Potential for bamboo as a feedstock for lignocellulosic biofuel production

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Declaration of Originality

I hereby declare that this thesis is originated entirely from me and has not been submitted in any form for any degree or diploma. Information derived from others has been clearly referenced in the thesis and all main sources of help have been acknowledged.

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Abstract

This thesis investigates the potential for bamboo to be a feedstock for the production of lignocellulosic biofuels. This was assessed from the perspectives of technology, economics and policy, and discusses the main drivers that could help or hinder the viability of such a process at the commercial scale. Three pretreatment technologies (liquid hot water, soaking in aqueous ammonia and dilute acid) and various enzyme loadings were explored as potential processing routes for converting bamboo into bioethanol; these conditions were compared based on the criteria of maximising sugar release and thus total ethanol production. Aspen Plus modelling software was used to simulate the conversion process at a scaled up level of 2,000 dry metric tonnes of bamboo per day. The generated mass and energy balances were used in a discounted cash flow analysis to yield the minimum production price for bamboo-derived bioethanol based on a reference year of 2011. Two case study scenarios (China and Colombia) were modelled as potential locations for establishing a bamboo to bioethanol process. Bioethanol from bamboo in China and Colombia was also compared with bioethanol derived from other lignocellulosic feedstocks (wheat straw and short-rotation coppice poplar) in various locations, and its competitiveness with fossil-based fuels at the pump was assessed. This research demonstrated that bioethanol from bamboo can be just as, if not more competitive than bioethanol from other lignocellulosic resources provided that certain criteria are fulfilled. The main determinants responsible for establishing a future in the alternative fuel market included maintaining a balance between the major factors relating to technology (achieving relatively high sugar yields with low enzyme usage), economics (obtaining low costs for feedstock and high credits for electricity generation), and policy (establishing these conditions in a location with strong policy support for bioethanol).

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Abbreviations

1G	1st generation (in reference to biofuels)
2G	2nd generation (in reference to biofuels)
ASE	Accelerated Solvent Extractor
CHP	Combined heat and power
COD	Chemical oxygen demand
CSL	Corn steep liquor
DA	Dilute acid
DAP	Diammonium phosphate
DM	Dry matter
EUR	Euro
FFV	Flex-fuel vehicle
FPU	Filter paper units
GBP	Great Britain Pounds
GHG	Greenhouse gas
HPLC	High performance liquid chromatography
IEA	International Energy Agency
ISBL	Inside-battery-limits
LHW	Liquid hot water
MESP	Minimum ethanol selling price
NREL	National Renewable Energy Laboratory
OECD	Organisation for Economic Co-operation and Development
PFD	Process flow diagram
SAA	Soaking in aqueous ammonia
TCI	Total capital investment
USD	US Dollars
VAT	Value-added tax
WWT	Wastewater treatment

Table of Contents

List of Figures **11**

List of Tables **15**

Chapter 1 Introduction and literature review **18**

1.1 Scope of research **19**

1.1.1 Purpose of study 20
1.1.2 Thesis outline 21

1.2 Feedstocks for biofuel production **23**

1.2.1 Conventional biofuels 23
1.2.2 Advanced biofuels 24
1.2.3 Composition of lignocellulose 24
1.2.4 Cell walls of the grass family 27
1.2.5 Bamboo biofuel potential 28

1.3 Biochemical conversion process **29**

1.3.1 Pretreatment 30
1.3.1.2 Pretreatments on bamboo 31
1.3.2 Enzymatic saccharification 32
1.3.3 Fermentation 33

1.4 Evaluation of economic feasibility **35**

1.5 Support schemes for biofuel production **38**

1.6 Case study scenarios **39**

1.6.1 China 39
1.6.2 Colombia 40

Chapter 2 Pretreatment and enzymatic saccharification of bamboo **42**

2.1 Introduction **43**

2.2 Materials and methods **44**

2.2.1 Plant material and preparation of biomass 44
2.2.2 Compositional analysis 45
2.2.3 Enzyme activity and enzymatic saccharification 45
2.2.4 Liquid hot water pretreatment 46
2.2.5 Soaking in aqueous ammonia pretreatment 47
2.2.6 Dilute acid pretreatment 47

2.3 Results and discussion **48**

2.3.1 Characterisation of bamboo biomass 48
2.3.2 Liquid hot water pretreatment 51
2.3.2.1 Effect of liquid hot water pretreatment on biomass composition 51
2.3.2.2 Effect of liquid hot water pretreatment on sugar release 53
2.3.2.3 Effect of enzyme loading on liquid hot water-pretreated bamboo 55
2.3.3 Soaking in aqueous ammonia pretreatment 57
2.3.3.1 Effect of soaking in aqueous ammonia pretreatment on biomass composition 57
2.3.3.2 Effect of soaking in aqueous ammonia pretreatment on sugar yields 58
2.3.3.3 Effect of enzyme loading on soaking in aqueous ammonia-pretreated bamboo 60
2.3.4 Dilute acid pretreatment 62
2.3.4.1 Effect of dilute acid pretreatment on biomass composition 62
2.3.4.2 Effect of dilute acid pretreatment on sugar yields 63
2.3.4.3 Effect of enzyme loading on dilute acid-pretreated bamboo 65
2.4 Conclusions 66

Chapter 3 Techno-economic assessment of bioethanol from bamboo 70

3.1 Introduction 71

3.2 Materials and methods 72

3.2.2 Bioethanol conversion process description 73
3.2.3 Process economics 83
3.2.3.1 Total capital investment 83
3.2.3.2 Fixed and variable operating costs 84
3.2.3.3 Discounted cash flow analysis 85
3.3 Results and discussion 86

3.3.1 Summary of simulation results for China base-case scenarios 86
3.3.2 Cost breakdown analysis 89
3.3.3 Effect of pretreatment on downstream processes 94
3.3.4 Sensitivity analysis on cost parameters 96
3.3.5 Comparison with other techno-economic analyses 99
3.4 Conclusions 102

Chapter 4 Competitiveness of bamboo-to-bioethanol case study scenarios 106

4.1 Introduction 107

4.2 Materials and methods 109

4.2.1 Feedstock composition 109
4.2.2 Process design and model assumptions 109
4.2.3 Economic analysis 112
4.2.4 Supply chain analysis 114
4.3 Results and discussion 116
4.3.1 Bioethanol policy development in China and Colombia 116
4.3.2 Comparison of MESPs in China and Colombia 119
4.3.3 Supply chain analysis for China and Colombia 122
4.3.3.1 Reference case scenarios for China and Colombia 123
4.3.3.2 Prospective scenarios for China and Colombia 126
4.3.4 Comparison of bioethanol from bamboo with wheat straw and poplar 130
4.3.4.1 Technology comparison 130
4.3.4.2 MESP comparison 131
4.3.4.3 Policy comparison 135
4.3.4.4 Prospects for advanced poplar feedstock 138
4.4 Conclusions 141
Chapter 5 General discussion 145
5.1 Technological feasibility of bamboo-to-bioethanol conversion 146
5.2 Economic performance of bamboo-based bioethanol 149
5.3 Role of policy on the competitiveness of bamboo-based bioethanol . 152
5.4 Comparison with other lignocellulosic feedstocks 155
5.5 Summary of main findings and conclusions 157
5.6 Future work 159
References 162
Appendix 176
Appendix A Process flow diagrams 177
Appendix B Discounted cash flow analysis (for LHW PT 10 FPU/g glucan scenario) 197

List of Figures

Figure 1.1 Diagrammatic representation of cellulose, hemicellulose and lignin structure in the plant cell wall (Zhu et al., 2013). ……...25

Figure 1.2 Structure of and bonding of cellulose (Harmsen et al., 2010). ..……..…25

Figure 1.3 Coniferyl, sinapyl and p-coumaryl building blocks of lignin (Pu et al., 2013). …..27

Figure 2.1 Time course assay of glucose release in enzymatic saccharification of *P. dulcis* over 144 hours, with standard error shown (n=3). ……..…..49

Figure 2.2 Complete mass closures of bamboo pretreated with liquid hot water as a percentage of dry matter (Figure also presented in Littlewood and Murphy, 2012)……..……..…52

Figure 2.3 Effect of liquid hot water pretreatment (PT) and enzymatic saccharification (ES) expressed as a percentage of dry matter under a standardised enzyme loading of 60 FPU/g glucan. Other sugars refer to the sum of galactose and arabinose solubilised during pretreatment. Theoretical maximum sugars indicated by the red dashed line and is equal to 64.2% of DM. *Optimal LHW pretreatment condition. …….53

Figure 2.4 Total sugar release from LHW pretreatment (190°C for 10 minutes) and enzymatic saccharification with five enzyme loadings. Sugar release is expressed as a percentage of the theoretical maximum. Red dashed line represents sugar release from pretreatment (43.0%). Error bars represent standard error (n=3). ……..……56

Figure 2.5 Complete mass closures of bamboo pretreated with soaking in aqueous ammonia expressed as a percentage of dry matter. ……57

Figure 2.6 Effect of soaking in aqueous ammonia pretreatment (PT) and enzymatic saccharification (ES) on total sugar release. Sugar release is expressed as a percentage of dry matter under a standardised enzyme loading of 60 FPU/g glucan. Other sugars refer to the sum of galactose and arabinose solubilised during pretreatment. Theoretical maximum sugars indicated by the red dashed line and is equal to 64.2% of DM. *Optimal SAA pretreatment condition. ……..……..59

Figure 2.7 Total sugar release from SAA pretreatment (15% NH_4OH for 25 hours) and enzymatic saccharification with five enzyme loadings. Sugar release is expressed as a percentage of the theoretical maximum. Red dashed line represents sugar release from pretreatment (43.0%). Error bars represent standard error (n=3). ……..…….61

Figure 2.8 Complete mass closures of bamboo pretreated with dilute acid expressed as a percentage of dry matter. ……..……62

Figure 2.9 Effect of dilute acid pretreatment (PT) and enzymatic saccharification (ES) on total sugar release. Sugar release is expressed as a percentage of dry matter under a standardised enzyme loading of 60 FPU/g glucan. Other sugars refer to the sum of galactose and arabinose solubilised during pretreatment. Theoretical maximum sugars indicated by the red dashed line and is equal to 64.2% of DM. *Optimal DA pretreatment condition. …….64

Figure 2.10 Total sugar release from DA pretreatment (0.2% H_2SO_4 at 160°C for 15 minutes) and enzymatic saccharification with five enzyme loadings. Sugar release is expressed as a percentage of the theoretical maximum. Red dashed line represents sugar release from the pretreatment stage (55.0%). Error bars represent standard error (n=3). ……..66

Figure 3.1 Overview of main areas (coded by letter A, B, C, etc.) in the bamboo-to-bioethanol conversion process modelled in Aspen Plus software (Figure also presented in Littlewood et al., 2013)……...72

Figure 3.2 Cost breakdown of bioethanol from bamboo with LHW pretreatment at an enzyme loading of 10 FPU/g glucan (MESP $0.484/litre) (Figure also presented in Littlewood et al., 2013)…….……..…….……90

Figure 3.3: Cost breakdown of bioethanol from bamboo with SAA pretreatment at an enzyme loading of 30 FPU/g glucan (MESP ($0.990/litre). ……91

Figure 3.4: Cost breakdown of bioethanol from bamboo with DA pretreatment at an enzyme loading of 10 FPU/g glucan (MESP $0.547/litre). . ……..…92

Figure 3.5 Cost breakdown summary for bioethanol from bamboo via LHW, SAA and DA pretreatment processes expressed in $/litre (FH: Feedstock handling; PT: Pretreatment; SF:

Saccharification & fermentation; PP: Product purification; WWT: Wastewater treatment; ST: Storage; CMB: Combustor/turbogenerator; UT: Utilities). ……..94

Figure 3.6 Sensitivity of MESP to variations in feedstock cost, enzyme cost and electricity credit in (a) LHW pretreatment with 10 FPU/g glucan (b) SAA pretreatment with 30 FPU/g glucan and (c) DA pretreatment with 10 FPU/g glucan scenarios. 98

Figure 3.7 Relationship between feedstock cost ($/tonne) and MESP ($/litre) from current study and other techno-economic models. MESP and feedstock costs have been updated to 2011 prices using the Consumer Price Index. Similar pretreatment processes are grouped such that: red represents acid pretreatments, blue represents hydrothermal pretreatments (LHW and steam) and green represents ammonia-based pretreatments. (1) Eggerman & Elander, 2005 (Liquid hot water (LHW) pretreatment); (2) Eggerman & Elander, 2005 (Dilute acid (DA) pretreatment); (3) Sendich et al., 2008 (Ammonia fibre explosion (AFEX) pretreatment, Consolidated bio-processing (CBP)); (4) Sendich et al., 2008 (AFEX, Simultaneous saccharification and co-fermentation (SSCF)); (5) Humbird et al., 2011 (DA); (6) Laser et al., 2009 (DA); (7) Macrelli et al., 2011 (Steam pretreatment); (8) Tao et al., 2011 (Soaking in aqueous ammonia (SAA) pretreatment); (9) Tao et al., 2011 (DA); (10) Tao et al., 2011 (LHW); (11) Hamelinck et al., 2005 (DA, Simultaneous saccharification and fermentation (SSF)); (12) Hamelinck et al., 2005 (Steam pretreatment; SSCF); (13) Hamelinck et al., 2005 (LHW, CBP); (14) Kazi et al., 2010 (LHW); (15) Kazi et al., 2010 (DA). ……100

Figure 4.1 MESPs of bioethanol from bamboo in China and Colombia in various pretreatment (SAA, LHW and DA pretreatments) and enzyme loading scenarios. ……..119

Figure 4.2 Support measures provided in the biofuel production supply chain (adapted from Global Subsidies Initiative (2008)). ……...122

Figure 4.3 Reference case scenarios for bioethanol at the pump in China (a-c) and Colombia (d-f) for five enzyme loading scenarios and via liquid hot water pretreatment (LHW PT), soaking in aqueous ammonia pretreatment (SAA PT) and dilute acid pretreatment (DA PT). Petrol prices are marked by the red cross and the fixed bioethanol prices are shown by the grey dashed line. All bioethanol prices are adjusted for comparison with petrol based on an energy equivalent. ……125

Figure 4.4 Prospective scenarios for bioethanol at the pump in China (a-c) and Colombia (d-f) for five enzyme loading scenarios and via liquid hot water pretreatment (LHW PT), soaking in aqueous ammonia pretreatment (SAA PT) and dilute acid pretreatment (DA PT). Petrol prices are marked by the red cross and the fixed bioethanol prices are shown by the grey dashed line. All bioethanol prices are adjusted for comparison with petrol based on an energy equivalent. ……..128

Figure 4.5 MESPs for bioethanol production via LHW and DA pretreatments from bamboo in China and Colombia, wheat straw in the UK and poplar from Sweden, Italy, France, Slovakia and Spain. (LHW: Liquid hot water pretreatment; DA: Dilute acid pretreatment; B: Bamboo; WS: Wheat straw; P: Poplar; Ch: China; Co: Colombia; Sw: Sweden; F: France; It: Italy; Sl: Slovakia; Sp: Spain). ……..132

Figure 4.6 Relationship between feedstock price ($/tonne) and MESP ($/litre) in sixteen bioethanol production scenarios. ……..133

Figure 4.7 Bioethanol pump prices in sixteen bioethanol production scenarios. National petrol prices in 2011 are marked by the red cross (B: Bamboo; WS: Wheat straw; P: Poplar; LHW: Liquid hot water pretreatment; DA: Dilute acid pretreatment; Ch: China; Co: Colombia: Sw: Sweden; Fr: France; It: Italy; Sl: Slovakia; Sp: Spain). ……..133

Figure 4.8 Bioethanol pump prices from poplar in France and Italy under reference case LHW and DA pretreatment scenarios compared with prospective scenarios with lignin-modified poplar. National petrol prices are marked by the red cross (P: Poplar; Fr: France; LHW: Liquid hot water pretreatment; DA: Dilute acid pretreatment; It: Italy; Pros: Prospective case). ……139

List of Tables

Table 1.1 Summary of pretreatment processes reported on bamboo in the literature……32

Table 1.2 Comparison of biochemical cellulosic ethanol production according to recent techno-economic studies. ……37

Table 2.1 Liquid hot water pretreatment conditions. ……...47

Table 2.2 Soaking in aqueous ammonia pretreatment conditions…….47

Table 2.3 Dilute acid pretreatment conditions. ……48

Table 2.4 Post-dilute acid pretreatment water wash conditions. ……...48

Table 2.5 Average composition of *P. viridiglaucescens* nodes and internodes (n=3). ……..50

Table 2.6 Average composition of *P. viridiglaucescens* and *P. dulcis* (n=3). ……..50

Table 2.7 Summary of composition and sugar release in bamboo under optimal LHW, SAA and DA pretreatment conditions. ……...67

Table 3.1 Liquid hot water pretreatment reactions and conversions. ……...74

Table 3.2 Soaking in aqueous ammonia pretreatment reactions and conversions. ……..75

Table 3.3 Dilute acid pretreatment reactions and conversions. ……..76

Table 3.4 Enzymatic saccharification operating conditions. ……..77

Table 3.5 Enzymatic saccharification reactions and conversions for LHW, SAA and DA pretreatments on bamboo. ……...78

Table 3.6 Seed train specifications. ……..….79

Table 3.7 Co-fermentation operating conditions. ……....79

Table 3.8 Co-fermentation reactions and conversions. ……..….80

Table 3.9 Summary of raw material costs. ……...84

Table 3.10 Summary of discounted cash flow analysis parameters. ……..…..85

Table 3.11 Summary of simulation results for five LHW pretreatment and enzyme loading scenarios. ……..…..86

Table 3.12 Summary of simulation results for five SAA pretreatment and enzyme loading scenarios. ……..…..87

Table 3.13 Summary of simulation results for five DA pretreatment and enzyme loading scenarios. ……88

Table 3.14 Summary of utilities consumption in three pretreatment scenarios. …….95

Table 3.15 Sensitivity analysis cost parameters. ……....97

Table 4.1 Chemical composition of various lignocellulosic feedstocks. ……...109

Table 4.2 Pretreatment and enzymatic saccharification conditions and yields for LHW and DA pretreatments on selected lignocellulosic feedstocks…….111

Table 4.3 Summary of economic cost parameters for bioethanol production from bamboo in China and Colombia. ……112

Table 4.4 Summary of variable operating costs for bioethanol production from wheat straw in the UK. ……....113

Table 4.5 Summary of main cost parameters for bioethanol production from poplar in selected European countries. ……..114

Table 4.6 Fuel pump price parameters ($US per litre) for cellulosic bioethanol production in various locations. ……...115

Table 4.7 Summary of process results of bioethanol production from lignocellulosic feedstocks. ……..130

Table 4.8 MESP comparison of reference case and prospective scenarios for bioethanol production from five European countries. ……..….138

Chapter 1 Introduction and literature review

1.1 Scope of research

1.1.1 Background

The use of liquid biofuels in the transport sector is becoming increasingly urgent due to environmental concerns of climate change, increasing oil prices and energy security. Of the total world primary energy consumption, 81% is accounted for by fossil fuels, and of this, transport accounts for over half of global oil consumption (IEA, 2012b, IEA, 2011b, IEA, 2013). Amongst the global carbon dioxide emissions generated in 2010, road transport comprised about 22%, demonstrating its significant contribution to global warming (IEA, 2012a). The International Energy Agency (IEA) has projected different outcomes for future energy demand and CO_2 emissions under various technology and policy scenarios. It was predicted that in the absence of new policies, current transport energy demand will double by 2050 and greenhouse gas (GHG) emissions will increase by more than 50% to 16 gigatonnes of CO_2 equivalent, indicating the urgency to invest in renewable and environmentally benign sources of fuel for the future (IEA, 2010). Alternatively, in the IEA *BLUE Map Scenario,* which targets a halving of 2005 global CO_2 emissions by 2050, biofuels are estimated to comprise approximately 26% of transport fuels in 2050, demonstrating the major contribution they can have towards energy security and climate change mitigation (IEA, 2010). Furthermore, it is expected that 90% of this proportion will come from advanced biofuels (from non-food feedstocks), and that more than half will be produced from non-OECD (Organisation for Economic Cooperation and Development) countries (IEA, 2010). This recognises the impact that advanced biofuels can have on the transport sector and the major role that non-OECD countries can have in enhancing global production and consumption.

Biofuels from plant biomass are said to be carbon neutral because the CO_2 released during their combustion is offset by CO_2 sequestered by the plant during photosynthesis; therefore their use should not increase the net carbon in the atmosphere (Naik et al., 2010). Many governments have realised this potential and have responded by investing in biofuel research and development, as well as setting mandates to boost production and consumption (e.g. the Renewable Fuel Standard in the US and the Renewable Energy Directive in the EU) (Al-Riffai et al., 2010). As a result, global biofuel production grew from 16 billion litres in 2000 to more than 100 billion litres in 2010, and today provides about 3% of the total road transport fuel on an energy basis (IEA, 2011a).

The production of conventional biofuels derived primarily from food crops such as sugarcane bioethanol in Brazil and corn bioethanol in the US have reached technological and market maturity and are currently produced at the commercial scale. However, controversial environmental and social impacts of conventional biofuels, such as potential competition with food crops and questionable GHG emission levels, are greater than previously anticipated, and have resulted in a shift towards advanced biofuels produced from lignocellulosic feedstocks (Sims et al., 2008, Sims et al., 2010). Advanced biofuels produced from waste residues, purpose-grown energy grasses or short-rotation forestry crops can be used on a much larger scale and can alleviate many of the above issues (Naik et al., 2010). Bamboo is suggested to be an ideal candidate for biofuel production based on its advantageous growth properties including a rapid growth rate and perennial nature, as well as low management requirements (Scurlock, 2000). Furthermore, it is estimated that approximately 3.2% of the world's total forest area (equivalent to 37 million hectares) is covered by bamboo, thereby potentially serving as an abundant resource for this purpose (Lobovikov et al., 2007). Provided that a technologically- and economically-viable process can be optimised and developed to scale, bamboo has the potential to serve as a feedstock for generating sustainable transport fuels such as bioethanol.

1.1.1 Purpose of study

The main aim of this thesis is to assess the technological potential and economic feasibility for bamboo to be used as a feedstock for bioethanol production. The main technological, economic and political drivers that could enable such a process to be viable at the commercial scale are considered. The specific research questions addressed in this thesis are:

1) What are the optimal processing technologies and conditions for maximising sugar release in bamboo?

2) From an economic perspective, which of these pretreatment processes and enzymatic saccharification conditions is the most favourable for producing bioethanol from bamboo?

3) How does the role of government policy impact the competitiveness of bamboo-derived bioethanol in different country case studies and amongst other lignocellulosic feedstocks?

These questions are addressed by generating novel experimental data on the optimal processing conditions to provide insight on how recalcitrant bamboo biomass is and how easily it can be converted into a more digestible substrate. Experimental results are combined with data collected from the literature to develop a techno-economic model generating the production price of bioethanol derived from bamboo in various case study scenarios. The specific approaches for addressing these questions are:

- To characterise the chemical composition of bamboo biomass.

- To explore experimentally the effect that three pretreatment technologies (liquid hot water, soaking in aqueous ammonia and dilute acid) have on bamboo cell wall composition and subsequent enzymatic saccharification.

- To determine experimentally the saccharification potential of pretreated bamboo by using different loadings of the commercial enzyme Cellic CTec2.

- To develop a process model using a chemical engineering process simulation software Aspen Plus to simulate the conversion of bamboo into bioethanol for different pretreatment and enzyme loading scenarios.

- To use a discounted cash flow analysis to generate the minimum ethanol selling price (MESP) for these scenarios and to assess the significant cost contributors in the process.

- To investigate the role that government policy has on the competitiveness of cellulosic bioethanol by using China and Colombia as potential production locations.

- To evaluate the competitiveness of bioethanol from bamboo with bioethanol from wheat straw in the UK and from short-rotation coppice (SRC) poplar in Europe.

1.1.2 Thesis outline

Chapter 1 presents a literature review, which introduces the different types of biofuels and feedstocks, describes the composition of lignocellulose and discusses features that make bamboo a potentially advantageous feedstock for producing biofuel. An overview of the biochemical conversion process is described. A review of techno-economic assessments is

presented, and the most common government policies that are implemented to establish a successful biofuels market in China and Colombia are addressed.

The following three chapters comprise experimental and modelling work, concluded by a final overall discussion chapter drawing together the main findings of the work. Each chapter includes a brief introduction, the methodology for conducting this work, the results and a discussion of the implications of these findings. As the thesis progresses, the scope of each chapter is broadened, beginning with: 1) a focus on the bamboo cell wall composition and a technology comparison of pretreatment processes, 2) a techno-economic assessment of these pretreatment processes at a scaled-up level, 3) a policy comparison of the competitiveness of bamboo in two locations with other lignocellulosic feedstocks.

Chapter 2 presents the experimental work conducted on bamboo. This includes characterising unpretreated bamboo followed by a comparison of three pretreatments (liquid hot water, soaking in aqueous ammonia and dilute acid) based on their effect on cell wall composition and saccharification potential. The effect of varying enzyme loadings and the influence on saccharification sugar yields is also explored.

A techno-economic assessment is presented in Chapter 3 to compare pretreatment technologies from an economic perspective. The minimum ethanol selling price of bioethanol is used to compare the economic viability of different pretreatment and enzyme loading process alternatives. A cost breakdown analysis reveals the major cost contributors in each process, and a sensitivity analysis demonstrates the influence of these parameters towards the overall price.

In Chapter 4, the optimal bamboo conversion processes based on experimental and economic results are selected and modelled using China and Colombia as case studies. This chapter focuses on the influence of costs and government policy on the economic competitiveness of bamboo-based bioethanol against petrol at the pump. The best case scenarios are then compared with other lignocellulosic feedstocks in various locations. These include wheat straw from the UK and short-rotation coppice poplar from Europe.

Chapter 5 summarises the main findings from this work in a broader context, discusses their implications and offers suggestions for future work.

1.2 Feedstocks for biofuel production

1.2.1 Conventional biofuels

Conventional or first-generation (1G) biofuels produced from food crops such as sugarcane ethanol in Brazil, corn ethanol in the US, palm oil biodiesel in Malaysia and rapeseed oil biodiesel in Germany, are characterised by mature technologies and commercialised markets (Sims et al., 2008). Although at a global production level, bioethanol is the most abundant biofuel due to significant contributions from the US and Brazil, other regions such as Europe are more focused on developing technologies for biodiesel production, which is also in demand (Luque et al., 2008). Generally, crops with high sugar and starch contents such as sugarcane and corn (maize) are the most common feedstocks used for conventional bioethanol production. A strong preference of these crops towards specific climatic and soil conditions in order to achieve high yields however, means that countries less endowed with favourable growth conditions are seeking out alternative resources such as potato, wheat, sugar beet and cassava (Havlík et al., 2011, Lee and Lavoie, 2013). The process of producing bioethanol from conventional feedstocks involves either the release of sugars from crops such as sugarcane or sugar beet, or enzymatic hydrolysis of starches from corn or wheat grain followed by fermentation of monomeric sugars with yeasts or bacteria into ethanol (IEA, 2007, Luque et al., 2008).

The production of biofuels from conventional feedstocks was initially believed to be a solution for problems relating to environmental pollution and oil price security. However, recent concerns have arisen regarding their actual environmental benefit and their possible impact on food prices. Many of the feedstocks used for fuel production have a shared purpose in the production of food, and therefore it has been suggested that the diversion of agricultural food crops away from food and towards biofuels has led to increasing food prices (Sims et al., 2008). An increasing global population combined with an escalating demand for food and energy has resulted in a complex nexus, raising debate as to how these limited resources should be divided between competing demands. Sceptics have further amplified this issue by criticising the actual environmental impact of biofuels, which, while initially claiming to be at least carbon neutral (if not negative), are subject to debate depending on the system boundaries taken into account when conducting environmental assessments (Murphy et al., 2011). Other controversial issues, such as the unintentional consequence of increased carbon emissions due to the direct and indirect expansion of croplands to meet greater biofuel demand, also strongly support the decision to transition from conventional to advanced biofuels from lignocellulosic feedstocks (Food and Agriculture Organisation of the United Nations, 2010, Sims et al., 2010).

1.2.2 Advanced biofuels

Many of the issues associated with conventional biofuels can be resolved by the production of advanced or 2nd-generation (2G) biofuels from lignocellulosic feedstocks. Cellulose, the main component of plant biomass, is the most abundant polymer on the planet, and with an estimated annual production of 10-50 billion dry tonnes, it represents a potentially abundant resource for biofuel production (Galbe, 2007, Naik et al., 2010). Lignocellulosic feedstocks typically fall into four main categories: 1) short-rotation forestry crops such as poplar and willow, 2) energy grasses including switchgrass and Miscanthus, 3) agricultural wastes such as sugarcane bagasse and 4) municipal solid wastes (Sims et al., 2010, Murphy et al., 2011). It is believed that where grasses and agricultural residues are abundant, there should be no requirement for additional land, and where energy crops can be grown on marginal or degraded lands, there would be no competition with food crops for agricultural lands (Sims et al., 2008).

The two primary routes for producing advanced biofuels are via the so-called thermochemical and biochemical pathways. The thermochemical route uses pyrolysis or gasification technologies to produce synthesis gas (a mixture of carbon monoxide and hydrogen), from which various long chain biofuels can be produced (Sims et al., 2008, Luque et al., 2008). In contrast, the biochemical conversion route involves the use of enzymes and microorganisms to hydrolyse cell wall sugars (cellulose, hemicellulose) into monomers, which are subsequently fermented by microorganisms into ethanol or other fuels (Sims et al., 2008). A detailed description of the biochemical process and the stages involved is given in Section 1.3. Despite significant research invested into the conversion process, progress has been hindered by numerous barriers – mainly, how to identify an economically, environmentally and energetically-favourable conversion process that can efficiently break down the recalcitrant plant cell wall into its monomeric components.

1.2.3 Composition of lignocellulose

The recalcitrance of lignocellulose is a property that terrestrial plants have developed as a structural and chemical mechanism for protection against degradation (Kristensen, 2009). This concept of recalcitrance is well known within the field of liquid biofuels, and plenty of research has been concentrated around the structure and composition of lignocellulose to seek ways to overcome this barrier. Lignocellulose is composed predominantly of three polymers – cellulose, hemicellulose and lignin, which are intimately associated with each other to form a highly complex structure (Wyman et al., 2005) (Figure 1.1). Cellulose,

hemicellulose and lignin are generally present in the ratios 30-45% cellulose, 25-30% hemicellulose and 25-30% lignin; however, the exact proportions of these components vary between different types of feedstocks and parts of the plant (Zhu, 2005, Hendriks and Zeeman, 2009, Zhao et al., 2012). Woody biomass sources have higher cellulose and lignin contents, whereas grasses are known to be more abundant in hemicellulose (primarily xylan), extractives and ash (Zhao et al., 2012).

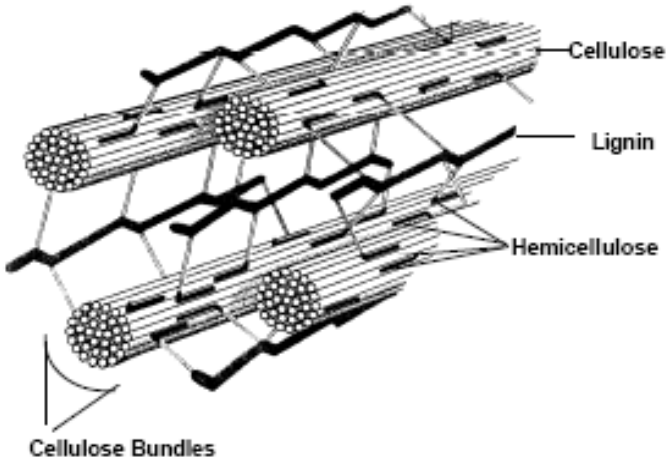


Figure 1.1 Diagrammatic representation of cellulose, hemicellulose and lignin structure in the plant cell wall (Zhu et al., 2013).

Cellulose $(C_6H_{10}O_5)_n$ is the most abundant constituent of the cell wall and is comprised almost entirely of β-1,4 linked D-glucose molecules. These glucose molecules are joined by hydrogen bonds to form microfibrils with a high degree of polymerisation ranging between 500 to 15,000 (Mittal et al., 2011). The degree of polymerisation represents the number of glucose units that make up one cellulose molecule (Harmsen et al., 2010). The β-1,4 orientation of glycosidic bonds results in the formation of intra- and inter-molecular hydrogen bonds by hydroxyl groups. This is one of the factors contributing to its highly crystalline form and it constitutes the recalcitrance of plant cell walls to enzymatic and microbial attack (Gomez et al., 2008).

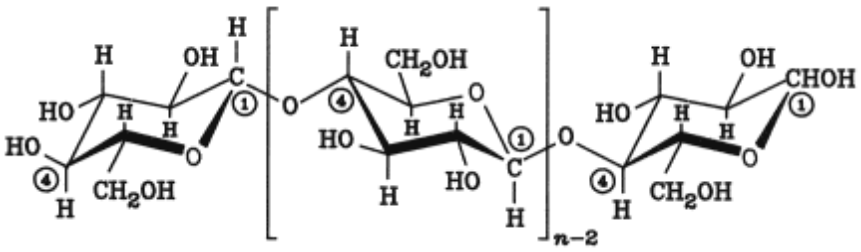
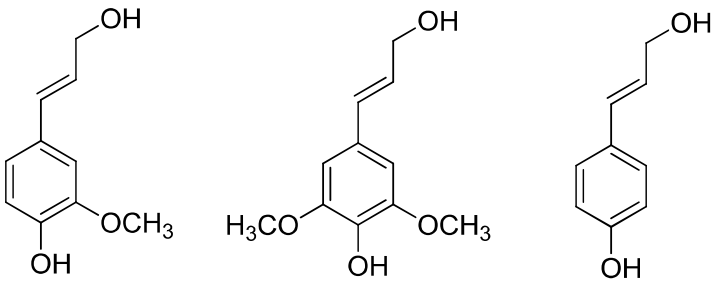


Figure 1.2 Chemical and bonding structure of cellulose (Harmsen et al., 2010).

Cellulose can exist in several crystalline polymorphs, referred to as polymorphs I, II, III and IV (Zhao et al., 2012). Native cellulose is typically cellulose I, however when subjected to chemical treatments it can be converted into other cellulose polymorphs. Non-crystalline regions of cellulose are known as amorphous cellulose, and this has been shown to be more susceptible to hydrolysis by cellulase enzymes (Mittal et al., 2011)

Hemicellulose is a highly branched polymer that provides a linkage between cellulose and lignin. Unlike cellulose, its composition and structure including linkages, side chains and degree of polymerisation differs depending on plant species and cell tissue (Zhao et al., 2012). Hemicellulose has a much lower degree of polymerisation compared with cellulose, ranging between 50 and 200 (Pu et al., 2008). In general, it is comprised of heterogeneous pentose and hexose sugars including xylose, galactose, mannose and arabinose (Gomez et al., 2008). The main polymer of grass hemicellulose is arabinoxylan, which consists of a xylan backbone with L-arabinose linked to xylose units. Various side chains are usually present, which include acetyl groups randomly attached by ester linkages to the hydroxyl group of sugar rings (Zhu, 2005). As a result of its branched and amorphous structure, it is more easily hydrolysed than the other cell wall components, which explains why hemicellulose is found to be removed more easily from cell walls during pretreatments at high temperatures and pressures (Wyman et al., 2005).

Lignin is a complex polymer formed by the polymerisation of phenyl propane units and constitutes the largest non-polysaccharide component in lignocellulose (Jørgensen et al., 2007, Brett and Waldron, 1996). It is known to surround cellulose and can covalently bond to some hemicelluloses, therefore playing a major role in protection against microbial and chemical degradation (Zhao et al., 2012). Lignin is comprised of three phenylpropanoid units as the precursors for its biosynthesis: coniferyl, sinapyl and *p*-coumaryl alcohols (Figure 1.3). These subunits make up guaiacyl (G), *p*-hydroxyphenyl (H) and syringyl (S) units, whose ratios vary depending on the type of biomass (Brett and Waldron, 1996). Hardwood species are more abundant in G and S monomers, softwoods have predominantly G monomers, whereas herbaceous plants typically contain all three of the monolignol units with additional peripheral groups such as hydroxycinnamic acids incorporated into the core structure (Chundawat et al., 2011, Pu et al., 2013)



coniferyl sinapyl p-coumaryl

Figure 1.3 Coniferyl, sinapyl and p-coumaryl building blocks of lignin (Pu et al., 2013).

1.2.4 Cell walls of the grass family

Bamboos are a group of giant, woody grasses belonging to the *Gramineae* family, Bambusoideae sub-family, comprising approximately 1,500 species within 87 genera (Liese, 1987b, El-Bassam, 1998, Kobayashi et al., 2004). Other members of this family include small grain staples (e.g. cereal straws), as well as crops grown for vegetative biomass and livestock feed (e.g. sugarcane and sorghum) (Stapleton et al., 2010). Although officially members of the *Gramineae* family, the wood-like features of bamboo which allow them to be used for construction, paper and pulping and various other "woody" applications, suggest that their unique cell walls may share similarities to both the grasses as well as wood species.

While the overall cell wall composition of grasses and dicots are broadly similar in that they contain a network of cellulose fibres encompassed by a matrix of non-cellulosic polysaccharides, the relative abundance and cross-linking of these polysaccharides as well as the presence of proteins and phenolics vary considerably (Vogel, 2008). Primary plant cell walls are classified as either Type I or Type II, based on the occurrence of matrix polysaccharides (Brett and Waldron, 1996). Type I cell walls, which are found in dicots, gymnosperms and non-graminaceous monocots, have higher levels of pectin and xyloglucan. Type II cell walls found in the Gramineae family, have lower levels of xyloglucan and pectin, and are instead richer in arabinoxylans (also known as glucoarabinoxylans or GAXs) and β-1,3, β-1,4 glucans (mixed glucans) (Gritsch, 2003, Vogel, 2008). Bamboo hemicellulose consists almost entirely (around 90%) of xylan, with different branching compared to dicots and gymnosperms (Gritsch, 2003). On the other hand, its xylan contains 6-7% acetyl groups, which is a feature common in hardwood species (Higuchi, 1985). The arabinose content in bamboo is also similar to that of softwoods, therefore demonstrating the complexity found in classifying bamboo cell walls.

Aromatic constituents such as lignins and low molecular weight phenolic acids, which in grasses, are in the form of *p*-coumaric and ferulic acid, severely limit the bioconversion of carbohydrates (Anderson and Akin, 2008). Grasses, wood and dicots all have rigid, lignified cell walls; however grasses have a higher content of phenolic acid (Baucher et al., 1998). These phenolic acids are known to form dimers and ether linkages to other aromatic components, resulting in the formation of a formidable barrier to enzymatic digestion (Anderson and Akin, 2008).

The composition of lignin in grasses based on its monomeric composition, types of linkages and organisation within the polymer, is another distinctive characteristic differentiating it from other plants. Bamboo lignin is typical of the *Gramineae* family, and is comprised of 3 aromatic alcohol precursors including *p*-coumaryl, coniferyl and sinapyl alcohols, which form the *p*-hydroxyphenyl (H), guaiacyl (G), and sinapyl (S) subunits to produce the final lignin polymer (Liese, 1987a). Gymnosperms have higher quantities of guaiacyl, dicots have relatively equal quantities of guaiacyl and sinapyl subunits, whereas monocots have a combination of all subunits and therefore lignin is called *p*-hydroxyphenyl-guaiacyl-syringyl, or “H-G-S” lignin (Brett and Waldron, 1996). The absolute lignin content however, varies widely amongst monocots ranging from 1.2% for herbaceous grasses, up to 26% for woody bamboos (Lewis and Yamamoto, 1990).

1.2.5 Bamboo biofuel potential

Bamboos are considered an ideal plantation crop because they can be grown in tropical, subtropical and temperate zones of all continents except the poles, and occupy 37 million hectares of land worldwide (Lobovikov et al., 2007). Bamboos have been utilised historically in over 1,500 different commercial applications ranging from construction, paper and pulping, textiles and agricultural tools (Scurlock, 2000). The plant consists of an underground root system (rhizome) from which aerial stems (culms) develop (El-Bassam, 1998). The rhizome branching system determines whether bamboos are classified as monopodial and leptomorph or sympodial and pachymorph (McClure, 1966). Monopodial bamboos are characterised by hollow cylindrical rhizomes that run horizontally and have buds that either develop into new rhizomes or culms (McClure, 1966). Sympodial bamboos have thicker solid rhizome structures that begin growing horizontally but can grow upwards through the ground to subsequently give rise to the culm (McClure, 1966).

The chemical composition of bamboos have been reported to contain approximately 40-48% cellulose, 24-28% hemicellulose and 20-26% lignin (as a percentage of dry matter), suggesting that with the appropriate technology there is an abundant pool of cell wall sugars available for bioethanol production (Yamashita et al., 2010). Bamboo stands are dense and productive, with an average above ground net biomass production in the order of 10 to 20 tonnes/ha/year (Isagi et al., 1997, Scurlock, 2000). Due to their high growth rate which has been reported to be the highest on the planet, reaching 120 cm in 24 hours, there is a fast turnover of harvest and re-growth from the same stand without damage to the plant (Tripathi and Khawlhring, 2010).

Bamboos have an extensive rhizome system allowing for: 1) efficient storage of nutrients year-round, limiting the amount of nutrient inputs required and reducing overall agricultural costs and environmental impact; 2) re-growth of new shoots during harvest, causing rapid regeneration over a short period of time; and 3) a reduction in soil erosion, which poses one of the largest environmental issues in countries such as China (Kobayashi et al., 2004, Potters et al., 2010). Their net-like root system creates an effective watershed protection mechanism by stitching soil together along riverbanks which can prevent the occurrence of landslides and floods (Tripathi and Khawlhring, 2010). Their ability to grow on marginal or degraded lands, which are unsuitable and economically unattractive for agricultural crops, not only brings degraded land back into production but also minimises potential indirect land-use change (this refers to the conversion of land from its original state to an altered due to biofuel feedstocks, and can result in changes in GHG emissions and carbon stock from that land) (Sanchez et al., 2012). The optimal harvesting season occurs over a 6-month period followed by the potential to store harvested bamboo for about a 3-month period, which provides a nearly year-round supply of biomass for bioethanol production (Potters et al., 2010).

1.3 Biochemical conversion process

The biochemical conversion of lignocellulose into ethanol involves three main stages: (1) pretreatment of biomass to yield an enzymatically-digestible material, (2) enzymatic saccharification to release cell wall sugars into monomeric components and (3) fermentation to convert monomeric sugars into a fuel such as ethanol.

1.3.1 Pretreatment

Due to the recalcitrant structure of lignocellulose, pretreatment is essential to ensure adequate yields of fermentable sugars from saccharification within an industrially acceptable set of conditions (Jørgensen et al., 2007). By using combinations of temperature, pH and pressure etc., pretreatment aims to improve saccharification yields by either solely or in combination:

- Increasing accessible biomass surface area and porosity
- Partially or fully removing lignin and/or hemicellulose
- Modifying the structure of lignin
- Reducing cellulose crystallinity
- Interrupting cell wall component interactions

After an effective pretreatment, the cellulose microfibrils become more accessible to enzymatic digestion, which increases the amount of monomeric sugars available for fermentation. The specific effect of pretreatment is dependent upon the pretreatment process, its operating conditions, and the feedstock used (Olofsson et al., 2008). In general, pretreatment efficacy is determined by maximising sugar release and production of useful by-products, whilst minimising sugar loss, energy use, environmental impact, chemical and capital expenditure and the formation of toxic compounds (sugar degradation products) that may inhibit downstream processes (Jørgensen et al., 2007). Pretreatment technologies fall into the categories: physical, chemical, physico-chemical and biological; these are described briefly below.

Physical or mechanical pretreatments involve chipping, grinding or milling the biomass into a smaller particle size (Kumar et al., 2009). After a physical pretreatment, enzymatic saccharification yields are improved due to the increased mass transfer and reduced cellulose crystallinity. However, high costs and energy requirements are needed to reduce the particle size significantly (Kumar et al., 2009, Harmsen et al., 2010). Chemical pretreatments using alkalis, acids, or organic solvents are alternative methods applied to improve enzymatic saccharification of cellulose. Depending on the chemical agent, the effect on biomass composition and structure varies significantly. However, high economic and environmental costs of using these chemicals and how to efficiently recover and recycle them within the process remains a critical technical, economic and environmental challenge (Zhao et al., 2009, Kumar et al., 2009). Similarly, physico-chemical pretreatments such as

steam explosion and ammonia fibre expansion (AFEX) use a combination of chemicals and high pressures to cause structural alteration of biomass (Brodeur et al., 2011). Biological pretreatments use microbial-secreted enzymes (from mainly brown, white and soft-rot fungi), to degrade lignin and hemicellulose whilst leaving cellulose intact (Ray et al., 2010). The operating conditions in biological pretreatments are usually mild, resulting in lower cost and lower energy requirements (Ray et al., 2010). However, due to the slow rates of lignin degradation, which can take up to several weeks, the industrial use of biological pretreatments has been limited up till now (Brodeur et al., 2011, Chiaramonti et al., 2012).

In the present research, three contrasting pretreatment processes expected to be effective on bamboo biomass – liquid hot water (LHW), soaking in aqueous ammonia (SAA) and dilute acid (DA) – were selected based on their impact on improving saccharification yields from herbaceous feedstocks, and were evaluated based on their abilities to improve sugar release from bamboo biomass.

1.3.1.2 Pretreatments on bamboo

Pretreatments previously attempted on bamboo include dilute sulphuric acid, biological pretreatment with white rot fungi, steam explosion, organosolv and alkali pretreatments (Table 1.1). While some pretreatments have been more effective than others, sugar yields are shown to be substantially improved after pretreatment, ranging from 60% to 96% of the initial sugar content, depending on the pretreatment type, severity and conditions. It was reported on *Phyllostachys heterocycla* that after pretreatment with 2% sulphuric acid (w/v), 69.4% of the cellulose was hydrolysed to glucose, and after pretreatment with 20% sodium hydroxide (w/w) this was increased to 95.5% conversion efficiency (Li et al., 2012a). A steam pretreatment with a sulphur dioxide catalyst was performed on *Bambusa balcooa,* which achieved an initial cellulose to glucose conversion efficiency of 62.7%, and was increased up to 91.3% when greater enzyme loadings were applied (García-Aparicio et al., 2011). A dilute acid pretreatment was previously tested on *Dendrocalamus asper*, however due to the relatively low yields reported, it was selected for further exploration in this work (Leenakul and Tippayawong, 2010, Tippayawong and Chanhom, 2011). Liquid hot water and soaking in aqueous ammonia pretreatments on the other hand, are two other processes that have shown potential for other feedstocks but have yet to be explored for bamboo.

Table 1.1 Summary of pretreatment processes reported on bamboo in the literature.

Bamboo species	Pretreatment	Sugar yield	Reference
Phyllostachys pubescens	Cellulose solvent- and organic solvent-based lignocellulose fractionation (COSLIF)	86.0% glucose yield, 82.6% xylose yield	Sathitsuksanoh et al. (2010)
Dendrocalamus asper	Dilute sulphuric acid	3.9-18.5 mg/mL total sugar yield	Leenakul and Tippayawong (2010)
Phyllostachys pubescens	White rot fungi (*Coriolus versicolor)*	37.0% of initial material	Zhang et al. (2007b)
Phyllostachys pubescens	Steam explosion/milling/ sodium hydroxide	75.8-90.1% glucose yield	Yamashita et al. (2010)
Bambusa balcooa	Sulphur-dioxide catalyst steam pretreatment	Up to 62.7% of glucose in pretreated material	García-Aparicio et al. (2011)
Bamboo (no named species)	Concentrated sulphuric acid	98.4% sugar recovery	Sun et al. (2011)
Sasa senanensis Rehd.	Catalysed steam	60-70% of initial sugar yield	Tsuda et al. (1998)
Phyllostachys heterocycla	Organosolv and alkali pretreatment	80.9-95.5% glucose yield	Li et al. (2012a)
Phyllostachys heterocycla	Microwave pretreatment with potassium hydroxide	20.9% glucose yield 63.1% xylose yield	Li et al. (2012b)
Dendrocalamus latiflorus Munro	Steam explosion with sulphuric acid catalyst	51.3% glucose yield	Wang et al. (2011)

1.3.2 Enzymatic saccharification

Enzymatic saccharification is considered to be a major technical bottleneck in the biochemical conversion process (Gomez et al., 2008). On the one hand the biochemical conversion of lignocellulose is often regarded to be advantageous over other alternatives (e.g. chemical hydrolysis) due to its potential for higher yields, lower energy costs and less severe operating conditions. However, the high and uncertain cost of enzyme presents a significant economic limitation in this process. Therefore the pretreatment stage prior to saccharification is believed to be essential to increase biomass digestibility, thereby decreasing enzyme loadings and consequently reducing the overall enzyme cost burden in the production of bioethanol (Jørgensen et al., 2007).

Many bacteria and fungi are capable of producing biomass-degrading enzymes (Yang et al., 2011). Commercially produced cellulase enzymes are typically sourced from a very limited range of fungi, with the cellulose-degrading fungus, *Trichoderma reesei* being widely used. Cellulolytic microorganisms are known to either evolve all the enzymes required for the degradation of lignocellulose, or may be part of a larger microbial community that synergistically degrades biomass (Himmel et al., 2007). Although cellulose is a homopolymer of glucose molecules, a number of different enzymes are required for its degradation (Kristensen, 2009). Three main types of cellulolytic enzymes are involved: endo-1,4-β-glucanases hydrolysing internal β-1,4-glycosidic bonds within the cellulose chain, exo-1,4-β-glucanases cleaving cellobiose from cellulose ends and 1,4-β-D-glucosidases converting cellobiose into glucose and cleaving glucose from cellooligosaccharides. The combined action of these enzymes can effectively hydrolyse cellulose by creating accessible enzyme binding sites, removing obstacles and relieving product inhibition (Jørgensen et al., 2007). *T. reesei* also produces a range of hemicellulases, however the heterogeneous nature of hemicellulose in different feedstocks means that the number and type needed in its breakdown is more variable. Therefore, understanding the biomass composition is essential for designing an optimal enzyme mixture that can effectively release sugars from plant cell walls.

Although it is known that the efficacy of enzymatic saccharification is determined by the types of enzymes used and the structure of lignocellulosic feedstocks, the mode of action taken by enzymes during saccharification is not yet completely understood (Himmel et al., 2007, Yang et al., 2011). Emerging biotechnology tools are currently being used in the development of new enzyme sources that can produce enzymes with traits such as improved specificity, thermal stability, greater resistance to environmental inhibitors, and with higher synergy with other enzyme combinations as ways to maximise sugar yields at lower costs (Yang et al., 2011). From an economic and energy perspective, maintaining a high solids concentration during saccharification is also a focus of biofuel research (Kristensen, 2009). This would result in higher sugar and therefore ethanol concentrations, minimising the amount of energy required in downstream processes, particularly in ethanol concentration (e.g. by distillation). However, higher substrate concentrations may also lead to greater concentrations of inhibitors which can hinder enzyme and fermentation activity, as well as possibly causing difficulties with stirring at higher viscosities (Kristensen, 2009).

1.3.3 Fermentation

Fermentation is a biological process where simple sugars (C5 and C6) are converted by

microorganisms into ethanol and CO_2 (Lin and Tanaka, 2006). Various yeasts, bacteria and fungi have been isolated with the ability to convert sugars into ethanol. The ideal fermentative microorganism should have a high ethanol yield and productivity, high tolerance for ethanol and inhibitors as well as the ability to utilise multiple sugars (Olofsson et al., 2008). Traditionally, the most common microorganism for ethanol fermentation is the yeast *Saccharomyces cerevisiae.* However, its inability to utilise C5 sugars such as xylose and arabinose has resulted in recent research focusing on engineering strains with improved traits, as well as investigating other types of microbes such as bacteria (e.g. *Zymomonas mobilis* and *Escherichia coli*) and xylose-fermenting yeasts (e.g. *Pichia stipites* and *Candida shehatae*) (Dien et al., 2003, Lin and Tanaka, 2006, Koppram et al., 2013).

When fermentation is initiated after saccharification this process is referred to as separate hydrolysis and fermentation (SHF). Alternatively, simultaneous saccharification and fermentation (SSF) describes the process where hydrolysis and fermentation occur simultaneously (Mosier et al., 2005b). From a cost standpoint, SSF is usually preferred over SHF due to the fewer number of reactors required and resultant lower capital costs, shorter processing times, and prevention of enzyme inhibition by maintaining glucose concentrations at relatively low levels (Lin and Tanaka, 2006, Chandel et al., 2007). The risk of contamination is also lower in SSF due to the presence of ethanol in the reactor. However, the main disadvantage of SSF relates to the need to compromise pH and temperature requirements for enzymes (45-50°C) and fermentation (28-35°C) culture conditions which often result in higher enzyme loadings and hence raw material costs (Dien et al., 2003). Optimisation of certain parameters such as substrate concentration, ratio of enzyme to substrate and yeast concentration are crucial in making this process economically and technically viable (Lin and Tanaka, 2006).

Another variant of SSF is known as simultaneous saccharification and co-fermentation (SSCF), which includes co-fermentation of both C5 and C6 sugars to ethanol. Here, the solid cellulose and solubilised hemicellulose streams derived from pretreatment are not separated, so that hemicellulose sugars are fermented to ethanol at the same time that SSF of cellulose occurs (Teixeira et al., 2000). While SSF requires two separate reactors to convert C5 and C6 sugars via different microorganisms, SSCF requires just one reactor and microorganism with the ability to process all sugars. The National Renewable Energy Laboratory (NREL) demonstrated the co-fermentation of glucose and xylose from dilute-acid pretreated yellow poplar with a metabolically engineered strain of *Z. mobilis,* and have modelled used this microorganism in their techno-economic study of bioethanol produced from corn stover (Humbird et al., 2011). The most integrative of these processes is the

development of consolidated bioprocessing (CBP), which represents the most combined saccharification and fermentation model yet. Unlike the other three processes where enzymes are provided externally, CBP uses a single reactor to produce ethanol as well as the required enzymes in the process (Chandel et al., 2007, Himmel et al., 2007). CBP demonstrates substantial economic and technical potential due to the elimination of operating and capital costs for enzyme purchase or production, however paths to identify an ideal organism which can efficiently carry out all these functions remains the biggest obstacle.

1.4 Evaluation of economic feasibility

Although the production of cellulosic ethanol on a commercial scale has yet to be successfully demonstrated, the number of global pilot- and demonstration-scale plants established over recent years has significantly increased. Various factors are known to influence the commercial viability of ethanol production such as feedstock availability and quality, ethanol distribution systems and government subsidies. However, the conversion process is one of the largest contributors to determining the overall cost (Slade et al., 2009). For bamboo there has yet to be the identification of an optimal conversion process for maximising sugar release and ethanol yields (Escobar et al., 2009). Market prices for fuels and feedstocks have undergone dramatic fluctuations over the past few decades. Oil prices have a direct impact on the economic feasibility of biofuels because as the international cost of oil rises, the competitiveness and profitability of biofuels also increases (Escobar et al., 2009). For example, between 2002 to 2008, petroleum prices varied from lows of $20 per barrel to highs of over $140 per barrel, and costs of feedstocks such as corn ranged from $2 per bushel to $4.20 per bushel in the US (Tao and Aden, 2009). These unpredictable price fluctuations have a significant impact on the economics of biofuel production.

Techno-economic models have been used to assess the commercial potential of biofuel processes (Tao and Aden, 2009). Although they provide a method to summarise the economic feasibility of a conversion process, many assumptions have to be made due to limited or extrapolated data, which increases uncertainty. Nevertheless, such evaluations are used extensively by companies, researchers and government bodies in the bioenergy sector, and are regarded as important for assessing the overall feasibility of biofuel production. Various techno-economic studies assessing the production economics of current and future conversion processes to generate bioethanol as well as biodiesel have been reported (Aden et al., 2002, Slade, 2009, Tao and Aden, 2009). For evaluating these

studies, chemical engineering simulation software tools such as Aspen Plus, Aspen HYSYS and gPROMS are often used (Wang, 2011). The techno-economic model developed by NREL in 2011 for the conversion of corn stover into bioethanol using dilute acid pretreatment uses Aspen Plus (Humbird et al., 2011). This model has been adopted as a basis for other techno-economic studies, and was modified and applied for the present work. Table 1.2 provides a summary of recent economic studies from the literature for cellulosic bioethanol production published between 2008 and 2010.

Each of these studies has defined specific process parameters such as plant size, feedstock type and cost as well as the processing conditions, resulting in a wide range of minimum ethanol selling prices (MESP) reported. The production prices for bioethanol from corn stover range from $0.39/litre (Aden et al., 2002) to $1.21/litre (Klein-Marcuschamer et al., 2010), despite the same feedstock and processing technologies being utilised in these different studies. This demonstrates the price variation that can occur as a result of setting assumptions based on parameters which have wide economic uncertainty (Gnansounou and Dauriat, 2010). Therefore, although comparisons between studies can be made, the MESP is more suited to: 1) comparison of process alternatives, e.g. pretreatment technologies (Sendich et al., 2008), 2) sensitivity analyses to identify major areas that require optimisation and 3) projecting the effects of developments in technology that might be reached in the future (Hamelinck et al., 2005, Humbird et al., 2011).

Table 1.2 Comparison of biochemical cellulosic ethanol production according to recent techno-economic studies (Humbird et al., 2011).

Feedstock	**Scale (dry tonne/day)**	**Feedstock cost ($/dry tonne)**	**Ethanol yield (l/dry tonne)**	**MESP ($/litre)**	**Processing technology**	**Reference**
Corn stover	850	45	295	0.49-0.58	AFEX pretreatment	Bals et al. (2010)
Straw, eucalyptus, poplar	1,760-2,200	57-127	265-318	0.56-0.77	Dilute acid pretreatment	Gnansounou and Dauriat (2010)
Aspen, poplar, corn stover, switchgrass	2,200	58-100	314-420	0.38-0.49	Dilute acid pretreatment	Huang et al. (2009)
Corn stover	2,200	75	159-273	0.90-1.17	Varying pretreatments	Kazi et al. (2010b)
Corn stover	1,700	60	197-280	0.93-1.21	Dilute acid pretreatment	Klein-Marcuschamer et al. (2010)
Switchgrass	5,000	44	367-397	0.17-0.22	AFEX pretreatment, Consolidated Bioprocessing (CBP)	Laser et al. (2009)
Corn stover	2,200	51	341	0.39	Dilute acid pretreatment, SSCF, electricity co-product	Aden et al. (2002)
Corn stover	2,200	59	299	0.57	Dilute acid pretreatment, SHF, electricity co-product	Humbird et al. (2011)
Hardwood	2,200	65	284	0.94-1.06	Dilute acid pretreatment	Piccolo and Bezzo (2009)
Corn stover	2,200	40	265	0.27-0.37 0.37-0.49	AFEX pretreatment, SSCF process and CBP process	Sendich et al. (2008)

1.5 Support schemes for biofuel production

With the appropriate technology, advanced biofuels have the potential to resolve issues relating to energy diversification and reduction of GHG emissions, and therefore policies should be tailored to support their deployment and speed up the rate of commercialisation (Carriquiry et al., 2010). It is believed that with suitable policy measures, advanced biofuel markets can become more economically attractive for relevant stakeholders including consumers, investors and producers, which would also increase their production and consumption on a global scale. However, there has been a historic dependence of alternative energy technologies on government support in order to compete with established fossil and other fuels in the marketplace (Rajagopal and Zilberman, 2007). A broad spectrum of policies such as excise tax exemptions, carbon taxes, mandatory blending requirements, and trade policies currently exist, however there is no "one size fits all" policy tool due to the diversity of factors and influences involved, meaning that each national situation needs to be assessed individually.

Energy and carbon policies are the most widely used instruments to help biofuels compete with fossil fuels. These include: 1) biofuels being exempted from excise taxes levied on fuels; 2) obligatory blending requirements mandating a certain amount of renewables to be used in domestic markets; 3) carbon taxes on fossil fuels to favour biomass as an energy source; and 4) subsidies or tax credits to support ethanol vehicles such as flex-fuel vehicles (FFVs) (Rajagopal and Zilberman, 2007). In addition, due to the significant contribution that feedstock has towards the cost of biofuel production, agricultural policies can be used to support farmers growing biofuel crops, which would lower overall costs. While trade policies such as taxation of exports serve to protect domestic producers in competing with the low costs of international markets, this may result in protection of less environmentally benign fuels. Lastly, government funding of research and development can improve conversion process technologies and crop yields, which have the potential to reduce supply chain costs and accelerate deployment of advanced biofuel production at the commercial scale (Sims et al., 2008).

1.6 Case study scenarios

1.6.1 China

Currently almost half of China's oil consumption is imported, and with the projection that the demand for fossil fuel oil will be 250 million tons per year by 2030, it has become crucial for the country to consider biomass alternatives as part of their renewable energy plan (Li and Chan-Halbrendt, 2009, Qiu et al., 2010). The exponential rise in private car ownership, which exceeded the United States in 2009 to become the world's largest auto market, is expected to double the present oil consumption. This has led to increased imports to meet demand and directly increases environmental pollution. Establishing a biofuel industry in China therefore provides an attractive solution for managing problems of energy insecurity, environmental pollution and rural development (Yang et al., 2005, Li and Chan-Halbrendt, 2009, Fang et al., 2010)

In China's 10th five-year plan (2001-2005), the government proposed the establishment of a biofuel industry aimed initially at utilising surplus grain stocks. However, due to concerns regarding food insecurity, government policy shifted towards promoting the use of non-food feedstocks grown on marginal and abandoned lands (Li and Chan-Halbrendt, 2009). Through the government's support for biofuel production using subsidies and the promotion of gasohol (low ethanol blends usually 10% ethanol, 90% gasoline, and also referred to as E10), China has become the third largest bioethanol producer in the world after the US and Brazil, with an overall fuel ethanol production capacity of 1.95 million tonnes (Qiu et al., 2010). Now, approximately 10% of the total fuel supply is accounted for by biofuels, and the recent increase in pilot plant projects in Henan, Anhui, Jiangsu amongst many other provinces, are positive indicators for a promising future for biofuels in China.

The Chinese government more recently issued an order to halt construction of manufacturing plants using food crops such as corn, which led to a shift in the production of fuel ethanol from corn and wheat to non-food crops that can be grown on marginal and abandoned lands (Li and Chan-Halbrendt, 2009). The Ministry of Agriculture has estimated the marginal and abandoned land area for energy crops in China ranges from 35-75 million hectares, of which 24 million hectares can be cultivated, suggesting a significant land area for growing biofuel crops (Tang et al., 2010). The failure to identify a key, non-food feedstock that can be grown on marginal or abandoned lands is a major constraint preventing the expansion of fuel ethanol (Cai et al., 2011). China's bamboo resources are one of the richest in the world. Its bamboo forests cover 7.6 million hectares of land and occupy 3% of the total

forest area distributed across 18 provinces (Maoyi, 2002, Zhou et al., 2011). The largest bamboo applications include edible shoot production, culms for material uses and paper and pulp production (Kleinhenz and Midmore, 2001). China's bamboo sector has increased by 54% since 1970 and the total forest area has grown at an annual rate of 3% since 1980 (Cao et al. 2011). These statistics supported by the fact that bamboo can potentially be grown on marginal lands, indicate a currently unrecognised industry for bamboos as a lignocellulosic feedstock for bioethanol production in China.

1.6.2 Colombia

Though Latin America is the second highest emitter of transport-derived CO_2 after North America, 2007 reports indicate that transport had the greatest relative share of CO_2 emissions compared to all other sectors (Schipper et al., 2010). In the last 10 years, demand for petrol has grown at a rate of 4.4% a year and this will no doubt contribute towards road emissions, which are also predicted to rise three-fold by 2030 due to increasing private car ownership (Consejo Nacional de Política Económica y Social República de Colombia, 2008, Schipper et al., 2010). While Brazil has the most well-known bioethanol industry in the continent, Colombia has recently emerged as a potential competitor, with a daily production of over a million litres, most of which is derived from sugarcane (Ministry of Mines and Energy, 2010, Janssen and Rutz, 2011).

The combination of suitable soil and climate conditions, available land resources and low labour costs make Colombia an ideal location for developing a biofuel industry (Janssen and Rutz, 2011). It is estimated that of the 114 million hectares of land, only 40,000 hectares are currently devoted to bioethanol production, therefore suggesting sufficient land availability for this purpose (Ministry of Mines and Energy, 2010). There are 13 sugar mills in Colombia, 95% of these allow for year-round sugarcane production, and 5 of these mills commercially produce bioethanol as a transport fuel (Toasa, 2009, Asocaña, 2012). Despite only being in operation for relatively few years, Colombia's bioethanol industry has already become the second most developed in the Western Hemisphere with an estimated total bioethanol production of over 336 million litres in 2011 (Toasa, 2009, Asocaña, 2012). These five plants with a recent addition of three more, produce enough bioethanol to meet the country's demand for 8% bioethanol blends with petrol (Asocaña, 2012). However, as domestic mandates rise each year, which, by 2015 aim to achieve 20% bioethanol blends with petrol, alternative feedstocks which can accelerate expansion of this bioethanol industry should not be overlooked (Toasa, 2009, Asocaña, 2012).

Latin America has approximately 11 million hectares of bamboo land, accounting for 39% of the world species total (Takahashi, 2006). Colombia, after Brazil and Venezuela, is reported to have the third richest bamboo resources with approximately 70 different species, with the majority belonging to the genera *Chusquea* and *Guadua* (Takahashi, 2006, Kleinn and Morales-Hidalgo, 2006). It was estimated that *Guadua* bamboo occupy approximately 51,500 hectares of land, of which 46,250 hectares are naturally regenerated forest (Londoño, 2001). However, currently less than 10% of the Colombian *Guadua* resources are used, and even less are sustainably managed (De Flander, 2005). Despite reports that bamboos are widely utilised in most of the Latin American countries, it is still considered a forest resource with little significance in many of the local economies (Muller and Rebelo, 2011). The possibility of using bamboo for bioethanol production offers a relevant application with the possibility of shifting its stigma of being merely a poor man's timber.

Chapter 2 Pretreatment and enzymatic saccharification of bamboo

2.1 Introduction

This chapter compares the technological feasibility of three pretreatment processes (liquid hot water, soaking in aqueous ammonia and dilute acid) as potential routes for maximising sugar release from bamboo. The effect of various pretreatment conditions were investigated in laboratory studies by assessing changes in cell wall composition and by measuring the amount of sugar released after enzymatic saccharification with a standardised enzyme loading. Enzymatic saccharification conditions were explored in a time course assay with different enzyme loadings to determine the optimal conditions for enhancing sugar release from bamboo.

Liquid hot water (LHW) pretreatment uses pressure to maintain water in a liquid state at high temperatures, which is shown to improve enzymatic digestibility of biomass by removing most of the hemicellulose content (Pérez et al., 2008, Hendriks and Zeeman, 2009). It was reported in the literature that up to 90% of glucan and 80% of xylan were released from corn stover; and for rye straw, yields were maximised to 92% of glucan and 95% of xylan after a LHW pretreatment at 190°C for 15 minutes (Ingram et al., 2009). The major advantage of LHW pretreatment is that it requires no additional chemical inputs. As a result, it is not only economically and environmentally favourable, but the low risk of equipment corrosion avoids the use of expensive materials of construction (Cybulska et al., 2009). However, the higher pressures and large amounts of water required may incur a greater energy consumption in order to make this process feasible at the industrial scale (Brodeur et al., 2011).

Soaking in aqueous ammonia (SAA) is a low severity (low temperature and pressure) process in which the biomass is soaked in aqueous ammonia (NH_4OH) from several hours to days at a time (Kim et al., 2009a). It was demonstrated on barley hull, that SAA pretreatment removed 50-66% of lignin, which resulted in glucose and xylose yields of 83% and 63%, respectively during saccharification (Kim et al., 2008). One major advantage of this treatment is that the majority of sugars can be preserved in the solid fraction, which minimises the amount of sugar degradation in the hydrolysate and consequently eliminates the need for a detoxification step prior to saccharification (Kim and Lee, 2005). The mild operating conditions required suggest that SAA pretreatment has the potential to be less energy-intensive than other high-temperature and pressure pretreatments. However, recovering and recycling ammonia remains to be one of the main challenges from a cost and environmental standpoint (Ko et al., 2009, Kim et al., 2009a).

Dilute acid (DA) pretreatment involves treating the biomass with a dilute acid solution at concentrations of 0.2-2.5% (w/w), at temperatures in the range of 130-210°C and for times of 5-40 minutes (Lloyd and Wyman, 2005, Mosier et al., 2005a, Qiu et al., 2010, Redding et al., 2011, Tippayawong and Chanhom, 2011). Although nitric, phosphoric and hydrochloric acid have been studied, sulphuric acid is the most common due to its low cost (Mosier et al., 2005b). Pretreatment of corn stover with 0.98% H_2SO_4 at 140°C for 40 minutes resulted in a total glucose and xylose conversion efficiency of 93% after significant hydrolysis of hemicellulose during pretreatment (Lloyd and Wyman, 2005). However, its limitations include: 1) the corrosive and toxic nature of acids, which mandate expensive materials of construction; 2) the requirement for neutralisation of the hydrolysate after pretreatment due to sensitive microorganisms and enzymes; and 3) possible sugar degradation into 5-hydroxymethylfurfural (HMF) and furfural, which not only reduces the potential sugar content for conversion into fuel, but can also inhibit microbial activity in downstream processes (Harmsen et al., 2010).

For each pretreatment process, a range conditions were tested and the following aspects were measured: 1) the effect of pretreatment on cell wall composition, 2) the effect of pretreatment on enzymatic saccharification and 3) the effect of varying enzyme loading on total sugar release from bamboo pretreated with the “optimal” pretreatment conditions.

2.2 Materials and methods

2.2.1 Plant material and preparation of biomass

Culms from two temperate, monopodial bamboo species, *Phyllostachys dulcis* and *Phyllostachys viridi-glaucescens* were harvested from the Royal Botanic Gardens, Kew. Leaves and branches were removed and each culm was separated into nodes and internodes and then left to air-dry in the laboratory for 2 weeks to reach a moisture content of approximately 10%.

Nodes and internodes were first ground separately using a Retsch AS2000 cutting mill with a 1 mm screen and then sieved to collect the material within the 850 and 180 µm size fraction. Moisture contents of each sample were measured gravimetrically by oven-drying samples at 105°C to allow dry matter (DM) to be calculated. This material was subsequently used as the ‘standardised’ bamboo biomass for all further experiments.

2.2.2 Compositional analysis

Compositional analysis of unpretreated (raw) and pretreated bamboo was performed to compare the effect of pretreatment on biomass composition. A two-step extraction using water followed by 95% ethanol was performed using a Dionex Accelerated Solvent Extractor (ASE) 200, following the National Renewable Energy Laboratory (NREL) protocol "Determination of extractives in biomass" (Sluiter et al., 2005). Samples were air-dried, re-weighed and moisture contents calculated to determine the percentage extractives in the biomass.

Compositional analysis procedures were based on the NREL protocol "Determination of structural carbohydrates and lignin in biomass" (Sluiter et al., 2008). Polymeric carbohydrates were hydrolysed into monomers and measured by High performance liquid chromatography (HPLC) (Agilent 1200 series) using a Bio-Rad Aminex HPX-87P column at 80°C with a flow rate of 0.6 ml/min water mobile phase. The lignin fractionates into acid-insoluble and acid-soluble material, which were assayed by gravimetric analysis and UV-Vis spectroscopy, respectively.

2.2.3 Enzyme activity and enzymatic saccharification

Prior to enzymatic saccharification, enzyme activity was measured according to the NREL protocol "Measurement of cellulase activities" (Adney and Baker, 2008). This determined cellulase enzyme activity in terms of "filter paper units" (FPU) per millilitre of original enzyme solution.

Enzymatic saccharification was conducted according to the NREL protocol "Enzymatic saccharification of lignocellulosic biomass" (Selig et al., 2008). Biomass samples equivalent to 0.1 g of cellulose (on a dry-weight basis) were weighed out into vials and each sample was performed in triplicate. To each vial the following were added: 5.0 mL of 0.1 M sodium citrate buffer (pH 4.8), 40 µL tetracycline, 30 µL cycloheximide and a calculated amount of distilled water to achieve a total volume of 10.0 mL in each vial, after the addition of enzyme. For each biomass sample, a corresponding control without enzyme was set-up to measure the concentration of soluble sugars released from the biomass, which could be subtracted from the total sugars to yield the amount released as a result of enzymatic hydrolysis. All samples were warmed in a shaking incubator set to 50ºC at a speed of 500 rpm. Two rounds

of enzymatic saccharification were performed and this determined the volume of enzyme added to each vial.

The first enzymatic saccharification was a standardised test to compare the effect of pretreatment on sugar release from bamboo with unpretreated bamboo. This was carried out for 72 hours with an enzyme loading of 60 FPU/g glucan of an enzyme mixture containing a 1:1 ratio of cellulase (Celluclast 1.5L) and β-glucosidase (Novozyme 188) as defined by the NREL protocol.

The sugar yield results from the first round of saccharification were used to determine the best pretreatment conditions, which served as the pretreated biomass for the second round of saccharification. All biomass samples were pretreated under the same conditions to determine the effect of saccharification time and enzyme loading on the total sugar release. Saccharification was carried out using a commercial enzyme mixture from Novozymes, Cellic® CTec2 (containing a blend of cellulase, β-glucosidase and hemicellulase enzymes) (Novozymes, 2010). Cellic CTec2 was used as it was the most recent commercial cellulase enzyme mixture released at the time the experiments were conducted. A time course assay was performed using Cellic CTec2 at loadings of 10, 30, 60, 100 and 140 FPU/g glucan, with samples harvested at intervals of 4, 8, 24, 48 and 72 hours.

Sugar standards were prepared at concentrations of 0.1, 1, 2 and 4 mg/mL for comparison against samples and HPLC analysis was used to measure glucose and xylose concentrations. To calculate the percentage digestibility of cellulose, soluble glucose (from controls without enzyme) concentrations were subtracted from enzyme-containing samples. A glucose anhydration correction factor (0.9) was used to correct for the water molecule added during hydrolysis of the cellulose polymer. The percentage sugar release was calculated by dividing grams of cellulose digested by the grams of cellulose added.

2.2.4 Liquid hot water pretreatment

LHW pretreatment was carried out using the Dionex ASE 200 machine. After a preliminary screen of pretreatment conditions, a second set of experiments within a suitable range were tested. Biomass (2.0g DM) was pretreated using a cell size of 22 mL in triplicate under the following conditions (Table 2.1).

Table 2.1 Liquid hot water pretreatment conditions

Parameter	Value
Temperature (°C)	170, 180, 190
Residence time (min)	10, 20 30
Pressure (kPa)	3,447
Heat-up time (min)	7, 8, 9
Purge time (sec)	120
Flush volume	100%

2.2.5 Soaking in aqueous ammonia pretreatment

A review of the literature suggested that Soaking in aqueous ammonia (SAA) pretreatment was successful in enhancing saccharification yields either with a combination of long pretreatment times at low temperatures, or with short times at high temperatures (Kim and Lee, 2005, Kim et al., 2008, Ko et al., 2009, Pallapolu et al., 2011). Preliminary trials of SAA pretreatment were first tested using a wide range of temperature and time parameters, which were indicative of a more suitable range of conditions for bamboo (Table 2.2). All SAA pretreatments were performed in triplicate on 2.0g DM. Biomass was pretreated in pressure tubes in a temperature-controlled oven. Tubes were cooled in a fume hood before performing vacuum filtration using Whatman 54 glass filter paper. Biomass was washed with approximately 2 litres of deionized water to neutralize the pH to approximately pH 5. The washed biomass was air-dried overnight and moisture contents were determined.

Table 2.2 Soaking in aqueous ammonia pretreatment conditions

Parameter	Value
Temperature (°C)	100
Time (min)	360, 840, 14,400
NH_4OH concentration (%w/w)	10, 15, 20, 25
Solid:liquid ratio	1:10

2.2.6 Dilute acid pretreatment

DA pretreatments using sulphuric acid were conducted in the Dionex ASE 200 machine. For each sample, approximately 2.0g of DM was used with a cell size of 22 mL under the following range of conditions (Table 2.3).

Table 2.3 Dilute acid pretreatment conditions

Parameter	Value
Temperature (°C)	120, 160, 200
Time (min)	5, 15
Pressure (kPa)	3,447
Sulphuric acid concentration (%v/v)	0.2, 1.0, 2.0
Heat-up time (min)	6, 8, 10
Purge time (sec)	120
Flush volume	100%

Following DA pretreatment, samples were washed through with distilled water using the Dionex ASE 200 to restore the pH to a suitable level (approximately pH 5) for enzymatic saccharification. The water-wash conditions are listed below in Table 2.4.

Table 2.4 Post-dilute acid pretreatment water wash conditions

Parameter	Value
Temperature (°C)	40
Time (min)	1
Pressure (kPa)	3,447
Heat-up time (min)	5
Flush volume	100%
Purge time (sec)	120

2.3 Results and discussion

2.3.1 Characterisation of bamboo biomass

A time course saccharification assay was performed on *P. dulcis* over a 144-hour (6 day) period to seek an optimal time point where the majority of glucose was released (Figure 2.1). This would reveal whether or not saccharification assay times could be reduced from the suggested level of 7 days (168 hours) (Selig et al., 2008). Total glucose is comprised of soluble glucose (stored carbohydrate reserves usually from starch in parenchyma cells) and "enzyme-derived" glucose (from enzymatic hydrolysis of glucan in the cell wall). It appeared that at 24 hours the enzyme-derived glucose reached a plateau though continued to increase slowly up to 72 hours where it reached a maximum of 6.7% of DM.

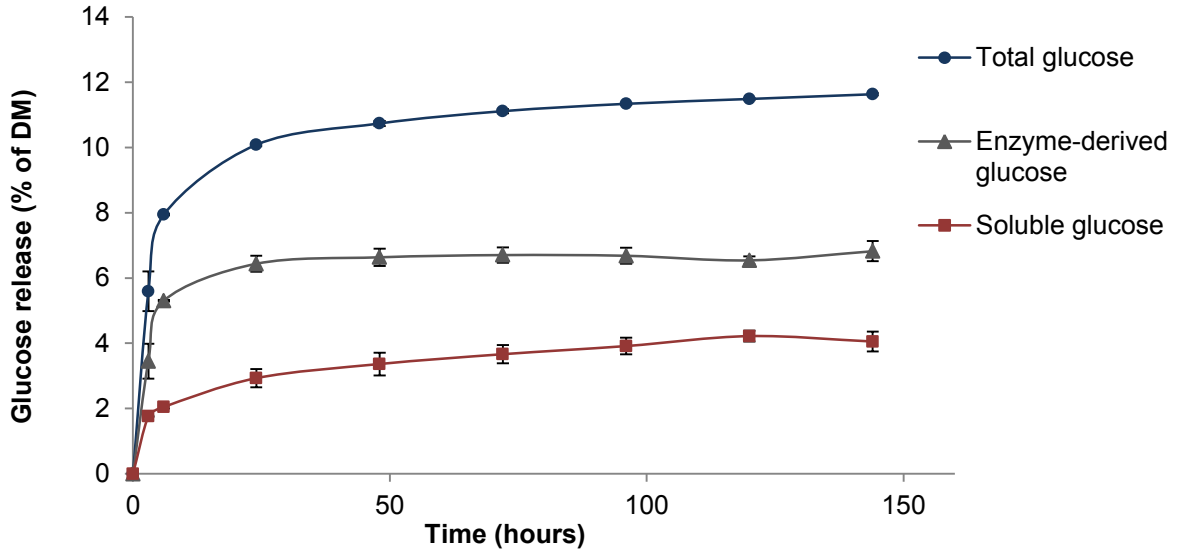


Figure 2.1 Time course assay of glucose release in enzymatic saccharification of *P. dulcis* over 144 hours, with standard error shown (n=3).

This "threshold" time point of 72 hours seems fairly consistent with other studies on bamboo saccharification rates, which have shown to reduce the suggested 7-day period to a range between 24-72 hours (Zhang et al., 2007a, Yamashita et al., 2010, Leenakul and Tippayawong, 2010). These results suggested that for future saccharification experiments, 72 hours would be an appropriate amount of time to ensure a close to maximal level of sugar release.

The chemical composition and saccharification yield (on extractive-free biomass) of nodes and internodes in *P. viridiglaucescens* were investigated to determine if there was any variation between different parts of the bamboo culm (Table 2.5). The variation in composition between nodes and internodes did not exceed 3% for any cell wall component. Glucan contents were 35-38% of DM, hemicellulose (xylan, galactan and arabinan) totalled 25-26% of DM, lignin was 20-22% of DM, ash was less than 1% of DM, and extractives were 13% of DM. Mass closures for nodes and internodes were 99.4% and 100.3%, respectively. The glucan to glucose sugar conversion was 4.5% of DM in the internodes compared with 3.8% of DM in the nodes. Single-factor ANOVA statistical analysis confirmed that there was no significant difference between the nodes and internodes for composition and saccharification at a p-value of 0.001. As a result, the whole bamboo culms (including nodes and internodes) for both species were combined for the following pretreatment and saccharification studies. It was assumed that bamboo waste residues used as the primary feedstock for the techno-economic model would be mainly comprised of the thin parts of the

upper section of the bamboo culm, and therefore would also have a very similar composition.

Table 2.5 Average composition of *P. viridiglaucescens* nodes and internodes (n=3)

Cell wall component	Node (% of DM)	Internode (% of DM)
Glucan	35.2 ± 0.8	38.4 ± 0.5
Xylan	21.0 ± 0.7	20.5 ± 0.5
Galactan	3.5 ± 0.3	3.6± 0.3
Arabinan	2.0 ± 0.2	1.8 ± 0.2
Lignin	22.5 ± 0.9	20.8 ± 0.5
Ash	0.6 ± 0.3	0.9 ± 0.2
Extractives	13.8 ± 1.7	13.5 ± 1.8
Mass closure	99.4 ± 0.2	100.3 ± 0.8

Compositional analysis of *P. viridiglaucescens* (Pv) and *P. dulcis* (Pd) showed that there was no significant difference in the composition between the two species (ANOVA, p-value>0.001 for all components except glucan which was significant at 0.05) (Table 2.6). The glucan to glucose conversion efficiency for Pd was 5.9% compared with 5.7% in Pv and these were not significantly different.

Table 2.6 Average composition of *P. viridiglaucescens* and *P. dulcis* (n=3)

Cell wall component	***P. dulcis* (% of DM)**	***P. viridiglaucesens* (% of DM)**
Glucan	36.1 ± 0.7	37.4 ± 0.3
Xylan	20.7 ± 0.5	21.0 ± 0.4
Galactan	2.4 ± 0.6	2.6 ± 0.2
Arabinan	4.0 ± 0.4	3.5 ± 0.5
Lignin	20.4 ± 0.5	21.7 ± 0.5
Ash	0.0	0.1 ± 0.1
Extractives	16.9 ± 1.8	14.4 ± 1.5
Mass closure	98.9 ± 1.4	101.3 ± 0.1

Because there were no significant differences in composition and saccharification yields between bamboo species and parts of the culm, the internode composition for *P. viridiglaucescens* was used to represent unpretreated bamboo material for comparison with pretreated bamboo. In this material, the glucan, xylan and lignin contents for nodes and internodes were all lower than the values reported in Scurlock (2000) for other members of

the *Phyllostachys* genus (40-48%, 23-27% and 25-30% of DM, respectively for glucan, xylan and lignin) as well as those reported in Higuchi (1957). This discrepancy seems to be due to the high extractives content of 13.5% in this work, which is significantly higher compared to the range of 0.3-3.1% in the aforementioned study, which would have had a proportionate impact on the composition of other constituents. The extractives content comprises non-structural components of the biomass such as proteins, ash, chlorophyll, waxes and sucrose (Sluiter et al., 2005). One reason for variation could have been related to differences in analytical procedures used for measuring the extractives. Additionally, it known that bamboo has a fairly high starch content of around 2-6% of DM, and this level can be influenced by seasonal variation, which could be a possible contributing factor responsible for inconsistencies between values reported in this work with other studies (Li, 2004). Further work could involve an analysis of the extractives composition to reveal whether a significant portion of this percentage was comprised of by starch. As a result, all compositional values reported are based on extractive-free bamboo biomass.

2.3.2 Liquid hot water pretreatment

2.3.2.1 Effect of liquid hot water pretreatment on biomass composition

Harsher LHW pretreatment conditions (higher temperatures and longer times) led to a greater proportion of biomass solubilised into the liquid hydrolysate (referred to as “mass loss” in Figure 2.2). Mass loss across the conditions ranged from 23.9% to 47.3% of DM. This was determined by the difference in dry weight between the pretreated and unpretreated biomass. Under the most severe pretreatment of 190°C for 30 minutes, the initial glucan content of 38.4% was reduced to 31.1% of DM (18.9% reduction). Although the absolute level of glucan has decreased after pretreatment, its proportion in the residual biomass is enriched through the selective solubilisation and removal of hemicellulose sugars and extractives from the biomass, and it now comprises about 60% of the composition in the residual solid material. The maximum lignin removal was observed after LHW pretreatment at 190°C for 10 minutes, where it decreased from 20.8% to 16.0% of the DM (23.3% reduction), but was found to increase at the 20 and 30 minute pretreatments at this temperature. Of the biomass components, hemicellulose (xylan, galactan and arabinan) was the most labile to pretreatment with LHW. This was shown by a complete removal of galactan and arabinan at 190°C, and a reduction in xylan from 20.4% to 1.4% of DM during pretreatment at 190°C for 30 minutes (93.3% reduction).

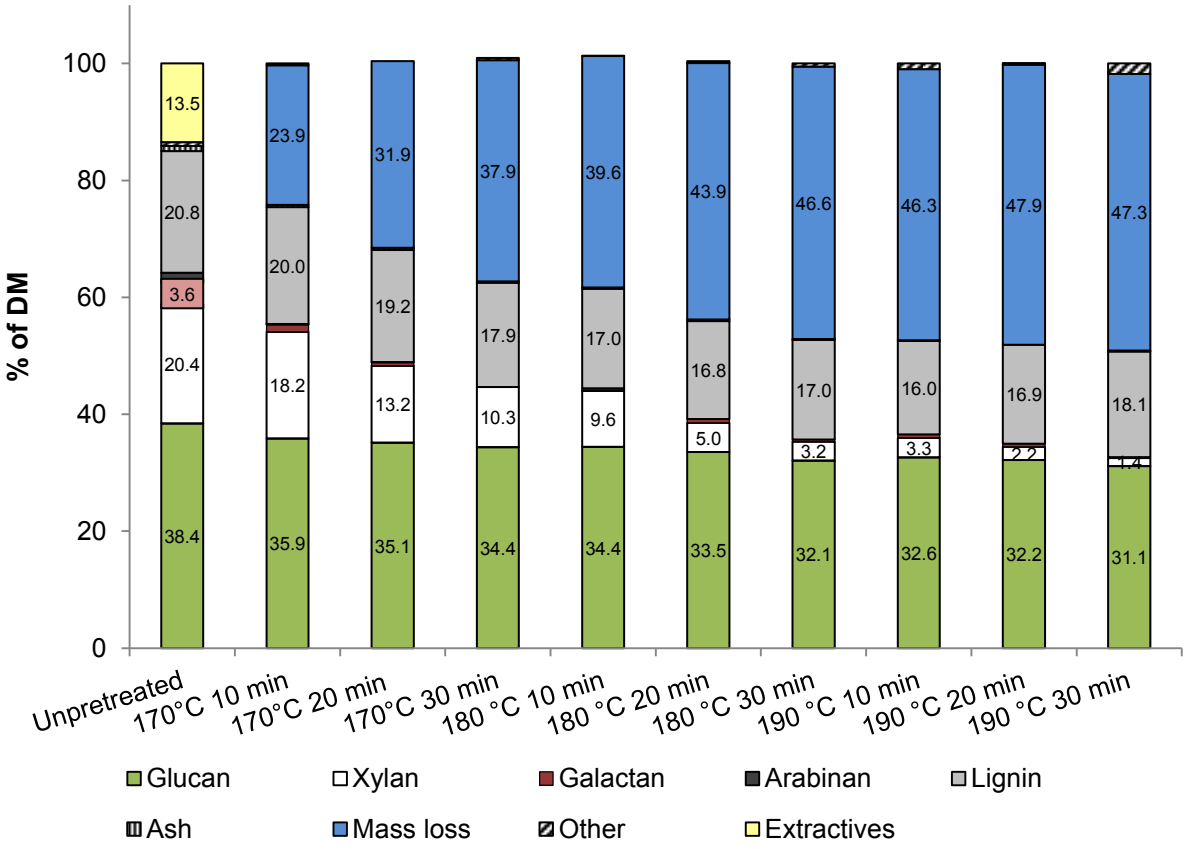


Figure 2.2 Complete mass closures of bamboo pretreated with liquid hot water as a percentage of dry matter (Figure also presented in Littlewood and Murphy (2012)).

The effect of LHW pretreatment on solubilisation of hemicellulose is consistent with other findings for bamboo as well as different biomass feedstocks (Pérez et al., 2008, García-Aparicio et al., 2011, Kim et al., 2011, Bondesson et al., 2013). The same response was also reported in a closely related bamboo species, where the increased severities during steam pretreatment resulted in an enhanced xylan removal of up to 68% of its initial content (García-Aparicio et al., 2011). It is known that during a hydrothermal pretreatment, fragmented lignin can react with itself or with other hemicellulose degradation products to form larger molecules known as “pseudo-lignin” (Sannigrahi et al., 2011). The formation of pseudo-lignin could explain the increased lignin content observed during the more severe pretreatments. The significant solubilisation of hemicellulose can be attributed to the auto-ionisation of water occurring during LHW pretreatment. This results in the formation of hydronium ions (H_3O^+) causing further hydrolysis of glycosidic linkages and deacetylation of hemicelluloses, which are less stable than cellulose at higher temperatures (Hashaikeh et al., 2007, Carvalheiro et al., 2008, Holopainen-Mantila et al., 2013). Although other reports have demonstrated that the sugars released during LHW pretreatment are predominately in oligomeric form, it is assumed in this work that these sugars are either in, or could easily be

converted into their monomeric form for direct fermentation into bioethanol (Cara et al., 2007, Kim and Lee, 2007).

2.3.2.2 Effect of liquid hot water pretreatment on sugar release

The sugars released from pretreatment and enzymatic saccharification are summed to evaluate pretreatment efficacy based on total release of cell wall sugars. The pretreatment (PT) sugar yield includes glucose, xylose and "other sugars" (arabinose and galactose), solubilised into the hydrolysate during pretreatment. The enzymatic saccharification (ES) sugar yields constitute glucose and xylose released from the residual glucan and xylan in the pretreated biomass. Total sugar release is presented as a percentage of the initial biomass DM, and also of the theoretical maximum sugar release (64.2% sugars in unpretreated bamboo). This approach reflects the relative contributions that pretreatment and enzymatic saccharification have towards total sugar release, as well as the contributions of sugars at each stage.

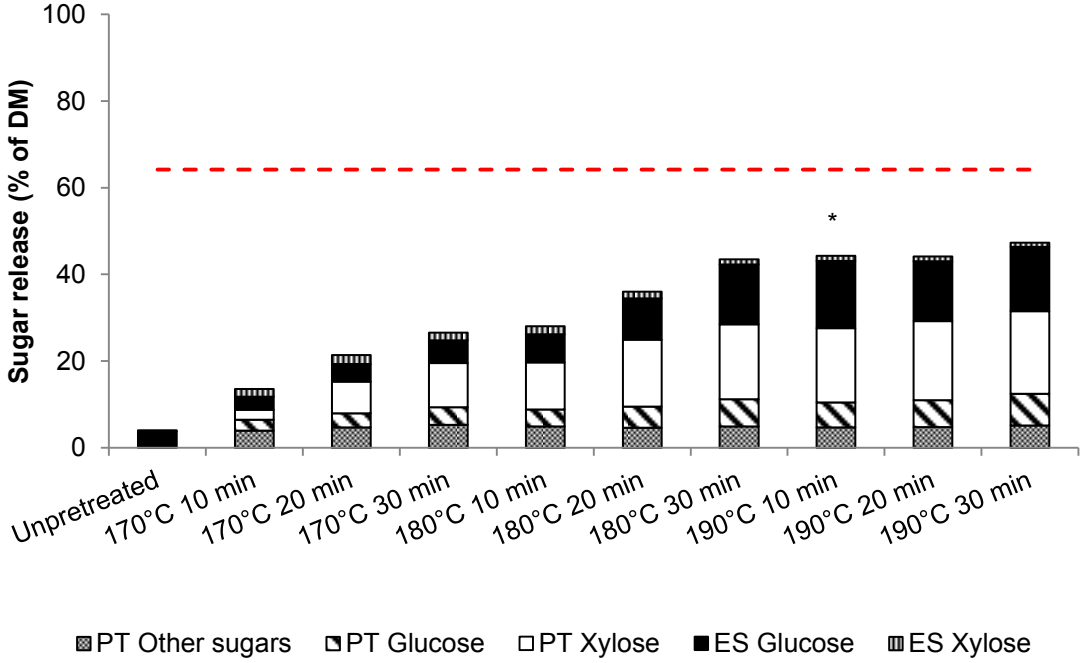


Figure 2.3 Effect of liquid hot water pretreatment (PT) and enzymatic saccharification (ES) on total sugar release. Sugar release is expressed as a percentage of dry matter under a standardised enzyme loading of 60 FPU/g glucan. Other sugars refer to the sum of galactose and arabinose solubilised during pretreatment. Theoretical maximum sugars indicated by the red dashed line and is equal to 64.2% of DM. *Optimal LHW pretreatment condition.

After LHW pretreatment the total sugar release ranged from 13.6% to 47.3% of DM (21.2% to 73.7% of the theoretical maximum) and was increased with pretreatment severity (Figure 2.3). However, there was no significant difference between the sugar release from LHW pretreatment at 190°C for 10, 20 or 30 minutes (ANOVA, $p>0.05$). The 190°C for 10 minutes condition had a sugar yield of 44.3% of DM (equivalent to 69.0% of the theoretical maximum) after pretreatment and enzymatic saccharification combined. This is an increase of over 6-fold from the unpretreated bamboo material. Under these pretreatment conditions, 84% of the initial xylan was solubilised during pretreatment, which resulted in 47% of the glucan hydrolysed during enzymatic saccharification (this was also the maximum glucose yield from saccharification amongst these conditions). While the maximum xylose release during pretreatment (93% of initial xylan) was achieved at the conditions of 190°C for 30 minutes, this did not lead to a significantly higher glucose release during saccharification. This demonstrates firstly, that continual xylan removal past a certain level failed to effectively improve glucan accessibility, and secondly suggests that factors other than xylan content may have continued to obstruct enzyme access to glucan during this stage.

In general it was observed that the more severe pretreatment conditions up to 190°C led to both a greater xylan (and hemicellulose) solubilisation, as well as higher glucose release during enzymatic saccharification. This demonstrates that solubilisation of xylan during pretreatment did have a substantial influence on enhancing glucan accessibility during enzymatic saccharification, and that it can be an indicator of a successful LHW pretreatment (Himmel et al., 2007). A similar outcome was reported for hydrothermally-pretreated wheat straw, where the highest saccharification yields were obtained with samples containing the highest cellulose and lowest xylan contents (Holopainen-Mantila et al., 2013). As mentioned in Section 1.2.2, hemicellulose chains of bamboo are extensively acetylated, and these acetyl groups have been shown to increase cell wall recalcitrance by inhibiting productive binding of enzyme to cellulose and obstructing access to β-1,4-glycosidic linkages (Pan et al., 2006, Selig et al., 2009). During LHW pretreatment it is understood that these hydrolysed acetyl groups further catalyse xylan depolymerisation. This helps to provide additional sites for enzyme binding as well as to reduce recalcitrance through the release of more easily hydrolysed xylo-oligomers, which increases the total xylose yield (Pu et al., 2013). Our findings are consistent with the results of Garcia-Aparicio et al. (2011), who found that bamboo subjected to a steam pretreatment had an improved glucose yield of 55.8%, suggesting that hydrothermal pretreatments such as LHW, can serve as an approach to increasing sugar conversion efficiencies in bamboo. Nevertheless, while many studies have reported this linear relationship between pretreatment xylan solubilisation and glucan accessibility in saccharification, it cannot be concluded that the increased glucan

accessibility is exclusively linked to selective xylan removal. Without selectively removing xylan in the absence of disruption to other biomass components, it is impossible to say whether one factor corresponds to the other, or whether this occurrence is simply (and more likely) a combination of other cell wall structural and chemical factors.

The predicted ethanol yield per dry tonne of biomass can be calculated from the total sugar yields. This calculation is based on the conversion of one glucose molecule into two molecules of ethanol, and assumes a 95% conversion efficiency of glucose into ethanol by the fermentative microorganism, *Zymomonas mobilis.* The xylose conversion into ethanol is based on the 85% conversion efficiency of 5 moles of xylose into 3 moles of ethanol (Aden et al., 2002). Predicted ethanol yields range from 56 to 246 litres of ethanol per dry tonne of biomass. It is worth pointing out that it was assumed in this study that solubilised xylan existed entirely as monomeric xylose and would be available directly for fermentation. However, other studies have shown that LHW-solubilised xylan is mainly oligomeric and not monomeric (Liu and Wyman, 2004, Kim et al., 2011). Some fermentative microorganisms such as one strain of *Geobacillus*, which has been patented by TMO Renewables (Cripps et al., 2011), are engineered to ferment oligomers directly to ethanol. Most other microorganisms however, would require additional hydrolysis to convert these oligomers into monomers in order to be fermented into ethanol. This suggests that a direct translation of the total sugar yields reported in this work into a predicted theoretical ethanol yield may be a slight overestimation.

2.3.2.3 Effect of enzyme loading on liquid hot water-pretreated bamboo

After identifying the optimal LHW pretreatment condition (which in this study was selected to be at 190°C for 10 minutes), five enzyme loadings (10-140 FPU/g glucan) of the commercial Novozymes enzyme mixture Cellic CTec2, were applied to generate different sugar yield scenarios. It is well recognised that enzyme cost is a significant factor within the overall process economics for lignocellulosic bioethanol production. Therefore it is suggested that by decreasing these loadings, the overall cost of production can be reduced which would be a major step towards commercialisation. Lower enzyme loadings were applied to assess whether this parameter could be reduced whilst maintaining sugar release at a sufficiently high level. Higher enzyme loadings were also investigated to see whether the total level of sugar release was hindered by insufficient levels of enzyme and whether the theoretical maximum could actually be reached. Sugar release from pretreatment and enzymatic saccharification combined is expressed as a percentage of the theoretical maximum in

Figure 2.4. After pretreatment, 43.0% of the theoretical maximum was released (due to sugar solubilisation during pretreatment), shown by the red dashed line in Figure 2.4. Total sugar release at each time point is calculated by adding the glucose and xylose release during saccharification to the original 43.0% released during pretreatment.

After 72 hours of saccharification, sugar release ranged from 59% to 76% of the theoretical maximum. However, there was only a slight improvement at the incremental increases in enzyme loading. The differences between enzyme loadings were also smaller at higher dosages, suggesting that a portion of the cell wall remained recalcitrant to enzymatic digestion even after an effective pretreatment. These findings support those reported by Cara et al. (2007), which showed that in other biomass feedstocks (e.g. olive tree), a portion of cellulose remained resistant to enzymes even at high loadings. This reinforces the theory that hemicellulose removal in bamboo is an effective route to enhance glucose release during saccharification, but only up to a certain point. Beyond this level, alternative routes may be required to fully maximise release of the remaining cell wall sugars.

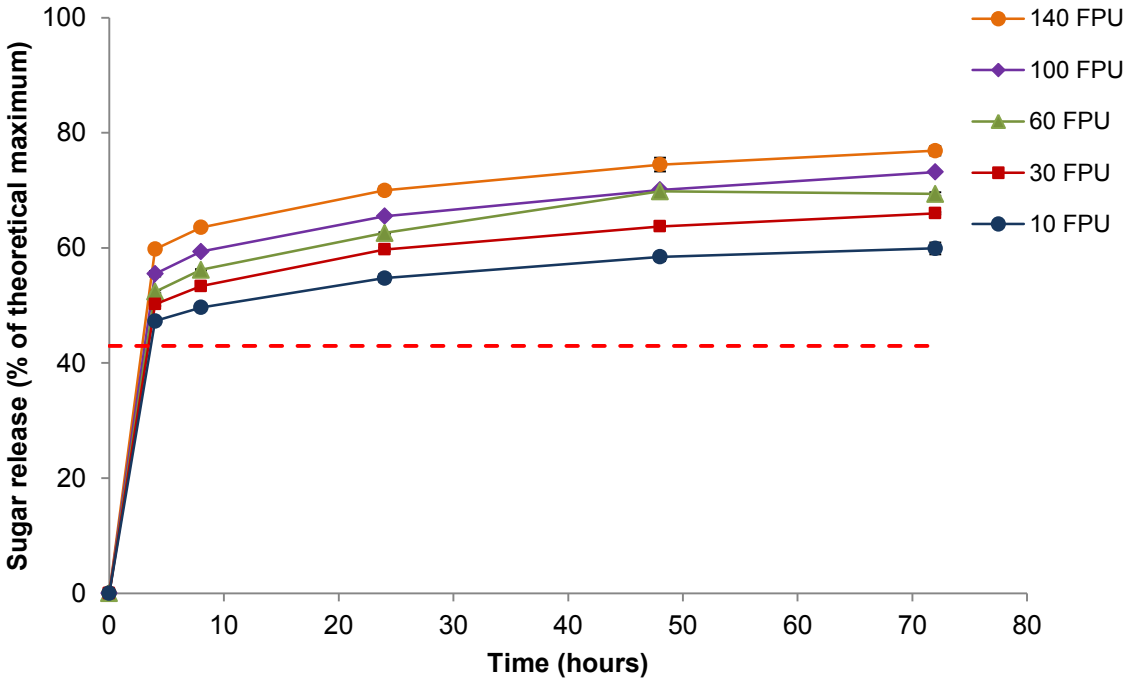


Figure 2.4 Total sugar release from LHW pretreatment (190°C for 10 minutes) and enzymatic saccharification with five enzyme loadings. Sugar release is expressed as a percentage of the theoretical maximum. Red dashed line represents sugar release from pretreatment (43.0%). Error bars represent standard error (n=3).

2.3.3 Soaking in aqueous ammonia pretreatment

2.3.3.1 Effect of soaking in aqueous ammonia pretreatment on biomass composition

Bamboo pretreated with SAA exhibited substantial delignification in comparison with unpretreated bamboo (Figure 2.5). Furthermore, there was a significant increase in the level of delignification with higher aqueous ammonia concentrations (ANOVA, p-value>0.05). The amount of mass loss during SAA pretreatment ranged from 23.1% to 29.8% of the initial biomass, and the majority of this was due to delignification. Despite this high level of delignification during SAA pretreatments, the majority of sugars were preserved within the bamboo material at all conditions. At the most severe pretreatment condition of 24 hours with 25% NH_4OH, glucan was only reduced from 38.4% to 37.2% and xylan from 20.4% to 18.3%; they were thus retained at 97% and 89% of their original contents, respectively. Under this condition, lignin was reduced from its initial level of 20.8% to 10.8% of DM, representing a 48.4% reduction. With the exception of lignin, there was relatively little variation in the biomass composition despite the range of pretreatment concentrations (10-25% w/w NH_4OH) and times (6-24 hours) tested.

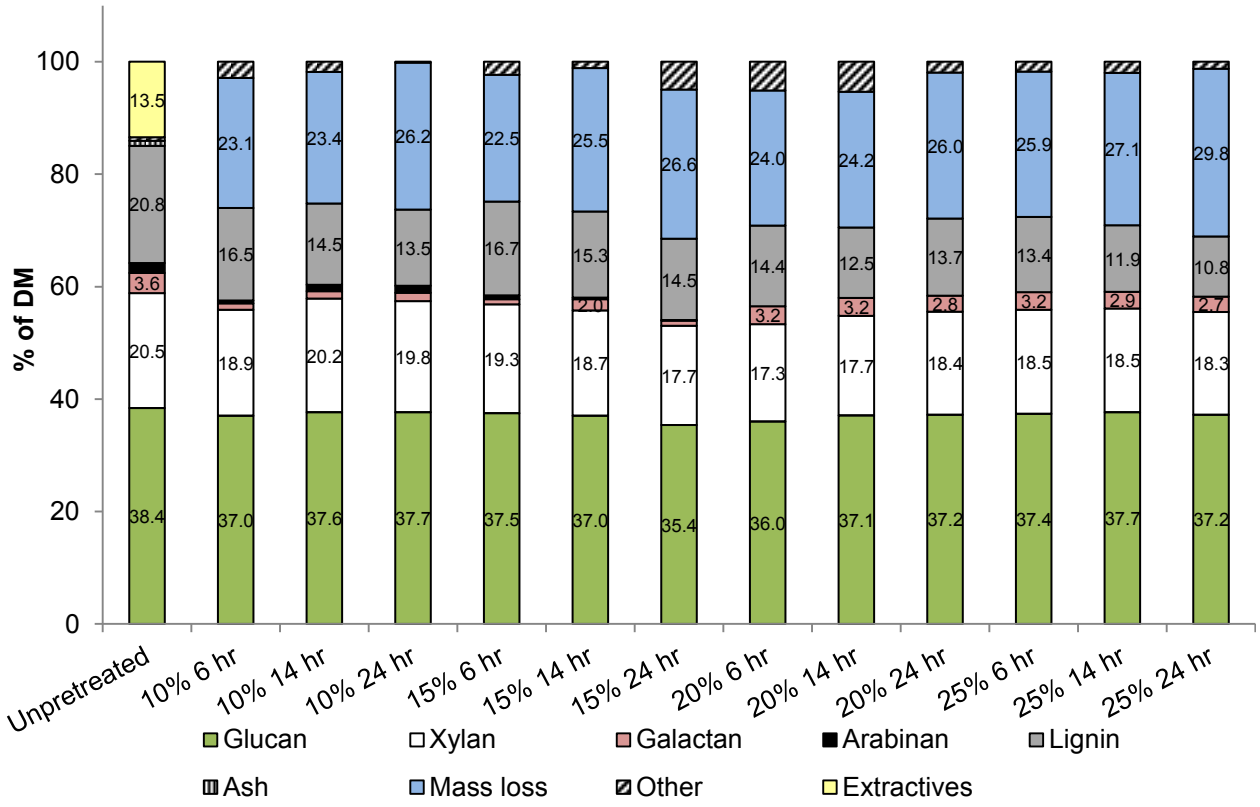


Figure 2.5 Complete mass closures of bamboo pretreated with soaking in aqueous ammonia expressed as a percentage of dry matter.

Levels of delignification reported in the literature vary significantly depending on the pretreatment conditions and feedstocks used, demonstrating that the optimal conditions have yet to be identified and/or these are linked to differences in biomass composition and structure. While almost half of the original lignin content was solubilised during the most severe SAA pretreatment, this amount is on the lower end of the range of 47% to 74% reported for other herbaceous feedstocks (Kim and Lee, 2005, Kim et al., 2008, Isci et al., 2008, Ko et al., 2009), and is substantially lower than the delignification levels achieved in corn stover (up to 80% of initial lignin content) (Cao et al., 1996, Kim et al., 2008). Although the amount of delignification achieved in this study is comparable to that reported for rice straw (47% delignification) (Ko et al., 2009), those results were achieved after 10 days of soaking compared to 24 hours used in this study. Despite these discrepancies, studies have been fairly consistent in demonstrating that after pretreatment, nearly 100% of glucan and around 85% of xylan are retained in the material – this post-pretreatment sugar-rich, low lignin composition was expected to be a major contributing factor towards increasing sugar release during enzymatic saccharification.

2.3.3.2 Effect of soaking in aqueous ammonia pretreatment on sugar yields

The sugar release from pretreatment and enzymatic saccharification in this section are reported in the same way as in Section 2.3.2.2. Following SAA pretreatment the total sugar release ranged from 21.8% to 30.6% of DM (equivalent to 34.0% to 47.7% of the theoretical maximum) (Figure 2.6). In terms of the predicted ethanol yield per dry tonne of biomass, this is equivalent to a range of 120 to 166 litres of bioethanol. At each aqueous ammonia concentration, there was an increase in total sugar release when time was raised from 6 to 14 to 24 hours. However, the difference between concentrations was not significant (ANOVA, p-value>0.05) with the exception of pretreatment with 15% NH_4OH for 24 hours, where total sugar release was maximised amongst the conditions tested. In contrast to LHW pretreatment, there was a greater proportion of sugar released from saccharification compared with pretreatment, such that this comprised approximately 67-82% of the total release. However, it was found that the pretreatment condition with the highest sugar release (15% NH_4OH for 24 hours) also had the greatest level of sugar solubilisation during pretreatment (16% compared with 6-11% of the theoretical maximum). At this condition, sugar release during saccharification (approximately 32% of the theoretical maximum) was exceeded by two other pretreatment conditions which reached 33% of the theoretical maximum.

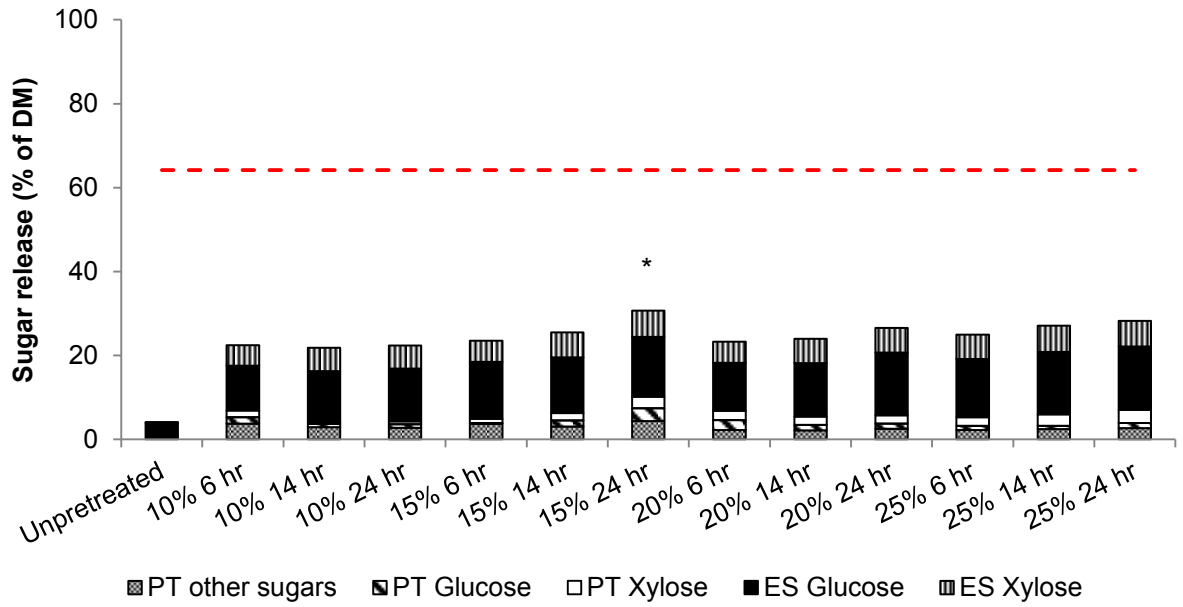


Figure 2.6 Effect of soaking in aqueous ammonia pretreatment (PT) and enzymatic saccharification (ES) on total sugar release. Sugar release is expressed as a percentage of dry matter under a standardised enzyme loading of 60 FPU/g glucan. Other sugars refer to the sum of galactose and arabinose solubilised during pretreatment. Theoretical maximum sugars indicated by the red dashed line and is equal to 64.2% of DM. *Optimal SAA pretreatment condition.

Delignification as a result of alkali pretreatment is viewed as an alternative approach to hemicellulose solubilisation for improving enzyme accessibility to cellulose. In general, although harsher pretreatment conditions led to enhanced delignification, this did not consistently produce the substrate most amenable to enzymatic digestion, as shown by sugar yields in saccharification (Figure 2.6). The highest saccharification yields amongst these conditions were achieved during pretreatments with 25% and 20% NH_4OH for 24 hours, which both released approximately 33% of the theoretical maximum. This was statistically higher (ANOVA, p-value<0.05) than the next best condition of pretreatment with 15% NH_4OH for 24 hours, which released 32% of the theoretical maximum. Between these conditions however, a substantial difference in the level of delignification was found, whereby pretreatment with 25% NH_4OH and 20% NH_4OH lost 48% and 31% from initial lignin contents, respectively. Although increased delignification was observed with higher aqueous ammonia concentrations and longer pretreatment times, these did not necessarily correspond to an enhanced sugar yield during saccharification when delignification exceeded 31% of initial lignin. Furthermore, there were other conditions which also achieved around 30% delignification during pretreatment that released significantly lower levels of sugar during saccharification. These variable results seem to suggest that there

does not seem to be a strong correlation between cell wall accessibility and the *absolute* level of lignin, but possibly stronger associations could be found between accessibility and other factors such as lignin chemistry and/or its physical or chemical linkage to other cell wall components.

On the one hand, numerous studies on herbaceous feedstocks have successfully demonstrated that progressive SAA-catalysed delignification enhances sugar release via greater enzyme accessibility to its substrate and reduced non-productive enzyme adsorption to lignin (Kim and Lee, 2007, Kim et al., 2008, Isci et al., 2008, Ko et al., 2009). However, other studies (Fan et al., 1981, Berlin et al., 2006, Zhu et al., 2008) have also contradicted this by showing that above a certain level of delignification, there is no further improvement in biomass digestibility. Two possible explanations have been proposed for the second argument: 1) extensive delignification results in a collapse of the cell wall structure, therefore reducing the available surface area for enzyme adsorption and preventing potential sugar release; and 2) the chemical composition of lignin is more important than absolute lignin content in reducing recalcitrance and thus enzymatic digestibility (Fan et al., 1981, Zhu et al., 2008, Moxley et al., 2012). Additionally, a recent finding on switchgrass revealed that while SAA pretreatment caused mild improvements in accessibility due to a lower lignin content, the remaining lignin was more evenly distributed (as opposed to being clustered) with a higher surface area to volume ratio, which actually resulted in hindering enzymes from effectively releasing cell wall sugars (Rollin et al., 2011). Though our results could possibly support this theory, further ultrastructural studies outside the scope of this investigation area would be required to establish whether bamboo biomass exhibits the same response during SAA-catalysed delignification.

2.3.3.3 Effect of enzyme loading on soaking in aqueous ammonia-pretreated bamboo

Bamboo pretreated with 15% NH_4OH for 24 hours generated the highest total sugar release from pretreatment and saccharification combined, and was therefore further investigated to determine whether these yields could be improved using different loadings of Cellic CTec2. Sugar release is expressed as a proportion of the theoretical maximum (Figure 2.7). After pretreatment, 15.8% of the theoretical maximum was released, shown by the red dashed line. After 72 hours of saccharification, sugar release ranged from 40-74% of the theoretical maximum, where the highest enzyme loading of 140 FPU/g glucan generated the greatest sugar yield. At lower enzyme loadings (10-60 FPU/g glucan) the difference in sugar release

between each increment was relatively larger (approximately 8-9%), compared to higher loadings where this diminished to approximately 5% of the theoretical maximum. This suggests that after pretreatment a substantial portion of easily hydrolysable sugars was remaining, which could be released by applying more enzyme. However, once these were removed with greater levels of enzyme, the remaining 25% of sugars were increasingly resistant to enzymatic digestion. Although SAA and LHW pretreatments were able to improve digestibility (despite the alternative routes to achieve this), sugar release did not exceed 74% in both processes, reinforcing the concept that additional physical or chemical factors may hinder accessibility beyond a certain level. Furthermore, it suggests that the optimal pretreatment on bamboo may require a combination of lignin and hemicellulose removal in order to overcome this recalcitrance barrier.

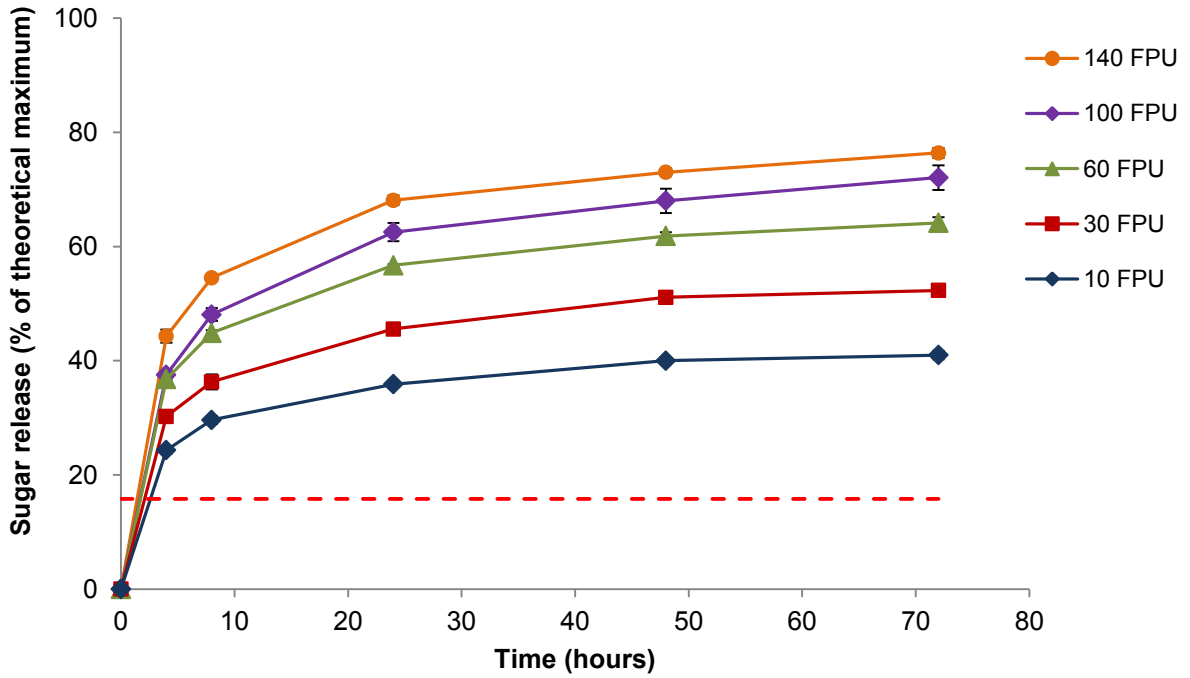


Figure 2.7 Total sugar release from SAA pretreatment (15% NH_4OH for 25 hours) and enzymatic saccharification with five enzyme loadings. Sugar release is expressed as a percentage of the theoretical maximum. Red dashed line represents sugar release from pretreatment (43.0%). Error bars represent standard error (n=3).

2.3.4 Dilute acid pretreatment

2.3.4.1 Effect of dilute acid pretreatment on biomass composition

Bamboo pretreated with DA exhibited a significant reduction in hemicellulose, which was greater at higher pretreatment severities (Figure 2.8). At stronger acid concentrations, the increase in time and temperature resulted in greater mass loss which ranged from 14.5% to 58.4% of DM. At the lower temperature of 120°C and with acid concentrations less than 2.0%, most of the relatively low levels of mass loss could be accounted for by sugar solubilisation, which included both hemicellulose as well as glucan content. Furthermore, pretreatments using acid concentrations of 2.0% also had a significant impact on lignin solubilisation, such that this decreased from its initial content of 20.8% to 16.0%, representing a 22.9% reduction under the most severe pretreatment condition. Under this condition, glucan was reduced from its initial content of 38.4% in unpretreated material to 24.5% (36.1% reduction), xylan from 20.5% to 2.0% (90.3% reduction), and galactan and arabinan were both removed entirely.

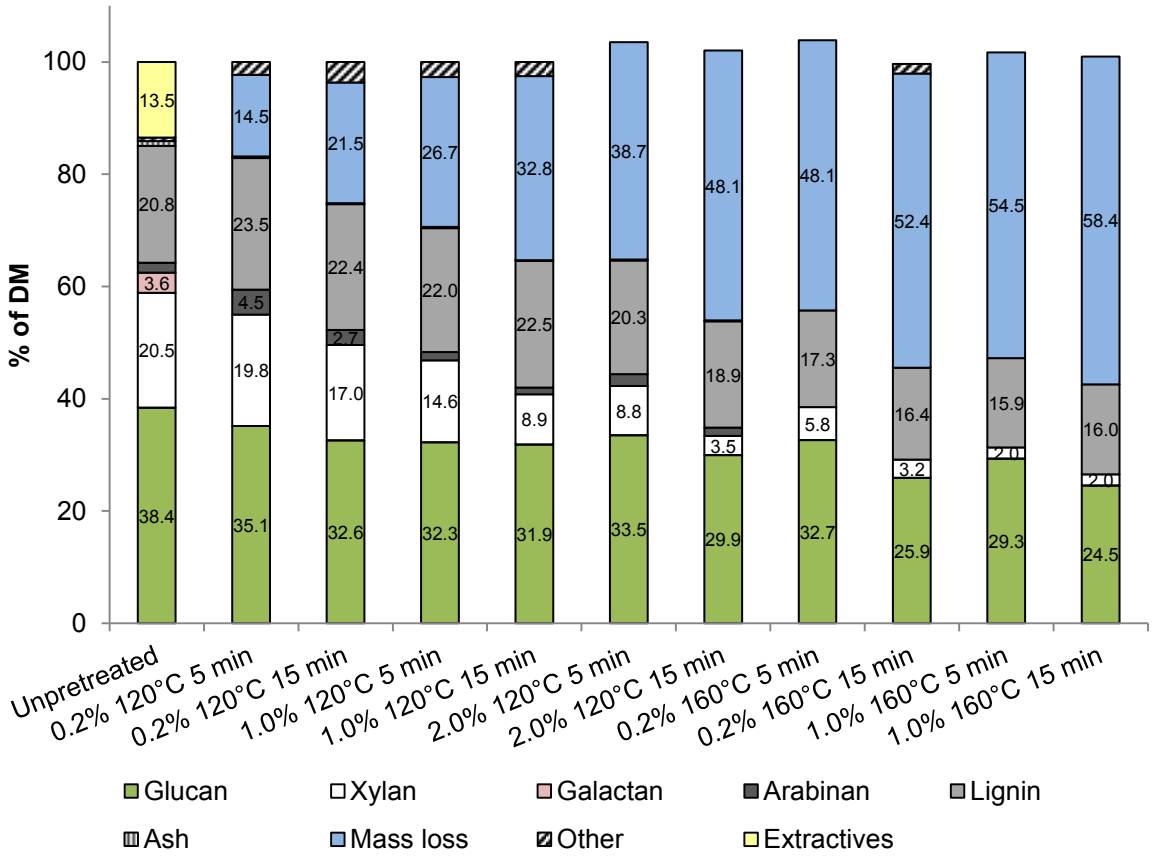


Figure 2.8 Complete mass closures of bamboo pretreated with dilute acid expressed as a percentage of dry matter.

Similar to the effect that LHW pretreatment had on bamboo, the relationship between enhanced hemicellulose solubilisation and higher severity pretreatments is also a well-reported feature of dilute acid pretreatments, which has been extensively documented for herbaceous grasses as well as softwood and hardwood species (Mosier et al., 2005b, Saha et al., 2005, Lloyd and Wyman, 2005, Cara et al., 2008, Redding et al., 2011, Moxley et al., 2012). According to Lloyd and Wyman (2005), the addition of small amounts of sulphuric acid results in further removal of xylan over 90%, which occurs either simultaneously or prior to depolymerisation of cellulose into glucose. While our results did not demonstrate this additional hemicellulose solubilisation in DA pretreatment compared with previous LHW pretreatments, depolymerisation of cellulose was clearly enhanced during DA pretreatment, which may have been influential in increasing sugar release during enzymatic saccharification. Solubilisation of sugars was the dominant reaction occurring during pretreatment, but the simultaneous degradation of lignin (20-30% removal of initial content) was also observed at increased pretreatment severities. While an alkali pretreatment is the most common approach for affecting lignin, other studies have highlighted structural and chemical effects that acid can have on lignin content, which are also linked with improving biomass digestibility (Moxley et al., 2012).

2.3.4.2 Effect of dilute acid pretreatment on sugar yields

Sugar release from pretreatment and enzymatic saccharification is reported in the same way as in Section 2.3.2.2. After DA pretreatment of bamboo, the total sugar release ranged from 9.5% to 50.8% of DM (14.8% to 79.1% of the theoretical maximum) (Figure 2.9). Therefore, at the best pretreatment condition, sugar release was more than seven times greater than unpretreated bamboo material. This level of sugar release is equivalent to a predicted ethanol yield from 42 to 270 litres of ethanol per dry tonne of biomass. The total sugar release showed a relatively constant increase up to the maximum level, which was reached after pretreatment with 0.2% H_2SO_4 at 160°C for 15 minutes. Once acid concentration increased to 1.0% (shown by the last two conditions in Figure 2.9), the sugar release decreased, suggesting that there was no improvement by raising the pretreatment severity past this level. This was consistent with the findings from Tippayawong and Chanhom (2011) who showed that increasing the acid concentration above a certain level did not improve the saccharification yields from *Dendrocalamus asper Backer* bamboo. At this selected "optimal" pretreatment condition, 69% of sugars were derived from pretreatment compared to only 31% from enzymatic saccharification, demonstrating the relatively larger contribution that sugar solubilisation during pretreatment has towards the total sugar release. At these

conditions, 84% of xylan was solubilised during pretreatment, which led to 57% of glucan hydrolysed during enzymatic saccharification. The optimal DA pretreatment with the greatest total sugar release did not have the highest xylan release during pretreatment. Instead, the highest xylan removal was achieved at the two most severe pretreatments (90% of xylan). This demonstrates that the additional acid-catalysed solubilisation of xylan during pretreatment above this point did not lead to improved glucose accessibility during saccharification.

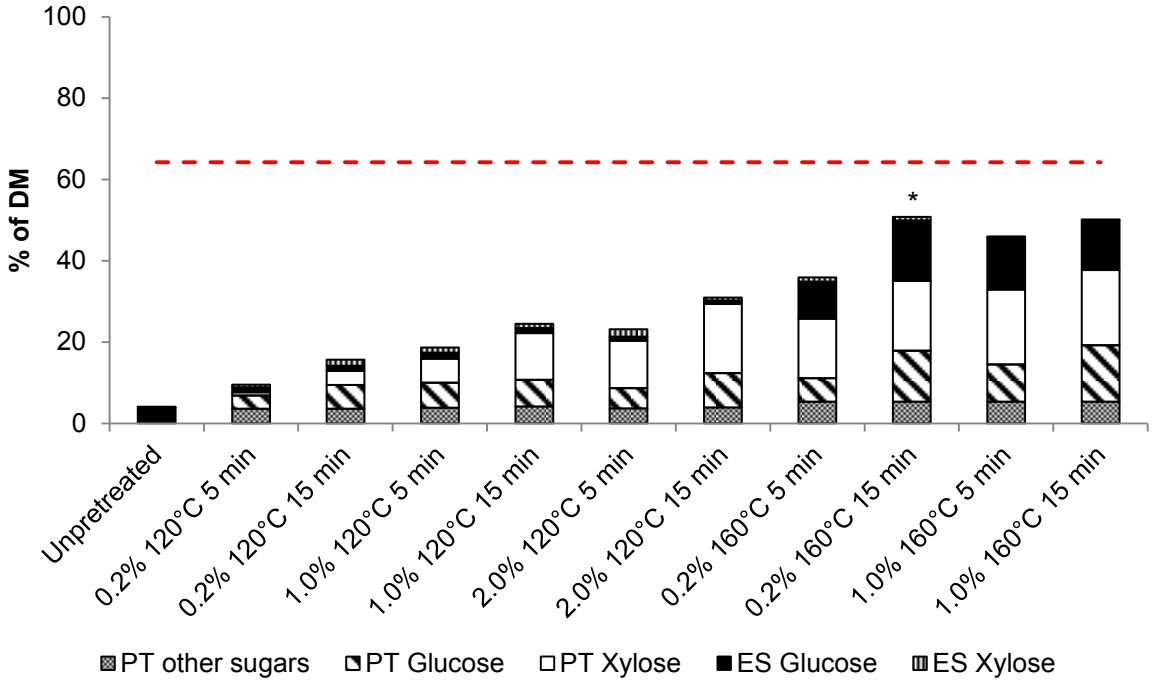


Figure 2.9 Effect of dilute acid pretreatment (PT) and enzymatic saccharification (ES) on total sugar release. Sugar release is expressed as a percentage of dry matter under a standardised enzyme loading of 60 FPU/g glucan. Other sugars refer to the sum of galactose and arabinose solubilised during pretreatment. Theoretical maximum sugars indicated by the red dashed line and is equal to 64.2% of DM. *Optimal DA pretreatment condition.

These results for DA pretreatment mirror the trends observed with LHW pretreatment, reinforcing some of the conclusions stated previously (e.g. hemicellulose solubilisation does improve glucan accessibility during enzymatic saccharification for bamboo). Although xylan solubilisation reached a higher level during LHW pretreatment than in DA pretreatment, it is likely that this was due to the higher pretreatment temperature used. Due to the heterogeneous structure of hemicellulose, it is less thermally stable compared with cellulose, which is reported to remain stable up to temperatures of around 150°C (Hill, 2007). In contrast, DA pretreatment was more effective in solubilising other cell wall components such

as glucan and lignin, which were removed by 21% and 32% of their initial contents respectively, and this was higher than the levels obtained during LHW pretreatment. It is quite possible that this was an influential parameter leading to higher saccharification yields for bamboo pretreated with dilute acid. Nevertheless, it has also been suggested that the relocalisation of lignin to a more concentrated distribution during DA pretreatment can substantially open up the cell wall structure and improve accessibility to cellulose, which may be just as important as lignin removal (Donohoe et al., 2008).

Interestingly, both LHW and DA pretreatments had the same level of xylan solubilisation during pretreatment (84% of initial xylan content) for the conditions generating the highest sugar release. While both pretreatment processes had conditions which removed more than 90% of xylan during pretreatment, neither of these were more successful in producing a substrate which had higher saccharification sugar release, suggesting that the additional xylan removal did not make cell wall glucan more accessible. These observations also support the findings in Moxley et al. (2012), which demonstrated in dilute sulphuric acid pretreated corn stover, that after a certain point, higher severity pretreatments produced solids with more recalcitrance. This is possibly due to a chemical or morphological change occurring in cellulose or lignin causing a reduced affinity of enzyme to cellulose.

2.3.4.3 Effect of enzyme loading on dilute acid-pretreated bamboo

Dilute acid pretreatment of bamboo with 0.2% H_2SO_4 at 160°C for 15 minutes generated the highest total sugar release from pretreatment and saccharification combined, and was selected to explore the effects of varied Cellic CTec2 enzyme loadings (Figure 2.10).

The total sugar yield after pretreatment was 55% of the theoretical maximum, and this was increased to 79% after saccharification with the previous standardised enzyme mixture. After 72 hours of saccharification with Cellic CTec2, the total sugar release ranged from 70-94% of the theoretical maximum. Although saccharification with 140 FPU/g glucan showed a faster initial rate of sugar release compared with 100 FPU/g glucan loading, by 48 hours there was no significant difference between these samples (t-test, $p>0.05$), suggesting that the remaining substrate may have been saturated with enzyme, and enhancing enzyme dosage led to no further increase in sugar release. These results are significantly higher than those achieved in Leenakul and Tippayawong (2010) for bamboo, which generated a maximum sugar yield of 70%, despite using a higher acid concentration and significantly longer pretreatment times (45-135 minutes compared with 15 minutes in this study).

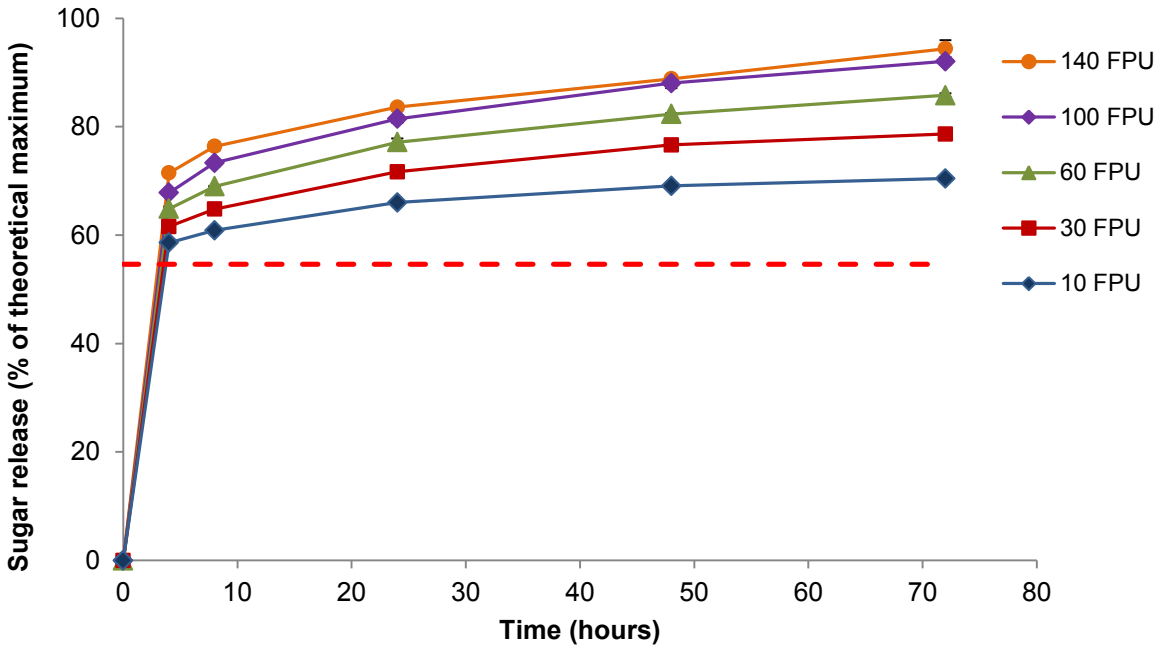


Figure 2.10 Total sugar release from DA pretreatment (0.2% H_2SO_4 at 160°C for 15 minutes) and enzymatic saccharification with five enzyme loadings. Sugar release is expressed as a percentage of the theoretical maximum. Red dashed line represents sugar release from pretreatment (55.0%). Error bars represent standard error (n=3).

2.4 Conclusions

The optimal conditions for each pretreatment were compared based on the following criteria: modification of bamboo cell wall composition, the total sugar release (from pretreatment, enzymatic saccharification and their combined total) as a percentage of the theoretical maximum under a standardised enzyme mixture, and the amount of sugar released using varied loadings of Cellic CTec2 (Table 2.7).

Bamboo pretreated with LHW and DA at the optimal conditions exhibited similar responses, in that 84% of xylan was solubilised during the pretreatment stage. This is in contrast to SAA pretreatment which resulted in a removal of 31% of the initial lignin content. Although the more severe LHW pretreatments explored in this study led to a higher level of xylan solubilisation compared with DA pretreatment (93.3% vs. 90.3%), this was most likely due to the higher temperatures applied (190°C vs. 160°C), which resulted in greater xylan removal. At the optimal conditions, the addition of sulphuric acid catalysed further removal of glucan in DA pretreatment, which significantly contributed towards total sugar release in pretreatment (55% vs. 43% of the theoretical maximum). SAA pretreatment, in comparison, had the

lowest pretreatment sugar release (16% of theoretical maximum), which was a combined result of the lower pretreatment temperatures (100°C), and the use of aqueous ammonia which selectively removed 31% of the lignin content instead.

Table 2.7 Summary of composition and sugar release in bamboo under optimal LHW, SAA and DA pretreatment conditions.

		LHW PT	**SAA PT**	**DA PT**
Optimal pretreatment condition		190°C for 10 minutes	100°C with 15% NH_4OH for 24 hours	0.2% H_2SO_4 at 160°C for 15 minutes
Main component solubilised (% of initial content)		Xylan, 84%	Lignin, 31%	Xylan, 84%
Standardised enzyme loading (% of theoretical maximum)	Total sugar release	69%	48%	79%
	Pretreatment	43%	16%	55%
	Enzymatic saccharification	26%	32%	24%
Varied enzyme loading saccharification (% of theoretical maximum)		59-76%	41-76%	70-94%

[a] LHW PT: Liquid hot water pretreatment
[b] SAA PT: Soaking in aqueous ammonia pretreatment
[c] DA PT: Dilute acid pretreatment

The effect of xylan or lignin solubilisation on subsequent enzymatic saccharification sugar yields is shown in Table 2.7. For all three pretreatments this ranged between 24-32% of the theoretical maximum, which is neither a wide nor a high range of values. SAA pretreatment generated the highest sugar release during saccharification (32% of theoretical maximum, compared to 26% and 24% for LHW and DA, respectively). However, when the actual amount of sugar present in the pretreated biomass was taken into account, which was significantly higher in the SAA pretreated bamboo, it meant that the actual accessibility of glucan in SAA pretreated material was lower (40% compared to 48% and 58% with LHW and DA pretreatments, respectively). This is indicative of three points: 1) higher enzymatic saccharification yields for SAA were merely a consequence of greater glucan/xylan *content* rather than improved accessibility; 2) lignin removal during pretreatment was not more effective than hemicellulose solubilisation in enhancing accessibility during saccharification; and 3) the preservation of sugars within the biomass during pretreatment to release more during saccharification, did not result in a higher total sugar release when compared with

solubilising these sugars during pretreatment (leading to less released in saccharification). Much of these findings agree with the conclusions discussed in Rollin et al. (2011) and Moxley et al. (2012), which discuss the influence of hemicellulose versus lignin removal on cell wall accessibility. Results from this study support the argument that an effective pretreatment does not focus solely on maintaining a balance between hemicellulose solubilisation, reduced cellulose crystallinity and minimal formation of sugar degradation products, but should also take into account the inevitable chemical and morphological changes that occur within cellulose and lignin components too (Moxley et al., 2012).

In the second experiment when different loadings of Cellic CTec2 were applied to the pretreated material, DA pretreatment had the highest total sugar yield from pretreatment and saccharification combined. At 140 FPU/g glucan, this reached up to 94% of the theoretical maximum, compared to 76% achieved in both SAA and LHW pretreatments. While this level of enzyme loading may be unrealistically high on a scaled-up level, even with a lower loading of 30 FPU/g glucan, 79% of the theoretical maximum was released, and above 60 FPU/g glucan, all loadings achieved more than 90% of the theoretical maximum**.** SAA pretreatments on the other hand, exhibited the widest range of sugar release (35% difference between the lowest and highest loadings), compared with the LHW and DA pretreatments (17% and 24%, respectively). This could be attributed to a substantial content of residual sugar left in the biomass after SAA pretreatment, which could only be hydrolysed when greater amounts of enzyme were applied. Two interesting observations can be made from these results. Firstly, a maximum of 76% of the theoretical maximum was released under the highest Cellic CTec2 loading in both SAA and LHW pretreatments, despite the contrasting methods used to achieve these yields, suggesting that the remaining 24% of cell wall sugars in bamboo remained recalcitrant to enzymatic digestion. Secondly, in both optimal DA and LHW pretreatment conditions, 84% of xylan was solubilised in pretreatment, however the maximum sugar release in DA after saccharification was almost 20% higher. One possible explanation suggested by Torget et al. (1991), is that lignin subjected to high temperature acid pretreatments undergoes chemical modification and recondenses as an altered lignin polymer. This recondensed lignin polymer was also shown to be present in a form which provided less of a barrier to enzymes during saccharification. Up to now however, a detailed explanation behind this proposition is unknown and further research on lignin structure would be required to fully investigate this hypothesis.

Each pretreatment process modifies the cell wall composition of the bamboo biomass in distinct ways and influences the amount of sugars released during saccharification. Based on the criterion of maximising sugar yields, it appears that DA pretreatment was the most

successful as it released 94% of sugars during pretreatment and saccharification combined. However, the importance of economic sustainability cannot be neglected, especially when commercial scale-up of these biomass-to-biofuel processes is considered. Whilst the DA pretreatment may be most ideal from the perspective of sugar yields, it is suspected based on evidence from other techno-economic analyses that the increased raw material cost of purchasing sulphuric acid and subsequent ammonia for its post-pretreatment neutralisation may outweigh the benefits of enhanced sugar yields (Humbird et al., 2011). SAA pretreatment appears to be less favourable in terms of technology, and possibly from economic and environmental standpoints as well, due to costs of ammonia (Kazi et al., 2010a, Schipper et al., 2010, Tao et al., 2011). On the other hand, although LHW pretreatment released less sugar compared with DA pretreatment, it lacks the requirement for chemical inputs which may bear further advantages with regards to lowering raw material costs, capital expenditure and minimising overall environmental impacts (Humbird et al., 2011, Kazi et al., 2010a, Schipper et al., 2010, Wang et al., 2012). The modelling of the economic feasibility for these processes is discussed in Chapter 3. However, it is clear from these laboratory-scale studies that when appropriate choices of pretreatment technology and conditions are used, high sugar release from bamboo can be achieved. The combination of these technical feasibility results with techno-economic modelling in the next chapters provides a more integrated picture of the potential viability of bamboo to bioethanol conversion process at scale.

Chapter 3 Techno-economic assessment of bioethanol from bamboo

3.1 Introduction

Research and development of cellulosic biofuel production over the last two decades has been guided by techno-economic assessments (Aden and Foust, 2009). Through process modelling followed by economic analysis, the potential of biomass-to-biofuel conversion processes can be evaluated from an economic standpoint. The minimum ethanol selling price (MESP) (also referred to as its production price) represents an absolute plant-gate price for ethanol, based on the conversion process and plant design assumptions. This value is defined as the lowest price at which bioethanol can be sold to maintain a set internal rate of return, while accounting for feedstock costs, capital and operating costs and other by- or co-products sold at market value (Sendich et al., 2008, Kazi et al., 2010a). Thus, techno-economic analyses can provide insight into possibilities for minimising costs and optimising production of ethanol derived from various lignocellulosic feedstocks. Industry leaders and policymakers have used this type of model to assess the market penetration potential of cellulosic ethanol compared with petroleum-derived fuels as well as ethanol derived from 1G technologies (Humbird et al., 2011).

The roles of technology and economics in the bioethanol conversion process are the main focus points of this chapter. Conversion efficiencies in pretreatment and enzymatic saccharification are based on experimentally derived data, whereas equipment efficiencies and capital costs are based on published literature, vendor quotations and costing software. There are currently a number of techno-economic models assessing the commercial potential for cellulosic biofuel production via alternative processing technologies and feedstocks. The Aspen Plus simulation model developed by the National Renewable Energy Laboratory (NREL) in 2002 and updated in 2011 for the production of bioethanol from corn stover using dilute acid pretreatment, is one of the most well recognised and widely used reports, and was therefore adapted in this present work (Humbird et al., 2011).

This chapter evaluates the economic viability of bioethanol produced from bamboo using three pretreatment processing technologies (liquid hot water, soaking in aqueous ammonia and dilute acid pretreatments) and five enzyme loading scenarios based on experimental data from Chapter 2. The major technical and economic contributors towards the MESP are identified and a sensitivity analysis is conducted to assess their impacts on the MESP. The effect of pretreatment on downstream processes and utilities consumption is discussed, and a comparison of these results with published studies is also made. All costs are reported in US dollars ($) and prices are based on the reference year 2011.

3.2 Materials and methods

3.2.1 Process overview

The bamboo-to-bioethanol techno-economic model was adapted from the 2002 and 2011 NREL technical reports, describing process design economics for dilute acid pretreatment and enzymatic hydrolysis of corn stover to bioethanol (Aden et al., 2002, Humbird et al., 2011). This model assumes a plant processing scale of 2,000 dry tonnes per day of biomass. It also uses "n^{th}-plant" economics which assumes several plants using the same technology are currently operating, thereby eliminating additional costs associated with pioneer plants (Humbird et al., 2011). Process flow diagrams (PFDs) were developed using Aspen Plus (v7.3) process simulation software to model the process from plant gate to pump. The example PFDs are provided in Appendix A. A brief overview of the process is described below and shown in Figure 3.1.

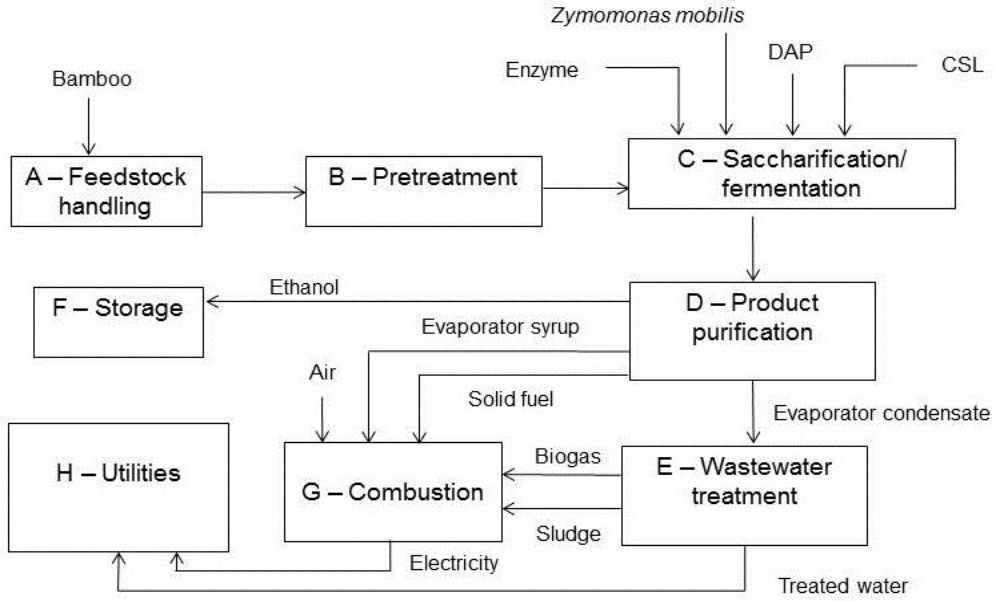


Figure 3.1 Overview of main areas (coded by letter A, B, C, etc.) in the bamboo-to-bioethanol conversion process modelled in Aspen Plus software (Figure also presented in Littlewood et al., 2013).

Harvested bamboo biomass is transported to the feedstock handling area (Area A) of the bioethanol plant. The bamboo is unloaded, washed and milled to a constant particle size and conveyed to pretreatment (Area B). Pretreatment (with liquid hot water, soaking in aqueous ammonia or dilute acid) prepares the biomass for enzymatic saccharification. Separate saccharification and fermentation (Area C) involves hydrolysis of cellulose into monomeric glucose followed by co-fermentation of glucose and xylose with recombinant *Zymomonas mobilis.* The ethanol product is sent to product purification (Area D) where it is concentrated

through distillation and molecular sieve adsorption to 99.6% and directed to storage (Area F). Either evaporation or lignin separation (depending on pretreatment process) is used to produce a solid cake consisting of mainly lignin and undigested residue, as well as a concentrated syrup which are both sent to the combustor (Area G). Wastewater treatment uses anaerobic and aerobic digestion to reduce the overall Chemical Oxygen Demand (COD) of dirty water. The treated water is then sent to utilities (Area H) to be combined with purchased well water and is suitable for plant process water requirements. Biogas and sludge (cell mass) generated from wastewater treatment, along with evaporator syrup and lignin cake, are burned in the combustor (Area G) for steam and electricity generation. Excess electricity not consumed by plant processes is sold to the grid, providing a co-product credit.

3.2.2 Bioethanol conversion process description

Area A – Feedstock Handling

The feedstock handling area was adapted from the NREL 2002 process, which assumes that feedstock will be processed, cleaned and milled on-site (Aden et al., 2002). Bamboo culms in bundles are transported to the plant gate at a rate of 91,667 kg/hr with a 10% moisture content based on a 2,000 dry metric tonne/day requirement. Trucks are used to transport bamboo bundles which are off-loaded by propane-fuelled forklifts. Bundles are first conveyed to an unwrapping system, next to a wash table for dirt removal, and then through a magnetic separator to remove ferrous metal contaminants. The washed bamboo is then sent for size reduction, which was assumed to be approximately 0.15-0.23 inches (0.41-0.58 cm) based on the values reported in the NREL model. Smaller particle sizes may minimise hydrolysis times and operating costs by allowing enzymes to access their sugar substrates more efficiently through an increased surface area. However, milling is a form of mechanical pretreatment which is highly energy-intensive, and therefore the costs and benefits of this process should be evaluated. Without investigating the consequence of incrementally scaling up lab-scale experiments (which adopted a particle size of 180-850 μm) to pilot and demonstration scale, it was not possible to know how the larger particle size used in industry may affect the experimental yields from Chapter 2. From the NREL report it was suggested that this size is appropriate for corn stover and it was assumed that it could also be applied for bamboo without having a significant compromise on sugar yields at this scale. Dirty wash water is sent to a clarifier-thickener, which uses a polymer to remove solids, followed by dewatering by a belt filter press. The solid underflow consisting mainly of fibres and dirt is

assumed to be disposed of via land application to fields, and recycled water is returned to the wash tables. It is assumed that 1% of water is evaporated and 3% is lost onto the bamboo and/or removed with solids.

Area B – Pretreatment

(a) Liquid hot water pretreatment

The pretreatment reactor system is adopted from the NREL 2011 process and includes a feedstock receiving system, a vertical pre-steamer vessel followed by a horizontal pretreatment reactor. The cleaned and milled bamboo is mixed with process water to control the total solids loading at 30% and is fed by conveyors into the pre-steamer. It should be noted that the total solids loading for pretreatment (as well as for saccharification) in this model is significantly higher than what was used in the experimental work of Chapter 2 (around 10% for pretreatment). At the industrial level, higher loadings are preferred due to their effect on increasing ethanol concentrations, thus reducing the amount of energy required in distillation for concentration, and therefore lowering operating and capital costs. Varying the solid loading was not a major focus of this work, and it was assumed that the results could be scaled up without compromising yields, however for potential industrial projects this is an area that should be investigated. High-pressure steam (13 atm) is injected to raise the temperature to 100°C for 7 minutes. The feedstock flows downwards into the pretreatment reactor, which is suitable for pretreatment at high temperatures. High-pressure steam is injected to raise the temperature to 190°C for 10 minutes. The main conversions occurring during pretreatment were based on the experimentally derived yields on bamboo from Chapter 2 (Table 3.1).

Table 3.1 Liquid hot water pretreatment reactions and conversions

Reaction	**% conversion of reactant**
Glucan + H_2O → Glucose	15.0%
Xylan + H_2O → Xylose	83.6%
Galactan + H_2O → Galactose	83.4%
Arabinan + H_2O → Arabinose	100.0%
Acetate → Acetic acid	100.0%

The pretreatment slurry is discharged to a blowdown tank for vaporisation of acetic acid and other inhibitory organics generated during pretreatment**.** This flash vapour is then condensed and sent to wastewater treatment for recycling.

(b) Soaking in aqueous ammonia pretreatment

The process design description for SAA pretreatment was adopted from Tao et al. (2011). The biomass is first pre-heated with low-pressure steam and is then sent to the pretreatment reactor with 15% NH_4OH (wt) and high-pressure steam, where it is diluted to a total solids loading of 20% and maintained at 100°C for 24 hours. The conversions and their efficiencies were based on experimental data from Chapter 2 (Table 3.2). Following pretreatment, a flash tank is used to flash vapour to recover 93% of the ammonia, which is sent to a centrifugal compressor followed by cooling. The recovered ammonia is sent to a day tank to be re-injected into the pretreatment reactor. The slurry from the flash tank is sent to a belt filter to separate solids from liquids. The solids are sent to a mix tank and are diluted to 20% total solids and then fed to saccharification/fermentation (Area C). Liquids from the filter are sent to an ammonia recovery column to concentrate 5% of the initial ammonia, which is recycled back to the day tank. It is therefore assumed that 2% of the ammonia is consumed within the pretreatment reactor – whereby 25% reacts with acetic acid to form ammonium acetate and the other 75% reacts with the biomass. The ammonia recovery column bottoms are combined with the solids from filtration and are together sent to the mix tank. Here, they are mixed with process water to a total solids concentration of 20% for saccharification.

Table 3.2 Soaking in aqueous ammonia pretreatment reactions and conversions

Reaction	**% conversion of reactant**
Glucan + H_2O → Glucose	7.9%
Xylan + H_2O → Xylose	13.7%
Galactan + H_2O → Galactose	73.7%
Arabinan + H_2O → Arabinose	95.2%
Acetate → Acetic acid	100%
Lignin → Soluble lignin	30.5%

(c) Dilute acid pretreatment

The DA pretreatment process design is based on the process from Humbird et al. (2011). The pretreatment reactor consists of an initial vertical vessel for pre-heating and acid impregnation of the biomass, followed by a horizontal reactor that heats up to the desired temperature. Biomass is first pre-steamed with low-pressure steam for up to 10 minutes, and is then conveyed to the horizontal vessel where H_2SO_4 is diluted to a concentration of 0.2% by process water. The mixture is held at 160°C for 15 minutes and then discharged into a

flash tank operating at atmospheric pressure. Following this flash, the hydrolysate slurry is conditioned with ammonia gas and dilution water to increase the pH to approximately 5 for saccharification/fermentation. The flash vapour is sent to wastewater treatment to reduce potentially inhibitory organics generated during acid pretreatment. The pretreatment conversions and efficiencies were derived from experimental results in Chapter 2 (Table 3.3). Additional reactions for HMF, furfural, lignin and acetate were adopted from the NREL 2011 process as these were not measured in the experimental section of this work.

Table 3.3 Dilute acid pretreatment reactions and conversions

Reaction	**% conversion of reactant**
Glucan + H_2O → Glucose	32.5%
Glucan → HMF + $2H_2O$	0.3%
Xylan + H_2O → Xylose	84.2%
Xylan → Furfural + $2H_2O$	5.0%
Galactan + H_2O → Galactose	100%
Arabinan + H_2O → Arabinose	100%
Acetate → Acetic acid	100%
Lignin → Soluble lignin	5.0%

Area C – Enzymatic saccharification and fermentation

Enzymatic saccharification and fermentation operate as batch processes. The pretreated slurry is cooled and diluted with process water and enzyme at loadings ranging from 10-140 FPU/g glucan (based on previous experiments) to reach 20% total solids loading for saccharification. Similarly to the pretreatment process, the total solids loading modelled in enzymatic saccharification is significantly higher than that used in the experimental work of Chapter 2 (10% compared to 2%). A loading of 20% is a realistic aim for industry and was assumed, however additional research should be conducted specifically for bamboo biomass for confirmation. Saccharification is initiated in a continuous reactor for 24 hours then batched to one of ten 950,000-gal vessels for an additional 48 hours. The saccharification conditions are the same for each pretreatment process and are listed in Table 3.4.

Table 3.4 Enzymatic saccharification operating conditions

Parameter	**Specification**
Temperature	48°C
Total solids loading	20%
Residence time	72 hours
Number and size of continuous vessels	8 (approx. 946,000 litres each)
Number and size of batch vessels	10 (approx. 3,600,000 litres each)

The amount of reactant converted into product is based on experimental data and this is summarised in Table 3.5. Only glucose and xylose yields were measured in our experimental data. Galactose and arabinose yields were therefore assumed to be the same as glucose and xylose, respectively. All conversions reported in Table 3.5 are expressed as a percentage of the content remaining in the pretreated bamboo material. In the case where 100% of arabinan or galactan was converted into their monomers during pretreatment, these are shown by “NA” conversion in Table 3.5.

After a total saccharification time of 72 hours, the slurry is cooled to 32°C for co-fermentation with the recombinant bacterium *Zymomonas mobilis.* This strain is demonstrated to simultaneously ferment 95% and 85% of glucose and xylose to ethanol, respectively (Humbird et al., 2011). *Z. mobilis* seed inoculum requires 10% of the saccharified slurry to be sent to seed production fermenters. The seed train specifications are listed in Table 3.6.

Table 3.5 Enzymatic saccharification reactions and conversions for LHW, SAA and DA pretreatments on bamboo.

% conversion of reactant															
Reaction/ Enzyme loading (FPU/g glucan)	**LHW pretreatment[a]**					**SAA pretreatment[b]**					**DA pretreatment[c]**				
	10	**30**	**60**	**100**	**140**	**10**	**30**	**60**	**100**	**140**	**10**	**30**	**60**	**100**	**140**
Glucan + H_2O → Glucose	26.3	37.8	44.3	51.4	58.7	22.7	38.7	55.1	66.1	73.3	36.5	56.1	73.1	87.9	89.5
Xylan + H_2O → Xylose	41.3	46.2	46.3	50.6	50.6	46.1	55.1	65.2	72.1	73.3	21.9	27.9	33.1	39.7	35.8
Galactan + H_2O → Galactose	26.3	37.8	44.3	51.4	58.7	22.7	38.7	55.1	66.1	73.3	NA	NA	NA	NA	NA
Arabinan + H_2O → Arabinose	NA [d]	NA	NA	NA	NA	46.1	55.1	65.2	72.1	73.3	NA	NA	NA	NA	NA

[a] LHW: Liquid hot water pretreatment
[b] SAA: Soaking in aqueous ammonia pretreatment
[c] DA: Dilute acid pretreatment
[d] NA indicates that 100% of this sugar was solubilised in pretreatment and there was therefore none to be hydrolysed during saccharification.

Table 3.6 Seed train specifications

Parameter	Specification
Inoculum level	10% vol. of vessel size
Batch time	24 hours
Turnaround time	12 hours
Number of trains	2
Number of fermenters	5
Corn steep liquor (CSL) loading	0.50% wt.
Diammonium phosphate (DAP) loading	0.67 g/L of whole slurry
Sorbitol	0.1% (w/v)

The majority of the sugars (90% of glucose and 85% of xylose) are converted into ethanol, which is recovered during a later stage. *Z. mobilis* only converts 4% of glucose and xylose into cell mass which is relatively low compared to the conversions found in *S. cerevisiae* and *E. coli*, meaning that a relatively higher amount of ethanol is produced. The seed is first pumped to a seed hold tank, then to the ethanol fermentation area. Fermentation operates in batch for 36 hours in 950,000-gal vessels. Corn steep liquor (CSL) and diammonium phosphate (DAP) are provided as nutrients for fermentation. It is also assumed that 3% of the sugars are lost to side products by contaminating microorganisms (Humbird et al., 2011). The fermentation operating conditions, reactions and conversions are listed in Tables 3.7 and 3.8.

Table 3.7 Co-fermentation operating conditions

Parameter	Specification
Organism	Recombinant *Zymomonas mobilis*
Temperature	32°C
Fermentation solids loading	19.8% total solids
Residence time	36 hours
Inoculum level	10% vol
Corn steep liquor (CSL) loading	0.25% wt
Diammonium phosphate (DAP) loading	0.33 g/L of whole slurry

Table 3.8 Co-fermentation reactions and conversions

Reaction	**% conversion of reactant**
Glucose → 2 Ethanol + 2 CO_2	95.0%
Glucose + 0.047 CSL + 0.018 DAP → 6 *Z. mobilis* + 2.4 H_2O	2.0%
Glucose + 2 H_2O → 2 Glycerol + O_2	0.4%
Glucose + 2 CO_2 → 2 Succinic Acid + O_{2+}	0.6%
3 Xylose → 5 Ethanol + 5 CO_2	85.0%
Xylose + 0.0039 CSL + 0.015 DAP → 5 *Z. mobilis* + 2 H_2O	1.9%
3 Xylose + 5 H_2O → 5 Glycerol + 2.5 O_2	0.3%
Xylose + H_2O → Xylitol + 0.5 O_2	4.6%
3 Xylose + 5 CO_2 → 5 Succinic acid + 2.5 O_2	0.9%

The resultant fermentation beer is stored in the beer well for 4 hours. An additional 0.5% of the ethanol is vented with CO_2 to the scrubber and returned back to the beer well.

Area D – Product Purification

The fermentation beer is fed to the product purification area where it is concentrated through two distillation columns and molecular sieve adsorption to approximately 99.5%. The first beer column removes CO_2 and a small portion of ethanol to the overhead and about 90% of water to the bottoms. Approximately 95% of CO_2 and 0.3% of ethanol are vented. The bottoms stream contains 0.9% of the initial ethanol concentration which is considered to be lost and sent to the evaporators. Over 99% of the ethanol is recovered in the scrubber with water vapour and is fed directly to the rectification column.

The rectification column concentrates the ethanol and sends the vapour to the molecular sieve for further dehydration. The rectification column bottoms have an ethanol concentration of 0.05% w/w and these are sent to wastewater treatment. The overhead vapour is sent to a molecular sieve adsorption unit, which act to selectively adsorb water from the mixture. The final ethanol vapour is 99.6% pure and is condensed and sent to storage.

The beer column overhead is combined with vents from seed and fermentation production to be sent to a water scrubber. The scrubber recovers 99% of the vented ethanol and returns it to the beer well. There are two alternative scenarios for dealing with the beer column bottoms depending on the pretreatment process.

Scenario (A) uses an evaporator system adopted from the 2002 NREL report, and this is only applied in the LHW pretreatment process (Aden et al., 2002). Here, a series of evaporators are used to concentrate the soluble organics as a syrup which is sent directly to the combustor. After the first evaporator, the remaining slurry is sent to a Pneumapress filter for solid-liquid separation producing a solid cake which is conveyed to the combustor for steam and electricity generation. The filtrate is returned to the second and third evaporators where the remaining water is removed to achieve a syrup with a moisture content of less than 40%. This is mixed with the lignin cake and sent to combustion.

Scenario (B) uses a lignin separation system and this is applied in the DA and SAA pretreatment processes which have a higher concentration of ammonium sulphate and ammonium acetate salts. Here, a press filter is used to separate solids which are sent directly to the combustor, while the liquor containing the salts is dealt with in the wastewater treatment area.

Area E – Wastewater Treatment

The wastewater treatment area reduces the amount of water discharged to the environment as well as minimises the fresh water requirement by treating wastewater streams and recycles them back into the process. The system includes anaerobic digestion, aerobic digestion and sludge dewatering and reverse osmosis (for DA/SAA pretreatment). Streams entering the anaerobic digestor include boiler blowdown, cooling tower blowdown, pretreatment flash vapour (LHW/DA pretreatment), evaporator condensate (LHW pretreatment), beer stillage water stream from lignin separation (SAA/DA pretreatment) and rectification column bottoms (LHW pretreatment). Anaerobic digestion converts organic material into biogas (methane and carbon dioxide) which is sent to the combustor. Aerobic digestion produces a clean water stream that is recycled back to the process and a sludge stream of cell mass that is also sent to combustion.

During anaerobic digestion, 91% of the organic components are digested. There is an 86% conversion of organic material to biogas and 5% to cell mass according to NREL's assumptions. For every kilogram of COD removed, 228 g of methane and 45 g of cell mass are produced (Humbird et al., 2011). All sulphates are converted to hydrogen sulphide (H_2S) in the biogas stream and are sent to the combustor where they are treated with flue gas desulphurisation.

The liquid from anaerobic digestion is pumped to aerobic lagoons where 96% of the remaining soluble organic matter is removed. Of this, 74% is comprised of water and CO_2 and 22% of cell mass. The total COD reduction after anaerobic and aerobic digestion is 99.6%.

For DA and SAA pretreatments, additional treatment is required to treat the ammonia present in the stream. Ammonium ions present in the stillage are converted into nitrate in aerobic digestion. The formation of nitric acid lowers the pH and caustic is therefore required to re-neutralise the pH. The digested material is sent to a membrane bioreactor for clarification and additional COD and silica particle removal takes place. The sludge stream from anaerobic and aerobic digestion is mixed in a sludge holding tank for dewatering and the remaining solid material (consisting primarily of cell mass) is sent to the combustor. The treated water is sent to a reverse osmosis for salt removal. The effluent is assumed to be pure and is mixed with process water for recycle within the process.

Area G – Combustor, boiler and turbogenerator

The combustor, boiler and turbogenerator system is used to burn organic by-product streams to generate steam and electricity. Combined Heat and Power (CHP) means the plant can reduce its solid waste disposal, be self-sufficient in energy and generate co-product credit through selling excess electricity to the grid. The streams entering the combustor include biogas from anaerobic digestion, sludge from aerobic digestion, lignin cake and evaporator syrup from the evaporator system (LHW pretreatment). The combined solid feed has a moisture content of 41%. Treated water entering the combustor is boiled and superheated to high-pressure steam, which drives a turbine and generator for electricity production. The boiler is assumed to have a thermal efficiency of 80% (the percentage of heating value converted to steam heat).

For DA/SAA pretreatments the level of sulphur in the flue gas requires flue gas desulphurisation. Lime is sprayed into the flue gas and this process converts 92% of the SO_2 into calcium sulphate. The flue gas is then sent to a baghouse, which separates the particulate ash which is landfilled with the calcium sulphate and the scrubbed gas is exhausted through a stack. The boiler feed water system includes a deaerator to remove air and non-condensables, a softener, and several other condensate surge tanks and pumps. Steam is extracted from the turbine at two points for the process and the rest is condensed and returned to the boiler feed water system. The excess electricity can then be sold to the grid for a co-product credit.

Area H – Utilities

The utilities area is responsible for the following systems and ensuring they are distributed to the rest of the plant: process water, cooling water, chilled water, clean-in-place (CIP), plant and instrument air and electricity usage. The CIP system includes chemicals for cleaning and sterilization of equipment throughout the process. Fresh water enters the plant to be mixed with treated waste water and is split into several streams to provide the plant with process water. Process water is distributed to the following areas: bamboo wash, pretreatment dilution, saccharification dilution, vent scrubber, boiler feed water, cooling tower makeup and CIP system.

3.2.3 Process economics

3.2.3.1 Total capital investment

Once mass and energy balances for the process have been generated, the economics of ethanol production can be determined. The Total Capital Investment (TCI) is determined from equipment purchased and installation costs estimated from process specifications. Equipment costs derived from NREL's vendor quotations reflect a baseline equipment size which can be scaled up or down according to the exponential scaling expression:

$$New\ cost = (Base\ cost)\left(\frac{New\ size}{Base\ size}\right)^{f\ scale} \qquad \text{(Eq. 1)}$$

The cost of each piece of purchased equipment reflects a quote made in a certain year. The NREL process costs are based on a reference year of 2007 and are therefore adjusted using the Plant Cost Index to a common year of 2011 using the formula:

$$2011\ Cost = (Base\ cost)\left(\frac{2010\ Cost\ index}{Base\ year\ index}\right) \qquad \text{(Eq. 2)}$$

Installed equipment costs can be calculated by multiplying installation factors for individual pieces of equipment adopted from NREL, with the estimated equipment cost. For other pieces of equipment, Aspen Process Economic Analyzer integrated with Aspen Plus was utilised to generate cost estimations based on mass and energy balances from the process design. All capital costs were therefore based on US data. The NREL study relied on consultations with US vendors, and the built-in Aspen costing software was also primarily US-based. To improve the specificity of the case study, a similar process could either be applied in China, or a scaling factor could be derived to scale capital costs up or down from

the US data – either method could have an major impact on the price results reported from this work.

Once the total equipment costs have been calculated in the year of interest, direct and indirect costs are added to yield the TCI. Direct costs include warehouse, site development and additional piping which comprise 4.0%, 9.0% and 4.5% of the Inside-battery-limits equipment costs (ISBL) (Areas B-D), respectively. The "Inside-battery-limits" deals with purchase and installation of process equipment, piping, instrumentation, controls, and process buildings, whereas "Outside-battery-limits" refers to utilities including power distribution, steam plant, instrument air systems, wastewater treatment, cooling towers etc. (Hamelinck et al., 2005). Indirect costs (as a proportion of the total direct costs) include prorateable costs (10%), field expenses (10%), home office and construction (20%), project contingency (10%) and other costs (10%).

3.2.3.2 Fixed and variable operating costs

The variable operating costs include raw materials, waste handling charges and by-product credits and are only incurred when the process is operating (Table 3.9).

Table 3.9 Summary of variable operating costs for China case study

Inputs	Price ($/tonne)	Reference
Bamboo[a]	44.6	Ding (2012)
Sulphuric acid	97.5	BAIINFO (2012b)
Ammonia	445.6	China fertilizer net (2012)
Lime ($Ca(OH)_2$)	120.0	Alibaba.com (2013)
Corn steep liquor	57.9	Humbird et al. (2011)
Diammonium phosphate (DAP)	502.5	BAIINFO (2012a)
Enzyme	507.0	Kazi et al. (2010a)
Sorbitol	1148.1	Humbird et al. (2011)
Caustic	317.5	Chemical Market Associates Inc. CMAI (2011)
Fresh water	0.3	Humbird et al. (2011)
Boiler feed water chemicals	5091.7	Humbird et al. (2011)
Cooling tower chemicals	3636.9	Humbird et al. (2011)
Electricity credit ($/kWh)	0.11	Sun (2012)

[a] Includes a transport cost of $0.05/tkm from Teravaninthorn and Raballand (2008).

The costs in Table 3.9 are based on China data whenever possible (referred to as the base-case model for all simulation results in this chapter), and are otherwise adopted from the NREL 2011 report. All costs are based on a reference year 2011.

Fixed operating costs include labour and various overhead items – these are incurred whether or not the plant is producing at full capacity. The number of employees for each area is adopted from the NREL 2011 report. Salaries are dependent on plant location, and were obtained via consultation with industry experts. Annual maintenance materials are estimated as 3% of the ISBL, and capital cost, local property tax and property insurance are assumed to be 0.7% of the fixed capital investment (Humbird et al., 2011).

3.2.3.3 Discounted cash flow analysis

A discounted cash flow rate of return analysis was used to determine the minimum ethanol selling price (MESP) from these processing scenarios. At this price, the net present value of the project is zero. In this analysis the discount rate, depreciation method, income tax rate, plant life and construction start-up duration have been adopted from the NREL 2011 report (Table 3.10). An example discounted cash flow analysis is given in Appendix B for the bioethanol production scenario using bamboo treated with LHW pretreatment at an enzyme loading of 10 FPU/g glucan.

Table 3.10 Summary of discounted cash flow analysis parameters

Parameter	Value
Plant life	30 years
Discount rate	10%
General plant depreciation	200% declining balance
General plant recovery period	7 years
Steam plant depreciation	150% declining balance
Steam plant recovery period	20 years
Corporate tax rate	25%
Financing	40% equity
Loan terms	10-year loan at 8% APR
Construction period	3 years
1st year expenditure	8%
2nd year expenditure	60%
3rd year expenditure	32%

Working capital	5% of fixed capital investment
Start-up time	3 months
Revenues	50%
Variable costs	75%
Fixed costs	100%

3.3 Results and discussion

3.3.1 Summary of simulation results for China base-case scenarios

Three pretreatment processes (LHW, SAA and DA pretreatment) and five enzyme loading scenarios (10-140 FPU/g glucan) were evaluated based on their MESP, ethanol production rate, ethanol yield and electricity generation. For LHW pretreatment on bamboo (190°C for 10 minutes), there was a progressive increase in ethanol production rate and ethanol yield with higher enzyme loadings (Table 3.11). The ethanol production rate ranges from 147 to 198 million litres per year. The ethanol yield, which represents the amount of bioethanol produced per tonne of biomass, ranges from 192 to 259 litres. Electricity generation decreases with enzyme loading due to a greater amount of biomass converted into bioethanol when more enzyme is applied, and varies between 46.4 to 54.4 MW. The MESPs for the scenarios range between $0.484 to $1.087/litre and increase with higher enzyme loadings. While it was expected that higher enzyme loadings would increase the amount of ethanol produced, resulting in greater sales and therefore a lower net MESP; instead, the benefit of greater ethanol production is significantly outweighed by the expensive cost of purchasing additional enzyme, and therefore leads to a higher MESP and a less economically favourable scenario.

Table 3.11 Summary of simulation results for five LHW pretreatment and enzyme loading scenarios.

Enzyme loading (FPU/g glucan)	MESP ($/litre)	Ethanol production rate (MMl/year)	Ethanol yield (litres/ tonne)	Electricity generation (MW)
10	0.484	147	192	54.4
30	0.589	160	209	52.2
60	0.742	172	225	50.2
100	0.913	188	245	48.7
140	1.087	198	259	46.4

For bamboo pretreated with SAA at the optimal pretreatment conditions (15% NH_4OH at 100°C for 24 hours) the ethanol production rate increases with higher enzyme loadings, and varies between 95 and 193 million litres per year (Table 3.12). The ethanol yield values range from 125 to 252 litres of ethanol per tonne of bamboo biomass and also increases with enzyme loading. Electricity generation decreases with the corresponding increase in ethanol production, ranging from a minimum of 44.3 MW to a maximum of 57.5 MW. The MESP ranges from \$0.990 to \$1.333/litre. On the one hand, previous experimental results demonstrated that increasing enzyme loadings led to higher sugar yields. However, the economic analysis revealed that the most economically viable scenario was with the second lowest enzyme loading (\$0.990/litre) (as opposed to the first as in LHW pretreatment scenarios), and that enzyme loadings above and below this level both led to higher costs. This suggested that with SAA pretreatment, the minimal sugar yields achieved at the lowest enzyme loading could not justify the enzyme cost at this level. Instead, the benefit of adding slightly more enzyme to improve sugar (and thus ethanol) yields was a more economically favourable option.

Table 3.12 Summary of simulation results for five SAA pretreatment and enzyme loading scenarios.

Enzyme loading (FPU/g glucan)	MESP ($/litre)	Ethanol production rate (MMl/year)	Ethanol yield (litres/ tonne)	Electricity generation (MW)
10	1.014	95	125	57.5
30	0.990	125	163	52.7
60	1.041	155	203	48.5
100	1.184	177	231	46.3
140	1.333	193	252	44.3

The ethanol production rate for bamboo pretreated with DA under the optimal conditions (0.2% H_2SO_4 at 160°C for 15 minutes) ranges from 179 to 243 million litres per year and increases with enzyme loading (Table 3.13). The ethanol yield also rises with increasing enzyme loading and varies between 234 and 318 litres of bioethanol per tonne of bamboo biomass. Electricity generation ranges from 32.8 MW to 43.9 MW and decreases with greater enzyme loadings. The MESP ranges between \$0.547 and \$0.882/litre and increases with enzyme loading. Similarly to LHW pretreatment, lower enzyme loadings in DA pretreatment led to more economically favourable scenarios due to the significant enzyme cost contribution, and therefore the lowest enzyme loading resulted in the most economically viable scenario with an MESP of \$0.547/litre.

Table 3.13 Summary of simulation results for five DA pretreatment and enzyme loading scenarios.

Enzyme loading (FPU/g glucan)	MESP ($/litre)	Ethanol production rate (MMl/year)	Ethanol yield (litres/ tonne)	Electricity generation (MW)
10	0.547	179	234	43.9
30	0.582	203	266	39.0
60	0.653	225	294	34.8
100	0.751	242	316	32.8
140	0.882	243	318	34.3

The techno-economic results showed that the lowest cost process for producing bioethanol from bamboo was with LHW pretreatment at 10 FPU/g glucan, followed by DA pretreatment at 10 FPU/g glucan and then with SAA pretreatment at 30 FPU/g glucan. However, it was also found that only at the lowest enzyme loading is LHW pretreatment more economically viable than DA pretreatment ($0.484/litre compared with $0.547/litre), because at all enzyme loadings greater than 10 FPU/g glucan, DA becomes the more economic option. At the lowest enzyme loading, although sugar yields from LHW pretreatment are minimal, so are the associated costs (e.g. capital, raw materials) in comparison with DA pretreatment. At a slightly higher enzyme loading, although the costs with DA pretreatment are still relatively higher, the sugar yields achieved are sufficiently greater to outweigh these costs and results in it being a more favourable scenario for producing bioethanol.

In general, despite a reduced level of ethanol production, lower enzyme loadings led to more economically favourable conditions for producing bioethanol from bamboo. In the LHW and DA pretreatments, the enzyme loading of 10 FPU/g glucan had the lowest MESP amongst the five enzyme loading scenarios, whereas for the SAA pretreatment process the second lowest enzyme loading of 30 FPU/g glucan was the most desirable scenario. The balance between enzyme cost and total ethanol sales (ultimately a consequence of pretreatment efficacy) was the main determinant of economic viability amongst these scenarios. For SAA pretreatment, although the enzyme cost contribution at 30 FPU/g glucan towards the MESP is much higher than with 10 FPU/g glucan, the 9% increase in sugar yield at 30 FPU/g glucan from 10 FPU/g glucan led to a significantly increased volume of ethanol produced, compensating for the costs of enzyme purchase. For both DA and LHW pretreatments, there was a smaller increase in sugar release of approximately 7% of initial sugar content, when 30 FPU/g glucan enzyme was used compared with 10 FPU/g glucan. It is not clear what the exact values required to make the extra enzyme purchase “worthwhile” are, but results from

these scenarios demonstrate that the cost of purchasing additional enzyme to release this extra amount of sugar, more often than not outweighs the benefits of producing more ethanol. This same increase in MESP is observed in enzyme loadings above 30 FPU/g glucan for SAA pretreatment.

3.3.2 Cost breakdown analysis

A cost breakdown analysis was performed for each scenario with the lowest MESP for the three pretreatment processes. Each pretreatment process is presented in Figures 3.2-3.4, where results are expressed as percentages of the MESP. Figure 3.5 then summarises the absolute cost values in $/litre, to allow comparisons between the different pretreatments to be made.

Bamboo pretreated with LHW and saccharified with an enzyme loading of 10 FPU/g glucan led to the most economically favourable scenario with the lowest MESP of $0.484/litre. The area with the largest contribution to the MESP is the feedstock handling area, of which raw materials & waste representing the cost of bamboo comprises 90.7% of this area, and accounts for 51% of the MESP (Figure 3.2). The second largest cost contributor is the cost of capital in the combustor/turbogenerator area, which makes up 22.3% of the MESP. The raw material cost in saccharification and fermentation corresponding to enzyme is also a significant contributor at 16.5% of the MESP. The negative credits gained from electricity generation from combusting bamboo residues also contribute a major portion of the MESP at -47.0% of the total price. The LHW pretreatment area comprises approximately 20% of the MESP, whereby 51.7% of this area comes from capital expenditure, 41.1% from energy consumption, and the remainder from other fixed costs.

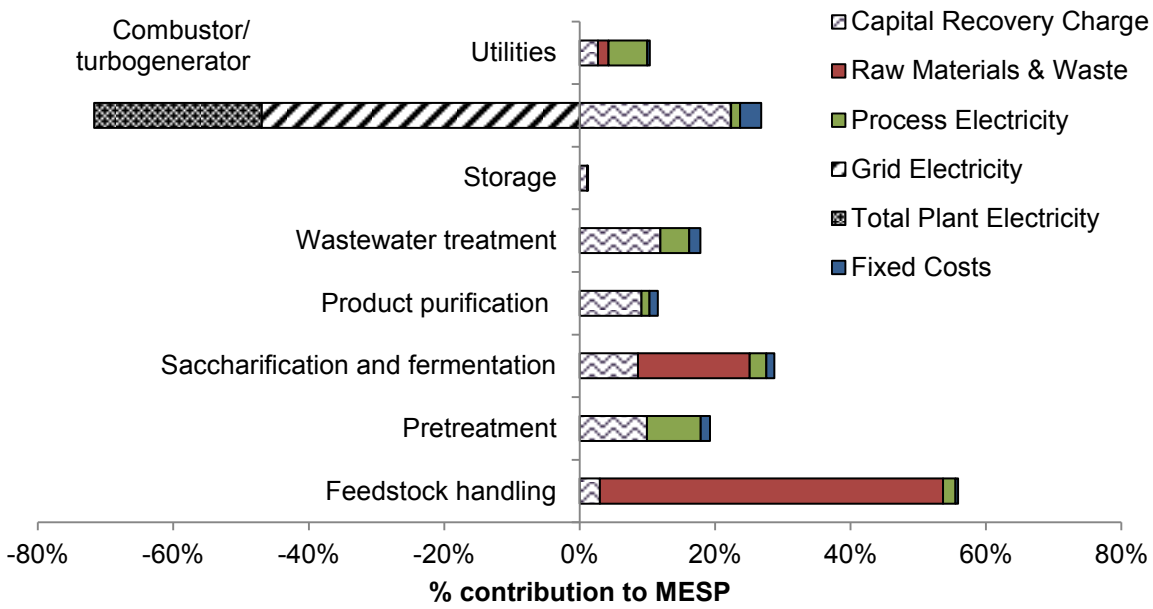


Figure 3.2 Cost breakdown of bioethanol from bamboo with LHW pretreatment at an enzyme loading of 10 FPU/g glucan (MESP $0.484/litre) (Figure also presented in Littlewood et al. (2013)).

Bamboo pretreated with SAA and treated with an enzyme loading of 30 FPU/g glucan during enzymatic saccharification was the most economically favourable scenario for this pretreatment process. It is evident that the largest cost contributor across all stages comes from raw materials & waste (Figure 3.3). In the feedstock handling area, the cost of bamboo makes up 29.1% of the MESP. The cost of enzyme in the saccharification and fermentation area is the second largest contributor and constitutes 28.4% of the MESP. Thirdly, the raw materials & waste contribution from the wastewater treatment area comprises 11.1% of the MESP. The cost of capital in the combustion area is the largest capital cost, representing 15.1% of the MESP; and this is also responsible for the substantial credit gained from electricity generation (-23.4% of the MESP). The contribution of the pretreatment area in this process accounts for approximately 15% of the MESP. In this stage, capital expenditure is the dominant cost, followed by energy consumption and then by raw materials and waste, making up 44.6%, 37.0% and 13.2% of the pretreatment area, respectively.

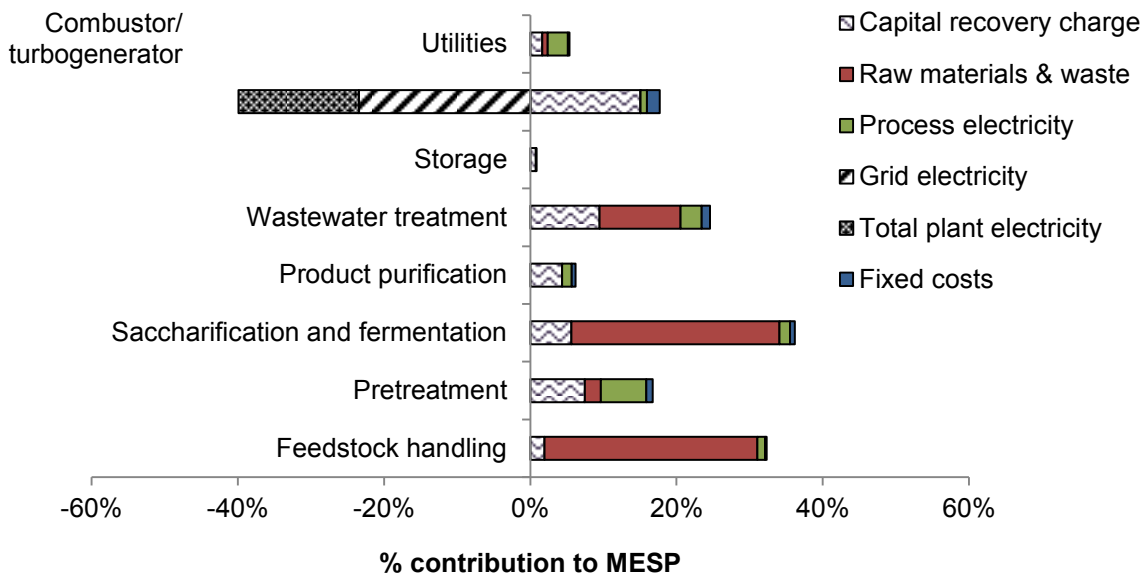


Figure 3.3: Cost breakdown of bioethanol from bamboo with SAA pretreatment at an enzyme loading of 30 FPU/g glucan (MESP ($0.990/litre).

Similar to the previous two pretreatment processes, the cost breakdown for bioethanol from bamboo using DA pretreatment with 10 FPU/g glucan enzyme showed that feedstock handling was the leading cost contributor to the MESP, with bamboo representing 36.8% of the total MESP (Figure 3.4). Here, the relative proportions of capital, raw materials, process electricity and fixed costs are similar between wastewater treatment, saccharification & fermentation and pretreatment areas, but the wastewater treatment area is the most expensive at 25.5% of the MESP, followed by pretreatment and saccharification and fermentation stages both comprising 19.8% of the MESP. The raw material costs in these three areas are significant due to the addition of sulphuric acid in pretreatment, caustic in wastewater treatment, and enzyme applied in saccharification and fermentation. The cost of capital in combustion is significant and accounts for 16.1% of the MESP and the generation of electricity contributes a negative credit of -20.9% towards offsetting the MESP.

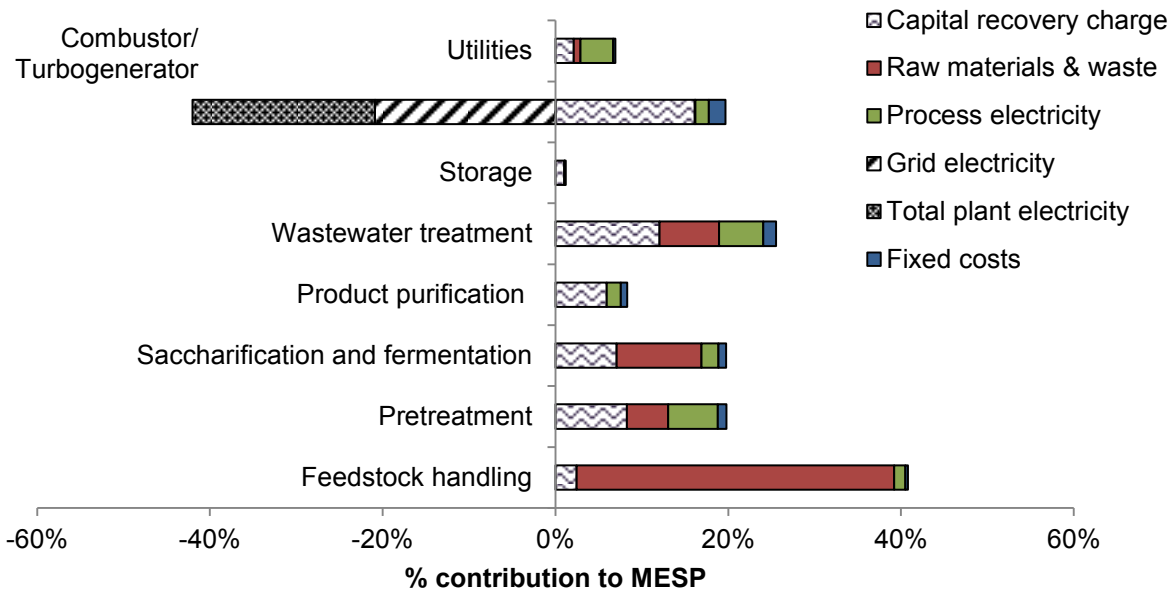


Figure 3.4: Cost breakdown of bioethanol from bamboo with DA pretreatment at an enzyme loading of 10 FPU/g glucan (MESP $0.547/litre).

In the cost breakdown analysis for each of the pretreatment scenarios, it is evident that at 29-51% of the MESP, feedstock is one of the major cost contributors. Despite bamboo prices of approximately $45/tonne used in this study, which are towards the low end of the price range in comparison to other published techno-economic analyses, (Kazi et al., 2010b, Tao et al., 2011, Macrelli et al., 2012) the feedstock handling area still has the largest contribution towards the MESP. Most techno-economic evaluations of biomass-to-bioethanol pathways suggest that there is a strong correlation between feedstock cost with the MESP, and that this relationship is consistent even at low feedstock costs (Humbird et al., 2011). Kumar and Murthy (2011) reported an average feedstock contribution of 46-56% of the MESP, whereas Gnansounou and Dauriat (2010) demonstrated approximately a 38% contribution from various feedstocks, which are also consistent with these results. While this could be an area for potential cost reduction (involving an assessment of potential field practices and logistical options which is out of the scope of this study), it has been suggested that mature technologies are defined by having raw material costs of approximately 70% of the overall manufacture costs, such an example being in the petroleum refining and corn wet milling industries (Sendich et al., 2008). Immature technologies on the other hand, have much higher processing costs with greater potential for optimisation. This theory supports these results, where feedstock costs in LHW and DA pretreatments were higher at 56% and 37% of the MESP, respectively, compared with SAA pretreatment which was much lower at only 29% of the MESP.

Enzyme cost was also a consistently large cost area within each pretreatment process. This accounted for 16% and 10% of the MESPs in the 10 FPU/g glucan LHW and DA pretreatment scenarios, respectively, compared to 28% in the SAA process which used 30 FPU/g glucan enzyme. The significant cost burden of enzymes is clearly demonstrated in Figure 3.5, which shows that the contribution of the saccharification/fermentation (SF) stage in the SAA process is more than double that in LHW and DA processes, and this was the area with the greatest cost discrepancy between the processes. Enzyme prices in this model were adopted from Kazi et al. (2010b) and seem to be relatively high compared to prices used in other economic analyses. However, prices for enzyme cocktails in large-scale cellulosic bioethanol projects are currently unknown and are based on hypothetical price projections. As an alternative, authors sometimes use a "top-down" measure and report enzyme contribution in dollars per litre, which is an aggregate assumption failing to account for variation in enzyme loading and actual cost (Kazi et al., 2010b, Klein-Marcuschamer et al., 2012). Nevertheless, this issue remains to be highly debated and is recognised to be an inconsistent parameter that hinders the robustness of techno-economic models.

In the future for China, the possibility for cost reductions may come from the application of modern enzymes produced locally, which could potentially be produced at significantly lower costs than those from some of the major enzyme manufacturers in Europe or the US. Assuming that enzyme loading is a major area for reducing the MESP, applying less enzyme is one approach to minimise costs, even though this would merely compromise total bioethanol production. Other alternatives without this consequence include: 1) optimising pretreatment technologies to improve saccharification yields, 2) advanced breeding of biomass feedstocks (e.g. increasing/decreasing cellulose or lignin contents, altering composition of cell wall components) to make them more digestible by enzymes, or 3) selection and breeding of naturally-occurring genotypes that are found to be more susceptible to enzymatic hydrolysis (Boerjan, 2005, Brereton et al., 2010).

The cost of the pretreatment area comprises approximately 16-21% of the MESP for all pretreatment technologies, and is ranked fourth in terms of its cost contribution within the conversion process, after feedstock handling, saccharification and fermentation and either wastewater treatment or combustion, depending on the scenario. Pretreatment in the DA process has the greatest cost contribution (% of MESP) amongst the processes due to the cost of sulphuric acid and the subsequent acid neutralisation with ammonia prior to enzymatic saccharification; yet its absolute cost ($/litre) is only slightly higher than the LHW process and is significantly lower than that of SAA pretreatment (Figure 3.5). These differences arise primarily from the cost of raw materials required with DA pretreatment

(sulphuric acid and ammonia for neutralisation prior to saccharification), which are not needed in LHW pretreatment. Figure 3.5 also demonstrates that the choice of pretreatment has a *direct* effect on the total pretreatment area cost, however the *indirect* economic consequences on downstream processes can be just as an important factor impacting the economic feasibility between alternative pretreatment processes. This is described in more detail in Section 3.3.3.

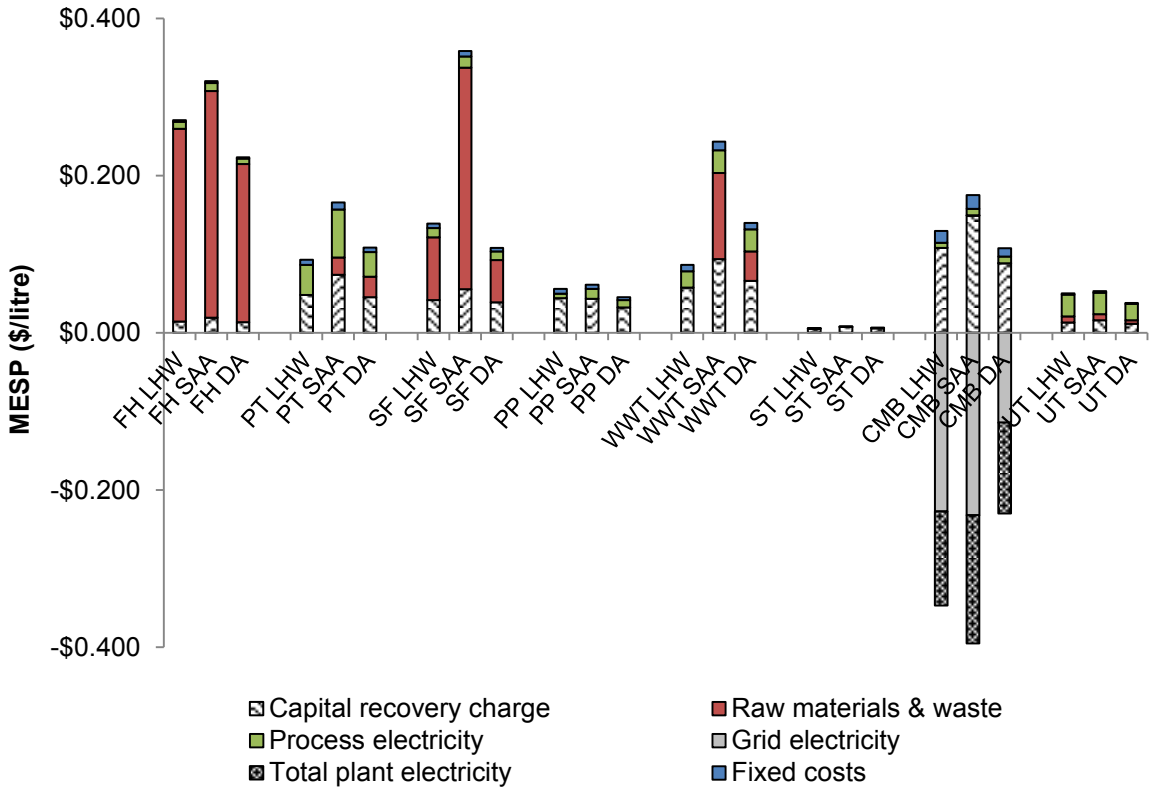


Figure 3.5 Cost breakdown summary for bioethanol from bamboo via LHW, SAA and DA pretreatment processes expressed in $/litre (FH: Feedstock handling; PT: Pretreatment; SF: Saccharification & fermentation; PP: Product purification; WWT: Wastewater treatment; ST: Storage; CMB: Combustor/turbogenerator; UT: Utilities).

3.3.3 Effect of pretreatment on downstream processes

The type of pretreatment process can have a significant impact on downstream processes as well as on the consumption of utilities including electricity, process water and heat. A summary of utilities consumption for the three most economically viable pretreatment scenarios is shown in Table 3.14. It was found that DA pretreatment had the highest electricity and process water consumption at 22.1 MW and 670,625 kg, respectively. LHW pretreatment had the highest heat consumption (128.3 kcal) and the lowest electricity

consumption (18.7 MW). SAA pretreatment had the second highest electricity and heat consumption (21.7 MW and 98.9 kcal, respectively) and the lowest process water consumption (593,591 kg). The LHW and SAA pretreatment processes both generated 36 MW of surplus electricity, whereas the DA pretreatment process, which consumed the most but generated the least electricity due to high sugar yields, was only left with 22 MW of excess electricity.

Table 3.14 Summary of utilities consumption in three pretreatment scenarios

Utility consumption	LHW	SAA	DA
Electricity (MW)	18.7	21.7	22.1
Process water (kg)	667,912	593,591	670,625
Total heat (kcal)	128.3	92.8	98.9

Electricity consumption was highest in the pretreatment, utilities and wastewater treatment areas for all three pretreatment scenarios. For both LHW and DA pretreatment processes, the same pretreatment reactor was modelled and this was based on the NREL 2011 model quotation. This reactor includes several energy-intensive conveyor belt systems for bringing the feedstock into the reactor, and this was the main cause of its high electricity consumption. For the SAA pretreatment process, the recovery and recycling of ammonia after pretreatment requires distillation columns, pumps and compressors which need significant amounts of energy; this is recognised to be a crucial area for improvement and optimisation from a technology perspective. For process water usage, the cooling tower makeup, pretreatment and saccharification areas are the highest consumers in all three pretreatment scenarios. The cooling tower makeup consumes a high amount of water due to the requirement for cooling down process heat streams and to cover the loss of water due to windage, evaporation and draw-off in the tower. LHW pretreatment consumed the second greatest amount of process water of the three scenarios (after DA pretreatment), which is one of the weaknesses of this pretreatment process that has also been reported in other techno-economic analyses (Eggeman and Elander, 2005, Kumar and Murthy, 2011). The heat requirement measured by cooling water usage is primarily attributed to the condensing turbine due to the high amount of electricity generated in the processes. In LHW and DA pretreatments, the product purification area is the second greatest cooling water consumer despite the implementation of two very different systems (evaporator vs. lignin separation systems). In the LHW pretreatment process, the evaporator condensate as part of the evaporation system requires a significant amount of cooling water; whereas in the DA pretreatment process, the rectification column condenser also has a high cooling water

requirement. In the SAA pretreatment process, the pretreatment area is the main stage that requires high amounts of cooling water due to the ammonia recovery column and compressor which consume 22% and 11%, respectively, of the total amount.

Aside from having an effect on the utilities consumption, the choice of pretreatment also impacts the function of specific equipment and therefore the overall process design. For the LHW pretreatment process, an evaporator system in product purification was used to concentrate the distillation column bottoms stream, producing an evaporator syrup and lignin cake which were sent to the combustor (Aden et al., 2002). In the SAA and DA processes however, the use of ammonia and sulphuric acid in pretreatment, required a change in this configuration, which affected downstream stages including wastewater treatment, combustion and product purification. The use of sulphuric acid in DA pretreatment meant that a neutralisation step was required to increase the slurry pH prior to saccharification, which resulted in the formation of ammonium acetate and sulphate salts in the hydrolysate. If the original evaporator system were to be used in these two pretreatment processes, the concentration of salts would be too high, resulting in possible fouling of the combustor system. Therefore, replacement of the evaporators with a lignin separation system was implemented in the SAA and DA pretreatment processes. Furthermore, the addition of a salt removal system in wastewater treatment as well as the requirement for caustic led to higher costs in wastewater treatment (Figure 3.5) particularly in the capital expenditure but also in the raw material and waste costs. The combustion area also required a flue gas desulphurisation step in SAA and DA pretreatment processes, which increased the total MESP. As a result of these additional costs incurred, bioethanol from bamboo using LHW pretreatment was the most economically favourable scenario when the lowest enzyme loading was applied.

3.3.4 Sensitivity analysis on cost parameters

From the cost breakdown analysis, it was evident that the feedstock cost, enzyme cost and price of renewable electricity are significant determinants of the MESP for the three pretreatment process scenarios. A sensitivity analysis was therefore conducted to investigate the sensitivity of the MESP to these economic parameters. Each parameter was varied by a range of 50% from their original value used in the reference case scenarios. This decision was based on other sensitivity analyses that typically used a range between 20-50% from the reference case price (Rismiller and Tyner, 2009, Slade et al., 2009, Kazi et al., 2010a, Wang, 2011, Hagman et al., 2012). Feedstock cost in the reference case was

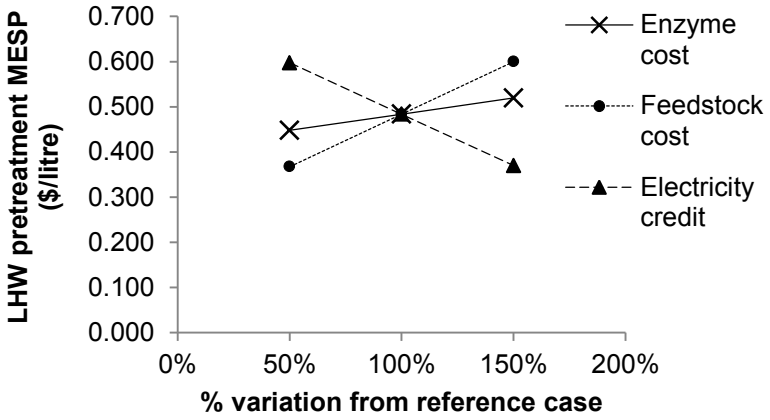
assumed to be $44.6/tonne, so this was varied from $22.3 to $66.9/tonne (Table 3.15). Likewise, enzyme cost was varied from $253.5 to $760.5/tonne, and electricity credit varied from $0.056 to $0.167/kWh. The gradient of the slope is indicative of the extent that these parameters have on the MESP, whereby a steeper slope will have a greater influence on the MESP and vice versa with a smaller slope. Lines increasing from left to right show a positive correlation between the economic parameter and the MESP, and lines decreasing from left to right show a negative correlation between the parameter and MESP.

Table 3.15 Sensitivity analysis cost parameters

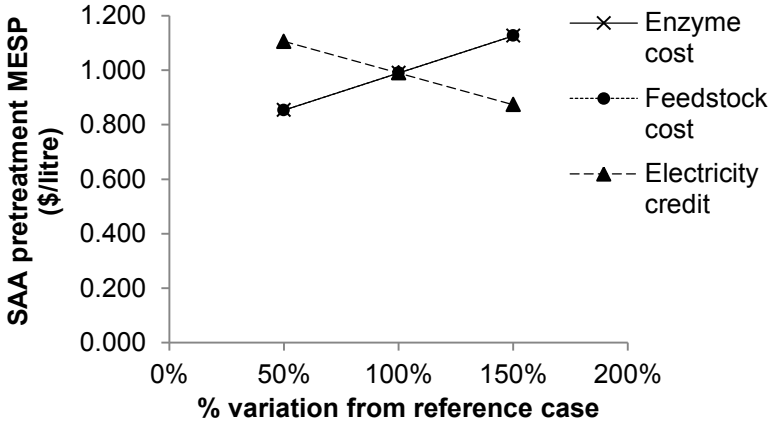
Parameter	Low	Reference case	High
Feedstock cost ($/tonne)	$22.3	$44.6	$66.9
Enzyme cost ($/tonne)	$235.5	$507.0	$760.5
Electricity credit ($/kWh)	$0.056	$0.11	$0.167

Unsurprisingly, there was a positive correlation between feedstock and enzyme costs with the MESP, whereas a negative correlation was found between electricity credit and the MESP (Figure 3.6). This means that higher enzyme and feedstock prices result in greater MESPs, and conversely lower electricity credit prices result in an increase in the MESP. The effect of these parameters on the MESP of bioethanol from bamboo with LHW pretreatment (Figure 3.6(a)) demonstrates that the MESP is most sensitive to feedstock price with a slope of 0.232, and this is closely followed by electricity credit with a negative slope of -0.227. Enzyme price is the least sensitive economic parameter with a slope of 0.071. With SAA pretreatment (Figure 3.6(b)), both enzyme and feedstock price have equal contributions towards the MESP with a slope of approximately 0.273, whereas electricity credit is also fairly sensitive at -0.232. For the DA pretreatment scenario, feedstock price also has the greatest economic impact on the MESP with a slope of 0.191, and this is followed by electricity credit at -0.114. The MESP by DA pretreatment is relatively less sensitive to enzyme cost with a slope of 0.046.

(a)



(b)



(c)

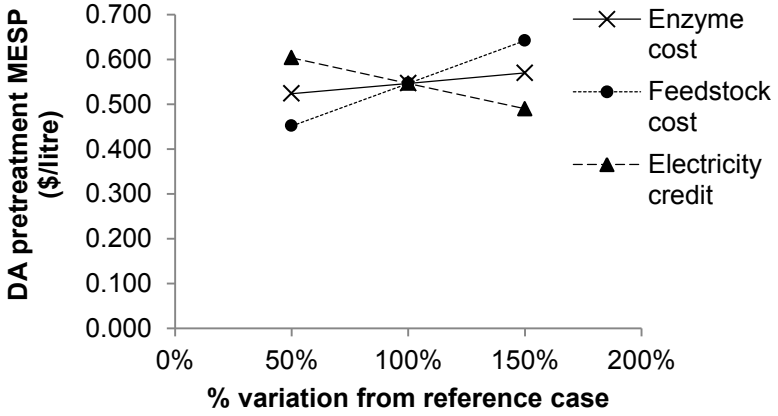


Figure 3.6 Sensitivity of MESP to variations in feedstock cost, enzyme cost and electricity credit in (a) LHW pretreatment with 10 FPU/g glucan (b) SAA pretreatment with 30 FPU/g glucan and (c) DA pretreatment with 10 FPU/g glucan scenarios.

All three of these scenarios demonstrate the importance for obtaining inexpensive feedstock costs, and how small cost variations can have larger impacts on the price of bioethanol production. These results reflect those presented in the cost breakdown analysis of Section 3.3.2 which also showed that the MESP was most sensitive to feedstock price. The generation of renewable electricity is an important product of the process, and selling this electricity to generate a co-product credit is shown to significantly reduce the MESP. The gradient of varying electricity credit in all scenarios was almost as steep as the feedstock price, demonstrating that price fluctuations, which are usually determined by government authorities, can have a major influence on the economic feasibility of bioethanol production processes. Finally, while the effect of enzyme price has already been shown to be a major driver of the MESP, it is the least influential of these economic parameters at 10 FPU/g glucan. Only at higher enzyme loadings (e.g. SAA pretreatment with 30 FPU/g glucan) does its role become as significant as the cost of feedstock in this analysis.

3.3.5 Comparison with other techno-economic analyses

Several techno-economic analyses evaluating the production of cellulosic ethanol via various biochemical processing pathways have recently been published in the literature. Many of these are derived from the original NREL processes (dilute acid pretreatment of corn stover) (Aden et al., 2002, Humbird et al., 2011) but with modifications in technology or parameters such as feedstock type/composition or experimental yields. Some studies have used other software to build-up new process, and are therefore based on entirely different assumptions. Due to the wide range of parameters, some of which can be easily controlled and others less so; an extensive range of MESP values for cellulosic bioethanol production currently exist, making comparisons between studies difficult. Based on previous results derived in this work as well as from other studies, it is accepted that the feedstock cost has a major influence on the MESP, and this correlation is plotted in Figure 3.7. The costs and MESPs in this present study from LHW, SAA and DA pretreatment processes are compared with similar pretreatment processes reported from other studies in the literature. MESP values and feedstock costs have been updated to 2011 $US equivalents using the Consumer Price Index.

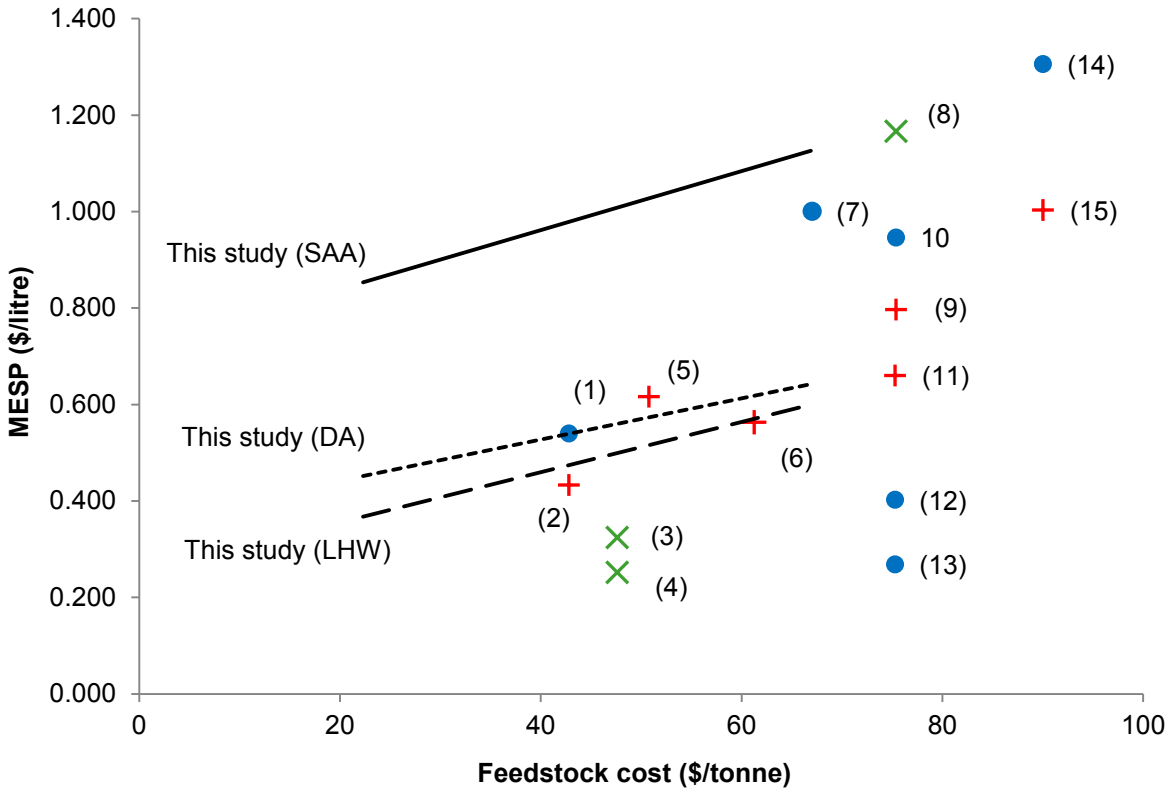


Figure 3.7 Relationship between feedstock cost ($/tonne) and MESP ($/litre) from this study and other techno-economic models. MESPs and feedstock costs have been updated to 2011 prices using the Consumer Price Index. Similar pretreatment processes are grouped such that: red represents acid pretreatments, blue represents hydrothermal pretreatments (LHW and steam) and green represents ammonia-based pretreatments. (1) Eggerman & Elander, 2005 (Liquid hot water (LHW) pretreatment); (2) Eggerman & Elander, 2005 (Dilute acid (DA) pretreatment); (3) Sendich et al., 2008 (Ammonia fibre explosion (AFEX) pretreatment, Consolidated bio-processing (CBP)); (4) Sendich et al., 2008 (AFEX, Simultaneous saccharification and co-fermentation (SSCF)); (5) Humbird et al., 2011 (DA); (6) Laser et al., 2009 (DA); (7) Macrelli et al., 2011 (Steam pretreatment); (8) Tao et al., 2011 (Soaking in aqueous ammonia (SAA) pretreatment); (9) Tao et al., 2011 (DA); (10) Tao et al., 2011 (LHW); (11) Hamelinck et al., 2005 (DA, Simultaneous saccharification and fermentation (SSF)); (12) Hamelinck et al., 2005 (Steam pretreatment; SSCF); (13) Hamelinck et al., 2005 (LHW, CBP); (14) Kazi et al., 2010 (LHW); (15) Kazi et al., 2010 (DA).

A range of MESPs from bamboo were generated from the sensitivity analysis when feedstock cost was varied by a range of +/-50% from the reference case and these were plotted here. At each feedstock cost, LHW pretreatment has the lowest MESP, followed by DA and SAA pretreatments. For DA pretreatments, the results generated in this study with bamboo are approximately within the same range of what is reported in the literature,

particularly studies by Eggeman and Elander (2005) (2), Humbird et al. (2011) (5), Laser et al. (2009) (6) and Hamelinck et al. (2005) (11). Studies by Tao et al. (2011) (9) and Kazi et al. (2010a) (15) are slightly higher which is mainly a result of the greater feedstock cost, but could also be related to other factors such as a slightly lower xylan to xylose yield (78.4% compared with 87.6% of xylan achieved in this present study), as well as a lower price for electricity credit which was only $0.04/kWh compared to $0.11/kWh in this study.

The MESPs for LHW pretreatment are the lowest among the pretreatment process explored in this study. However, with exception of Eggeman and Elander (2005) (1), most of the results from the literature are highly scattered and are not well correlated with each other nor with this work. Studies by Macrelli et al. (2012) (7) Tao et al. (2011 (10), Kazi et al. (2010b) (14) are significantly higher, whereas studies by Hamelinck et al. (2005) (12, 13) are significantly lower than our results. The lower price outliers were based on Hamelinck et al. (2005) who chose to model SSF and CBP processes representing mid- and long-term technologies, which are therefore considerably lower than most of the “current” prices. The DA pretreatment results from Hamelinck et al. (2005) (11) represent a short-term technology, and are more comparable to the results from this study. The study by Tao et al. (2011) (10) is approximately more than $0.200/litre higher than results from this work. While their glucan to glucose conversion efficiency was higher, their xylan to xylose conversion of 33.0% was significantly lower than the 87.4% adopted in this study and could have been an important parameter accounting for some of price discrepancies observed.

The numbers of studies reporting the use of ammonia-based pretreatments as a route to produce bioethanol are less common, so AFEX as well as SAA pretreatments were included for comparison in Figure 3.7. Studies by Sendich et al. (2008) (3, 4) model the price for two types of AFEX processing scenarios – scenario 3 represents AFEX pretreatment with a simultaneous saccharification and co-fermentation pathway (SSCF), whereas scenario 4 uses AFEX pretreatment but with a more advanced consolidated bio-processing (CBP) approach. Both processes are shown to decrease costs significantly due to the reduced capital and operating costs as a result of requiring just one microorganism to carry out all these functions in a single reactor. CBP was also adopted in Hamelinck et al. (2005) as one of the long-term technologies, which suggests that while this pathway is modelled and expected to have significant potential for cost reductions in the future, it may not be feasible at present. Although a much higher feedstock cost is used in Tao et al. (2011) for SAA pretreatment, an extrapolation of the results from this study would lead to an MESP in a similar price range of $1.167/litre. These economic results are fairly conclusive in

demonstrating that from an economic perspective, SAA pretreatment is much more expensive and would not currently be cost competitive at the commercial scale.

Some of the difficulties in comparing results between techno-economic analyses have been highlighted in this section. Many of these discrepancies have been discussed, such as the variable yields achieved in pretreatment and enzymatic saccharification, which affects the overall ethanol yield and therefore total sales. Even within the same pretreatment type, conditions such as solid loading, temperature, time and pressure can affect energy usage, thereby impacting the net electricity credit that is sold to generate additional profit. The process combinations of saccharification and fermentation are also shown to affect capital and operating costs, whereby technologies that combine these stages together in one step (e.g. CBP) are modelled to be more cost-effective than the alternative of keeping them separate (e.g. SHF).

Other factors affecting the economics of these processes include solid loading and cellulase enzyme production. The total solids for pretreatment and saccharification in this study were modelled to be 30% and 20% respectively; however this is known to vary between 10-30% based on other studies, which will influence the total volume of process water required. Additionally, the production of enzymes, whether on- or off-site, can also significantly affect the MESP. In this study it was assumed that enzymes would be purchased from outside suppliers, which on the one hand, may have reduced capital costs; while on the other, may have increased raw material costs due to high enzyme prices. Several studies comparing the economics of on-site versus off-site enzyme production have reached conflicting conclusions regarding these options. In Humbird et al. (2011), the additional cost of an on-site enzyme production facility resulted in a MESP increase of approximately $0.089 to $0.132/litre. However, Barta et al. (2010) demonstrated that, under specific enzyme production scenarios, on-site enzyme production could be viable, but that there was still a great level of uncertainty regarding the economic contribution to this process. Therefore, other than recognising that the enzyme area is a highly uncertain cost, the improved transparency of these costs needs to be addressed in order to identify what the most economical solution is.

3.4 Conclusions

The techno-economic feasibility of bioethanol produced from this base-case for bamboo in China was assessed via three processing pathways (LHW pretreatment, SAA pretreatment

and DA pretreatment) and five enzyme loading scenarios (10-140 FPU/g glucan Cellic Ctec2). It was found that within each pretreatment process, lower enzyme loadings generally led to more economically favourable scenarios with reduced MESPs. For LHW and DA pretreatment this was with 10 FPU/g glucan ($0.484/litre and $0.547/litre, respectively), whereas for SAA pretreatment the best-case scenario was with 30 FPU/g glucan ($1.014/litre). It is believed that under LHW and DA pretreatments, a sufficient level of sugar release was achieved at the lowest enzyme loading, and the costs of using more enzyme were greater than the benefit of releasing more sugar (and thereby ethanol). The same pattern was observed with SAA pretreatment, but because this process was less effective in releasing cell wall sugars, an additional increase in enzyme led to a more economically viable scenario.

A cost breakdown analysis was performed on the optimal scenarios for each pretreatment process, to identify the major cost contributors towards the MESP. For all three pretreatment processes, feedstock was consistently ranked as one of the most significant costs, comprising 29-51% of the MESP. The cost of enzyme was also a major contributor towards the MESP, where it accounted for 10-28% of the MESP even at relatively low dosages (equal to or less than 30 FPU/g glucan). Credits generated from the combustion of bamboo residues led to a negative contribution of -21 to -47% towards the MESP, offsetting the gross price by a considerable amount. The pretreatment area comprised around 16-21% of the MESP but was not one of the highest costing stages within the conversion process due to higher capital and operating costs found in feedstock handling, saccharification and fermentation, wastewater treatment and combustion areas. However, the lack of chemical requirement in LHW pretreatment was one of the key advantages which kept raw material and downstream (wastewater treatment, combustion) costs to a minimum, and thus resulted in it being the most economic process at the lowest enzyme loading.

The cost breakdown analysis also revealed the impact that the type of pretreatment can have on downstream processes and utility consumption, including electricity, water and heat. The processes with the highest water consumption were DA pretreatment followed closely by LHW pretreatment. Electricity consumption was greatest in the DA and SAA pretreatment processes. With SAA pretreatment this was mainly attributed to the process design of ammonia recovery in pretreatment, which involved a number of energy-intensive columns, pumps and compressors. Furthermore, the use of ammonia and sulphuric acid causing a build-up of ammonium and sulphate salts in combustion and wastewater treatment of SAA and DA processes, resulted in: 1) the replacement of the evaporator system in the LHW process with a lignin separation system, and 2) the requirement for caustic in wastewater

treatment and flue gas desulphurisation in combustion which contributed towards additional capital and operating costs in these processes (Humbird et al., 2011).

A sensitivity analysis was conducted to evaluate the impact of varying three economic parameters (feedstock cost, enzyme cost and price of electricity credit) on the MESP. For DA and LHW processes the MESP was most sensitive to the feedstock cost, followed by electricity credit and then enzyme cost. Only for SAA pretreatment was the cost of enzyme just as significant as the feedstock cost due to the higher loading used in that particular scenario, which increased its overall cost burden.

Lastly, the relationship between feedstock cost and MESP was compared between this work on bamboo and with other studies using different lignocellulosic feedstocks modelling bioethanol production via similar pretreatment processes. There was a wide range of variation in the MESPs for hydrothermal pretreatments. The studies with lower prices were often based on long-term technologies combining saccharification and fermentation processes into a single stage (e.g. SSF and CBP), whereas higher prices could be related to the enhanced sugar yields as well as the increased electricity credits assumed in this work (Hamelinck et al., 2005, Kazi et al., 2010b, Tao et al., 2011, Macrelli et al., 2012). The DA pretreatment results plotted against other studies showed a clear correlation between the increase in MESP with feedstock cost and these were within approximately the same range. SAA pretreatment results were compared with ammonia-based pretreatments (AFEX and SAA), and revealed that prices reported here were comparable to the two SAA price results, but were significantly higher than the MESPs using AFEX pretreatment (most likely due to their use of CBP and SSF technologies) (Isci, 2008, Sendich et al., 2008, Tao et al., 2011).

This chapter has described the results and findings of the economic viability of bamboo-derived bioethanol produced via several combinations of pretreatment processing and enzyme loading scenarios. It was shown that bamboo pretreated with liquid hot water and saccharified with 10 FPU/g glucan could produce bioethanol at the lowest cost (about $0.484/litre) due to its simple processing technology. At higher enzyme loadings, dilute acid would become more viable due to the enhanced sugar yields achieved during pretreatment and enzymatic saccharification. A cost breakdown and sensitivity analysis highlights the major economic contributions of feedstock cost, enzyme cost and electricity credit, which could potentially decrease (or increase) bioethanol production costs significantly. Pretreatment with soaking in aqueous ammonia was demonstrated to be the least economically competitive due to the poor sugar yields and high costs of capital and chemicals as well as its energy-intensive process. It is evident that the cost of bioethanol at

the plant gate is highly dependent on techno-economic parameters such as sugar conversion efficiencies, costs of chemical and raw materials and utilities consumption. However, when the supply-chain boundaries are extended to evaluate the price of bioethanol at the pump, additional factors such as government policy are believed to be just as (if not more) critical to determine whether bioethanol can be competitive with petroleum-based fuels at the pump. This aspect is considered in the following chapter.

Chapter 4 Competitiveness of bamboo-to-bioethanol case study scenarios

4.1 Introduction

In this chapter, China-based bioethanol from bamboo is first compared with Colombian-based bioethanol to assess how varying costs and policies in the two countries can influence the economic feasibility. The most viable bamboo technology scenarios are then compared against two other potential lignocellulosic feedstocks (wheat straw and short-rotation coppice poplar) in six different countries (UK, Sweden, Italy, France, Slovakia and Spain) to evaluate the relative competitiveness of bioethanol from bamboo on a broader scale. The bioethanol supply chain is used to generate a theoretical bioethanol pump price to compare with fossil-based fuels. Reference case scenarios reflect current policies and indicate their contributions toward helping or hampering the competitiveness of bioethanol; whereas prospective scenarios explore whether or not bioethanol could be competitive in the future with possible policy amendments or with plant biotechnology advances.

Colombia and China are selected as ideal locations for establishing bamboo-to-bioethanol industries based on their natural abundance of bamboo resources. Colombia has the second highest bamboo diversity of the Latin American countries, with approximately 19 genera and 95 species (Londoño, 2001). One tropical, sympodial species, *Guadia angustifolia* is well known for its role in traditional culture and economy, and its forest cover is estimated at 51,500 hectares (Londoño et al., 2002, De Flander, 2005, Muller and Rebelo, 2011). Most of the bamboo in Colombia is concentrated in the Valle de Cauca and Eje Cafetero (Coffee region). In Eje Cafetero, *Guadua* provides approximately 28,000 hectares of forest cover and serves as an important refuge of biodiversity (Camargo et al., 2010). China on the other hand, boasts a richer bamboo diversity containing over 500 different species belonging to 39 genera and has 4.21 million hectares of natural bamboo forests and plantations (Maoyi and Yang, 2004). The genus *Phyllostachys* includes 76 known monopodial bamboo species, of which *P. edulis* (Moso bamboo) is one of the most prominent, due to its hard culms and tough walls rendering it ideal for timber and skin use (Maoyi and Yang, 2004). Moso bamboo is one of the most economically valuable bamboo species in China; it covers about 3 million hectares of land area and its resource has doubled in the last 30 years (Fu, 2001).

For the feedstock study, wheat straw in the UK and poplar in Europe are chosen for comparison with bamboo. Agricultural wastes such as wheat straw have gained significant interest over recent years to serve as feedstocks for bioethanol production, due to the assumption that they are cheap, available and abundant. The UK produces around 11.9 million tonnes of cereal straw annually, of which there is a surplus of 4.9 million tonnes (Barker, 2009). From the cereal straw varieties produced in the UK, wheat straw accounts

for 54% by mass of the total (Renewable Fuels Agency (RFA), 2010). Wheat straw production area in the UK covers 1.8 million hectares and is third highest amongst the EU 27 countries (European Commission, 2011a, The National Non-Food Crops Centre, 2008). The UK has invested in advanced bioethanol production and introduced regulatory measures such as the Renewable Transport Fuels Obligation, which requires that fossil fuel producers incorporate renewables into their energy mix (aimed at 5% by April 2013) (UK Government, 2012). The EU on the other hand, has set a supranational mandate (Renewable Energy Directive (2009/28/EC)) stating that by 2020, 10% of transport fuel should be derived from renewable sources (European Commission, 2011b). Poplars (*Populus* spp.) are of interest due to their: 1) potential for management under short- (and very short) rotation coppice (SRC) harvest cycles, 2) low nutrient demand, 3) high biomass yield on different types of land and 4) natural abundance, shown by a total of 131,400 hectares of land covered by indigenous poplar in Europe (Coaloa and Nervo, 2010).

The criteria for bioethanol to be cost-competitive and economically viable requires that conversion costs be at least equivalent or lower than the current price of petrol (Chandel et al., 2007). Government intervention in the form of tax exemptions, subsidies and mandates are commonly implemented to reduce bioethanol costs and to enhance its competitiveness with fossil-based fuels. In this chapter, the potential for bioethanol production from selected feedstocks, technologies and countries is evaluated, thereby providing a framework to facilitate dialogue between scientists, producers and policy-makers on how to address the major bottlenecks within the supply chain which hinder the economic viability of cellulosic bioethanol.

4.2 Materials and methods

4.2.1 Feedstock composition

The composition of bamboo was based on the experimental results derived from Chapter 2. The baseline wheat straw composition was averaged and normalised from research literature (Ballesteros et al., 2006, Pérez et al., 2008), whereas the baseline poplar composition was derived from the NREL database for Hybrid Poplar Caudina (*P. deltoides* x *P. nigra var. caudina*) (US Department of Energy, 2004) (Table 4.1). The composition of genetically-modified poplar with a reduced lignin content was obtained from Coleman et al. (2008), and a brief description for generating this transgenic poplar line is described in the footnote of Table 4.1. Sweden, Italy, Slovakia, Spain and France were selected as five European countries for comparison in this study.

Table 4.1 Chemical composition of various lignocellulosic feedstocks

Cell wall component (% of DM)	Bamboo	Wheat straw	Hybrid Poplar Caudina	Lignin-modified poplar (C3H-14)[a]
Glucan	38.4	34.6	45.3	55.1
Xylan	20.5	21.1	15.5	22.8
Galactan	3.6	0.9	1.0	1.0
Arabinan	1.8	2.3	1.0	0.5
Mannan	--	--	2.1	1.8
Lignin	20.8	18.0	28.2	11.3
Ash	0.9	5.6	2.0	2.2
Extractives	13.5	15.4	5.0	5.4
Reference	This study	Ballesteros et al. (2006); Pérez et al. (2008)	US Department of Energy (2004)	Coleman et al. (2008)

[a] The lignin-modified poplar is down-regulated for coumaroyl shikimate/quinate 3'-hydroxylase, an enzyme involved in lignin biosynthesis. This genetically modified variety has 56% less lignin compared to its non-modified control (Coleman et al., 2008)

4.2.2 Process design and model assumptions

A techno-economic analysis for bamboo-based bioethanol was conducted to first compare the economic potential for bamboo-based bioethanol in China and Colombia. Based on these results, the two best technology scenarios for bamboo (using LHW and DA pretreatments) were compared with wheat straw and poplar in various locations. For the

wheat straw and poplar scenarios, the NREL model in Aspen Plus (described in Section 3.2.2) was used to generate mass and energy balances for the economic analysis (Humbird et al., 2011). The process parameters and conversions were adopted from empirical data in published literature and publically available databases. These were compared with the experimental data for bamboo and are listed in Table 4.2. The poplar scenarios were based on work conducted by the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) team evaluating the effects of LHW and DA pretreatments on poplar wood (Wyman et al., 2009).

Several assumptions were made in the process design for the information that was not reported in the literature. For example, there was no data on the formation of 5-hydroxymethylfurfural (HMF) from C6 sugars and furfural from C5 sugars; therefore it was assumed that none of these compounds were generated during pretreatment. When not reported, the pretreatment conversions of arabinan and galactan were also assumed to be the same as xylan and glucan, respectively. C5 and C6 sugar yields during enzymatic saccharification were also assumed to be the same as xylose and glucose, respectively. Where only the glucose yield was reported, the same conversion efficiencies were assumed for other polysaccharides.

Table 4.2 Pretreatment and enzymatic saccharification conditions and yields for LHW and DA pretreatments on selected lignocellulosic feedstocks.

	Bamboo LHW[a]	**Bamboo DA[b]**	**Wheat straw LHW**	**Wheat straw DA**	**Poplar LHW**	**Poplar DA**
Pretreatment conditions	190˚C, 10 min	160˚C, 15 min, 0.2% H_2SO_4	188˚C, 40 min	121˚C, 90 min, 2% (w/v) H_2SO_4	200˚C, 10 min	190˚C, 1.1 min, 2.0% H_2SO_4
Pretreatment reactions (fraction of reactant converted to product)						
Glucan + H_2O → Glucose	15.0%	32.5%	0%	12%	2%	24%
Glucan → Glucose oligomers + H_2O	NA[c]	NA	NA	NA	2%	NA
Glucan → HMF + H_2O	NA	NA	NA	2.7%	NA	NA
Xylan + H_2O → Xylose	83.6%	84.2%	91%	85%	4%	62%
Xylan → Xylose oligomers + H_2O	NA	NA	NA	NA	54%	NA
Xylan → Furfural + $2H_2O$	NA	NA	NA	0.2%	NA	NA
Arabinan + H_2O → Arabinose	0%	0%	NA	100%	NA	NA
Mannan + H_2O → Mannose	NA	NA	NA	NA	NA	NA
Galactan+ H_2O → Galactose	26.3%	0%	NA	NA	NA	NA
Lignin → Soluble lignin	0%	0%	19%	16%	25%	NA
Enzymatic saccharification conditions	10 FPU cellulase/g glucan, 72h	10 FPU cellulase/ g glucan, 72h	(15 FPU cellulase + 15 IU β-glucosidase) /g dry solid in treated biomass, 72h	(20 FPU cellulase + 40 IU β-glucosidase)/ g glucan, 72h	(15 FPU cellulase + 40 CBU β-glucosidase) /g glucan in original biomass,72h	(15 FPU cellulase)/ g glucan in original biomass, 72h
Enzymatic saccharification yields	26.3% glucose yield, 41.3% xylose yield	36.5% glucose yield, 21.9% xylose yield	76.3% glucose yield, 20.5% xylose yield	62.2% glucose yield, 73.9% xylose yield	55.0% glucose yield, 89.8% xylose yield	82.5% glucose yield, 24.7% xylose yield
References	This study	This study	Pérez et al. (2008)	Qi et al. (2010)	Wyman et al. (2009); Kim et al. (2009b)	Wyman et al. (2009)

[a] LHW: Liquid hot water pretreatment
[b] DA: Dilute acid pretreatment.
[c] NA: Data was not reported and assumptions were applied in the process design (Section 4.2.2).

4.2.3 Economic analysis

The processing parameters for the Colombia bamboo to bioethanol scenario were assumed to be the same as for the China bamboo scenario (Section 3.2.4). Costs for Colombia were obtained from techno-economic studies, online quotations or through consultation with industry experts, and are based on a reference year of 2011 (Table 4.3). The discounted cash flow analysis parameters were the same as those listed in Section 3.2.3.3 with the exception of corporate tax rate.

Table 4.3 Summary of economic cost parameters for bioethanol production from bamboo in China and Colombia.

Material	China cost	Colombia cost	Reference
Bamboo ($/tonne)	44.6	54.5	Ding (2012); Camargo and Berjan (2012); Lobovikov et al. (2007)
Transport cost (USD/t/km)	0.05	0.035	Teravaninthorn and Raballand (2008)
Aqueous ammonia ($/tonne)	445.6	457.2	China fertilizer net (2012); Humbird et al. (2011)
Caustic ($/tonne)	317.5	455	Chemical Market Associates Inc. CMAI (2011)
Sulphuric acid ($/tonne)	97.5	96.1	BAIINFO (2012b); Vida (2011)
Diammonium phosphate ($/tonne)	502.5	617.8	BAIINFO (2012a); World Bank (2013)
Lime ($/tonne)	120.0	110.0	Alibaba.com (2013); Miller (2011)
Landfill tax ($/tonne)	4.5	2.7	Nanning Federation of Industry and Commerce (2011); UN Habitat (2010)
Electricity credit ($/kWh)[a]	0.11	0.10	Sun (2012); Borzychowski et al. (2012)
Corporate tax rate	25%	15%	Pinzon (2012); Government of Colombia - Proexport (2011)

[a] Amount that renewable electricity generators receive from selling excess electricity to the grid or to other suppliers/distributors.

For the UK wheat straw comparison study, UK variable operating costs are listed in Table 4.4. These costs were updated to a reference year of 2011. For poplar it was assumed there

would be no variation between the European countries, and costs were adopted from the NREL model and scaled to a reference year of 2011. For wheat straw it was assumed that the biomass would be transported by lorry from a 50 km distance of the bioethanol plant and an average transportation and handling charge of $11.9 for distances up to 113 km was adopted from Hess et al. (2007). For poplar an average transportation and handling charge of $0.103/km/tonne of poplar was adopted from Neuvonen (2010).

The discounted cash flow parameters are the same as those listed in Table 3.10 of Chapter 3 with the exception of the corporate tax rate, which for the UK was set at 26% in 2011. For the poplar study, the main cost parameters are listed in Table 4.5. Labour costs of European countries were scaled up or down relative to the average salary in the UK in 2011 (Wang et al., 2012, Eurostat, 2012).

Table 4.4 Summary of variable operating costs for bioethanol production from wheat straw in the UK.

Materials/chemicals/energy	**Cost**	**Reference**
Wheat straw ($/tonne) [a]	79.7	FarmingUK (2011); Hess et al. (2007)
Sulphuric acid ($/tonne)	86.4	ICIS (2011a)
Ammonia ($/tonne)	457.2	Humbird et al. (2011)
Lime ($Ca(OH)_2$) ($/tonne)	203.2	Humbird et al. (2011)
Corn steep liquor ($/tonne)	57.9	Humbird et al. (2011)
Diammonium phosphate (DAP) ($/tonne)	500.7	The World Bank (2011)
Enzyme ($/tonne)	507.0	Kazi et al. (2010a)
Sorbitol ($/tonne)	1148.1	Humbird et al. (2011)
Caustic ($/tonne)	152.4	ICIS (2011b)
Fresh water ($/tonne)	0.26	Humbird et al. (2011)
Boiler feed water chemicals ($/tonne)	4539.1	Humbird et al. (2011)
Cooling tower chemicals ($/tonne)	3636.9	Humbird et al. (2011)
Ash landfill ($/tonne)	37.8	Wang et al. (2012); HM Revenue & Customs (2010)
Surplus electricity ($/kWh)	0.147	Department of Energy & Climate Change (DECC) (2010)

[a] Includes a transportation cost of $11.1/tonne up to distances of 113 km.

Table 4.5 Summary of main cost parameters for bioethanol production from poplar in selected European countries.

	Sweden	France	Italy	Slovakia	Spain	Reference
SRC poplar ($/tonne)[a]	44.5	101.1	45.9	112.0	76.2	Spinelli et al. (2005); Christersson (2010); Spinelli et al. (2008); Petráš (2012); Vega-Nieva et al. (2009)
Landfill cost ($/tonne)	83.6	26.7	115.6	2.3	43.7	Confederation of European Waste-to-Energy Plants CEWEP (2011); Speck and Markovic (2001)
Electricity credit ($/kWh)[b]	0.04[c]	0.17[d]	0.33[d]	0.12[d]	0.17[d]	Europe's Energy Portal (2012)
Income tax	26.3%	34.3%	27.5%	19.0%	30.0%	Organisation for Economic Co-operation and Development (OECD) (2011)
Labour ratio	1.95	1.7	1.33	0.42	1.02	Wang et al. (2012); Eurostat (2012)

[a] Includes a transportation cost for a distance of approx. 50 km (Neuvonen, 2010).
[b] Electricity credit refers to the amount that renewable electricity generators can receive from selling their excess electricity to the grid.
[c] Price of electricity certificate given to renewable electricity producers per kWh of electricity generated.
[d] Price of electricity from renewable sources is regulated based on a fixed feed-in tariff system.

4.2.4 Supply chain analysis

A supply chain analysis was conducted to model a theoretical pump price for bioethanol for comparison with petrol. The final pump price includes the bioethanol production cost (MESP), a fuel tax (often referred to as excise tax, indirect tax or global tax depending on the country), value-added tax (VAT) and a fuel distribution cost. Distribution costs are those associated with the distribution of fuel from terminals to stations and retail and include (but are not limited to) fees, insurance, depreciation, profit and advertising of the fuel (The California Energy Commission, 2013). For all scenarios, a distribution cost of $0.032/tkm was adopted from Slade et al. (2009). The energy content of petrol is greater than bioethanol (31.2 MJ/litre compared to 21.2 MJ/litre), such that 1 litre of bioethanol was assumed to be equivalent to approximately 0.68 litres of petrol (Wang et al., 2012). Therefore all bioethanol pump prices in this chapter have been adjusted to their petrol equivalent on an energy basis. For all scenarios, the reference case reflects the competitiveness of bioethanol at the pump

based on policies and costs in 2011. Prospective scenarios for bamboo evaluated the effect of complete removal of government support systems for bioethanol; whereas for the feedstock comparison this was based on demonstrated improvements in plant biotechnology for SRC poplar biomass.

Petrol prices for the UK scenario were based on unleaded petrol, while Europe petrol prices were based on Euro Super-95 petrol. All prices were converted into US Dollars ($) based on the average 2011 exchange rate for Great Britain Pounds (GBP) or Euros.

Table 4.6 Fuel pump price parameters ($US per litre) for cellulosic bioethanol production in various locations.

	Petrol pump price	**Fuel tax**	**Value-added tax**	**Bioethanol policy**
China	1.240	5%	17%	Full exemption and receives subsidy of $0.16/litre. Fixed bioethanol price of $1.130/litre.
Colombia	1.345	39%	16%	Full exemption. Fixed bioethanol price of $1.260/litre.
UK	1.965	0.893	20.0%	No exemption
Sweden	2.036	0.818	25.0%	Full exemption
France	2.003	0.817	19.6%	Partial exemption ($0.187/litre)
Italy	2.074	0.832	21.0%	No exemption
Slovakia	1.929	0.737	20.0%	Full exemption
Spain	1.761	0.568	18.0%	Exempt from hydrocarbon tax but not VAT and subjected to an indirect tax on retail sales of hydrocarbons (additional national and regional rate of $0.032/litre)
Reference	European Commission (2012); The World Bank (2012); Bolton (2013)	Scott and Junyang (2012); European Commission (2012); HM Revenue & Customs (2011)	Scott and Junyang (2012); Government of Colombia - Proexport (2011); European Commission (2012)	Yang et al. (2005); Pelkmans et al. (2008); Bacovsky et al. (2009); Government of Colombia - Proexport (2011); European Commission (2012); REN21 (2013); Scott and Junyang (2012)

4.3 Results and discussion

4.3.1 Bioethanol policy development in China and Colombia

China has long been a supporter of research and development of liquid biofuels since the 1980's; however only since 2001 did it actively introduce its own national bioethanol programme (Qiu et al., 2012). Although the initial motivation was to utilise surplus stock in leading grain production provinces, this then led to a series of policies during 2001 to 2005, aimed to promote bioethanol production and distribution of E10 blends (10% ethanol, 90% gasoline) as a fuel. In the 10th Five-Year Plan (2001-2005) a specific bioethanol policy was introduced with the aim of testing out various production, marketing and support schemes for bioethanol produced from surplus stale wheat and maize stocks (Li and Chan-Halbrendt, 2009). In 2002, two more national standards for fuel ethanol and E10 blends were issued and four bioethanol production facilities were approved for development. Continuing on from this success, the programme was further developed in 2004, which not only expanded the use of E10 blends to five provinces and 27 cities in an additional four provinces, but also included subsidies for producers each year for their losses incurred per tonne of bioethanol produced. The following year, the Renewable Energy Law of China was passed and came into effect on the 1st of January 2006 (Global Subsidies Initiative, 2008). This law fully established China's commitment towards biofuels and set a Renewable Energy Fund as a strategy to financially aid biofuel technology R&D, demonstration projects, domestic equipment manufacturing, and evaluation of raw material resources. Additionally, support policies were also formulated which included: a 5% waiver on consumption tax on bioethanol under the E10 program, a value-added tax (VAT) exemption on all bioethanol production, and a fixed direct subsidy incentive provided for bioethanol plants (Global Subsidies Initiative, 2008, Scott and Junyang, 2012).

Although bioethanol production was intended to utilise cereals at least 2 years old, new cereals were being exploited – this was believed to have reduced the total agricultural land area available for food production as well as to have increased food prices, resulting in concerns over food security in China (Qiu et al., 2010). As a method to tackle these issues, it was decided in 2006 that any new bioethanol plants must be approved by the Central Government before being constructed. The previous policy that provided financial support for bioethanol plants utilising cereal crops was also removed and updated so that only non-cereal bioethanol plants would receive support in the form of low interest loans and direct subsidies (Li and Chan-Halbrendt, 2009, Qiu et al., 2012). Furthermore, non-cereal feedstocks grown on marginal lands would receive a one-off subsidy of 2,700 RMB (approx.

USD 355.1 in 2007 dollars) per hectare, as a tactic to promote the use of low-quality or degraded lands. In 2007, the Middle and Long Term Development Plan for renewable energy stated that biofuels "must not compete with grain over land, it must not compete with the food that consumers demand, it must not compete with feed for livestock, and it must not inflict harm on the environment", therefore clearly re-defining many of the nation's initial biofuel policies (Qiu et al., 2012). From then on, a cassava-based bioethanol plant was approved, and the potential for other non-cereal feedstocks were also investigated, including sweet sorghum, sweet potato and sugarcane.

Although there are currently no commercial-scale production facilities for cellulosic ethanol in China, significant technological progress has been achieved, and many believe that China is already becoming a leader in cellulosic technology. By the end of 2009, eight pilot- and demonstration-scale cellulosic ethanol plants were in operation with a combined capacity of 280,500 tonnes of bioethanol per year, and 20 other cellulosic ethanol plants across the country are being built with an estimated capacity of 2 million tonnes (Qiu et al., 2012). It is therefore believed that China is on the right path for developing a bioethanol industry which can contribute to global energy security and reduction of GHG emissions, however, it is clear that the challenges relating to feedstock choice, technology and government policy are major areas that will require clear direction in order to sustain future growth.

In Colombia, support for bioethanol as a transport fuel also began in 2001 with the establishment of Law 693, which mandated that by 2006, cities with more than 500,000 inhabitants require 10% bioethanol blends with petrol (Econergy International Corporation, 2007). However, it was only in 2005 that the production of sugarcane ethanol began to take-off, and between 2005 and 2007 production increased from 0 to 278 million litres a year (Toasa, 2009). In December 2004, Law 939 was further established to provide a framework for biofuel production; this authorised the Ministry of Energy to regulate environmental and technical requirements and to establish a price structure for biofuel blends (Econergy International Corporation, 2007).

Two sugar mills, Incauca and Providencia, were the first to initiate bioethanol production, and by February 2006, three more ethanol plants owned by the Risaralda, Mayaguez and Manuelita mills also started production, allowing the capital city of Bogotá to receive E10 blends. By 2007, several other Colombian Departments also began blending ethanol with gasoline and by April 2008, 70% of petrol was mixed as a 10% blend with sugarcane ethanol. This was then planned to increase to a 25% ethanol blend in 20 years within the Bogotá, Valle de Cauca and Eje Cafetero regions (Toasa, 2009). A new ethanol plant owned

by the Riopaila mill with a production capacity of almost 300,000 litres per day began production in 2009, which produces almost enough bioethanol to fulfil a 10% blend for the entire country (Toasa, 2009). In 2011, the government issued the decree 4892, which establishes a biofuel blend in vehicles ranging from E8 to E10 and for flex-fuel vehicles this can range from E25 to E85 (Pinzon, 2012). Colombia's sugarcane and ethanol industries are supported by a number of interconnected institutions. One of the leading organisations for ethanol production is ASOCAÑA, which represents the all sugar mills and ethanol plants and aims to promote and sustain development of these industries (Toasa, 2009). Other support policies include incentives for biofuel projects established under the Single Enterprise Free Trade Zone regime, which are offered a reduction on income tax rate from 33% to 15%, as well as agricultural subsidies and tax exemptions for palm oil plantations (Government of Colombia - Proexport, 2011, Marin et al., 2011).

Currently, there are five ethanol facilities owned by the 14 sugar mills producing ethanol from sugarcane, and one facility producing ethanol from cassava (Pinzon, 2012). However, due to the low yields from cassava, production levels are low compared to sugarcane and account for only 2% of the total ethanol produced (Pinzon, 2012). The majority of Colombia's sugarcane plantations and ethanol refineries are located in the Cauca Valley and Eje Cafetero (coffee) region due to the rich soil and weather conditions that have consistently allowed for a year round supply of sugarcane for more than 140 years. These favourable conditions allow for high growth rates and make Colombia one of the most productive countries in the world for sugarcane production (Toasa, 2009). Of the total crop area (approx. 400,000 hectares), half is used for commercial sugarcane production and 20% of this is dedicated towards bioethanol production (Toasa, 2009). Most of these ethanol production facilities are not only energetically self-sufficient due to the combustion of bagasse for electricity generation, but also benefit from an additional credit from selling the excess electricity to the national grid (Asocaña, 2012). In 2011, the country's installed energy capacity was 190 MW and this is expected to rise to 333 MW (with 145 MW surplus) by 2015 (Asocaña, 2012). In 2011, total sugarcane bioethanol production reached 1,075,000 litres per day. However, under current capacity, the initial biofuel blend mandate has not yet been reached, and domestic mandates are continuing to rise each year. In 2015 the national target has been extended to 20% bioethanol blends with petrol; therefore in order to achieve these aims, alternative feedstocks that can potentially accelerate bioethanol deployment are being seriously considered (Toasa, 2009, Asocaña, 2012, Pinzon, 2012).

4.3.2 Comparison of MESPs in China and Colombia

The techno-economic analysis results for bioethanol from bamboo in China and Colombia demonstrate two distinct patterns: firstly, the cost of bioethanol production in Colombia is on average, higher than the cost of production in China at the same enzyme and pretreatment process conditions; and secondly, the pretreatment processes and enzyme loading conditions that generate the lowest costs in China are also the lowest in Colombia (Figure 4.1). It is evident that bioethanol produced via SAA pretreatment is significantly higher in comparison to LHW and DA pretreatments, even at its lowest MESP of $0.990/litre at 30 FPU/g glucan in China. With SAA pretreatment, there is a significant cost difference in the MESPs between China and Colombia at each enzyme loading of approximately $0.098/litre. Therefore the cost of producing bioethanol in China at a range of enzyme loadings (up to 60 FPU/g glucan) is less costly than any of the enzyme loading scenarios in Colombia. The lowest MESP for bioethanol production amongst all scenarios is in China using LHW pretreatment at the lowest enzyme loading, which yields a MESP of $0.484/litre. This is followed by the same pretreatment and enzyme loading condition in Colombia, which has the second lowest MESP of $0.540/litre. At 30 FPU/g glucan the MESPs for bioethanol from LHW and DA pretreatments in China and Colombia are similar, (DA pretreatment is marginally lower by $0.007/litre and $0.010/litre in China and Colombia scenarios, respectively) demonstrating that there is no clear preference in country or processing technology for producing bioethanol at this enzyme loading. However, at enzyme loadings above this, the cost differences between DA and LHW pretreatment increase progressively, such that DA pretreatment becomes significantly lower cost at the higher enzyme loadings.

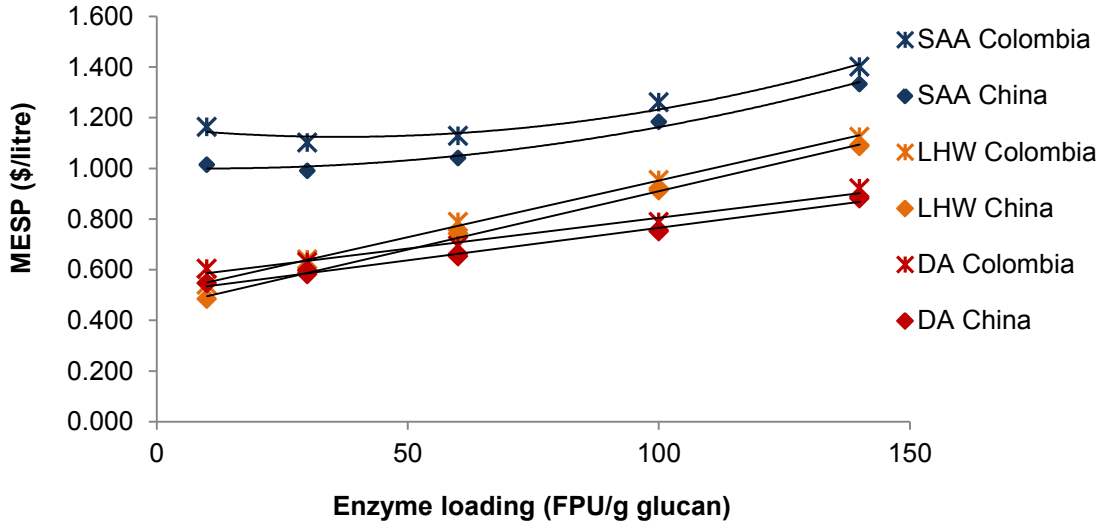


Figure 4.1 MESPs of bioethanol from bamboo in China and Colombia in various pretreatment (SAA, LHW and DA pretreatments) and enzyme loading scenarios.

Most of the discrepancies between the two countries can be accounted for by raw material cost differences in China and Colombia. A comparison of these costs was presented in Table 4.3, demonstrating that prices for aqueous ammonia, caustic and diammonium phosphate are more expensive in Colombia, as well as the electricity credit which is lower at $0.10/kWh in Colombia compared to $0.11/kWh in the China scenarios. Other costs were found to be lower in Colombia such as transport ($0.035/tonne/km compared to $0.05/tonne/km in China) as well as costs for sulphuric acid and lime used in the DA and SAA pretreatment processes. The landfill tax in Colombia was also almost half of that in China at $2.6/tonne compared with $4.5/tonne, suggesting that on average, most of these economic parameters would be largely balanced out between the two countries. Most of these chemical and commodity costs are affected by market price fluctuations, while others are determined by government policies such as landfill tax and electricity, and therefore can potentially be regulated to improve the economic viability of the bioethanol process. Although these variations in material prices may justify some of the cost differences, based on the volume of bamboo processed on a daily basis, the feedstock price, which was previously demonstrated to be the leading contributor towards the MESP, is the believed to be the leading determinant.

Feedstock cost has consistently been shown to be the major cost determinant in the biochemical conversion process for biomass to biofuel processes (Humbird et al., 2011, Wang et al., 2012). In this study, the difference between bamboo prices in China ($44.6/tonne) and Colombia ($54.5/tonne) is approximately ten dollars. Like other commodities, feedstock cost is determined by the interaction between demand and supply. The location of harvested bamboo as well as the part of the culm utilised (thereby affecting its size/diameter and final purpose) are two key factors influencing the price of bamboo, and were taken into consideration for this model. Prices corresponding to waste residues of *Phyllostachys edulis* (Moso bamboo) in China's Nanjing province and *Guadua angustifolia* (Guadua bamboo) in the Eje Cafetero region of Colombia were therefore adopted in this economic analysis.

In both China and Colombia, the total area of bamboo forests could potentially provide sufficient amounts of biomass to support a bioethanol industry of this scale. However in reality, many of these resources would be diverted towards the production of higher value products. Moso bamboo covers approximately 3 million hectares of land and is the most important bamboo species in China. It is used for applications such as timber, shoot production, soil or water conservation, landscaping and for new products such as bamboo juice, beer and charcoal (Fu, 2001). Currently, the three largest bamboo sectors include

handicrafts, bamboo shoots and industrial processing (Marsh and Smith, 2006). The industrial processing sector is further divided into sub-sectors ranging from low-value products such as paper and pulp, to high-value products such as flooring and laminated furniture (Marsh and Smith, 2006). Based on consultation with bamboo experts in China, prices of bamboo ranging from 6-9 cm in diameter as well as prices for pulp and waste material, which were significantly cheaper, were obtained (Ding, 2012).

Historically in China, factories used to purchase whole bamboo culms and were forced to deal with large amounts of wasted residues. A solution for this problem resulted in the "pre-processing bamboo revolution" where culms are now separated into their various sections for different supply chains as a way to potentially utilise 100% of the material with near zero wastage (Marsh and Smith, 2006). However, a recent Chinese bamboo study recently reported that despite these efforts, the average utilisation rate of bamboo in industry is less than 50%, and can even be as low as 20% in some industries (e.g. the timber industry utilises approximately 40% of bamboo, leaving 60% as waste residues (Zeng et al., 2011). These wastes include leaves, branches and thin culms that fail to meet industrial size requirements for processing. It was reported that most waste is currently used as a fuel for boiler burning, however a need for turning these wastes into higher-value products via chemical or biological treatment is being explored (Yang et al., 2012).

A similar approach is taken in Colombia for processing Guadua bamboo. Guadua bamboo is an important resource for native and rural communities in Colombia and is used on a daily basis for shelter, food, transport, musical instruments and arts and crafts (Londoño, 2001). After harvest, the bamboo culm is divided into four main sections, which, from bottom to top are known as *cepa, basa, sobre basa* and *puntal,* and the residual waste material (Camargo et al., 2010). The price and use of these sections vary: *cepa* is used mainly for domestic products (e.g. fences), *basa* is the most valuable and is traded amongst farmers and small companies, *sobre basa* is often flattened for weaving mats and *puntal* is used to supporting crops such as plantains and bananas (Camargo et al., 2010). Prices for bamboo are therefore based on the size and part of culm, and reflect its potential end product. It is estimated that on average, 30% of the bamboo routed for industrial processing is wasted, thereby representing a potential biomass resource for bioethanol production in Colombia. Therefore, the decision made in this analysis was that rather than competing with these existing markets for expensive bamboo culms used to produce higher value products, the waste residues generated as a by-product of these industries, which have no major purpose, can be sold at a significantly reduced cost to bioethanol producers for the production of sustainable fuels.

4.3.3 Supply chain analysis for China and Colombia

The MESP represents a theoretical value for the production of bioethanol at the plant gate based on defined technological and economic assumptions. However, in order to determine whether bioethanol is cost competitive at the pump, other factors such as taxes and distribution costs need to be accounted for, as they can comprise over 50% of the fuel price at the pump in some countries (Deloitte, 2011). In order to improve the competitiveness of bioethanol, especially in regions where technologies are still immature, governments can intervene to provide support at different stages of the supply chain (Figure 4.2).

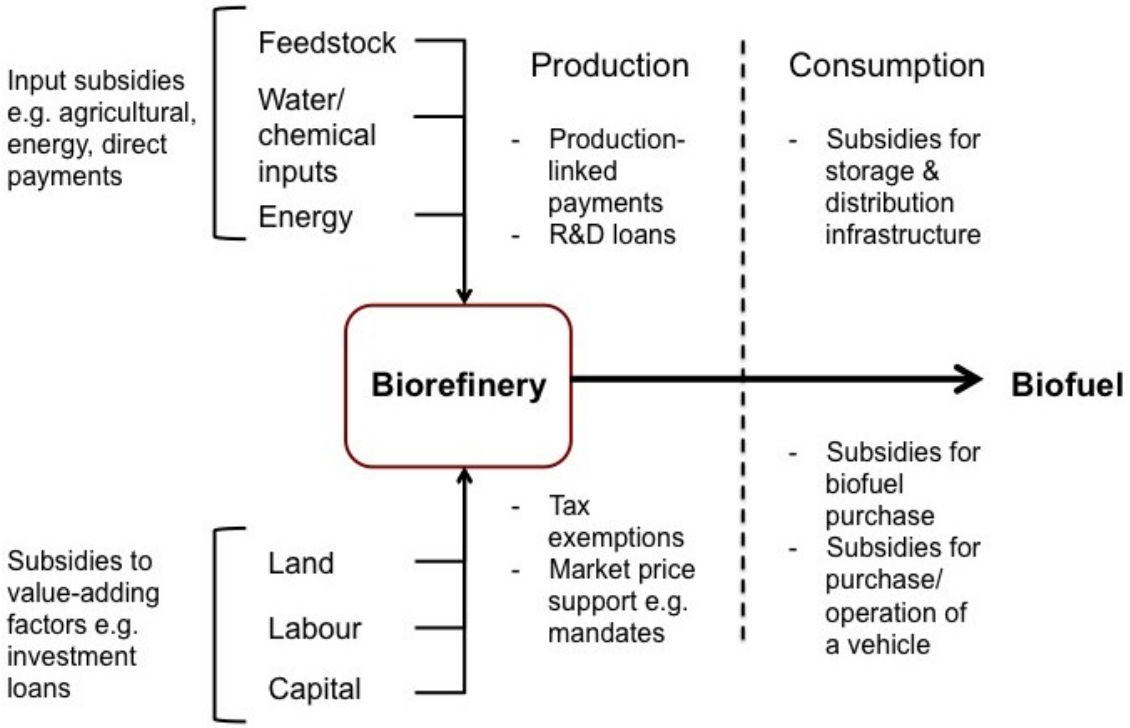


Figure 4.2 Support measures provided in the biofuel production supply chain (adapted from Global Subsidies Initiative (2008)).

The main inputs for biofuel production include feedstock, water/chemicals and energy used in the conversion process. Policies fixed at the input level include financial support for farmers such as agricultural subsidies to grow cellulosic feedstocks, tax reductions for land, reduced prices for fertilizers, as well as energy subsidies (e.g. capping electricity prices) (Global Subsidies Initiative, 2008, Jung et al., 2010). Other input factors such as land, labour and capital can also be given financial aid through investment loans for demonstration projects. The main output of this process – biofuel – is often supported through various demand and supply policies. Governments may choose to incentivise biofuel production by: 1) providing subsidies to boost their competitiveness, especially if losses are incurred during

the production, storage or distribution stage, 2) exempting them from VAT or consumption/fossil fuel taxes, and 3) setting mandates ensuring a certain level of fuel production or consumption, which acts as a form of market price support by guaranteeing demand for the product (Mabee and Saddler, 2005, Global Subsidies Initiative, 2008, Jung et al., 2010). Many of these measures are passed on directly or indirectly to the consumer benefitting from the subsidised fuel, which allows it to become more cost-competitive at the pump. Some governments may additionally subsidise costs for purchasing or operating biofuel-run vehicles (e.g. flex-fuel vehicles) as a way to further boost consumption.

4.3.3.1 Reference case scenarios for China and Colombia

Based on the support for bioethanol in China, which currently allows it to be exempted from all taxes and offers a subsidy of $0.16/litre, its pump price in the reference case scenario consists of only two components – the fuel production cost (MESP) and a distribution cost (Figure 4.3). For bamboo pretreated with LHW, the pump prices range from $0.584 to $1.471/litre with enzyme loadings of 10-140 FPU/g glucan (Figure 4.3(a)). Only at enzyme loadings of 60 FPU/g glucan and lower, is the price of bioethanol lower than petrol. The price of fuel in China is regulated by the government (grey dashed line in Figure 4.3), and this is fixed at a price of 0.911 times the price of RON (research octane number) 90 gasoline (Global Subsidies Initiative, 2008). Based on this guideline, the price margin between the theoretical and fixed bioethanol price is assumed to be the amount of profit that could be potentially received by producers. At an enzyme loading of 10 FPU/g glucan the theoretical pump price of $0.583/litre is $0.546/litre lower than the fixed bioethanol price of $1.130/litre. However this margin decreases with higher enzyme loadings and at 100 FPU/g glucan it exceeds the fixed bioethanol price, therefore becoming uncompetitive. Bioethanol at the pump produced via SAA pretreatment ranges from $1.328 to $1.832/litre (Figure 4.3(b)). At all enzyme loadings the pump price of bioethanol is higher than both petrol ($1.250/litre) and the fixed bioethanol price ($1.130/litre). The closest scenario to being competitive is at an enzyme loading of 30 FPU/g glucan, which at $1.328/litre is still not an economically viable nor a competitive option for bioethanol production. Pump prices for bioethanol produced via DA pretreatment range from $0.676 to $1.169/litre across the enzyme loading scenarios (Figure 4.3(c)). Although the lowest bioethanol price amongst the DA pretreatment scenarios is higher than the lowest price with LHW pretreatment ($0.676/litre compared with $0.584/litre), DA pretreatment offers a wider range of scenarios that allow bioethanol to compete at the pump (10-100 FPU/g glucan, rather than 10-160 FPU/g glucan with LHW pretreatment scenarios). Only at the highest enzyme loading does bioethanol produced from

DA pretreatment become uncompetitive, demonstrating that it is a highly favourable process for producing bioethanol.

Similar trends are observed for bioethanol pump prices in Colombia via the various pretreatment and enzyme loading scenarios. Theoretical pump prices range from $0.826 to $1.685/litre when LHW pretreatment is used, however only at enzyme loadings of 60 FPU/g glucan and below is bioethanol competitive with petrol ($1.345/litre) (Figure 4.3(d)). The greatest profit margin is achieved at the lowest enzyme loading of 10 FPU/g glucan, which results in a pump price of $0.826/litre. Bioethanol produced via SAA pretreatment in Colombia is uncompetitive at the pump at all enzyme loadings including the lowest price achieved of $1.651/litre (Figure 4.3(e)). These pump prices increase from this value to a maximum of $2.092/litre, which is $0.747/litre greater than the petrol price. Theoretical bioethanol pump prices for the DA pretreatment scenarios range from $0.919 to $1.385/litre and with the exception of the highest enzyme loading, are all competitive with petrol and lower than the fixed bioethanol price ($1.260/litre) (Figure 4.3(f)).

Due to the similar support systems established for bioethanol in China and Colombia, the patterns observed with different pretreatments were found to be highly similar. It was previously demonstrated that the average production costs in China were lower than those in Colombia due to cheaper bamboo feedstock prices. However, the price for petrol and the fixed bioethanol price are also lower in China than in Colombia, and therefore the level of competitiveness in the respective countries is relatively equal. As a result, bioethanol is economically competitive at the pump in China and Colombia under the same processing scenarios. These include: LHW pretreatment with enzyme loadings of 10-60 FPU/g glucan and DA pretreatment with enzyme loadings of 10-100 FPU/g glucan, but not with SAA pretreatment under any enzyme loading scenarios.

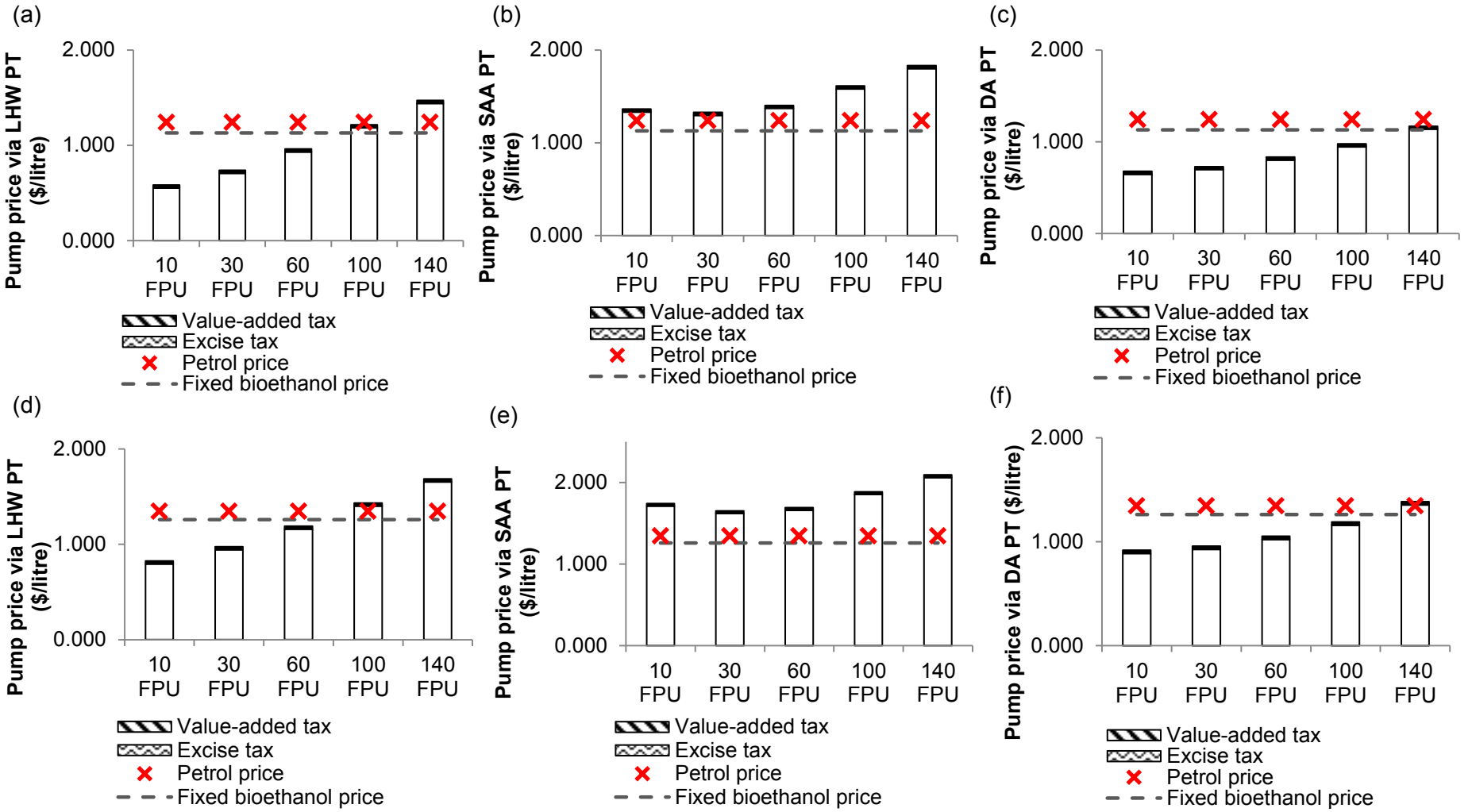


Figure 4.3 Reference case scenarios for bioethanol at the pump in China (a-c) and Colombia (d-f) for five enzyme loading scenarios and via liquid hot water pretreatment (LHW PT), soaking in aqueous ammonia pretreatment (SAA PT) and dilute acid pretreatment (DA PT). Petrol prices are marked by the red cross and the fixed bioethanol prices are shown by the grey dashed line. All bioethanol prices are adjusted for comparison with petrol based on an energy equivalent.

4.3.3.2 Prospective scenarios for China and Colombia

Although the Chinese government has provided a substantial level of support for bioethanol since 2001, financial losses incurred as a direct consequence of these policies have been significant. Reports have shown that in 2004 government subsidies for biofuels totalled $100 million and this increased progressively to $107 million and $114 million in years 2005 and 2006 (Global Subsidies Initiative, 2008). If subsidy provisions were to continue at this rate, the total losses in 2020 from subsidies are estimated to reach $616 million, of which $328 million would come from consumption tax exemption, $261 million from VAT exemption, $119 million from direct subsidies and $8.9 million from low interest loans (Huang et al., 2007). Consequently, bioethanol subsidies are gradually being scaled back each year from what was originally $0.20 per litre in 2008, now to $0.16 per litre in 2011 (Scott and Junyang, 2012). Assuming that these levels of support will continue to diminish, the cost of bioethanol production will need to be reduced to allow bioethanol to compete with petrol.

Prospective scenarios were modelled to evaluate whether bioethanol from bamboo could be economically feasible without any form of government support. In the prospective scenarios subsidies, tax exemptions and the fixed bioethanol pricing were removed from the original reference case scenario (Figure 4.4). When bioethanol was not protected by government policy, there was a noticeable increase in its pump prices in both China and Colombia, which lowered its competitiveness with petrol. Bioethanol in China produced via LHW pretreatment generated theoretical pump prices ranging from $0.914 to $2.003/litre at the various enzyme loadings (Figure 4.4(a)). Without government support, the maximum of enzyme loading before bioethanol is uncompetitive is reduced from 60 FPU/g glucan in the reference case to 30 FPU/g glucan. This is a result of the enhanced pump prices due to the removal of subsidies and the addition of tax payments, causing prices across the enzyme loadings to rise by an average of $0.424/litre. Bioethanol produced via SAA pretreatment in China was not competitive in any of the reference case scenarios, which was supported by tax exemptions, and therefore becomes even less competitive in the prospective scenario (Figure 4.4(b)). Pump prices range between $1.828 and $2.448/litre, which are approximately $0.541/litre higher than the reference case scenarios. With DA pretreatment, bioethanol at the pump is competitive with petrol in three of the five scenarios, which is a reduction from the reference case where four of the five enzyme loadings led to economically competitive bioethanol prices. With DA pretreatment, pump prices vary from $1.028 to $1.633/litre and are around $0.397/litre higher than the reference case scenarios. On average, the bioethanol pump price in China in the prospective scenarios can be broken

down into its constituents with following proportions: production cost at 79.0%, taxes at 18.6% and distribution at 2.4% of the total pump price.

In the prospective scenarios for Colombia, bioethanol pump prices increased substantially when all forms of government protection mechanisms were removed. It was also assumed in these scenarios that bioethanol projects would pay a higher corporate tax rate of 33% instead of the reduced rate of 15% for projects established under the Single Enterprise Free Trade Zone (which was an assumption of the reference case). Pump prices for bioethanol produced via LHW pretreatment vary between $1.200 and $2.630/litre, which is almost double ($0.639/litre higher on average) the reference case prices. Moreover, only the lowest enzyme loading of 10 FPU/g glucan allows bioethanol to be competitive with petrol, which is a substantial reduction in the number of potential production scenarios from the reference case. Similar to China, bioethanol produced using SAA pretreatment was uncompetitive in the reference case, and therefore becomes even less competitive in the prospective scenario with pump prices of $2.809 to $3.374/litre, which are on average $1.110/litre higher than the reference case. The number of scenarios leading to economically competitive bioethanol via DA pretreatment was reduced from four enzyme loading scenarios to zero in the prospective cases. Here, bioethanol pump prices are in the range of $1.481 to $2.233/litre, and are approximately $0.674/litre higher than the respective reference cases. The bioethanol pump price in the Colombia prospective cases consists of the following components and their proportions: production cost at 62.0%, taxes at 38.0% and distribution at 1.10% of the total pump price.

Similar patterns were observed in China and Colombia for the reference case scenarios, however when all forms of government intervention were removed in the prospective scenario, Colombia-based bamboo became significantly less competitive at the pump. This was mainly due to the differing tax rates between these countries, whereby the fuel tax in China was just 5% compared to the global tax rate on fossil fuels of 39% in Colombia. This was evident in the pump price breakdown which revealed that the proportion of the price comprised of taxes in China was 19%, in contrast to 38% in Colombia, which is almost double. This comparison demonstrates that if government support were to be eliminated, bioethanol in China could still be competitive with LHW and DA pretreatment at the lower range of enzyme loadings. However, in Colombia it would only be viable with LHW pretreatment at the lowest possible enzyme loading. Therefore, without protection from taxes and assuming that tax rates remain at the present level, producers in Colombia need to seek alternative approaches to further reduce costs within the bamboo to bioethanol conversion process.

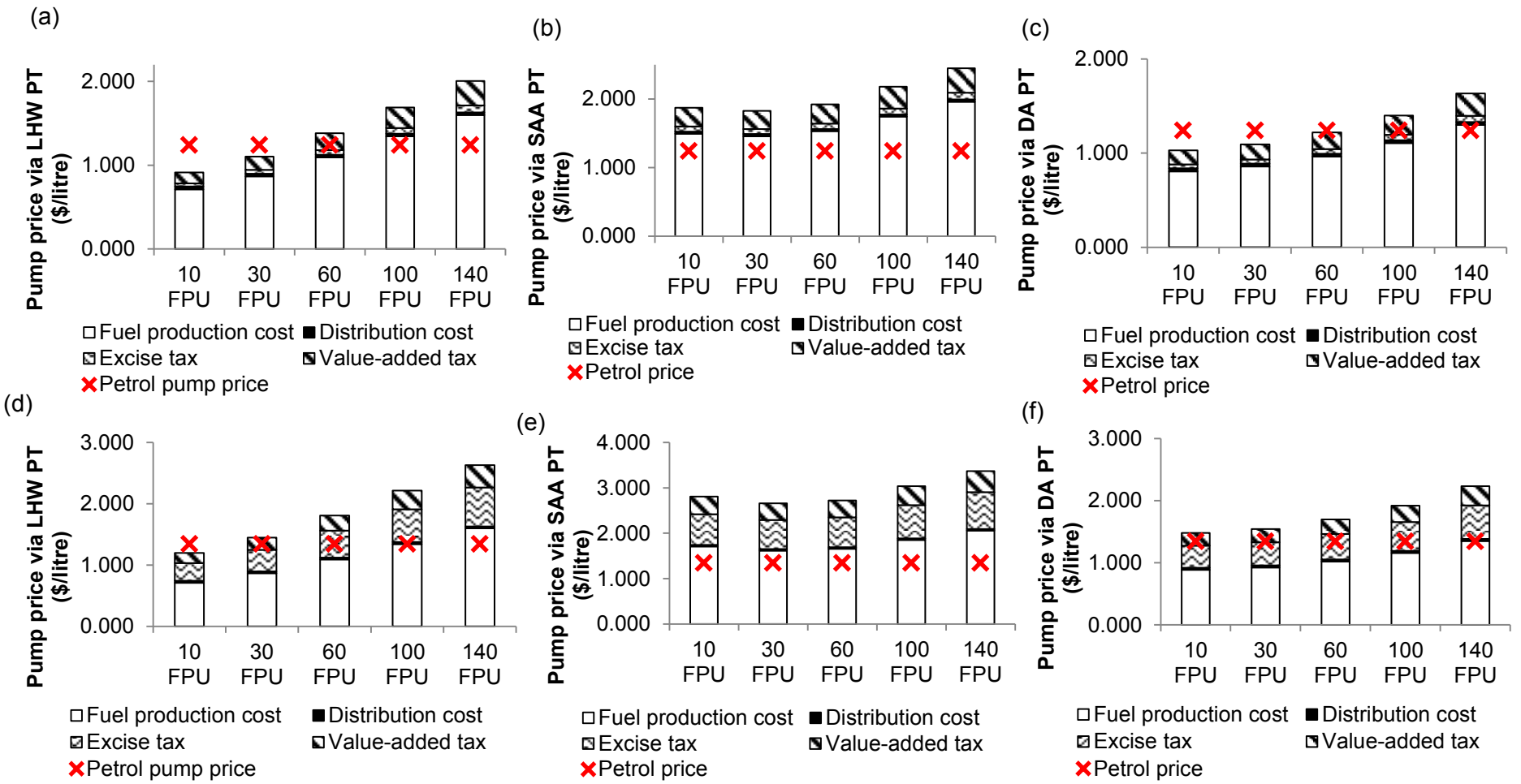


Figure 4.4 Prospective scenarios for bioethanol at the pump in China (a-c) and Colombia (d-f) for five enzyme loading scenarios and via liquid hot water pretreatment (LHW PT), soaking in aqueous ammonia pretreatment (SAA PT) and dilute acid pretreatment (DA PT). Petrol prices are marked by the red cross and the fixed bioethanol prices are shown by the grey dashed line. All bioethanol prices are adjusted for comparison with petrol based on an energy equivalent.

On the one hand it is apparent that an immature biofuel industry can be somewhat of a financial burden due to its high costs and over-reliance on the country's finances in order to remain commercial and cost-competitive. However, the potential socio-economic impact that biofuels may have in the long run can be significant and may bring benefits to multiple areas. China and Colombia are both developing nations with large rural economies that could significantly benefit from a booming biofuel industry. The management of bamboo plantations can create new businesses – harvesting, processing, storage and transport can provide skillsets and offer jobs for farmers, and selling these feedstocks can increase farmer's incomes directly (Qiu et al., 2012). These benefits have been demonstrated in the sugarcane industry in Brazil, which employs about 1 million workers, as well as the bioethanol industry in the United States, which is responsible for creating more employment in rural areas than any other activity (Dufey, 2006). In China, the biofuel programme is estimated to generate up to 9.26 million jobs, and in Colombia, the government predicted that every farming family could earn two times the minimum salary through bioethanol production (Dufey, 2006). Furthermore, the taxes paid by sugar mills and ethanol plants in Colombia provide a major source of revenue for most of the municipalities located in the Cauca Valley region. In 2007, tax payments in 34 municipalities accounted for 11% of the budget on average, but reached 20% and even 50% in some municipalities, demonstrating the significant economic contribution bioethanol can potentially make (Toasa, 2009). In terms of poverty alleviation, a recent study by Huang et al. (2007) demonstrated that in a country like China where the poor have access to land and earn most of their income from agriculture, biofuels have the potential to raise returns and alleviate poverty levels. These optimistic predictions supported by successes achieved in more developed countries indicate the possible socio-economic impact that biofuels can have on less developed countries such as China or Colombia seeking to establish such an industry.

At the same time, other stakeholders have raised potential issues relating to negative environmental and social impacts in Colombia (Janssen and Rutz, 2011). Ecological consequences of biofuels have led to the need to define agricultural land management strategies in order to preserve existing forest area and to prevent them from being turned into biomass growing areas, especially when this leads to cutting down forest to cultivate biofuel crops (Cortés-Marín and Ciro-Velázquez, 2011). Another is related to land tenure issues, where the biofuel was previously blamed for re-allocating land and cultivation of biofuel crops to land formally owned by Afro-Colombians. In many situations, small-scale farmers were forced to leave their land and any opposition was met with violence and abuse of human rights. Experiences such as these, make it essential that land tenure rights of

indigenous populations are handled more carefully in future sustainability schemes to avoid any negative social impacts (Janssen and Rutz, 2011).

4.3.4 Comparison of bioethanol from bamboo with wheat straw and poplar

4.3.4.1 Technology comparison

For each feedstock (wheat straw, poplar and bamboo), two pretreatment technologies (LHW and DA pretreatment) were modelled to investigate the techno-economic potential in each bioethanol production scenario. The optimal conditions for each pretreatment derived from literature data, were based on the composition of the respective feedstocks. A techno-economic model was used to determine the average ethanol production and yield, electricity generation and consumption for bioethanol produced via these various processes (Table 4.7).

Table 4.7 Summary of process results of bioethanol production from lignocellulosic feedstocks.

Feedstock / pretreatment process[a]	Ethanol production (MMl/year)	Ethanol yield (litres/dry tonne)	Electricity generated (MW)	Electricity consumed (MW)
B LHW	147	192	54	19
B DA	172	234	44	22
WS LHW	208	281	46	20
WS DA	201	271	37	23
P LHW	179	233	56	21
P DA	228	298	46	21

[a] LHW: Liquid hot water pretreatment; DA: Dilute acid pretreatment; B: Bamboo; WS: Wheat straw; P: Poplar.

The total ethanol production rate amongst the six scenarios ranges from 147 to 228 MMl/year, and this corresponds to an ethanol yield of 192-298 litres per dry tonne of biomass. The highest ethanol production rate is achieved with DA pretreatment on poplar (228 MMl/year), followed by LHW pretreatment on wheat straw (208 MMl/year). The lowest rates of ethanol production are found for both pretreatments on bamboo (147 MMl/year and 172 MMl/year with LHW and DA, respectively) as well as for LHW pretreatment on poplar. For bamboo and poplar biomass, DA pretreatment seems to be a more favourable route compared with LHW pretreatment for maximising ethanol production, based on the selected conditions. The greatest electricity generation is achieved for the LHW pretreatment on

poplar scenario (56 MW) followed by LHW pretreatment on bamboo (54 MW), whereas the lowest is generated using pretreatment with DA on wheat straw (37 MW). All six scenarios consume a similar amount of electricity ranging from 19-23 MW, however for the same feedstock, LHW pretreatment on average generates more electricity than DA pretreatment, whereas DA pretreatment has a greater level of consumption.

For the highest ethanol producing scenarios (e.g. DA pretreatment on poplar), there are two primary factors responsible for this outcome. The first is an enhanced sugar yield during enzymatic saccharification, and the second is the high feedstock cellulose content. In the scenario for DA pretreatment on poplar, the glucose yield from saccharification reaches 82.5% of glucan, which is the highest value amongst the scenarios modelled (ranging from 26-76% of glucan), and the cellulose content of poplar is also the highest at 45.3% of DM, compared to 38.4% and 34.6% of DM in bamboo and wheat straw, respectively (Table 4.1). In comparison, LHW and DA pretreatment on bamboo scenarios have the lowest level of glucose release during saccharification (26% and 37% of glucan in LHW and DA, respectively), and while the cellulose content is not the lowest, it is only several percentage points above wheat straw and therefore does not overcome these low saccharification yields (determined experimentally at small scale, see Chapter 2). Both wheat straw scenarios achieve fairly high levels of glucose release during saccharification (62-76% of glucan) and this is also reflected by the ethanol production rate of these scenarios, which are second and third highest. These observations support the theory that maximising bioethanol production from a technology perspective involves targeting feedstocks or genotypes with high cellulose contents, and identifying effective routes to release cell wall sugars during their biochemical conversion.

4.3.4.2 MESP comparison

The technology scenarios for bamboo, wheat straw and poplar were modelled in eight locations to investigate the effect of local costs and policies on the economic feasibility of bioethanol production (Figure 4.5). Countries were selected based on the accessibility and abundance of feedstocks and included bamboo in China and Colombia, wheat straw in the UK and SRC poplar in Sweden, Italy, Spain, Slovakia and France. Economic results demonstrate that amongst these technologies, feedstocks and countries, the lowest MESP and thus most economically viable scenario for producing bioethanol is with poplar in Italy using LHW pretreatment ($0.368/litre). This is followed closely by bioethanol from bamboo in China (LHW and DA at $0.484/litre and $0.547/litre, respectively), bamboo in Colombia

(LHW and DA at \$0.540/litre and \$0.603/litre, respectively), and poplar in Italy with DA pretreatment (\$0.520/litre). Several scenarios have significantly higher MESPs and are thus considered to be highly uneconomic, such as bioethanol from poplar in Slovakia using both LHW and DA pretreatments (\$0.972/litre and \$0.913/litre, respectively), poplar in France under LHW and DA pretreatments (\$0.888/litre and \$0.866/litre, respectively), as well as from poplar in Sweden with LHW pretreatment (\$0.844/litre).

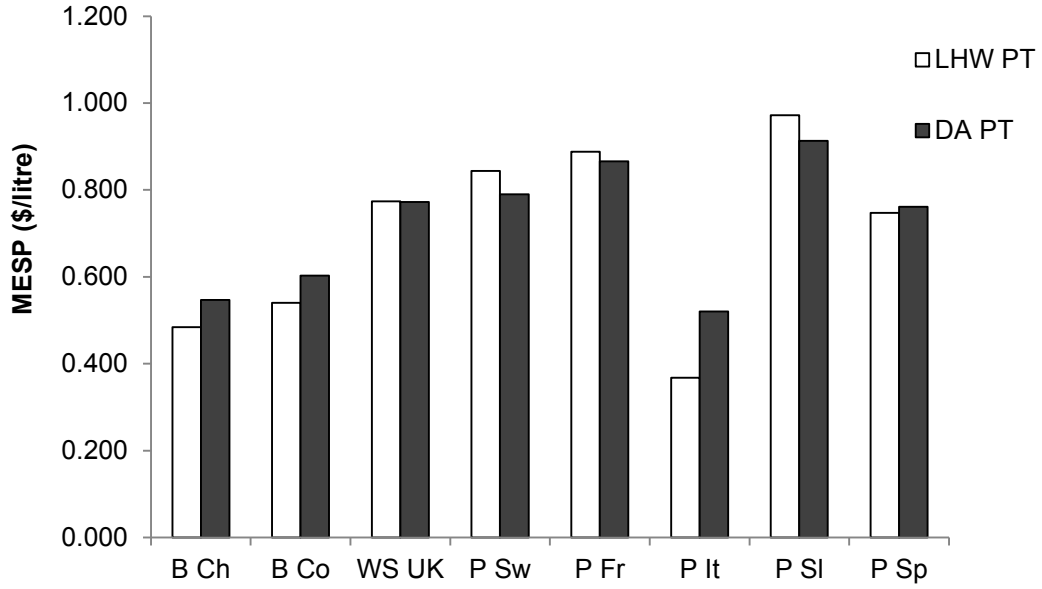


Figure 4.5 MESPs for bioethanol production via LHW and DA pretreatments from bamboo in China and Colombia, wheat straw in the UK and poplar from Sweden, Italy, France, Slovakia and Spain. (LHW: Liquid hot water pretreatment; DA: Dilute acid pretreatment; B: Bamboo; WS: Wheat straw; P: Poplar; Ch: China; Co: Colombia; Sw: Sweden; F: France; It: Italy; Sl: Slovakia; Sp: Spain)

From the technology comparison (Section 4.3.4.1), it was revealed that for two of the three feedstocks, DA pretreatment was a preferred route for maximising ethanol production compared to LHW pretreatment. Interestingly, when costs are taken into account, there is an even split between DA and LHW pretreatments for being most economically viable. LHW pretreatment is the preferred processing route for bioethanol from bamboo in China and Colombia and poplar in Italy and Spain, whereas for DA pretreatment is favoured for wheat straw in the UK and poplar in Sweden, Slovakia and France. For the techno-economic evaluation of bioethanol from bamboo, it was demonstrated in Chapter 3 that under the lowest enzyme loading scenario, LHW pretreatment was more competitive than DA pretreatment. This was due to the higher cost of raw materials with DA pretreatment, which did not correspond to a significantly greater sugar release (although this trend changed at higher enzyme loadings). For wheat straw, although the MESP for DA pretreatment is

$0.002/litre less than with LHW pretreatment, it is recognised that this techno-economic model does not take into account potential error or variation in its cost estimates, and therefore the actual difference between these scenarios is likely to be insignificant. In the poplar study, bioethanol in Sweden, France and Slovakia are shown to be more economical using DA pretreatment, and the reverse is found in Spain and Italy. What is more surprising is that all five EU countries adopted the same technological assumptions in LHW and DA pretreatments, yet there was greater variation in the MESPs amongst these countries than between the countries of other feedstocks. This suggests that the contribution of technology within the techno-economic model may not play as significant a role as the cost parameters in determining the economic potential of biomass-to-bioethanol processes.

These results indicate that while ethanol is the main product, the pathway producing the most ethanol is not necessarily the most cost competitive. The major cost factors within the techno-economic model that vary between countries and are often determined by government policy include feedstock cost, electricity credit, landfill/ash disposal, labour costs and income tax. Of these, the most significant are feedstock cost and electricity credit. Based on the previous review of other literature studies showing the strong relationship between feedstock cost and MESP (Section 3.3.5), this is also plotted in Figure 4.6 for the current scenarios.

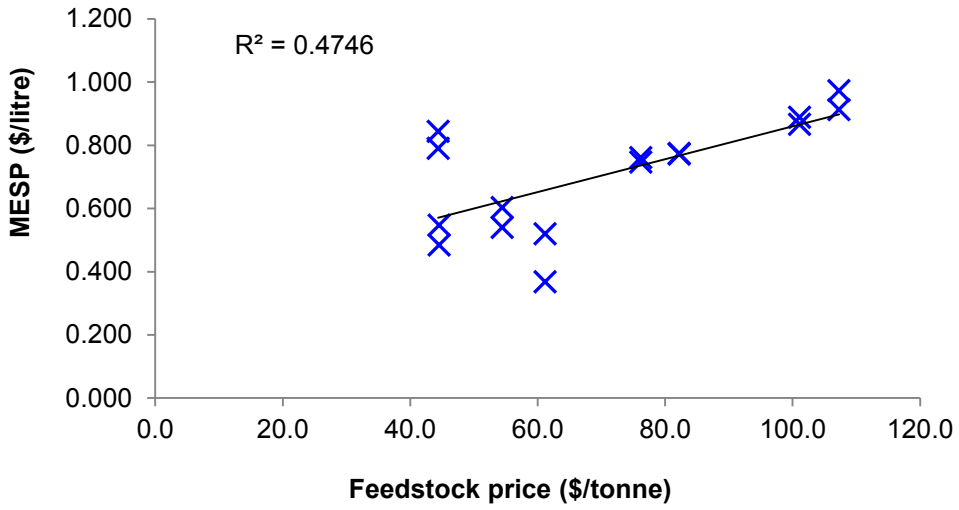


Figure 4.6 Relationship between feedstock price ($/tonne) and MESP ($/litre) in sixteen bioethanol production scenarios.

The linear best-fit line has an R^2 = 0.48, which suggests that the fit of the curve is less than 50% and therefore not highly correlated. However, there are several high and low outliers within this data, which heavily skew the correlation. The feedstock cost in Sweden is the lowest at $44.4/tonne, which, based on this positive linear relationship, should yield the

lowest MESP. Surprisingly however, Sweden has one of the highest MESPs for both pretreatment processes, and is the most costly amongst the EU countries, suggesting that other cost factors are also responsible for determining the production cost. The lower outlier data points in Figure 4.6 represent bioethanol from poplar in Italy. At a poplar cost of $61.1/tonne this is a relatively middle/low feedstock price among these scenarios, but the MESPs for both pretreatment processes are one of the lowest, and are significantly below expectation based on the feedstock price. By eliminating these two scenarios from the graph and re-plotting the best-fit curve, the relationship between feedstock cost and MESP is much more significant with a R^2 value of 0.97.

The electricity credit has a major influence on the bioethanol production cost particularly in the LHW pretreatment scenarios which have lower levels of bioethanol and higher levels of electricity production, and therefore is responsible for skewing the data in Figure 4.6. Support systems for renewable electricity generation vary between different countries, but include feed-in tariffs, quota obligations, feed-in premiums and tradable electricity certificates. Feed-in tariffs are generally the most common and effective approach, and operate by fixing the price of electricity per kWh sold back to the grid (Ragwitz et al., 2012).

Amongst these scenarios, Sweden is the only country to utilise a combination of electricity certificates and a quota obligation to reach a 17.9% share of renewables in the electricity sector by 2011 (Ericson, 2010). These certificates are worth a certain amount and are received by producers and then sold to generate income from their electricity production (Ericson, 2010). However, at a rate of only $0.04/kWh, Swedish generators earn little for their renewable electricity compared with other countries that generally receive between $0.10/kWh (Colombia) to $0.17/kWh (Spain), and especially compared with Italy, with an exceptionally high rate of $0.33/kWh. For wheat straw and bamboo scenarios, the difference in electricity generation between the LHW and DA pretreatment processes is small (2 MW and 10 MW for wheat straw and bamboo, respectively), whereas for poplar, the LHW pretreatment process generates 35 MW more electricity than with DA (as a result of compromised ethanol yields). Therefore, at the scale of 2,000 dry tonnes of feedstock processed per day modelled in these scenarios, the difference in electricity credit between Sweden and Italy is equivalent to approximately $86 million per year. This large sum highlights the significance of electricity credit on the cost structure of bioethanol production, and demonstrates its impact of acting as a buffer to significantly reduce the MESP in Italy, compared to only a slight reduction in Sweden, for the same amount of electricity generated. The desirable combination of this high electricity credit with a relatively low feedstock cost are the two primary determinants accounting for the most cost-effective bioethanol

production scenario found with poplar in Italy using a LHW pretreatment process. The other cost factors such as ash/landfill disposal, income taxes and labour rates have a relatively minor impact on the MESP.

4.3.4.3 Policy comparison

The influence of government fuel pricing policy was investigated amongst the sixteen feedstock, technology and country production scenarios to assess how competitive bioethanol could be at the pump. In the supply chain analysis, bioethanol in each scenario was compared against its respective national petrol price in 2011 (Figure 4.7). Certain countries offered some form of government support to incentivise bioethanol production; these policies were listed previously in Table 4.6.

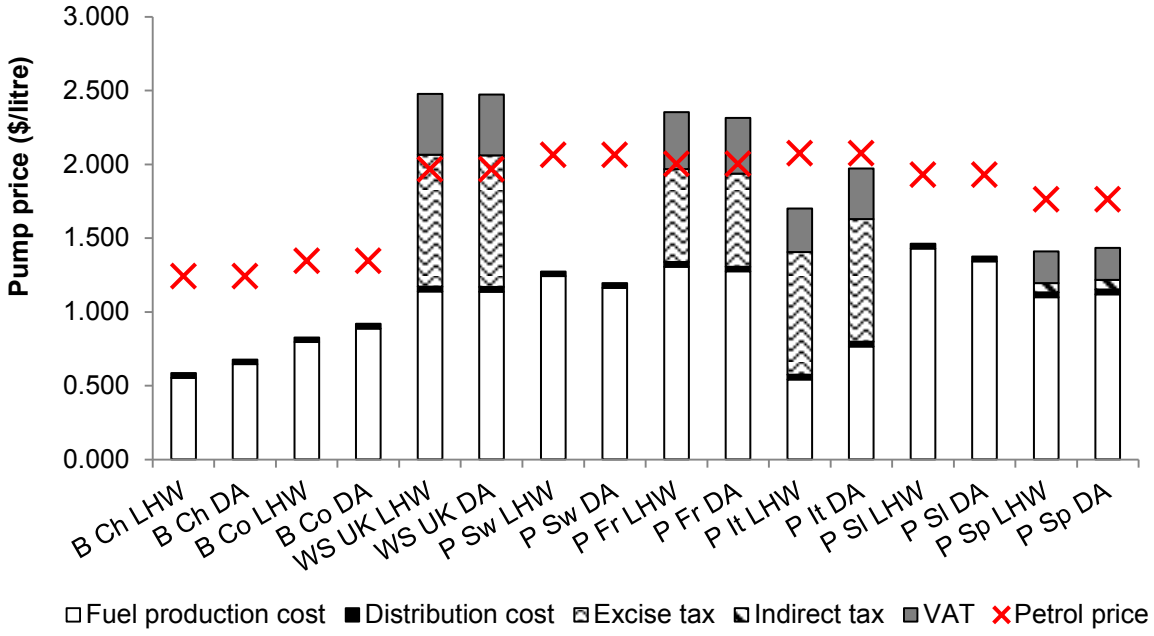


Figure 4.7 Bioethanol pump prices in sixteen bioethanol production scenarios. National petrol prices in 2011 are marked by the red cross (B: Bamboo; WS: Wheat straw; P: Poplar; LHW: Liquid hot water pretreatment; DA: Dilute acid pretreatment; Ch: China; Co: Colombia: Sw: Sweden; Fr: France; It: Italy; Sl: Slovakia; Sp: Spain).

With the exception of bioethanol from wheat straw in the UK (both LHW and DA pretreatments) and from poplar in France (LHW and DA pretreatments), all production scenarios are competitive with petrol at the pump. Bioethanol pump prices range from \$0.584 to \$2.472/litre amongst the different scenarios. The lowest pump prices are found from bamboo in China and Colombia (\$0.584 to \$0.919/litre). The highest prices are from

wheat straw in the UK ($2.476 and $2.472/litre) and poplar in France ($2.353 and $2.315/litre), which are both higher than their national petrol prices of $1.965 and $2.003/litre, respectively. The pump prices of bioethanol from poplar in Italy are also high at $1.700 and $1.971/litre for LHW and DA pretreatments, respectively, which are slightly lower than the national petrol price of $2.074/litre. Bioethanol from bamboo has been discussed in detail earlier and this section will therefore focus mainly on bioethanol from poplar and wheat straw.

On the one hand, retail petrol prices in the UK are relatively high amongst the European countries, making it easier for bioethanol to compete with fossil fuels. However, fuel duties are also the highest and therefore without any subsidy or exemption, bioethanol has a low chance of being a cost-effective alternative to petrol (FTA, 2011). Fiscal incentives in the UK previously provided biofuel production with a selective advantage over petrol through a duty derogation of £0.20/litre (approximately $0.308/litre). Yet even this scheme, which was described as “pale in comparison” to some of the other EU countries that offer 100% tax exemptions, was removed in April 2010 in favour of an equivalent fuel duty to petrol (Charles and Wooders, 2012). Although fuel duty rates have decreased since then from £0.5819 to £0.5795/litre (approximately $0.897 to $0.893/litre), VAT rates have been progressively raised from 15% to 17.5% and to 20% in 2011, creating additional barriers for bioethanol producers. The production costs of bioethanol from wheat straw in the UK are on average the third highest amongst the modelled scenarios. However, once these additional cost factors are accounted for in the pump price calculation, the overall cost is significantly increased, exceeding the other scenarios, demonstrating the restrictive (as well as positive) role that government policy can have on the competitiveness of alternative fuels.

Similar conclusions can be drawn from the poplar country scenarios. Sweden in particular, had one of the higher costs for bioethanol production, however due to its national policies which exempt it fully from all taxes, the pump price of bioethanol is the lowest amongst the poplar scenarios and competitive with petrol at prices of $1.273 and $1.194/litre for LHW and DA processes, respectively. The same is found for bioethanol from poplar in Slovakia, which is also highly competitive with petrol at the pump. Even though VAT and one form of indirect tax are imposed on bioethanol production in Spain, the combination of a relatively inexpensive poplar price with the lowest VAT rate (18%) means that bioethanol would be competitive with petrol, and of the tax-paying countries, it is the most competitive country. In contrast, Italy had the lowest production costs amongst all European scenarios, however the lack of tax exemption means that its pump price is more than double its initial production cost, and consequently its bioethanol pump prices exceed those in Sweden, Slovakia and

Spain. Despite these high tax duties, bioethanol in Italy under both LHW and DA pretreatment processes is still competitive with petrol due to its sufficiently low feedstock price and generous electricity credit rates. The situation in France on the other hand is just as unattractive as in the UK. Although bioethanol producers benefit from a partial tax exemption of $0.187 per litre, which is also only applied to a certain quota of bioethanol each year, (in 2010 this was 867,000 tonnes) it is still subject to the full VAT rate. France consequently has the highest pump prices for SRC poplar of $2.353 and $2.315/litre for LHW and DA processes, respectively, which are both greater than the price for petrol in 2011 ($2.003/litre). Due to these vastly differing support schemes, a varied range of results exist for the theoretical pump prices for bioethanol amongst the European countries.

The current policy in Sweden and Slovakia offers bioethanol full exemption from all taxes and without such schemes, bioethanol would not be competitive with petrol in either country. Although a number of producers in France receive a partial tax relief, the amount of bioethanol benefiting from this is progressively decreased each year to minimise the country's fiscal losses for the government and to reduce the burden on taxpayers (Jung et al., 2010). In 2008, the estimated cost of borne by the government was around $225 million, ranked second in the EU after Sweden at approximately $301 million (Jung et al., 2010). In the short run, government support is likely to be required in order for cellulosic bioethanol production from poplar and other feedstocks to be competitive at the pump. However, as the industry matures, it is expected that tax relief will be continually scaled back, emphasising the urgency to seek out alternative approaches to sustain and promote the long-term growth of the advanced bioethanol market.

Comparatively speaking, bioethanol from bamboo in either China or Colombia remains a highly competitive option to petrol due to low costs, and significant government support; it is therefore a leading option for bioethanol production. Though the abundance of wheat straw in the UK could justify a wheat straw-to-bioethanol market, the high costs and policy situation have created unlikely conditions for such a market to fully develop. It is therefore difficult to imagine whether other resources may be more suitable, or whether without fundamental policy changes, the possibilities for establishment of a successful bioethanol industry are limited. For poplar in the EU, there are several possibilities whereby bioethanol could be a competitor to fossil-based fuels; however this is mostly only feasible in countries with major support schemes, unless feedstock prices and electricity credits are cost-effective to generate a low enough production cost where bioethanol could still be competitive on its own.

4.3.4.4 Prospects for advanced poplar feedstock

A prospective scenario for poplar was modelled based on the work of the CEC FP7 EnergyPoplar project and literature data (Coleman et al., 2008). A SRC poplar feedstock with projected lignin modifications induced by genetic engineering and/or advanced breeding science was modelled. This lignin-modified poplar is down-regulated for coumaroyl shikimate/quinate 3'-hydroxylase – an enzyme involved in lignin biosynthesis. As a result, the genetically modified variety not only has 56% less lignin compared to its non-modified control, but is also assumed to achieve glucose yields of 80% without any form of pretreatment (Coleman et al., 2008). Therefore, in this prospective scenario, the pretreatment area was removed from the process design, and all polysaccharides were assumed to have the same enzymatic saccharification yields as glucose and with a reduced enzyme loading. Under these prospective scenarios, the MESP is reduced in four of the five countries by approximately 41% from the reference scenario (Table 4.8). Italy is an exception to this trend, where the high electricity tariff, which favours electricity generation and hence higher lignin contents, results in a reduced MESP of 31% from the DA pretreatment process and only 6% from the LHW pretreatment process.

Table 4.8 MESP comparison of reference case and prospective scenarios for bioethanol production from five European countries

	MESP ($/litre)		
	Liquid hot water pretreatment	**Dilute acid pretreatment**	**Prospective scenario**
Sweden	0.844	0.790	0.425
France	0.888	0.866	0.540
Italy	0.368	0.520	0.346
Slovakia	0.972	0.913	0.559
Spain	0.747	0.761	0.463

In the countries where bioethanol production was exempt from taxes in the reference case scenarios, (e.g. Sweden and Slovakia), bioethanol pump prices were competitive with petrol and therefore the prospective scenario only enhances its competitiveness further. France and Italy are two countries where bioethanol is either not competitive or only has a minor economic advantage over petrol, and these two scenarios are plotted in Figure 4.8 against the reference case LHW and DA pretreatment scenarios as a comparison. In France, due to substantial taxes imposed on bioethanol, it is neither competitive via LHW nor DA

pretreatment routes. The improvements to the SRC poplar feedstock would change this and allow it to be competitive with petrol ($2.003/litre) at a pump price of $1.742/litre. In Italy, although bioethanol is competitive at the pump, the prospective scenario would still enhance this significantly from the DA pretreatment process ($1.971/litre at pump) and slightly from the LHW process ($1.700/litre) at a pump price of $1.661 per litre.

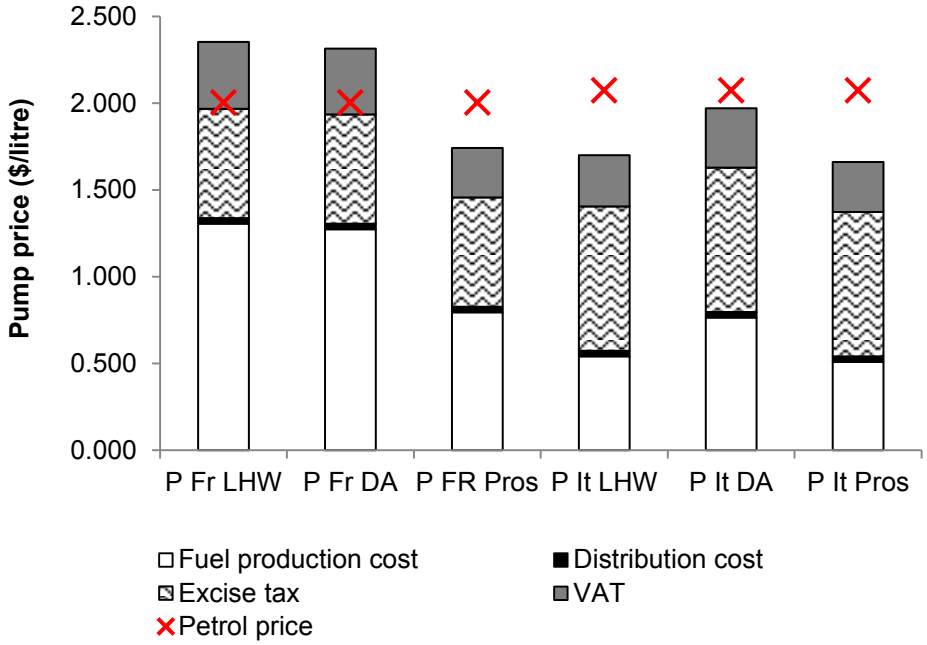


Figure 4.8 Bioethanol pump prices from poplar in France and Italy under reference case LHW and DA pretreatment scenarios compared with prospective scenarios with lignin-modified poplar. National petrol prices are marked by the red cross (P: Poplar; Fr: France; LHW: Liquid hot water pretreatment; DA: Dilute acid pretreatment; It: Italy; Pros: Prospective case).

These cost savings associated with the prospective poplar scenario arise from two main areas. Firstly, it has been demonstrated experimentally that as a result of the reduced lignin content in this poplar feedstock, the cell wall is more accessible to enzymes without any form of pretreatment (Mansfield et al., 2012). Therefore, removal of the pretreatment stage in the process design leads to considerable cost savings in capital, utilities and raw material expenditure from this area. Secondly, glucose yields of 80% of glucan were achieved during enzymatic saccharification with reduced enzyme loadings of 10 FPU/g glucan, which significantly cuts enzyme costs, particularly in comparison to the LHW pretreatment scenario (Mansfield et al., 2012).

Despite these advantages, criticism can be directed towards how this diminished lignin content may affect the growth phenotype of poplar trees. Various studies have observed and documented an inverse relationship between lignin content and growth, where trees with reduced lignin contents are significantly smaller than their wildtype counterparts. It is understood that the role of lignin within the cell wall structure is fundamental in providing support, water transport and defence against enzymatic and microbial attack, therefore it is important to establish that its decreased content is not in any way associated with a negative impact on plant survival or health (Coleman et al., 2008, Hisano et al., 2009). To emphasise this concern, it was demonstrated on 8-month old glasshouse grown transgenic poplar trees, that the C3'H-14 line with the lowest lignin content had a diminished tree volume of 73% compared with the wildtype (Mansfield et al., 2012). This demonstrates that although these transgenic trees might have exhibited improved accessibility – a desirable trait for bioethanol production – the less desirable effect on decreasing tree volume needs to be addressed. Probably the most direct economic consequence of this is that trees will either need to be planted at higher densities within the same plot of land, or greater land areas will be required to yield an equal amount of biomass on a volume basis. Feedstock prices may be affected as a consequence, which, if are significantly increased, may substantially undermine the cost savings achieved from capital and enzyme cost reductions.

In regard to these limitations, current biotechnology research is focused on overcoming the problems associated with low lignin content and establishing alternative routes to breed plants with enhanced phenotypic traits for bioethanol production. It is believed that the issue of diminished biomass yields with low lignin trees can be attributed to the effect that low lignin content has on xylem vessels. Two options explaining this could be a collapse of vessels and/or increased embolism from air bubbles entering water-conducting cells, or possibly the formation of tyloses blocking vessels and reducing the efficiency of water transport (Kitin et al., 2010). Scientific research aims to target this problem by using promoters to selectively drive transgene expression in fibres, so that the reduced lignin content is concentrated in fibres only and not in vessels (Simmons et al., 2008). Additionally, as an alternative to down-regulating lignin biosynthesis, the composition of existing lignin can be modified (for example by modifying ratios of *p*-hydroxyphenyl/guaicyl/syringyl units or cinnamaldehyde/cinnamyl alcohol in lignin), which has also demonstrated potential to improve biomass digestion during pretreatment and saccharification (Chen and Dixon, 2007). Lastly, the vast genetic variability in chemical composition and saccharification yields of poplars even of the same species, offers the potential for improvement via advanced breeding programmes. The screening and sexual crossing of plant genotypes with

favourable traits could potentially produce an elite F1 generation for genetic modification and offers a promising route for the future of the biofuel industry.

4.4 Conclusions

The potential for establishing a bioethanol industry from bamboo in China and Colombia was compared to investigate the effect that local prices and policies can have on the competitiveness of bioethanol. Techno-economic analyses were used to generate the MESP for bamboo-derived bioethanol using three pretreatment processes (liquid hot water, soaking in aqueous ammonia and dilute acid pretreatments). These results showed firstly, that the average cost of bioethanol production in Colombia was higher than the cost of production in China under the same processing conditions; and secondly that the pretreatment and enzyme loading conditions that yielded the most economically viable bioethanol production scenarios in China were also the most economical in Colombia.

It was revealed that liquid hot water and dilute acid pretreatments at low enzyme loadings were significantly more competitive compared with bioethanol produced with soaking in aqueous ammonia pretreatment. The lowest MESP was achieved for bioethanol from bamboo in China using LHW pretreatment with a 10 FPU/g glucan enzyme loading ($0.484/litre). However, at enzyme loadings higher than this, DA pretreatment became the more economically favourable option. Although there were minor variations in local costs between the two countries, the main discrepancy resulting in the MESP differences was attributed to the bamboo feedstock price, which was about ten dollars (~20%) less expensive in China. Relatively inexpensive prices of bamboo corresponding to bamboo waste materials were assumed in both countries, as an approach to minimise potential competition with existing bamboo industries and to reduce overall feedstock costs, thereby adding value to these potential “waste” residues.

Reference case and prospective supply chain analyses were used to evaluate the competitiveness of bioethanol from bamboo with petrol at the pump. Reference cases reflected the present (based on 2011) policy status and established whether or not government support schemes were currently helping or hindering the competitiveness of bioethanol with petrol. It was found that policies in China and Colombia both provided bioethanol full exemptions from the value-added tax as well as national fuel taxes, and China also offered an additional subsidy per litre bioethanol to further incentivise its production. As a result, it was demonstrated that bioethanol in China and Colombia was competitive under

the same processing conditions: LHW pretreatment at enzyme loadings of 10-60 FPU/g glucan and DA pretreatment at enzyme loadings of 10-100 FPU/g glucan, but not under any conditions using SAA pretreatment. Under prospective scenarios where all forms of government support were removed, results revealed substantial differences between the countries, whereby bioethanol from Colombia was significantly less competitive from the reference case and in comparison to China. Here, the number of competitive scenarios was reduced from seven in the reference case to just one in the prospective scenario (LHW pretreatment with the lowest enzyme loading). This finding was almost entirely attributed to the high fuel tax in Colombia, which, if paid in full, would constitute double the tax percentage in China. Bioethanol in China remained to be competitive with petrol across a range of scenarios despite removal of government support.

Bioethanol from bamboo in China and Colombia under the two most competitive processing technologies – LHW pretreatment with 10 FPU/g glucan and DA pretreatment with 10 FPU/ g glucan – were compared with wheat straw in the UK and poplar in the EU. The roles of technology, economics and policy were explored based on processing conditions and conversion efficiencies reported in the literature, and the main determinants influencing commercialisation of these processes have been discussed. Amongst the six technology scenarios, (LHW and DA pretreatments on bamboo, wheat straw and poplar) DA pretreatment was favoured for two of the three feedstocks based on higher ethanol yields. As a result, electricity generation was consistently higher in LHW pretreatments for each feedstock. These results support the theory that the variation in cell wall composition and structure of different feedstocks are accountable for the specific responses exhibited during pretreatments. From a technology perspective, the goal of maximising ethanol production can be achieved through enhancing saccharification sugar yields via low-energy and –cost pretreatment technologies.

Despite pretreatment with DA being preferred from a technology standpoint, results from the economic analysis demonstrated that once location-dependent cost factors were taken into consideration, there was an equal divide in preference between LHW and DA pretreatment amongst the sixteen scenarios evaluated. LHW pretreatment was favoured for bioethanol production from bamboo in China and Colombia and poplar in Italy and Spain, whereas DA pretreatment was preferred for bioethanol production for wheat straw in the UK and poplar in Sweden, Slovakia and France. The lowest MESP and thus most economically favourable scenario was for poplar in Italy with LHW pretreatment, ($0.368/litre) and the highest MESP was for poplar in Slovakia via both LHW and DA pretreatments ($0.888/litre and $0.866/litre, respectively). It appeared that the two major cost factors that contributed towards these

scenarios were prices for feedstock and electricity credit. There was a strong correlation between feedstock cost and MESP amongst these scenarios, which supported previous findings. However, the high price that generators in Italy receive for their renewable electricity combined with a low feedstock cost were the main determinants that accounted for this scenario being the most economically competitive of those assessed.

Theoretical bioethanol pump prices were modelled to highlight the influence of government fuel pricing policy on the competitiveness with petrol. Many countries use forms of policy support to exempt alternative fuels as a mechanism to reduce costs and improve competitiveness. Bioethanol in all scenarios except wheat straw in the UK and poplar in France was competitive with petrol. Bioethanol from bamboo in China and Colombia had the lowest pump prices due to inexpensive production costs and significant policy support in the form of full tax exemption, and a 16 cent per litre subsidy in China. The UK and France with few or no fiscal incentives to support bioethanol, would not be ideal locations for establishing such an industry. Provided that a cheap feedstock price can be obtained for poplar in Italy, the high credit for renewable electricity generation provides a buffer for the expensive taxes levied on fuel, allowing bioethanol in Italy to be competitive. Countries with high feedstock costs and low electricity credits, such as Sweden and Slovakia, were seen to be uneconomic simply from the perspective of production costs. However their full exemption from taxes shifted this outlook and resulted in the lowest pump prices among the poplar scenarios. These results demonstrate that optimising conversion processes and identifying low material costs are important, however support policy measures have the capacity to eventually make or break the competitiveness of bioethanol and should be a top priority for producers.

A prospective case was modelled for the five poplar scenarios to reveal how advances in genetic engineering may enhance the commercial potential for bioethanol from lignocellulose. Data was modelled from a recent study demonstrating that genetically engineered poplar with a 56% reduction in lignin content could achieve 80% glucose yields during saccharification without any form of pretreatment and a low enzyme loading. For four of the five European countries, the bioethanol production cost was reduced by 41% from the reference scenarios. Italy, which has a higher electricity credit and therefore benefits from feedstocks with higher lignin contents, exhibited reductions in production costs from the reference cases. These major cost savings were attributed to decreased capital costs and operating costs from removal of the pretreatment area and the decreased enzyme loading. As a result, bioethanol in all European countries would be competitive with petrol. Although these modified poplars showed improved accessibility to enzymes, it was also found that their tree volume was diminished significantly compared with the wildtype, which could

potentially negate all cost savings in this scenario if more biomass is required to compensate for this loss. Current biotechnology research is therefore focused on overcoming these issues by establishing alternative pathways to breed plants with enhanced phenotypic traits for bioethanol production.

Using locally-sourced bamboo in China or Colombia is shown to be a highly feasible option for bioethanol production, at least compared with the alternative options of wheat straw in the UK or poplar in Europe. On the one hand from a technology standpoint, sugar (and therefore ethanol) yields were the lowest on bamboo and could be improved through optimisation of the ideal pretreatment process; however, the economic analysis revealed that despite these drawbacks, its cost of production was also one of the lowest compared to the other scenarios. The significant policy support measures adopted in both locations cushioned fuel pump prices through tax exemptions and subsidies, which further reduced costs. Compared to the UK and European countries, which either had low production costs and high tax rates, or high production costs and low tax rates, no other feedstock, technology or country scenario was found to be as favourable for bioethanol production.

Chapter 5 General discussion

The aim of this thesis was to investigate the potential for bamboo as a feedstock for bioethanol production from the perspectives of technology, economics and government policy. Three main questions were posed in the outset of the research and were explored in detail throughout the study:

1) What are the optimal processing technologies and conditions for maximising sugar release in bamboo?

2) From an economic perspective, which of these pretreatment processes is the most viable for producing bioethanol from bamboo?

3) How does the role of government policy impact the competitiveness of bamboo-derived bioethanol in different country case studies and amongst other lignocellulosic feedstocks?

To address these topics, new experimental work examined the efficacy of different pretreatment technologies for maximising sugar yields from bamboo, simulations of the biochemical conversion process at a scaled-up level were conducted and economic analyses performed for different case study scenarios and feedstocks. The following discussion summarises and integrates these findings and suggests areas for future research.

5.1 Technological feasibility of bamboo-to-bioethanol conversion

A comparison of three pretreatments (liquid hot water, dilute acid and soaking in aqueous ammonia) was made based on the criterion of maximising sugar release from bamboo. Pretreatment with liquid hot water and dilute acid was shown to generate a higher level of sugar release compared with bamboo pretreated with soaking in aqueous ammonia. Under LHW and DA pretreatments, approximately 84% of xylan was solubilised during the pretreatment stage in contrast to 31% of lignin removed during SAA pretreatment. It was found that the effect of high temperature and short times used (190°C for 10 minutes and 160°C for 15 minutes with 0.2% H_2SO_4 in LHW and DA, respectively) was much more effective than lower temperatures and longer times (100°C for 24 hours with 15% NH_4OH in SAA pretreatment) in improving cell wall accessibility and total sugar release from bamboo.

The impact of removing certain cell wall components during pretreatment had variable effects on saccharification sugar yields. During saccharification, 24-32% of the theoretical

maximum sugars were released after the different pretreatments. However, considering that there was a significantly higher residual sugar content in the SAA pretreated bamboo material, 32% of theoretical maximum was not a particularly high value. In fact, upon a more detailed examination of these values, it was realised that despite releasing a greater proportion of sugars in SAA pretreatment, glucan in the pretreated material was still less accessible than that from other pretreatments (under the standardised loading, 40% of glucan in pretreated material was saccharified compared with 48% and 58% in LHW and DA pretreatments, respectively). These observations were not consistent with other findings on feedstocks reported in the literature such as barley hull, which showed that a lignin removal of 50-66% was sufficient to release around 83% of glucan (Kim et al., 2008). This work demonstrated instead, that by solubilising a significant portion of sugars (mainly xylan but others too) during pretreatment (by LHW and DA pretreatments), glucan accessibility was significantly improved and that this was a more effective approach for maximising total sugar release from bamboo than lignin removal by SAA pretreatment.

Several studies have discussed the impact of hemicellulose versus lignin removal on plant cell wall accessibility, and while these results appeared to support hemicellulose removal as being a more effective approach, it should also be recognised that it is all but impossible to selectively remove one cell wall component without causing effects on other cell wall constituents. The cell wall contains a complex matrix of cellulose, hemicellulose and lignin, which are physically and chemically linked with one another such that removal or alteration of one component will either directly or indirectly influence others and therefore the entire structure. Thus, even during DA pretreatment where 84% of xylan was removed (alone, this would have severely impacted other cell wall functions), in conjunction with a 15% removal of glucan and 22% of lignin, other chemical, structural and morphological changes would have also occurred within the cell wall and amongst its components. These potential impacts were not studied in detail and may have been crucial factors, in addition to the very substantial removal of xylan, affecting sugar release potential from bamboo.

Low and high loadings of the commercial enzyme mixture Cellic CTec2 were applied to the pretreated bamboo material to generate a range of sugar release data. From a technology perspective, bamboo pretreated with DA achieved the highest level of sugar release at each enzyme loading. At the highest loading (140 FPU/g glucan), DA pretreatment had a combined pretreatment and saccharification yield of 94% of the theoretical maximum, compared to just 76% from SAA and LHW pretreated material. At lower enzyme loadings too, such as 30 FPU/g glucan, 79% of the theoretical maximum was released, and above 60 FPU/g glucan all loadings released more than 90% of the theoretical maximum. From the

range of sugar release at these enzyme loadings, it was evident that different pretreatment processes resulted in varying levels of accessibility. The variability in sugar release between high and low loadings was much larger in SAA pretreated material (35% difference) compared with LHW and DA pretreated material (17% and 24% difference, respectively). With SAA pretreated material, it seemed that progressively higher loadings were required in order to effectively penetrate the cell wall and hydrolyse sugars, as opposed to being easily released at lower enzyme levels, as found with LHW pretreated bamboo. At the highest enzyme loading however, sugar release from both pretreatments levelled off at 76% of the theoretical maximum sugars, despite the alternative routes used to achieve this. This level was exceeded by almost 20% with DA pretreatment. It could be suggested that lignin subjected to high temperature acidic conditions in DA pretreatment, is chemically modified and recondensed as an altered polymer in such a way that allows glucan to be more amenable to enzymatic saccharification (Torget et al., 1991).

These experimental results provide insight into how bamboo biomass can be broken down into sugars and converted into ethanol via different pretreatment processes and enzymatic saccharification. From a technology perspective alone, the highest enzyme loadings would, as expected, release the most sugar. While sugar yields can be viewed as the most important indicator of a successful pretreatment, other factors such as potential formation of sugar degradation products also add considerable weight to determining the “optimal” pretreatment conditions, and should be explored when taking this research further. There are currently only a limited number of studies reporting the effect of pretreatment on bamboo in terms of its saccharification potential. This work therefore provides a new contribution towards this area of research by showing that pretreatment with LHW and DA was more successful than pretreatment with SAA. However, upon examination of these studies, it is evident that there is a wide range of variation amongst the pretreatment data, demonstrating that this area is still being explored from a very broad perspective. Wider investigation into other types of pretreatments may still be necessary; and for selected promising technologies, further optimisation trials will be required to optimise sugar yields to those found from other feedstocks considered for commercialisation. A better grasp of the bamboo cell wall and its response to pretreatments would enhance this understanding and guide scientific research in the right direction. Due to the large number of bamboo species existing globally, it would also be helpful to perform some screening of the different varieties (e.g. temperate, tropical, sympodial, monopodial) to identify whether certain species have higher saccharification potential than others.

5.2 Economic performance of bamboo-based bioethanol

Simulation software AspenPlus™ was used to model the conversion of bamboo into bioethanol via LHW, SAA and DA pretreatments and enzyme loadings, at a scale of 2,000 dry tonnes per day. Mass and energy balances were used in an economic analysis to generate a theoretical bioethanol production price (MESP) under alternative processing scenarios to compare its commercialisation potential and to determine the major cost contributors. It was found that for all pretreatment pathways, lower enzyme loadings were more economically favourable, as the cost of using more enzyme outweighed the benefit of producing more bioethanol under these conditions. For LHW and DA pretreatments, 10 FPU/g glucan led to the lowest bioethanol production prices ($0.484/litre and $0.547/litre, respectively). For SAA pretreatment, 30 FPU/g glucan was most economical as there was simply not enough ethanol produced at the lowest enzyme loading due to the ineffective pretreatment and consequently, low sugar yields. However, at this loading, the production price of $1.014/litre was still significantly higher than the best-case options for the other pretreatments and therefore not competitive in comparison.

A consistent finding among the pretreatment scenarios was that the cost of feedstock, which was for bamboo waste residues, emerged as the major contributor towards the overall production cost (29-51% of the total). Although prices used in this study were fairly inexpensive, this finding was consistent with the high contribution feedstock costs make to the overall ethanol production cost, that has been reported by other techno-economic studies, even when different processing technologies are modelled (Gnansounou and Dauriat, 2010, Kumar and Murthy, 2011). The theory that more developed processing technologies result in higher feedstock contributions was reflected in the results from this work, particularly when comparing LHW with SAA pretreatment. Ideally, in order to understand the feedstock cost better, a breakdown of the feedstock supply chain could be conducted to identify the main contributing stages. In general, the bamboo supply chain is known to be comprised of growing, harvesting, pre-processing, storage and transport stages. Lowering the cost of transport is said to be a major obstacle for the feedstock supply chain, and is dependent on factors such as distance and mode of transportation, biomass bulk density, moisture content, loading costs and load capacity of vessels (Sampson et al., 2012). In contrast to other bioenergy crops, bamboo is still predominantly harvested manually instead of mechanically. Although this is time-consuming and laborious, it could still be economically feasible in poor countries where labour rates are low (e.g. China and Colombia); and it is also viewed to be beneficial from the perspectives of energy

consumption and GHG emissions, as well as having socio-economic benefits such as providing skills for workers managing these plantations.

Although the system boundaries in this model were limited to feedstock cost at the plant gate, if a life-cycle analysis assessing the environmental impact of bamboo-derived bioethanol were to be conducted, these could be expanded to include the establishment or management of large-scale bamboo plantations. The establishment of bamboo plantations has been fairly well documented for different species and locations, and has included factors such as water consumption, herbicide/pesticide use, nutrient inputs and biomass yields – all of which affect the final feedstock cost. Identifying the most suitable bamboo species is important not just for traits mentioned in previous chapters (high cellulose content and saccharification potential), but also for considering growth properties such as their ability to thrive in different climates or soil conditions with high growth rates and yields. While it is claimed that bamboos can be grown on poor quality soils, this will undoubtedly have a negative impact on their productivity. Its economic value will therefore be affected whether this is due to reduced biomass yields, smaller culms, or the burden of having to apply additional nutrient inputs to achieve acceptable yields. Inter-cropping of bamboo stands with crops such as maize and cassava has also been shown to increase productivity (JiangHua and QingPing, 2012). This system could be economically beneficial for farmers based on the ability to generate increased returns from growing alternative crops, getting better use of existing equipment for more than one crop, improving productivity and soil quality, and providing a year-round source of revenue due to the different optimal harvest times.

The other major cost contributors within each pretreatment pathway included enzyme (10-28% of the production price) and the credits from combustion of bamboo residues for electricity generation (-21 to -47% of the total price), which offset the gross price significantly. On the one hand, the total cost of pretreatment was not one of the leading contributors towards the production price (it accounted for approximately 16-21% which was lower than the costs for feedstock handling, saccharification and fermentation, wastewater treatment and combustion areas). However, its indirect impact on capital, operating and utility costs of downstream processes was recognised to be significant. One example of this was the use of sulphuric acid and ammonia, present in DA and SAA pretreatment processes. If the same evaporator system used in the LHW pretreatment process was used, the level of ammonium acetate and sulphate ions present in the hydrolysate would have been too concentrated, possibly fouling pieces of equipment. Therefore the switch to the lignin separation system in the product recovery area, the addition of a salt removal system in wastewater treatment,

and the requirement for caustic, were some of the major changes needed that influenced the total cost in these processes.

The relationship between feedstock cost and ethanol production price from this work was compared with other published techno-economic studies based on the same pretreatment processes. However, comparisons between studies are challenging due to the varying assumptions made within the models including sugar yields and conditions from pretreatment and saccharification as well as processing options (e.g. simultaneous or separate saccharification and fermentation or consolidated bioprocessing, which are often modelled as short-, medium- and long-term alternatives). These differences were apparent in findings presented in Section 3.3.5, which showed that although DA pretreatment results were relatively in-line with the literature (Eggeman and Elander, 2005, Hamelinck et al., 2005, Laser et al., 2009, Humbird et al., 2011), there were major contradictions in the trends for LHW and ammonia-based pretreatments. Part of this was related to the fact that models for ammonia pretreatments (especially SAA pretreatment) were scarce, and these results were consequently compared with AFEX pretreatment as the closest alternative, which is a more developed process. Furthermore, many of these studies modelled the conversion into bioethanol using CBP and SSCF saccharification/fermentation pathways, which reduced overall costs significantly below results from this work. Similar discrepancies were also found amongst the LHW pretreatment results. It was therefore more accurate to draw conclusions by comparing LHW, SAA and DA pretreatment and enzyme scenarios within this study rather than between studies.

Another major weakness of this model was related to the assumptions made with enzyme cost and its resultant impact on the economics of bioethanol production. At both high and low loadings, the enzyme cost was recognised to be a leading contributor to the production price, and deriving a reliable value is recognised as a limitation in this type of techno-economic model (Kazi et al., 2010b, Klein-Marcuschamer et al., 2012). As a way to resolve this issue, authors have based enzyme prices either on on-site enzyme production facilities, or on industry documents reporting enzyme contribution in dollars per litre (Humbird et al., 2011, Klein-Marcuschamer et al., 2012). While the former option is possibly more robust, it would involve building an enzyme production area, which has been demonstrated to be less economical due to additional capital and operating costs (Kazi et al., 2010a, Humbird et al., 2011, Klein-Marcuschamer et al., 2012). The latter option however, is less reliable as it merely sets a generic enzyme contribution, ignoring the fact that enzyme contribution varies significantly with different feedstocks, pretreatment technologies and thus loadings applied. In this research, although it was assumed that enzymes were purchased and not produced

on-site, costs were derived based on enzymes produced on-site from another study (Kazi et al., 2010b). It is believed that this was likely to be an overestimation of commercial enzyme prices available in reality, and the sensitivity analysis confirmed that price variations in enzyme cost, especially at higher loadings of 30 FPU/g glucan, would lead to substantial shifts in the price results. For the future, using more modern enzymes and/or utilising enzymes manufactured locally in China, may offer potential areas for significant cost reductions within the biochemical ethanol production pathway.

These findings have demonstrated that producers need to seek an appropriate balance between technology and economics. The main aim of scientific research has been focused on maximising sugar and thus ethanol production from bioenergy feedstocks through screening and optimising pretreatment technologies – three of which were explored in this study. From a technology standpoint, certain criteria carry more weight, such as sugar yields and degradation product formation. At the same time, other aspects of the conversion process including use of chemicals as well as water and energy consumption need to be accounted for when considering large-scale or commercialised production of bioethanol. These results revealed that minimal enzyme loadings were preferred, as long as this released a "sufficient" level of sugar release. Unfortunately a one-size-fits-all value for this level cannot be determined as it depends specifically on the assumptions made within each scenario. In the case of SAA pretreatment, this was found at 30 FPU/g glucan; in contrast, LHW and DA pretreatment processes showed that the lowest enzyme loadings (10 FPU/g glucan) were most preferred. While it could be suggested that the enzyme loadings could have been further reduced below this level, based on the scale of the experimental design this would have led to increased error in the results. A more reliable method could be to simply extrapolate these results based on other published data to establish whether a decreased loading would still maintain the balance between technology and costs to yield an economically favourable scenario.

5.3 Role of policy on the competitiveness of bamboo-based bioethanol

Two bamboo-to-bioethanol case study scenarios were modelled using China and Colombia as potential locations based on a reference year of 2011. The bioethanol pump price was used to compare the competitiveness of bioethanol from bamboo with fossil-based fuels under the current policy scenario. This evaluation method accounted for existing support mechanisms for bioethanol including tax exemptions, subsidies and mandates, which are implemented to effectively reduce bioethanol prices and enhance competitiveness with petrol. It was found that on average, the cost of bioethanol production in Colombia was

higher than the cost of production in China under the same processing conditions. Amongst all the scenarios tested (two countries, three pretreatments and five enzyme loadings), the most economically viable scenario for producing bioethanol was in China with LHW pretreatment and a low enzyme loading of 10 FPU/g glucan ($0.484/litre). Results indicated that there was no one leading pretreatment for all enzyme scenarios, but rather that LHW pretreatment was preferred at lower loadings, and DA pretreatment at higher loadings.

The main reason for a lower average bioethanol production price in China was due to the feedstock cost, which was cheaper than that in Colombia. However, without doing a cost breakdown analysis of the feedstock cost (as mentioned in Section 5.2), the precise reasons for differences were unknown. Within Asia and South America respectively, China and Colombia are considered to be the leading bamboo producers with both high volume and species diversity. For China, costs were modelled on *Phyllostachys edulis,* whereas in Colombia these were based on the popular species for construction, *Guadua angustifolia*. It has been shown that the area of bamboo forests in China grew at a rate of 11.2% between years 2004-2008 (2.25% annual growth rate), and a similar pattern of growth was also reported for natural forest area (State Forestry Administration People's Republic of China, 2010, Lugt et al., 2012). This not only demonstrates that the land area for bamboo resources is growing, but also that existing resources are being sustainably managed to respond to the increase in demand for bamboo. This was also supported by the rise in establishment of permanent plantations due to high domestic and international market growth for bamboo-based products. It was assumed that the addition of these plantations for bioethanol production would not come at the expense of natural forests. Currently, a major portion of bamboo resources are derived from industrial provinces (e.g. Zhejiang and Anhui), so it seems plausible that development of bioethanol industries in these regions could be successful (State Forestry Administration People's Republic of China, 2010, Lugt et al., 2012).

It is generally found that the production costs of renewable energy are higher than conventional sources mainly due to the enhanced level of risk associated with new technologies, which deters investors. In both China and Colombia, respective government parties have established mechanisms to financially support the production and dissemination of bioethanol within the country. Aside from the bioethanol production cost, distribution costs and taxes (VAT, indirect fuel taxes) also comprised the final ethanol pump price, demonstrating that obtaining a low cost of production is just one component of many to ensure bioethanol is competitive. In China and Colombia there are many supply- and demand-side policies to promote production of the industry (Alexander and Torres, 2011).

Although each country faces its own social, economic and political obstacles, based on the example of Brazil, which has been hailed as the pioneer of the ethanol industry, several drivers including strong governmental support, research and development, abundant raw materials and labour have been vital in its history of success (Alexander and Torres, 2011). From the standpoint of having abundant raw materials and labour, China and Colombia show good prospects for the future. With respect to government support, there has been a significant surge in biofuel policies over the last several decades (Section 4.3.1), which has already made an impact on fuel production and consumption at a national level. This further demonstrates the government's recognition that establishing a profitable industry can contribute to combating energy insecurity and rising GHG emissions. In China there is a large amount of research being conducted at various institutes and companies regarding topics such as feedstock improvement, biomass conversion and production processes. Collaborative initiatives have been established between China and other countries internationally, to improve scientific and industrial development (Yuan et al., 2011). Colombia has focused most of its efforts on producing ethanol from sugarcane and therefore it does not have an established cellulosic bioethanol industry. However, most recently it was announced that the National Renewable Energy Laboratory in the US would be working with Ecopetrol (the largest oil company in Colombia) to process sugarcane bagasse into ethanol (National Renewable Energy Laboratory (NREL), 2013)). With this forthcoming infrastructure developing, the introduction of bamboo as additional biomass resource could bring unforeseen economic benefits to Colombia.

Although the price for bioethanol has been fixed in both Colombia and China, even without this, bioethanol from bamboo could compete with petrol under the current cost and policy situation. The reference case scenarios showed that with a LHW pretreatment, enzyme loadings ranging from 10-60 FPU/g glucan would result in a pump price lower than the petrol price, with DA pretreatment this was even higher (10-100 FPU/g glucan), and with SAA pretreatment none of these scenarios would be a competitive option. At the same time, it was also recognised that enzyme loadings greater than 30 FPU/g glucan are not likely to be used at the commercial scale. These high loadings were simply modelled to see how high sugar yields could be maximised to, and at what level these would become uneconomical. With the expectation that biofuels should become self-sustaining without government support, prospective scenarios with all forms of government intervention removed, were investigated. These showed that bioethanol in China would still be highly competitive from a number of scenarios (LHW pretreatment at 10-30 FPU/g glucan and DA pretreatment at 10-60 FPU/g glucan). In Colombia, competitiveness was significantly reduced such that only

pretreatment with LHW and 10 FPU/g glucan enzyme would be economically viable, due to the high tax rates levied on fuels.

5.4 Comparison with other lignocellulosic feedstocks

Bioethanol from bamboo in China and Colombia was compared with that from wheat straw in the UK and short-rotation coppice poplar in five European countries (Sweden, Slovakia, France, Spain, Italy). LHW and DA pretreatments were selected as the main pretreatment routes for producing bioethanol; sugar yields and process conditions were derived from the literature. It was found that the conditions for producing bioethanol from bamboo in both locations were highly favourable compared to the other scenarios modelled. Despite the relatively low ethanol yields achieved as a result of incomplete process optimisation during lab-scale experiments, bioethanol from bamboo via LHW and DA pretreatment was one of the most competitive options due to the low feedstock costs and strong government support in China and Colombia. This was in contrast to the UK and Europe, where the undesirable combination of either a high production cost or high tax rate was not ideal for yielding a competitive bioethanol market.

Amongst the six technology scenarios tested (LHW/DA pretreatment on three feedstocks), the higher ethanol yields achieved with DA pretreatment resulted in it being the preferred route for two out of three feedstocks (excluding wheat straw) and emphasised the importance for maximising sugar release during the conversion process. As a result, LHW pretreatment consistently produced a higher amount of electricity, which in some cases led to a more economical scenario when credits for renewable electricity were substantially higher. It was found that the highest ethanol yields were attributed to both an increased sugar yield during saccharification, as well as a higher cellulose content of the feedstock. This was particularly true for poplar, with a cellulose content of 45.3% of DM compared with just 38.4% and 34.6% of DM in bamboo and wheat straw, respectively. However, the ethanol yields achieved with wheat straw, which were second and third highest were reflective of the high saccharification yields achieved in these scenarios. These results supported the concept that for farmers or producers, main priorities should focus on identifying feedstocks with enhanced cellulose contents, followed by screening for varieties with easily accessible sugars, and then optimising conversion processes to maximise release during saccharification.

Although DA pretreatment was favoured for two of the three feedstocks from a technology perspective, results indicated that when locations (and therefore cost considerations) were

integrated into the model, there was an equal split in preference between LHW and DA pretreatment. For bamboo-derived bioethanol in China and Colombia, LHW pretreatment was more economically viable, and this was also true for poplar-based bioethanol in Italy and Spain. The case study for Italy was exceptional in that the significantly high credit for renewable electricity generation offset a large proportion of the costs, which not only led to the lowest production price of $0.368/litre, but also showed how processes with greater electricity generation (e.g. LHW pretreatment) could be economically advantageous in certain cases. The influence of feedstock cost as the other critical determinant of ethanol production was evident by the highest production price found from poplar in Slovakia ($0.888/litre with LHW pretreatment), which had the most expensive feedstock cost amongst those modelled. The relationship between feedstock cost and ethanol production cost was demonstrated to be highly correlated. Despite the wide range of support systems employed for renewable electricity generation in the EU, including feed-in tariffs, quota obligations, feed-in premiums and tradable electricity certificates, prices for credits ranged on average between $0.10-0.17/kWh. It was only in certain "extreme" circumstances where electricity credits were substantially higher (Italy) or lower (Sweden), did the results skew the linear relationship.

Theoretical pump prices for bioethanol production based on different feedstocks, technologies and countries, revealed that all were competitive with petrol with the exception of wheat straw in the UK and poplar in France. The lowest pump prices were found for bioethanol from bamboo in both China and Colombia as a result of the full tax exemptions and the additional subsidy per litre ethanol in China. The two uncompetitive scenarios in the UK and France were due to the lack of government support which was either non-existent or minimal in the respective locations. The significant role that fuel taxes can have on the competitiveness of bioethanol was evident in the examples of Sweden and Slovakia, which had high production costs, yet were still economically viable at the pump due to full exemption from taxes. The opposite outcome was observed for Italy, which had a pump price close to that of petrol; however, the high electricity credits protected it from exceeding this level.

A prospective scenario for poplar was modelled based on a recent study that demonstrated a 56% reduction in lignin content after genetic modification, which also led to reduced enzyme loadings during saccharification and complete removal of the pretreatment area. With these improvements, the bioethanol production price was reduced by approximately 41% from four countries (with the exception of Italy which was less), due to savings achieved in capital, utilities and raw material expenditure. On the downside, these studies also found that plants

with significantly lower lignin contents had weakened growth phenotypes due to the diminished presence of lignin which is vital for plant support, water transport and defence against microbial and enzymatic attack (Coleman et al., 2008, Hisano et al., 2009). Current research into plant biotechnology is focused on these issues via mechanisms such as targeting lignin reduction in certain cell types or altering the composition of lignin, however the ideal solution is yet to be identified (Chen and Dixon, 2007, Simmons et al., 2008).

5.5 Summary of main findings and conclusions

- While the importance of optimising the bioethanol production process and identifying cheap feedstock costs were demonstrated, the role of policy support measures (through tax exemptions and subsidies) and their ability to make or break bioethanol competitiveness should be a top priority for producers.

- Feedstock cost was the single greatest cost contributor to the production cost in all three technology scenarios, followed by the credits from exported surplus electricity (offsetting other costs) and then the cost of enzymes. This was despite feedstock costs being based on prices for bamboo waste residues. These results also highlighted the uncertainties surrounding enzyme cost estimates due to the lack of accurate commercial enzyme prices.

- Reducing enzyme loadings resulted in lower bioethanol production costs for all three pretreatment processes. However, the balance between maximising ethanol yields whilst minimising enzyme loadings needed to be maintained.

- Bioethanol from bamboo in China and Colombia could be more competitive compared with bioethanol from wheat straw and poplar in the UK and Europe. This was primarily due to a highly favourable combination of cheap costs of bamboo residues, strong government support for bioethanol and low costs of inputs (e.g. materials and labour).

- Under the current policy conditions in China and Colombia, bioethanol at the pump would be competitive with petrol under a number of different liquid hot water and dilute acid enzyme loading scenarios. However, in a prospective case with diminished government support, competitiveness was significantly reduced in

Colombia due to the high tax rates. This reflected how support schemes could be a major help or hindrance towards the economic feasibility of bioethanol production.

- Bioethanol production costs were significantly reduced when prospects in plant genetic engineering were integrated into the economic model. However, the undesirable side effects of these modifications such as reduced tree volume, have yet to be fully regulated, which remains to be an aim for future plant biotechnology research.

- The lowest theoretical cost for bioethanol production was generated using liquid hot water pretreatment, followed by dilute acid and then soaking in aqueous ammonia pretreatments. Bioethanol produced via liquid hot water and dilute acid pretreatments could be competitive with petrol at low enzyme loadings, however soaking in aqueous ammonia was not competitive under any circumstance. This was due to the use of chemicals required in pretreatment, which directly influenced the cost of the pretreatment stage and this had an indirect impact on many downstream processes (e.g. equipment choices).

- From a technology standpoint, dilute acid pretreatment had the highest sugar release under the standardised enzyme loading, (79% of the theoretical maximum sugars compared to 69% and 48% in liquid hot water and soaking in aqueous ammonia pretreatments, respectively). This was maximised to 94% of the theoretical maximum at the highest enzyme loading with the commercial enzyme Cellic CTec2.

- Although hemicellulose removal during pretreatment seemed to be more effective than lignin removal on releasing sugar during saccharification, without further ultra-structural studies of the bamboo cell wall structure, these assumptions could not be confirmed.

- Optimal pretreatment conditions were derived for bamboo using liquid hot water, soaking in aqueous ammonia and dilute acid pretreatments. These were determined to be: 190°C for 10 minutes, 100°C for 24 hours with 15% NH_4OH and 160°C for 15 minutes with 0.2% H_2SO_4 respectively, based on lab-scale experiments.

5.6 Future work

The goal of this thesis was to evaluate the major areas of the bamboo to bioethanol conversion to determine whether bamboo has potential to serve as a feedstock for this purpose. Technological, economic and policy drivers that play a large role towards commercialisation have been thoroughly discussed. However, additional areas of research could be explored to contribute towards further development of this research area.

- The bamboo composition data used in this work was grounded on the assumption that there is little variation in cell wall composition between different bamboo species (Scurlock, 2000). Data from two Chinese temperate species, *Phyllostachys viridiglaucescens* and *Phyllostachys dulcis* were used for the experimental and economic modelling work. While this was most relevant for the China case study scenario, investigation of other Colombian bamboo species (e.g. *Guadua angustifolia*) would benefit the specificity of these case study results. Additionally, an initial screening process of different bamboo species (tropical and temperate) could help identify whether varieties exist with enhanced saccharification potential or higher cellulose contents, especially as it is not known whether or not similar cellulose contents are necessarily indicative of accessibility to enzyme.

- Other factors such as culm age and time of harvest could also potentially influence the cell wall composition. Although bamboo is generally harvested once it reaches maturity (approx. 5 years), studies have found a significant difference in the level of certain cell wall components such as cellulose and extractives at different ages, which could affect the total sugar yield during pretreatment and saccharification (Li et al., 2007). As mentioned in Chapter 2, starch contents are also known to vary with season, such that autumn and winter have lower contents than spring and summer (Okahisa et al., 2006). Therefore, the time of harvest could be another aspect of investigation.

- The content of extractives was noted in Chapter 2 to be significantly higher (13.5% of DM) than other reported values of 0.3-3.1% of DM, which is speculated to be attributed to the high starch content of bamboo (possibly 2-6% of DM). It would be useful to analyse the composition of these extractives to determine what the actual starch content was, as this would have contributed towards the theoretical maximum sugar content present in bamboo biomass, thereby affecting total ethanol yields and the production price of bioethanol.

- The concentration of inhibitors (HMF, furfural, acetic acid) generated from pretreatments (specifically high-temperature LHW and DA pretreatments) could be quantified to determine whether a large amount of sugar degradation had occurred during the higher severity pretreatments. Insight into this area would be valuable for identifying the optimal pretreatment conditions and for deciding whether the modelled microorganism *Zymomonas mobilis* would be suitable for fermentation.

- The effect of pretreatment on cell wall composition and saccharification yield was investigated in Chapter 2. However, it was not known as to how different pretreatments affected other aspects of the cell wall. Ultra-structural studies such as pore shape, size and spatial distribution, cellulose crystallinity, and the final outcome of solubilised components (e.g. redistribution or re-deposition of degradation compounds on cell wall surfaces) could be explored via microscopic techniques.

- The feedstock cost was consistently identified to be the greatest contributor towards the cost of bioethanol production from bamboo. Therefore it would be useful to examine the biomass supply chain to identify what the major costs were (e.g. production, collection, harvest, processing, storage and transport) and whether these could potentially be reduced or optimised under scenario alternatives.

- It was assumed in this model that bamboo biomass would be delivered to the plant to meet the 2,000 dry tonne requirement without any consideration as to how much land area would be needed to support this volume. By taking into account biomass yields/productivity, the total land area could be calculated. It could then be investigated as to how growing bamboo on marginal or degraded lands (with resultant lower biomass yields), would affect the land requirement for a bioethanol project of this scale.

- In addition, a sensitivity analysis could be performed with respect to the effect of plant size on the MESP. The processing capacity of 2,000 dry metric tonnes adopted here was based on the NREL 2002 report (Aden et al., 2002) which determined this value based on the trade-off between economies of scale and the increased cost of delivering feedstock. It is not known whether this assumption would apply to bioethanol production from bamboo in China or Colombia.

- A life-cycle analysis could be conducted on a cradle-to-grave basis, to assess the overall environmental impact of alternative pretreatments in the bamboo to bioethanol

pathway. A LCA would also account for bamboo plantation management factors such as nutrient and water inputs, which are believed to be extremely favourable for bamboo biomass due to its efficient rhizome storage system. The prospects for commercialisation of bioethanol from bamboo would be enhanced by a comprehensive evaluation of the environmental and social impacts of the process of producing this fuel to complement the techno-economic modelling accomplished in this thesis.

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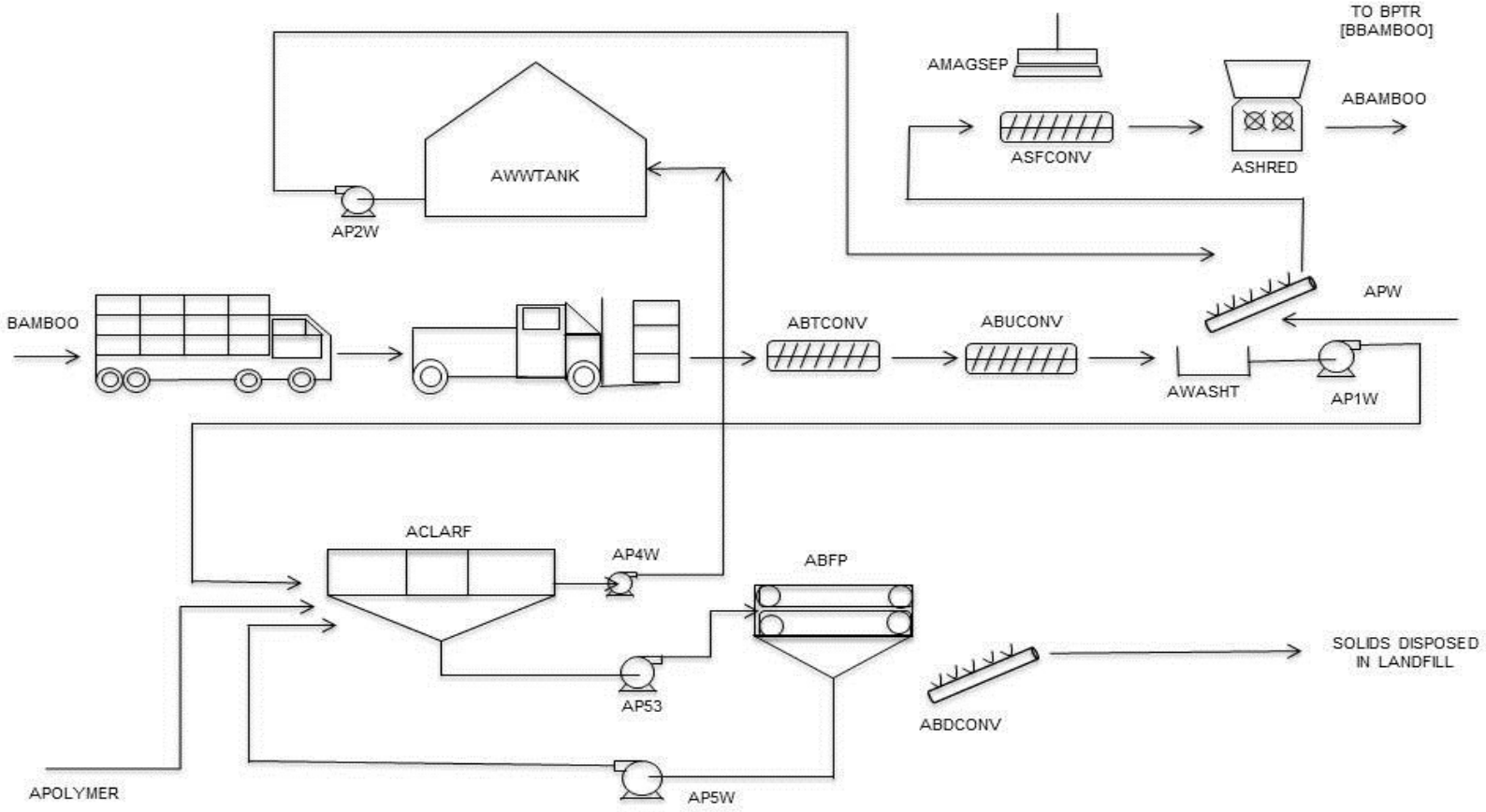
ZHU, L., O'DWYER, J. P., CHANG, V. S., GRANDA, C. B. & HOLTZAPPLE, M. T. 2008. Structural features affecting biomass enzymatic digestibility. *Bioresource Technology,* 99**,** 3817-3828.

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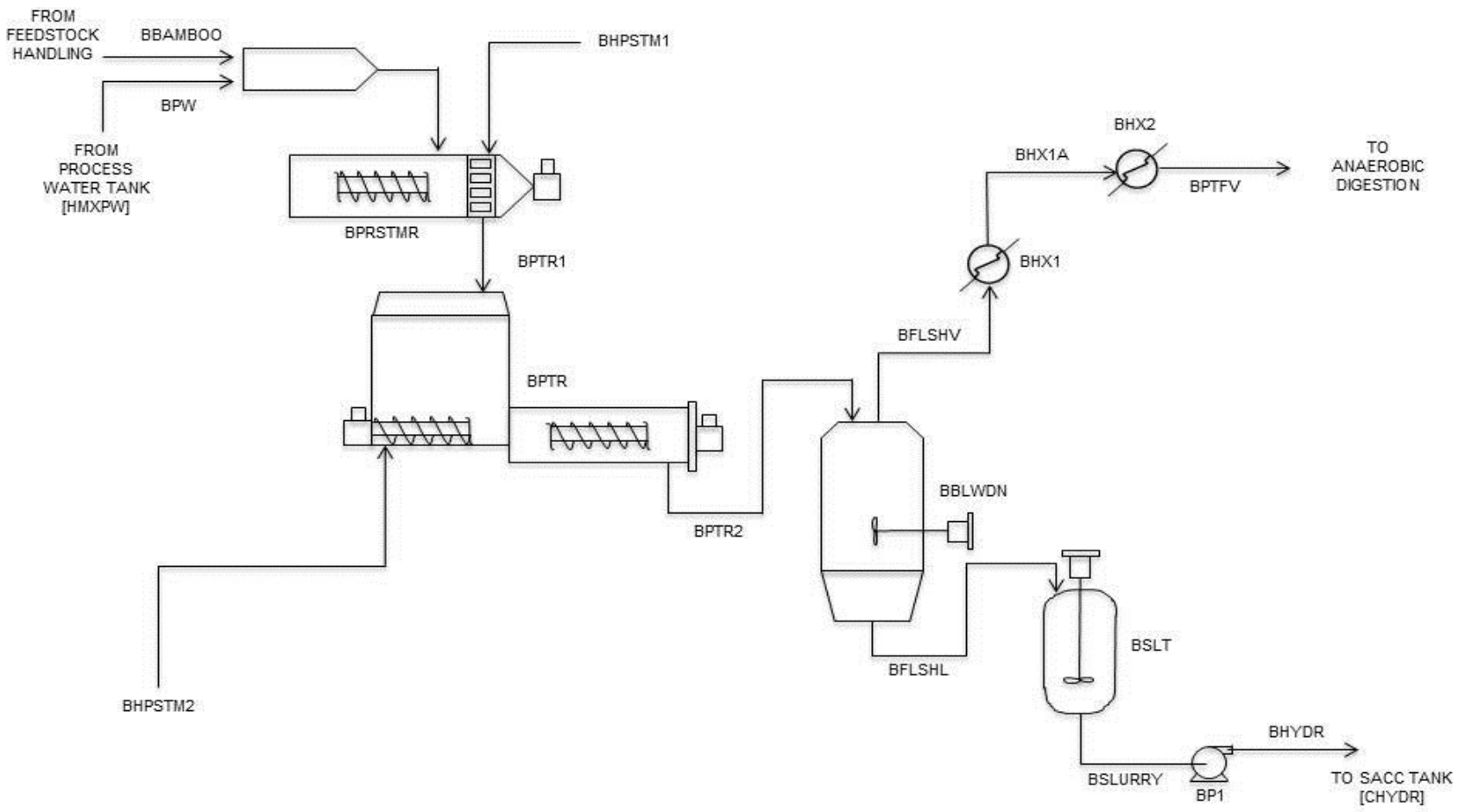
Appendix

Appendix A Process flow diagrams

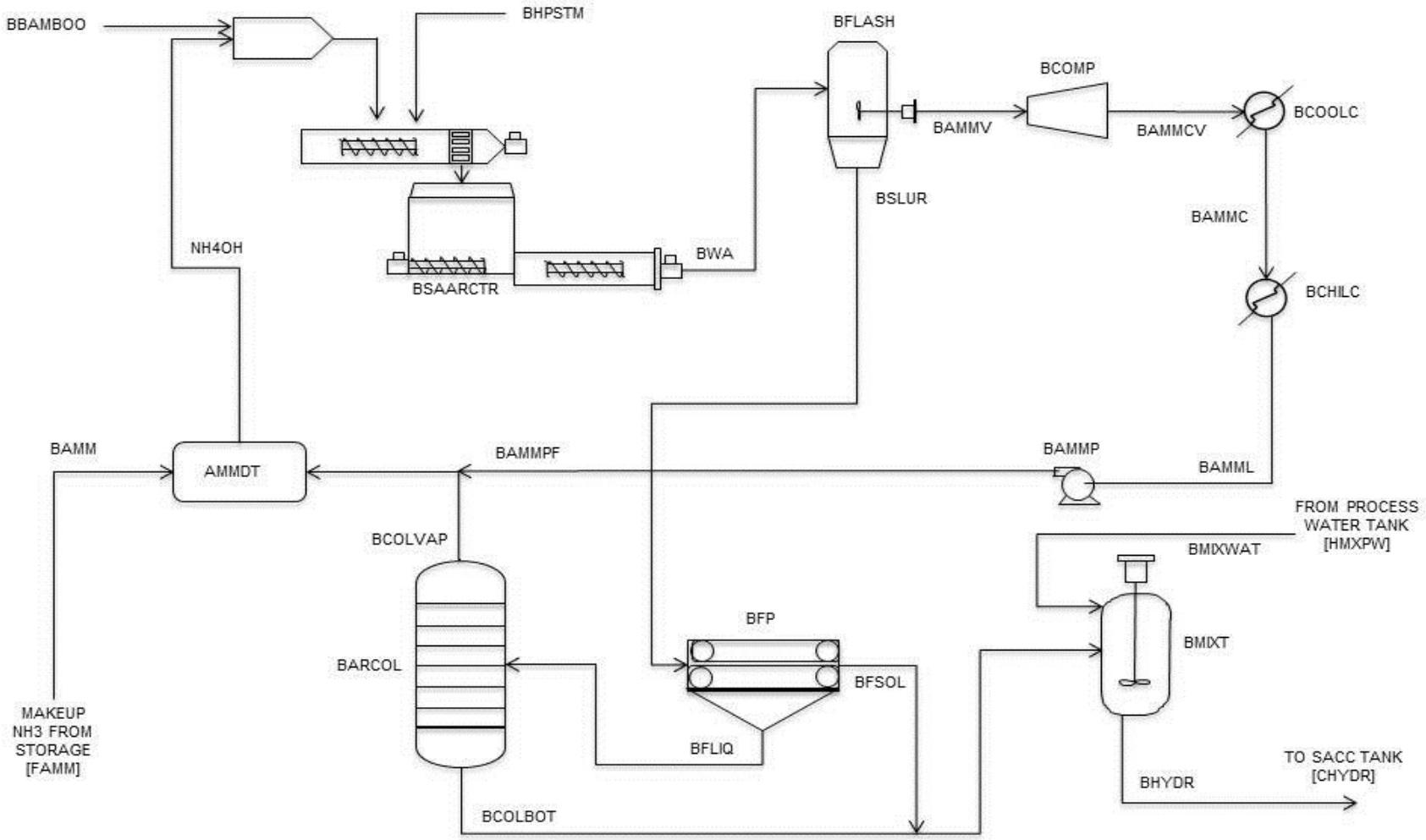
AREA A – FEED HANDLING



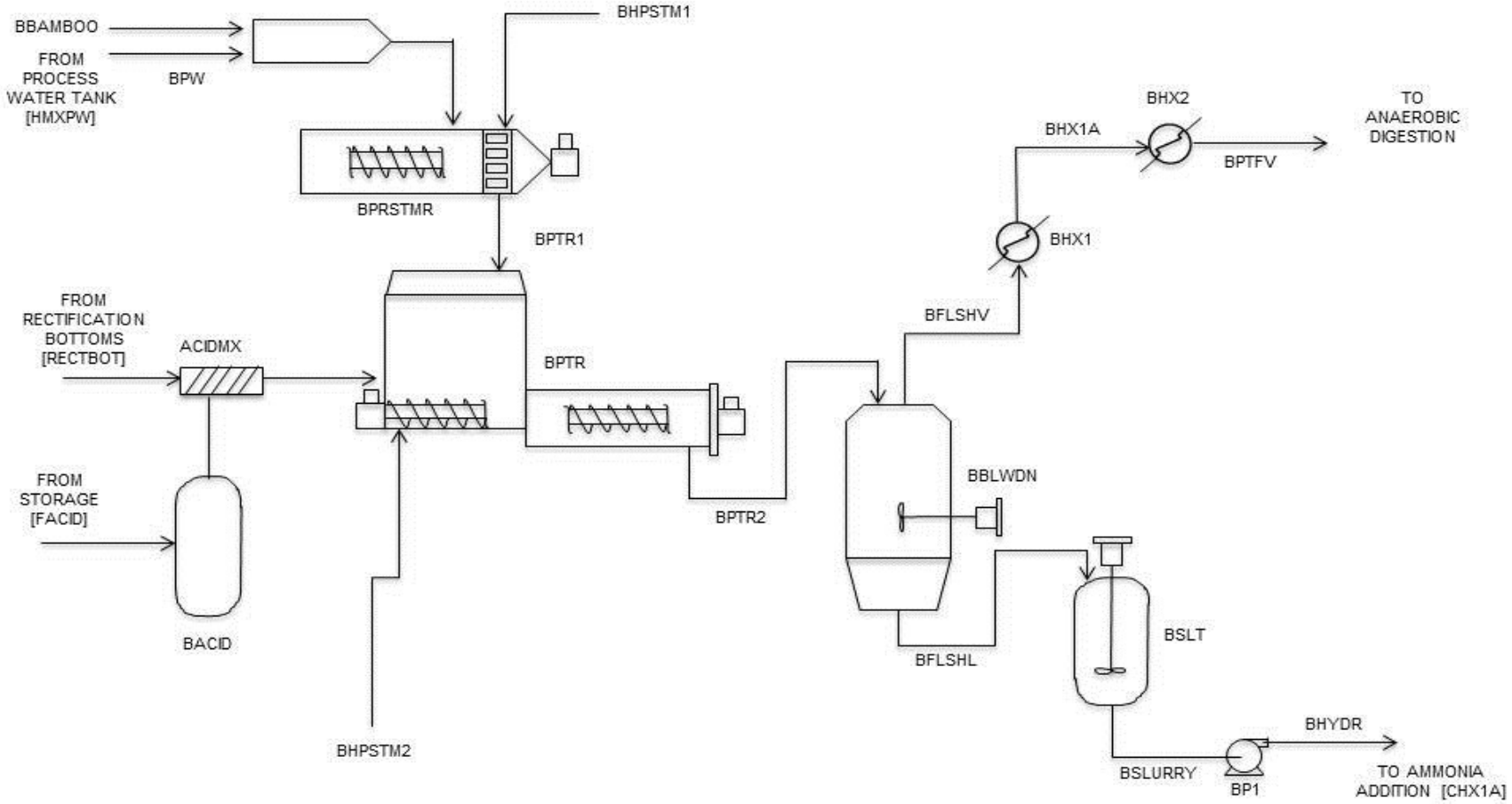
AREA B – LHW PRETREATMENT



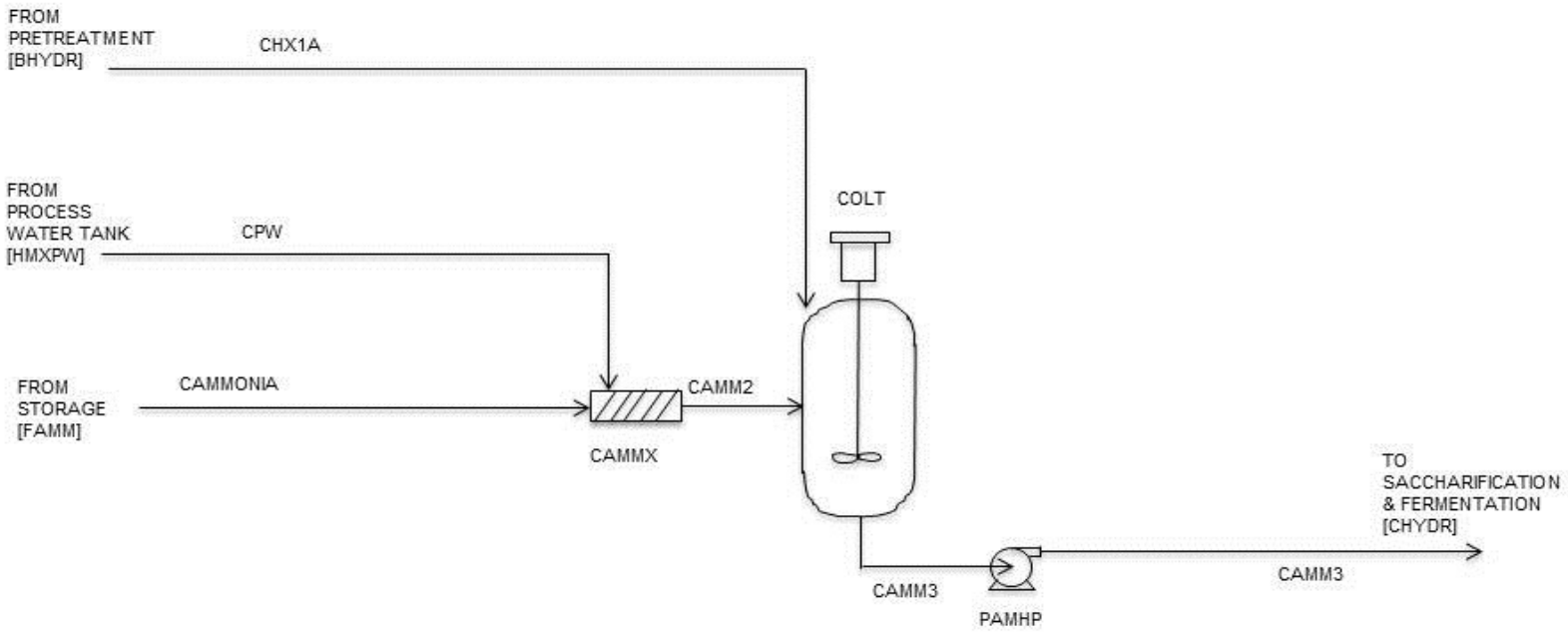
AREA B – SAA PRETREATMENT



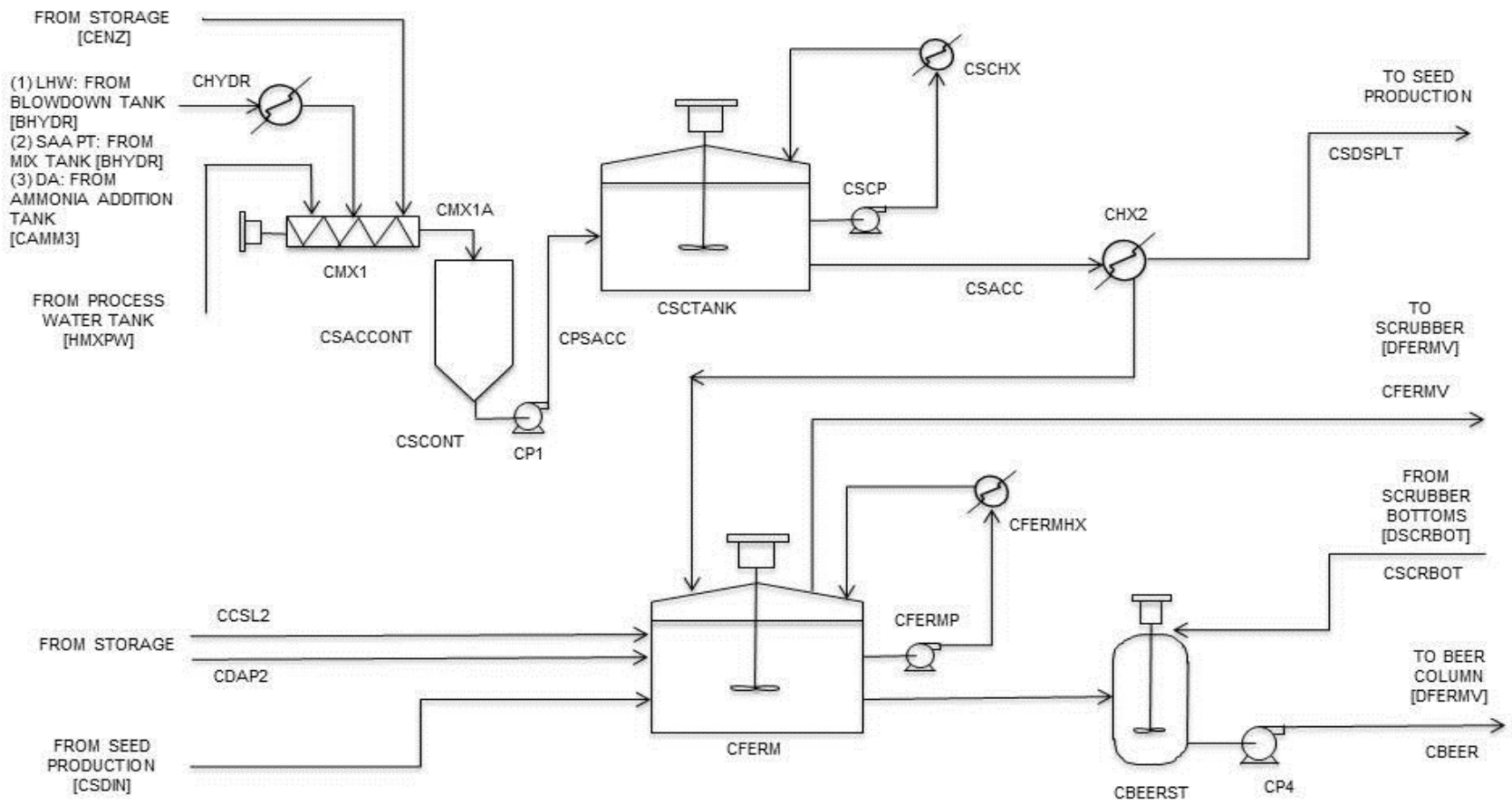
AREA B – DA PRETREATMENT



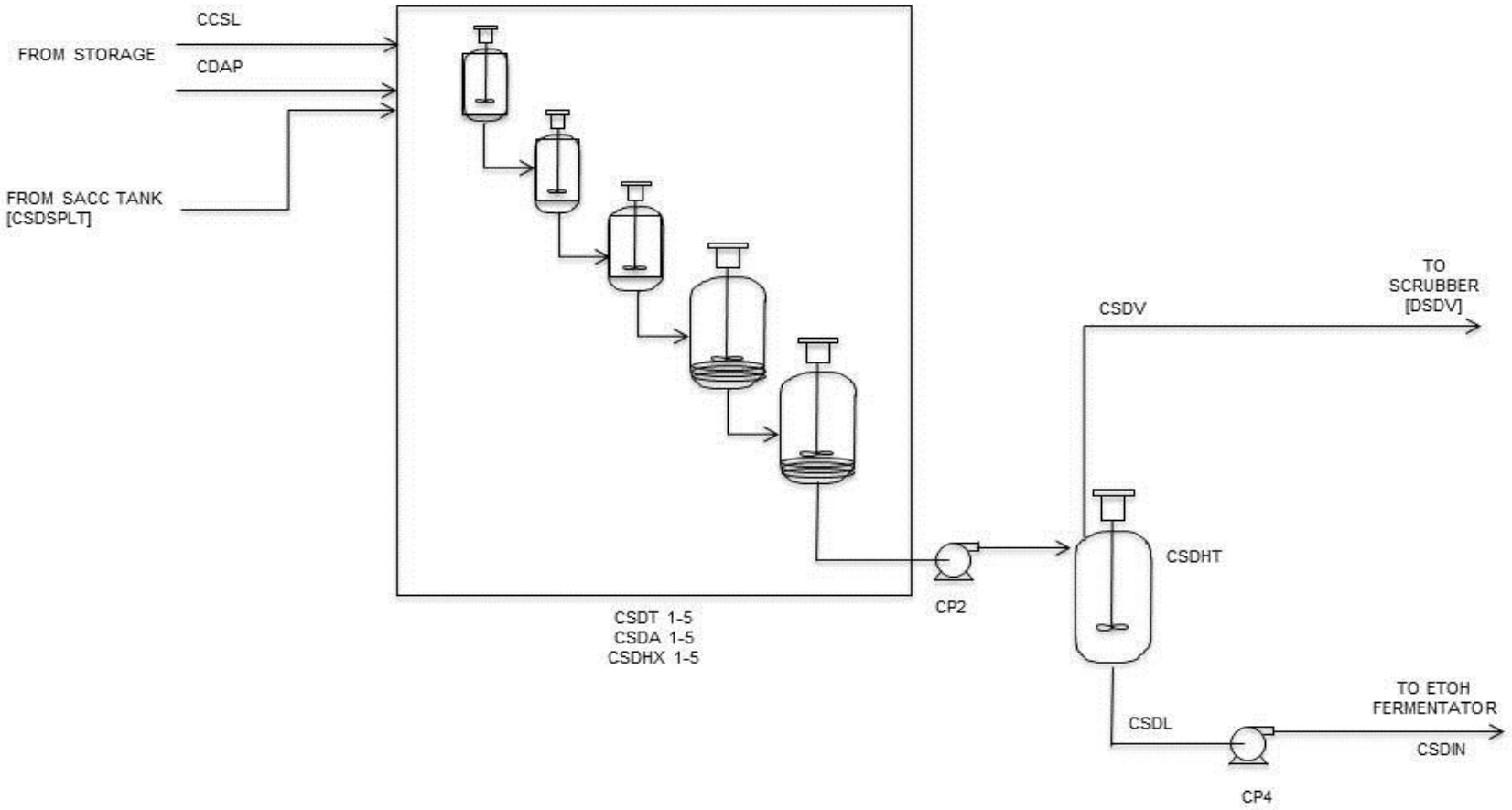
DA PRETREATMENT – AMMONIA ADDITION

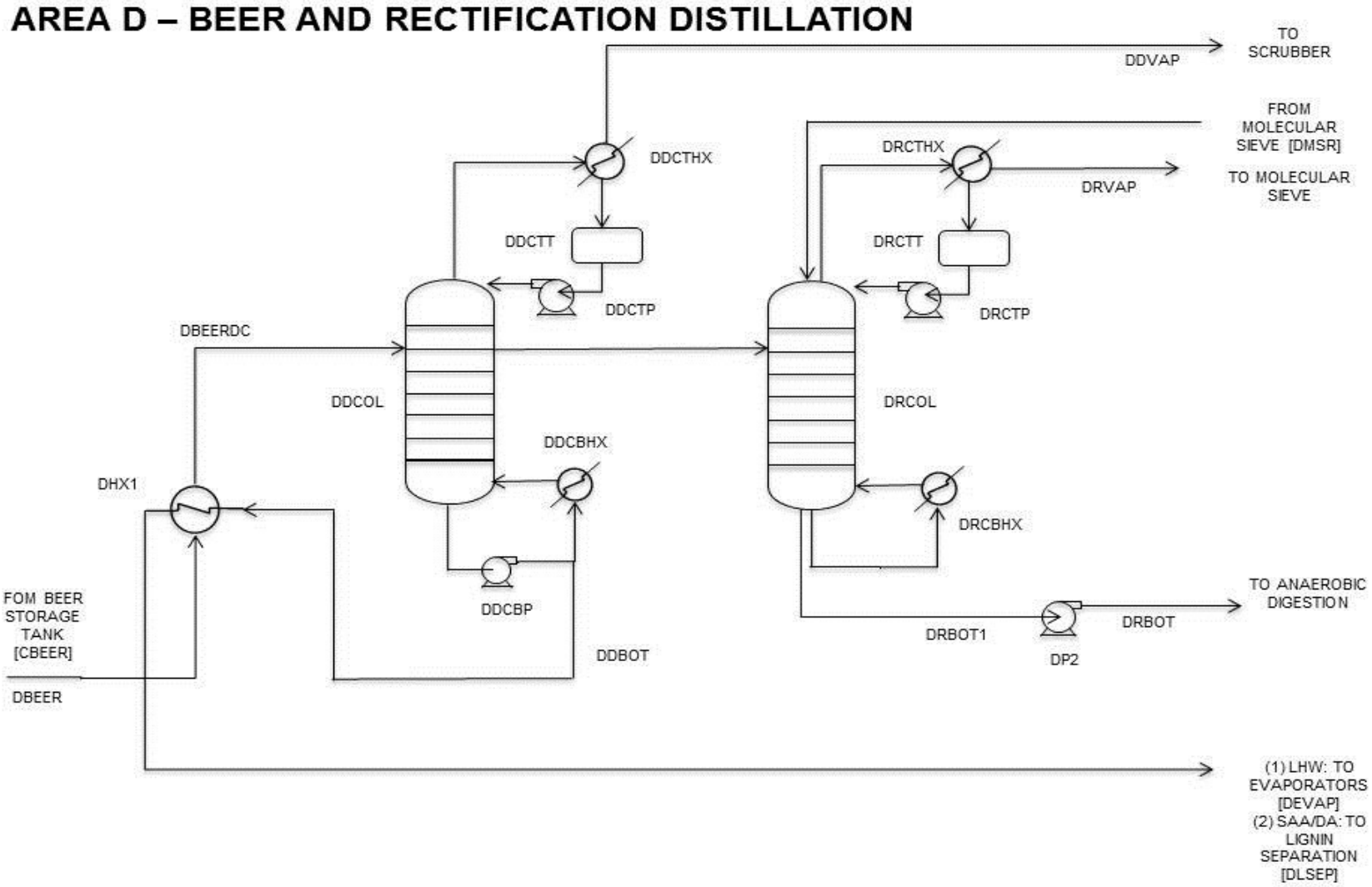


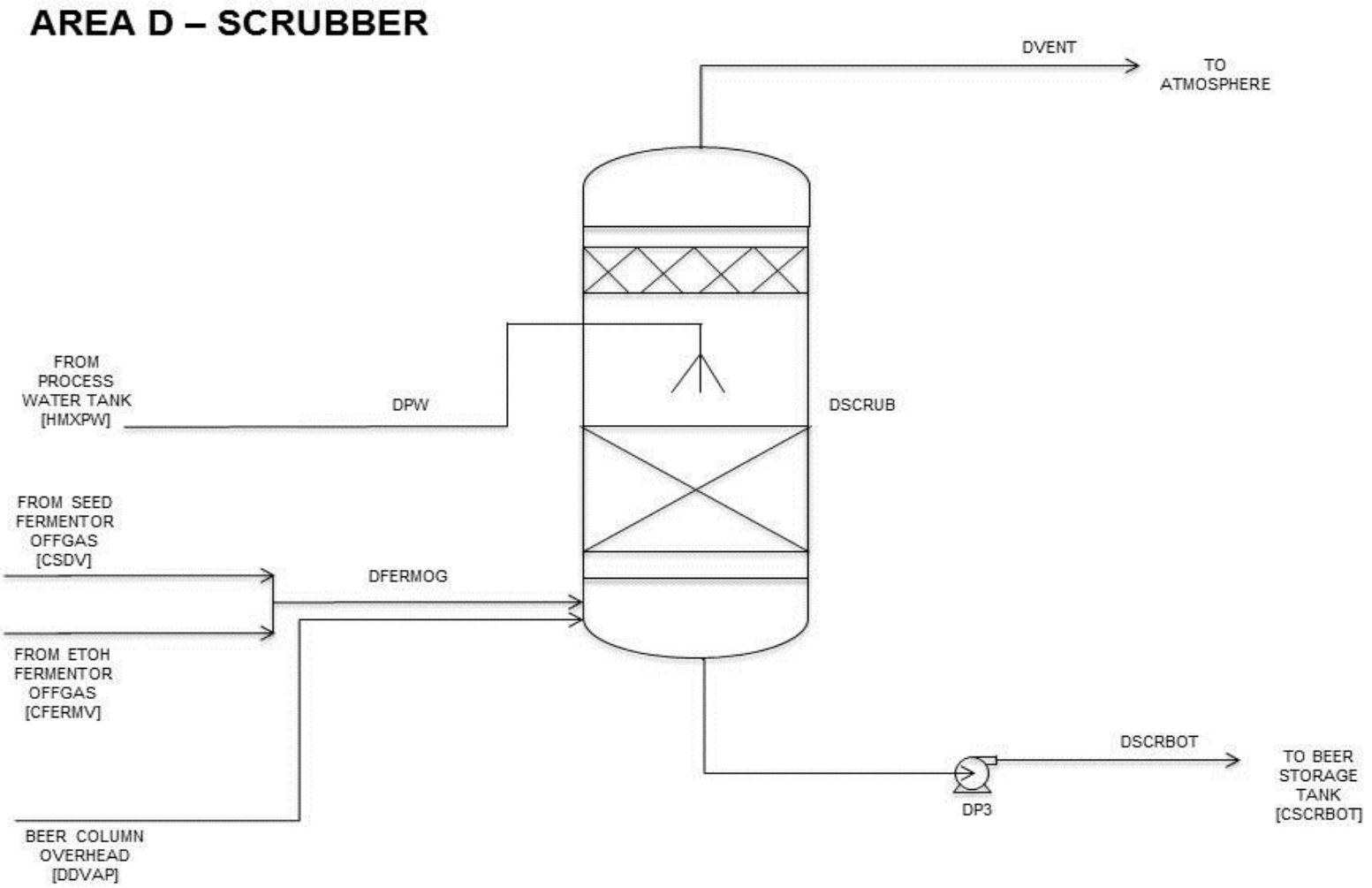
AREA C – SACCHARIFICATION & CO-FERMENTATION



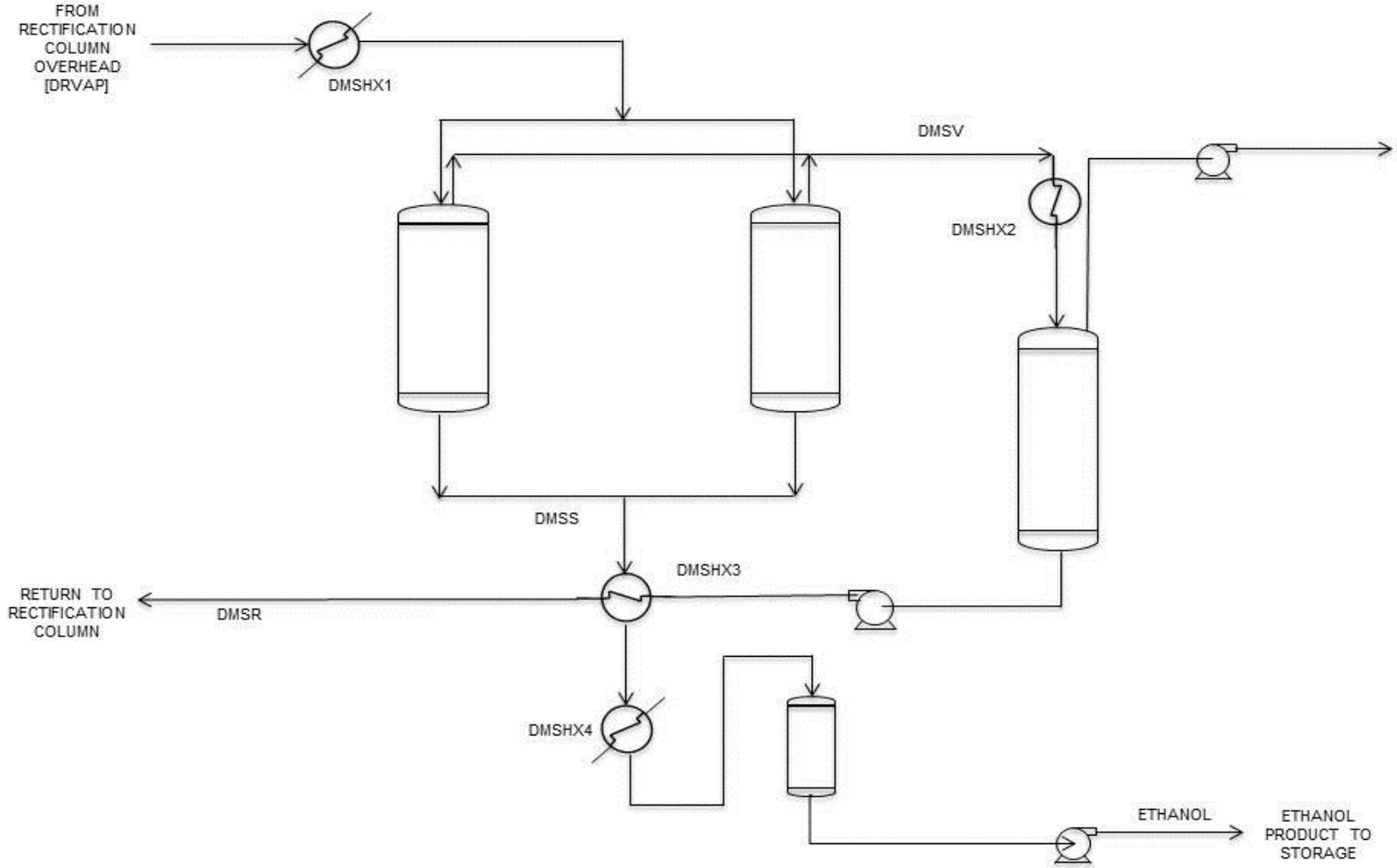
AREA C – SEED PRODUCTION

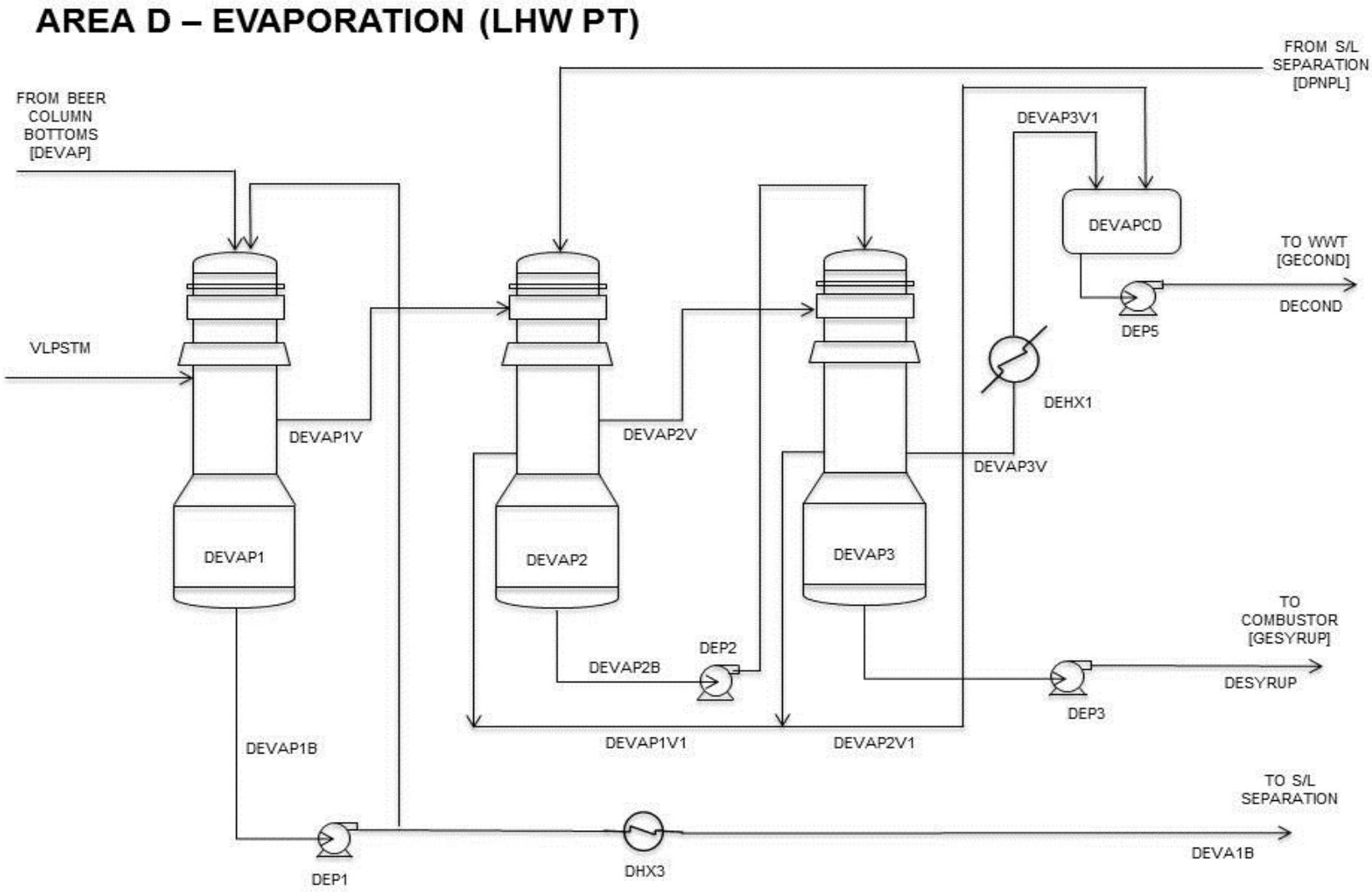


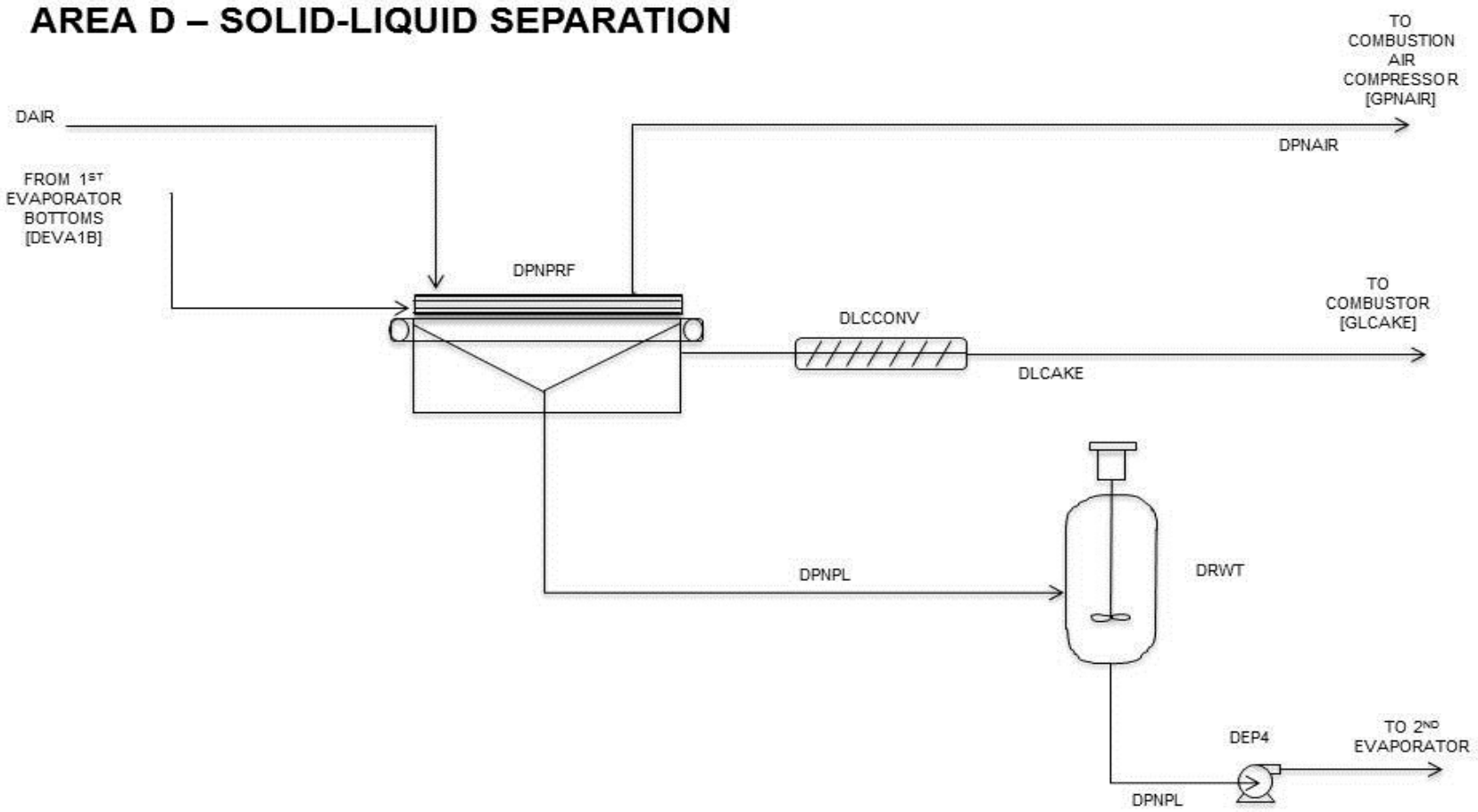

AREA D – BEER AND RECTIFICATION DISTILLATION
DDVAP
TO SCRUBBER
FROM MOLECULAR SIEVE [DMSR]
TO MOLECULAR SIEVE
DRVAP
DDCTHX
DRCTHX
DDCTT
DRCTT
DDCTP
DRCTP
DBEERDC
DDCOL
DRCOL
DDCBHX
DRCBHX
DHX1
DDCBP
FOM BEER STORAGE TANK [CBEER]
DBEER
DDBOT
DRBOT1
DP2
DRBOT
TO ANAEROBIC DIGESTION
(1) LHW: TO EVAPORATORS [DEVAP]
(2) SAA/DA: TO LIGNIN SEPARATION [DLSEP]


AREA D – SCRUBBER
DVENT
TO
ATMOSPHERE
FROM
PROCESS
WATER TANK
[HMXPW]
DPW
DSCRUB
FROM SEED
FERMENTOR
OFFGAS
[CSDV]
DFERMOG
FROM ETOH
FERMENTOR
OFFGAS
[CFERMV]
DSCRBOT
TO BEER
STORAGE
TANK
[CSCRBOT]
DP3
BEER COLUMN
OVERHEAD
[DDVAP]

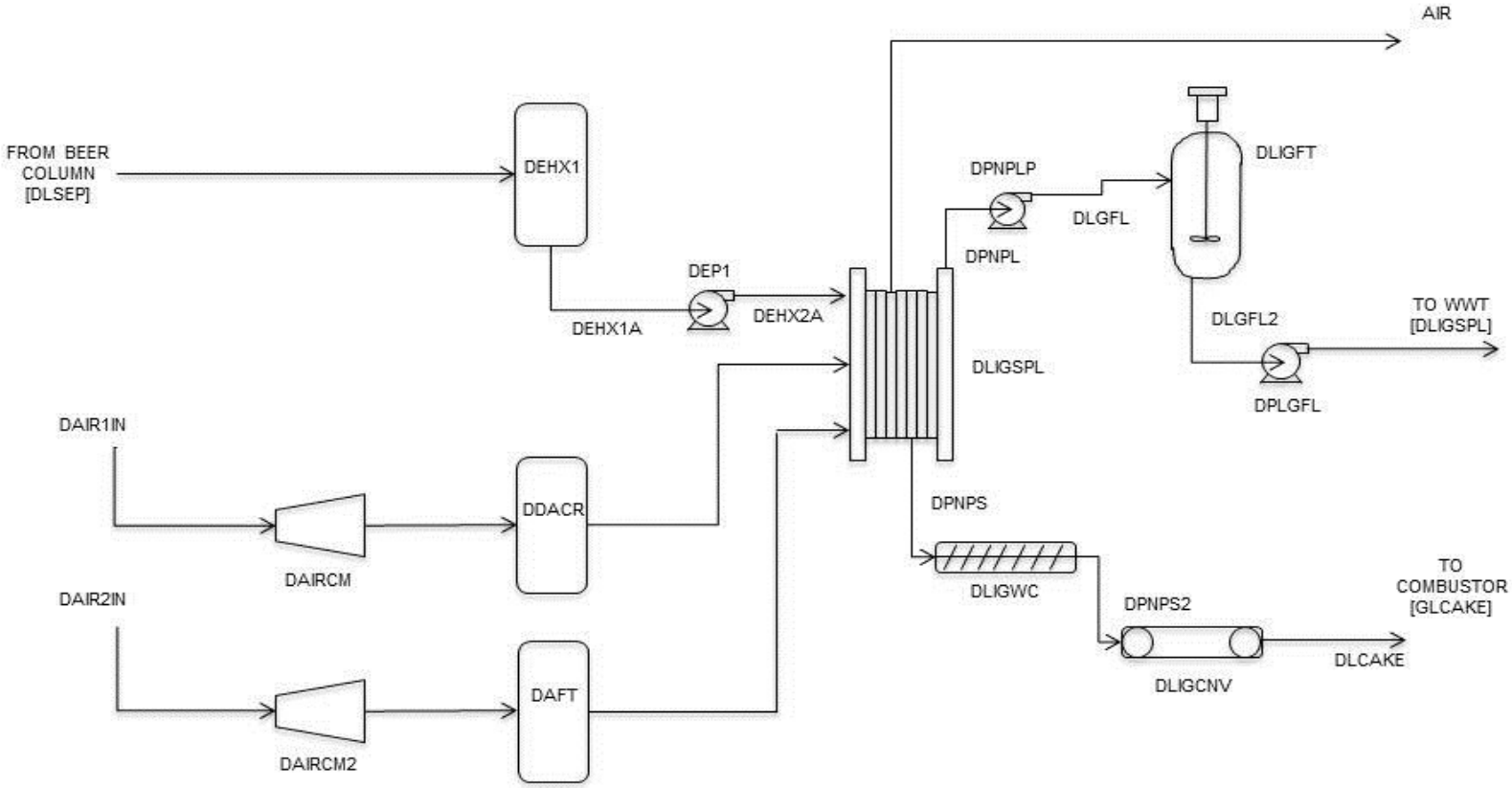
AREA D – MOLECULAR SIEVE ADSORPTION



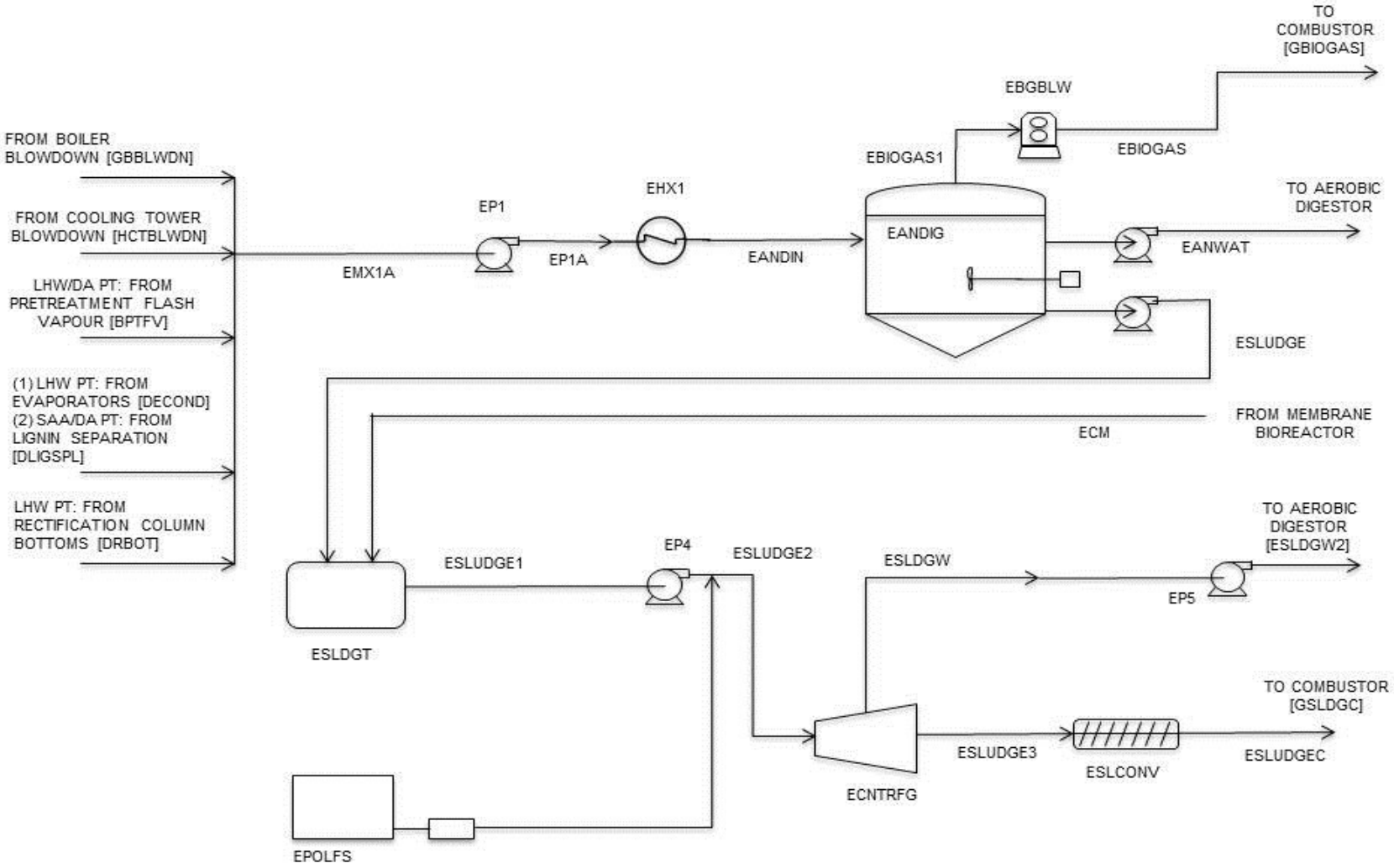

AREA D – EVAPORATION (LHW PT)
FROM BEER COLUMN BOTTOMS [DEVAP]
VLPSTM
FROM S/L SEPARATION [DPNPL]
DEVAP3V1
DEVAPCD
TO WWT [GECOND]
DECOND
DEP5
DEHX1
DEVAP1V
DEVAP2V
DEVAP3V
DEVAP1
DEVAP2
DEVAP3
TO COMBUSTOR [GESYRUP]
DESYRUP
DEP3
DEP2
DEVAP2B
DEVAP1V1
DEVAP2V1
DEVAP1B
TO S/L SEPARATION
DEVA1B
DEP1
DHX3


AREA D – SOLID-LIQUID SEPARATION
TO COMBUSTION AIR COMPRESSOR [GPNAIR]
DAIR
DPNAIR
FROM 1ST EVAPORATOR BOTTOMS [DEVA1B]
DPNPRF
TO COMBUSTOR [GLCAKE]
DLCCONV
DLCAKE
DPNPL
DRWT
DEP4
TO 2ND EVAPORATOR
DPNPL

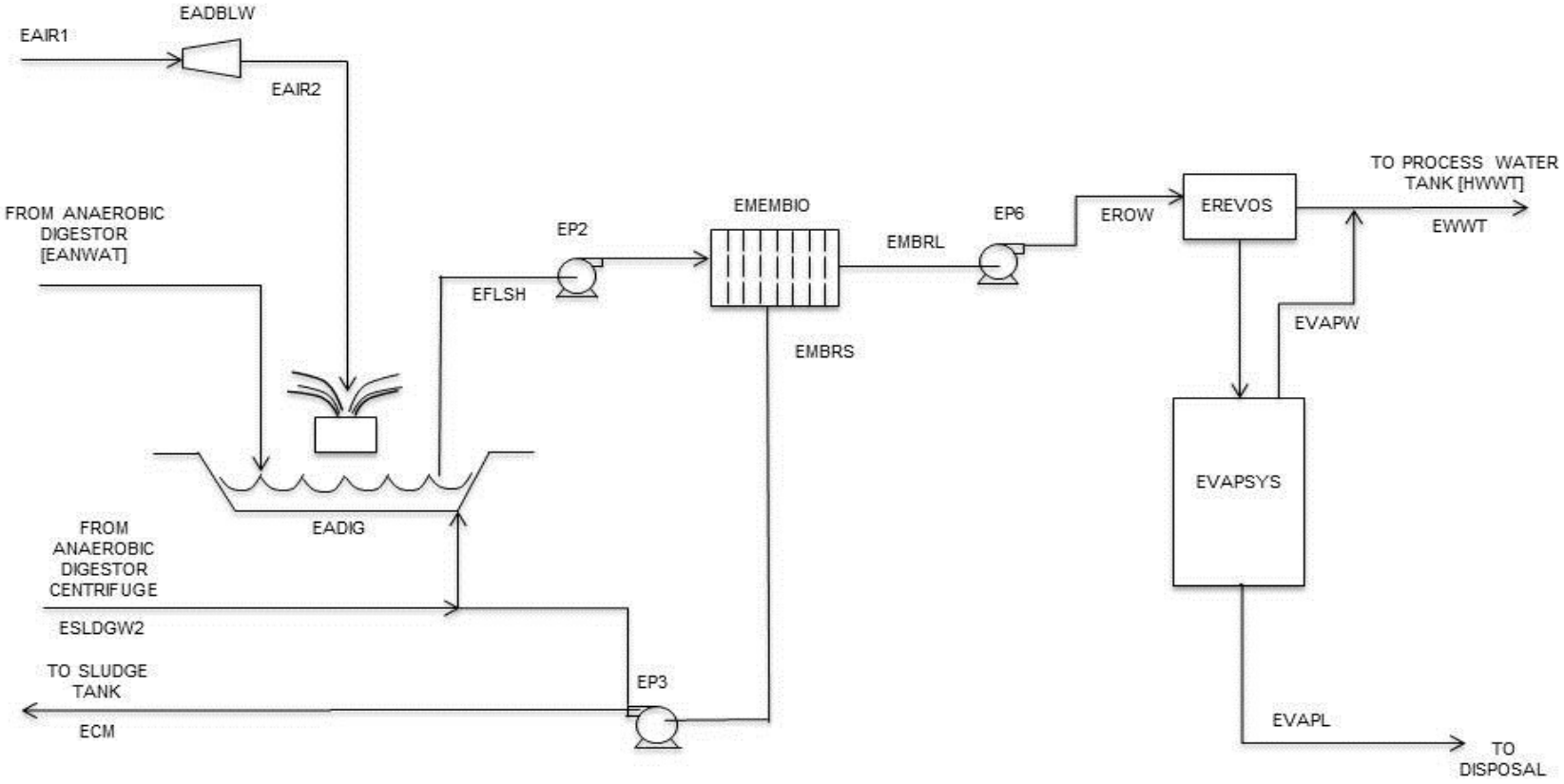
AREA D – LIGNIN SEPARATION (FOR SAA/DA PT)

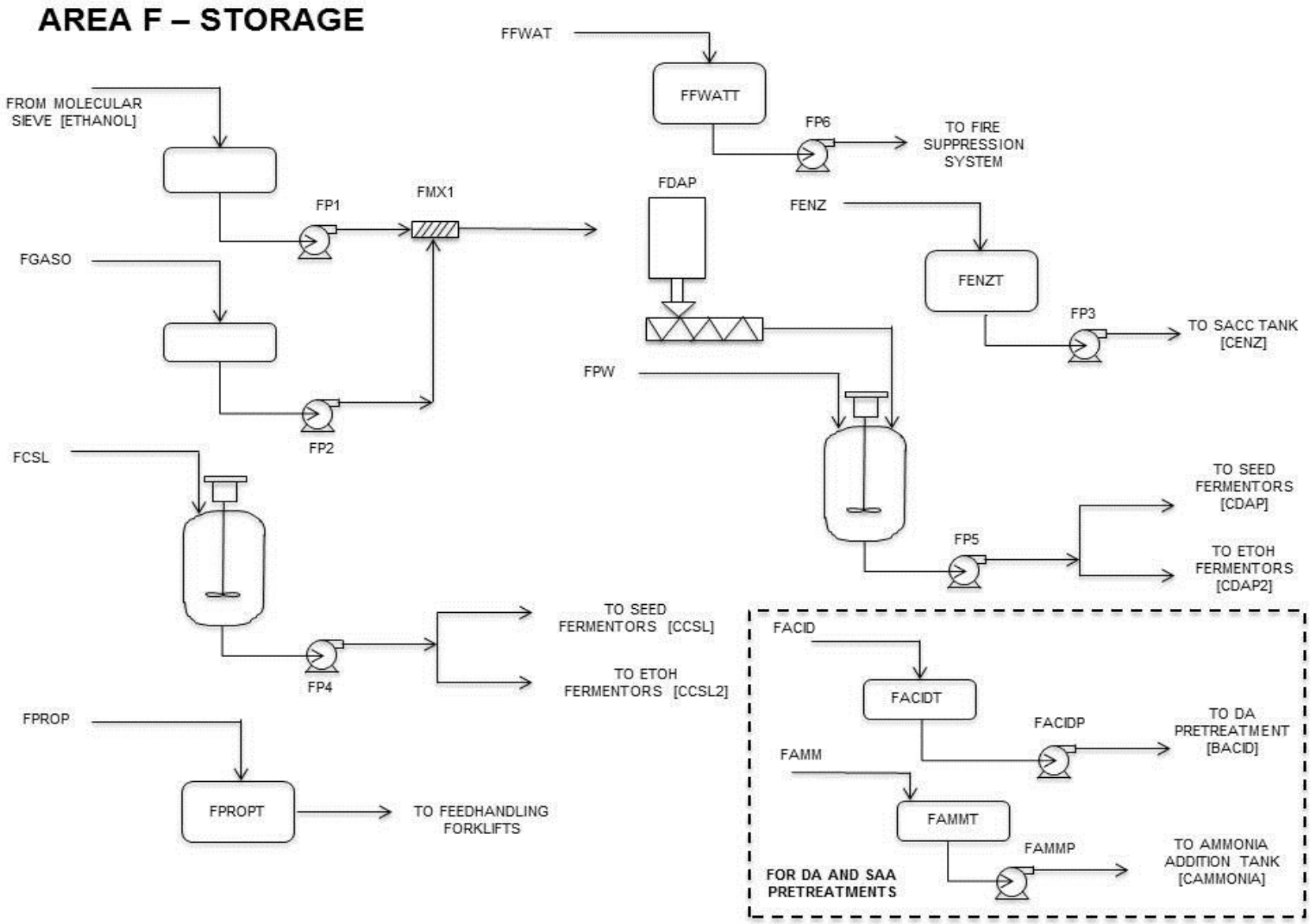


AREA E – ANAEROBIC DIGESTION

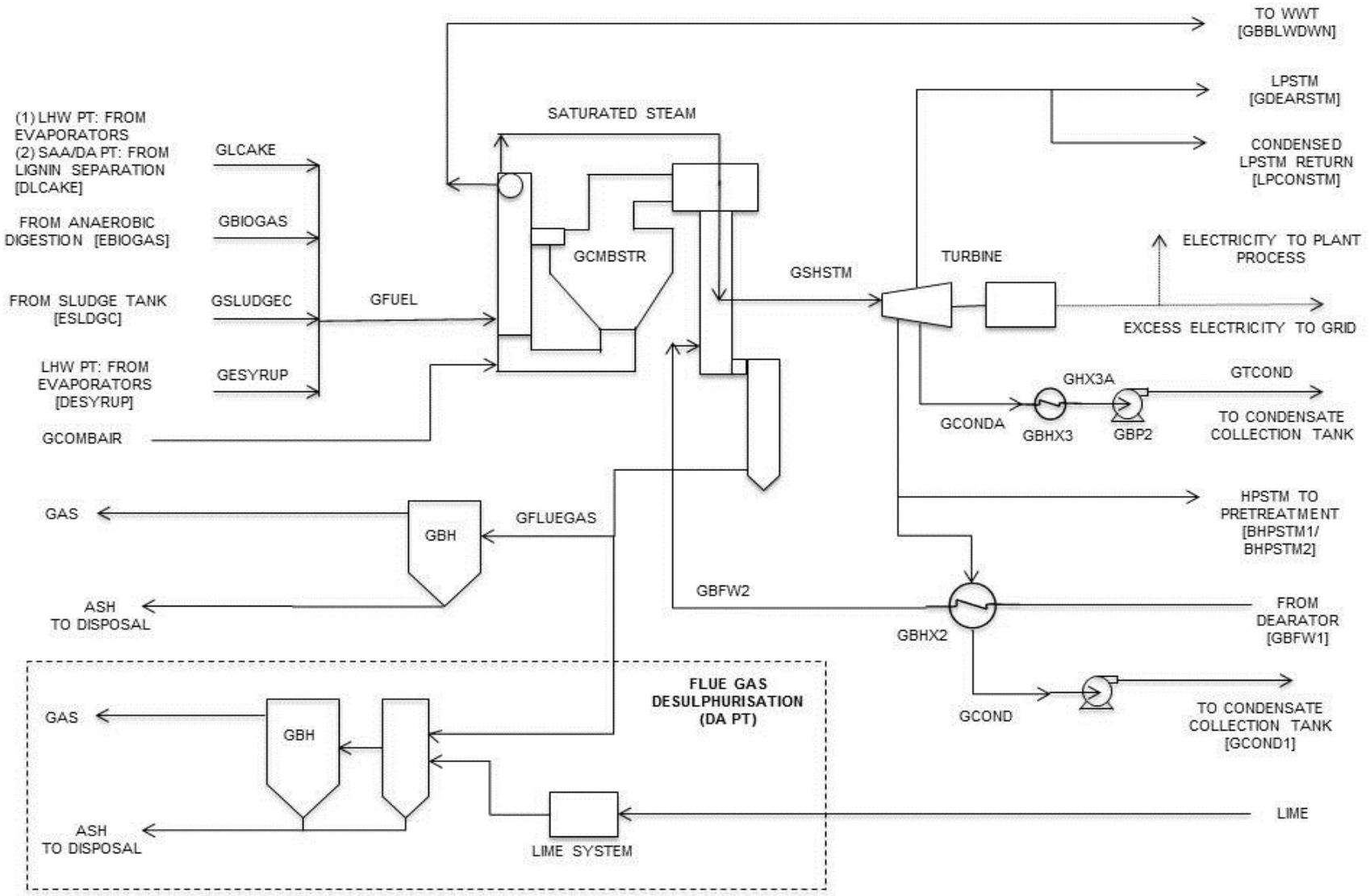


AREA E – AEROBIC DIGESTION

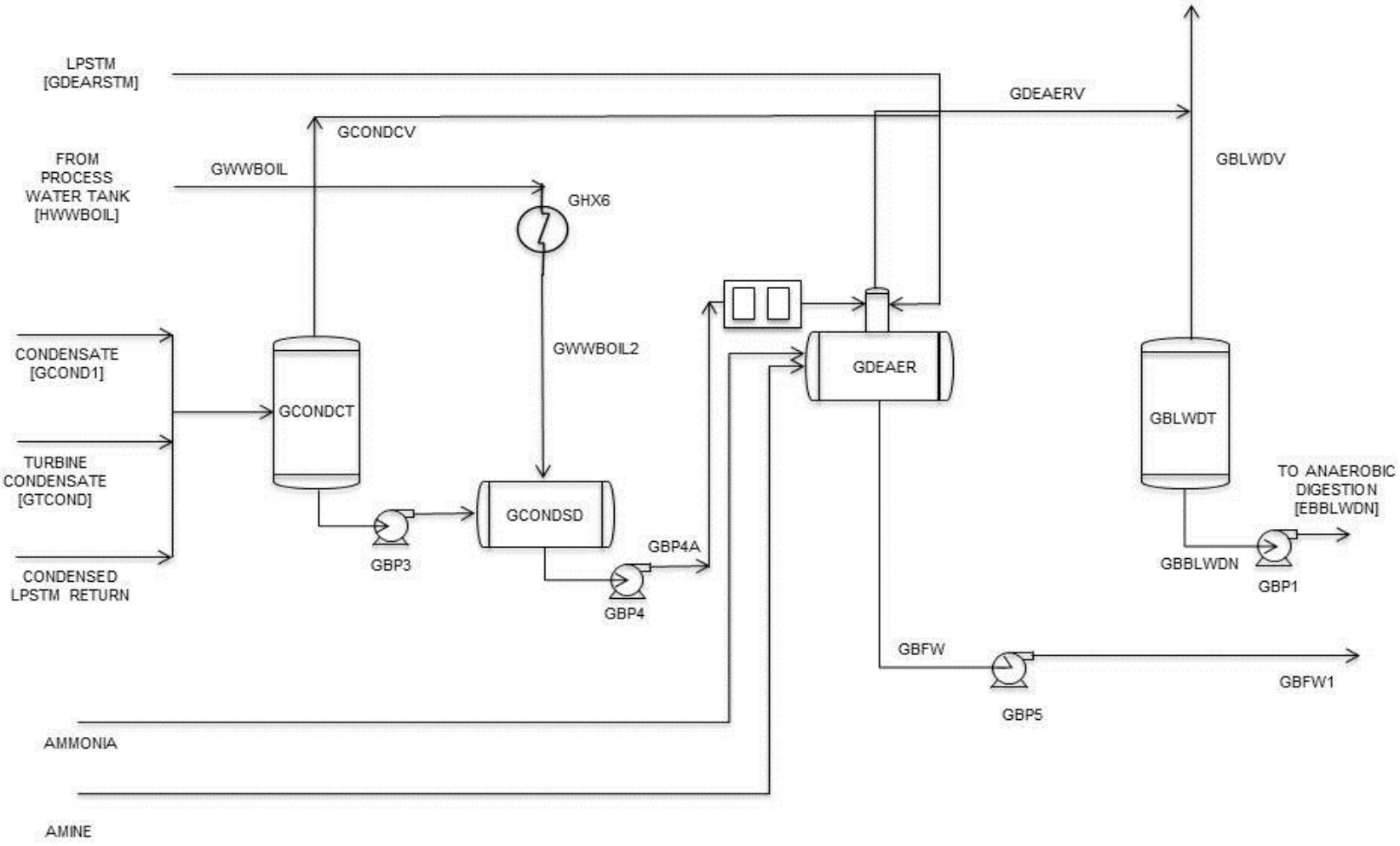



AREA F – STORAGE
FFWAT
FFWATT
FP6
TO FIRE SUPPRESSION SYSTEM
FROM MOLECULAR SIEVE [ETHANOL]
FP1
FMX1
FDAP
FENZ
FENZT
FP3
TO SACC TANK [CENZ]
FGASO
FP2
FPW
FCSL
TO SEED FERMENTORS [CDAP]
TO ETOH FERMENTORS [CDAP2]
FP5
TO SEED FERMENTORS [CCSL]
TO ETOH FERMENTORS [CCSL2]
FP4
FACID
FACIDT
FACIDP
TO DA PRETREATMENT [BACID]
FAMM
FAMMT
FAMMP
TO AMMONIA ADDITION TANK [CAMMONIA]
FOR DA AND SAA PRETREATMENTS
FPROP
FPROPT
TO FEEDHANDLING FORKLIFTS

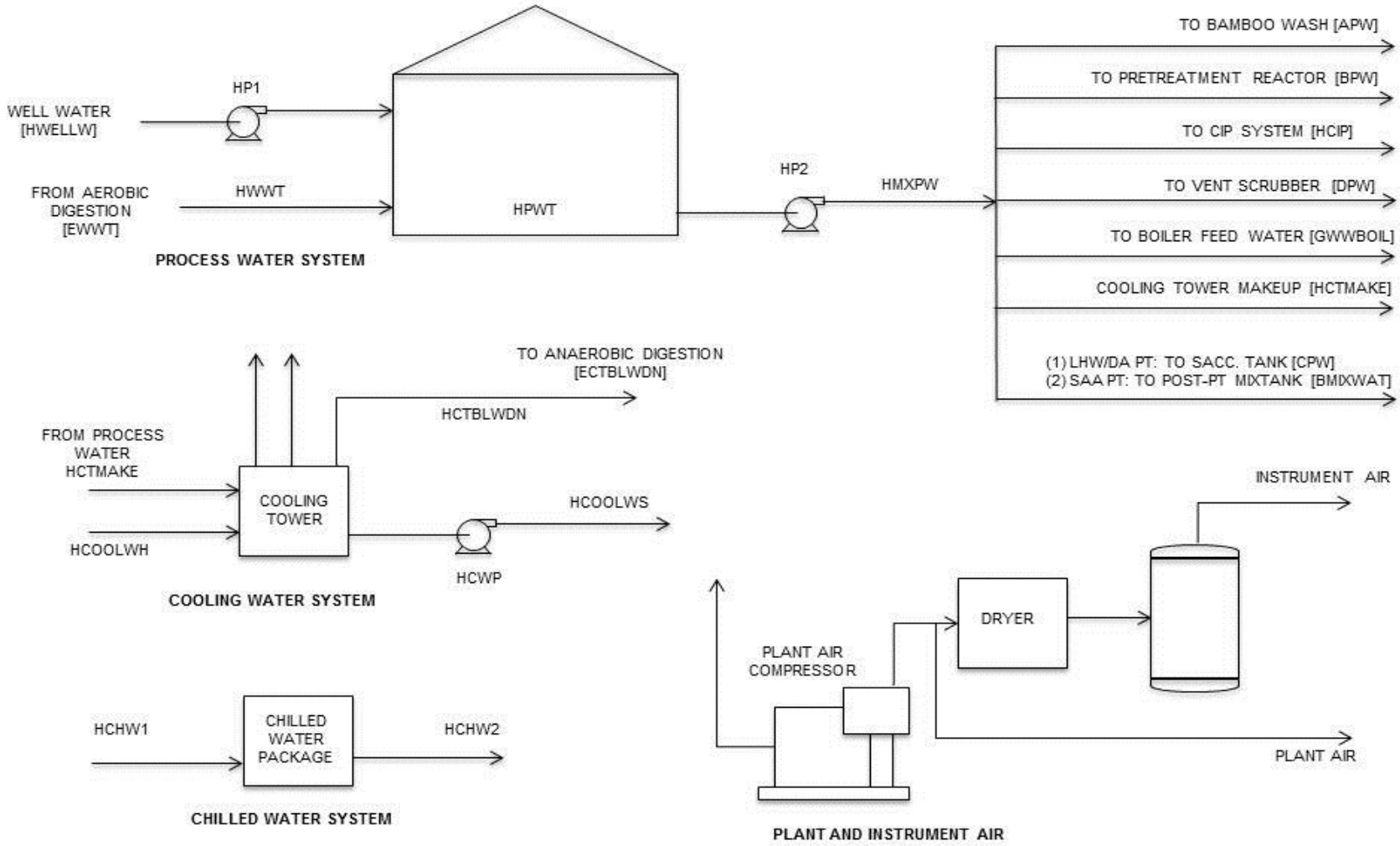
AREA G – COMBUSTOR/BOILER/TURBOGENERATOR



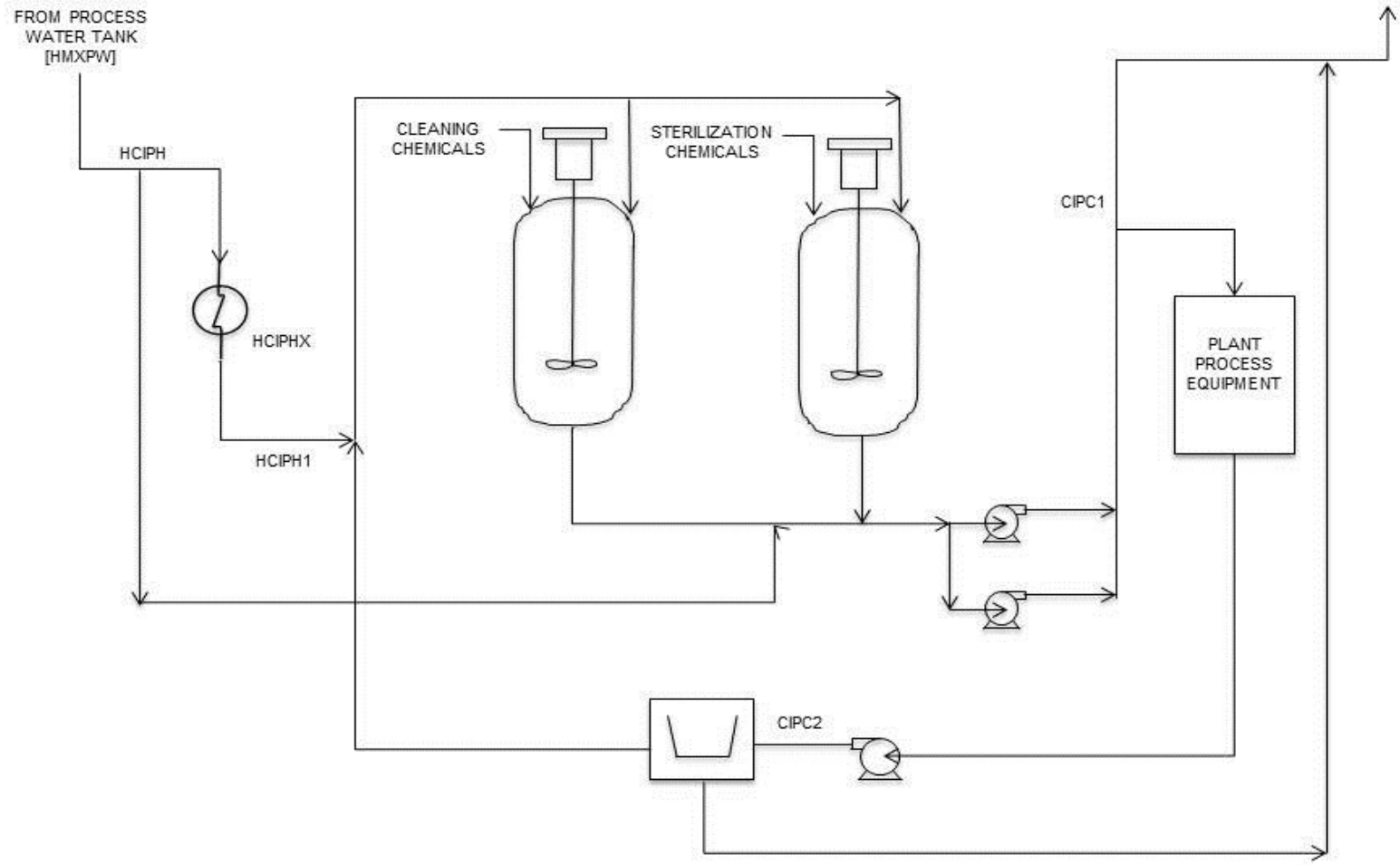
AREA G – BOILER FEED WATER PREPARATION



AREA H – UTILITIES



AREA H – CIP SYSTEM



Appendix B Discounted cash flow analysis (for LHW PT 10 FPU/g glucan scenario)

Year		-2	-1	0	1	2	3	4
Fixed capital investment		$13,370,244	$100,276,834	$53,480,978				
Land		$1,848,000						
Working capital				$20,891,007				
Loan payment					$37,360,513	$37,360,513	$37,360,513	$37,360,513
Loan interest payment		$1,604,429	$13,637,649	$20,055,367	$20,055,367	$18,670,955	$17,175,790	$15,561,013
Loan principal		$20,055,367	$170,470,618	$250,692,086	$233,386,939	$214,697,381	$194,512,658	$172,713,158
Ethanol sales					$62,146,259	$71,024,296	$71,024,296	$71,024,296
By-product credit					$29,205,242	$33,377,419	$33,377,419	$33,377,419
Total annual sales					$91,351,501	$104,401,715	$104,401,715	$104,401,715
Annual manufacturing cost								
Feedstock					$31,500,643	$36,000,735	$36,000,735	$36,000,735
Baghouse bags					$519,687			
Other variable costs					$12,047,800	$12,850,987	$12,850,987	$12,850,987
Fixed variable costs					$6,763,897	$6,763,897	$6,763,897	$6,763,897
Total product cost					$50,832,027	$48,851,722	$48,851,722	$48,851,722
Annual depreciation								
General plant writedown					14%	24.49%	17.49%	12.49%
Depreciation charge					$48,378,039	$82,909,599	$59,211,470	$42,284,234
Remaining value					$290,166,671	$207,257,071	$148,045,602	$105,761,367
Steam plant writedown					3.75%	7.22%	6.68%	6.18%
Depreciation charge					$2,972,829	$5,722,894	$5,293,221	$4,896,844
Remaining value					$76,302,604	$70,579,711	$65,286,490	$60,389,647
Net revenue					-$30,886,761	-$51,753,455	-$26,130,488	-$7,192,097
Losses forward						-$30,886,761	-$82,640,216	-$108,770,703
Taxable income					-$30,886,761	-$82,640,216	-$108,770,703	-$115,962,801
Income tax					$0	$0	$0	$0
Annual cash income					$3,158,961	$18,189,480	$18,189,480	$18,189,480
Discount factor		1.2100	1.1000	1.0000	0.9091	0.8264	0.7513	0.6830
Annual present value	$236,708,711				$2,871,782	$15,032,628	$13,666,025	$12,423,659
Total capital investment + interest		$20,355,435	$125,305,932	$94,427,352				
Net present worth				$0				
NPV of income tax		$48,099,270			$0	$0	$0	$0
NPV of ethanol income		$667,956,028			$56,496,599	$58,697,765	$53,361,605	$48,510,550

Year	5	6	7	8	9	10	11	12
Fixed capital investment								
Land								
Working capital								
Loan payment	$37,360,513	$37,360,513	$37,360,513	$37,360,513	$37,360,513	$37,360,513	$0	$0
Loan interest payment	$13,817,053	$11,933,576	$9,899,421	$7,702,533	$5,329,895	$2,767,445	$0	$0
Loan principal	$149,169,697	$123,742,759	$96,281,666	$66,623,686	$34,593,068	$0	$0	$0
Ethanol sales	$71,024,296	$71,024,296	$71,024,296	$71,024,296	$71,024,296	$71,024,296	$71,024,296	$71,024,29
By-product credit	$33,377,419	$33,377,419	$33,377,419	$33,377,419	$33,377,419	$33,377,419	$33,377,419	$33,377,419
Total annual sales	$104,401,715	$104,401,715	$104,401,715	$104,401,715	$104,401,715	$104,401,715	$104,401,715	$104,401,715
Annual manufacturing cost								
Feedstock	$36,000,735	$36,000,735	$36,000,735	$36,000,735	$36,000,735	$36,000,735	$36,000,735	$36,000,735
Baghouse bags		$519,687					$519,687	
Other variable costs	$12,850,987	$12,850,987	$12,850,987	$12,850,987	$12,850,987	$12,850,987	$12,850,987	$12,850,987
Fixed variable costs	$6,763,897	$6,763,897	$6,763,897	$6,763,897	$6,763,897	$6,763,897	$6,763,897	$6,763,897
Total product cost	$48,851,722	$49,371,408	$48,851,722	$48,851,722	$48,851,722	$48,851,722	$49,371,408	$48,851,722
Annual depreciation								
General plant writedown	8.93%	8.92%	8.93%	4.46%				
Depreciation charge	$30,232,043	$30,198,188	$30,232,043	$15,099,094				
Remaining value	$75,529,325	$45,331,137	$15,099,094	$0				
Steam plant writedown	5.71%	5.29%	4.89%	4.52%	4.46%	4.46%	4.46%	4.46%
Depreciation charge	$4,529,005	$4,189,707	$3,874,983	$3,584,835	$3,537,270	$3,536,477	$3,537,270	$3,536,477
Remaining value	$55,860,641	$51,670,935	$47,795,951	$44,211,116	$40,673,846	$37,137,369	$33,600,100	$30,063,623
Net revenue	$6,971,892	$8,708,836	$11,543,547	$29,163,531	$46,682,828	$49,246,071	$51,493,037	$52,013,516
Losses forward	-$115,962,801	-$108,990,908	-$100,282,072	-$88,738,525	-$59,574,995	-$12,892,166	$0	$0
Taxable income	-$108,990,908	-$100,282,072	-$88,738,525	-$59,574,995	-$12,892,166	$36,353,904	$51,493,037	$52,013,516
Income tax	$0	$0	$0	$0	$0	$9,088,476	$12,873,259	$13,003,379
Annual cash income	$18,189,480	$17,669,793	$18,189,480	$18,189,480	$18,189,480	$9,101,004	$42,157,047	$42,546,614
Discount factor	0.6209	0.5645	0.5132	0.4665	0.4241	0.3855	0.3505	0.3186
Annual present value	$11,294,236	$9,974,138	$9,334,079	$8,485,527	$7,714,115	$3,508,831	$14,775,788	$13,556,662
Total capital investment + interest								
Net present worth								
NPV of income tax	$0	$0	$0	$0	$0	$3,504,001	$4,511,999	$4,143,277
NPV of ethanol income	$44,100,500	$40,091,363	$36,446,694	$33,133,358	$30,121,235	$27,382,941	$24,893,582	$22,630,529

Year	13	14	15	16	17	18	19	20
Fixed capital investment								
Land								
Working capital								
Loan payment	$0	$0	$0	$0	$0	$0	$0	$0
Loan interest payment	$0	$0	$0	$0	$0	$0	$0	$0
Loan principal	$0	$0	$0	$0	$0	$0	$0	$0
Ethanol sales	$71,024,296	$71,024,296	$71,024,296	$71,024,296	$71,024,296	$71,024,296	$71,024,296	$71,024,29
By-product credit	$33,377,419	$33,377,419	$33,377,419	$33,377,419	$33,377,419	$33,377,419	$33,377,419	$33,377,419
Total annual sales	$104,401,715	$104,401,715	$104,401,715	$104,401,715	$104,401,715	$104,401,715	$104,401,715	$104,401,715
Annual manufacturing cost								
Feedstock	$36,000,735	$36,000,735	$36,000,735	$36,000,735	$36,000,735	$36,000,735	$36,000,735	$36,000,735
Baghouse bags				$519,687				
Other variable costs	$12,850,987	$12,850,987	$12,850,987	$12,850,987	$12,850,987	$12,850,987	$12,850,987	$12,850,987
Fixed variable costs	$6,763,897	$6,763,897	$6,763,897	$6,763,897	$6,763,897	$6,763,897	$6,763,897	$6,763,897
Total product cost	$48,851,722	$48,851,722	$48,851,722	$49,371,408	$48,851,722	$48,851,722	$48,851,722	$48,851,722
Annual depreciation								
General plant writedown								
Depreciation charge								
Remaining value								
Steam plant writedown	4.46%	4.46%	4.46%	4.46%	4.46%	4.46%	4.46%	4.46%
Depreciation charge	$3,537,270	$3,536,477	$3,537,270	$3,536,477	$3,537,270	$3,536,477	$3,537,270	$3,536,477
Remaining value	$26,526,353	$22,989,876	$19,452,606	$15,916,129	$12,378,859	$8,842,382	$5,305,112	$1,768,635
Net revenue	$52,012,723	$52,013,516	$52,012,723	$51,493,830	$52,012,723	$52,013,516	$52,012,723	$52,013,516
Losses forward	$0	$0	$0	$0	$0	$0	$0	$0
Taxable income	$52,012,723	$52,013,516	$52,012,723	$51,493,830	$52,012,723	$52,013,516	$52,012,723	$52,013,516
Income tax	$13,003,181	$13,003,379	$13,003,181	$12,873,457	$13,003,181	$13,003,379	$13,003,181	$13,003,379
Annual cash income	$42,546,812	$42,546,614	$42,546,812	$42,156,849	$42,546,812	$42,546,614	$42,546,812	$42,546,614
Discount factor	0.2897	0.2633	0.2394	0.2176	0.1978	0.1799	0.1635	0.1486
Annual present value	$12,324,296	$11,203,853	$10,185,369	$9,174,559	$8,417,660	$7,652,383	$6,956,744	$6,324,283
Total capital investment + interest								
Net present worth								
NPV of income tax	$3,766,558	$3,424,196	$3,112,858	$2,801,639	$2,572,610	$2,338,772	$2,126,124	$1,932,869
NPV of ethanol income	$20,573,209	$18,702,917	$17,002,652	$15,456,956	$14,051,778	$12,774,344	$11,613,040	$10,557,309

Year	21	22	23	24	25	26	27	28
Fixed capital investment								
Land								
Working capital								
Loan payment	$0	$0	$0	$0	$0	$0	$0	$0
Loan interest payment	$0	$0	$0	$0	$0	$0	$0	$0
Loan principal	$0	$0	$0	$0	$0	$0	$0	$0
Ethanol sales	$71,024,296	$71,024,296	$71,024,296	$71,024,296	$71,024,296	$71,024,296	$71,024,296	$71,024,29
By-product credit	$33,377,419	$33,377,419	$33,377,419	$33,377,419	$33,377,419	$33,377,419	$33,377,419	$33,377,419
Total annual sales	$104,401,715	$104,401,715	$104,401,715	$104,401,715	$104,401,715	$104,401,715	$104,401,715	$104,401,715
Annual manufacturing cost								
Feedstock	$36,000,735	$36,000,735	$36,000,735	$36,000,735	$36,000,735	$36,000,735	$36,000,735	$36,000,735
Baghouse bags	$519,687					$519,687		
Other variable costs	$12,850,987	$12,850,987	$12,850,987	$12,850,987	$12,850,987	$12,850,987	$12,850,987	$12,850,987
Fixed variable costs	$6,763,897	$6,763,897	$6,763,897	$6,763,897	$6,763,897	$6,763,897	$6,763,897	$6,763,897
Total product cost	$49,371,408	$48,851,722	$48,851,722	$48,851,722	$48,851,722	$49,371,408	$48,851,722	$48,851,722
Annual depreciation								
General plant writedown								
Depreciation charge								
Remaining value								
Steam plant writedown	2.23%							
Depreciation charge	$1,768,635							
Remaining value	$0							
Net revenue	$53,261,672	$55,549,993	$55,549,993	$55,549,993	$55,549,993	$55,030,307	$55,549,993	$55,549,993
Losses forward	$0	$0	$0	$0	$0	$0	$0	$0
Taxable income	$53,261,672	$55,549,993	$55,549,993	$55,549,993	$55,549,993	$55,030,307	$55,549,993	$55,549,993
Income tax	$13,315,418	$13,887,498	$13,887,498	$13,887,498	$13,887,498	$13,757,577	$13,887,498	$13,887,498
Annual cash income	$41,714,889	$41,662,495	$41,662,495	$41,662,495	$41,662,495	$41,272,730	$41,662,495	$41,662,495
Discount factor	0.1351	0.1228	0.1117	0.1015	0.0923	0.0839	0.0763	0.0693
Annual present value	$5,636,957	$5,118,070	$4,652,791	$4,229,810	$3,845,282	$3,463,007	$3,177,919	$2,889,017
Total capital investment + interest								
Net present worth								
NPV of income tax	$1,799,320	$1,706,023	$1,550,930	$1,409,937	$1,281,761	$1,154,336	$1,059,306	$963,006
NPV of ethanol income	$9,597,554	$8,725,049	$7,931,863	$7,210,784	$6,555,258	$5,959,326	$5,417,569	$4,925,063

Year	29	30
Fixed capital investment		
Land		-$1,848,000
Working capital		-$20,891,007
Loan payment	$0	$0
Loan interest payment	$0	$0
Loan principal	$0	$0
Ethanol sales	$71,024,296	$71,024,296
By-product credit	$33,377,419	$33,377,419
Total annual sales	$104,401,715	$104,401,715
Annual manufacturing cost		
Feedstock	$36,000,735	$36,000,735
Baghouse bags		
Other variable costs	$12,850,987	$12,850,987
Fixed variable costs	$6,763,897	$6,763,897
Total product cost	$48,851,722	$48,851,722
Annual depreciation		
General plant writedown		
Depreciation charge		
Remaining value		
Steam plant writedown		
Depreciation charge		
Remaining value		
Net revenue	$55,549,993	$55,549,993
Losses forward	$0	$0
Taxable income	$55,549,993	$55,549,993
Income tax	$13,887,498	$13,887,498
Annual cash income	$41,662,495	$41,662,495
Discount factor	0.0630	0.1486
Annual present value	$2,626,379	$6,192,864
Total capital investment + interest		-$3,380,009
Net present worth		
NPV of income tax	$875,460	$2,064,288
NPV of ethanol income	$4,477,330	$10,557,309