis to evaluate the environmental impacts of PHAs and PLA based on over 30 studies in the period 2000 – 2011. The environmental impacts were analysed from cradle to factory gate thus taking into consideration the cultivation of renewable resources to the preparation of plastic granules (Essel 2012). The impact categories were climate change measured in the form of greenhouse gas emissions in CO_2-equivalents per kilogram polymer (CO_2-eq./kg) and fossil resource depletion in terms of fossil resources

for energy and materials in megajoule per kilogram polymer (MJ/kg). Their results are presented in Figure 2-6.

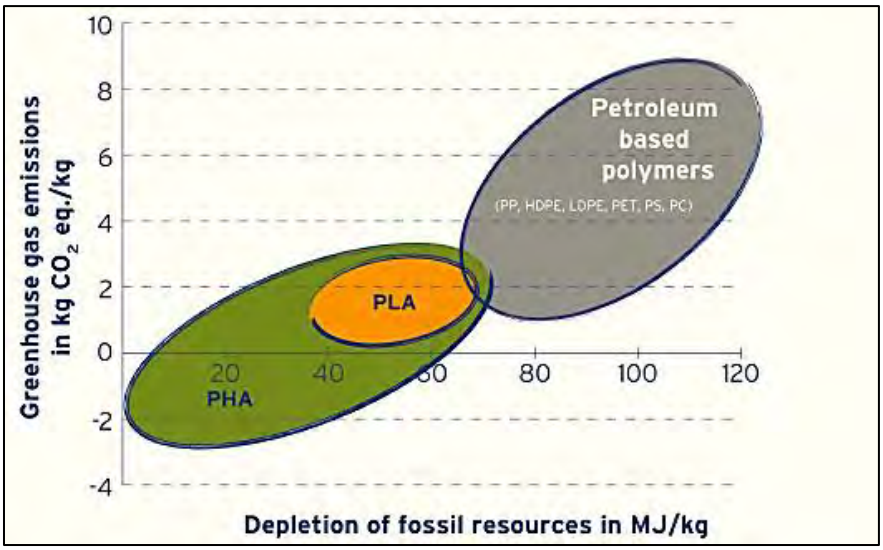


Figure 2-6: Comparison of PHAs (green circle) and PLA (orange circle) to petroleum plastics (grey circle) in two environmental impacts: climate change and fossil resource depletion (Essel 2012)

The general conclusions that can be drawn are that both PHAs and PLA possess ecological advantages when compared to petroleum plastics in terms of reduced gas emissions and depletion of fossil resources (Essel 2012). Recent LCA results seem to suggest that while PHAs can be more beneficial in some environmental impact categories (cogeneration power plant and greenhouse gas credits) they have lower environmental scores than petroleum plastics in other impact categories (eutrophication and acidification) (Chanprateep 2010).

Harding (2008) observed that the electricity requirement of PHA production was the largest contributor to the following LCA categories: abiotic depletion, global warming, ozone layer depletion and photochemical oxidation. A sixth LCA category, eutrophication was mostly influenced by the carbon dioxide released during microbial growth and the chemical oxygen demand of the wastewater (Harding 2008). Incidentally, water usage, raw materials selection and the electricity usage were found to be of high importance in the assessment of the environmental burden of PHAs (Fernandez-Docosta *et al.* 2015). On this note, it is important to highlight that the production process of PHAs is far from optimised as opposed to the production of petrochemicals polymers, thus suggesting that further decrease of the environmental burden of PHAs is attainable (Essel 2012).

2.4.3 PHA economics as a tool to inform reduction of costs for PHA production

Several factors can determine the viability of the development of cost effective PHA production such as the microorganism used, type of substrate used, the yield, the cultivation method, its productivity and the downstream recovery process. Van Wegen *et al.* (1998) recommended an economic assessment across the whole process to identify key areas for optimisation to reduce PHA production cost. Emphasis is put on high productivity, reduced culture volume, and enhanced downstream processing, reduced wastewater, and lower production costs together with reduced equipment costs (Harding 2008, Rehm 2009). For emerging technologies, the confidentiality of process data is critical, thus a generic approach is required to estimate the economics of PHAs in view of limited or not-yet generated data from current/future pilot plants (Hermann & Patel 2006).

2.4.4 Previous economic assessment on large scale production of PHAs

Previous economic assessments on the large scale production of PHAs are summarised in Table 2-12. For comparison, the prices were escalated to the year 2014 using CEPCI indices[1]. The simulations were undertaken with the main focus on the microorganisms used, the carbon substrates and the recovery processes used. The findings are summarised in Table 2-13. For the studies conducted by Choi & Lee (1997), Choi & Lee (1998), Lee & Choi (1998) and Garcia *et al.* (2011) the production costs of PHAs was limited to the direct production costs of PHAs excluding sales cost and margins, shown in Equation 2-1.

$$Production\ costs\ of\ PHAs\ (\$/kg) = \frac{Direct\ fixed\ capital\ dependent\ items + raw\ materials + utilities + labour + waste\ disposal}{total\ amount\ of\ PHAs\ produced\ per\ year} \quad 2\text{-}1$$

The general findings summarised in Table 2-13 were that PHA productivity, yield, recovery process and cost of substrate affect the production costs of PHAs significantly. Tackling the issue of high cost of pure substrates, Van Wegen *et al.* (1998) first demonstrated that the use of a low cost carbon substrate can decrease PHA production costs. Choi & Lee (1999) also gives an overview of potential low cost susbrates that could be used for PHA production but nonetheless pointed out that resulting PHA content and productivity were tyically lower, challenging the viability of the overall process economics. Choi & Lee (1999) undertook a sensitivity analysis to show that PHA productivity only affected equipment related costs while PHA cellular content influenced various components of process economics. Water, energy usage and greenhouse gas emissions were not reported in these studies.

[1] The CEPCI indices used can be found in Table 3-2

Table 2-12: Summary of feasibility studies conducted on the large scale production of PHAs

Study conducted by	Simulation package used	Annual production rate and type of PHAs produced (tonnes/ year)	Microorganism used	Carbon source used	Type of recovery process used	Final cost of production of PHAs ($/kg)	Final cost of production of PHAs ($/kg) estimated for the year 2014
Choi & Lee (1997)	SuperPro	2850 (PHB)	*(i) Cupriavidus necator* (ii) *Alcaligenes latus* (iii) *Methylobacterium organophilum* *(iv)* recombinant *Escherichia coli*	(i) Glucose (ii) Sucrose (iii) Methanol (iv) Glucose	a) surfactant-hypochlorite digestion b) chloroform and hypochlorite	(i) a: 5.58, b: 9.16 (ii) a: 8.16, b:11.96 (iii) a: 7.34, b: 10.95 (iv) a: 6.14, b: 9.45 (cost calculated for the year 1996)	(i) a: 8.23, b: 13.5 (ii) a: 12, b:17.6 (iii) a: 10.8, b: 16.2 (iv) a: 9.06, b: 13.9
Hazenberg & Witholt (1997)	n.m	1000 (mcl PHA)	*Pseudomonas oleovorans*	Octane	n. m	10	14.8
Choi & Lee (1998)	SuperPro	100 000 (PHB)	recombinant *Escherichia coli*	Glucose	Sodium hydroxide	3.66 (cost calculated for the year 1996)	5.52
Lee & Choi (1998)	BioPro Designer	100 000 (PHB)	*Alicaligenes latus*	Sucrose	Surfactant-hypochlorite digestion	2.6	3.83
Van Wegen *et al.* (1998)	BioPro designer	4300 (PHB)	recombinant *Escherichia coli*	(i) Glucose (ii) Diary whey	Sodium hypochlorite	(i) 6.08 (ii) 3.60 to 5.63	(i) 8.97 (ii) 5.31 to 8.30
Garcia *et al.* (2011)	SuperPro	2000 (i) PHA (ii) PHBV	(i) recombinant *Escherichia coli* (ii) *Haloferax mediterrenei* iii) *Cupriavidus necator*	(i) Whey (ii) Rice bran (iii) Wheat	(i) surfactant-hypochlorite digestion (ii) Lysis of cells (iii) Enzymatic cell lysis using enzymes	(i) 3.42 (ii) 6.29 (iii) 2.58	(i) 3.36 (ii) 6.19 (iii) 2.54
Fernandez-Docosta *et al.* (2015)	ASPEN Plus softwarre	1500 (PHB)	Microbial enrichment culture	Wastewater	(i) allkali-surfactant (ii) surfactant-hypochlorite (iii) dichloromethane solvent	(i) 1.57 (ii) 1.75 (iii) 2.18	(i) 1.57 (ii) 1.75 (iii) 2.18

Table 2-13: Summary of findings from feasibility studies conducted on the large scale production of PHAs

Study conducted by	Principal investigation	Findings
Choi & Lee (1997)	Process analysis and economic evaluation of PHB production	(i) PHB productivity, content and yield affect the final cost of production of PHB (ii) The cost of carbon susbtrate significantly affects the final price of PHB (iii) Increasing the efficiency of the recovey process can lower the price of PHB
Hazenberg & Witholt (1997)	Economic evaluation of mcl PHAs	(i) PHA content is a key factor in downstream processing (ii) Optimisation of PHA production depends on volumetric PHA productivity as welll as final PHA content in the cell (iii) Nitrogen limitation, media optimisation results in higher PHA content in continous cultures
Choi & Lee (1998)	Recovery of PHB by simple digestion with chemicals	(i) A simple sodium hydroxide digestion method was developed to recover PHB at 98.5% purity
Lee & Choi (1998)	Effect of cultivation performance on the final production cost of PHB	(i) Higher cellular PHB content led to a decrease in the downstream recovery cost (ii) Higher cellular PHB content led to a decrease in raw material costs due to higher yield of PHB on carbon source (iil) Direct fixed capital–dependent costs decreased with increasing productivity
Van Wegen *et al.* (1998)	Economic analysis on the production of PHB	(i) Final PHB production costs is dependent on PHB expression level and recovery process (ii) Media cost and biomass yield moderaly affect the cost of production of PHB (iil) Maximum cell density had little effect on the process design (iv) The partial substitution of glucose by diary whey can decrease the PHB production costs significantly
Garcia *et al.* (2011)	Techno-economic evaluation of PHA production from industrial food wastes and agricultural feedstocks	(i) Final production costs of PHB depends on production capacity of plant, renewable raw material, cultivation strategy and downstream processing (ii) There needs to be an upstream processing stage where the food waste is processed into fermentation feedstock
Fernandez-Docosta *et al.* (2015)	Techno-economic and environmental assessmnet of microbial community-based PHAs from wastewater	(i) Alkaline treatment as a recovery process is more cost-effective and environment friendly compared to solvent based recovery processes (ii) Final PHB prices are not yet competitive with petroleum plastics

Garcia *et al.* (2011) and Fernandez-Docosta *et al.* (2015) investigated the use of agricultural wastes and waste water as potential carbon sources for PHA production, which resulted in lower production costs of PHAs. Using waste water as raw material and assuming that the final effluent was suitable to enter the water cycle, no waste water treatment costs were included in the production costs (Fernandez-Docosta, *et al.* 2015). Instead, since the PHA plant was essentially treating waste water, economic credits in the form of avoided waste water treatment costs were deducted from the final PHA production costs. Fernandez-Docosta *et al.* (2015) also performed a sensitivity analysis on the process design and concluded that higher cell rupture efficiency in the downstream processing resulted in lower production costs. However no sensitivity analysis were done on the actual PHA production in terms of PHA productivity, content, cultivation time or concentration.

2.4.5 Reviewing simulation packages for assessing large scale production of PHAs

While SuperPro and ASPEN were mainly used for previous economic assessments on PHA production, Gosling (2005) suggested that these packages must be used for detailed simulation requiring unique solutions and when it is reasonable to invest in them. Due to the confidentiality of plant data associated with emerging technologies such as the production of PHAs, it is more appropriate to use a generic approach allowing an estimation of production costs (Hermann & Patel 2006).

In view of establishing a basis for process selection in early stage process development, simplified modeling tools with low cost of acquisition and non-specialist knowledge such as MS Excel can provide rapid and useful guidelines for investigating the process economics for bio-products such as PHAs (De Beer, 2010). A comparison between different commercial software is presented in Table 2-14 showing that MS Excel can deliver the material and energy inventories, which are the most important data to acquire in order to estimate equipment sizes and further compute the final production costs. Similarly, energy, water usage and carbon dioxide emissions can all be found from material and energy inventories. Using MS Excel, Harding (2008) developed such a tool for bioprocess simulation called the CeBER Bioprocess Modeller which generates material and energy inventories to simulate bioprocesses at a conceptual and developmental stage (De Beer 2010). The model outputs can further be used in economic and environmental assessment. The following section will give an overview of this tool.

Table 2-14: Common commercial software used for process simulation adapted from Gosling (2005)

	MS Excel	Aspen Batch (Plus)	SuperPro
Material Balance	✓	✓	✓
Material and Energy Balance	✓	✓	✓
Detailed Description	✓	✓	✓
Rate-Based Models		✓	✓
CFD & Rate Based Models		✓	✓

2.5 LARGE SCALE PRODUCTION OF BIO-PRODUCTS USING THE CEBER BIOPROCESS MODELLER AS SIMULATION TOOL

The CeBER Bioprocess Modeller was developed within the Centre for Bioprocess Engineering Research (CeBER) at the UCT Department of Chemical Engineering as a PhD study. It is a spreadsheet model developed in MS Excel that can simulate the aerobic or anaerobic microbial production of intra- or extracellular products using batch or continuous processes (Harding 2008). The main drive behind the development of this tool was to provide a simplified bioprocess model during early development phases that can supply material and energy inventories for Life Cycle Assessment (LCA) when limited data is available (Harding 2008). The model was built based on a stoichiometric approach, first principles and heuristics (Harding 2008). Table 2-15 gives an overview of the user inputs needed for the model and the corresponding simulation outputs. On specifying the amount of product and raw materials, the mass balance is done using the stoichiometric approach given in Equation 2-2:

$$(Carbon\ source\ 1+2)+Oxygen\ source+(Nitrogen\ source\ 1+2)+Sulphur\ source+ Phosphorus\ source \rightarrow Biomass+Product+Water+Carbon\ dioxide+Nitrogen\ waste+ Sulphur\ waste+Phosphorus\ waste \quad 2\text{-}2$$

Figure 2-7 illustrates the generic process diagram, with sterilisation, inoculation, microbial growth and product formation as upstream processes. Steps such as solid-liquid separation, cell disruption, centrifugation amongst others are available as downstream processing depending on the user's inputs. There can be a maximum of six concentration/purification units for the downstream processes followed by a final product formulation step. The model allows for specific user inputs. Where detailed inputs cannot be provided, the model uses default values, which are aggregated values from different bioprocess flowsheets using literature or actual process data. Default values are available for yield

coefficients, material compositions and densities, operating temperatures, pressures and recovery values (Harding 2008).

Table 2-15: User input requirements and corresponding simulation outputs from the CeBER Bioprocess Modeller generic flowsheet adapted from Harding (2008) and De Beer (2010)

Operation	User inputs	Simulation outputs
Overall process	-Option for batch or continuous operation -Option for solid or liquid product and whether it is an intra- or extracellular product -Option for anaerobic or aerobic biomass growth User defined quantity of product	-Material and energy needed -Waste materials -Product purity and recovery
Bioreaction	-Requires yield coefficients, growth rates, biomass concentration -Option to include maintenance calculations	-Predicts microbial growth -Agitation and amount of antifoam needed
Sterilisation	-Media sterilisation -Option for steaming out vessels, backing steam and space heating -Option for pre-heating during sterilisation	-Material and energy needed
Cooling	-Include relevant cooling (bioreactor, post bioreactor cooling)	-Material and energy needed
Downstream	-Specify downstream units from built-in models -Addition of reacting and non-reacting chemicals at each step Input parameters (e.g. temperature, % recovery, wash ratio)	-Material and energy needed
Waste treatment	-Option for waste treatment	-Chemical Oxygen Demand (COD)

The model also consists of a database compiled from literature which offers the use of a variety of carbon, nitrogen, sulphur and phosphorus sources as raw materials together with relevant constants and physical data (Harding 2008). The use of default values provides the user an estimate of material and energy inventories based on very limited data and minimal inputs. Using process specific data available from pilot plants or literature, the models delivers more accurate material and energy inventories (De Beer 2010).

For validation purposes, Harding (2008) investigated the production of four bio-products namely penicillin, cellulose, biodiesel and PHAs. The simulation outputs from these models were further used to undertake LCA studies. While the model was developed to generate adequate data for use in environmental assessment, De Beer (2010) demonstrated the use of the material and energy inventories obtained from the CeBER Bioprocess Modeller to undertake preliminary equipment sizing to perform an economic assessment of the large scale production of citric acid. In sections 2.5.1 and 2.5.2 process design considerations and costing calculations are addressed in view of using material and energy inventories as a starting point to compute production costs.

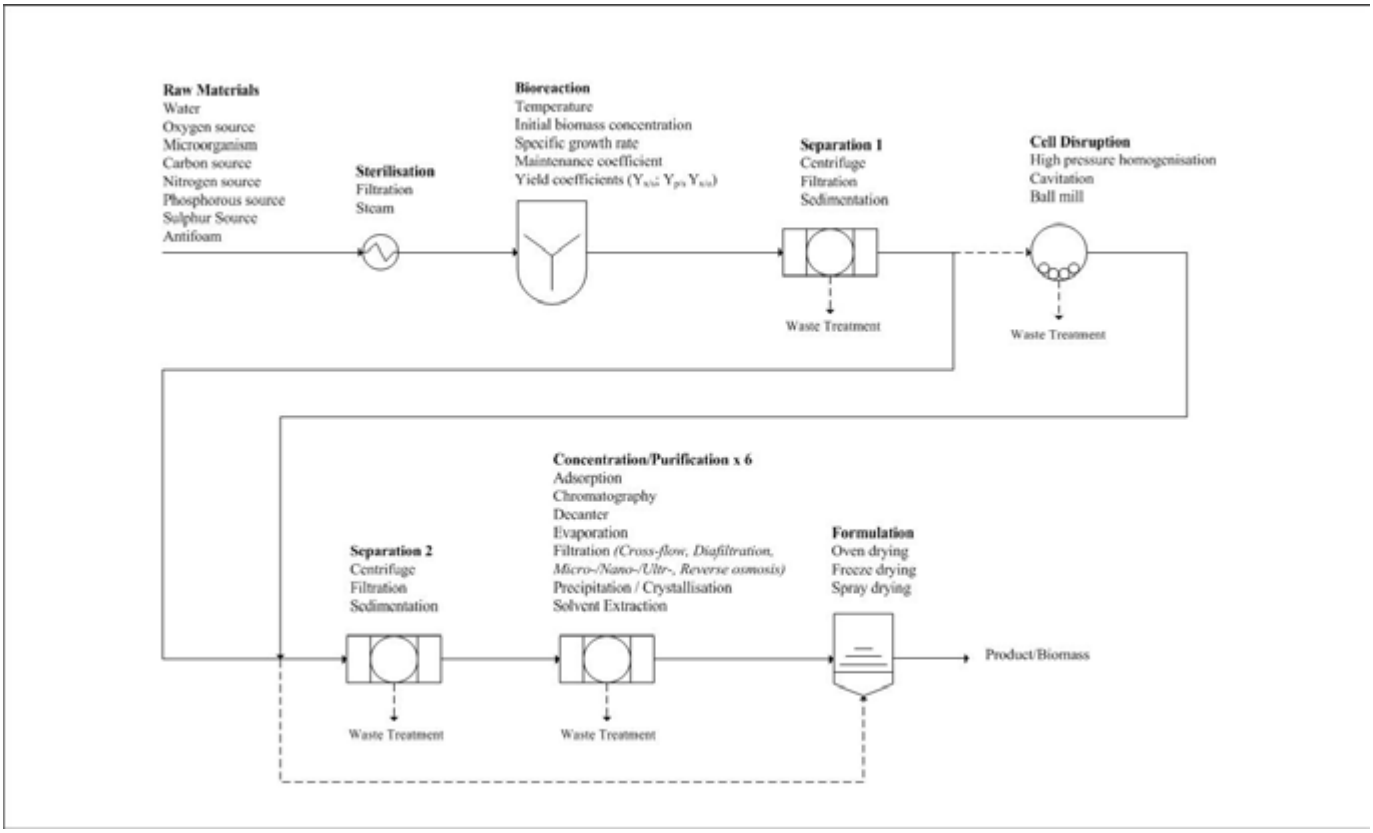


Figure 2-7: Representation of the generic process flow diagram of the CeBER Bioprocess Modeller generic flowsheet (Harding 2008)

2.5.1 Process design considerations for equipment sizing

There are basic engineering approaches and rules of thumbs that can be used to perform preliminary equipment sizing which can further be used for process economics. Equipment is usually selected and sized based on the following factors: volumetric throughput rate, limited residence time, solids size, loading and removal efficiency, interface area for mass transfer, number of equilibrium stages and heat transfer area amongst others (Asenjo 1990). Table 2-16 presents the major variables usually used for the design and sizing of specific equipment (Peters & Timmerhaus 1991). The maximum scale-up ratio along with the overdesign factor is also presented.

Table 2-16: Factors influencing equipment scale-up and design adapted from Peters & Timmerhaus (1991)

Type of equipment	Major variables for operational design (other than flow rate)	Major variables characterizing size or capacity	Approximate recommended safety or over-design factor %
Batch reactor	Reaction rate Equilibrium state	Volume Residence time	20
Continuous reactor	Reaction rate Equilibrium state	Volume Residence time	20
Evaporators	Latent heat of vapourisation Temperatures	Flow rate Heat transfer area	15
Nozzle-discharge centrifuges	Discharge method	Flow rate Power input	20 20
Sedimentation centrifuges	Discharge method	Flow rate Power input	20 20
Reciprocating compressors	Compression ratio	Flow rate Power input Piston displacement	10 10 10
Reboilers	Temperature Viscosities	Flow rate Heat transfer area	15
Tube-and-shell heat exchangers	Temperatures Viscosities Thermal conductivities	Flow rate Heat transfer area	15

Once the equipment has been sized, its cost can be calculated, using one of three ways of estimation methods (NMSU Chemical Engineering 2012):

i. Request a quote directly from the vendor. This is the most accurate costing since it is based on detailed design and engineering
ii. The cost can be extrapolated from the cost of similar equipment and scaled for time and size (six-tenths rule). It is considered to be reasonably accurate with decreasing accuracy with large extrapolation
iii. Cost estimating charts can be used and scaled for time. It is the most convenient way of estimating equipment cost in preliminary design but is the least accurate

Cost estimates can be categorised based on level of development and accuracy as detailed in Table 2-17. The first three estimates involve early stage project design, which is usually developed using minimum amount of detailed engineering to give an estimate of the cost of a product. This facilitates process interrogation and analysis prior to detailed design such that optimisation can be sought.

Table 2-17: Categories of cost estimates and corresponding accepted accuracy (Whitesides 2012)

Cost estimate	Accuracy
Order of Magnitude (OME) estimate	± 50%
Study estimate	± 30%
Preliminary (budget, scope) estimate	± 20%
Definitive estimate	± 10%
Detailed estimate	± 5%

The relationship between the costs of two (or more) items of equipment can be related based on their ratio in terms of quantity and amount or size (Whitesides 2012). This forms the foundation of the six-tenths rule typically used in preliminary cost estimates, within 20 % accuracy (Whitesides 2012). Thus, if the cost of a similar equipment of different size or capacity is known, approximate costs of other equipment can be estimated using the Equation 2-3 (Whitesides 2012):

$$\boldsymbol{C_B = C_A \left(\frac{S_B}{S_A}\right)^N} \qquad 2\text{-}3$$

where C_B = the approximate cost ($) of equipment having size S_B (m^3, kW, m^3/hr amongst others); C_A = known cost ($) of equipment with corresponding size S_A (same units as S_B); S_B/S_A = ratio based on size, dimensionless; N = size exponent

The size factor N varies from 0.3 to 1. An average of 0.6 is normally used, hence name of the sixth tenths rule. For a higher level of accuracy different N values can be used for different equipment, some of which are given in Table 2-18:

Table 2-18: Process equipment and size exponent (N) (Whitesides 2012)

Equipment	Size exponent (N)
Air compressor, single stage	0.67
Air compressor, multiple stage	0.75
Boiler	0.50
Tanks and vessels	0.68
Reactor	0.68
Heat exchanger	0.62
Pumps	0.70

2.5.2 Calculation of PHA production costs

The production costs of a commodity product like PHAs fall into three categories as shown in Table 2-19. The direct manufacturing costs pertain to operating expenses of the plant and are directly linked to production rate. The fixed manufacturing costs represent plants costs which are independent of

production rate. The general expenses include administrative costs, sales and research functions (Turton *et al.* 2012).

Table 2-19: Factors affecting the cost of manufacturing a product (Turton *et al.* 2012)

Factor	Description of factor
1. Direct costs	**Factors that vary with the rate of production**
a) Raw materials	-Costs of chemical feedstocks required by process
b) Waste treatment	-Cost of waste treatment to product environment
c) Utilities	-Costs of utility streams required by process
d) Operating labour	-Costs of personnel required for plant operations
e) Direct supervisory and clerical labour	-Costs of administrative, engineering and support personnel
f) Maintenance and repairs	-Costs of labour and materials associated with maintenance
g) Operating supplies	-Costs of miscellaneous supplies
h) Laboratory changes	-Costs of routine and special laboratory tests
i) Patents and royalties	-Costs of using patented or licensed technology
2. Fixed costs	**Factors not affected by the level of production**
a) Depreciation	-Costs associated with the direct fixed capital (DFC) investment of the plant
b) Local taxes and insurance	-Costs associated with property taxes and liability insurance
c) Plant overhead costs	-All costs associated with operations of auxiliary facilities supporting the manufacturing process. Costs involve payroll, accounting services, employee benefits amongst others
3. General Expenses	**Costs associated with management-level and administrative activities not directly related to the manufacturing process**
a) Administration costs	-Costs for administration
b) Distribution and selling costs	-Costs of sales and marketing required to sell products
c) Research and development	-Costs of research activities related to the process and product

The manufacturing costs correspond to the expenses required to make a product only and consists of the direct and fixed costs. The raw materials, utilities and waste disposal costs can be calculated based on the material and energy inventories of the process. Preliminary estimates for the rest of the manufacturing costs can be found as a function of the direct fixed capital (DFC) and operating labour (OL) of the plant as shown in Table 2-20.

The operating labour can be calculated based on the total number of operators needed to run the plant. The DFC consists of the costs associated with building the plant and is based on the total costs of the equipment purchased using multipliers called 'Lang factors' to estimate installation and accessory costs. Table 2-21 gives a range and the most commonly used values for multipliers. According to Petrides (2015), commodity biochemical plants fall on the low end of the range while biopharmaceutical plants fall on the high end with the average value corresponding to relatively large plants that produce medium to high value products.

Table 2-20: Manufacturing costs breakdown as a function of direct fixed capital (DFC) and operating labour (OL)

Manufacturing costs (Fixed and direct costs)		**Reference**
Depreciation of fixed capital investment	For preliminary cost estimates, the fixed capital investment can be depreciated linearly over a 10- year period ~ 10 % of DFC	Ulrich (1984), Petrides (2015)
Maintenance and repair	4.5 % of DFC (maintenance material: 2.7 % of DFC and maintenance labour: 1.8% of DFC)	Silla (2003), Sinnott (2005)
Insurance cost	1% of DFC	Ulrich (1984), Sinnott (2005), Heinzle *et al.* (2006), Petrides (2015)
Local tax	2 % of DFC	Ulrich (1984), Sinnott (2005), Heinzle *et al.* (2006), Petrides (2015)
Direct supervisory and clerical labour	15 % of OL	Silla (2003), Peters et al. (2004), Sinnott (2005), Petrides (2015)
Laboratory charges	15 % of OL	Silla (2003), Peters et al. (2004), Sinnott (2005), Petrides (2015)
Operating supplies	15 % of maintenance and repair	Ulrich (1984), Peters et al. (2004)
Plant overhead costs	60 % of operating labour, supervision and maintenance labour	Ulrich (1984), Creese *et al.* (1992), Peters *et al.* (2004)

Table 2-21: Average Lang factors used to calculate the direct fixed capital (DFC) investment of a plant (Petrides 2015)

Cost Item	**Most commonly used multiplier**	**Range of multiplier values**
Total plant direct cost (TPDC)		
Equipment purchase cost (PC)	1 x PC	-
Installation	0.50 x PC	0.2 – 1.5
Process piping	0.40 x PC	0.3 – 0.6
Instrumentation	0.30 x PC	0.2 – 0.6
Insulation	0.03 x PC	0.01 – 0.05
Electrical	0.15 x PC	0.1 – 0.05
Buildings	0.45 x PC	0.1 – 0.2
Yard improvement	0.15 x PC	0.05 – 0.2
Auxiliary facilities	0.50 x PC	0.2 – 1.0
Total plant indirect cost (TPIC)		
Engineering	0.25 x TPDC	0.2 – 0.3
Construction	0.35 x TPDC	0.3 – 0.4
Total plant cost (TPC)	TPDC + TPIC	
Contractor's fee	0.05 x TPC	0.03 – 0.08
Contingency	0.10 x TPC	0.07 – 0.15
Direct Fixed Capital (DFC) = TPC + Contractor's fee and contingency		

2.6 SYNTHESIS OF LITERATURE REVIEW

Bio-degradable and bio-based PHAs have been portrayed as versatile bio-plastics owing to their broad range of properties facilitating many applications. They may be tailored through the microorganisms, the carbon substrates and process conditions. A major hurdle preventing wide spread commercialisation of PHAs is their high costs of production. For an emerging technology like PHAs, early stage economic analysis can inform optimisation of PHAs by identifying key process components for improvement.

There have been a number of studies dedicated to the economic assessment of PHAs. Before doing the latter, the large scale production of PHAs must be simulated to yield material and energy inventories. A review of most software packages used for the simulation of PHAs showed that expensive and specialist packages were mostly used, while for early stage cost estimation there is no need for such intensive simulation packages. For early process simulation based on minimum input data, a generic tool like the CeBER Bioprocess Modeller can be used to simulate the production of a commodity product like PHAs. The material and energy inventories can then be used to compute early-stage production estimation costs.

A major factor affecting the production costs of PHAs is the ability of the microorganism to produce PHAs characterised by their intracellular PHA content, cell concentration and cultivation time. Another factor is the high costs associated with the purification and recovery of PHAs during downstream processing. The efficiency of the cell disruption influences the amount of PHB that can be produced and the centrifugation steps are considered energy intensive steps. The cost of the carbon substrate is a major contributor to the PHA production costs; hence many experimental studies have focussed on using cheaper sources of carbon such as waste carbon streams high in organic content. Typically the microorganism cannot metabolise these low cost carbon sources as efficiently as tailored media leading to low PHA production, hence a trade-off is needed between the cost of carbon substrates and resulting PHA productivity. Recent environmental assessments show that PHA production is beneficial over the production of petroleum plastics in terms of greenhouse gases and abiotic depletion. However electricity usage and water usage still remain major contributors to environmental burden. A systematic inquiry of potential factors influencing the economics and sustainability of large scale production of PHAs can drive process optimisation by providing a starting platform for directing both academic research and attracting early investors.

2.7 THESIS OBJECTIVES

The main objective of this study is to use early stage process costing as a tool to inform the reduction of production costs of PHAs. To obtain cost related process information, the use of a generic non-specialist simulation tool, the CeBER Bioprocess Modeller was used to simulate the large scale production of PHAs to provide material and energy inventories. A base case scenario was established and corresponding process economics calculations were used to interrogate the production costs of PHAs as well as the process energy, water requirement and carbon dioxide emissions.

Based on the information collected in the literature review, four process factors were investigated: the energy efficiency of the centrifuges and the efficiency of the cell disruption in the downstream processing; the type of PHA purification and recovery processes; the PHA productivity achieved by the microorganism, dependent on the PHA content, cell concentration and cultivation time; the trade-off between using a low cost carbon substrate and its influence on overall PHA production. Through these considerations and comparison to the base case scenario, this study provides insight into specific factors or combination of factors important for the minimisation of the cost of production of PHAs together with lowering the energy, water usage and emissions of the overall process. A systematic approach is used to conduct this study with the following key questions being addressed:

i. How can material and energy inventories from early stage process simulation be used to compute the production costs of PHAs for the base case scenario? What are the major cost contributions?
ii. What is the effect of the postulated factors on the material and energy inventories and equipment requirements for PHA production?
iii. What is the relative importance of the postulated factors with respect to the overall optimisation of the PHA production system? Are there interactions between these factors?
iv. What are the subsequent effects on PHA production costs, energy usage, water usage and carbon dioxide emissions?
v. Which of the postulated factors or combination of factors result in lower production costs and what is the corresponding optimisation potential of the investigated PHA production system with respect to the base case scenario?

3 CONCEPTUAL APPROACH TO PHA PRODUCTION FLOWSHEET

3.1 INTRODUCTION

Chapter 3 explains the methodology behind the construction of the base case scenario and the four scenarios dedicated to the investigation of the process factors found to affect PHA production. As mentioned in Section 2.5, as part of his study Harding (2008) simulated the production of PHAs and his work was used as a basis to construct the base case scenario for this study. The Harding (2008) model is introduced in Section 3.2.1. Section 3.2.2 deals with modifications that were made to simulate the large scale production of PHAs. Large scale equipment considerations were used to guide the preliminary sizing of equipment and are addressed in Section 3.2.3. The method used to calculate the production cost of PHAs is explained in Section 3.3. The methodology used to build different process flow sheets for the investigation of the four process factors is explained in Section 3.4.

3.2 CONSTRUCTING THE BASE CASE SCENARIO FOR THE LARGE SCALE PRODUCTION OF PHAS USING THE CEBER BIOPROCESS MODELLER

3.2.1 The Harding (2008) model for the production of the PHA monomer, PHB

The PHA process flowsheet used as a basis for this study was established from the ICI Bioproducts process with the laboratory data of Harrison (1990). The initial model was compiled by Harding (2008) and introduced in Section 2.5. The process flow diagram is shown in Figure 3-1. The type of PHAs produced was PHB. Raw materials, the microorganism, *Cupriavidus necator* and antifoam were fed to an aerated reactor where the microorganism was allowed to grow to a designated biomass concentration under balanced nutrient supply. Onset of nitrogen or phosphorus limitation resulted in PHB accumulation under fed-batch conditions. This was followed by cell rupture in a high pressure homogeniser and the cell debris was removed by centrifugation. The purification and recovery process was enzymatic digestion where the ruptured cells were re-suspended in an alkaline serine protease, Optimase L660, with the pH being controlled by potassium hydroxide. Once the non-polymeric cell matter was digested, the PHB was treated with a non-ionic detergent Synperonic NP8, in a stirred tank reactor to remove lipid contents. The PHB was further purified using repeated cycles of water washing and centrifugation. After being treated with hydrogen peroxide and a final water wash and centrifuge cycle, the PHB was spray dried.

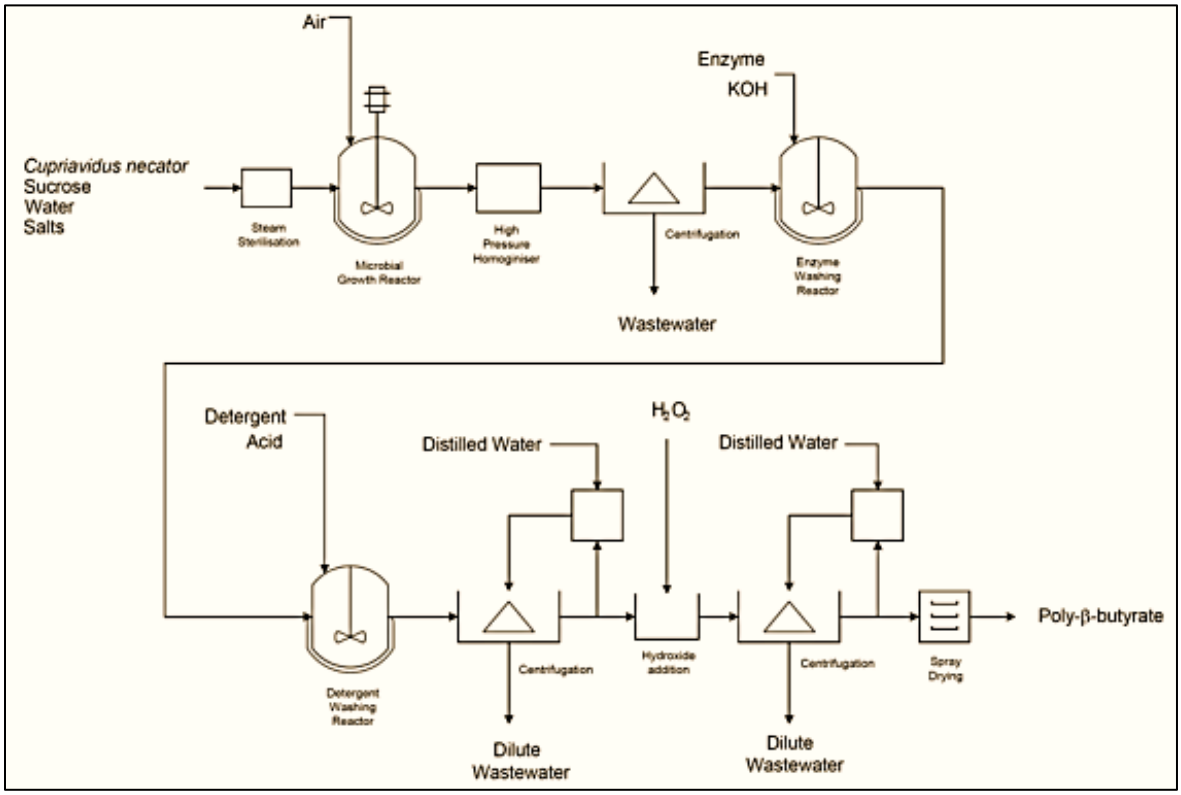


Figure 3-1: Process flow diagram for the production of 1000 kg of PHB per run using an enzymatic digestion purification recovery process developed by Harding (2008) simulated in the CeBER Bioprocess Modeller

Using a limited set of inputs to illustrate the suitability of the model for early stage design, three scenarios (Scenarios 1 to 3), shown in Table 3-1, were investigated by Harding (2008). The material and energy inventories were compared to the scaled-up PHB production system using Harrison's (1990) data. Harding (2008) identified three main differences between his model outputs and the Harrison (1990) inventories. Firstly, PHB production was triggered by nitrogen limitation in the Harrison (1990) model but physical limitation cannot be incorporated in the generic flowsheet. Thus the stoichiometric amount of nitrogen was calculated based on the final biomass concentration leading to an overestimation of the actual nitrogen required. Secondly, the difference in overall mass inventories was attributed to the omission of trace elements in the Harding (2008) model while they were present in Harrison (1990) inventories. Thirdly, Harrison (1990) did not report the electrical energy required for steam compression which led to a lower electrical energy requirement compared to Harding (2008) model. Harding (2008) further investigated the differences in the Life Cycle Assessment (LCA) of PHB

production using material and energy inventories from both his model and Harrison (1990) data, hence establishing the suitability of his model for use as an early PHB production simulation model.

3.2.2 Adaptation of the Harding (2008) PHB model for this study

The main objective of this study was to scale up the Harding (2008) model for large scale production, use the material and energy inventories to estimate equipment sizes and thereafter compute the production costs of PHB. Sensitivity analysis can be conducted to identify the key variables or "hot spots" in the production system by using this base case scenario. These are then used to identify the most beneficial interventions in lowering the cost of PHB production and minimising energy, water usage and carbon dioxide emissions.

Choi *et al.* (1997) did a comprehensive study on the economics of the large scale production of PHB (20 tonnes of PHB per run) and followed on with further studies as shown in Table 2-12. Due to limited information on large scale production of PHAs with respect to process description and equipment costs, it was decided to use Choi *et al.* (1997) equipment sequencing to establish the base case along with the PHB model proposed by Harding (2008). PHB production is usually a fed-batch process whereby the PHB is only collected at the end of the cultivation. For the different microoganims investigated by Choi *et al.* (1997), PHB cultivation along with bioreactor downtime took between 51 and 82 hours. In order to maximise the number of runs per year and prevent bottle necks in the process, Choi & Lee (1997) decoupled the upstream and downstream processing by collecting the microbial culture in a storage tank after cultivation, and proceeded to size the downstream processing equipment in the same 51 to 82 hours time frame. Thus, on completion of one batch of downstream processing, another batch of microbial culture was ready to be processed. The centrifuges and washing cycles were performed continuously. The same approach was used for this study. The Harding (2008) PHB model was scaled up from 1000 kg to produce 20 tonnes of crude PHB per run. Most of the user inputs were kept the same as the original PHB model with only a few changes shown in Table 3-1:

Table 3-1: Input data for the large scale production of PHB based on the Harding (2008) PHB model under nitrogen limitation

	Scenario PHB1	Scenario PHB2	Scenario PHB3	This study
Steam sterilisation: Include additional steam requirements (steam out vessel, backing steam, space heating)				
Reactor temperature (maintain reactor temperature) (°C)	30	[37]	[37]	30
Preheat feed with sterilized media; Preheat temperature (°C)	65	[60]	[60]	65
Heat transfer efficiency	100	[80]	[80]	[80]
Microbial growth conditions (batch production of polybutyrate ($CH_{0.13}O_{0.67}$) from *Cupriavidus necator*				
Product: Biomass ratio	2.4	2.4	2.4	2.4
Carbon source (excess): Glucose (%)	0	0	[1]	0
Nitrogen source (excess): Ammonium sulphate (%)	0	0	[5]	0
Oxygen source (vvm): Air	0.6	0.6	0.6	0.6
Compression:	Single stage reciprocating compressor			Two stage compression with intercooling
Final biomass concentration (g/L)	44[#]	44[#]	44[#]	44[#]
Yield coefficients: $Y_{p/s}$ (g/g)	0.44	0.44	0.44	0.44
Antifoam addition	0.1	0.1	[1]	0.1
Agitation				
Number of tanks	1	[5]	[5]	1
Power per unit volume (Energy: Electricity) (kW/m^3)	0.5	0.5	[0.95]	0.5
Efficiency	0.8	[0.9]	[0.9]	0.8
Cell disruption: High pressure homogenisation				
Energy efficiency (Energy: Electricity) (mg/J)	0.8	[1.25]	[1.25]	0.8
Cell disruption efficiency (%)	100	[90.25]	[90.25]	[90.25]
Centrifugation				
Number of wash/spin cycles	1	1	1	1
Energy required (Energy: Electricity) (kJ/m^3)	7600	7600	7600	7600
Efficiency	1	[0.6]	[0.6]	[0.6]
Solid fraction removed	100	100	[98]	[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	Such that vol. equals 1/3 original vol
Enzyme addition: Optimase L660 (MKC) – protease				
Protease volume added (%v/v)	0.8	0.8	0.8	0.8
Potassium hydroxide added ** (%v/v)	10	10	10	10
Agitation energy (Energy: Electricity) (MJ/m^3)	5.76	5.76	5.76	5.76

Detergent addition: Synperonic NP8 (ICI Ltd)				
Detergent volume added (%v/v)	10	10	10	10
Agitation energy (Energy: Electricity) (MJ/m^3)	5.76	5.76	5.76	5.76
Centrifugation				
Number of wash/spin cycles (re-suspension with H_2O)	5^	5^	5^	5^
Energy required (Energy: Electricity) (kJ/m^3)	7600	7600	7600	7600
Efficiency	1	[0.6]	[0.6]	[0.6]
Solid fraction retained (%)	100	100	[98]	[98]
Liquid fraction retained ^	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	Such that after 5 wash cycles vol. remaining equals vol. entered [0.76]
Hydrogen peroxide addition				
H_2O_2 volume added (%v/v)	1.2	1.2	1.2	1.2
Centrifugation				
Number of wash/spin cycles (re-suspension with H_2O)	2	2	2	2
Energy required (Energy: Electricity) (kJ/m^3)	7600	7600	7600	7600
Solid fraction retained (%)	100	100	[98]	[98]
Liquid fraction retained^	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	Such that after 2 wash cycles vol. remaining equals vol. entered [0.65]
Spray drying (Energy: Natural gas)				(Energy: Electrical)
Solid fraction retained (%)	100	[99]	[99]	[99]
Liquid fraction retained (%)	100	100	[99]	[99]
Electricity (MJ/m^3)	-	-	-	3250

*Values available from literature and not specific to this flowsheet design
** The amount of potassium hydroxide 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 Excel model but used to calculate other information for the data input here
[] Default values calculated or assumed in the MS-Excel model when no explicit inputs are given

In the model, the PHB concentration was expressed as a function of the final non-PHB biomass concentration and was calculated from the difference of the non-PHB biomass and final cell concentration. Similarly, the PHB content was expressed as the ratio of the PHB concentration to the non-PHB biomass concentration. The liquid fractions with the symbol ^ in Table 3-1 were calculated using MS Excel to meet the desired specifications. The input to the number of wash cycles in the

centrifuges was done in terms of addition of excess water to the process; that is for 2 wash cycles, 200 % (v/v) of water was added to the specific unit operation.

The enzyme (Optimase L660) and detergent (Synperonic NP8) described in Harding (2008) and Harrison (1990) could not be sourced at present time since these products may have been discontinued or had their names changed over the years. As such, alternative chemicals that perform the same action and have similar properties were sourced:

- Non-ionic detergent Propylene Glycol ,99.5%min ($1300 – 1660/ton)[2]
- TTX Keratinase as a high quality alkaline protease ($30 – 35/kg)[3]

Harding (2008) modelled the spray dryer to use natural gas only. Typically for large scale spray dryers some of the energy needed to evaporate the water is also supplied as electrical energy (Baker & McKenzie 2007). Thus, for this study, an overall value of 3250 MJ/m^3 was used as the total energy required for evaporating water using a spray dryer (Baker & McKenzie 2007).

3.2.3 Process considerations to address for large scale production

A typical process plant can run for a maximum of 350 days comprising of 8400 hours/year (Nguyen & Demirel 2013; Pascall & Adams 2013; Okorafor & Davis 2015). One run produces 20 tonnes of crude PHB and the amount of PHB that can be produced in one year can be calculated from the total number of runs possible in 8400 hours, which was dependent on the operating time for one run. In terms of equipment sizing, some process design considerations for scale up was introduced in Section 2.5.1 and in order to get an appreciation of large scale processes, common large scale equipment along with their proposed preliminary design used in bioprocessing plants were looked into.

Industrial scale continuous sterilisers can handle a throughput of up to 100 m^3/hr and scale up is straightforward and linear (Junker *et al.* 2006). Compressors can usually be sized from their horsepower in the range of 10 to 30 000 Hp (Seider *et al.* 2009). In industry, submerged or liquid fermentation (for e.g large scale production of citric acid) typically takes place in either 150 – 200 m^3 stirred tank reactors or in 300 – 500 m^3 (up to 1000 m^3) bubble column reactors (Batt & Tortorello 2014). The size of the stirred tank reactor is constrained by the energy input through the impeller shaft, required for gas-liquid mass transfer. While larger stirred tanks reactors have been reported, for the highly aerobic culture in this study at high cell concentration, the gas-liquid mass transfer demand is high. Thus, the maximum

[2] http://www.alibaba.com/product-detail/Non-ionic-detergents-Used-Propylene-Glycol_1672118417.html
[3] http://www.alibaba.com/product-detail/TTX-Keratinase-as-a-high-quality_1875798290.html

stirred reactor size was limited to 200 m^3 and if the cultivation volume was larger, multiple reactors of 200 m^3 were used. Large bioreactors usually have cleaning-in-place (CIP) system which consists of using a mixture of chemicals, heat and water to clean bioreactors and pipes without having to dismantle the plant (Process Worldwide 2011). A CIP time of 3.5 hours was considered a reasonable time to allocate to 200 m^3 bioreactors (Alfa Laval 2015).

Centrifuges are available as either batch or continuous and can process microbial cultures at various different flow rates from a few litres per hours up to 200 m^3/hr (Asenjo 1990). Centrifuge scale up is usually done via the sigma factor, Σ which is the equivalent settling area of a centrifuge and has units of m^2 (Shukla *et al.* 2007). Centrifuge selection can be done using the following diagram of settling velocity under gravity (Stokes' Terminal Settling Velocity – TSV) and the volumetric throughput shown in Figure 3-2.

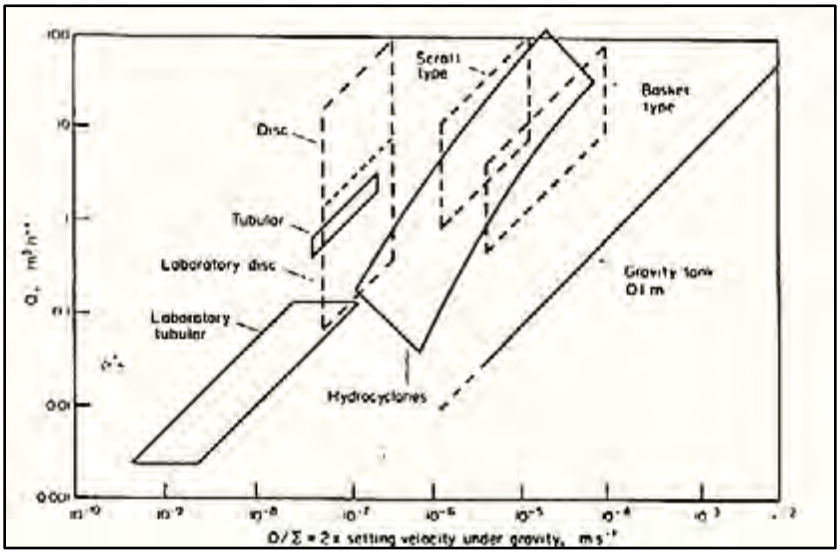


Figure 3-2: Settling velocity under gravity (Stokes' Terminal Settling Velocity-TSV) and the volumetric throughput (Dunn 1998)

Sigma, Σ can be used for preliminary centrifuge sizing. It can be defined as shown in Equation 3-1:

$$Q = 2u_0\Sigma \qquad 3\text{-}1$$

where Q: volumetric throughput (m^3/s) and u_0 is the settling velocity under gravity (ms^{-1}).

For small particle sizes like bacterial cells and cell debris (0.1 to 2 µm), continuous disk stack centrifuges are usually used in large scale bioprocessing plants (Cannell 1998; Am Ende 2011). According to Figure 3-2, the average settling velocity for a disk-stack centrifuge is 10^{-7} ms^{-1}, and this can be used along with

the volumetric flow rate through the centrifuge to find the sigma factor using Equation 3-1. The sigma factor can then be used as a scaling variable to estimate the cost of the centrifuge based on previous costs data of centrifuges.

High pressure homogenisation for cell disruption can be scaled up based on flow rates (Asenjo 1990). A number of suppliers offer industrial high pressure homogenisers which can process up to 60 m^3/hr (SPX Corporation 2013; Direct Industry 2015). Spray dryers are usually custom built according to the need of the manufacturer. Capacities for industrial drying plants in the dairy industry can range from 500 to over 10 000 kg/h of water evaporated (Mujumdar 1995). Some commercial suppliers offer spray dryers with up to 2000 - 3000 kg/h of evaporative capacity for use for polymer drying (YIBU Drying Leading 2008; Changzhou 2015). For the sake of this early cost estimation, in-depth equipment design was not done. The cost was estimated from the evaporative load of the spray dryer, which is shown using Equation 3-4.

3.3 PROCESS COSTING FOR THE LARGE SCALE PRODUCTION OF PHB

While the labour and raw materials costs were calculated for the year 2014, the cost calculations for the equipment, utilities and waste treatment were all done using data from previous years. In order to report this cost accurately for the year 2014, Equation 3-2 from Turton *et al.* (2009) was used to take changing economic condition (inflation) into account using the annual Chemical Engineering Plant Cost Index (CEPCI) data in Table 3-2.

$$Cost_{present} = Cost_{past} \times \left(\frac{Index_{present}}{Index_{past}}\right) \qquad 3\text{-}2$$

Table 3-2: Chemical Engineering Plant Cost Index (CEPCI) compiled from Business News (2014) and Chemical Engineering Essentials for the CPI Professional (2015)

Year	CEPCI	Year	CEPCI
2014	576.1	2002	395.6
2010	585.7	2001	394.3
2009	550.8	2000	394.1
2008	521.9	1999	390.6
2007	575.4	1998	389.5
2006	499.6	1997	386.5
2005	402.0	1996	381.7
2004	395.6	1995	381.1
2003	394.3	1985	325.0

3.3.1 Approach to equipment costing

The six-tenths rule, along with specific size exponential values for N, introduced in Section 2.5.1 were used to compute the cost of equipment. Equipment costs for this study was calculated by adjusting the

scaling variable and cost index based on equipment costs in Table 3-3 reported by Choi & Lee (1997) in 1996.

Table 3-3: Equipment costs reported by Choi & Lee (1997)

Equipment	Size	Cost 1996 ($)
Compressor	478.95 (kW)	350000
Blending Tank	52.5 (m^3)	55000
Air filter	0.7 (m^3/s)	8000
Bioreactor	185.37 (m^3)	974000
Disk-stack centrifuge	27611.37 (m^2)	108000
Flat bottom tank	176.98 (m3)	74000
Spray dryer	D = 0.80 m; H = 2.39 m	74000
Heat steriliser	D = 0.10 m; H = 7.42 m	319000

For the calculation of the volumetric flow rate through the air filters (m^3/s), Equation 3-3 from Nienow (2012) was used, where the volume of air per unit of medium per unit of time (vvm) in the model was 0.6:

$$v_s = (vvm/60)(volume\ of\ broth, m^3) \quad 3\text{-}3$$

Choi & Lee (1997) did not make use of a high pressure homogeniser in their process, therefore other studies were consulted for cost information. Van Wegen (1998) reported the cost of a high pressure homogeniser processing 65 m^3/hr of throughput to be $ 430,000 in 1995. This was used as a basis to cost the homogeniser. Similarly, the costs of the continuous steriliser and spray dryer in Choi & Lee (1997) were based on their diameter and height. Since the output from the CeBER Bioprocess Modeller can only estimates the volume of these units, alternative scaling variables were considered. For a continuous steriliser with insulated 316 stainless steel pipes, including heat exchangers, processing a flow rate of 40.4 m^3/hr, the price of $ 163,000 was reported in 2007 (Arifeen *et al.* 2007). For the spray dryer, typically the area of dryer chamber or the dimensions of the chamber are used to calculate the cost but this includes an in-depth design of the spray dryer. Thus, for the preliminary costing of the spray dryer, the cost was calculated from the evaporative load, E (kg/h) using Equation 3-4. The cost includes a stainless steel dryer body, access platform, support steel, burner, air-heater shell, feeding shell and instrumentation, and applies for evaporative loads in the range of 181 – 38,780 kg/h (Mujumdar, 1995).

$$Cost\ (\$\,1995) = 13793.8 \times E^{0.435} \quad 3\text{-}4$$

In order to validate the costing calculations performed using the six-tenth rule, the cost of current equipment prices with similar sizes were computed using an online cost estimator tool developed by Peters *et al.* (2015). However, large scale bioreactors and homogeniser costs could not be computed in this cost estimator tool. Thus, for comparison, bioreactors and homogeniser costs were extrapolated from a recent techno-economic assessment performed for the microbial production of oil (Koutinas *et al.* 2014). The comparison between the calculated equipment costs and recent equipment prices are shown in Section 4.2.3.

3.3.2 Raw materials costs

Accurate commercial prices for raw materials are usually available for business subscribers only and not for university purposes (Heriot - Watt Information Services Guides 2015). Since there is no specific way of finding accurate prices for bulk raw materials for early stage feasibility studies, the raw materials prices, shown in Table 3-4, were found from suppliers' websites.

Table 3-4: Raw materials costs for the large scale production of PHB using the CeBER Bioprocess Modeller

Raw material	Price (2014)
Glucose	0.36 $/kg[1]
Ammonium sulphate	0.12 $/kg[2]
Enzyme (TTX Keratinase)	32.5 $/kg[3]
Potassium hydroxide	0.50 $/kg[4]
Detergent (Non-ionic detergent Propylene Glycol)	1.48 $/kg[5]
Hydrogen peroxide	0.52 $/kg[6]
Antifoam	2.5 $/kg[7]
Surfactant (Triton X -100)	1.5$/kg[8]
Sodium hypochlorite	0.4 $/kg[9]
Sodium hydroxide	0.44 $/kg[10]

[1] http://www.alibaba.com/product-detail/glucose_529979205.html?s=p
[2] http://www.alibaba.com/product-detail/ammonium-sulfate-industrial-grade-_1847599317.html?s=p
[3] http://www.alibaba.com/products/F0/serine_alkaline_protease.html?qrwKey=serine_al
[4] http://www.alibaba.com/product-detail/Factory-price-for-Potassium-hydroxide_1242327329.html?s=p
[5] http://www.alibaba.com/trade/search?fsb=y&IndexArea=product_en&CatId=&SearchText=non-ionic+detergent
[6] http://www.alibaba.com/product-detail/Factory-directly-2013-best-price-industrial_1498879667.html
[7] http://www.alibaba.com/product-detail/This-298-Easy-Usage-Antifoam-For_1891009721.html
[10] from Yup Lee (1997)
[9] http://www.alibaba.com/showroom/sodium-hypochlorite-price.html
[10] http://www.alibaba.com/product-detail/market-price-for-industrial-sodium-hydroxide_1694108604.html?s=p

3.3.3 Utilities costs

The costs of the utilities were calculated using costs data from Coulson and Richardson (2005) reported for the year 2004, shown in Table 3-5. Only the cost of the process water was reported. The cooling water needed after sterilisation was assumed to be recycled in the plant since typical large scale

facilities have their own cooling tower to control the flow of recycled water throughout the plant (Turton *et al.* 2012).

Table 3-5: Cost of utilities used for the large scale production of PHB using the CeBER Bioprocess Modeller from Coulson and Richardson (2005)

Electricity	0.015	$/MJ
Steam	12	$/t
Cooling water	0.01	$/t
Process water	0.5	$/t

3.3.4 Labour costs

The number of operators required per shift, N_{OL} can be estimated by Equation 3-5:

$$N_{OL} = \left(6.29 + 31.7P^2 + 0.23N_{np}\right)^{0.5} \quad 3\text{-}5$$

where P: number of processing steps involving particulate solids, for example transportation and distribution, particulate size control and particulate solids; N_{np}: number of other non-particulate processing steps and includes compression, heating and cooling, mixing and reaction

Each of the N_{OL} operators was assumed to work 8-hour shift with a total of 40 hours per week (Seider *et al.* 2009; National Career Service 2012). For a plant running 24 hours a day, for every operator, 4.5 operators must be hired to account for 3 shifts per day and 3 weeks of leave that are generally allocated to each operator per year, thus working for a total of 49 weeks per year. Using an hourly wage of $ 26/hour from the Bureau of Labour Statistics (2014), the operational labour of the plant can be found based on the total number of operators each working for a total of 2080 hours per year.

3.3.5 Waste treatment costs

The water effluent of the PHB production process had a high carbon content which was reported as the chemical oxygen demand (COD). COD can be used as a measure of the amount of organic pollutants in wastewater (MANTECH 2015). For the purpose of waste treatment cost, a cost of $ 0.33/kg organic compound was used (Seider *et al.* 2009).

3.3.6 Computing the production costs of PHB

Once all the costs were computed, the direct fixed capital (DFC) and annual operating costs of the PHB plant could be calculated as outlined in Section 2.5.2. It is important to recall that the simulation for the base case model was done for 20 tonnes of crude PHB per run and depending on the time taken for one

run, the total number of runs possible in 8400 hours can be calculated. Additionally, the total amount of PHB produced annually could be calculated. Table 3-6 presents the average Lang factors specific for bioprocess plants from Petrides (2015) that were used to calculate the DFC of the plant. From there, the information in Table 2-20 was used to calculate the annual direct production costs.

Table 3-6: Calculation of annual operating costs of a PHB production plant based on its direct fixed capital (DFC) and Lang factors adapted from Choi & Lee (1997)

Fixed Capital Estimate	
A. Total Plant Direct Cost (TPDC) (physical cost)	
a) Equipment Purchase Cost (PC)	(1.00 x PC)
b) Installation	(0.50 x PC)
c) Process Piping	(0.35 x PC)
d) Instrumentation	(0.40 x PC)
e) Insulation	(0.03 x PC)
f) Electrical	(0.10 x PC)
g) Buildings	(0.45 x PC)
h) Yard Improvement	(0.15 x PC)
i) Auxiliary Facilities	(0.40 x PC)
B. Total Plant Indirect Cost (TPIC)	
a) Engineering	(0.25 x TPDC)
b) Construction	(0.35 x TPDC)
C. Other Costs (OTC)	
a) Contractor's fee	(0.05 x (TPDC + TPIC)
b) Contingency	(0.10 x (TPDC + TPIC)
D. Direct Fixed Capital (DFC) = TPDC + TPIC + OTC	
Direct Annual Production Cost	
A. DFC-Dependent items	
a) Depreciation	(0.10 x DFC)
b) Maintenance material	(0.03 x DFC)
c) Insurance	(0.01 x DFC)
d) Local taxes	(0.02 x DFC)
B. Labour-dependent items	
a) Operating labour	Dependent on total number of operators, hourly wage and total number of working hours
b) Maintenance labour	(0.02 x DFC)
c) Supervision	(0.20 x B(a))
d) Operating supplies	(0.15 x (A(b) + B(b))*
e) Laboratory	(0.15 x a)
C. Administration and overhead expense	(0.6 x (B(a) +B(b) + B(c))
D. Raw materials	Prices computed based on mass inventory
E. Other consumables	Include expensive consumables such as membrane or filter cloth
F. Utilities	Prices computed based on mass inventory
G. Waste treatment/disposal	Prices computed based on mass inventory
Annual direct production costs of PHB ($/kg) $= \frac{A+B+C+D+E+F+G}{Total\ amount\ of\ PHA\ produced\ annually}$	

* 0.15 x (Maintenance material + maintenance labour)

This calculated cost of production referred to the large scale production cost of PHB using an enzymatic digestion recovery process simulated in the CeBER Bioprocess Modeller. This was considered as the base case scenario for this study. Different scenarios were built to systematically inquire the different factors that can potential affect the PHB production system. The energy usage reported was limited to the electrical energy required by the equipment and took into consideration the equivalent energy needed for the steam sterilisation. A steam enthalpy of 2724.66 KJ/kg was used for the temperature of 140°C attained in the model during sterilisation (The Engineering ToolBox 2015).

3.4 CONSTRUCTION OF DIFFERENT SCENARIOS FOR INVESTIGATING THE PHB PRODUCTION SYSTEM

The construction of different flowsheets to investigate the four process factors is explained in this section. Along with the production cost, the energy usage (electricity consumption for equipment and energy required to produce steam), process water (without cooling water which was assumed to be recycled) and carbon dioxide emissions from the bioreactors due to catabolism of the carbon source were computed for each different scenario as well.

3.4.1 Effect of energy efficiency of centrifuges and the homogeniser efficiency

In Table 2-10 the centrifugation stages were identified to be energy intensive and the efficiency of cell disruption was recognised to affect the cost of recovery and. To investigate these effects on the PHB production system, the cell disruption efficiency for the homogeniser was varied ± 5 % and the energy efficiency of the centrifuges varied ± 10%. The effect of these changes, on the cost of production of PHB, water and energy usage and carbon dioxide emissions are discussed in Section 4.3.

3.4.2 Effect of PHB purification and recovery process for downstream processing

To investigate the effect of the purification and recovery process, the enzymatic digestion downstream processing was replaced by two different processes with one using sodium hypochlorite-surfactant and the other using sodium hydroxide only. These two processes were chosen because they did not require any chlorinated solvents and achieved high purity (> 90%) and recovery (> 90%) of PHB. In order to compare the effects of downstream processing, the upstream process was kept the same as for the enzymatic digestion process used in the base case scenario.

The first investigated process was based on the study conducted by Choi & Lee (1997). At the start of downstream processing, once the cells were harvested by centrifugation, a surfactant solution was added to the biomass and mixed for one hour as a pre-treatment step; this was followed by a two hour sodium hypochlorite treatment. After cell lysis, centrifugation was used to remove the non-cellular

polymeric matter, rinsing with water and the product was finally spray-dried. A process flow sheet incorporating the recovery and purification process of PHB shown in Figure 3-3 was built and is shown in Section 4.4.1.

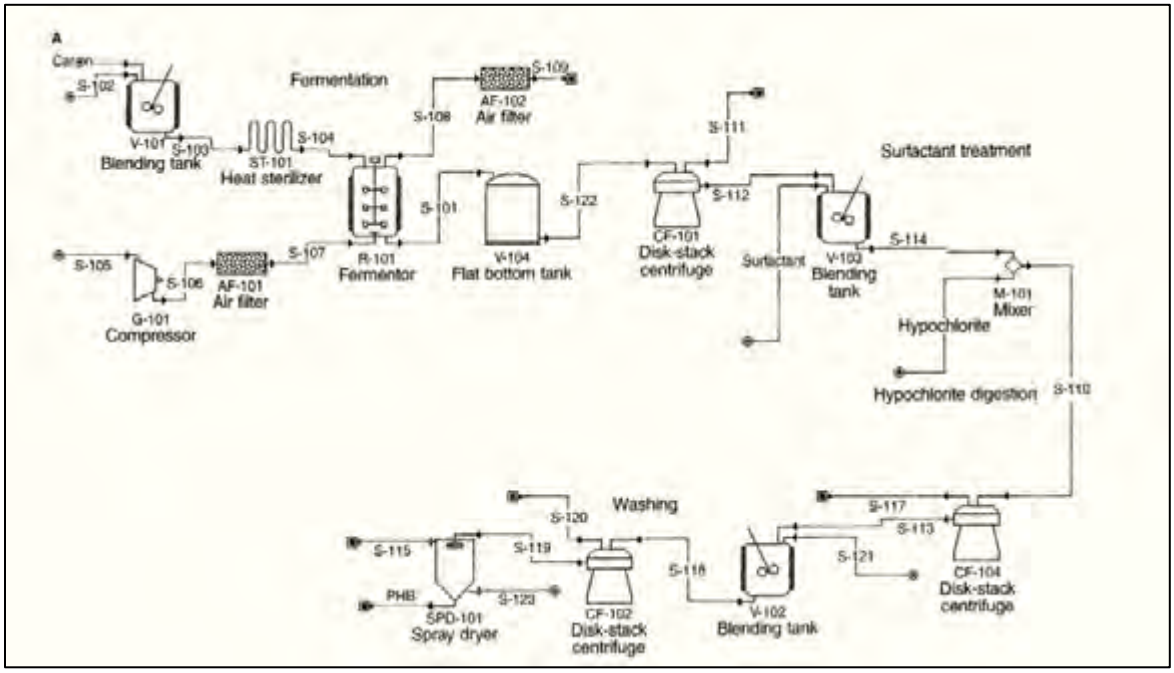


Figure 3-3: Process flowsheet for PHB production using a surfactant-sodium hypochlorite purification and recovery method (Choi & Lee 1997)

In Choi & Lee (1997), they used 1 % w/v of surfactant but the CeBER Bioprocess Modeller requires w/w input. Since the solution was so dilute, a value of 1 % v/v of surfactant was assumed in the model. There was no mention of the amount of sodium hypochlorite needed for the cell lysis, however Heinrich *et al.* (2012) stated that a sodium hypochlorite concentration of 13 % (v/v) was sufficient to effectively digests the non-PHB biomass with minimum heat generation and this amount was used in the model. The purity and recovery of PHB achieved for this process reported by Choi *et al.* (1997) was 99 % and 95 % respectively. In order to achieve the same purity and recovery in the model, the solid and liquid fraction in the downstream processing steps (centrifuge and spray dryer) were adjusted as well as the fraction of product retained in the mixing tanks. Table 3-7 shows the input data for the sodium hypochlorite-surfactant recovery process modelled in the CeBER Bioprocess Modeller.

Table 3-7: List of input data for the CeBER Bioprocess Modeller for the sodium hypochlorite-surfactant purification and recovery process

	Surfactant-sodium hypochlorite recovery process
Steam sterilisation: Include additional steam requirements (steam out vessel, backing steam, space heating)	
Reactor temperature (maintain reactor temperature) (°C)	30
Preheat feed with sterilized media; Preheat temperature (°C)	65
Heat transfer efficiency	[80]
Microbial growth conditions (batch production of polybutyrate ($CH_{0.13}O_{0.67}$) from *Cupriavidus necator*	
Product: Biomass ratio	2.4
Carbon source (excess): Glucose (%)	0
Nitrogen source (excess): Ammonium sulphate (%)	0
Oxygen source (vvm): Air	0.6
Compression: Single stage reciprocating compressor	Two stage compression with intercooling
Final biomass concentration (g/L)	44[#]
Yield coefficients: $Y_{p/s}$ (g/g)	0.44
Antifoam addition	0.1
Agitation	
Number of tanks	1
Power per unit volume (Energy: Electricity) (kW/m^3)	0.5
Efficiency	0.8
Cell disruption: High pressure homogenisation	
Energy efficiency (Energy: Electricity) (mg/J)	0.8
Cell disruption efficiency (%)	[90.25]
Centrifugation	
Number of wash/spin cycles	1
Energy required (Energy: Electricity) (kJ/m^3)	7600
Efficiency	[0.6]
Residence time (hrs)	4h25
Solid fraction removed	[98]
Liquid fraction removed	Such that vol. equals 1/3 original vol
Surfactant addition: Triton X-100	
Triton X-100 volume added (%v/v)	1
Electricity (MJ/m^3)	5.76
Residence time (hrs)	1
Sodium hypochlorite addition:	
Volume added (%v/v)	13
Electricity (MJ/m^3)	5.76
Residence time (hrs)	2
Centrifugation	

Number of wash/spin cycles (re-suspension with H_2O)	1
Energy required (Energy: Electricity) (kJ/m^3)	6420
Efficiency	[0.6]
Residence time (hrs)	1h45 min
Solid fraction retained (%)	0.99
Liquid fraction retained (%)	0.9
Centrifugation	
Number of wash/spin cycles (re-suspension with H_2O)	2
Energy required (Energy: Electricity) (kJ/m^3)	6420
Efficiency	[0.6]
Residence time (hrs)	0.75 hrs x 2
Solid fraction retained (%)	0.99
Liquid fraction retained	0.46
Spray drying (Energy: Electricity)	
Solid fraction retained (%)	[0.99]
Liquid fraction retained (%)	[0.99]
Electricity (MJ/m^3)	3250

The second investigated process was a sodium hydroxide digestion method (using 0.19 % w/v sodium hydroxide) reported by Choi & Lee (1998). It was similar to the sodium hypochlorite-surfactant process with one less processing step. The corresponding purity and recovery of PHB achieved were 91 % and 90 %. Table 3-8 shows the input data for the sodium hydroxide process modelled in the CeBER Bioprocess Modeller. Similar to the sodium hypochlorite-surfactant process, the inputs to the downstream processing were modified to achieve these final values. A process flow sheet incorporating the sodium hydroxide recovery process is shown in Section 4.4.2. The effect of these two different recovery processes on the final cost of production of PHB, water and energy usage and carbon dioxide emissions are discussed in Section 4.4.

Table 3-8: List of input data for the CeBER Bioprocess Modeller for the sodium hydroxide purification and recovery process

Assumptions	**Sodium hydroxide recovery process**
Steam sterilisation: Include additional steam requirements (steam out vessel, backing steam, space heating)	
Reactor temperature (maintain reactor temperature) (°C)	30
Preheat feed with sterilized media; Preheat temperature (°C)	65
Heat transfer efficiency	[80]
Microbial growth conditions (batch production of polybutyrate ($CH_{0.13}O_{0.67}$) from *Cupriavidus necator*	
Product: Biomass ratio	2.4
Carbon source (excess): Glucose (%)	0

Nitrogen source (excess): Ammonium sulphate (%)	0
Oxygen source (vvm): Air	0.6
Compression: Single stage reciprocating compressor	Two stage compression with intercooling
Final biomass concentration (g/L)	44[#]
Yield coefficients: $Y_{p/s}$ (g/g)	0.44
Antifoam addition	0.1
Agitation	
Number of tanks	1
Power per unit volume (Energy: Electricity) (kW/m^3)	0.5
Efficiency	0.8
Cell disruption: High pressure homogenisation	
Energy efficiency (Energy: Electricity) (mg/J)	0.8
Cell disruption efficiency (%)	[90.25]
Centrifugation	
Number of wash/spin cycles	1
Energy required (Energy: Electricity) (kJ/m^3)	7600
Efficiency	[0.6]
Residence time (hrs)	4
Solid fraction removed	[98]
Liquid fraction removed	Such that vol. equals 1/3 original vol
Sodium hydroxide addition:	
Sodium hydroxide volume added (%v/v)	0.38
Electricity (MJ/m^3)	5.76
Residence time (hrs)	1
Centrifugation	
Number of wash/spin cycles (re-suspension with H_2O)	1
Energy required (Energy: Electricity) (kJ/m^3)	6420
Efficiency	[0.6]
Residence time (hrs)	4h25
Solid fraction retained (%)	[0.98]
Liquid fraction retained (%)	0.8
Centrifugation	
Number of wash/spin cycles (re-suspension with H_2O)	2
Energy required (Energy: Electricity) (kJ/m^3)	6420
Efficiency	[0.6]
Residence time (hrs)	2
Solid fraction retained (%)	0.97
Liquid fraction retained	0.32
Spray drying (Energy: Electricity)	
Solid fraction retained (%)	0.97
Liquid fraction retained (%)	[0.99]
Electricity (MJ/m^3)	3250

3.4.3 Effect of microbial properties

There are three main microbial properties that can be investigated; the cell and associated PHB concentration (g/L), PHB content (% of final cell concentration) and the cultivation time (hours). It is important to recall that in the CeBER Bioprocess Modeller, the PHB concentration is expressed as a function of the final non-PHB biomass concentration, where the PHB concentration is calculated from the difference of the non-PHB biomass and final cell concentration. Similarly, the PHB content is expressed as the ratio of the PHB concentration to the non-PHB biomass concentration.

It was decided to take a statistical approach to investigate these properties in view of finding out which of these microbial properties affected the final cost of production of PHB the most. The effect of these on the energy, water usage and carbon dioxide emissions was also investigated. The statistical approach used was a factorial design investigation, more specifically, a 2^3 factorial design where the three factors were varied between a low and high value of their original values for the base case shown in Table 3-9 with the response being the PHB cost of production in $/kg. The statistical software Design-Expert® 9 was used.

Table 3-9: Design of factorial design investigation of microbial properties on the large scale production of PHB

Natural variables in CeBER Bioprocess Modeller	Coded variables in Design-Expert® 9	Low value input in Design-Expert® 9	High value input in Design-Expert® 9	Base case value
Final non-PHB biomass conc (g/L)	E1	40	50	44.1
PHB biomass ratio	E2	2	3	2.4
Culture time (hrs)	E3	40	50	44.4

The factorial design experiment consists of examining a normal probability plot of the estimates of the effects of the coded variables (CHE5030Z 2013). The effects that are negligible are normally distributed with mean zero and variance σ^2 and will tend to fall along a straight line on this plot, whereas significant effects will have non-zero means and will not lie along the straight line (CHE5030Z 2013). Thus, the model from the software was specified to contain effects that are apparently non-zero, based on the normal probability plot, to identify significant coded variables that affect the production cost of PHB. The apparently negligible effects of the other coded variables were combined as an estimate of error CHE5030Z (2013). The results are shown in Section 4.5.

3.4.4 Effect of carbon source

The investigation in this section was a follow-up of the previous section with main aim to see what kind of trade-offs exist between the decrease of the carbon price and corresponding decrease in PHB productivity (usually reported in units of g/L/h) which usually happens when microorganisms are fed with waste carbon substrates. The effect of decreasing the price of the carbon substrate (glucose) from $ 0.36/kg as reported in Table 3-4 to $ 0/kg was investigated for the factorial design analysis presented in Section 3.4.3 to see which impacts the final cost of production of PHB the most; the microorganism properties or the cost of the carbon substrate. As an additional investigation, a PHB model using a waste carbon substrate was constructed to analyse the economics of PHB production. Referring to Table 2-6, the various waste carbon substrates that *Cupriavidus necator* can metabolise were summarised in Table 3-10 and it was decided to use starch as the waste carbon substrate in the model since it was shown to achieve a significantly high cell concentration.

Table 3-10: Production of PHAs from waste carbon substrates using *Cupriavidus necator*

Carbon source	PHA monomer	Average %PHA content	Average PHA productivity (g/L/h)	Fermentation time (hours)	Average PHA conc. (g/L)	Average cell conc. (g/L)	Reference
Potato starch, saccharified waste	PHB	46	1.47	72	106	230	Haas et al. (2008)
Molasses	PHB	37.5	0.1	75	7.5	20	Beaulieu *et al.* (1995)
Waste glycerol	PHB	38	0.84	45	37.8	99.5	Cavalheiro *et al.* (2009)
Olive oil	PHB	79	0.0472	72	3.40	4.3	Fukui & Doi (1998)
Corn oil	PHB	81	0.0405	72	2.92	3.6	Fukui & Doi (1998)
Oleic oil	PHB	82	0.0467	72	3.36	4.1	Fukui & Doi (1998)
Soybean oil (Wild strain)	PHB	74	0.940	96	90.3	122	Kaha *et al.* (2004)
Soybean oil (Recombinant oil)	PHB, PHV	72.5	1.00	96	96.4	133	Kahar *et al.* (2004)

3.4.5 Development of an optimised scenario for the large scale production of PHB

Based on the results from the different scenarios investigated, insight was given on how the four process factors influenced the PHB production system. From this, an optimum scenario was developed in the CeBER Bioprocess Modeller and compared to the base case scenario to identify the optimisation potential of the process.

3.5 CONCLUDING REMARKS

This chapter outlined the methodology used to investigate the production cost of the PHA monomer, PHB. The Harding (2008) PHB model was scaled up and modified to be used as the base case scenario for this study. The material and energy inventories were used with an early process sizing approach. Previous equipment costs were used to cost the equipment for the base case scenario using the sixth-tenths rule. Lang factors were then used to compute the direct production costs of PHB for the base case model. From there, different scenarios were constructed to systematically inquire their effects on the PHB production system. The results are shown in Chapter 4 where the different scenarios were compared with the results of the base to establish their relative importance for minimising the final cost of production of PHB, energy usage, water usage and carbon dioxide emissions.

4 RESULTS AND DISCUSSIONS

4.1 INTRODUCTION

The results presented in this chapter are divided into two sections, meeting two sets of objectives. The first objective is to set up the base case scenario, which represents an adapted version of the PHB flowsheet set up by Harding (2008) representing the experimental study of Harrison (1990). The mass and energy inventories established from the CeBER Bioprocess Modeller were used to further compute process economics. Preliminary equipment sequencing, sizing and the process economic evaluation, carried out as specified in Section 3.3, are addressed in Section 4.2. Along with the production costs of PHB ($/kg), the energy usage (MJ/kg), water usage (kg/kg PHB) and carbon dioxide emissions (kg/kg PHB) are also reported. In addition, key variables or "hot spots" in the process are identified.

The second objective is to create scenarios derived from the base case scenario to investigate the relative importance of the four main factors postulated to affect the large scale production of PHB: the efficiency of the centrifuge from an energy perspective, the homogeniser efficiency, the PHB purification and recovery process, the microbial properties, and the carbon source in terms of purity and cost. The results are presented in Sections 4.3 to 4.6. Based on the findings, a scenario was built using optimised conditions and is presented in Section 4.7. Along with the production costs of PHB ($/kg), the energy usage (MJ/kg PHB), water usage (kg/kg PHB) and carbon dioxide emissions (kg/kg PHB) are reported and compared to the base case scenario.

All simulations were done for 20 tonnes of crude PHB per run and scaled accordingly to get the amount of PHB produced per year for the total operating hours of the plant. Depending on the number of runs per year, the annual costs of raw materials, utilities, electricity and waste disposal could be scaled up from the material and energy inventories for the production of 20 tonnes of crude PHB per run. The annual labour and direct fixed capital (DFC) dependent costs were independent of the total number of runs per year.

4.2 BASE CASE SCENARIO SIMULATION OUTPUT FROM THE CEBER BIOPROCESS MODELLER: MATERIAL AND ENERGY INVENTORIES

Henceforth the PHB model developed using the inputs in Table 3-1 is referred to as the base case scenario. The default values for the investigated factors are shown in Table 4-1. As mentioned

previously, the model input for the PHB: non-PHB biomass ratio is used as a representation of PHB content and it can be used along with the specified final non-PHB biomass concentration to calculate the final PHB concentration as explained in Section 3.4.3.

Table 4-1: Original values for investigated factors for the base case scenario

Energy efficiency of centrifuge (%)	60.0
Homogeniser breakage efficiency (%)	90.3
Recovery method for PHB purification	Enzymatic digestion
PHB: final non-PHB biomass ratio	2.4
Final non-PHB biomass concentration (g/L)	44.1
Cultivation time (hours)	44.4
Type of carbon substrate	Glucose at $ 0.40 /kg

4.2.1 Process description, flow sheet and output for the base case scenario

The process description shown in Figure 4-1 is similar to the Harding (2008) model, in Figure 3-1. Auxiliary equipment such as blending and storage tanks has been added to consider the flow and storage of material through a large scale process. Raw materials were blended in the blending tank (BL-100) before being sterilised by the continuous steriliser (SX-100). The sterilised raw materials, the microorganism, *Cupriavidus necator* and antifoam were fed to the two reactors (RX-100 and RX-101). The reactors were supplied with air from the compressor (CM-100). The production process was a fed-batch onewhere the microbial culture was only collected at the end of PHB production. The microbial culture from both the reactors, (RX-100 and RX-101) was stored in the storage tank (ST-100). This was followed by cell rupture in the high pressure homogeniser (HP-100) and the cell debris was removed by the centrifuge (CF-100). The PHB extraction and purification process used was the enzymatic digestion of proteinaceous biomass and lipid dissolution by detergent. The particulate ruptured cell debris and liberated PHB were re-suspended with an alkaline serine protease in the mixing tank (RX-102), with the pH being controlled by potassium hydroxide. Once the non-polymeric proteinaceous cell matter was digested, the PHB was treated with a non-ionic detergent in stirred tank reactor (RX-103) to remove lipid and associated components. The PHB was further purified using repeated cycles of water washing in the centrifuge (CP-101). This was followed by a hydrogen peroxide treatment in (RX-104) to further remove any residual cell debris by oxidation. After a final water wash in the centrifuge (CP-102), the slurry containing PHB was stored in the tank (ST-101). The PHB was then sent to the spray dryer (SD-100) for continuous spray drying. The complete list of equipment used in the process is shown in Table 4-2.

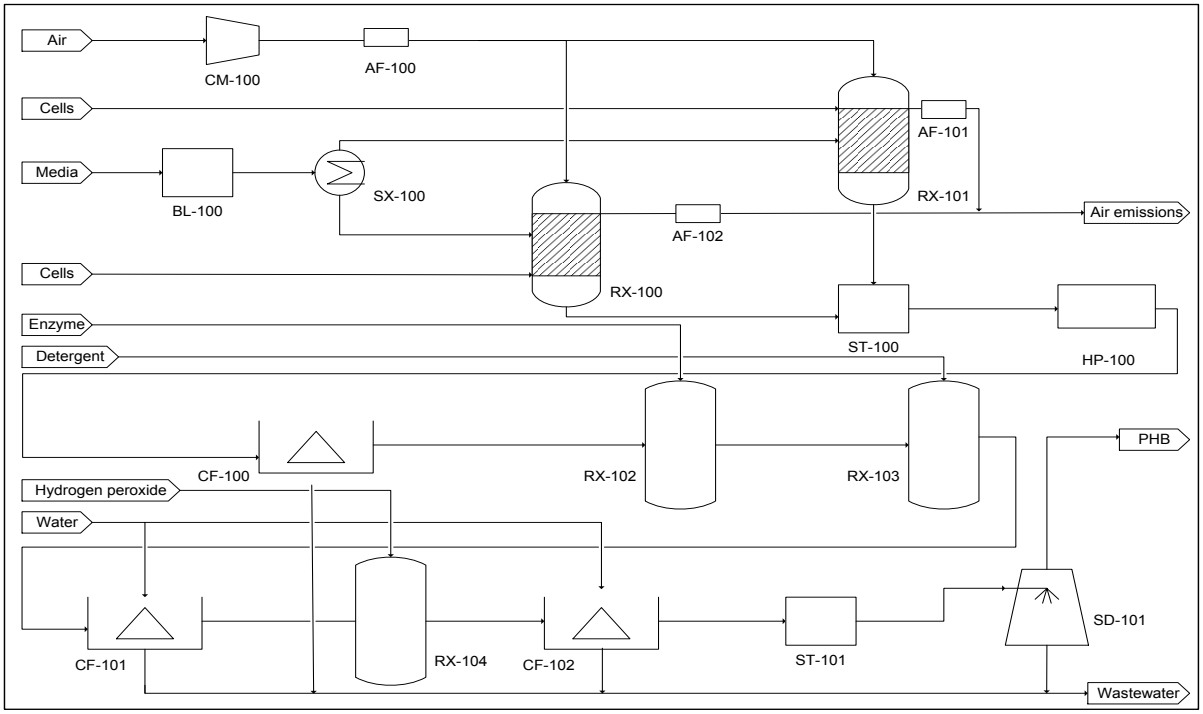


Figure 4-1: Process flow diagram for the simulation 20 tonnes of crude PHB per run for the base case scenario

Table 4-2: List of equipment and description for the simulation of 20 tonnes of crude PHB per run for the base case scenario

List of equipment	Description
AF-100	Compressor air filter
AF-101	Bioreactor 1 air filter
AF-102	Bioreactor 2 air filter
BL-100	Raw materials blending tank
CF-100	Centrifuge 1
CF-101	Centrifuge 2 with wash cycles
CF-102	Centrifuge 3 with wash cycles
CM-100	Compressor
HP-100	High pressure homogeniser
RX-100	Bioreactor 1
RX-101	Bioreactor 2
RX-102	Enzyme digestion tank
RX-103	Detergent addition tank
RX-104	Hydrogen peroxide addition tank
SD-101	Continuous spray dryer
ST-100	Storage tank 1
ST-101	Storage tank 2
SX-100	Continuous steriliser

The mass and energy balances calculated using the model led to the inventories presented in Table 4-3 and Table 4-5 for the simulation of the large scale production of 20 tonnes of crude PHB per run. There were no waste carbon and nitrogen sources, namely glucose and ammonium sulphate respectively since

the model is built on a stoichiometric approach. Therefore the exact amount of raw materials needed to produce 20 tonnes of crude PHB per run was calculated with the original purity and recovery from the Harding (2008) model maintained. For each unit operation, the model also gave an estimation of the batch volume required and the corresponding energy usage, shown in Table 4-5. The estimated batch volume refers to the total volume of material that passes through a specific unit process in order to get the final desired product mass (Harding 2008). The final product stream specification is shown in Table 4-4.

Table 4-3: Mass balance and utility requirements for the simulation of 20 tonnes of crude PHB for the base case scenario

IN		**OUT**	
Process water (m^3)	636	Total pure PHB (kg)	19270
Antifoam (kg)	196	Water (m^3)	665
Glucose (kg)	73540	Antifoam (kg)	196
O_2 (kg)	528300	*Cupriavidus necator* (kg)	11780
N_2 (kg)	1987000	Enzyme (kg)	488
Ammonium sulphate (kg)	13920	Detergent (kg)	6096
Enzyme (kg)	488	Hydrogen peroxide (kg)	1209
Detergent (kg)	6100	Potassium hydroxide (kg)	7891
Hydrogen peroxide (kg)	1213	Waste PHB (kg)	1411
Potassium hydroxide (kg)	7896	Air emissions:	
		O_2 (kg)	503700
		N_2 (kg)	1987000
		CO_2 (kg)	42800
		SO_2 (kg)	6751
Total Mass (ton)	3255	Total Mass (ton)	3255

Note: Chemical oxygen demand (COD) of wastewater was 27680 kg

Table 4-4: Final product stream composition exiting the spray dryer for the base case scenario

Total pure PHB (kg)	19270
Water (kg)	195
Enzyme (kg)	0.273
Detergent (kg)	3.41
Hydrogen peroxide (kg)	4.23
Water (kg)	504
Potassium hydroxide (kg)	4.41
Total Product Mass (Crude PHB) (kg)	19983
Purity (%)	96.4
Recovery (%)	93.2

Table 4-5: Estimated volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the base case scenario simulating the production of 20 tonnes of crude PHB

Unit operation	Description	Estimated volume of culture required (m^3)	Electricity (MJ)	Steam (kg)	Cooling water (m^3)
Sterilisation	Steam sterilisation	245	13630	36350	
Microbial growth	Bioreactor	245			
Aeration			342800		
Agitation			30650		
Post fermentation cooling					119800
Cell disruption	High pressure homogeniser	245	43380		
Solid-liquid separation 2	Centrifugal spin/washing	245	2956		
Conc./Purification 1	Enzyme mixing tank	81	466		
Conc./Purification 2	Detergent mixing tank	87	502		
Conc./Purification 3	Centrifugation with 5 washes	151 (for 1 wash)	4966		
Conc./Purification 4	H_20_2 addition tank	87.07	502		
Conc./Purification 5	Centrifugation with 2 washes	150 (for 1 wash)	2789		
Formulation	Spray drying	87 (19.3 m^3 of water lost)*	63430		
Final product volume out	-	16.5			
Total			506100	36350	119800

* Water lost between Conc./purification 5 and formulation steps

4.2.2 Approach to preliminary equipment sizing

The approach used is that of a semi-continuous process, which is frequently used in large scale PHB production as reported by Byrom (1990) and Choi at al. (1997) in order to minimise reactor turn-around time and maximise the use of time consuming unit processes such as the steriliser, homogeniser, centrifuges and spray dryer. In Section 3.2.3, homogenisers processing throughput of up to 60 m^3/hr were reported. Since the homogeniser was the first step in the downstream processing, it was decided to fix the maximum flow rate as 60 m^3/hr where continuous processing was desired. Thus, in conjunction with the estimated volume of flow given in Table 4-5, the residence time for continuous sterilising, filling and emptying of vessels, homogenisation and centrifugation could be calculated. Based on the Harding (2008) model, two hours each were allocated, for the enzyme mixing (RX-102), detergent addition (RX-103) and hydrogen peroxide treatment (RX-104) steps. While more detailed in-depth equipment sizing and process optimisation might lead to improved efficiency of the PHB production

system, this approach was considered robust enough for a feasibility study estimate since the inventories from the CeBER Bioprocess Modeller are applicable for early process stage simulation only. As discussed in Section 3.2.3, the maximum bioreactor size was limited to 200 m^3, therefore in order to handle 245 m^3 microbial culture required for the base case, two bioreactors of volume 153 m^3 each (RX-100 and RX-101) were used. This volume included the 20% overdesign factor required for bioreactors. The steriliser was restricted to fill the reactors one at a time. Medium from the continuous steriliser (SX-100) was collected into the bioreactors with 4.25 hours being allocated to fill the bioreactors at a flow rate of 57.6 (~58) m^3/hr which was below the maximum fixed flow rate limit of 60 m^3/hr. Adhering to a flow rate of 58 m^3/hr, it took 2.11 hours to empty the two bioreactors simultaneously into the storage tank (ST-100). Adding in the 3.5 hours for the cleaning and sterilising of the bioreactors specified in Section 3.2.3, the total bioreactor downtime was 9.86 hours used for filling up, emptying and cleaning. Therefore the total upstream processing time including the production time and the reactors downtime was 55 hours (44.4 + 9.86= 54.3 ~ 55 hours).

The homogeniser and centrifuges were assumed to be continuous with a throughput of 58 m^3/hr. The microbial culture flowed continuously through the homogeniser (HP-100), taking 4.22 hours to process 245 m^3 of microbial culture. This flowed to the centrifguge (CF-100), which took another 4.22 hours. The output from centrifuge (CF-100) flowed continously to the enzyme digestion tank (RX-102), which acted as a storage tank as well. Once the centrifugation was over, all the microbial culture was collected in tank (RX-102). The culture stayed for an additional 2 hours in mixing tank (RX-102) for enzyme digestion. Adhering to a flow rate of 58 m^3/hr, it took only 1.4 hours to empty the contents to the detergent washing tank (RX-103) since the culture volume leaving reactors (RX-100 and RX-101) decreased after being processed by the homogeniser (HP-100) and the centrifuge (CF-100). The tank (RX-103) also acted as an intermediate storage tank. The culture was held in this tank for another 2 hours. The centrifuge (CF-101) was used to process five wash cycles. Since one centrifuge wash took 2.61 hours for a throughput of 58 m^3/hr, the total centrifugation time was 13.1 hours. During the final wash, the output of the centrifuge (CF-101) was transferred continously to the hydrogen peroxide tank (RX-104), acting as an intermediate storage tank. The suspension was held for another 2 hours for oxidation with hydrogen peroxide. The centrifuge (CF-102) was used for two wash cycles of 2.58 hours each at 58 m^3/hr, thus requiring a total residence time of 5.16 hours. The culture was transferred into the storage tank (ST-101) where it was continuously fed to the spray dryer (SD-100). This allowed for the decoupling of the spray dryer from the rest of the downstream processing. The total downstream processing time from leaving the reactor to entering the spray dryer was 34.1 hours. Therefore in order to complete the overall

downstream processing during the next batch of PHB production, the spray dryer (SD-100) could work continuously for 20.9 hours (55 – 34.1 hours). The Gantt chart for the base case scenario is shown in Figure 4-2. The spray dryer was found to be among the most expensive and energy consuming equipment items in the downstream processing as it will be demonstrated in Section 4.2.5. By keeping a high flow rate of 58 m^3/hr across all centrifuges even if the culture volume was decreased, the residence time for the centrifuges could be decreased leading to an increase in operational time for the spray dryer. Subsequently, a lower water evaporation rate was required leading to a lower spray dryer cost.

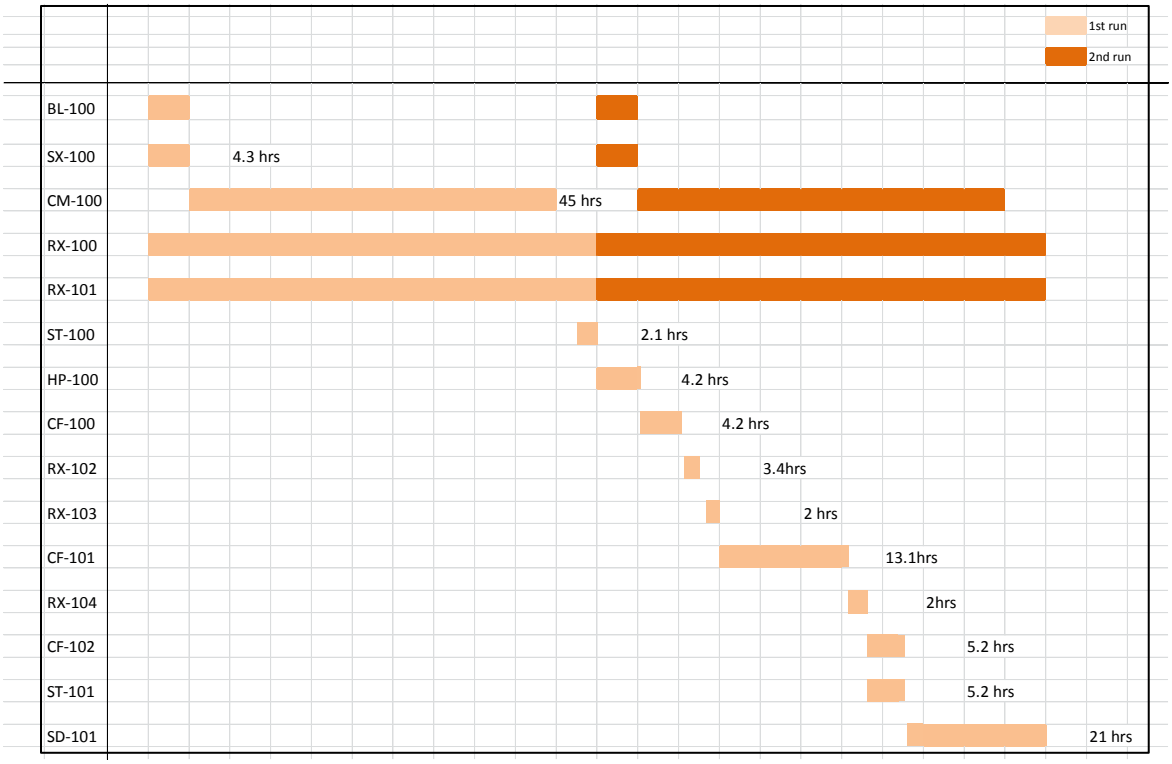


Figure 4-2: Gantt chart for the simulation of 20 tonnes of crude PHB per run for the base case scenario

4.2.3 Equipment sizing and costing results for the base case scenario

Using the information in Table 3-3, the costing of the equipment was performed as detailed in Section 3.3.1., using an overdesign factor as reported in Table 2-16 for safety purposes. The cost of equipment for the base case in the year 2014 is shown in Table 4-6. As mentioned in Section 3.3.1, to validate the equipment prices calculated using the six-tenths rule, equipment prices of similar size were computed using the online cost estimator tool by Peters *et al.* (2015) for the compressor, centrifuges, storage tanks and spray dryer together with a recent techno-economic study by Koutinas *et al.* (2014) for the bioreactor and homogeniser, shown in Table 4-7. The maximum percentage difference in the calculated prices from recent equipment prices was 22 %, which was considered acceptable for a first estimate

study, thus demonstrating that the costing approach and calculations were sufficiently accurate for preliminary cost estimates within accuracy of ± 30 %, normally expected for a study estimate as reported in Table 2-17.

Table 4-6: Equipment cost for the simulation of 20 tonnes of crude PHB per year for the base case scenario

	Scaling variable	**Size including overdesign factor**	**Cost (1000 $, 2014)**
Compressor (CM-100)	Power (kW)	2382	1760
Air filters (AF-100, AF-101, AF-102)	Volumetric flow rate (m^3/s)	3	58.5
Blending tank (BL-100)	Volume (m^3)	306	275
Steriliser (SX-100)	Flowrate (m^3/hr)	68	244
Bioreactors (RX-100, RX-101)	Volume (m^3)	153	4400
Storage tank (ST-100)	Volume (m^3)	306	162
Homogeniser (HP-100)	Flowrate (m^3/hr)	73	675
Centrifuge (CF-100)	Sigma (m^2)	100694	354
Enzyme washing tank (RX-102)	Volume (m^3)	101	130
Detergent washing tank (RX-103)	Volume (m^3)	109	136
Centrifuge (CF-101)	Sigma (m^2)	100694	354
H_2O_2 tank (RX-104)	Volume (m^3)	109	136
Centrifuge (CF-102)	Sigma (m^2)	100694	354
Storage tank (ST-101)	Volume (m^3)	109	80.2
Spray dryer (SD-100)	Water evaporation rate (kg/hr)	1276	468
Total			**9590**

Table 4-7: Comparison of calculated equipment costs with recent equipment costs calculated from an online cost estimator tool developed by Peters *et al.* (2015) and a recent techno-economic study by Koutinas *et al. (*2014)

	Size	**Units**	**Cost (1000 $, 2014)**	**Reference for method of calculation**	**% difference from calculated costs in Table 4-6**
Compressor (CM-100)	2382; 3	kW; m^3/s	1790	Peters *et al.* (2015)	+ 1
Centrifuge (CF-100)	100694;194*	kw	431	Peters *et al.* (2015)	+ 21
Storage tank (ST-101)	306	m^3	165	Peters *et al.* (2015)	+2
Enzyme washing tank (RX-102)	101	m^3	153	Peters *et al.* (2015)	+ 18
Spray dryer (SD-100)	1276	kg/hr	516	Peters *et al.* (2015)	+ 10
Bioreactors (RX-100, RX-101)	153	m^3	5393	Koutinas *et al.* (2014)	+ 22
Homogeniser (HP-100)	73	m^3/hr	590	Koutinas *et al.* (2014)	- 13

* Centrifuge power was calculated using its energy requirement and residence time

Note: % difference for the costs of (CF-101), (CF-102) were also 21 % ; % difference for the costs of (RX-100), (RX-101) were also 18 %

4.2.4 Calculation of annual PHB production costs

The model simulated the production of 20 tonnes of crude PHB per run. Using the mass and energy inventories, the equipment was sized and their costs along with the costs of raw materials, utilities, and electricity and waste treatment were computed as explained in Section 3.3. These are presented in Table 4-8.

Table 4-8: Equipment costs along the costs associated with raw materials, utilities, electricity, waste disposal for the simulated production of 20 tonnes of crude PHB per run for the base case scenario

	Cost (1000 $)
Cost of equipment	9590
Raw materials	54.1
Utilities (steam and water)	2.53
Electricity	9.85
Waste disposal	9.57

As mentioned in Section 3.2.3, the plant was designated to run for 8400 hours. Since one run took 55 hours, there could be 152 runs a year, leading to the production of 2929 tonnes of pure PHB (3040 tonnes of crude PHB) per year. The direct annual production (DFC) costs were calculated as detailed in Table 3-6 and the results are shown in Table 4-9. The Lang factor method estimate offers accuracy of ± 30 %. A major limitation in this approach is that most of the Lang factors come from plants in the United States and the same applies for the calculation of the operating labour. Thus said, if more detailed economic analysis is required, Lang factors and labour data specific to the country where the plant is to be built should be used. Nonetheless this method of calculation is suited for preliminary economic studies (Ereev & Patel 2011). Therefore for the production of 2929 tonnes of pure PHB, the direct PHB production costs were calculated to be $ 8.4/kg of purified PHB. This falls on the higher end of commercial PHA price estimates as reported in Section2.4.2.2. Choi & Lee (1997) and Van Wegen (1998) pointed out the high costs associated with an enzymatic digestion purification process due to the use of expensive chemicals as raw materials and more complex downstream processing which involves extensive centrifugation.

Table 4-9: Calculation for direct production costs of 2929 tonnes of pure PHB per year for the base case scenario

Calculations of direct fixed capital (DFC)			
A.	*Total plant direct cost (TPDC)*	Calculation factors	Cost (1000 $,2014)
	a. Equipment purchase cost (PC)	(1.00 x PC)	9590
	b. Installation	(0.50 x PC)	4790
	c. Process Piping	(0.35 x PC)	3360
	d. Instrumentation	(0.40 x PC)	3840
	e. Insulation	(0.03 x PC)	288
	f. Electrical	(0.10 x PC)	959
	g. Buildings	(0.45 x PC)	4310
	h. Yard improvements	(0.15 x PC)	1440
	i. Auxiliary facilities	(0.40 x PC)	3840
	Total		**32400**
B.	*Total plant indirect cost (TPIC)*		
	a. Engineering	(0.25 x TPDC)	8100
	b. Construction	(0.35 x TPDC)	11300
	Total		**19400**
C.	Other costs (OTC)		
	a. Contractor' fee	(0.05 x (TPDC + TPIC)	2590
	b. Contingency	(0.10 x (TPDC + TPIC)	5190
	Total		**7780**
D.	**Direct fixed capital (DFC)**		**59600**
Annual operating cost			
A.	*DFC dependent items*		
	a) Depreciation	(0.10 x DFC)	5960
	b) Maintenance material	(0.03 x DFC)	1610
	c) Insurance	(0.01 x DFC)	596
	d) Local taxes	(0.02 x DFC)	1190
	Total		**9360**
B.	*Labour-dependent items*		
	a) Operating labour	$ 26/hour x 2080 hours/year x 13 operators	708
	b) Maintenance labour	(0.02 x DFC)	1070
	c) Supervision	(0.20 x B(a))	106
	d) Operating supplies	(0.15 x (A(b) + B(b))*	403
	e) Laboratory	(0.15 x B(a))	106
	Total		**2400**
C.	*Plant overheads*	(0.6 x (B(a) +B(b) +B(c))	**1130**
D.	*Raw material*	Raw materials costs for 20 tonnes/run of crude PHB x 152 runs	**8220**
E.	*Utilities*	Utilities costs for 20 tonnes/run of crude PHB x 152 runs	**1880**
F.	*Waste disposal*	Waste disposal costs for 20 tonnes/run of crude PHB x 152 runs	**1460**
	Total direct annual operating costs		**24400**

* 0.15 x (Maintenance material + maintenance labour)

4.2.5 Analysis of breakdown of costs, electricity usage and equipment costs for the base case scenario

A percentage breakdown of the annual production costs obtained in Figure 4-3 showed that the direct fixed capital (DFC) dependent costs were the major contributor to the direct annual production costs, including plant overheads (43%) followed by the raw materials (34 %) shown in Figure 4-3. Based on the individual raw material costs used, glucose accounted for 49 % of the total costs, shown in Figure 4-4. This demonstrates the need to find cheaper carbon sources to decrease the production costs of PHB. The labour costs contributed to 10 %. Utilities (including electricity) and waste disposal each contributed to 8 % and 6 % respectively. For comparison, the costs breakdown of Choi & Lee (1997) and Van Wegen (1998) are presented in Figure 4-5 and Figure 4-6 respectively.

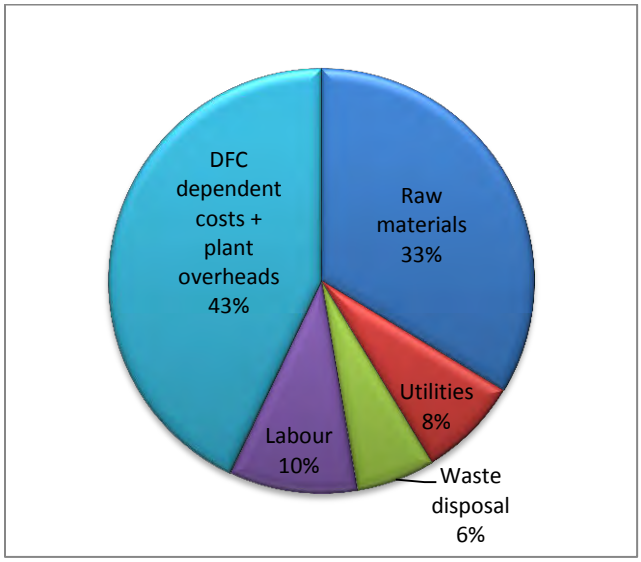


Figure 4-3: Total annual production costs breakdown for the production of 2929 tonnes of pure PHB per year for the base case scenario in which a total production cost of $ 24.4 million was estimated in 2014

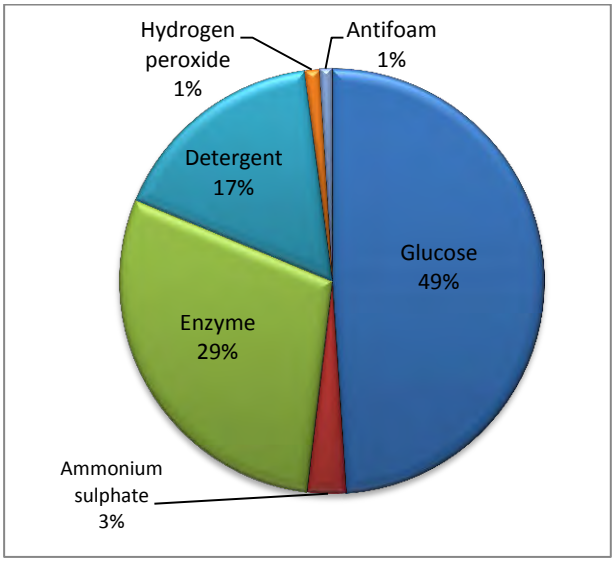


Figure 4-4: Raw material costs breakdown for the production of 2929 tonnes of pure PHB per year for the base case scenario where raw materials costs of $ 8.22 million was estimated in 2014

For the costs breakdown reported by Choi & Lee (1997), shown in Figure 4-5, the raw materials costs were larger when compared to the DFC costs. The lower DFC dependent costs of Choi & Lee (1997) were attributed to less equipment needed for the surfactant-sodium hypochlorite purification and recovery process used in their simulation compared to the enzymatic digestion process. Figure 4-6 shows the total annual cost breakdown for the study done by Van Wegen *et al.* (1998) which included the costs associated with start-up; however, the costs of the media including glucose and direct fixed capital costs remained the major costs contributing to the final production costs of PHB, which was line with the

results of this study. The DFC costs in Figure 4-3 were calculated from the equipment costs. Figure 4-7 gives the equipment costs breakdown showing that the most costly piece of equipment was the bioreactors followed by the compressor. However the most energy intensive piece of equipment was the compressor, followed by the spray dryer, as shown in Figure 4-8.

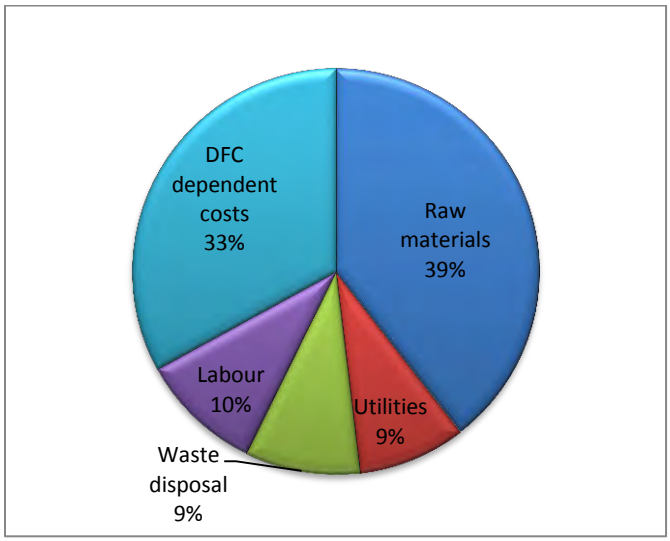


Figure 4-5: Total annual production costs breakdown for the 2850 tonnes of pure PHB per year with a surfactant-sodium hypochlorite recovery process simulated in SuperPro in which a total production cost of $ 17.5 million was estimated in 1996 (Choi & Lee 1997)

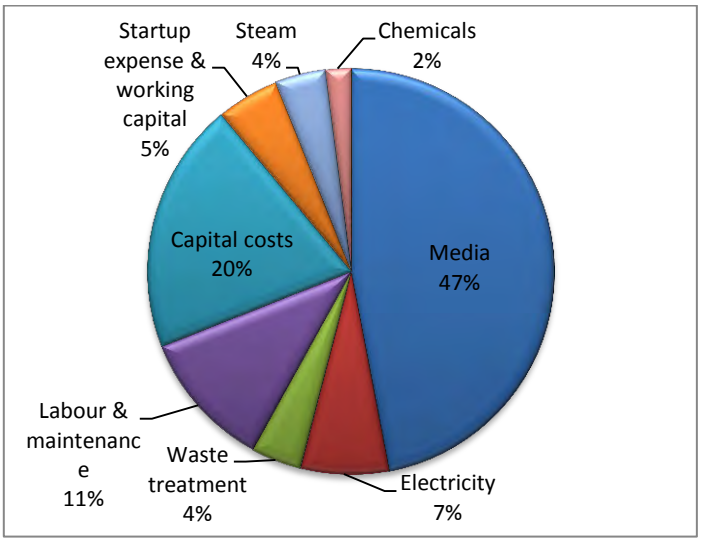


Figure 4-6: Total annual production costs breakdown for the net production of 4300 tonnes of crude PHB per year using a sodium hypochlorite purification and recovery process in which a total production cost of $ 19.7 million was estimated in 1995 (Van Wegen *et al.* 1998)

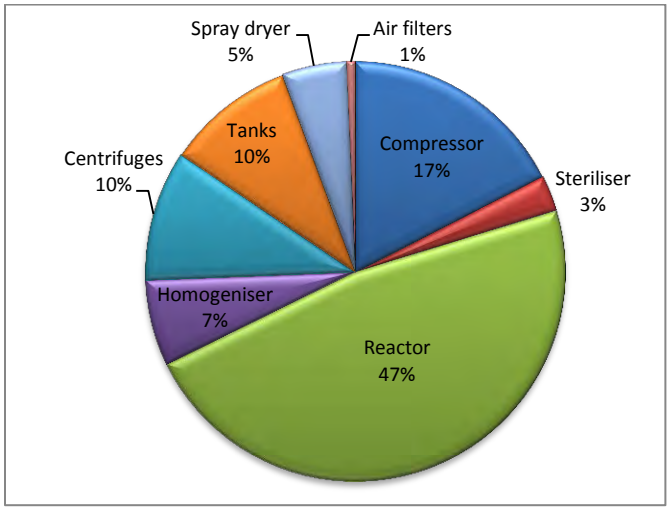


Figure 4-7: Equipment costs breakdown for the net production of 2929 tonnes of pure PHB per year for the base case scenario where the total equipment costs were estimated at $ 9.6 million in 2014

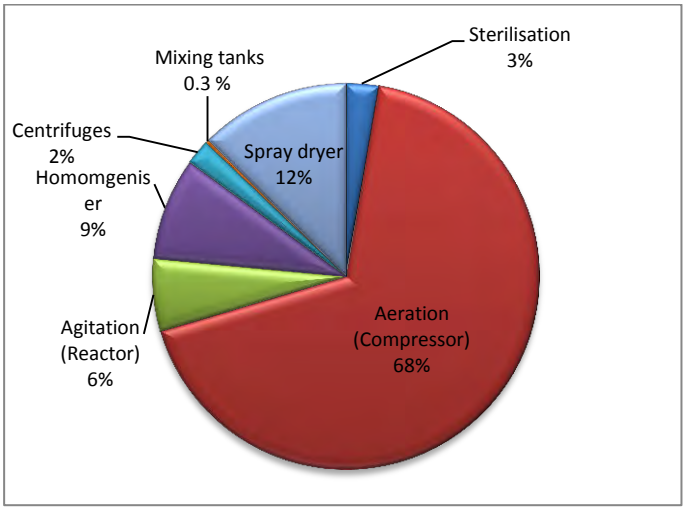


Figure 4-8: Electricity usage breakdown for the net production of 2929 tonnes of pure PHB per year for the base case scenario where the total electricity usage was 506100 MJ

4.2.6 Process summary for the base case scenario

Figure 4-9 illustrates the process summary for the base case scenario with production costs of $ 8.4 /kg PHB, total energy usage of 31 MJ/kg PHB, total carbon dioxide emissions of 2.2 kg/kg PHB and a total water usage of 33 kg/kg PHB. These values were obtained from the mass and energy inventories shown in Table 4-3, Table 4-4 and Table 4.5 as a function of the total amount of pure PHB produced. Table 4-10 gives the total cost breakdown for the base case with the major cost contributors being the DFC dependent costs (including plant overheads) and the raw materials costs. The results in this section set the base case scenario process summary in terms of the amount of pure PHB produced annually with respect to the productivity achieved by the microorganism and its subsequent recovery, the production costs, the energy and water usage and the carbon dioxide emissions. From the costs breakdown, it was shown that the DFC dependent costs and raw materials were the largest contributors to the production costs of PHB. The DFC dependent costs relate directly to the total equipment costs of the process, thus lower equipment costs must be targeted to lower the DFC dependent costs. Similarly, with the carbon substrates being such a significant contributor to the production costs, a cheaper carbon source is needed to reduce the production costs significantly. Since Harding (2008) concluded that the energy requirement of the process resulted in a significant environmental footprint, the energy usage of the production system must be minimised. Lower carbon dioxide emissions are also desired along with decreased water use, viewed as a natural resource.

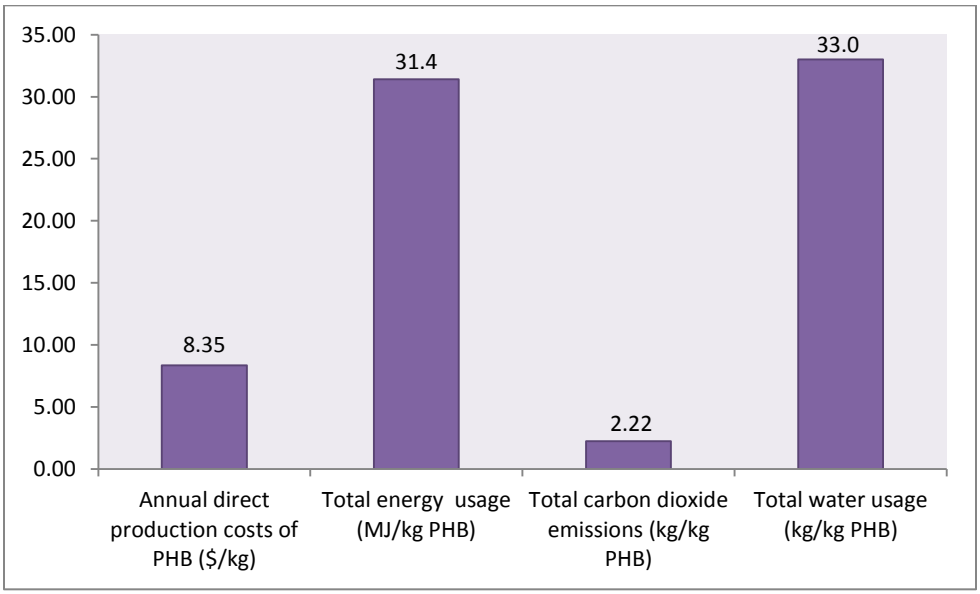


Figure 4-9: Process summary for the production of 2929 tonnes of pure PHB per year for the base case scenario illustrating the cost of production of PHB ($/kg), total energy usage of the process (MJ/kg PHB), total water usage (kg/kg PHB)

Table 4-10: Total production cost breakdown per kg PHB as estimated for the production of 2929 tonnes of pure PHB per year for the base case scenario

Raw materials ($/kg)	2.81
Utilities ($/kg)	0.13
Electricity ($/kg)	0.51
Labour ($/kg)	0.82
Waste disposal ($/kg)	0.50
DFC dependent costs + plant overheads ($/kg)	3.58
Annual direct production costs of PHB ($/kg)	**8.35**

4.3 INVESTIGATING THE EFFECT OF THE ENERGY EFFICIENCY OF THE CENTRIFUGES AND THE HOMOGENISER EFFICIENCY ON PHB PRODUCTION SYSTEM

In this section, the effect of varying the centrifuge energy efficiency across the three centrifuges and the homogeniser efficiency was investigated with respect to the base case scenario. The mass and energy inventories, along with the product specification and equipment costs are shown in Appendix A-1. The direct annual production costs of PHB were calculated in a similar fashion to the base case scenario presented in Table 4-9. The model used and detailed production costs calculations are presented in the accompanying CD-ROM. The findings are summarised in Figure 4-10.

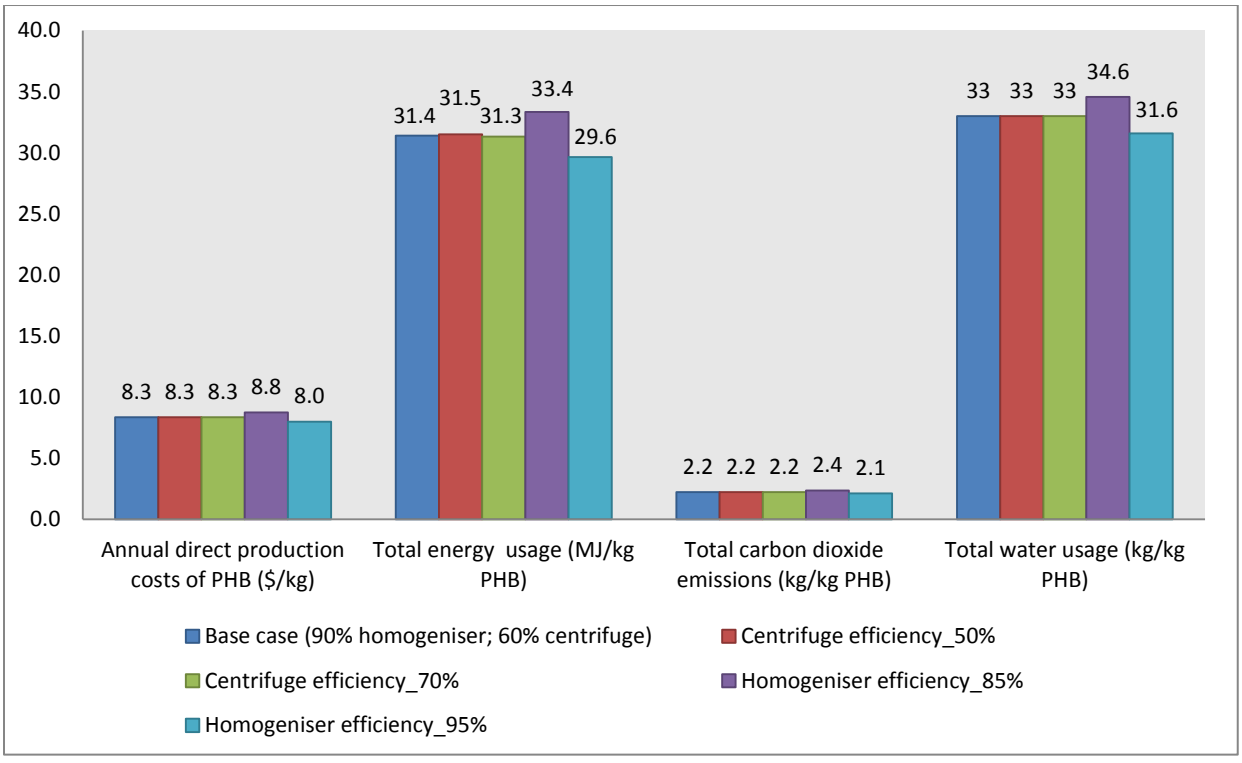


Figure 4-10: Effect of varying the energy efficiency of the centrifuges and the homogeniser efficiency on the annual large scale production of PHB using the CeBER Bioprocess Modeller

Varying the energy efficiency of the centrifuges had very little effect on the overall PHB production in terms of cost, energy use, water use and carbon dioxide emissions as shown. An increase in the energy efficiency of the centrifuge from 60 % to 70 % only made the overall process slightly less energy intensive with no significant decrease in the cost of production of PHB. The only change was on the total energy usage of the process, with less efficient centrifuges (50 %) leading to a 0.4 % increase in total electricity usage and more efficient centrifuges (70 %) resulting in a 0.3 % decrease in total electricity usage.

On the other hand, varying the efficiency of the homogeniser had a significant effect on the overall process. A decrease in homogeniser efficiency from 90 % to 85 % led to a 5 % increase in PHB production cost, a 6 % increase in energy consumption, a 5 % increase in water consumption and a 6 % increase in carbon dioxide emissions. Increasing the homogeniser efficiency to 95 % lowered the PHB production costs by 4 %, decreased the energy consumption, water consumption and carbon dioxide emissions by 6 %, 4 % and 5 % respectively. A more efficient homogeniser ruptured more cells and more PHB could be released from the cells as shown in Table 4-11. While the efficiency of the homogeniser varied, the centrifuges were assumed to work in similar fashion to the base case, therefore the final product recovery was still 93.2 % while the purity increased with a more efficient homogeniser and decreased for a less efficient one.

Table 4-11: Effect of varying the energy efficiency of the centrifuges and efficiency of the homogeniser on the annual large scale production of PHB process simulated in the CeBER Bioprocess Modeller

	Total amount time of one run (hours)	Amount of pure PHB produced in one run (kg)	Amount of pure PHB produced per year (tonnes)	Purity (%)
Base case	55	19270	2929	96.4
Energy efficiency centrifuges _50%	55	19270	2929	96.4
Energy efficiency centrifuges _70%	55	19270	2929	96.4
Homogeniser efficiency _85 %	56	19220	2883	96.2
Homogeniser efficiency _95 %	55	19320	2937	96.7

This demonstrates the importance of optimising the cell disruption step in PHB production where a more efficient disruption leads to a lower cost of production of PHB. A similar trend, as reported in Figure 4-10, can be applied to Table 4-12. The energy efficiency of the centrifuge barely affected the total annual production costs with the most noticeable change being in the electricity usage costs. A

lower homogeniser efficiency resulted in higher raw materials costs, electricity costs, waste disposal costs and annual operating costs and the opposite applies for a higher homogeniser efficiency since the homogeniser efficiency affected the amount of PHB produced as seen in Table 4-11. Fernandez *et al.* (2005) also observed that an increase in homogeniser efficiency led to lower production costs. A similar observation can be reported for this study, show in Table 4-12.

Table 4-12: Effect of varying the energy efficiency of the centrifuges and efficiency of the homogeniser on the large scale production of PHB simulated in the CeBER Bioprocess Modeller

	Raw materials ($/kg)	Utilities ($/kg)	Electricity ($/kg)	Labour ($/kg)	Waste disposal ($/kg)	DFC dependent costs + plant overheads ($/kg)	Annual direct production costs of PHB ($/kg)
Base case	2.81	0.131	0.511	0.818	0.497	3.58	8.35
Centrifuge efficiency_50%	2.81	0.131	0.513	0.818	0.497	3.58	8.35
Centrifuge efficiency_70%	2.81	0.131	0.509	0.818	0.497	3.58	8.35
Homogeniser efficiency_85%	2.95	0.139	0.544	0.843	0.562	3.72	8.76
Homogeniser efficiency_95%	2.68	0.125	0.481	0.800	0.439	3.46	7.99

4.4 INVESTIGATING THE EFFECT OF PURIFICATION AND RECOVERY PROCESSES ON PHB PRODUCTION

In this section, two processes for the purification and recovery of PHB are compared to the enzymatic digestion used in the base case scenario. Their effects on PHB production cost, energy usage, water usage and carbon dioxide emissions are considered. The direct annual production costs of PHB were calculated in a similar fashion to the base case scenario presented in Table 4-9. The models used and detailed production cost calculations for each process are presented in the accompanying CD-ROM.

4.4.1 Surfactant-sodium hypochlorite purification and recovery method

The process diagram in Figure 4-11 was developed based on the flowsheet for the surfactant sodium hypochlorite process explained in Section 3.4.2. This process had one less processing step than the base case flowsheet in Figure 4-1. The upstream processing, PHB production, homogeniser efficiency and energy efficiency of the centrifuges remained the same as the base case. The mass and energy inventories are shown in Table 4-13, the estimated batch volume (m^3), electricity, steam and cooling requirements are shown in Table 4-15 and the final product composition is shown in Table 4-14. The major differences between the inventories, in Table 4-13 were the amount of water required and the

PHB lost in the wastewater. With less water-intensive centrifugation steps, less water was required for the surfactant-sodium hypochlorite recovery process. Due to the higher recovery and purity achieved for the surfactant-sodium hypochlorite recovery process more pure PHB could be recovered than the base case scenario, as seen in Table 4-14, resulting in less PHB lost in the wastewater.

The model calculated the amount of microbial culture needed to produce the desired amount of product. A slightly higher amount of microbial culture, 247 m^3 compared to 245 m^3 for the base case scenario, was required to get the desired 20 tonnes of crude PHB at the specified purity of 99 % with a recovery of 95 %. The difference in the volume of the microbial culture required explains the major differences in Table 4-15 , when compared to the base case scenario in Table 4-5. However, due to overall less processing steps, the surfactant-sodium hypochlorite recovery process required less total energy for its unit operations (487200 MJ) than the base case scenario, which required 506100 MJ.

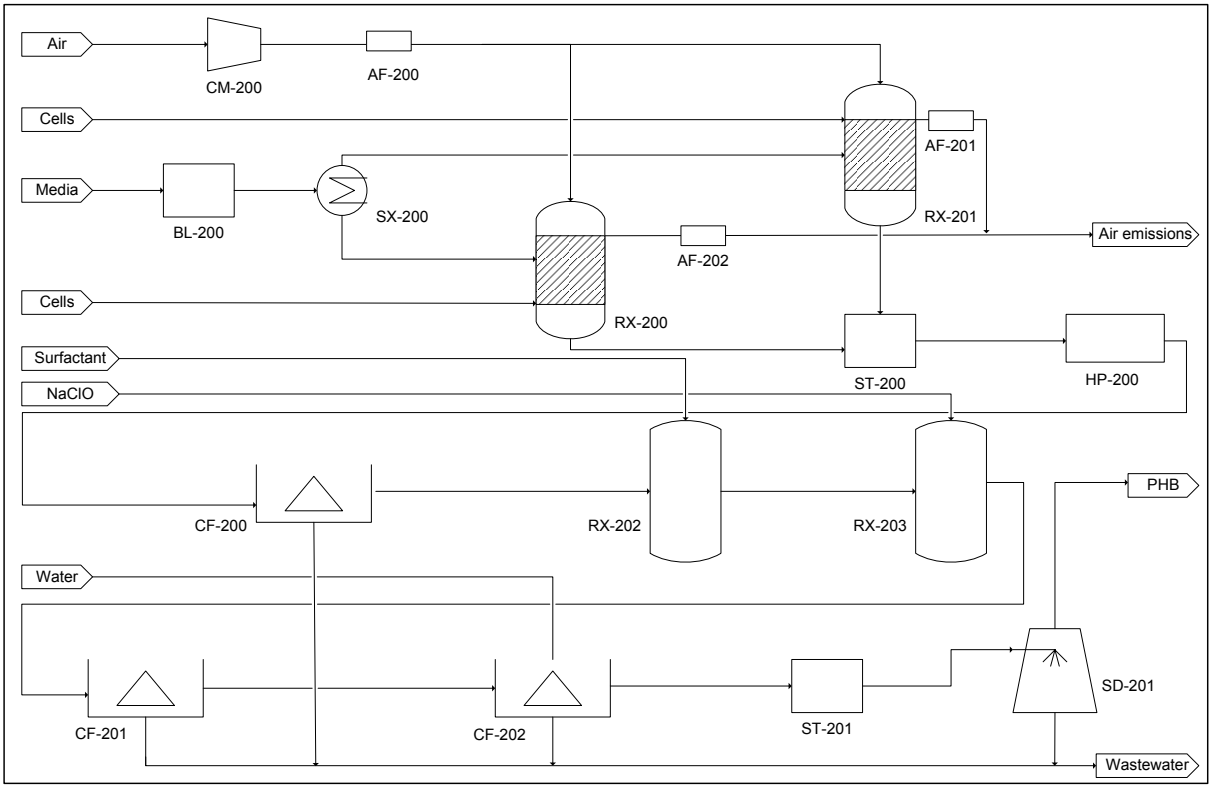


Figure 4-11: Process flowsheet for PHB production using a surfactant-sodium hypochlorite purification and recovery method developed in the CeBER Bioprocess Modeller simulating 20 tonnes of crude PHB per run

Table 4-13: The effect of PHB recovery process on the mass and energy inventories for the simulation of 20 tonnes of crude PHB per run for a surfactant-sodium hypochlorite process and the enzymatic digestion process used for the base case scenario

IN			**OUT**		
	Surfactant-NaOCl process	Base case (Enzymatic digestion)		Surfactant-NaOCl process	Base case (Enzymatic digestion)
Process water (m^3)	221	636	Total pure PHB (kg)	19820	19270
Antifoam (kg)	197	196	Water (m^3)	251	665
Glucose (kg)	74110	73540	Antifoam (kg)	197	196
O_2 (kg)	520900	528300	*Cupriavidus necator* (kg)	11870	11780
N_2 (kg)	1959000	1987000	Triton X 100 (kg)	692	-
Ammonium sulphate (kg)	14030	13920	Sodium hypochlorite (kg)	9988	-
Triton X 100 (kg)	692	-	Enzyme (kg)	-	488
Sodium hypochlorite (kg)	9988	-	Detergent (kg)	-	6096
Enzyme (kg)	-	488	Hydrogen peroxide (kg)	-	1209
Detergent (kg)	-	6100	Potassium hydroxide (kg)	-	7891
Hydrogen peroxide (kg)	-	1213	Waste PHB (kg)	1024	1411
Potassium hydroxide (kg)		7896	Air emissions:		
			O_2 (kg)	496200	503700
			N_2 (kg)	1959000	1987000
			CO_2(kg)	43130	42800
			SO_2 (kg)	6803	6751
Total Mass (ton)	2800	3255	Total Mass (ton)	2800	3255

Note: Chemical oxygen demand (COD) of wastewater was 21770 kg for surfactant-sodium hypochlorite process and 27680 kg for the enzymatic digestion process

Table 4-14: Final product stream composition for the simulation of 20 tonnes of crude PHB per run using a surfactant-sodium hypochlorite purification and recovery process using the CeBER Bioprocess Modeller

Total pure PHB (kg)	19820
Water (kg)	180
Total Product mass (crude PHB) (kg)	20000
Purity (%)	99.1
Recovery (%)	95.1

Table 4-15: Estimated batch volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the simulation of 20 tonnes of crude PHB per run with a surfactant-sodium hypochlorite purification and recovery process using the CeBER Bioprocess Modeller

Unit operation	Description	Batch volume (m^3)	Electricity (MJ)	Steam (kg)	Cooling water (m^3)
Sterilisation	Steam sterilisation	247	11270	30040	
Microbial growth	Bioreactor	247			
Aeration	Bioreactor High pressure homogeniser		338000		
Agitation			30220		
Post fermentation cooling					120800
Cell disruption		247	43710		
Solid-liquid separation 2	Centrifugal spin/washing	247	2979		
Conc./Purification 1	Surfactant mixing tank	81.6	470		
Conc./Purification 2	NaClO mixing tank	91.6	528		
Conc./Purification 3	Centrifugation	91.6	980.0		
Conc./Purification 4	Centrifugatiion with 2 washes	33.3 (for 1 wash)	532		
Formulation	Spray drying	34.4 (17.8 m^3 of water lost)*	58530		
Final product volume out	-	16.4			
Total	**-**		487200	30040	120800

* Water lost between Conc./purification 4 and formulation steps

The same design approach highlighted in Section 4.2.2 was used to calculate the residence time for each piece of equipment with a maximum continuous flow rate of 60 m^3/h. The total upstream time was 55 hours and the total downstream time up till the spray dryer was 15.7 hours, hence 39.3 hours were available for the spray dryer for the final product. The Gantt chart for the process is shown in Figure 4-12 and equipment costs are shown in Table 4-16.

Since the surfactant-sodium hypochlorite process had fewer processing steps, the equipment costs of $ 9.3 million were lower than base case scenario with equipment costs $ 9.6 million. The cost of the spray dryer for the surfactant-sodium hypochlorite process was $ 320 000, i.e lower than the price of $ 480 000 reported for the base case scenario. Since the downstream processing up to the spray dryer took only 15.6 hours compared to 34 hours for the base case, the spray dryer residence time for surfactant-sodium hypochlorite process was higher, resulting in lower evaporation rate. With the cost of the spray dryer calculated from the evaporation rate, this resulted in lower spray dryer equipment costs.

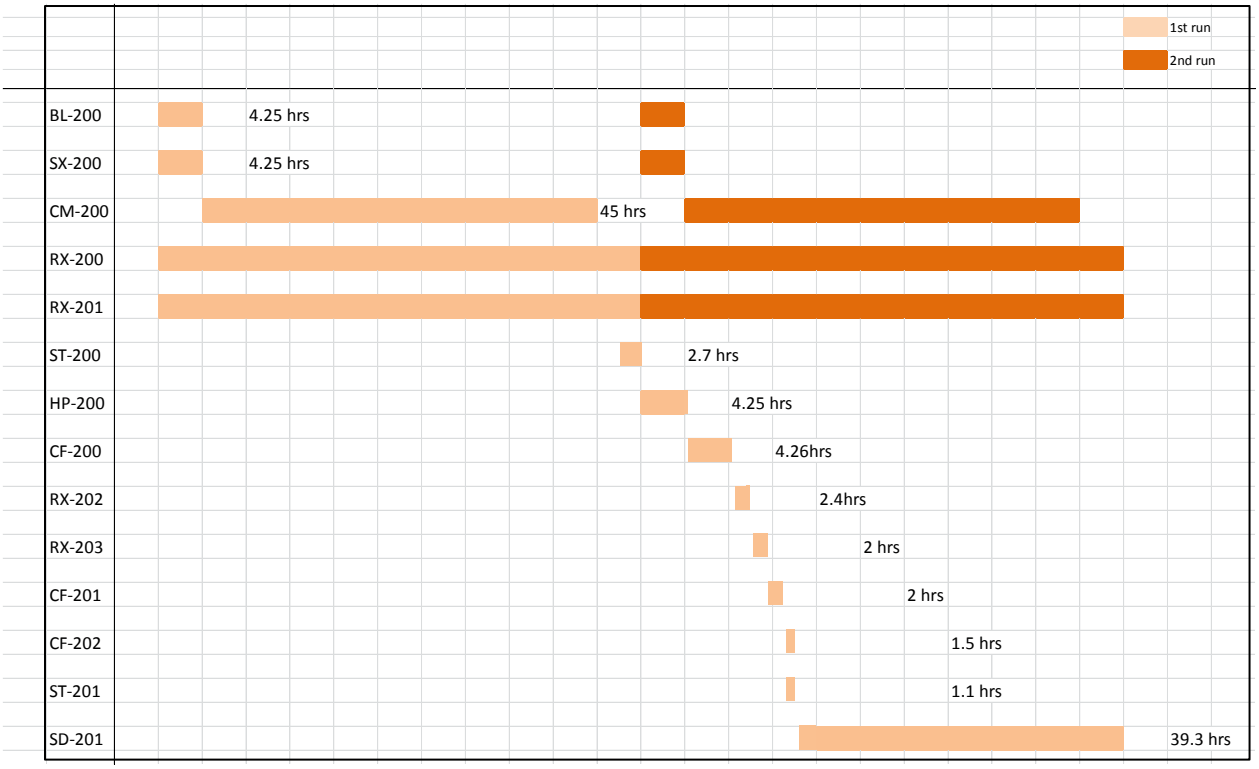


Figure 4-12: Gantt chart for the simulation of 20 tonnes of crude PHB per run with a sodium hypochlorite-surfactant purification and recovery process simulated in the CeBER Bioprocess Modeller

Table 4-16: Equipment costs for the simulation of 20 tonnes of crude PHB per run with a surfactant-sodium hypochlorite recovery process using the CeBER Bioprocess Modeller

	Scaling variable	**Size including overdesign factor**	**Cost (1000 $,2014)**
Compressor (CM-200)	Power (kW)	2349	1610
Air filters (AF-200, AF-201, AF-202)	Volumetric flow rate (m^3/s)	3	58.8
Blending tank (BL-200)	Volume (m^3)	309	277
Steriliser (SX-200)	Flowrate (m^3/hr)	73	254
Bioreactors (RX-200, RX-201)	Volume (m^3)	154	44300
Storage tank (ST-200)	Volume (m^3)	309	163
Homogeniser (HP-200)	Flowrate (m^3/hr)	73	675
Centrifuge (CM-200)	Sigma (m^2)	100694	354
Surfactant washing tank (RX-202)	Volume (m^3)	102	130
NaClO washing tank (RX-203)	Volume (m^3)	114	141
Centrifuge (CM-201)	Sigma (m^2)	100694	354
Centrifuge (CM-202)	Sigma (m^2)	100694	354
Storage tank (ST-201)	Volume (m^3)	43	42.7
Spray dryer (SD-200)	Water evaporated rate (kg/hr)	533	320
Total			9280

4.4.2 Sodium hydroxide recovery method

A second flowsheet using a sodium hydroxide recovery process, presented in Figure 4-13, was built using the procedure outlined in Section 3.4.2. Compared to the base case scenario, the sodium hydroxide process has two less processing steps. The mass and energy inventories are shown in Table 4-17. The estimated batch volume (m^3), electricity, stream and cooling requirements are shown in Table 4-19 and final product composition shown in Table 4-18.

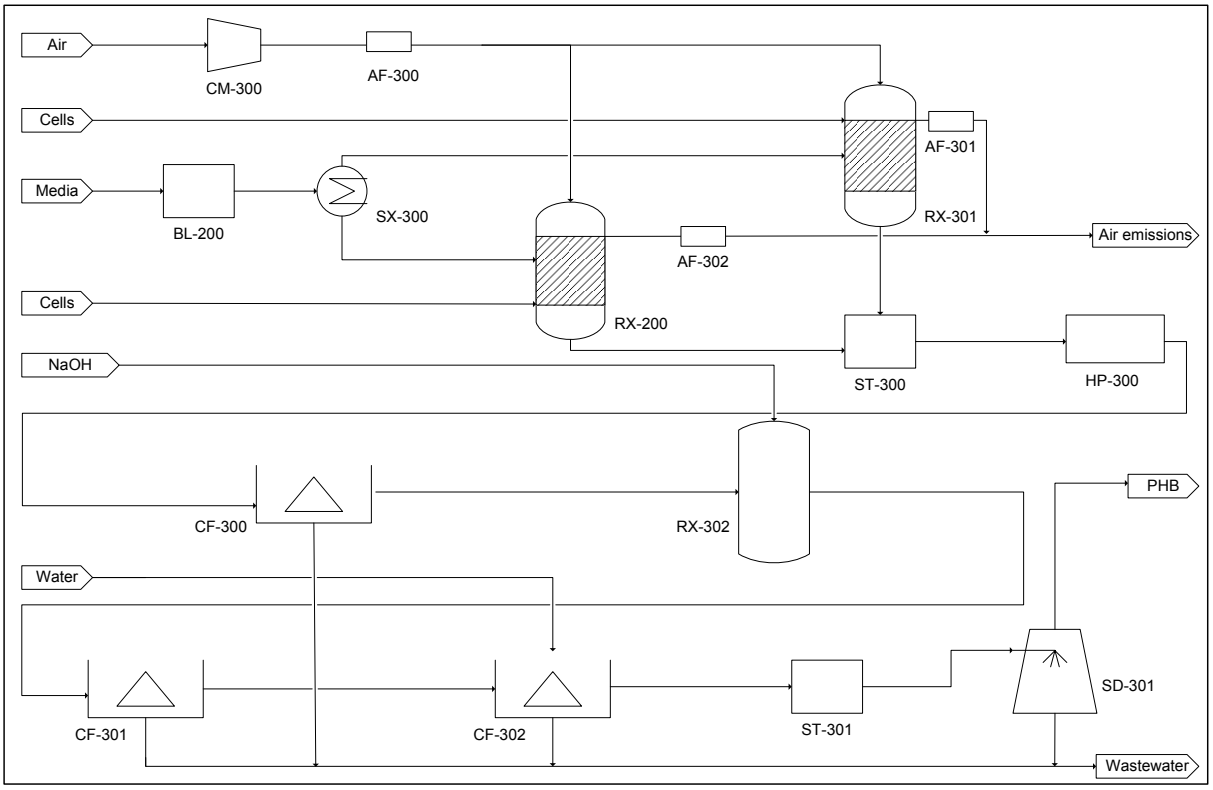


Figure 4-13: Process flowsheet for PHB production with a sodium hydroxide purification and recovery method developed in the CeBER Bioprocess Modeller simulating 20 tonnes of crude PHB per run

Similar to the surfactant-sodium hypochlorite process, the sodium hydroxide process required significantly less amount of water than the base case, resulting in an overall lower mass inventory. However, the PHB lost in the wastewater was higher than the base case since the recovery was only 90 % shown in Table 4-18. In order to produce 20 tonnes of crude PHB with 98 % purity at 90 % recovery, a larger microbial volume (256 m^3) than the base case scenario (245 m^3) was required. This is shown in Table 4-17. This translated to larger equipment and utilities needed. The same approach similar to the base case in Section 4.2.2 was applied to construct a Gantt chart for the sodium hydroxide recovery process, shown in Figure 4-14. With two less processing steps than the base case and one less step than the surfactant-sodium hypochlorite process, the sodium hydroxide process had the shortest

downstream time up till the spray dryer (13.3 hours), thus leaving 42.7 hours for the spray dryer processing. Although the sodium hypochlorite process had one less processing step than the surfactant-sodium hypochlorite recover process, its equipment costs were higher at $9.5 million shown in Table 4-20 compared to $9.3 million for the sodium hypochlorite process and $ 9.6 million for the base case scenario. This can be explained by larger equipment needed to process the higher volume of culture, arising due to the low recovery.

Table 4-17: Mass balance and utility requirements for the simulation of 20 tonnes of crude PHB per run with a sodium hydroxide process and the enzymatic digestion process used for the base case

IN			**OUT**		
	NaOH process	Base case (Enzymatic digestion)		NaOH process	Base case (Enzymatic digestion)
Process water (m^3)	229	636	Total pure PHB (kg)	19650	19270
Antifoam (kg)	205	196	Water (m^3)	260	665
Glucose (kg)	77320	73540	Antifoam (kg)	205	196
O_2 (kg)	540100	528300	*Cupriavidus necator* (kg)	12390	11780
N_2 (kg)	2032000	1987000	Sodium hydroxide (kg)	582	-
Ammonium sulphate	14640	13920	Enzyme (kg)	-	488
Sodium hydroxide	582	-	Detergent (kg)	-	6096
Enzyme (kg)	-	488	Hydrogen peroxide (kg)	-	1209
Detergent (kg)	-	6100	Potassium hydroxide (kg)	-	7891
Hydrogen peroxide (kg)	-	1213	Waste PHB (kg)	2095	1411
Potassium hydroxide (kg)		7896	Air emissions:		
			O_2 (kg)	514400	503700
			N_2 (kg)	2032000	1987000
			CO_2(kg)	45000	42800
			SO_2 (kg)	7097	6751
Total Mass (ton)	2894	3255	Total Mass (ton)	2894	3255

Note: Chemical oxygen demand (COD) of wastewater was 27110 kg for sodium hydroxide process and 27680 kg for the enzymatic process

Table 4-18: Final product stream composition for the simulation of 20 tonnes of crude PHB per run with a sodium hydroxide purification and recovery process using the CeBER Bioprocess Modeller

Total pure PHB (kg)	19650
Water (kg)	351
Total Product mass (crude PHB) (kg)	20000
Purity (%)	98.2
Recovery (%)	90.4

Table 4-19: Estimated batch volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the simulation of 20 tonnes of crude PHB per run with a sodium hydroxide purification and recovery process using the CeBER Bioprocess Modeller

Unit operation	Description	Batch volume (m^3)	Electricity (MJ)	Steam (kg)	Cooling water (m^3)
Sterilisation	Steam sterilisation	256	11640	31030	
Microbial growth	Bioreactor	256			
Aeration	Bioreactor High pressure homogeniser		350500		
Agitation			31340		
Post fermentation cooling					126000
Cell disruption		246	45600		
Solid-liquid separation 2	Centrifugal spin/washing	256	3088		
Conc./Purification 1	Sodium hydroxide tank	84.6	487		
Conc./Purification 2	Centrifugation	84.6	487		
Conc./Purification 3	Centrifugation with 2 washes	34.5 (for 1 wash)	549		
Formulation	Spray drying	40 (23.1 m^3 of water lost)*	76100		
Final product volume out	-	16.4			
Total			519800	310300	126000

* Water lost between Conc./purification 3 and formulation steps

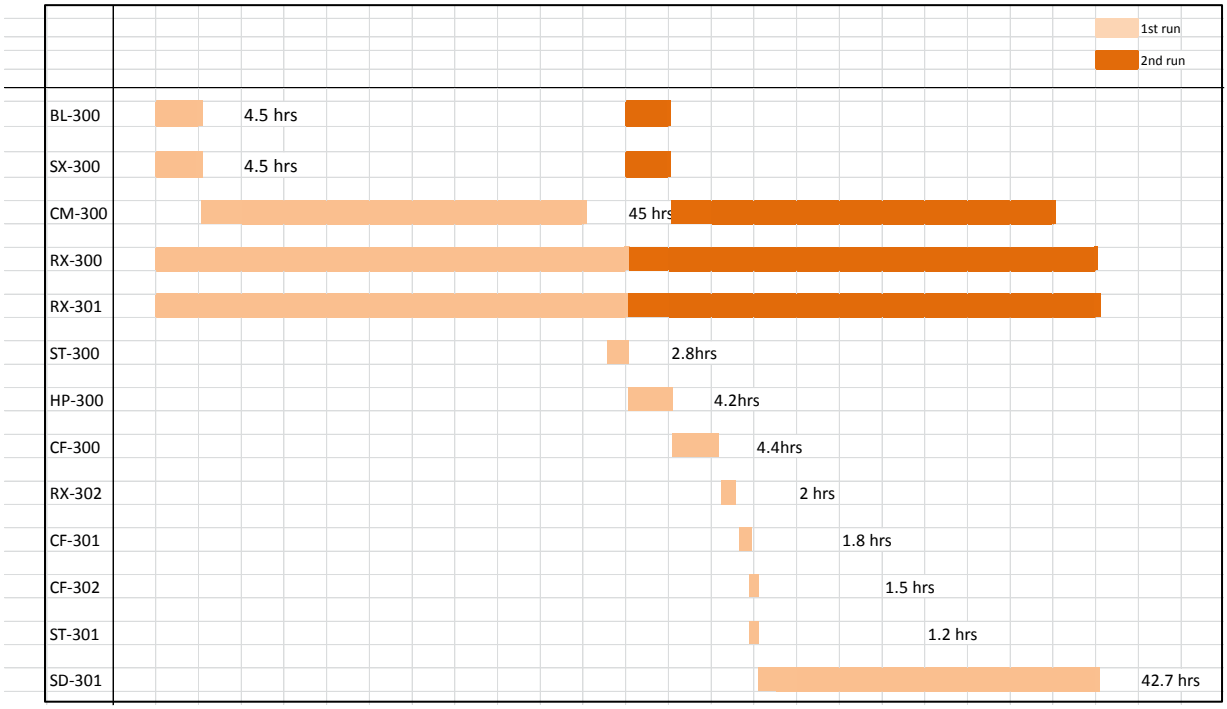


Figure 4-14: Gantt chart for the simulation of 20 tonnes of crude PHB per run with a sodium hydroxide purification and recovery process simulated in the CeBER Bioprocess Modeller

Table 4-20: Equipment costs for the simulation of 20 tonnes of crude PHB per run with a sodium hydroxide purification and recovery process using the CeBER Bioprocess Modeller

	Scaling variable	Size including overdesign factor	Cost (1000 $,2014)
Compressor (CM-300)	Power (kW)	2436	1790
Air filters (AF-300, AF-301, AF-302)	Volumetric flow rate (m^3/s)	3.2	60.1
Blending tank (BL-300)	Volume (m^3)	320	284
Steriliser (SX-300)	Flowrate (m^3/hr)	71	242
Bioreactors (RX-300, RX-301)	Volume (m^3)	160	4540
Storage tank (ST-300)	Volume (m^3)	308	163
Homogeniser (HP-300)	Flowrate (m^3/hr)	73	675
Centrifuge (CM-300)	Sigma (m^2)	100694	354
NaOH washing tank (RX-302)	Volume (m^3)	106	134
Centrifuge (CM-301)	Sigma (m^2)	100694	354
Centrifuge (CM-302)	Sigma (m^2)	100694	354
Storage tank (ST-301)	Volume (m^3)	50	47.3
Spray dryer (SD-300)	Water evaporated rate (kg/hr)	636	346
Total			9460

4.4.3 Process comparison among the three purification and recovery processes

As shown in Figure 4-15, both the surfactant-sodium hypochlorite and sodium hydroxide processes resulted in a lower production cost of PHB of $ 6.86 and $ 6.92 compared with $ 8.35 kg for the base case scenario, equivalent to a reduction of 18 % and 17 % respectively. The total energy usage was lowered by 9 % and 3 % for the surfactant-sodium hypochlorite and sodium hydroxide recovery processes respectively.

The water usage was significantly lower for the surfactant-sodium hypochlorite (11.2 kg/kg PHB) and sodium hydroxide processes (11.6 kg/kg PHB) when compared to the base case (33 kg/kg PHB), owing to a reduction in centrifugation and washing steps. The carbon dioxide emissions were the same for the surfactant-sodium hypochlorite recovery process and the base case scenario (2.2 kg/kg) and slightly higher (2.3 kg/kg) for the sodium hydroxide process. This was due to the low recovery of the sodium hydroxide process shown in Table 4-21 where a larger microbial culture was needed resulting in more carbon dioxide emissions during microbial growth. Table 4-21 shows the purification and recovery for each process and the corresponding total amount of PHB produced per year, demonstrating that a higher recovery and purity result in a higher annual PHB production.

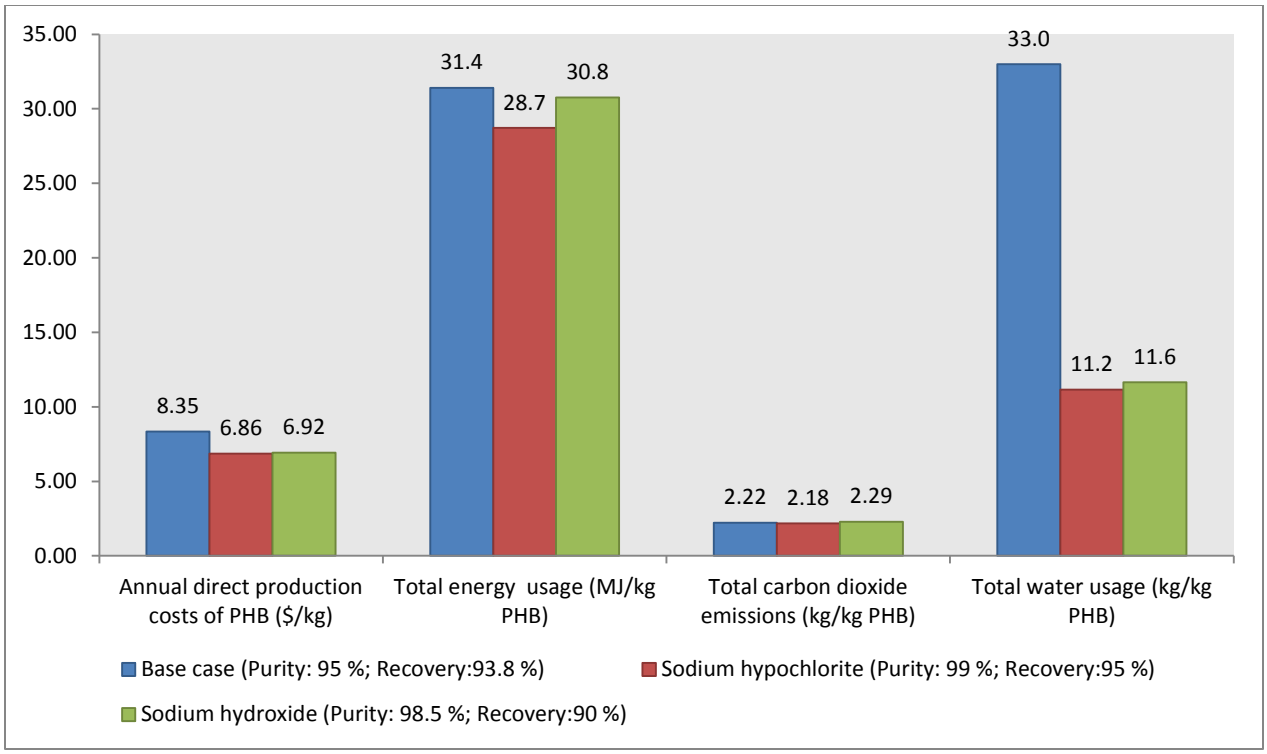


Figure 4-15: Effect of different purification and recovery processes on the annual large scale production of PHB simulated in the CeBER Bioprocess Modeller

Table 4-21: Effect of purification and recovery process on the annual large scale production of PHB simulated in the CeBER Bioprocess Modeller

Type of recovery process	Total time of one run (hours)	Volume of microbial culture for 1 run (m^3)	Total number of runs in one year	Amount of pure PHB produced in one run (kg)	Amount of pure PHB produced per year (tonnes)	Purity (%)	Recovery (%)
Base case (Enzymatic digestion)	55	245	152	19270	2929	96.4	93.2
Surfactant-sodium hypochlorite	55	247	152	19820	3013	99.1	95.1
Sodium hydroxide	56	256	150	19650	2948	98.2	90.4

From Table 4-22, it can be observed that the raw material costs for the surfactant-sodium hypochlorite and sodium hydroxide process were lower than the base case by 39 % and 46 % respectively since the costs of the surfactant, sodium hypochlorite and sodium hydroxide were lower than the high costs of the enzyme and detergent needed for the base case scenario. The DFC dependent (including plant

overheads) costs were 6 % lower for the surfactant-sodium hypochlorite process and 3 % lower for the sodium hydroxide process. The electricity costs were slightly lower for the surfactant-sodium hypochlorite recovery at $ 0.48/kg and the same for the sodium hydroxide process at $ 0.51/kg. With the DFC being lower for both processes investigated, the maintenance labour was lower resulting in a reduction cost of 5 % and 2 % in overall labour costs for the surfactant-sodium hypochlorite and sodium hydroxide processes respectively. The waste disposal costs were 24 % lower for the surfactant-sodium hypochlorite process since the recovery and purity of the final PHB were higher than the base case resulting in lower residual biomass, but only 2 % lower for the sodium hydroxide process because although higher purity of PHB was achieved, the overall recovery was lower (90 %) than the base case (93 %) resulting in higher residual biomass.

Table 4-22: Total annual production costs breakdown for the large scale production of PHB using either a surfactant-sodium hypochlorite or sodium hydroxide process instead of the enzymatic process used for the base case

Type of recovery process	Raw materials ($/kg)	Utilities ($/kg)	Electricity ($/kg)	Labour ($/kg)	Waste disposal ($/kg)	DFC dependent costs + plant overheads ($/kg)	Annual direct production costs of PHB ($/kg)
Base case (Enzymatic digestion)	2.81	0.13	0.51	0.82	0.50	3.58	8.35
Sodium hypochlorite	1.71	0.11	0.48	0.78	0.38	3.38	6.86
Sodium hydroxide	1.52	0.12	0.51	0.80	0.48	3.47	6.92

4.5 INVESTIGATING THE EFFECT OF MICROBIAL PROPERTIES ON PHB PRODUCTION

The investigated properties of the microorganism were the PHB content, final cell concentration and culture time related to growth rate and product formation rate. Their effects on the production cost of PHB were investigated through the use of factorial design analysis using the statistical software package Design-Expert® 9, previously explained in Section 3.4.3. In the approach used in the CeBER Bioprocess Modeller, the PHA concentration is expressed as a function of the final non-PHA biomass concentration. The PHA concentration is calculated from the difference of the non-PHA biomass and final cell concentration. Similarly, the PHA content is expressed as the ratio of the PHA concentration to the non-PHA biomass concentration. The three variables E_1 (final non-PHB biomass), E_2 (PHB: biomass ratio) and E_3 (culture time) were varied between a low and high level relative to the base case scenario values in Table 4-1 with the response being the calculated annual direct production costs of PHB ($/kg) using a 2^3

factorial design approach specified in Table 4-23. The mass and energy inventories along with the product specification and equipment costs are shown in Appendix B-1. The direct annual production costs of PHB were calculated in similar fashion to Table 4-9. All the models and associated costing calculations can be found in the accompanying CD-ROM.

Table 4-23: Coded and natural variables for 2^3 factorial design experiments to investigate the effects of the microbial properties on the production costs of PHB for the production of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

	Coded variable			Natural variables			Final PHB conc. (g/L)	Final cell conc. (g/L)	PHB content (%)	Productivity (g/L/h)	Response (Y) PHB production costs($/kg)
	X_1	X_2	X_3	E_1 (Final non-PHB biomass conc (g/L)	E_2 (PHB: Biomass ratio)	E_3 (Culture time (hours)					
Scenario 1	-1	-1	-1	40	2	40	80	120	67	2	9.64
Scenario 2	1	-1	-1	50	2	40	100	150	67	2.5	8.44
Scenario 3	-1	1	-1	40	3	40	120	160	75	3	6.79
Scenario 4	1	1	-1	50	3	40	150	200	75	3.75	6.49
Scenario 5	-1	-1	1	40	2	50	80	120	67	1.6	10.56
Scenario 6	1	-1	1	50	2	50	100	150	67	2	9.28
Scenario 7	-1	1	1	40	3	50	120	160	75	2.4	7.88
Scenario 8	1	1	1	50	3	50	150	200	75	3	7.19

Figure 4-16 illustrates the result of the 2^3 factorial design experiments. Unimportant effects usually have a normal distribution centered near zero while important effects have a normal distribution centered at their respective effect values (Engineering Statistics Handbook, 2003). The fitted line represents the expected location of the effects where they are insignificant (Minitab, 2015). Four effects were chosen since these did not conform to the line representing insignificance, resulting in the most significant model. The detailed ANOVA results for the 2^3 factorial design experiment are shown in Appendix B-1 in Table B-33. The highest effect was the PHB: biomass ratio (B) followed by the culture time (C) and final non-PHB biomass concentration (A). The positive effects for the PHB: biomass ratio and final non-PHB biomass concentration meant that at their higher levels, a lower response in the form of lower production costs of PHB was obtained while at lower level, higher production costs were attained. The contrary applied for the culture time which means that a higher cultivation time directly translated into higher production costs PHB and vice-versa. Interestingly the combination effect of AB i.e. the interaction between the PHB: biomass ratio and non-PHB biomass concentration was also significant. The eight scenarios presented in Table 4-23 were further analysed to consider the effects of the

microbial properties on energy usage, water usage and carbon dioxide emissions, reported in Figure 4-17. The breakdown of the production costs for the different scenarios is also presented in Table 4-25.

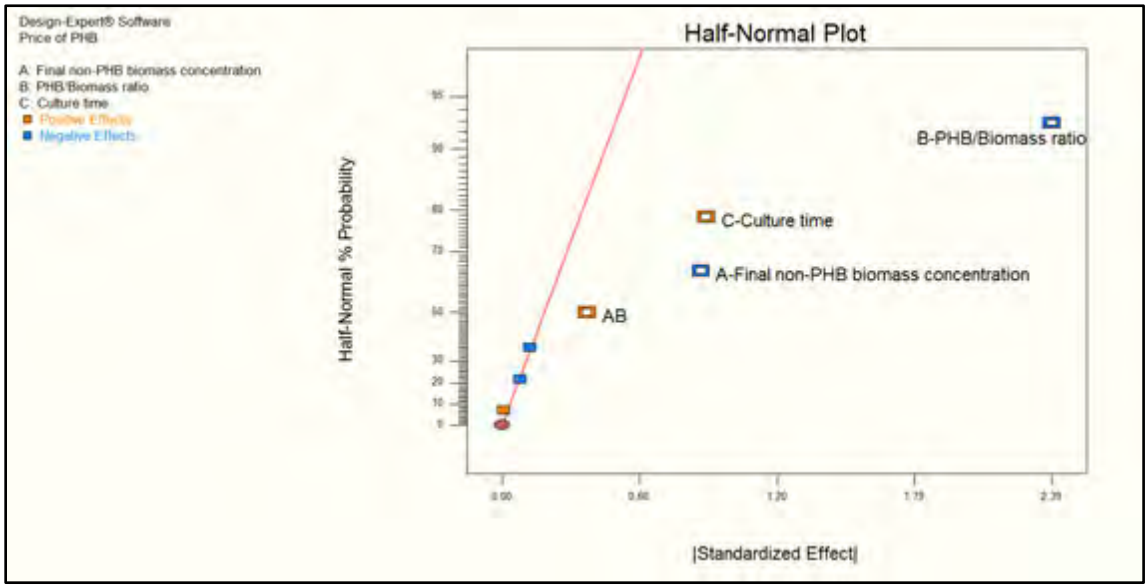
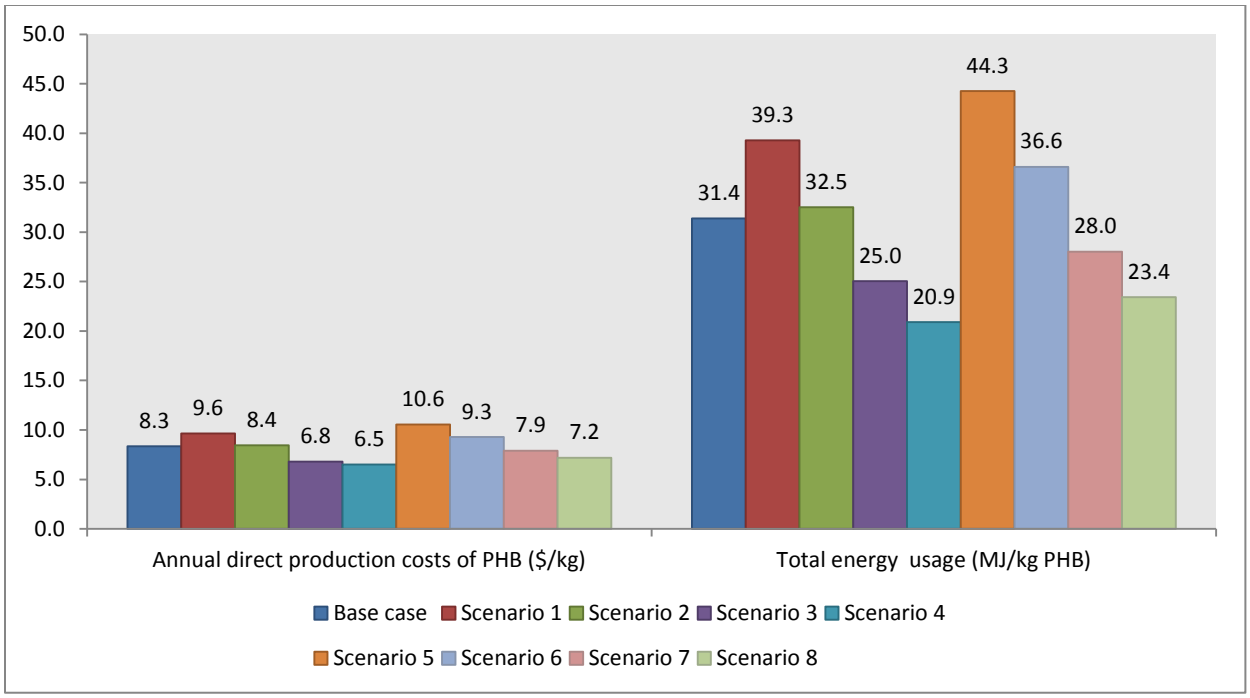


Figure 4-16: Half normal probability plot displaying the absolute values of the three effects, PHB biomass ratio, final non-PHB biomass (g/L), ratio and culture time (hours) with respect to production costs

In terms of PHB production costs, Scenario 4 was the best scenario with the highest PHB: biomass ratio (3), highest final non-PHB biomass (50 g/L) and lowest culture time (40 hours). Scenario 5 was the worst scenario with the lowest PHB: biomass ratio (2), lowest final non-PHB biomass (40 g/L) and highest culture time (50 hours). Scenario 4 resulted in a 22 % decrease in production costs of PHB, a 33 % decrease in energy usage, a 30 % decrease in water usage and a 7 % decrease in carbon dioxide emissions with respect to the base case scenario. On the other hand, Scenario 5 resulted in an increase of 27 % increase in production costs PHB, 41 % increase in energy usage, a 33 % increase in water usage and a 7 % increase in carbon dioxide emissions. According to Choi & Lee (1999), a lower PHB content resulted in more raw materials being converted to other metabolites. This increased the non-PHB biomass which consequently led to higher recovery costs of the pure PHB and higher waste disposal costs (Choi & Lee 1999). A lower PHB content meant more cells were needed to produce the same amount of pure PHB when compared to higher PHB containing cells, thus leading to an increase in size of bioreactors and subsequent downstream equipment and direct fixed capital costs (Choi & Lee 1999). Choi & Lee (1997) put forward that a high PHB productivity shortened the run time required, corresponding to more production runs in the given total annual operating time. This is demonstrated in Table 4-24 with Scenario 4 having the largest number of runs per year resulting in the highest annual

production rate of PHB. While the recovery remained the same across the eight scenarios, the purity varied with the scenarios having a higher PHB content resulting in higher final PHB purity. Table 4-25 illustrates the breakdown of the production costs for the eight scenarios. Since the PHB: biomass ratio had the largest effect on the production costs of PHB, Scenarios 3 ,4, 7 and 8 which had a high PHB: biomass ratio of 3 resulted in lower production costs than Scenarios 1, 2, 5 and 6 having a PHB: biomass ratio of 2. This observation was consistent across the raw materials, utilities costs and DFC costs, which varied with the PHB: biomass ratio. While previous studies by Choi & Lee (1998) postulated the effects of microbial properties by undertaking a sensitivity analysis upon comparing the PHB content and productivity to the final PHB production costs, the factorial design experiment done in this study added statistical evidence to support these observations. In addition, the relative importance of the different microbial properties with respect to decreasing PHB production costs was established in this study.



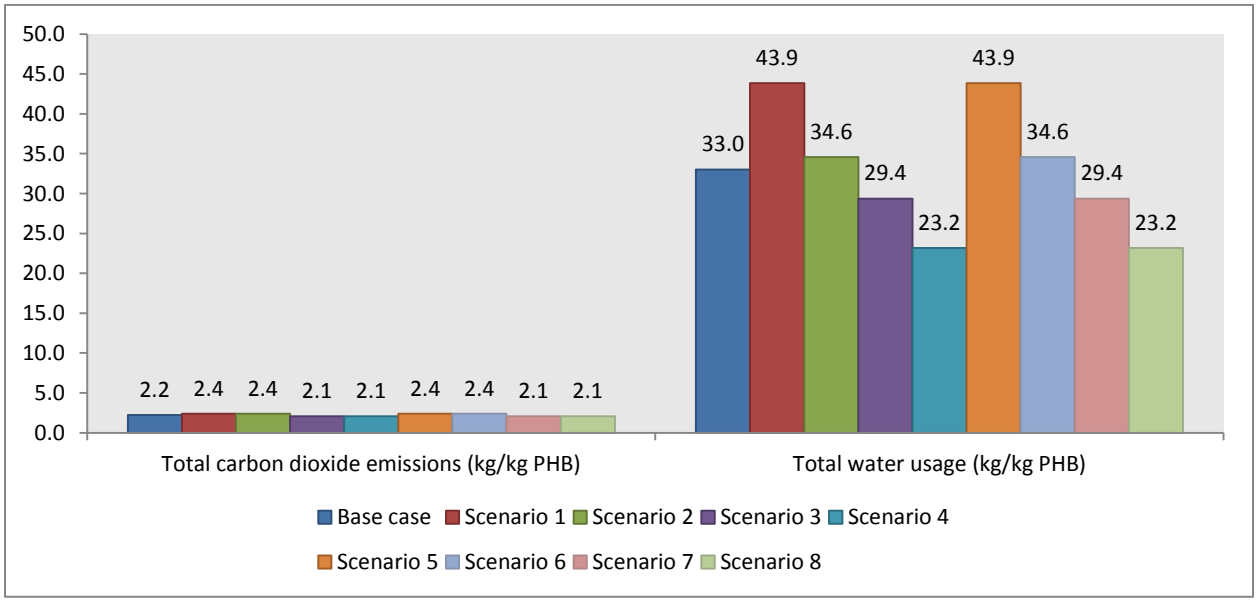


Figure 4-17: Effect of microbial properties for the annual large scale production of PHB simulated in the CeBER Bioprocess Modeller

Table 4-24: Effect of microbial properties on the annual large scale production of PHB simulated in the CeBER Bioprocess Modeller

	Total amount of time for one run (hours)	Volume of microbial culture for 1 run (m^3)	Total number of runs in one year	Amount of pure PHB produced in one run (kg)	Amount of pure PHB produced per year (tonnes)	Purity (%)	Recovery (%)
Base case	55	245	152	19270	2929	96.4	93.2
Scenario 1	53	313	158	19020	3005	95.2	93.2
Scenario 2	51	259	164	19220	3152	96.2	93.2
Scenario 3	50	219	168	19370	3254	96.9	93.2
Scenario 4	49	182	171	19510	3336	97.6	93.2
Scenario 5	63	313	133	19020	2530	95.2	93.2
Scenario 6	61	259	137	19220	2633	96.2	93.2
Scenario 7	60	219	140	19590	2743	98	93.2
Scenario 8	59	182	142	19510	2770	97.6	93.2

Table 4-25: Total annual production costs breakdown for the large scale production of PHB with different microbial properties

	Raw materials ($/kg)	Utilities ($/kg)	Electricity ($/kg)	Labour ($/kg)	Waste disposal ($/kg)	DFC dependent costs + plant overheads ($/kg)	Annual direct production costs of PHB ($/kg)
Base case	2.81	0.13	0.51	0.82	0.50	3.58	8.35
Scenario 1	3.32	0.15	0.64	0.88	0.58	4.07	9.61
Scenario 2	2.95	0.14	0.53	0.78	0.56	3.48	8.42
Scenario 3	2.58	0.12	0.40	0.70	0.43	2.85	7.24
Scenario 4	2.34	0.11	0.33	0.65	0.42	2.65	6.49
Scenario 5	3.32	0.15	0.74	1.04	0.58	4.74	10.56
Scenario 6	2.95	0.14	0.61	0.93	0.56	4.10	9.28
Scenario 7	2.55	0.12	0.45	0.81	0.42	3.52	7.88
Scenario 8	2.34	0.11	0.38	0.77	0.42	3.17	7.19

4.6 INVESTIGATING THE EFFECT OF CARBON SOURCES ON PHB PRODUCTION

As shown in the cost breakdown of the base case in Figure 4-4, a major cost in PHB production was the cost of the carbon substrate. Table 4-26 illustrates the impact of decreasing the price of the carbon substrate for the base case scenario resulting in a maximum reduction of 17 % in the production costs of PHB. While a variety of low cost substrates can be considered as potential substitutes to high cost pure carbon substrates, these are typically associated with reduced PHA content and lower productivity. Choi & Lee (1997) postulated that this could make PHA economics challenging. To investigate this issue, this

section extends with the scenarios presented in Section 4.5 whereby for each scenario the cost of the carbon substrate, namely glucose was decreased from $ 0.36 kg to a final $ 0/kg, i.e. assuming waste carbon substrate could be obtained free of costs. The results are shown in Table 4-27. This analysis was carried out to determine the relative importance of the microbial properties and the cost of carbon substrates.

Table 4-26: Effect of the carbon substrates' price on the annual production costs of PHB for the base case scenario

Price of C ($/kg)	PHB cost ($/kg)
0.36	8.35
0.30	8.12
0.20	7.74
0.10	7.35
0.00	6.97

Table 4-27: Effect of the carbon substrates' price on the annual production costs of PHB with the scenarios presented in Section 4.5

Scenario 1		**Scenario 2**		**Scenario 3**		**Scenario 4**	
Price of C ($/kg)	PHB cost ($/kg)	Price of C ($/kg)	PHB cost ($/kg)	Price of C ($/kg)	PHB cost ($/kg)	Price of C ($/kg)	PHB cost ($/kg)
0.36	9.64	0.36	8.44	0.36	6.79	0.36	6.49
0.30	9.39	0.30	8.20	0.30	6.57	0.30	6.28
0.20	8.99	0.20	7.80	0.20	6.21	0.20	5.92
0.10	8.59	0.10	7.39	0.10	5.85	0.10	5.56
0.00	8.18	0.00	6.99	0.00	5.49	0.00	5.20
Scenario 5		**Scenario 6**		**Scenario 7**		**Scenario 8**	
Price of C ($/kg)	PHB cost ($/kg)	Price of C ($/kg)	PHB cost ($/kg)	Price of C ($/kg)	PHB cost ($/kg)	Price of C ($/kg)	PHB cost ($/kg)
0.36	10.60	0.36	9.28	0.36	7.88	0.36	7.19
0.30	10.30	0.30	9.04	0.30	7.67	0.30	6.97
0.20	9.91	0.20	8.64	0.20	7.31	0.20	6.61
0.10	9.51	0.10	8.23	0.10	6.96	0.10	6.24
0.00	9.11	0.00	7.83	0.00	6.60	0.00	6.25

Scenario 4 illustrates that with a waste carbon substrate obtained free of costs, a maximum possible reduction of 38% in the production costs of PHB with respect to the base case scenario could be achieved. However this could only be achieved when the microorganism properties were favourable to minimise the production cost of PHB i.e., a high PHB content and cell concentration along with a low cultivation time which was the case for Scenario 4. The worst case considered, i.e Scenario 5, had a lower PHB content and cell concentration with a higher cultivation time. Together with a hypothetical waste carbon source provided free of cost, the PHB production costs was $ 9.1 /kg, higher than $ 8.4/kg obtained for the base case scenario, for an average performing microorganism with glucose as carbon

substrate. This demonstrates that the low cost of carbon substrates does not present an isolated solution to lower the cost of production of PHAs; a good cell and PHA concentration are essential to ensure the economic viability of the large scale production of PHAs. In terms of metabolising waste carbon sources by the microorganism *Cupriavidus necator*, the highest productivity reported was obtained for potato starch by Haas *et al.* (2008). A scenario was thus built using starch as waste carbon source to investigate its effects on the PHB production system. The same base model was used, with the inputs shown in Table 3-1 but with the following changes: starch was used as carbon source instead of glucose, the cultivation time was 72 hours instead of 44.4 hours, the PHB: biomass ratio was calculated to be 0.85 and the non-PHB biomass concentration was 124 g/L, calculated using data from Haas *et al.* (2008) as detailed in Table 3-10. The starch was assumed to be free of cost. To be comparable to the base case scenario, the purity and recovery of the downstream processing were kept similar. The mass and energy inventories together with the equipment costs are shown in Appendix C-1. The direct annual production costs of PHB were calculated in similar fashion to Table 4-9. All the models and associated costing calculations can be found in the accompanying CD-ROM.

A significant increase in total energy usage of the process utilising starch as waste carbon source was observed in Figure 4-18, owing to the large microbial culture needed to produce the desired 20 tonnes of crude PHB per run, due to their low PHB content. This translated in greater carbon dioxide emissions as well. The water usage was lower for the starch process since the volume of PHB containing culture was lower and thus less water was needed for the centrifugation steps for washing. The direct production costs of PHB using starch as a carbon source, obtained free of cost, was $ 10.7 /kg PHB, which was 27 % higher than the base case. Since the starch process had a longer cultivation time, only 101 runs can be achieved during the total operating time of the plant compared to the 152 runs possible for the base case as illustrated in Table 4-28.

As shown in Table 4-29, the raw materials costs are significantly lower when using a waste carbon substrate but all remaining operating costs increased, especially the DFC costs (including plant overheads), owing to the larger equipment needed to process the cells. Similarly the waste disposal costs were higher as well because of the large amount of residual non-PHB biomass. This confirms the need for a microorganism that can accumulate enough PHB to make the large scale production economically feasible when coupled with a waste carbon substrate.

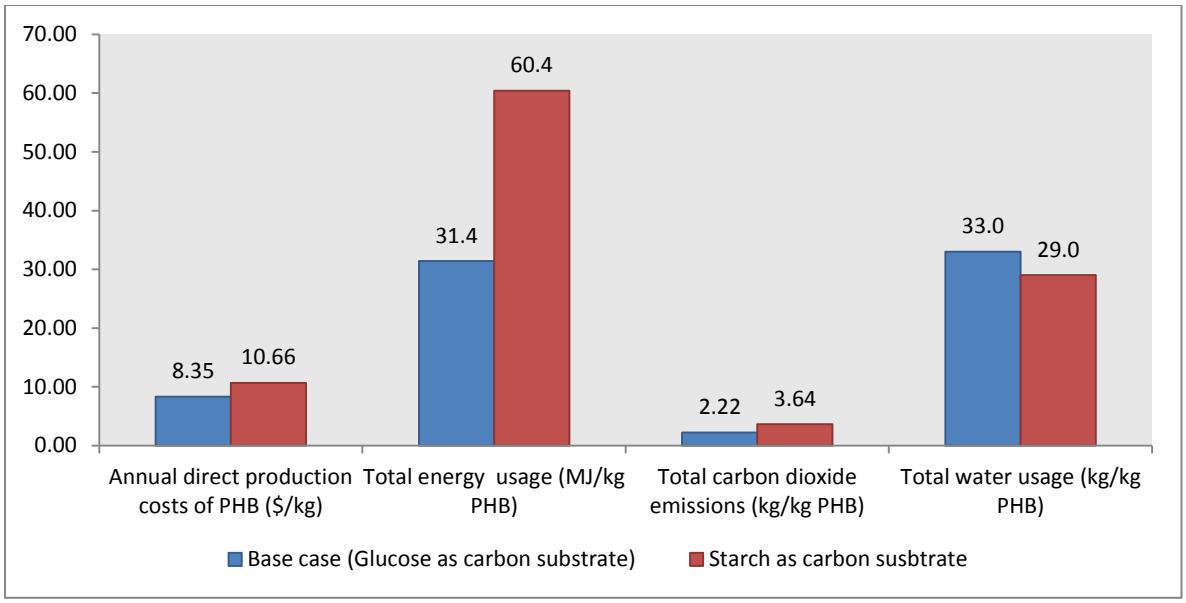


Figure 4-18: Effect of using starch as waste carbon source for the large scale production of PHB

Table 4-28: Effect of using starch as waste carbon source on the annual large scale production of PHB simulated in the CeBER Bioprocess Modeller

Type carbon source	Total amount of time for one run (hours)	Volume of microbial culture for 1 run (m^3)	Total number of runs in one year	Amount of pure PHB produced in one run (kg)	Amount of pure PHB produced per year (tonnes)	Purity (%)	Recovery (%)
Base case (glucose as carbon source)	55	245	152	19270	2929	96.4	93.2
Starch as waste carbon source	83	262	101	19220	1941	96.2	93.2

Table 4-29: Total costs breakdown for the production of PHB using starch waste as carbon source with an enzymatic digestion purification and recovery process

	Raw materials ($/kg)	Utilities ($/kg)	Electricity ($/kg)	Labour ($/kg)	Waste disposal ($/kg)	DFC dependent costs + plant overheads ($/kg)	Annual direct production costs of PHB ($/kg)
Base case (Glucose as carbon substrate)	2.81	0.13	0.51	0.82	0.50	3.58	8.35
Starch as waste carbon source	1.28	0.18	1.06	1.29	1.06	5.80	10.66

4.7 OPTIMISED CASE SCENARIO FOR PHB PRODUCTION

To integrate the findings, an optimised case scenario was developed for the production of 20 tonnes of crude PHB per year with a PHB: biomass ratio of 3, a final non-PHB concentration of 50 g/L and a culture time of 40 hours. It was assumed that a waste carbon source could be obtained free of cost delivering the same process dynamics and performance as that of the glucose process. The energy efficiency of the centrifuge and the homogeniser efficiency were assumed to be 70 % and 95 % respectively. The surfactant-sodium hypochlorite purification and recovery process was used for the extraction and purification of PHB. The mass and energy inventories together with the equipment costs are shown in Appendix D-1. The direct annual production costs of PHB were calculated in similar fashion to Table 4-9. All the models and associated costing calculations can be found in the accompanying CD-ROM. Figure 4-19 shows the optimisation potential of the base case integrating the findings of this study. As a result, the production costs of PHB were lowered to $ 4/kg, a 51 % decrease with respect to the base case process. Energy usage was decreased by 41 %, carbon dioxide emissions decreased by 14 % and the water usage was lowered by 77 %.

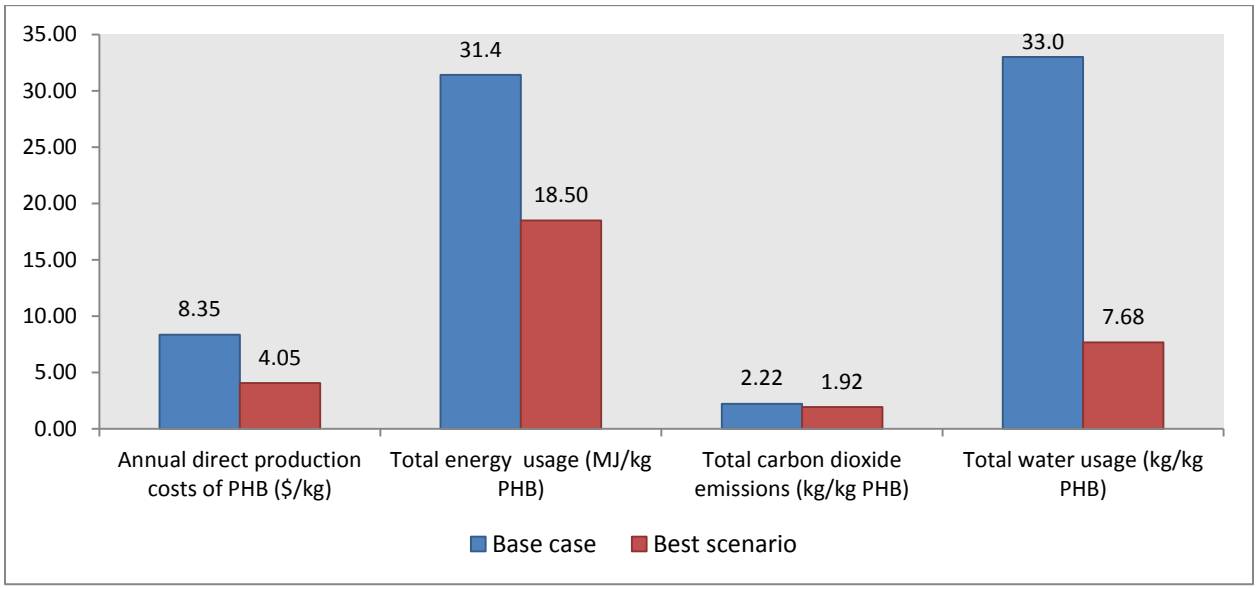


Figure 4-19: Investigation of an optimised case scenario on the large scale production of PHB with a PHB productivity of 3.8 g/L/h , a sodium hypochlorite-surfactant purification and recovery process with a cell rupture efficiency of 95 % and an energy efficiency of 70 % for the centrifuges, and a hypothetical carbon source assumed to be free of cost

The effects of a shorter cultivation time and higher PHB content and cell concentration resulted in a greater production of pure PHB per year, shown in Table 4-30. Table 4-31 shows the significant decrease in raw material costs due to free carbon source. Since the PHB content and concentration of the cells

were high, a smaller amount of cells was needed to produce 20 tonnes of crude PHB per run, resulting in smaller size equipment and thus lower DFC dependent costs. With a higher homogeniser efficiency, less non-PHB residual biomass was discarded as waste, thus explaining the lower costs for the waste disposal. With a more efficient and less water demanding recovery process, the best case scenario required less utilities and electricity costs.

Table 4-30: Investigation of an optimised case scenario on the annual large scale production of PHB with a PHB productivity of 3.8 g/L/h , a sodium hypochlorite-surfactant purification and recovery process with a cell rupture efficiency of 95 % and an energy efficiency of 70 % for the centrifuges and, a hypothetical carbon source assumed to be free of cost

	Total time of one run (hours)	Volume of microbial culture for 1 run (m^3)	Total number of runs in one year	Amount of pure PHB produced in one run (kg)	Amount of pure PHB produced per year (tonnes)	Purity (%)	Recovery (%)
Base case	55	245	152	19270	2929	96.4	93.2
Best case scenario	49	172	171	19880	3399	99.4	95.0

Table 4-31: Total costs breakdown for the production of PHB for an optimised case with a PHB productivity of 3.8 g/L/h , a sodium hypochlorite-surfactant purification and recovery process with a cell rupture efficiency of 95 % and an energy efficiency of 70 % for the centrifuges and, a hypothetical carbon source assumed to be free of cost

	Raw materials ($/kg)	Utilities ($/kg)	Electricity ($/kg)	Labour ($/kg)	Waste disposal ($/kg)	DFC dependent costs + plant overheads ($/kg)	Annual direct production costs of PHB ($/kg)
Base case	2.81	0.13	0.51	0.82	0.50	3.58	8.35
Best scenario	0.26	0.09	0.30	0.62	0.28	2.48	4.05

5 CONCLUSIONS

5.1 PHAS AS PROMISING ALTERNATIVES TO PETROLEUM PLASTICS

The literature review revealed the potential of PHAs as alternatives to petroleum plastics, owing to their versatility and suitability in various applications. The possibility of producing different PHAs with varying physical properties, based on the incorporation of different monomers, along with their bio-compatibility and bio-degradability in both land and sea set PHAs apart from other bioplastics such as PLA. However the high costs production of $ 4 - 9/kg has prevented PHAs from achieving economies of scale. Various factors affect the microbial production of PHAs on a large scale. The main factors were found to be the efficiency of cell disruption and the energy required for centrifugation, the type of purification and recovery used for PHAs, the microbial properties associated with PHA productivity and the high cost of the carbon substrate.

5.2 IMPORTANCE OF EARLY STAGE PROCESS SIMULATION AND ECONOMICS

Process simulation and economic assessment are powerful tools to identify approaches to lower the cost of production of PHAs so that investment can be directed to promising process options at pilot scale and further on to large scale. Process simulations are typically time consuming and expensive, demanding heavy investment and requiring the user to have prior knowledge of the simulation tool. In addition, most simulation tools require specific data inputs to function properly. These are proprietary knowledge of companies before product commercialisation. The aim of this study was to demonstrate the use of early stage process simulation and economics to investigate the large scale production of PHAs. This simulation at an early stage facilitated the informing of potential process options and optimisation that are necessary to lower the costs of production of PHAs, as well as energy usage, water usage and carbon dioxide emissions. The use of a generic PHA production flowsheet developed by Harding (2008) using experimental data from Harrison (1990) and simulated in the CeBER Bioprocess Modeller, was modified to simulate the large scale production of PHAs. This was used as the base case scenario. The type of PHA simulated was PHB using glucose as the carbon source and the microorganism C*upriavidus necator*. The type of purification and recovery process was the enzymatic digestion of PHB, followed by detergent and hydrogen peroxide treatment. It was decided to simulate 20 tonnes of crude PHB per run. The CeBER Bioprocess Modeller allowed calculations of material and energy inventories along with estimated volumes for each unit operation as simulation outputs. Engineering principles and

rules of thumbs were used for preliminary sizing of the equipment. The equipment was sized such that downstream processing could be completed during the next batch of PHB production which took 55 hours. With a plant running 8400 hours a year, 152 runs were possible resulting in the production of 2929 tonnes of pure PHB per year. The six-tenths rule was used to cost the equipment from previous economic studies on similar equipment. CEPCI indices were used to estimate the equipment costs in 2014. Using Lang factors, the direct fixed capital (DFC) could be calculated. The production costs of PHB for the base case scenario was calculated to be $ 8.4/kg with an energy usage of 31.4 MJ/kg PHB, a water usage of 33 kg/kg PHB and carbon dioxide emissions of 2.22 kg/kg PHB. The major costs were found to be the DFC dependent costs, which included the plant overheads costs (43 %) and the raw material costs (34 %) of which 50 % was allocated to the cost of glucose. With electricity being a significant contributor to the environmental footprint of the process as reported by Harding (2008), lower energy usage and lower carbon dioxide emissions are also desired. Fernandez-Docosta *et al.* (2015) also expressed the need to manage the water usage of the process to limit environmental burden due to water being a natural resource.

5.3 RELATIVE IMPORTANCE OF KEY FACTORS FOR THE LARGE SCALE PRODUCTION OF PHAS

The effect of the four factors specified in Section 5.1 were investigated and compared to the base case scenario in order to provide insight into their relative importance with respect to lowering the cost of PHB production along with minimising energy usage, water usage and carbon dioxide emissions.

Varying the energy efficiency of the centrifuge from 60 % to 50 or 70 % had no effect on the production costs of PHB. There was only a slight variation in the total energy usage of the process with more energy efficient centrifuges leading to a decrease in energy requirement. Varying the cell disruption efficiency in the homogeniser had a significant effect on the cost of PHB production. A more efficient homogeniser at 95 % yielded a PHB cost of production of $8/kg with lower energy usage, water usage and carbon dioxide emissions per year, since more PHB could be released from the cells. A less efficient homogeniser at 85 %, on the other hand increased the production costs to $ 8.8/kg with an increase in energy usage, water usage and carbon dioxide emissions per year.

The enzymatic digestion purification and recovery process, used in the base case scenario, was a water intensive one with its two centrifugation and washing steps. Both the surfactant-sodium hypochlorite and sodium hydroxide processes had a significantly lower water usage. With less processing steps than the base case, the equipment costs were lower with reduced DFC dependent costs, resulting in a lower

PHB production cost of $ 6.86/kg for the surfactant-sodium hypochlorite process and the $ 6.92/kg for the sodium hydroxide recovery process. The raw materials costs were also lower since the chemicals needed were less expensive than the enzyme and detergent used for the base case scenario. Energy usage was reduced due to less equipment being required and the volumes processed being reduced. The purification and recovery achieved also influenced performance. The sodium hydroxide process had a PHB recovery of 90% which required more culture to produce the desired amount of PHB when compared to the surfactant-sodium hypochlorite process with a recovery of 95 %. Thus, although the final production costs were similar, due to an increased cultivation volume, the sodium hydroxide process required 7.3 % more energy, 3.6 % more water and emitted 4.5 % more carbon dioxide than the surfactant-sodium hypochlorite process. Thus, along with lower PHB production costs, lower energy requirement, water usage and lower carbon dioxide emissions must be targeted in order to ensure that the overall production of PHB has the least possible negative impact on the environment.

The efficiency of the PHB production stage was recognised as critical with respect to both production costs and environmental impact. A 2^3 factorial design was carried out in which the PHB content varied between 67 % and 75 %, the non-PHB biomass concentration between 40 and 50 g/L and the culture time between 40 and 50 hours, yielding a total biomass concentration between 120 and 200 g/L with a volumetric PHB concentration between 80 and 150 g/L. The 2^3 factorial design analysis showed that the PHB: biomass ratio (PHB content), final non-PHB biomass (measure of cell concentration) and the culture time had significant effects on the cost of PHB production. The statistically significant variables were the PHB: biomass ratio (defining the amount of non-PHB biomass produced and impacting raw materials used) followed by the culture time (defining the equipment size and duration of run impacting electricity required) and final non-PHB biomass concentration (defining the volume of the system, impacting water and energy requirement and equipment size) as well as the interaction between the non-PHB biomass concentration and PHB: biomass ratio. A combination of high PHB: biomass ratio of 3, high final non-PHB biomass concentration of 50 g/L and low culture time of 40 hours resulted in a low cost of production of $ 6.5/kg. The energy and water usage along with the carbon dioxide emissions were decreased as well. This combination resulted in more PHB contained in the cells, thus smaller equipment sizes were needed to achieve the desired amount of PHB for a run. This resulted in more PHB produced yearly as well as lower waste disposal costs due to the smaller amount of residual biomass and lower energy costs due to the reduction in equipment size.

While the carbon source is a major contributor to production costs, a low cost carbon substrate must be coupled with the microorganism achieving sufficient PHB productivity in order to make the large scale production of PHB economically favourable. The lowest productivity in the 2^3 factorial design for the investigation of microbial properties was 1.6 g/L/h. This was considerably higher than the productivity reported for many studies on waste carbon sources. One of the highest productivities recorded for a waste carbon source was potato starch reported by Haas *et al.* (2008). This scenario was modelled in the CeBER Bioprocess Modeller. While the raw materials costs decreased substantially, the PHB production costs ($10.7/kg) was 28 % higher than the base case scenario owing to longer cultivation time resulting in less PHB produced yearly and high amount of residual biomass, increasing the waste disposal costs. There was an increase in equipment costs and subsequent energy usage since larger equipment were needed to process more cells with lower PHB content to achieve the desired amount of PHB per run. Thus if a waste carbon substrate is used in place of pure substrate, the overall process must be optimised to achieve sufficient PHB content to ensure good productivity to make PHB economics favourable.

5.4 KEY FINDINGS TOWARDS A COST EFFECTIVE LARGE SCALE PRODUCTION OF PHAS

With the DFC (including plant overheads) being the largest contributor to PHB production costs, production systems with lower equipment cost result in more cost effective PHB production as well as reduced energy usage, water usage and carbon dioxide emissions. From this study, it was found that the productivity defined by the microorganism's properties had the largest impact PHB production. High productivity was ensured by a high PHB content, high cell concentration and low cultivation time. This translated to a higher PHB annual production rate and lower equipment costs. Subsequently energy, water usage and carbon dioxide emissions were reduced. Secondly the purification and recovery process affected the equipment costs as well as energy use and water use significantly. With the carbon source being the second largest contributor to the production costs, cheaper carbon sources could lower the PHB production costs significantly. This can only be achieved if a good PHB productivity is maintained to ensure lower production costs. Lastly, a high homogeniser efficiency released more PHB leading to a higher production rate of PHB. The energy efficiency of the centrifuges has the least effect on production costs but it influenced the energy requirement of the overall process.

The optimum values for the four process factors investigated were used to develop an optimised scenario for the large scale production of PHB which resulted in a 51 % decrease for production cost of PHB from $ 8.4/kg to $ 4/kg, a 41 % decrease in energy usage from 31.4 MJ/kg to 18.5 MJ/kg PHB, a 77

% decrease in water usage from 33 kg/kg to 7.7 kg/kg PHB and a 14 % decrease in carbon dioxide emissions from 2.2 kg/kg to 1.9 kg/kg PHB. This shows the extent of optimisation on the overall PHB production process should these combined conditions be sustained in the process. While this model is a hypothetical process, it shows the path towards identifying the key areas that need optimisation in the large scale production of PHB. A high PHB performance production system in the form of high PHB content, concentration and low culture time, is essential to ensure high volumetric productivity of PHB, requiring less cells to achieve a desired amount of PHB. This translates into smaller bioreactors and downstream processing and thus lowers the DFC costs of the process. This ensures less energy usage as well. A lower cell requirement also means that less carbon dioxide is released during microbial growth. For similar reasons, optimising the cell rupture step can benefit the whole production system. If the high PHB productivity is coupled with a simple purification and recovery process, further costs can be reduced. Water usage can be decreased if the purification and recovery process can recover the PHB granules with the minimum number of washes in the centrifuges. High purity and recovery is also desired in order to maximise the amount of PHB produced and minimise residual biomass waste. Replacing the expensive pure carbon substrate by a waste carbon source free of cost may further decrease the costs of raw materials. However reduced production costs can only be achieved if the waste carbon source can yield a PHB productivity that can ensure commercial success.

5.5 IMPACT OF STUDY AND RECOMMENDATIONS FOR FUTURE WORK

This study was conducted for the most common type of PHA, PHB but the findings can be extended to other PHAs. This study helps to establish a platform to further drive the optimisation of PHA production. Insight has been given into the relative importance of various process factors with respect to the optimisation of the overall PHA production system. This can be useful to guide laboratory scale experiments since unfavourable PHA production systems can be identified upfront based on the PHA content and concentration achieved along with the required cultivation time and corresponding purification and recovery process. Similarly, promising waste carbon substrates that can potentially deliver favourable process economics can be targeted based on their productivity. Essentially, the findings of this study can be used as a screening tool to select promising PHA production systems from both an economic and environmental perspective. This study was limited to the most common PHA producing microorganism *Cupriavidus necator*. This approach can be extended to other microorganisms. Further process optimisation and sequencing of unit operations can deliver a more efficient production system. Further process economics in the form of a profitability analysis can inform on the return of

investment and break even time of the PHA facility. LCA studies can be performed to deliver further environmental assessment. These additional investigations can lead to a more robust platform to drive PHA optimisation.

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APPENDIX

A-1: EFFECT OF EQUIPMENT EFFICIENCY ADDITIONAL DATA

Table A-1: Mass balance and utility requirements for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller with a centrifuge energy efficiency of 50 %

IN		**OUT**	
Process water (m^3)	492	Total pure PHB (kg)	19270
Antifoam (kg)	196	Water (m^3)	521
Glucose (kg)	73540	Antifoam (kg)	196
O_2 (kg)	528300	*Cupriavidus necator* (kg)	11780
N_2 (kg)	1987000	Enzyme (kg)	488
Ammonium sulphate (kg)	13920	Detergent (kg)	6096
Enzyme (kg)	488	Hydrogen peroxide (kg)	1209
Detergent (kg)	6100	Water for downstream (kg)	143900
Hydrogen peroxide (kg)	1213	Potassium hydroxide (kg)	7891
Water for downstream (kg)	144400	Waste PHB (kg)	1411
Potassium hydroxide (kg)	7896	Air emissions:	
		O_2 (kg)	503700
		N_2 (kg)	1987000
		CO_2 (kg)	42800
		SO_2 (kg)	6751
Total Mass (ton)	3255	Total Mass (ton)	3255

Note: Chemical oxygen demand (COD) of wastewater was 27680 kg

Table A-2: Estimated batch volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller with a centrifuge energy efficiency of 50 %

Unit operation	**Description**	**Batch volume (m^3)**	**Electricity (MJ)**	**Steam (kg)**	**Cooling water (m^3)**
Sterilisation	Steam sterilisation	245	13630	36350	
Microbial growth	Bioreactor	245			
Aeration			342800		
Agitation			30650		
Post fermentation cooling					119800
Cell disruption	High pressure homogeniser	245	43380		
Solid-liquid separation 2	Centrifugal spin/washing	245	3547		
Conc./Purification 1	Enzyme mixing tank	81	466.4		
Conc./Purification 2	Detergent mixing tank	87.1	501.5		
Conc./Purification 3	Centrifugation with 5 washes	151 (for 1 wash)	5959		
Conc./Purification 4	H_20_2 addition tank	87.1	501.5		
Conc./Purification 5	Centrifugation with 2 washes	150 (for 1 wash)	3347		
Formulation	Spray drying	87.1 (19.3 m^3 of water lost)*	63430		
Final product volume out	-	16.5			
Total	**-**		508200	36350	119800

* Water lost between Conc./purification 5 and formulation steps

Table A-3: Final product stream composition for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller with a centrifuge energy efficiency of 50 %

Total pure PHB(kg)	19270
Water (kg)	195
Enzyme (kg)	0.273
Detergent (kg)	3.41
Hydrogen peroxide (kg)	4.23
Water (kg)	504
Potassium hydroxide (kg)	4.41
Total product mass (crude PHB) (kg)	19983
Purity (%)	96.4
Recovery (%)	93.2

Table A-4: Equipment cost for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller with a centrifuge energy efficiency of 50 %

	Scaling variable	**Size including overdesign factor**	**Cost ($,2014)**
Compressor (CM-100)	Power (kW)	2382	1760
Air filters (AF-100, AF-101, AF-102)	Volumetric flow rate (m^3/s)	3	58.5
Blending tank (BL-100)	Volume (m^3)	306	275
Steriliser (SX-100)	Flowrate (m^3/hr)	68	244
Bioreactors (RX-100, RX-101)	Volume (m^3)	153	4400
Storage tank (ST-100)	Volume (m^3)	306	162
Homogeniser (HP-100)	Flowrate (m^3/hr)	73	675
Centrifuge (CF-100)	Sigma (m^2)	100694	354
Enzyme washing tank (RX-102)	Volume (m^3)	101	130
Detergent washing tank (RX-103)	Volume (m^3)	109	136
Centrifuge (CF-101)	Sigma (m^2)	100694	354
H_2O_2 tank (RX-104)	Volume (m^3)	109	136
Centrifuge (CF-102)	Sigma (m^2)	100694	354
Storage tank (ST-101)	Volume (m^3)	109	80.2
Spray dryer (SD-100)	Water evaporated rate (kg/hr)	1276	468
Total			9590

Table A-5: Mass balance and utility requirements for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller with a centrifuge energy efficiency of 70 %

IN		OUT	
Process water (m^3)	492	Total pure PHB (kg)	19270
Antifoam (kg)	196	Water (m^3)	521
Glucose (kg)	73540	Antifoam (kg)	196
O_2 (kg)	528300	*Cupriavidus necator* (kg)	11780
N_2 (kg)	1987000	Enzyme (kg)	488
Ammonium sulphate (kg)	13920	Detergent (kg)	6096
Enzyme (kg)	488	Hydrogen peroxide (kg)	1209
Detergent (kg)	6100	Water for downstream (kg)	143900
Hydrogen peroxide (kg)	1213	Potassium hydroxide (kg)	7891
Water for downstream (kg)	144400	Waste PHB (kg)	1411
Potassium hydroxide (kg)	7896	Air emissions:	
		O_2 (kg)	503700
		N_2 (kg)	1987000
		CO_2 (kg)	42800
		SO_2 (kg)	6751
Total Mass (ton)	3255	Total Mass (ton)	3255

Note: Chemical oxygen demand (COD) of wastewater was 27680 kg

Table A-6: Estimated batch volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller with a centrifuge energy efficiency of 70 %

Unit operation	Description	Batch volume (m^3)	Electricity (MJ)	Steam (kg)	Cooling water (m^3)
Sterilisation	Steam sterilisation	245	13630	36350	
Microbial growth	Bioreactor	245			
Aeration			342800		
Agitation			30650		
Post fermentation cooling					119800
Cell disruption	High pressure homogeniser	245	43380		
Solid-liquid separation 2	Centrifugal spin/washing	245	2534		
Conc./Purification 1	Enzyme mixing tank	81	466.4		
Conc./Purification 2	Detergent mixing tank	87.1	501.5		
Conc./Purification 3	Centrifugation with 5 washes	151 (for 1 wash)	4256		
Conc./Purification 4	H_20_2 addition tank	87.1	501.5		
Conc./Purification 5	Centrifugation with 2 washes	150 (for 1 wash)	2390		
Formulation	Spray drying	87.1	63430		
Final product volume out	-	16.5 (19.3 m^3 of water lost)*			
Total	**-**		504500	36350	119800

* Water lost between Conc./purification 5 and formulation steps

Table A-7: Final product stream composition for the simulation of 20 tonnes of crude PHB per using the CeBER Bioprocess Modeller with a centrifuge energy efficiency of 70 %

Total pure PHB (kg)	19270
Water (kg)	195
Enzyme (kg)	0.273
Detergent (kg)	3.41
Hydrogen peroxide (kg)	4.23
Water (kg)	504
Potassium hydroxide (kg)	4.41
Total product mass (crude PHB) (kg)	19983
Purity (%)	96.44
Recovery (%)	93.2

Table A-8: Equipment cost for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller with a centrifuge energy efficiency of 70 %

	Scaling variable	**Size including overdesign factor**	**Cost (1000 $,2014)**
Compressor (CM-100)	Power (kW)	2382	1760
Air filters (AF-100, AF-101, AF-102)	Volumetric flow rate (m^3/s)	3	58.5
Blending tank (BL-100)	Volume (m^3)	306	275
Steriliser (SX-100)	Flowrate (m^3/hr)	68	244
Bioreactors (RX-100, RX-101)	Volume (m^3)	153	4400
Storage tank (ST-100)	Volume (m^3)	306	162
Homogeniser (HP-100)	Flowrate (m^3/hr)	73	675
Centrifuge (CF-100)	Sigma (m^2)	100694	354
Enzyme washing tank (RX-102)	Volume (m^3)	101	130
Detergent washing tank (RX-103)	Volume (m^3)	109	136
Centrifuge (CF-101)	Sigma (m^2)	100694	354
H_2O_2 tank (RX-104)	Volume (m^3)	109	136
Centrifuge (CF-102)	Sigma (m^2)	100694	354
Storage tank (ST-101)	Volume (m^3)	109	80.2
Spray dryer (SD-100)	Water evaporated rate (kg/hr)	1276	468
Total			9590

Table A-9: Mass balance and utility requirements for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller with a homogeniser efficiency of 85 %

IN		OUT	
Process water (m^3)	514	Total pure PHB (kg)	19220
Antifoam (kg)	207	Water (m^3)	546
Glucose (kg)	77650	Antifoam (kg)	207
O_2 (kg)	557800	*Cupriavidus necator* (kg)	13650
N_2 (kg)	2098000	Enzyme (kg)	507
Ammonium sulphate (kg)	14700	Detergent (kg)	6339
Enzyme (kg)	507	Hydrogen peroxide (kg)	1257
Detergent (kg)	6343	Water for downstream (kg)	149600
Hydrogen peroxide (kg)	1262	Potassium hydroxide (kg)	8205
Water for downstream (kg)	150200	Waste PHB (kg)	1407
Potassium hydroxide (kg)	8210	Air emissions:	
		O_2 (kg)	531900
		N_2 (kg)	2098000
		CO_2 (kg)	45190
		SO_2 (kg)	7128
Total Mass (ton)	3429	Total Mass (ton)	3429

Note: Chemical oxygen demand (COD) of wastewater was 31220 kg

Table A-10: Estimated batch volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller with a homogeniser efficiency of 85 %

Unit operation	Description	Batch volume (m^3)	Electricity (MJ)	Steam (kg)	Cooling water (m^3)
Sterilisation	Steam sterilisation	259	14320	38170	
Microbial growth	Bioreactor	259			
Aeration			361900		
Agitation			32370		
Post fermentation cooling					126500
Cell disruption	High pressure homogeniser	259	47310		
Solid-liquid separation 2	Centrifugal spin/washing	259	3109		
Conc./Purification 1	Enzyme mixing tank	85.9	492.8		
Conc./Purification 2	Detergent mixing tank	91.9	529.4		
Conc./Purification 3	Centrifugation with 5 washes	159 (for 1 wash)	5181		
Conc./Purification 4	H_20_2 addition tank	91.9	529.4		
Conc./Purification 5	Centrifugation with 2 washes	156 (for 1 wash)	2900		
Formulation	Spray drying	91.9 (20.8 m3 of water lost)*	68360		
Final product volume out	-	16.5			
Total	**-**		537000	38170	126500

* Water lost between Conc./purification 5 and formulation steps

Table A-11: Final product stream composition for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller with a homogeniser efficiency of 85 %

Total pure PHB (kg)	19220
Water (kg)	210
Enzyme (kg)	0.293
Detergent (kg)	3.67
Hydrogen peroxide (kg)	4.51
Water (kg)	537
Potassium hydroxide (kg)	4.744
Total product mass (crude PHB) (kg)	19982
Purity (%)	96.2
Recovery (%)	93.2

Table A-12: Equipment cost for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller with a homogeniser efficiency of 85 %

	Scaling variable	**Size including overdesign factor**	**Cost (1000 $,2014)**
Compressor (CM-100)	Power (kW)	2515	1830
Air filters (AF-100, AF-101, AF-102)	Volumetric flow rate (m^3/s)	3.2	60.5
Blending tank (BL-100)	Volume (m^3)	323	286
Steriliser (SX-100)	Flowrate (m^3/hr)	68	243
Bioreactors (RX-100, RX-101)	Volume (m^3)	161	4570
Storage tank (ST-100)	Volume (m^3)	323	168
Homogeniser (HP-100)	Flowrate (m^3/hr)	73	675
Centrifuge (CF-100)	Sigma (m^2)	100694	354
Enzyme washing tank (RX-102)	Volume (m^3)	107	135
Detergent washing tank (RX-103)	Volume (m^3)	115	141
Centrifuge (CF-101)	Sigma (m^2)	100694	310
H_2O_2 tank (RX-104)	Volume (m^3)	115	141
Centrifuge (CF-102)	Sigma (m^2)	100694	354
Storage tank (ST-101)	Volume (m^3)	115	83.3
Spray dryer (SD-100)	Water evaporated rate (kg/hr)	1194	455
Total			9810

Table A-13: Mass balance and utility requirements for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller with a homogeniser efficiency of 95 %

IN		OUT	
Process water (m^3)	471	Total pure PHB (kg)	19320
Antifoam (kg)	186	Water (m^3)	499
Glucose (kg)	69850	Antifoam (kg)	186
O_2 (kg)	501700	*Cupriavidus necator* (kg)	10100
N_2 (kg)	1887000	Enzyme (kg)	470
Ammonium sulphate (kg)	13220	Detergent (kg)	5878
Enzyme (kg)	471	Hydrogen peroxide (kg)	1166
Detergent (kg)	5881	Water for downstream (kg)	138800
Hydrogen peroxide (kg)	1170	Potassium hydroxide (kg)	7608
Water for downstream (kg)	139200	Waste PHB (kg)	1414
Potassium hydroxide (kg)	7613	Air emissions:	
		O_2 (kg)	478400
		N_2 (kg)	1887000
		CO_2 (kg)	40650
		SO_2 (kg)	6411
Total Mass (ton)	3098	Total Mass (ton)	3098

Note: Chemical oxygen demand (COD) of wastewater was 24500 kg

Table A-14: Estimated batch volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller with a homogeniser efficiency of 95 %

Unit operation	Description	Batch volume (m^3)	Electricity (MJ)	Steam (kg)	Cooling water (m^3)
Sterilisation	Steam sterilisation	233	13020	34710	
Microbial growth	Bioreactor	233			
Aeration			325500		
Agitation			29110		
Post fermentation cooling					113800
Cell disruption	High pressure homogeniser	233	39830		
Solid-liquid separation 2	Centrifugal spin/washing	233	2819		
Conc./Purification 1	Enzyme mixing tank	76.8	442.5		
Conc./Purification 2	Detergent mixing tank	82.7	476.4		
Conc./Purification 3	Centrifugation with 5 washes	144 (for 1 wash)	4772		
Conc./Purification 4	H_20_2 addition tank	82.7	476.4		
Conc./Purification 5	Centrifugation with 2 washes	144 (for 1 wash)	2688		
Formulation	Spray drying	82.7 (18 m^3 of water)*	59020		
Final product volume out	-	16.5			
Total	**-**		478200	34710	113800

* Water lost between Conc./purification 5 and formulation steps

Table A-15: Final product stream composition for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller with a homogeniser efficiency of 95 %

Total pure PHB (kg)	19320
Water (kg)	182
Enzyme (kg)	0.254
Detergent (kg)	3.18
Hydrogen peroxide (kg)	3.98
Water (kg)	474
Potassium hydroxide (kg)	4.112
Total product mass (crude PHB) (kg)	19984
Purity (%)	96.7
Recovery (%)	93.2

Table A-16: Equipment cost for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller with a homogeniser efficiency of 95 %

	Scaling variable	**Size including overdesign factor**	**Cost (1000 $,2014)**
Compressor (CM-100)	Power (kW)	2262	1690
Air filters (AF-100, AF-101, AF-102)	Volumetric flow rate (m^3/s)	2.9	56.7
Blending tank (BL-100)	Volume (m^3)	291	266
Steriliser (SX-100)	Flowrate (m^3/hr)	68	245
Bioreactors (RX-100, RX-101)	Volume (m^3)	145	4250
Storage tank (ST-100)	Volume (m^3)	290	157
Homogeniser (HP-100)	Flowrate (m^3/hr)	73	675
Centrifuge (CF-100)	Sigma (m^2)	100694	354
Enzyme washing tank (RX-102)	Volume (m^3)	96	125
Detergent washing tank (RX-103)	Volume (m^3)	103	132
Centrifuge (CF-101)	Sigma (m^2)	100694	354
H_2O_2 tank (RX-104)	Volume (m^3)	103	132
Centrifuge (CF-102)	Sigma (m^2)	100694	354
Storage tank (ST-101)	Volume (m^3)	103	77.5
Spray dryer (SD-100)	Water evaporated rate (kg/hr)	952	412
Total			9280

B-1: EFFECT OF MICROBIAL PROPERTIES ADDITIONAL DATA

Table B-1: Scenario 1 mass balance and utility requirements for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

IN		OUT	
Process water (m^3)	647	Total pure PHB (kg)	19020
Antifoam (kg)	250	Water (m^3)	678
Glucose (kg)	76820	Antifoam (kg)	250
O_2 (kg)	654800	*Cupriavidus necator* (kg)	13510
N_2 (kg)	2463000	Enzyme (kg)	633
Ammonium sulphate (kg)	14770	Detergent (kg)	7913
Enzyme (kg)	633	Hydrogen peroxide (kg)	1566
Detergent (kg)	7917	Water for downstream (kg)	186400
Hydrogen peroxide (kg)	1571	Potassium hydroxide (kg)	10240
Water for downstream (kg)	187100	Waste PHB (kg)	1393
Potassium hydroxide (kg)	10250	Air emissions:	
		O_2 (kg)	628700
		N_2 (kg)	2463000
		CO_2 (kg)	45170
		SO_2 (kg)	7159
Total Mass (ton)	4064	Total Mass (ton)	4064

Note: Chemical oxygen demand (COD) of wastewater was 31750 kg

Table B-2: : Scenario 1 estimated batch volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

Unit operation	Description	Batch volume (m^3)	Electricity (MJ)	Steam (kg)	Cooling water (m^3)
Sterilisation	Steam sterilisation	313	16560	44150	
Microbial growth	Fermentor	313			
Aeration			424900		
Agitation			35190		
Post fermentation cooling					125200
Cell disruption	High pressure homogeniser	313	45160		
Solid-liquid separation 2	Centrifugal spin/washing	313	3793		
Conc./Purification 1	Enzyme mixing tank	103	595.3		
Conc./Purification 2	Detergent mixing tank	111	640.8		
Conc./Purification 3	Centrifugation with 5 washes	196 (for 1 wash)	6424		
Conc./Purification 4	H_20_2 addition tank	111	640.9		
Conc./Purification 5	Centrifugation with 2 washes	195 (for 1 wash)	3615		
Formulation	Spray drying	111 (27.2 m^3 of water lost)*	89340		
Final product volume out	-	16.5			
Total	**-**		626859	44150	125200

* Water lost between Conc./purification 5 and formulation steps

Table B-3: Scenario 1 final product stream composition for the simulation of 20 tonnes of crude PHB using the CeBER Bioprocess Modeller

Total pure PHB (kg)	19020
Water (kg)	275
Enzyme (kg)	0.380
Detergent (kg)	4.75
Hydrogen peroxide (kg)	5.58
Water (kg)	664
Potassium hydroxide (kg)	6.14
Total product mass (crude PHB) (kg)	19975
Purity (%)	95.2
Recovery (%)	93.2

Table B-4: Scenario 1 equipment cost for the simulation of 20 tonnes of crude PHB per run the CeBER Bioprocess Modeller

	Scaling variable	**Size including overdesign factor**	**Cost (1000 $,2014)**
Compressor (CM-100)	Power (kW)	3279	2240
Air filters (AF-100, AF-101, AF-102)	Volumetric flow rate (m^3/s)	3.9	67.8
Blending tank (BL-100)	Volume (m^3)	391	325
Steriliser (SX-100)	Flowrate (m^3/hr)	68	245
Bioreactors (RX-100, RX-101)	Volume (m^3)	195	5200
Storage tank (ST-100)	Volume (m^3)	391	191
Homogeniser (HP-100)	Flowrate (m^3/hr)	73	675
Centrifuge (CF-100)	Sigma (m^2)	100694	354
Enzyme washing tank (RX-102)	Volume (m^3)	129	153
Detergent washing tank (RX-103)	Volume (m^3)	139	161
Centrifuge (CF-101)	Sigma (m^2)	100694	354
H_2O_2 tank (RX-104)	Volume (m^3)	139	161
Centrifuge (CF-102)	Sigma (m^2)	100694	354
Storage tank (ST-101)	Volume (m^3)	139	94.8
Spray dryer (SD-100)	Water evaporated rate (kg/hr)	2969	676
Total			11200

Table B-5: Scenario 2 mass balance and utility requirements for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

IN		**OUT**	
Process water (m^3)	514	Total pure PHB (kg)	19220
Antifoam (kg)	207	Water (m^3)	546
Glucose (kg)	77630	Antifoam (kg)	207
O_2 (kg)	542000	*Cupriavidus necator* (kg)	13660
N_2 (kg)	2039000	Enzyme (kg)	507
Ammonium sulphate (kg)	14920	Detergent (kg)	6340
Enzyme (kg)	508	Hydrogen peroxide (kg)	1257
Detergent (kg)	6343	Water for downstream (kg)	149700
Hydrogen peroxide (kg)	1262	Potassium hydroxide (kg)	8206
Water for downstream (kg)	150200	Waste PHB (kg)	1407
Potassium hydroxide (kg)	8211	Air emissions:	
		O_2 (kg)	515600
		N_2 (kg)	2039000
		CO_2 (kg)	45650
		SO_2 (kg)	7235
Total Mass (ton)	3354	Total Mass (ton)	3354

Note: Chemical oxygen demand (COD) of wastewater was 31230 kg

Table B-6: Scenario 2 estimated batch volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

Unit operation	**Description**	**Batch volume (m^3)**	**Electricity (MJ)**	**Steam (kg)**	**Cooling water (m^3)**
Sterilisation	Steam sterilisation	259	14180	37800	
Microbial growth	Fermentor	259			
Aeration			351700		
Agitation			29130		
Post fermentation cooling					126500
Cell disruption	High pressure homogeniser	259	45640		
Solid-liquid separation 2	Centrifugal spin/washing	258	3109		
Conc./Purification 1	Enzyme mixing tank	85.6	492.9		
Conc./Purification 2	Detergent mixing tank	91.9	529.5		
Conc./Purification 3	Centrifugation with 5 washes	159 (for 1 wash)	5182		
Conc./Purification 4	H_20_2 addition tank	91.9	529.5		
Conc./Purification 5	Centrifugation with 2 washes	156 (for 1 wash)	2901		
Formulation	Spray drying	91.9 (20.8 m^3 of water lost)*	68370		
Final product volume out	-	16.5			
Total	**-**		506100	37800	126500

* Water lost between Conc./purification 5 and formulation steps

Table B-7: Scenario 2 final product stream composition for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

Total pure PHB (kg)	19220
Water (kg)	210
Enzyme (kg)	0.293
Detergent (kg)	3.67
Hydrogen peroxide (kg)	4.51
Water (kg)	537
Potassium hydroxide (kg)	4.75
Total product mass (crude PHB) (kg)	19982
Purity (%)	96.2
Recovery (%)	93.2

Table B-8: Scenario 2 equipment cost for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

	Scaling variable	**Size including overdesign factor**	**Cost (1000 $,2014)**
Compressor (CM-100)	Power (kW)	2714	1940
Air filters (AF-100, AF-101, AF-102)	Volumetric flow rate (m^3/s)	3.2	60.5
Blending tank (BL-100)	Volume (m^3)	324	286
Steriliser (SX-100)	Flowrate (m^3/hr)	68	244
Bioreactors (RX-100, RX-101)	Volume (m^3)	161	4570
Storage tank (ST-100)	Volume (m^3)	323	168
Homogeniser (HP-100)	Flowrate (m^3/hr)	73	675
Centrifuge (CF-100)	Sigma (m^2)	100694	354
Enzyme washing tank (RX-102)	Volume (m^3)	107	135
Detergent washing tank (RX-103)	Volume (m^3)	115	141
Centrifuge (CF-101)	Sigma (m^2)	100694	354
H_2O_2 tank (RX-104)	Volume (m^3)	115	141
Centrifuge (CF-102)	Sigma (m^2)	100694	354
Storage tank (ST-101)	Volume (m^3)	115	83.3
Spray dryer (SD-100)	Water evaporated rate (kg/hr)	1758	538
Total			10000

Table B-9: Scenario 3 mass balance and utility requirements for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

IN		**OUT**	
Process water (m^3)	438.6	Total pure PHB (kg)	19370
Antifoam (kg)	174.8	Water (m^3)	467
Glucose (kg)	69600	Antifoam (kg)	175
O_2 (kg)	390100	*Cupriavidus necator* (kg)	9923
N_2 (kg)	1468000	Enzyme (kg)	439.5
Ammonium sulphate (kg)	12950	Detergent (kg)	5494
Enzyme (kg)	440	Hydrogen peroxide (kg)	1090
Detergent (kg)	5497	Water for downstream (kg)	129800
Hydrogen peroxide (kg)	1094	Potassium hydroxide (kg)	7112
Water for downstream (kg)	130200	Waste PHB (kg)	1418
Potassium hydroxide (kg)	7116	Air emissions:	
		O_2 (kg)	367400
		N_2 (kg)	1468000
		CO_2 (kg)	40030
		SO_2 (kg)	6278
		Chemical Oxygen Demand:	
		COD (kg)	23980
Total Mass (ton)	2523	Total Mass (ton)	2523

Note: Chemical oxygen demand (COD) of wastewater was 23980 kg

Table B-10: Scenario 3 estimated batch volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the simulation of 20 tonnes of crude PHB per run with an enzymatic digestion recovery process using the CeBER Bioprocess Modeller

Unit operation	**Description**	**Batch volume (m^3)**	**Electricity (MJ)**	**Steam (kg)**	**Cooling water (m^3)**
Sterilisation	Steam sterilisation	219	12260	32680	
Microbial growth	Fermentor	219			
Aeration			253100		
Agitation			24600		
Post fermentation cooling					113400
Cell disruption	High pressure homogeniser	219	41190		
Solid-liquid separation 2	Centrifugal spin/washing	219	2647		
Conc./Purification 1	Enzyme mixing tank	72.3	416.4		
Conc./Purification 2	Detergent mixing tank	77.8	448		
Conc./Purification 3	Centrifugation with 5 washes	135 (for 1 wash)	4467		
Conc./Purification 4	H_20_2 addition tank	77.8	448		
Conc./Purification 5	Centrifugation with 2 washes	135 (for 1 wash)	2514		
Formulation	Spray drying	77.8 (16.4 m^3 of water lost)*	53750		
Final product volume out	-	16.5			
Total	**-**		395840	32680	113400

* Water lost between Conc./purification 5 and formulation steps

Table B-11: Scenario 3 final product stream composition for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

Total pure PHB (kg)	19370
Water (kg)	165
Enzyme (kg)	0.232
Detergent (kg)	2.90
Hydrogen peroxide (kg)	3.71
Water (kg)	442
Potassium hydroxide (kg)	3.76
Total product mass (crude PHB) (kg)	19986
Purity (%)	96.9
Recovery (%)	93.2

Table B-12: Scenario 3 equipment cost for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

	Scaling variable	**Size including overdesign factor**	**Cost (1000 $,2014)**
Compressor (CM-100)	Power (kW)	1953	1520
Air filters (AF-100, AF-101, AF-102)	Volumetric flow rate (m^3/s)	2.7	54.7
Blending tank (BL-100)	Volume (m^3)	273	255
Steriliser (SX-100)	Flowrate (m^3/hr)	68	244
Bioreactors (RX-100, RX-101)	Volume (m^3)	136	4070
Storage tank (ST-100)	Volume (m^3)	273	150
Homogeniser (HP-100)	Flowrate (m^3/hr)	73	675
Centrifuge (CF-100)	Sigma (m^2)	100694	354
Enzyme washing tank (RX-102)	Volume (m^3)	90.4	120
Detergent washing tank (RX-103)	Volume (m^3)	97.2	126
Centrifuge (CF-101)	Sigma (m^2)	100694	354
H_2O_2 tank (RX-104)	Volume (m^3)	97.2	126
Centrifuge (CF-102)	Sigma (m^2)	100694	354
Storage tank (ST-101)	Volume (m^3)	97.2	74.3
Spray dryer (SD-100)	Water evaporated rate (kg/hr)	1097	438
Total			8920

Table B-13: Scenario 4 mass balance and utility requirements for the simulation of 20 tonnes of crude PHB using the CeBER Bioprocess Modeller

IN		OUT	
Process water (m^3)	347	Total pure PHB (kg)	19510
Antifoam (kg)	145	Water (m^3)	375
Glucose (kg)	70100	Antifoam (kg)	145.1
O_2 (kg)	323900	*Cupriavidus necator* (kg)	9994
N_2 (kg)	1218000	Enzyme (kg)	353
Ammonium sulphate (kg)	13040	Detergent (kg)	4413
Enzyme (kg)	353	Hydrogen peroxide (kg)	878
Detergent (kg)	4415	Water for downstream (kg)	104500
Hydrogen peroxide (kg)	881	Potassium hydroxide (kg)	5712
Water for downstream (kg)	104900	Waste PHB (kg)	1428
Potassium hydroxide (kg)	5715	Air emissions:	
		O_2 (kg)	301000
		N_2 (kg)	1218000
		CO_2 (kg)	40320
		SO_2 (kg)	6323
Total Mass (ton)	2089	Total Mass (ton)	2089

Note: Chemical oxygen demand (COD) of wastewater was 23580 kg

Table B-14: Scenario 4 estimated batch volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

Unit operation	Description	Batch volume (m^3)	Electricity (MJ)	Steam (kg)	Cooling water (m^3)
Sterilisation	Steam sterilisation	1812	10620	28300	
Microbial growth	Fermentor	182			
Aeration			210100		
Agitation			20420		
Post fermentation cooling					114200
Cell disruption	High pressure homogeniser	182	41490		
Solid-liquid separation 2	Centrifugal spin/washing	181.9	2176		
Conc./Purification 1	Enzyme mixing tank	60	345.8		
Conc./Purification 2	Detergent mixing tank	64.5	371.2		
Conc./Purification 3	Centrifugation with 5 washes	110 (for 1 water wash)	3612		
Conc./Purification 4	H_20_2 addition tank	64.5	371.2		
Conc./Purification 5	Centrifugation with 2 washes	108 (for 1 water wash)	2023		
Formulation	Spray drying	64.5 (12 m^3 of water lost)*	39440		
Final product volume out	-	16.5			
Total	**-**		330969	28300	114200

* Water lost between Conc./purification 5 and formulation steps

Table B-15: Scenario 4 final product stream composition for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

Total pure PHB (kg)	19510
Water (kg)	121
Enzyme (kg)	0.173
Detergent (kg)	2.16
Hydrogen peroxide (kg)	2.97
Water (kg)	354
Potassium hydroxide (kg)	2.79
Total product mass (crude PHB) (kg)	19990
Purity (%)	97.6
Recovery (%)	93.2

Table B-16: Scenario 4 equipment cost for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

	Scaling variable	**Size including overdesign factor**	**Cost (1000 $,2014)**
Compressor (CM-100)	Power (kW)	1621	1320
Air filters (AF-100, AF-101, AF-102)	Volumetric flow rate (m^3/s)	2.3	48.9
Blending tank (BL-100)	Volume (m^3)	273	225
Steriliser (SX-100)	Flowrate (m^3/hr)	68	244
Bioreactors (RX-100, RX-101)	Volume (m^3)	113	3590
Storage tank (ST-100)	Volume (m^3)	227	132
Homogeniser (HP-100)	Flowrate (m^3/hr)	73	675
Centrifuge (CF-100)	Sigma (m^2)	100694	354
Enzyme washing tank (RX-102)	Volume (m^3)	75	106
Detergent washing tank (RX-103)	Volume (m^3)	80.6	111
Centrifuge (CF-101)	Sigma (m^2)	100694	354
H_2O_2 tank (RX-104)	Volume (m^3)	97.2	111
Centrifuge (CF-102)	Sigma (m^2)	100694	310
Storage tank (ST-101)	Volume (m^3)	80.6	65.4
Spray dryer (SD-100)	Water evaporated rate (kg/hr)	692	359
Total			8000

Table B-17: Scenario 5 mass balance and utility requirements for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

IN		OUT	
Process water (m^3)	647	Total pure PHB (kg)	19020
Antifoam (kg)	250	Water (m^3)	678.3
Glucose (kg)	76820	Antifoam (kg)	250
O_2 (kg)	785800	*Cupriavidus necator* (kg)	13510
N_2 (kg)	2956000	Enzyme (kg)	633
Ammonium sulphate (kg)	14770	Detergent (kg)	7913
Enzyme (kg)	633	Hydrogen peroxide (kg)	1566
Detergent (kg)	7917	Water for downstream (kg)	186400
Hydrogen peroxide (kg)	1571	Potassium hydroxide (kg)	10240
Water for downstream (kg)	187100	Waste PHB (kg)	1393
Potassium hydroxide (kg)	10250	Air emissions:	
		O_2 (kg)	759600
		N_2 (kg)	2956000
		CO_2 (kg)	45170
		SO_2 (kg)	7159
Total Mass (ton)	4688	Total Mass (ton)	4688

Note: Chemical oxygen demand (COD) of wastewater was 31750 kg

Table B-18: Scenario 5 estimated batch volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

Unit operation	Description	Batch volume (m^3)	Electricity (MJ)	Steam (kg)	Cooling water (m^3)
Sterilisation	Steam sterilisation	313	16880	45010	
Microbial growth	Fermentor	313			
Aeration			509800		
Agitation			42230		
Post fermentation cooling					125200
Cell disruption	High pressure homogeniser	313	45160		
Solid-liquid separation 2	Centrifugal spin/washing	313.	3793		
Conc./Purification 1	Enzyme mixing tank	103	595.3		
Conc./Purification 2	Detergent mixing tank	111	640.9		
Conc./Purification 3	Centrifugation with 5 washes	196 (for 1 wash)	6424		
Conc./Purification 4	H_20_2 addition tank	111	640.9		
Conc./Purification 5	Centrifugation with 2 washes	195 (for 1 wash)	3615		
Formulation	Spray drying	111 (27.2 m^3 of water lost)*	89340		
Final product volume out	-	16.5			
Total	**-**		719119	45010	125200

* Water lost between Conc./purification 5 and formulation steps

Table B-19: Scenario 5 final product stream composition for the simulation of 20 tonnes of crude PHB using the CeBER Bioprocess Modeller

Total pure PHB (kg)	19020
Water (kg)	275
Enzyme (kg)	0.380
Detergent (kg)	4.75
Hydrogen peroxide (kg)	5.8
Water (kg)	664
Potassium hydroxide (kg)	6.14
Total product mass (crude PHB) (kg)	19975
Purity (%)	95.2
Recovery (%)	93.2

Table B-20: Scenario 5 equipment cost for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

	Scaling variable	**Size including overdesign factor**	**Cost (1000 $,2014)**
Compressor (CM-100)	Power (kW)	3147	2170
Air filters (AF-100, AF-101, AF-102)	Volumetric flow rate (m^3/s)	3.9	67.8
Blending tank (BL-100)	Volume (m^3)	391	325
Steriliser (SX-100)	Flowrate (m^3/hr)	68	245
Bioreactors (RX-100, RX-101)	Volume (m^3)	196	5300
Storage tank (ST-100)	Volume (m^3)	391	191
Homogeniser (HP-100)	Flowrate (m^3/hr)	73	675
Centrifuge (CF-100)	Sigma (m^2)	100694	354
Enzyme washing tank (RX-102)	Volume (m^3)	129	153
Detergent washing tank (RX-103)	Volume (m^3)	139	161
Centrifuge (CF-101)	Sigma (m^2)	100694	354
H_2O_2 tank (RX-104)	Volume (m^3)	139	161
Centrifuge (CF-102)	Sigma (m^2)	100694	354
Storage tank (ST-101)	Volume (m^3)	139	94.8
Spray dryer (SD-100)	Water evaporated rate (kg/hr)	1697	530
Total			1100

Table B-21: Scenario 6 mass balance and utility requirements for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

IN		OUT	
Process water (m^3)	514	Total pure PHB (kg)	19220
Antifoam (kg)	207	Water (m^3)	546
Glucose (kg)	77630	Antifoam (kg)	207
O_2 (kg)	650400	*Cupriavidus necator* (kg)	13660
N_2 (kg)	2447000	Enzyme (kg)	507
Ammonium sulphate (kg)	14920	Detergent (kg)	6340
Enzyme (kg)	507.5	Hydrogen peroxide (kg)	1257
Detergent (kg)	6343	Water for downstream (kg)	149700
Hydrogen peroxide (kg)	1262	Potassium hydroxide (kg)	8206
Water for downstream (kg)	150200	Waste PHB (kg)	1407
Potassium hydroxide (kg)	8211	Air emissions:	
		O_2 (kg)	624000
		N_2 (kg)	2447000
		CO_2 (kg)	45650
		SO_2 (kg)	7235
Total Mass (ton)	3870	Total Mass (ton)	3870

Note: Chemical oxygen demand (COD) of wastewater was 31230 kg

Table B-22: Scenario 6 estimated batch volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the simulation of 20 tonnes of crude PHB per run using CeBER Bioprocess Modeller

Unit operation	Description	Batch volume (m^3)	Electricity (MJ)	Steam (kg)	Cooling water (m^3)
Sterilisation	Steam sterilisation	259	14500	38660	
Microbial growth	Fermentor	259			
Aeration			422000		
Agitation			34950		
Post fermentation cooling					126500
Cell disruption	High pressure homogeniser	259	45640		
Solid-liquid separation 2	Centrifugal spin/washing	259	3109		
Conc./Purification 1	Enzyme mixing tank	85.6	492.9		
Conc./Purification 2	Detergent mixing tank	91.9	529.5		
Conc./Purification 3	Centrifugation with 5 washes	159 (for 1 water wash)	5182		
Conc./Purification 4	H_20_2 addition tank	91.9	529.5		
Conc./Purification 5	Centrifugation with 2 washes	156 (for 1 water wash)	2901		
Formulation	Spray drying	91.9 (20.8 m^3 of water lost)*	68370		
Final product volume out	-	16.5			
Total	**-**		598204	38660	126500

* Water lost between Conc./purification 5 and formulation steps

Table B-23: Scenario 6 final product stream composition for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

Total pure PHB (kg)	19220
Water (kg)	210
Enzyme (kg)	0.293
Detergent (kg)	3.67
Hydrogen peroxide (kg)	4.51
Water from downstream (kg)	537
Potassium hydroxide (kg)	4.74
Total product mass (crude PHB) (kg)	19982
Purity (%)	96.2
Recovery (%)	93.2

Table B-24: Scenario 6 equipment cost for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

	Scaling variable	**Size including overdesign factor**	**Cost (1000 $,2014)**
Compressor (CM-100)	Power (kW)	2605	1880
Air filters (AF-100, AF-101, AF-102)	Volumetric flow rate (m^3/s)	3.2	60.5
Blending tank (BL-100)	Volume (m^3)	324	286
Steriliser (SX-100)	Flowrate (m^3/hr)	67	244
Bioreactors (RX-100, RX-101)	Volume (m^3)	162	4570
Storage tank (ST-100)	Volume (m^3)	323	168
Homogeniser (HP-100)	Flowrate (m^3/hr)	73	675
Centrifuge (CF-100)	Sigma (m^2)	100694	354
Enzyme washing tank (RX-102)	Volume (m^3)	107	135
Detergent washing tank (RX-103)	Volume (m^3)	139	141
Centrifuge (CF-101)	Sigma (m^2)	100694	354
H_2O_2 tank (RX-104)	Volume (m^3)	115	141
Centrifuge (CF-102)	Sigma (m^2)	100694	354
Storage tank (ST-101)	Volume (m^3)	115	83.3
Spray dryer (SD-100)	Water evaporated rate (kg/hr)	1024	425
Total			9870

Table B-25: Scenario 7 mass balance and utility requirements for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

IN		OUT	
Process water (m^3)	439	Total pure PHB (kg)	19370
Antifoam (kg)	175	Water (m^3)	467
Glucose (kg)	69600	Antifoam (kg)	175
O_2 (kg)	468100	*Cupriavidus necator* (kg)	9923
N_2 (kg)	1761000	Enzyme (kg)	440
Ammonium sulphate (kg)	12950	Detergent (kg)	5494
Enzyme (kg)	439.8	Hydrogen peroxide (kg)	1090
Detergent (kg)	5497	Water for downstream (kg)	129800
Hydrogen peroxide (kg)	1094	Potassium hydroxide (kg)	7112
Water for downstream (kg)	130200	Waste PHB (kg)	1418
		Air emissions:	
		O_2 (kg)	445500
		N_2 (kg)	1761000
		CO_2 (kg)	40030
		SO2 (kg)	6278
Total Mass (ton)	2895	Total Mass (ton)	2895

Note: Chemical oxygen demand (COD) of wastewater was 23980 kg

Table B-26: Scenario 7 estimated batch volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

Unit operation	Description	Batch volume (m^3)	Electricity (MJ)	Steam (kg)	Cooling water (m^3)
Sterilisation	Steam sterilisation	219	12580	33540	
Microbial growth	Fermentor	219			
Aeration			303700		
Agitation			29520		
Post fermentation cooling					113400
Cell disruption	High pressure homogeniser	219	41190		
Solid-liquid separation 2	Centrifugal spin/washing	219	2647		
Conc./Purification 1	Enzyme mixing tank	72.3	416.4		
Conc./Purification 2	Detergent mixing tank	77.8	448		
Conc./Purification 3	Centrifugation with 5 washes	135 (for 1 wash)	4467		
Conc./Purification 4	H_20_2 addition tank	77.8	448		
Conc./Purification 5	Centrifugation with 2 washes	135 (for 1 wash)	2514		
Formulation	Spray drying	77.8 (16.6 m^3 of water lost)*	53750		
Final product volume out	-	16.5			
Total	**-**		451680	33540	113400

* Water lost between Conc./purification 5 and formulation steps

Table B-27: Scenario 7 final product stream composition for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

Total pure PHB (kg)	19370
Water (kg)	165
Enzyme (kg)	0.232
Detergent (kg)	2.90
Hydrogen peroxide (kg)	3.71
Water (kg)	443
Potassium hydroxide (kg)	3.76
Total product mass (crude PHB) (kg)	19990
Purity (%)	98
Recovery (%)	93.2

Table B-28: Scenario 7 equipment costs for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

	Scaling variable	**Size including overdesign factor**	**Cost (1000 $,2014)**
Compressor (CM-100)	Power (kW)	1874	1479
Air filters (AF-100, AF-101, AF-102)	Volumetric flow rate (m^3/s)	2.7	54.7
Blending tank (BL-100)	Volume (m^3)	273	255
Steriliser (SX-100)	Flowrate (m^3/hr)	67	244
Bioreactors (RX-100, RX-101)	Volume (m^3)	137	4070
Storage tank (ST-100)	Volume (m^3)	273	150
Homogeniser (HP-100)	Flowrate (m^3/hr)	73	675
Centrifuge (CF-100)	Sigma (m^2)	100694	35.4
Enzyme washing tank (RX-102)	Volume (m^3)	90.4	120
Detergent washing tank (RX-103)	Volume (m^3)	97.2	126
Centrifuge (CF-101)	Sigma (m^2)	100694	354
H_2O_2 tank (RX-104)	Volume (m^3)	97.2	126
Centrifuge (CF-102)	Sigma (m^2)	100694	354
Storage tank (ST-101)	Volume (m^3)	97.2	74.3
Spray dryer (SD-100)	Water evaporated rate (kg/hr)	707	362
Total			8790

Table B-29: Scenario 8 mass balance and utility requirements for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

IN		OUT	
Process water (m^3)	347	Total pure PHB (kg)	19510
Antifoam (kg)	145	Water (m^3)	375
Glucose (kg)	70100	Antifoam (kg)	145.1
O_2 (kg)	388600	*Cupriavidus necator* (kg)	9994
N_2 (kg)	1462000	Enzyme (kg)	353
Ammonium sulphate (kg)	13040	Detergent (kg)	4413
Enzyme (kg)	353	Hydrogen peroxide (kg)	878
Detergent (kg)	4415	Water for downstream (kg)	104500
Hydrogen peroxide (kg)	881	Potassium hydroxide (kg)	5712
Water for downstream (kg)	104900	Waste PHB (kg)	1428
Potassium hydroxide (kg)	5715	Air emissions:	
		O_2 (kg)	365800
		N_2 (kg)	1462000
		CO_2 (kg)	40320
		SO_2 (kg)	6323
Total Mass (ton)	2397	Total Mass (ton)	2397

Note: Chemical oxygen demand (COD) of wastewater was 23580 kg

Table B-20: Scenario 8 estimated batch volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

Unit operation	Description	Batch volume (m^3)	Electricity (MJ)	Steam (kg)	Cooling water (m^3)
Sterilisation	Steam sterilisation	182	10940	29160	
Microbial growth	Fermentor	182			
Aeration			252200		
Agitation			24500		
Post fermentation cooling					114200
Cell disruption	High pressure homogeniser	182	41490		
Solid-liquid separation 2	Centrifugal spin/washing	182	2176		
Conc./Purification 1	Enzyme mixing tank	60	345.8		
Conc./Purification 2	Detergent mixing tank	64.5	371.2		
Conc./Purification 3	Centrifugation with 5 washes	110 (for 1 wash)	3612		
Conc./Purification 4	H_20_2 addition tank	64.5	371.2		
Conc./Purification 5	Centrifugation with 2 washes	108 (for 1 wash)	2023		
Formulation	Spray drying	64.5 (12 m^3 of water lost)*	39440		
Final product volume out	-	16.5			
Total	**-**		377469.2	29160	114200

* Water lost between Conc./purification 5 and formulation steps

Table B-31: Scenario 8 final product stream composition for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

Total pure PHB (kg)	19510
Water (kg)	121
Enzyme (kg)	0.173
Detergent (kg)	2.16
Hydrogen peroxide (kg)	2.971
Water from downstream (kg)	354
Potassium hydroxide (kg)	2.79
Total product mass (crude PHB) (kg)	19990
Purity (%)	97.6
Recovery (%)	93.2

Table B-32: Scenario 8 equipment costs for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller

	Scaling variable	**Size including overdesign factor**	**Cost (1000 $,2014)**
Compressor (CM-100)	Power (kW)	1557	1289
Air filters (AF-100, AF-101, AF-102)	Volumetric flow rate (m^3/s)	2.3	48.9
Blending tank (BL-100)	Volume (m^3)	227	225
Steriliser (SX-100)	Flowrate (m^3/hr)	68	244
Bioreactors (RX-100, RX-101)	Volume (m^3)	113	3590
Storage tank (ST-100)	Volume (m^3)	227	132
Homogeniser (HP-100)	Flowrate (m^3/hr)	73	675
Centrifuge (CF-100)	Sigma (m^2)	100694	354
Enzyme washing tank (RX-102)	Volume (m^3)	75	106
Detergent washing tank (RX-103)	Volume (m^3)	80.6	111
Centrifuge (CF-101)	Sigma (m^2)	100694	354
H_2O_2 tank (RX-104)	Volume (m^3)	80.6	111
Centrifuge (CF-102)	Sigma (m^2)	100694	354
Storage tank (ST-101)	Volume (m^3)	80.6	65.4
Spray dryer (SD-100)	Water evaporated rate (kg/hr)	451	298
Total			7950

Table B-33: ANOVA results for the 2^3 factorial design analysis to investigate the effects of microbial properties on the final cost of production of PHB for the simulation of 20 tonnes of crude PHB per run with an enzymatic digestion purification and recovery process using the CeBER Bioprocess Modeller

	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Source					
Model	14.80	4	3.70	268.33	0.0004
A-Final non-PHB biomass concentration	1.50	1	1.50	108.55	0.0019
B-PHB/Biomass ratio	11.45	1	11.45	830.47	< 0.0001
C-Culture time	1.58	1	1.58	114.47	0.0017
AB	0.27	1	0.27	19.85	0.0210
Residual	0.041	3	0.014		
Cor Total	14.84	7			

Std. Dev.	0.12	R-Squared	0.9972
Mean	8.28	Adj R-Squared	0.9935
C.V. %	1.42	Pred R-Squared	0.9802
PRESS	0.29	Adeq Precision	44.664

C.1: EFFECT OF USING STARCH AS CARBON SOURCE ADDITIONAL DATA

Table C-1: Mass balance and utility requirements for the simulation of 20 tonnes of crude PHB per run with an enzymatic digestion process using the CeBER Bioprocess Modeller with starch as the carbon source

IN		**OUT**	
Process water (m^3)	436	Total pure PHB (kg)	19220
Antifoam (kg)	210	Water (m^3)	472
Starch (kg)	101300	Antifoam (kg)	210
O_2 (kg)	1293000	*Cupriavidus necator* (kg)	29120
N_2 (kg)	4864000	Enzyme (kg)	409
Ammonium sulphate (kg)	23340	Detergent (kg)	5108
Enzyme (kg)	409	Hydrogen peroxide (kg)	1019
Detergent (kg)	5111	Water for downstream (kg)	121400
Hydrogen peroxide (kg)	1024	Potassium hydroxide (kg)	6612
Water for downstream (kg)	121900	Waste PHB (kg)	1407
Potassium hydroxide (kg)	6616	Air emissions:	
		O_2 (kg)	1251000
		N_2 (kg)	4864000
		CO_2 (kg)	69890
		SO_2 (kg)	11320
Total Mass (ton)	6853	Total Mass (ton)	6853

Note: Chemical oxygen demand (COD) of wastewater was 58950 kg

Table C-2: Estimated batch volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the simulation of 20 tonnes of crude PHB per run with an enzymatic digestion process using the CeBER Bioprocess Modeller with starch as the carbon source

Unit operation	Description	Batch volume (m^3)	Electricity (MJ)	Steam (kg)	Cooling water (m^3)
Sterilisation	Steam sterilisation	262	15910	42420	
Microbial growth	Bioreactor	262			
Aeration			839000		
Agitation			49110		
Post fermentation cooling					183400
Cell disruption	High pressure homogeniser	262	64980		
Solid-liquid separation 2	Centrifugal spin/washing	263	2957		
Conc./Purification 1	Enzyme mixing tank	86.7	499.5		
Conc./Purification 2	Detergent mixing tank	91.8	528.9		
Conc./Purification 3	Centrifugation with 5 washes	146 (for 1 wash)	4400		
Conc./Purification 4	H_20_2 addition tank	91.8	528.9		
Conc./Purification 5	Centrifugation with 2 washes	127 (for 1 wash)	2353		
Formulation	Spray drying	91.8 (19.8 m^3 of water lost)*	65100		
Final product volume out	-	16.5			
Total			1045367	42420	183400

* Water lost between Conc./purification 5 and formulation steps

Table C-3: Final product stream composition for the simulation of 20 tonnes of PHB per run with an enzymatic digestion process using the CeBER Bioprocess Modeller with starch as the carbon source

Total pure PHB (kg)	19220
Water (kg)	200
Enzyme (kg)	0.282
Detergent (kg)	3.53
Hydrogen peroxide (kg)	4.59
Water (kg)	546
Potassium hydroxide (kg)	4.57
Total Product mass (crude PHB) (kg)	19983
Purity (%)	96.2
Recovery (%)	93.2

Table C-4: Equipment cost for the simulation of 20 tonnes of crude PHB per run with an enzymatic digestion recovery process using the CeBER Bioprocess Modeller with starch as the carbon source

	Scaling variable	Size including overdesign factor	Cost (1000 $,2014)
Compressor (CM-100)	Power (kW)	3597	2400
Air filters (AF-100, AF-101, AF-102)	Volumetric flow rate (m^3/s)	3.3	61
Blending tank (BL-100)	Volume (m^3)	328	289
Steriliser (SX-100)	Flowrate (m^3/hr)	68	244
Bioreactors (RX-100, RX-101)	Volume (m^3)	164	4610
Storage tank (ST-100)	Volume (m^3)	328	170
Homogeniser (HP-100)	Flowrate (m^3/hr)	73	675
Centrifuge (CF-100)	Sigma (m^2)	100694	354
Enzyme washing tank (RX-102)	Volume (m^3)	108	136
Detergent washing tank (RX-103)	Volume (m^3)	115	141
Centrifuge (CF-101)	Sigma (m^2)	100694	354
H_2O_2 tank (RX-104)	Volume (m^3)	115	141
Centrifuge (CF-102)	Sigma (m^2)	100694	354
Storage tank (ST-101)	Volume (m^3)	103	83.2
Spray dryer (SD-100)	Water evaporated rate (kg/hr)	472	302
Total			10300

D-1: EFFECT OF OPTIMISATION ADDITIONAL DATA

Table D-1: Mass balance and utility requirements for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller using an optimised case scenario with a PHB productivity of 3.8 g/L/h, a sodium hypochlorite-surfactant purification and recovery process with a cell rupture efficiency of 95 % and an energy efficiency of 70 % for the centrifuges, and a hypothetical carbon source assumed to be free of cost

IN		OUT	
Process water (m^3)	153	Total pure PHB (kg)	19880
Antifoam (kg)	137	Water (m^3)	179
Glucose (kg)	66320	Antifoam (kg)	137
O_2 (kg)	306400	*Cupriavidus necator* (kg)	8358
N_2 (kg)	1153000	Triton X 100 (kg)	480
Ammonium sulphate (kg)	12340	Sodium hypochlorite (kg)	6932
Triton X 100 (kg)	480	Waste PHB (kg)	1027
Sodium hypochlorite (kg)	6932	Air emissions:	
		O_2 (kg)	284800
		N_2 (kg)	1153000
		CO_2(kg)	38150
		SO_2 (kg)	5983
Total Mass (ton)	1698	Total Mass (ton)	1698

Note: Chemical oxygen demand (COD) of wastewater was 15990 kg

Table D-2: Estimated batch volume (m^3), electricity (MJ), steam (kg) and cooling water (m^3) required for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller using an optimised case scenario with a PHB productivity of 3.8 g/L/h , a sodium hypochlorite-surfactant purification and recovery process with a cell rupture efficiency of 95 % and an energy efficiency of 70 % for the centrifuges and a hypothetical carbon source assumed to be free of cost

Unit operation	Description	Batch volume (m^3)	Electricity (MJ)	Steam (kg)	Cooling water (m^3)
Sterilisation	Steam sterilisation	171	8350	22260	
Microbial growth	Bioreactor	171			
Aeration			198800		
Agitation			19320		
Post fermentation cooling					108100
Cell disruption	High pressure homogeniser	172	37880		
Solid-liquid separation 2	Centrifugal spin/washing	172	1774		
Conc./Purification 1	Surfactant mixing tank	56.7	326.8		
Conc./Purification 2	NaClO mixing tank	63.7	366.7		
Conc./Purification 3	Centrifugation	63.7	456.9		
Conc./Purification 4	Centrifugation with 2 washes	33.3 (for 1 wash)	456.9		
Formulation	Spray drying	28.6 (12 m^3 of water lost)*	39470		
Final product volume out	-	16.4			
Total	-		307201	22260	108100

* Water lost between Conc./purification 4 and formulation steps

Table D-3: Final product stream composition for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller using an optimised case scenario with a PHB productivity of 3.8 g/L/h, a sodium hypochlorite-surfactant purification and recovery process with a cell rupture efficiency of 95 % and an energy efficiency of 70 % for the centrifuges and a hypothetical carbon source assumed to be free of cost

Total pure PHB (kg)	19880
Water (kg)	122
Total product mass (crude PHB) (kg)	20000
Purity (%)	99.4
Recovery (%)	95.1

Table D-1: Equipment cost for the simulation of 20 tonnes of crude PHB per run using the CeBER Bioprocess Modeller using optimised case scenario with a PHB productivity of 3.8 g/L/h, a sodium hypochlorite-surfactant purification and recovery process with a cell rupture efficiency of 95 % and an energy efficiency of 70 % for the centrifuges and a hypothetical carbon source assumed to be free of cost

	Scaling variable	**Size including overdesign factor**	**Cost (1000 $,2014)**
Compressor (CM-200)	Power (kW)	1534	1260
Air filters (AF-200, AF-201, AF-202)	Volumetric flow rate (m^3/s)	2.1	47.2
Blending tank (BL-200)	Volume (m^3)	214	216
Steriliser (SX-200)	Flowrate (m^3/hr)	67	242
Bioreactors (RX-200, RX-201)	Volume (m^3)	107	3450
Storage tank (ST-200)	Volume (m^3)	214	127
Homogeniser (HP-200)	Flowrate (m^3/hr)	73	675
Centrifuge (CM-200)	Sigma (m^2)	100694	354
Surfactant washing tank (RX-202)	Volume (m^3)	71	102
NaClO washing tank (RX-203)	Volume (m^3)	80	110
Centrifuge (CM-201)	Sigma (m^2)	100694	354
Centrifuge (CM-202)	Sigma (m^2)	100694	354
Storage tank (ST-201)	Volume (m^3)	36	37.6
Spray dryer (SD-200)	Water evaporated rate (kg/hr)	384	278
Total			7610