Modeling, Optimization, and Life Cycle Assessment of Bioethanol Production

by

© Jalil Shadbahr

A thesis submitted to the

School of Graduate Studies

in partial fulfilment of the requirements for the degree of

Doctor of Philosophy

Faculty of Engineering and Applied Science

Memorial University of Newfoundland

July 2017

St. John's Newfoundland

Dedicated to

My wife and my parents

For their endless support and faith

ABSTRACT

Second generation bioethanol produced primarily from lignocellulosic biomass resources has attracted great attention over the past two decades due to its numerous advantages such as: (i) potential to reduce environmental impacts in comparison to fossil fuels (ii) capability to mix with gasoline and use in vehicles without modifications in regular engines, and (iii) not competing with food resources that are being used in first generation ethanol.

Simultaneous Saccharification and Fermentation (SSF) approach was proffered for this research over the Separate Hydrolysis and Fermentation (SHF) method to mitigate the inhibition impacts of hydrolysis products and reduce the capital costs of process. SSF process was experimentally studied in a batch media at various levels of enzyme loading and sugars concentration to investigate the interactive influences of sugars concentration and enzyme loading on the final ethanol yield and concentration. Results indicate that cellulase inhibition by cellobiose and glucose is remarkable when enzyme loading is increased from intermediate to high level, particularly at high initial sugars concentrations. The acquired experimental data from batch SSF reactions were consequently applied to determine five major kinetic parameters (k1, k2, Keq, λ, and μ) of kinetic models which incorporate the synergistic effects of supplementing β-glucosidase with cellulase on cellulose conversion and end-product inhibitions. The accuracy and reliability of the derived kinetic parameters were then verified by the good agreement between experimental results and the simulation concentration profiles of sugars and ethanol using tuned parameters under different reaction conditions.

Multi-objective optimization of the SSF process based on mechanistic kinetic and reaction models was carried out in this study to further improve the SSF performance by simultaneously maximizing the ethanol yield/concentration and minimizing enzyme loading. Controlled elitist genetic algorithm, a variant of NSGA II, was used for bi-objective optimization of three case studies with a varied combination of objectives and constraints. The optimized objectives in each case were validated by experiments at the corresponding operating parameters. Comparing the results with non-optimized experiments proved that optimization is capable of improving the objectives.

Lower environmental impact is an important criterion when selecting the best technology for lignocellulose to bioethanol conversion. In this study, the influence of pretreatment process design on the environmental performance of the chained ethanol production process was evaluated by life cycle analysis. Resulting substrates by two pretreatment designs led to significant differences in final ethanol concentration. The amount of produced ethanol as the functional unit in the life cycle analysis of a bioethanol production plant will significantly affect the environmental performance of the system. LCA was performed in small scale (pretreatment unit) and large scale (bioethanol plant) for both scenarios and results confirmed that pretreatment process leading to higher final ethanol concentration helps to mitigate the environmental impacts of the whole production process in most environmental impact categories

ACKNOWLEDGEMENTS

I would like to express my deepest appreciation to my supervisors, Dr. Yan Zhang and Dr. Faisal Khan, whose expertise, support, generous guidance, and understanding helped me complete this thesis.

I was fortunate to have Dr. Yan Zhang as my advisor, who always encouraged me to look at the challenges from a different point of view. Her patience, suggestions, valuable comments and experience have been always helpful to overcome the challenges to the completion of this dissertation. I am indebted to her for her advice and thoughtful feedback.

I am honored to work with Dr. Faisal Khan, who always aimed at moving me forward. I am so grateful to him for insightful comments, prompt feedback and helpful discussion at various stages of my study. His support and advice were essential for this work.

I am also grateful to Dr. Syed Imtiaz as a member of my supervisory committee for his encouragement and useful comments.

Support of this research from the Natural Sciences and Engineering Research Council (NSERC) of Canada, Newfoundland Centre for Forest Science and Innovation and Memorial University of Newfoundland are gratefully acknowledged.

Finally, I deeply thank my parents and my beloved wife for their endless love and patience. None of this would have been possible without their love, support, concern, and strength.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND SYMBOLS

$[B]$	concentration of cellobiose (g/L)
$[C]$	concentration of cellulose (g/L)
$[E]$	concentration of ethanol (g/L)
$[E]_m$	Ethanol concentration above which cells do not grow (g/L)
$[E']_m$	Ethanol concentration above cells do not produce ethanol (g/L)
E_{1B}	Bound concentration of CBH and EG (g/kg)
E_{2B}	Bound concentration of β-glucosidase (g/kg)
E_{2F}	Concentration of β-glucosidase in solution (g/kg)
$[EC]$	Cellulase enzyme concentration (g/L)
$[G]$	concentration of glucose (g/L)
K_{eq}	cellulase adsorption saturation constant (g/L)
K_E	ethanol inhibition constant for the microorganism (g/L)
K_G	glucose saturation constant for the microorganism (g/L)
K_L	constant for β-glucosidase adsorption to lignin (g/L)
K_m	cellobiose saturation constant for β-glucosidase (g/L)
K_{1B}	inhibition constant of cellulase by cellobiose (g/L)
K_{1E}	inhibition constant of cellulase by ethanol (g/L)
K_{1G}	inhibition constant of cellulase by glucose (g/L)
K_{2G}	inhibition constant of β-glucosidase by glucose (g/L)
K_{XE}	Inhibition of cell growth by ethanol (g/L)

K_{MO}	Monod constant (g/L)
K_S	Monod constant for growth on glucose or xylose (g/L)
K'_S	Monod constant for product formation from glucose or xylose (g/L)
K_i	Inhibition constant for growth on glucose or xylose (g/L)
K'_i	Inhibition constant for product formation from glucose or xylose (g/L)
K_{iIG}	Inhibition constants for glucose (g/kg)
K_{iIB}	Inhibition constants for cellobiose (g/kg)
K_{iIX}	Inhibition constants for xylose (g/kg)
K_{3M}	Substrate (cellobiose) saturation constants (g/kg)
$[L]$	concentration of lignin (g/L)
$[M]$	concentration of mannose (g/L)
R_S	Substrate reactivity
S	Substrate concentration (glucose or xylose) (g/L)
T	time (h)
$[X]$	concentration of cell mass (g/L)
Y_{XG}	yield coefficient of cell mass from glucose (g/g)
Y_{EG}	Yield of ethanol cells per gram of glucose
enzc	cellulase activity concentration (FPU/g cellulose)
enzg	β-glucosidase activity concentration (IU/g cellulose)
k_1	maximum specific rate of cellulose hydrolysis to cellobiose (h^{-1})
k_1'	lumped specific rate of cellulose hydrolysis to cellobiose (h^{-1})
k_2	specific rate of cellobiose hydrolysis to glucose (g/[IU h])

k_2'	lumped specific rate of cellobiose hydrolysis to glucose (g/[L·h])
k_{endo}	Hydrolysis rate constant of endoglucanase (h^{-1})
k_{exo}	Hydrolysis rate constant of exoglucanase (h^{-1})
k_{ir}	Reaction rate constants (kg/g.h)
m_s	specific rate of substrate consumption for maintenance requirements (h^{-1})
r_G	volumetric rate of glucose consumption (g/[L·h])
r_M	volumetric rate of mannose consumption (g/[L·h])
r_X	volumetric rate of cell mass production (g/[L·h])
r_1	volumetric rate of cellulose hydrolysis to cellobiose (g/[L·h])
r_2	volumetric rate of cellobiose hydrolysis to glucose (g/[L·h])
α	Constant in product inhibition model
β	beta glucosidase concentration (g/L)
λ	rate of decrease in cellulose specific surface area (h^{-1})
μ_m	maximum specific growth rate of the microorganism (h^{-1})
σ_{endo}	Endoglucanase enzyme capacity
σ_{exo}	Exoglucanase enzyme capacity
τ	Delay constant of exoglucanase hydrolysis (h)
v	Specific rate of product formation (h^{-1})
v_m	Maximum specific rate of product formation (h^{-1})
γ	Constant in product inhibition model

1 INTRODUCTION

1.1 Research Background and Problem Statement

Ethanol produced from inexpensive and abundant lignocellulosic biomass has been considered as one of the most attractive and promising renewable energy sources [1]. Lignocellulosic biomass refers to inedible plant materials made up primarily of cellulose, hemicelluloses, and lignin. Biochemical conversion of lignocellulosic biomass, which involves the release of monomeric sugars from cellulose and hemicellulose and their fermentation into ethanol, is currently the dominant technology for bioethanol production [2]. Although the cost of biochemical ethanol production has been reduced remarkably due to the advances in enzyme biotechnology, there are still economic, technical and environmental challenges for implementing lignocellulosic ethanol on the industrial scale.

Overcoming the natural recalcitrance of lignocellulosic biomass by chemical, physicochemical or biological pretreatments is necessary to efficiently convert biomass into ethanol. The alterations of macroscopic and microscopic structures of biomass occurred during pretreatment basically include removal of lignin, decrease in the crystallinity of cellulose, and increase in the surface area and porosity of the biomass. Due to the variety of biomass sources, there is no stand-alone pretreatment method for all biomass. Selection of pretreatment method and its conditions depends on the nature of biomass, processing efficiency and cost. Moreover, an unavoidable problem encountered in pretreatment step is the generation of lignocellulose-derived by-products that act as inhibitors for enzymes and fermenting microorganisms in the subsequent conversion steps

[3, 4]. Development of cheap detoxification methods to remove and/or neutralize inhibitors and strategies to lower the pretreatment cost deserves further study.

Pretreated biomass comprising water-insoluble solids (mainly cellulose and lignin) and a liquid fraction composed of partially hydrolyzed hemicellulosic sugars typically undergoes enzymatic hydrolysis and fermentation to finally convert into ethanol. These operations can be carried out consecutively (separate hydrolysis and fermentation), simultaneously (simultaneous saccharification and fermentation) or fully consolidated (consolidated bioprocessing) [5]. Although separate hydrolysis and fermentation (SHF) allows two reactions to be operated under their optimal conditions (temperature, pH, nutrient composition, solid loading), severe inhibition of cellulolytic enzyme activity by released glucose or cellobiose is the main problem which significantly reduces the depolymerization efficiency. Consolidated bioprocessing (CBP) is a one-step process in which lignocellulosic biomass is directly converted into ethanol by special microorganism or microbial consortium without pretreatment. However, lacking of suitable and efficient microorganism/microbial consortium makes CBP somewhat risky [6]. Simultaneous saccharification and fermentation (SSF) is capable of attenuating inhibition of cellulolytic enzymes by intermediately consuming glucose released from cellulose. Nonetheless, SSF carried out at enzyme suboptimal temperatures slows the hydrolysis rate, careful optimization of SSF conditions must be done for balanced hydrolysis and fermentation rates [7-9].

Although a good ethanol yield is usually achieved in SSF, final ethanol concentration and productivity is still quite low compared with starch- and sugar-based ethanol production

processes. High substrate loadings, which are inevitable for achieving ethanol concentration, are hardly achievable due to the limitations of mixing and mass transfer caused by the high viscosity of the medium and high toxicity due to the pre-concentration of inhibitors [10,11]. Moreover, SSF performance (i.e., ethanol yield or concentration) is also highly influenced by the amount of enzyme used. Higher enzyme concentrations can increase the conversion of cellulose into glucose, and consequently, the concentration of ethanol, but also increases the operating cost [12]. Understanding the interactions between enzyme and substrate loadings is therefore important in optimizing the SSF performance. So far, most of the optimization studies of SSF process are based on statistically designed experiments, which are valid only in range of parameters studied and therefore cannot be applied to wider ranges directly [13-16]. Systematic optimization of SSF based on experimentally validated kinetic model is highly needed for more accurate and widely applicable optimization results.

As environmental awareness increases, industries and businesses are assessing how their activities affect the environment. Life cycle assessment (LCA) has been a strong tool to analyse the environmental impacts of any process and product and been implemented in recent years for LCA of bioethanol. Major objectives of performed studies on LCA of bioethanol have been concentrated on either contrasting the environmental impacts of bioethanol and conventional fossil fuels (mainly gasoline) [17, 18], or comparing the LCA results of bioethanol production from different sources of biomass [19,20]. The significance of influence of process design on the environmental performance of the bioethanol production has been discounted in literature and requires more consideration.

Pretreatment as a key process for an ethanol plant could be considered for LCA study at different process designs. Research on how process design and technology improvements in an ethanol plant affect the environmental performances of the system is required.

1.2 Objectives

This PhD study addresses the technical and environmental challenges of second generation bioethanol production by incorporating the modeling, simulation and optimization of batch SSF process as well as LCA assessment of ethanol plant with different process designs. Specifically, the objectives of this study are to i) identify the interactions of enzyme and substrate loadings on SSF; ii) determine the kinetic parameters over wide initial sugars concentrations and enzyme loadings; iii) perform multi-objective optimizations of SSF process to simultaneously maximize ethanol yield and minimize enzyme consumptions; and iv) analyze the environmental impacts of second-generation ethanol plant via LCA analyses with different process designs.

1.3 Thesis Contribution

This thesis contributes to the better understanding of SSF process and highlights the importance of process design on output of an ethanol plant and accordingly environmental impacts of the ethanol production process. It implements a kinetic model which alongside the hydrolysis of cellulose, considers the simultaneous fermentation of glucose and mannose. This model uses a wider range of fermentable sugars concentration to tune five

kinetic parameters based on the batch SSF experiments and offers a domain for each parameter depending on the enzyme usage and sugars concentration. The good agreement among modeling and experimental results confirms the reliability of the model for further application in process optimization. Moreover, this study firstly attempted a multi-objective optimization of SSF process based on the experimentally-verified mechanistic kinetic model. In contrast to extensive researches on single objective optimization of SSF, this work offers an optimization intending to optimize two objectives by providing a set of solutions that reflects the trade-offs between the objectives. This set of solutions unlike the one solution in single objective optimization enables the decision makers to perform the reaction in optimum condition at various situation of process.

Furthermore, the originality of this thesis also contributes to offer the life cycle assessment of an ethanol production plant at two different pretreatment designs and two levels of analysis. To the best of my knowledge, no research has been so far investigated the environmental performance of an ethanol plant with a certain type and amount of feedstock but different process designs. Based on two pretreatment scenarios, LCA study compares the environmental impacts of pretreatment unit in the first level and whole ethanol plant in the second level. This approach also helps the decision makers to alongside the economic issues, compare the environmental influences of each process for large scale production of bioethanol.

1.4 Proposed Methodology and Scope of Work

The scope of this study covers the simultaneous saccharification and fermentation process in the batch media as well as environmental performance of an ethanol plant at two pretreatment designs through life cycle assessment.

The interactive influence of the initial concentrations of fermentable sugars and enzyme loading on the batch SSF process was explored by experiments firstly. Impacts of initial sugars concentration and enzyme loading on the ethanol yield and final concentration were investigated. Product inhibition impact and the role of enzyme loading on the SSF efficiency were investigated by performing SSF experiments in a batch reactor and results are discussed in details.

The acquired experimental results then were implemented for kinetic modeling of the SSF process. The applied kinetic model reported by Philippidis et al. [21] and Pettersson et al. [22] was chosen and developed to achieve the kinetic parameters at various combinations of sugars concentration and enzyme loading based on the experimental data. Five major kinetic parameters were determined by least square fitting of experimental measurements. The validity of the derived kinetic parameters was verified by comparing the simulated ethanol concentration profiles estimated using kinetic parameters with experimental results of batch SSF under different operating conditions.

In order to render the process economically, optimization of multi-objectives have been considered. Objectives such as ethanol yield or cellulose conversion must be maximized while the amount of enzyme loading and required enzyme per unit of produced ethanol

need to be minimized in favor of feasible production of ethanol. The kinetic model was implemented to optimize the process in different cases of optimization. In each case two objectives were assigned to be optimized regarding to subjected constraint and defined range of decision variables. The results of optimization are presented as optimal solutions diagram and in each case of optimization, results have been validated experimentally regarding to the specified operation parameters.

Investigating the importance of the pretreatment process design on the environmental performance of a bioethanol production plant through life cycle analysis is also in the scope of this thesis. The bioethanol production plant designed by National Renewable Energy Laboratory (NREL) [23] was selected for this study which uses dilute acid pretreatment. Two scenarios for pretreatment unit were studied and some modifications based on the Wayman et al. (2009) [24] and Humbird et al. (2011) [25] were applied in the new scenario. The effectiveness of different scenarios¬ of pretreatment units on the life cycle analysis results of the individual unit as well as of the whole production plant was studied. Comparative LCA study based on two pretreatment designs, provide an insightful tool to compare the environmental impacts of each process in limited and large perspective of an ethanol production plant.

1.5 Organization of the Thesis

A comprehensive literature review of cellulosic ethanol production, different technologies for pretreatment, hydrolysis and fermentation approaches, modeling of SSF process, and optimization of SSF process have been presented in chapter 2. Chapter 3 investigates the

interactions of major components involved in the SSF process through various experiment runs and analyze the role of enzyme loading amount and sugars concentration on final ethanol concentration. Chapter 4 focuses on a kinetic model that kinetic parameters are tuned by using experimental results. Inhibition impacts of produced product for each process have been discussed and reliable kinetic model with adjusted parameters for each range of operation parameters were achieved. Chapter 5 presents the multi-objective optimization of SSF process. Optimization was performed for three case studies with different objectives and constraints combination and results were validated experimentally. Chapter 6 analyzes the environmental impacts of pretreatment unit and whole bioethanol plant at two pretreatment scenarios. Base scenario was modified by some changes in process designs. Life cycle analysis at two levels for two pretreatment scenarios was performed and environmental impacts of two cases were compared. Chapter 7 makes a summary of the thesis and illustrates the main conclusions of this work. Recommendations for future studies on this subject has been provided at the end of this chapter.

1.6 References

1. Conde-Mejia, C., Jimenez-Gutierrez, A., & El-Halwagi, M. (2012). A comparison of pretreatment methods for bioethanol production from lignocellulosic materials. Process Safety and Environmental Protection, 90, 189–202.
2. Kumar, S., Singh, S. P., Mishra, I. M., & Adhikari, D. K. (2009). Recent advances in production of bioethanol from lignocellulosic biomass. Chemical Engineering Technology, 32, 517–526.

3. Wang, K., Jiang, J. X., Xu, F., & Sun, R.C. (2009). Influence of steaming explosion time on the physico-chemical properties of cellulose from Lespedeza stalks. Bioresoure Technology, 100, 5288-5294.
4. Kumar, P., Barrett, D. M., Delwiche, M. J., & Stroeve, P. (2009). Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Ind Eng Chem Res, 48 (8), 3713-3729.
5. Balat, M. (2011). Production of bioethanol from lignocellulosic materials via the biochemical pathway: A review. Energy Conversion and Management, 52, 858-875.
6. Olson, D. G., McBride, J. E., Shaw, A. J., & Lynd, L.R. (2012). Recent progress in consolidated bioprocessing. Current Opinion in Biotechnology, 23(3), 396-405.
7. Hoyer, K., Galbe, M., & Zacchi, G. (2009). Production of fuel ethanol from softwood by simultaneous saccharification and fermentation at high dry matter content. J ChemTechnol Biotechnol, 84, 570-577.
8. Shin, D., Yoo, A., Kim, S. W., & Yang, D. R. (2006). Cybernetic modeling of simultaneous saccharification and fermentation for ethanol production from steam-exploded wood with Brettanomyces custersii. Journal of Microbiology and Biotechnology, 16(9), 1355-1361.
9. van Zyl, J. M., van Rensburg, E., van Zyl, W. H., Harms, T. M., & Lynd, L. R. (2011). A Kinetic Model for Simultaneous Saccharification and Fermentation of Avicel with Saccharomyces cerevisiae. Biotechnology and Bioengineering, 108, 924-933.

10. Zhu, J. Y., & Pan, X. J. (2010). Woody biomass pretreatment for cellulosic ethanol production: technology and energy consumption evaluation. Bioresource Technology, 101, 4992–5002.
11. Koppram, A., & Olsson, L. (2014). Combined substrate, enzyme and yeast feed in simultaneous saccharification and fermentation allow bioethanol production from pretreated spruce biomass at high solids loadings. Biotechnology for Biofuels, 7, 1-9.
12. Paulova, L., Patakova, P., Branska, B., Rychtera, M., & Melzoch, K. (2015). Lignocellulosic ethanol: Technology design and its impact on process efficiency. Biotechnology Advances, 33(6), 1091-1107.
13. Betiku, E., & Taiwo, A.E. (2015). Modeling and optimization of bioethanol production from breadfruit starch hydrolyzate vis-à-vis response surface methodology and artificial neural network. Renewable Energy, 74, 87-94.
14. Shanavas, S., Padmaja, G., Moorthy, S.N., Sajeev, M.S., & Sheriff, J.T. (2011). Process optimization for bioethanol production from cassava starch using novel eco-friendly enzymes. Biomass and Bioenergy, 35(2), 901-909.
15. Sivamani, S., & Baskar, R. (2015). Optimization of bioethanol production from cassava peel using statistical experimental design. Environ. Prog. Sustain. Energy, 34, 567-574.
16. Wahono, S. K., Rosyidaa, V. T., Darsiha, C., Pratiwia, D., Frediansyaha, A., & Hernawana. (2015). Optimization of Simultaneous Saccharification and Fermentation Incubation Time Using Cellulose Enzyme for Sugarcane Bagasse on The Second-Generation Bioethanol Production Technology. Energy Procedia, 65, 331-36.

17. Von Blottnitz, H., & Curran, M. A. (2007). A review of assessments conducted on bioethanol as a transportation fuel from a net energy, greenhouse gas, and environmental life cycle perspective. Journal of Cleaner Production, 15, 607–619.
18. Hedegaard, K., Thyo, K. A., & Wenzel, H. (2008). Life cycle assessment of an advanced bioethanol technology in the perspective of constrained biomass availability. Environmental Science Technology, 42, 7992–7999.
19. Davis, S. C., Anderson-Teixeira, K. J., & DeLucia, E. H. (2009). Life-cycle analysis and the ecology of biofuels. Trends in Plant Science, 14, 140–146.
20. Gonzalez-Garcia, S., Moreira, M. T., & Feijoo, G. (2010). Comparative environmental performance of lignocellulosic ethanol from different feedstocks. Renewable and Sustainable Energy Reviews, 14, 2077–2085.
21. Philippidis, G. P., Spindler, D. D., & Wyman, C. E. (1992). Mathematical modeling of cellulose conversion to ethanol by the simultaneous saccharification and fermentation process. Applied Biochemistry and Biotechnology, 34-5, 543-556.
22. Pettersson, P. O., Eklund, R., & Zacchi, G. (2002). Modeling Simultaneous Saccharification and Fermentation of Softwood. Applied Biochemistry and Biotechnology, 98-100, 733-746.
23. Wooley, R., Ruth, M., Sheehan, J., Ibsen, K., Majdeski, H., & Galvez, A. (1999). NREL/TP-580-26157. Golden: Colorado National Laboratory of the U.S. Department of Energy. Lignocellulosic Biomass to Ethanol Process Design and Economics Utilizing Co-Current Dilute Acid Prehydrolysis and Enzymatic Hydrolysis Current and Futuristic Scenarios.

24. Wyman, C. E., Dale, B. E., Elander, R. T., Holtzapple, M., Ladisch, M. R., Lee, Y. Y., Mitchinson, C., & Saddler, J. N. (2009). Comparative sugar recovery and fermentation data following pretreatment of poplar wood by leading technologies. Biotechnology Progress, 25, 333–339.

25. Humbird, D., Davis, R., Tao, L., Kinchin, C., Hsu, D., Aden, A., Schoen, P., Lukas, J., Olthof, B., Worley, M., Sexton, D., & Dudgeon, D. (2011). NREL/TP-5100-47764. Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol Dilute-Acid Pretreatment and Enzymatic Hydrolysis of Corn Stover.

2 LITERATURE REVIEW

Modern societies have a high and increasing dependency on fossil fuels [1] and the scarcity of these resources in the future will cause serious problems as above 80% of required energy is provided by fossil resources [2, 3]. Additionally, because of the increasing rate of the world's population, many concerns have been debated regarding environmental issues caused by fossil fuels' consumption such as acid rain and global warming [4-7]. Thus, investigation of alternative sources of energy such as renewable fuels is obligatory.

Biofuels are produced in the forms of solid, liquid, and gas from renewable biomass and include bioethanol, biogas, biobutanol, and biodiesel [5, 8-10]. Bioethanol is one of the most important and prevalent biofuels in the market and replaces some portion of fossil fuels [11, 12].

Current bioethanol, which is blended with gasoline for the market, is generated from sugar cane and corn, is called first generation bioethanol. Due to the competition of the sources with food resources, this first generation has been questioned as a sustainable source. For this reason, second generation of biofuels which could be produced by lignocellulosic biomass has attracted the interest of researchers [13-16].

There are two major pathways for producing bioethanol from lignocellulosic biomass: (1) biochemical conversion and (2) thermochemical conversion [17, 18]. In the thermochemical conversion route, raw biomass is first gasified at the temperature of about 800°C and then the produced syngas (hydrogen, carbon dioxide, and carbon monoxide) will be converted to a mixture of alcohols in the presence of a catalyst. Separation of

produced alcohols will be performed in the next step through the distillation process. The biochemical conversion pathway includes four major steps: pretreatment, hydrolysis, fermentation, and product recovery [17-21]. The biochemical conversion route is the main subject of this thesis and will be discussed in details in the following sections.

2.1 Lignocellulosic Biomass

Lignocellulosic biomass has attracted many researchers in the last decades as one of the most promising and sustainable sources for producing bioethanol [22]. Forestry and agricultural residues, the most abundant sources of lignocellulosic materials, are low-cost and sustainable feedstocks for bioethanol production [23]. Three major components create the structure of lignocellulosic biomass: cellulose, hemicellulose, and lignin. Cellulose, hemicellulose, and lignin contents of lignocellulosic materials are different in amount and their entangled structure. These components create a complex matrix where lignin and hemicellulose surround cellulose and protect it from access to the enzyme which causes recalcitrance of enzymatic hydrolysis of cellulose [24-29]. Different lignocellulosic biomass sources have various amount of cellulose, hemicellulose, and lignin. Lignin is a random aromatic compound which hinders cellulosic bioethanol production due its strong linkages to cellulose and hemicellulose. The complex nature of lignin polymerization makes a challenge for cellulose separation and further depolymerisation. Prior to cellulose hydrolysis, the lignin content of biomass must be removed in order to provide access for the enzyme to reach the cellulose. Lignin content of softwood is higher than for other biomass which means that cellulose and hemicellulose amount of softwood is lower which

makes the softwood pretreatment more severe. In addition to this, amongst the same categories of biomass, hemicellulose content also varies in sub-components. While xylan is a major part of hemicellulose in agriculture residues and even hardwoods, the majority of hemicellulose content in softwoods is mannan. These different compositions in major components will cause a different output combination of the pretreated product and highly influence the subsequent ethanol production units, hydrolysis and fermentation [30-36].

2.2 Biochemical Conversion of Lignocellulosic Biomass to Ethanol

The process of biochemical conversion of lignocellulosic biomass into bioethanol consists of different steps. As is shown in Figure 2.1, the main steps start with pretreatment and then are followed by enzymatic hydrolysis (saccharification) and fermentation and finally, the product will be purified using separation processes such as distillation or dehydration. The pretreatment process treats the raw biomass to release cellulose and hemicellulose by cleaving the bonds that lignin has created around their polymeric chains. Most of the hemicellulose and some portion of the cellulose are hydrolysed in the pretreatment step and monomeric sugars are released. Unconverted cellulose is hydrolysed in the hydrolysis process to liberate glucose. Potentially, the produced glucose and released sugars from pretreatment could be fermented to ethanol in the fermentation process. Purification of the produced bioethanol takes place in the last step [37-39]. In the following sections, three main steps including pretreatment, hydrolysis, and fermentation are further discussed in details.

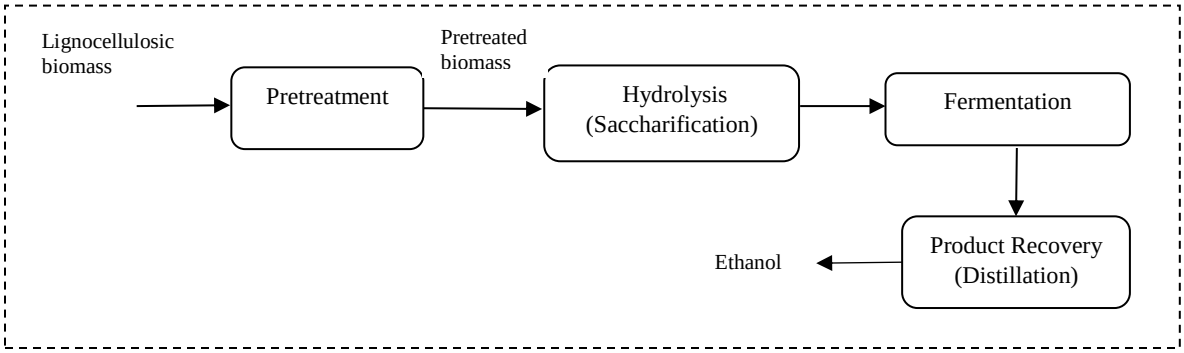


Figure 2.1. Main steps of bioethanol production from lignocellulosic biomass

2.3 Pretreatment of Lignocellulosic Biomass

Lignocellulosic biomass is an abundant and affordable resource for bioethanol production, but to convert this rich polysaccharide content resource into feasible ethanol, a pretreatment process is unavoidable. The recalcitrant character of lignocellulosic biomass enables it to resist any kind of deformation in the crystalline structure of biomass and therefore makes it more difficult to hydrolyze the cellulose fiber into monomeric sugars [40-42]. The main purpose of the pretreatment process is to make the cellulose available for enzyme hydrolysis and this occurs by diminishing the recalcitrance of lignocellulosic biomass through breaking the strong linkage between hemicellulose and lignin to cellulose and removing them [41, 42].

Numerous pretreatment methods have been investigated. Regarding the composition of the raw biomass, different technologies might be more effective in disrupting the lignocellulosic structure of biomass. In general they can be categorized into three main approaches: biological pretreatment, physical pretreatment, and chemical pretreatment. The biological pretreatment method uses microbes for removing lignin and despite low

energy consumption, the slow rate of reaction and degradation of materials prevent its being a promising methodology [11].

Physical methods of pretreatment include ball milling, extrusion, and comminution and are mostly applied for size reduction and to fractionate the lignocellulosic biomass to increase the accessible surface area of the biomass and enhance the enzymatic hydrolysis. The main obstacle of using these methods is that their energy consumption is high and also they are not feasible for some lignocellulosic biomass such as softwood, although implementing them alongside the chemical pretreatment methods is recommended and enhances the performance of the pretreatment. This is called physicochemical pretreatment [43-48].

Chemical pretreatments are the most promising pretreatment technologies. These methods use chemical substances to release cellulose from lignocellulosic biomass by removing hemicellulose or lignin. According to agent used, chemical pretreatments can be categorized as three major types: Acid pretreatment, Alkaline pretreatment, and Organosolv pretreatment.

2.3.1 Acid pretreatment

Acid pretreatment, which is also called acid hydrolysis, is the most cited and traditional method of pretreatment for lignocellulosic biomass. In this approach several organic (fumaric acid and maleic acid ($C_4H_4O_4$)) and inorganic acids (sulfuric acid (H_2SO_4), hydrochloric acid (HCl), phosphoric acid (H_3PO_4), and nitric acid (HNO_3)) are used [49-55]. However, due to the better efficiency and lower cost, sulfuric acid is the most common

agent for pretreatment of the wide range of biomass [56, 57]. Acid pretreatment enhances the cellulose accessibility by hydrolyzing the hemicellulose and amorphous cellulose fractions of the biomass. Dilute acid (0.2-5 wt %) with a residence time of lower than an hour is usually preferred to concentrated acid due to the corrosive and toxic properties of concentrated acid which increase the cost of an operation to provide a corrosion resistance reactor and recover the used acid [58-60]. The major problem with acid pretreatment is the reaction conditions such as temperature, pH, and residence time, which must be carefully chosen to prevent the components such as hydrolyzed sugars from hemicellulose from converting to furfural and HMF (5-hydroxymethylfurfural), which are inhibitors of the hydrolysis process [61, 62]. SO_2 was also studied as an acid catalyst by Monavari et al. (2009) [63] for softwood pretreatment and showed promising method for this type of biomass.

Among all the pretreatment methods, steam explosion, which is a physicochemical method, is one of the most investigated approaches for pretreatment of lignocellulosic biomass. This method can be applied in the presence or absence of an acid catalyst (H_2SO_4 or SO_2) when biomass material is exposed to high pressure saturated steam (160-230˚C) for a few minutes. Quick release of the pressure causes the removal of hemicellulose from the structure of the lignocellulosic biomass, but removal of lignin is not so considerable. This method has the capability to be used for most biomass such as agricultural residue and softwood. In the case of softwood, an acid catalyst is mandatory to improve the efficiency of the pretreatment process [43, 44, 46, and 64]. Two key parameters in this method are temperature and retention time. Degradation of hemicellulose to fermentable sugars

depends on the duration of the retention time of the substrate. A high retention time causes the degradation of sugars into inhibitory substances such as furfural and 5-hydroxylmethyl-furfural (HMF), which are inhibitors of the hydrolysis process [65, 66].

2.3.2 Alkaline Pretreatment

Alkaline pretreatment is mainly implemented for delignification of low lignin content biomass such as hardwood or agricultural residue. In this method various alkali agents such as ammonia, sodium hydroxide, and calcium hydroxide have been investigated to break the linkage between polysaccharides and lignin and to dissolve the lignin in order to make the cellulose and hemicellulose more accessible. Alkaline pretreatment uses a lower temperature and pressure in comparison to other pretreatment technologies; however, the reaction time could be extended to days [64, 66-69]. The main advantage of alkaline pretreatment over acid pretreatment is milder reaction conditions (temperature and pressure), although conversion of alkali to salts causes problems for an ethanol production plant [38]. Recovering the alkali, the treatment of produced salts as well as the precipitation of salts in the utility sections of the plant are the challenges of alkaline pretreatment [70].

Ammonia fiber explosion (AFEX) is one of the most studied physicochemical techniques of alkaline pretreatment. In this method, the biomass is soaked in ammonia at a temperature of approximately 100^{o}C and under high pressure for 5-60 min and then this is followed by a sudden decrease of the pressure which causes breakage of the lignin linkages and reduction of the lignin content [71-73]. The moderate reaction conditions and higher pH of

reaction media prevent the recovered sugars from converting to undesired inhibitors, in comparison to the steam explosion technique. However, the higher price of ammonia and requirement of the system for ammonia recovery alongside the inefficiency of AFEX for high lignin content biomass are comparative drawbacks of this method [38, 70].

2.3.3 Organosolv pretreatment

Organosolv pretreatment is the technique which uses the organic mixture of an alcohol such as methanol or ethanol with an acid such as acetic or formic acid to remove lignin from the lignocellulosic biomass. Lower molecular weight alcohols for this technique are preferred due to their low boiling points which makes the solvent recovery process easier. This method can be implemented with or without an acid catalyst such as sulfuric or hydrochloric acid to solubilize lignin, hydrolyze hemicellulose and achieve cellulose rich feed for the hydrolysis process [74-76].

The main advantages of organosolv pretreatment are the easy recovery of solvents and the capability of the technique to separate the lignin content of biomass, which can be considered as a by-product of a biorefinery plant. The main problem associated with this technique is the requirement to remove and recover the solvent in order to make the process economically feasible, which increases the energy consumption of the plant. Also, the hydrolysate product must be washed thoroughly to remove the existing solvent that would act as an inhibitor in enzymatic hydrolysis and fermentation processes [77-79].

2.4 Enzymatic Hydrolysis (Saccharification) and Fermentation

Producing bioethanol from lignocellulosic biomass will be followed by two key processes after the pretreatment step: hydrolysis of cellulose (and hemicellulose) and fermentation of released sugars to ethanol. The following sections will describe two processes individually and compare two major approaches to performing hydrolysis and fermentation: Separate Hydrolysis and Fermentation (SHF) and Simultaneous Saccharification and Fermentation (SSF).

2.4.1 Separate Hydrolysis and Fermentation (SHF)

After the pretreatment process, hydrolysis of pretreated materials to fermentable sugars is the next important process. Hydrolysis of the cellulose into fermentable sugars is still the main barrier in the commercial-scale cellulosic ethanol production process, due to the high cost of enzymes [80-82] and low efficiency of the process which is inevitably caused by end product inhibition [83, 84]. The hydrolysis process of cellulose polymers to produce glucose consists of three major sub-processes which are operated by three classes of enzymes. Endo-1,4-beta-glucanases (EC 3.2.1.4) enzyme breaks 1,4-beta-linkages of the amorphous structure of cellulose . Free ends of the cellulose polymer will be hydrolyzed to cellobiose by exo-1,4-beta-glucanases (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21) and then reacts on cellobiose molecules to cleave them into glucose molecules [85-87].

Fermentation is the subsequent process of hydrolysis in order to ferment the fermentable sugars such as glucose, mannose, and xylose into ethanol. These hydrolyzed sugars are coming from either hydrolysis or pretreatment. Different types of microorganisms are currently being used for this purpose but the most common and frequently used

microorganism is Saccharomyces cerevisiae or baker's yeast [88, 89]. The advantages of saccharomyces cerevisiae over the other microorganisms include: high tolerance of ethanol and inhibitors coming from pretreated hydrolysate [90-93], having GRAS (generally regarded as safe) status, mature process technology, and wide usage in industry [94]. On the other hand, a major drawback of the S. cerevisiae is that it cannot utilize the pentose sugars such as xylose existing in pretreated hydrolysate [95, 96]. To overcome this barrier, recombinant genetically engineered microorganisms have recently been shown to be a promising solution [97-100].

The Separate Hydrolysis and Fermentation (SHF) process occurs in sequentially separate reactors for hydrolysis and fermentation; therefore, each process can be performed at its own optimum condition. For instance, the optimum temperature for the hydrolysis process is 45-50˚C, while for the fermentation process 30-37˚C is the best range of temperature [101-103]. Another advantage of SHF is that due to the separate units, lignin removal after the hydrolysis is conceivable; thus, the fermentation process can be performed in continuous mode with cell recycling [104]. Nevertheless, there are some drawbacks involved in performing the SHF process. The largest obstacle of SHF is the inhibitory roles that glucose and cellobiose play in the reaction media as the end-product and intermediate product of hydrolysis, respectively. Both cellobiose and glucose have inhibitory impacts on cellulase and; additionally, glucose effectively inhibits the β-glucosidase [105-107]. To overcome this problem, solids loading must be lowered and enzyme loading must be increased, which may not make the process economically feasible [108]. The other major disadvantage of SHF is the high possibility of contamination. The hydrolysis processing

time is lengthy and due to the existence of produced sugars, might be a suitable environment for growing undesired microbes in the reaction media [103].

2.4.2 Simultaneous Saccharification and Fermentation (SSF)

Integration of hydrolysis and fermentation processes to one step is called Simultaneous Saccharification and Fermentation (SSF). In this approach, the pretreated biomass serves as the substrate; therefore, there are some fermentable sugars already in the liquid fraction which will be fermented immediately and the solid portion consists of cellulose. Cellulose will be hydrolyzed in the reaction media and the produced glucose will be consumed quickly by microorganisms to produce ethanol. Extensive research has demonstrated that SSF improves the biomass conversion by decreasing the inhibitory impact of converted sugars. A higher enzymatic hydrolysis rate of reaction and higher ethanol yield are reported for SSF due to the diminishment of the product inhibition impact of glucose on enzymes [105, 109-111]. Another major advantage of SSF over SHF is the lower risk of contamination, mainly due to lower available sugars and the presence of ethanol in the reactor [103]. In addition, decreasing the cost of the operation by implementing one vessel for both reactions must be considered as an economic advantage of SSF [103]. Nevertheless, compromising the reaction conditions of hydrolysis and fermentation are the major challenges in implementing SSF. As is mentioned in the previous section, each hydrolysis and fermentation has optimum operation conditions. The differences between optimum reaction conditions for hydrolysis and fermentation make the optimal operation condition (pH: 5 and temperature: 37°C) not flexible for SSF in order to compromise between enzyme and yeast requirements [112, 113].

Accumulation of produced ethanol in the reaction media can also cause another disadvantage with the SSF process, due to its inhibitory impact on microorganisms and enzyme. Studies show that ethanol is capable to significantly decrease the enzyme activity [103, 114].

2.5 Kinetic Modeling of SSF Process

Industrialization of ethanol production from lignocellulosic biomass by relying on the experimental data to establish the basic concepts of the process would cost significant resources and time [115]. Many researchers in recent years have been attracted to developing kinetic models to simulate and analyze the behavior of the parameters involved in the SSF process during ethanol production [115-118]. Moreover, optimization of the SSF process without implementing a kinetic model alongside reliably tuned parameters is impossible. Although several mathematical models have been proposed and developed for enzymatic hydrolysis and fermentation individually [119-125], developing the kinetic models for the SSF process and scaling up the application of kinetic models are still required [126-129]. Kadam et al. (2004) [119] proposed a kinetic model based on the Langmuir isotherm for hydrolysis process on pretreated corn stover. The details of proposed model by Kadam et al. are presented in Table 2.1.

Table 2.1. Kinetic model for hydrolysis reaction proposed by Kadam et al. [119]

Reaction	Formulation
Cellulose to cellobiose reaction with glucose, cellobiose, and xylose inhibition	$r_1 = \frac{k_{1r} E_{1B} R_S S}{1 + \frac{B}{K_{1IB}} + \frac{G}{K_{1IG}} + \frac{X}{K_{1IX}}}$
Cellulose to glucose reaction with glucose, cellobiose, and xylose inhibition	$r_2 = \frac{k_{2r} (E_{1B} + E_{2B}) R_S S}{1 + \frac{B}{K_{2IB}} + \frac{G}{K_{2IG}} + \frac{X}{K_{2IX}}}$
Cellobiose to glucose reaction with glucose and xylose inhibition	$r_3 = \frac{k_{3r} E_{2F} B}{K_{3M}(1 + \frac{G}{K_{3IG}} + \frac{X}{K_{3IX}}) + B}$

This proposed model assumes that cellulose can be hydrolyzed into two products: glucose and cellobiose. The produced cellobiose can be further cleaved into glucose. This kinetic model also considers the products inhibition (glucose and cellobiose) as well as substrate inhibition (xylose) on the hydrolysis reaction and K_{iIG}, K_{iIB}, and K_{iIX} represent the inhibition constants for glucose, cellobiose, and xylose, respectively. [119].

A kinetic model of glucose and xylose fermentation was performed by Krishnan et al. (1999) [120] using recombinant saccharomyces cerevisiae to compare the growth rate of glucose, xylose, and a mixture of both. The fermentation kinetic model is demonstrated in Table 2.2.

Table 2.2. Kinetic model for fermentation reaction proposed by Krishnan et al. [120]

Reaction	**Formulation**
Growth rate of microorganism	$\mu = \frac{\mu_m S}{K_S + S + S^2/K_i}(1-(\frac{[E]}{[E]_m})^{\alpha})$
Production rate of ethanol	$\nu = \frac{\nu_m S}{K'_S + S + S^2/K'_i}(1-(\frac{[E]}{[E']_m})^{\gamma})$

Depending on the type of the used substrate (S), this model can be used for both glucose and xylose fermentation. Kinetic parameters of the model, however, are determined through single substrate experiments. Substrate inhibition (glucose and xylose (S)) and product inhibition (ethanol (E)) were incorporated in the proposed model to analyze the inhibition impacts on the growth rate of microorganism (yeast) and the production rate of ethanol [120].

The proposed kinetic model by van Zyl et al. (2011) [129] for SSF process considers the glucose, cellobiose, and ethanol inhibition on the hydrolysis process and ethanol inhibition on the fermentation process. The model is illustrated in Table 2.3.

Table 2.3. Kinetic model for SSF process proposed by van Zyl et al. [129]

Reaction	Formulation
Cellulose hydrolysis:	$\frac{d[C]}{dt} = -(k_{endo}(\frac{[EC]_{endo}}{1+\sigma_{endo}}) + \tanh(\frac{t}{\tau}) k_{exo} \frac{[EC]_{exo}}{1+\sigma_{exo}})(\frac{K_{1B}}{[B]+K_{1B}})(\frac{K_{1E}}{[E]+K_{1E}})$
Cellobiose formation:	$\frac{d[B]}{dt} = -1.055\frac{d[C]}{dt} - \frac{k'_2[B][\beta]}{K_m(1+\frac{[G]}{K_{1G}})+[B]}$
Glucose formation:	$\frac{d[G]}{dt} = -1.111\frac{d[C]}{dt} - 1.052\frac{d[B]}{dt} - \frac{1}{Y_{XG}}\frac{\mu_m[X][G]}{[G]+K_{MO}}(1-\frac{[E]}{K_{XE}})$
Ethanol production:	$\frac{d[E]}{dt} = \frac{Y_{EG}}{Y_{XG}}\frac{\mu_m[X][G]}{[G]+K_{MO}}(1-\frac{[E]}{K_{XE}})$

The model is based in the study done by South et al. (1995) [116] which is based on the created complex between enzyme and substrate (EC). Cellobiose (B), glucose (G), and ethanol (E) inhibition impacts on SSF reaction are investigated in this model. In this study, cellulose is the only primary substrate and other pretreatment products such as recovered sugars from hemicellulose are neglected. The ramp function ($\tanh(t/\tau)$) in the cellulose hydrolysis reaction is accounted for the delay that occurred in the first 10 hours of the reaction due to the non-productive bonding of the exoglucanase enzyme and substrate. Nevertheless, more investigation of this phenomenon is required. Moreover, in this model the Langmuir isotherm is considered for cellobiose formation as does Kadam et al. [119] but glucose formation is modeled based on Michaelis-Menten kinetics [129]. Depending on the regional interest and the type of the available biomass, these proposed models are applicable in a narrow range of substrate and operational conditions.

One of the comprehensive kinetic models for the SSF process is proposed by Philippidis et al. [105, 106, 126, and 127] and later developed by Pettersson et al. [130]. In the first version of the model, glucose is produced either directly from the hydrolysis of cellulose or through cleavage of a cellobiose molecule into two glucose molecules. The primary model also considered the cellulose as substrate and therefore only the inhibition effects of cellobiose, glucose, and ethanol are studied [126]. Pettersson et al. [130] later developed the model by incorporating mannose as the substrate for the SSF process. In the developed model, ethanol production from fermentation of mannose as well as the inhibitory impact of mannose on yeast performance have been taken into account [130]. Three kinetic parameters are tuned by Pettersson et al. [130] at a certain concentration of fermentable sugars.

Although in the developed model, in addition to glucose fermentation, mannose fermentation is also considered [130], two main drawbacks still need to be considered. The first problem is that for tuning the kinetic parameters, only concentrations of ethanol and glucose are considered, while there are other substances that could be influential in modeling, such as cellobiose and mannose concentration. The other disadvantage is that kinetic parameters are tuned in a certain range of sugars concentration, while the achieved hydrolysate from pretreatment could be diluted and mixed with solid fraction in different amounts, depending on the other operational conditions of the SSF process, which can be determined by optimization the of process. These two drawbacks need to be considered to analyse the interactive influence of the initial concentrations of fermentable sugars and enzyme loading, to provide an insight into SSF process optimization. More details of this

model and recommendations to improve its performance for a wider concentration range of glucose and mannose are discussed in chapter four of this thesis.

2.6 Multi-Objective Optimization of SSF Process

Feasible production of cellulosic ethanol is not achievable unless the optimization of the process is performed and due to the decisive role of the SSF process, optimization of this process seems to be unavoidable. Numerous studies have used a single objective such as ethanol productivity [131, 132] or ethanol yield [133, 134]; however, ethanol production optimization, like all industrial cases, involves more than one objective, which in most situations might be in conflict.

Optimization studies on the SSF process mainly rely on the experiments' results. Triwahyuni et al. (2015) [135] optimized the SSF process by means of a series of experiments performed with different amounts of enzyme loading to maximize the ethanol yield. Various reaction times are studied by Wahono et al. (2015) [131] to maximize the final ethanol concentration. Response surface methodology is implemented in some cases, after acquiring experimental data to extract a regression model from experimental results [134, 136, and 137]. These studies have mainly investigated the effects of the key parameters such as cellulose loading, yeast amount, enzyme dosage, solution pH, temperature, and reaction time on ethanol production. Nevertheless, these optimization studies are limited to the specific set of the experiments and may not be applicable to a wider range of parameters of the SSF process.

Kinetic behaviour of parameters involved in the SSF process, such as sugars concentration, cellulose amount, and enzyme loading, determine the performance of the process. The kinetic model analyzes the interactions among the components and provides a perspective of the outcomes of the interactions such as inhibition and deactivation of the enzyme and yeast by the products and primary substrate. Optimization of the SSF process without considering the complex reactions and interactions among the components using a reliable kinetic model does not provide a comprehensive result.

2.6.1 Single Objective vs Multi-Objective Optimization

Despite the extensive studies on optimization of ethanol production using response surface methodology, implementing a reliable kinetic model to optimize the SSF process is rarely investigated. In recent reported studies, Wang et al. (2016) [138] optimized the cell, enzyme, and substrate amount based on a strict kinetic model to maximize the final ethanol concentration. Unrean et al. (2016) [139] optimized the SSF process for maximum ethanol concentration by integrating a dynamic metabolic model of yeast with a hydrolysis model in order to optimize the substrate and yeast loadings.

As can be seen, all these studies have optimized a single objective whilst optimizing one objective will not necessarily lead to the best solution that satisfies other objectives. Therefore, to achieve a cost-effective approach for cellulosic ethanol production, optimizing multi-objectives which may also be in conflict is crucial. Multi-objective optimization offers a set of optimal solutions that illustrates trade-offs among different

objectives. In the case of the SSF process, while maximum ethanol yield or highest ethanol concentration as the product is the first objective, enzyme consumption the second objective, must be the lowest in order to have an optimized process, despite the fact that under certain circumstances, increasing the amount of enzyme may improve the ethanol yield or final ethanol concentration.

In a general form, the multi-objective optimization problem can be formulated as follow:

Objectives: to be minimized/ maximized: $f_n(x)$; $i=1,2,\ldots, N$

Constraints: to confine the results: $g_m(x) \geq 0$; $m=1,2,\ldots, M$

$h_k(x)=0$; $k=1,2,\ldots, K$

Decision variables: Lower bound$\leq x_i \leq$ Upper bound; $i=1,2,\ldots, J$

For a multi-objective problem, a solution will be defined as a vector that consists of J decision variables. Objectives ($f_n(x)$) will be optimized regarding defined constraints ($g_m(x)$) and the limitation of operation parameters (x_i) [140].

2.6.2 Genetic Algorithms for Multi-Objective Optimization

Traditional techniques of multi-objective optimization use a weighing method for multiple objectives and transform the problem into a single objective optimization. Weighing factors of objectives are determined in advance, regarding preferences and considerations. Each set of weighing factors will provide a single solution for the optimization problem and varying the factors would result in a new solution. The achieved set of solutions creates a pareto solution for the multi-objective optimization problem.

According to Deb (2001) [141], traditional procedures can be categorized as preference-based multi-objective optimization, which requires additional information in order to convert the problem into a single objective optimization. The optimal solution will then be achieved by solving the single objective optimization. On the contrary, a second category, which is called ideal multi-objective optimization, does not depend on the higher level information to produce optimal solutions; however, high level information can be later implemented to select the most desired solution from the set of pareto optimal solutions.

In addition, preference solutions are time consuming, due to the necessity of apply different weighing factors for each run. Also, by increasing the number of objectives, preference techniques require more information and constraints for the user to solve the multi-objective optimization problem [141].

Nowadays, evolutionary optimization algorithms have been implemented for multi-objective optimization, due to their approach of using a population based method to develop new population of solutions in each iteration from one solution in an iteration. The major reasons for using evolutionary techniques in recent years are their applicability for wide ranges of operations, simplicity to use in different applications, and flexibility for different case studies [141, 142]. Recently, evolutionary (Genetic) optimization algorithms have been widely used for multi-objective problems as a set of pareto optimal solutions is required for this kind of problems and can be provided by these approaches in a single run [141-145].

Multi-objective problems in recent decades have been optimized by means of several variants of genetic algorithms such as: Strength Pareto Evolutionary Algorithms (SPEA) [146] and SPEA2 [147], Vector Evaluated Genetic Algorithms (VEGA) [148], Niched Pareto Genetic Algorithms (NPGA) [149], and Non-dominated Sorting Genetic Algorithms (NSGA) [150].

Among different procedures of evolutionary optimization algorithms, the Non-dominated Sorting Genetic Algorithm II (NSGA II) has attracted more attention [140]. The main advantages of NSGA II over other genetic algorithms are the search through the main domain for global optimum solutions, a reduction of the computational complexity, and also an increase in the diversity of the population, by introducing the crowded comparison operator [151]. In general, the main aspects of the NSGA II are: (i) implementing elitism that can store all non-dominated solutions and then improving convergence properties, (ii) guaranteeing the diversity and distribution of solutions, and (iii) applying a non-dominated procedure in order to sort the individuals regarding the level of non-domination [143, 151, and 152].

2.7 Life Cycle Analysis of Bioethanol Production

The process of compilation and evaluation of the inputs and outputs of a product or system including materials, products, energy, and by-products to quantify the environmental impacts of the system is called life cycle assessment/analysis (LCA). LCA methodology provides a quantified tool for investors and decision makers to compare the results of an LCA study of different cases or among different products to assess the products and designs from the environmental point of view. Each LCA study considers four main phases which

must be carefully determined regarding the analyzed system or product: (I) Goal and scope of the study as well as the system boundary, (II) Life cycle inventory to identify the inputs and outputs, (III) Quantification of the life cycle impacts, and (IV) Interpretation of the assessment results [153, 154]. Regarding the objective of the study, all inputs and outputs of the system must be normalized based on the functional unit, which acts as a reference for all involved materials and energy of the system. The system boundary would also be defined to clarify the limits of the considered system which determines that which units and processes are included in the assessment and which must be excluded [155-157].

Life cycle analysis has been practiced in great deal of researches for bioethanol production from different points of view. In some cases, bioethanol as a fuel has been compared with current fossil fuels at different ratios of combination [158-160]. Patrizi et al. (2013) [160] studied the impact of replacing 10% of required gasoline (E10) with second generation bioethanol through LCA and 6% diminishment in total CO_2 emission was achieved. Increasing the proportion of ethanol in the mixture of ethanol-gasoline has always shown a significant decrease in GHG emissions. Morales et al. (2015) [161] compared the greenhouse gas (GHG) emissions of various blends of ethanol and gasoline and GHG emission reduces more than 40% in the case of using E85 (85% ethanol). The amount of reduction highly depends on the raw material used for ethanol production and as the ethanol portion in mixture increases, the role of raw biomass in production stage becomes more significant. Generally, in all case studies, GHG emissions decreased compared with conventional fossil fuels; however, the reduction amounts highly depend on the sources of the biomass. [161-163].

Different types of biomass as sources of ethanol production have been studied. Munoz et al.(2014) [162] compared the life cycle of ethanol from four agricultural feedstocks with fossil-based ethanol and in all bio-based cases GHG emission diminished; however, for some other environmental impacts, such as land use, fossil-based ethanol presented a better performance. LCA results for each type of biomass have been shown to be highly dependent on the method of the biomass production, cultivation technologies, geographical location of biomass, fertilizers used, and the sources of the consumed energy [165-168].

The most considered impact in LCA studies is for greenhouse gases (GHG) emissions, which has been analyzed for different combinations of ethanol-gasoline, when the blended ethanol was achieved from various sources of biomass. Nevertheless, few research has addressed other environmental impact categories such as acidification, ozone layer depletion, respiratory organics/inorganics, and carcinogens. These impact categories are mainly caused by raw materials preparation, e.g. using fertilizers in agricultural practices [169-171].

Comparison of the LCA results for ethanol production from a specific feedstock, which reflects the importance of the process design on the environmental performance of the ethanol production plant, has yet to be considered.

2.8 References

1. U.S. Energy Information Administration. 2011. International Energy Outlook 2011. DOE/EIA-0484.
2. Soetaert, W., Vandamme E. J. (2009). Biofuels. John Wiley & Sons, Ltd.
3. Arundel, A., Sawaya, D. (2009). The bioeconomy to 2030: Designing a policy agenda.
4. IEA. 2009. Transport Energy and CO2: Moving Towards Sustainability. OECD Publishing,
5. Demirbas, A. (2009). Biofuels securing the planet's future energy needs. Energy conversion and management. 50, 2239-2249.
6. Demirbas, A., Fatih, M., Balat, M., & Balat, H. (2009). Potential contribution of biomass to the sustainable energy development. Energy Conversion and Manage, 50(7), 1746-1760.
7. Fiona, J.W., Egginton, P., Barrow, E., Desjarlais, C., Hengeveld, H., Lemmen, D., S., & Simonet, G. (2007). Background Information: Concepts, Overviews and Approaches. From Impacts to Adaptation: Canada in a Changing Climate, Chapter 2.
8. Berktay, A. & Nas, B. (2008). Biogas production and utilization potential of wastewater treatment sludge. Energy sources Part A, 30, 179-188.
9. Chhetri, A. B. & Islam, M. R. (2008). Towards producing a truly green biodiesel. Energy sources Part A, 30, 754-764.

10. Konwer, D., Kataki, R., & Saikia, P. (2007). Pyrolysis of some indigenous tree species of northeast India: effect of pyrolysis temperature and heating rate on the products yield and char quality. Energy sources Part A, 29, 1433-1442.
11. Goldemberg, J. (2008). The Brazilian biofuels industry. Biotechnol Biofuels, 1 (6), 1- 7.
12. Gray, K. A., Zhao, L., & Emptage, M. (2006). Bioethanol. Current Opinion Chemical Biology, 10 (2), 141-146.
13. Börjesson, P. (2009). Good or bad bioethanol from a greenhouse gas perspective what determines this?. Applied Energy, 86 (5), 589-594.
14. Carolan, J. E., Joshi, S. V., & Dale, B. E. (2007). Technical and Financial Feasibility Analysis of Distributed Bioprocessing Using Regional Biomass Pre-Processing Centers. Journal of Agricultural & Food Industrial Organization, 5(10): 1027.
15. Lynd, L. R., Zyl, Van Zyl, W. H., McBride, J. E., & Laser, M. (2005). Consolidated bioprocessing of cellulosic biomass: an update. Current Opinion in Biotechnology, 16, 577-583.
16. Aristidou, A., Penttila, M. (2000). Metabolic engineering applications to renewable resource utilization. Current Opinion Chemical Biology, 11, 187-198.
17. Limayem, A., & Ricke, S. C. (2012). Lignocellulosic biomass for bioethanol production: Current perspectives, potential issues and future prospects. Progress in Energy and Combustion Science, 38, 449-467.

18. Wagner, H., & Kaltschmitt, M. (2013). Biochemical and thermochemical conversion of wood to ethanol—simulation and analysis of different processes. Biomass Conversion and Biorefinery, 3(2), 87-102.
19. Foust, T. D., Aden, A., Dutta, A., & Phillips, S. (2009). An economic and environmental comparison of a biochemical and a thermochemical lignocellulosic ethanol conversion processes. Cellulose, 16, 547-565.
20. Chandel AK, Chan E, Rudravaram R, Narasu ML, Rao LV, Ravindra P. (2007). Economics and environmental impact of bioethanol production technologies: an appraisal. Biotechnol Mol Biol Rev, 2, 14-32.
21. Gamage J, Howard L, Zisheng Z. (2010). Bioethanol production from lignocellulosic biomass. J Biobased Mater Bioenerg, 4, 3-11.
22. Zhu, Z. S., Zhu, M. J., Xu, W. X., & Liang, L. (2012). Production of Bioethanol from Sugarcane Bagasse Using NH4OH-H2O2 Pretreatment and Simultaneous Saccharification and Co-fermentation. Biotechnology and Bioprocess Engineering, 17, 316-325.
23. Jones, R. A., Gandier, J. A. Thibault, J., & Tezel, F. H. (2011). Enhanced Ethanol Production through Selective Adsorption in Bacterial Fermentation. Biotechnology and Bioprocess Engineering, 16, 531-541.
24. Chundawat, S. P. S., Beckham, G. T., Himmel, M. E., & Dale, B. E. (2011). Deconstruction of lignocellulosic biomass to fuels and chemicals. Annu Rev Chem Biomol Eng, 2,121–145.

25. Zhao, X. Q., Zi, L. H., Bai, F. W., Lin, H. L., Hao, X. M., Yue, G. J., & Ho, N. W. Y. (2012). Bioethanol from Lignocellulosic Biomass. Adv Biochem Engin/Biotechnol, 128, 25–51.
26. Van Dyk, J. S., & Pletschke, B. I. (2012). A review of lignocellulose bioconversion using enzymatic hydrolysis and synergistic cooperation between enzymes Factors affecting enzymes, conversion and synergy. Biotechnology Advances, 30(6), 1458-1480.
27. Cho, D. H., Shin, S. J., Sang, B. I., Eom, M. H., & Kim, Y. H. (2013). ABE Production from Yellow Poplar through Alkaline Pre-hydrolysis, Enzymatic Saccharification, and Fermentation. Biotechnology and Bioprocess Engineering, 18, 965-971.
28. Fan, L.T., Yong-Hyun, L., & Beardmore, H. D. (2006a). Major chemical and physical features of cellulosic materials as substrates for enzymatic hydrolysis. Advances in Biochemical Engineering/Biotechnology, 14(1980), 101-117.
29. Lin, Y., & Tanaka, S. (2006). Ethanol fermentation from biomass resources: current state and prospects. Applied Microbiology and Biotechnology, 69(6), 627-642.
30. Linde, M., Jakobsson, E. L., Galbe. M., & Zacchi, G. (2008). Steam pretreatment of dilute H_2SO_4 -impregnated wheat straw and SSF with low yeast and enzyme loadings for bioethanol production. Biomass and Bioenergy, 32 (4), 326-332.
31. Esteghlalian, A., Hashimoto, A. G., Fenske, J. J., & Penner, M. H. (1997). Modeling and optimization of the dilute-sulfuric-acid pretreatment of corn stover, poplar and switchgrass. Bioresour Technol, 59 (2),129-136

32. Rudolf, A., Baudel, H., Zacchi, G., Hahn-Hägerdal, B., & Lidén, G. (2008). Simultaneous saccharification and fermentation of steam-pretreated bagasse using Saccharomyces cerevisiae TMB3400 and Pichia stipitis CBS6054. Biotechnol Bioeng, 99 (4), 783-790.
33. Söderström, J., Pilcher, L., Galbe, M., & Zacchi, G. (2003). Two-step steam pretreatment of softwood by dilute H2SO4 impregnation for ethanol production. Biomass and Bioenergy, 24 (6), 475-486.
34. Frederick Jr, W., Lien, S., Courchene, C., DeMartini, N., Ragauskas, A., & Iisa, K. (2008). Production of ethanol from carbohydrates from loblolly pine: A technical and economic assessment. Bioresour Technol, 99 (11), 5051-5057.
35. Sassner, P., Galbe, M., & Zacchi, G. (2008). Techno-economic evaluation of bioethanol production from three different lignocellulosic materials. Biomass and Bioenergy, 32 (5), 422-430.
36. Scordia, D., Cosentino, S. L., Lee, J. W., & Jeffries, T. W. (2011). Dilute oxalic acid pretreatment for biorefining giant reed (Arundo donax L.). Biomass and Bioenergy, 35 (7), 3018-3024.
37. Wyman, C.E. (2008). Cellulosic ethanol: A unique sustainable liquid transportation fuel. MRS Bulletin, 33, 381-383.
38. Zheng, Y., Pan, Z., & Zhang, R. (2009). Overview of biomass pretreatment for cellulosic ethanol production. Int. Journal of Molecular Sciences, 2(3), 51-68.
39. Wooley, R., Ruth, M., Sheehan, J., Ibsen, K., Majdeski, H., & Galvez, A. (1999). NREL/TP-580-26157. Golden: Colorado National Laboratory of the U.S.

Department of Energy. Lignocellulosic Biomass to Ethanol Process Design and Economics Utilizing Co-Current Dilute Acid Prehydrolysis and Enzymatic Hydrolysis Current and Futuristic Scenarios.

40. Fujii, T., Fang, X., Inoue, H., Murakami, K., & Sawayama, S. (2009). Enzymatic hydrolyzing performance of Acremonium cellulolyticus and Trichoderma reesei against three lignocellulosic materials. Biotechnology and Biofuels, 2, 24.
41. Zhao, X., Zhang, L., & Liu, D. (2012a). Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. Biofuels, Bioproducts and Biorefining, 6 (4), 465–482.
42. García, V., Päkkilä, J., Ojamo, H., Muurinen, E., & Keiski, R. L. (2011). Challenges in biobutanol production: How to improve the efficiency? Renewable and Sustainable Energy Reviews, 15, 964-980.
43. Alvira, P., Tomás-Pejó, E., Ballesteros, M., & Negro, M. (2010). Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis. Bioresour Technol, 101 (13), 4851-4861.
44. Galbe, M., & Zacchi, G. (2007). Pretreatment of lignocellulosic materials for efficient bioethanol production. Biofuels, 41-65.
45. Hendriks, A., & Zeeman, G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour Technol, 100 (1), 10-18.
46. Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y., Holtzapple, M., & Ladisch, M. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol, 96 (6), 673-686.

47. Fan, L. T., Yong-Hyun, L., & Gharpuray, M. M. (2006b). The nature of lignocellulosics and their pretreatments for enzymatic hydrolysis. Advances in Biochemical Engineering/Biotechnology, 23, 157-187.
48. Mais, U., Esteghlalian, A., Saddler, J. N., & Mansfield, S. D. (2002). Enhancing the enzymatic hydrolysis of cellulosic materials using simultaneous ball milling. Applied Biochemistry and Biotechnology, 99(1-3), 815-832.
49. Shuai, L., Yang, Q., Zhu, Y. J., Luc, F. C., Weimer, P. J., Ralpha, J., & Pana, X. J. (2010). Comparative study of SPORL and dilute-acid pretreatments of spruce for cellulosic ethanol production. Bioresource Technology, 101(9), 3106-3114.
50. Digman, M. F., Shinners, K. J., Casler, M. D., Dien, B. S., Hatfield, R. D., Jung, H. J., Muck, R. E., Weimer, P. J. (2010). Optimizing on-farm pretreatment of perennial grasses for fuel ethanol production. Bioresour Technol, 101(14), 5305-14.
51. Wyman, C. E., Dale, B. E., Elander, R. T., Holtzapple, M., Ladisch, M. R., Lee, Y. Y., Mitchinson, C., & Saddler, J. N. (2009). Comparative sugar recovery and fermentation data following pretreatment of poplar wood by leading technologies. Biotechnology Progress, 25(2), 333-339.
52. Wang, Z., Keshwani, D. R., Redding, A. P., & Cheng, J. J. (2010). Sodium hydroxide pretreatment and enzymatic hydrolysis of coastal Bermuda grass. Bioresource Technology, 101(10), 3583-3585.
53. Marzialetti, T., Olarte, M. B. V., Sievers, C., Hoskins, T. J. C., Agrawal, P. K., & Jones, C. W. (2008). Dilute acid hydrolysis of loblolly pine: a comprehensive approach. Industrial and Engineering Chemistry Research, 47(19), 7131-7140.

54. Himmel, M. E., Adney, W. S., Baker, J. O., Elander, R., McMillan, J. D., Nieves, R. A., Sheehan, J. J., Thomas, S. R., Vinzant, T. B., & Zhang, M. (1997). Advanced bioethanol production technologies: a perspective. Fuels and Chemicals from Biomass. 666: 2-45.

55. Brodeur, G., Yau, E., Badal, K., Collier, J., Ramachandran, K. B., & Ramakrishnan, S. (2011). Chemical and Physicochemical Pretreatment of Lignocellulosic Biomass. Enzyme Research Volume, 2011, 1-17.

56. Nguyen, Q. A., Tucker, M. P., Keller, F. A., & Eddy, F. P. (2000). Two-stage dilute-acid pretreatment of softwoods. Applied Biochemistry and Biotechnology, 84-86, 561-576.

57. Kim, J. S., Lee, Y. Y., & Park, S. C. (2000). Pretreatment of wastepaper and pulp mill sludge by aqueous ammonia and hydrogen peroxide. Applied Biochemistry and Biotechnology, 84-86, 129-139.

58. Sun, X. F., Xu, F., Sun, R. C., Wang, Y. X., Fowler, P., & Baird, M. S. (2004). Characteristics of degraded lignins obtained from steam exploded wheat straw. Polymer Degradation and Stability, 86, 245-256.

59. Zhu, S., Wu, Y., Yu, Z., Liao, J., & Zhang, Y. (2005). Pretreatment by microwave/alkali of rice straw and its enzymic hydrolysis. Process Biochemistry, 40, 3082-3086.

60. Kootstra, A. M. J., Beeftink, H. H., Scott, E. L., & Sanders, J. P. M. (2009). Optimization of the dilute maleic acid pretreatment of wheat straw. Biotechnology for Biofuels, 2(31), 1-14.

61. Kabel, M. A., Bos, G., Zeevalking, J., Voragen, A. G., & Schols, H. A. (2007). Effect of pretreatment severity on xylan solubility and enzymatic breakdown of the remaining cellulose from wheat straw. Bioresource Technology, 98, 2034-2042.

62. Liu, Z. L., Slininger, P. J., & Gorsich, S. W. (2005). Enhanced biotransformation of furfural and hydroxymethylfurfural by newly developed ethanologenic yeast strains. Applied Biochemistry and Biotechnology, 121, 451-460.

63. Monavari, S., Galbe, M., & Zacchi, G. (2009). Impact of impregnation time and chip size on sugar yield in pretreatment of softwood for ethanol production. Bioresource Technology, 100, 6312-6316.

64. Kumar, P., Barrett, D. M., Delwiche, M. J., & Stroeve, P. (2009). Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Ind Eng Chem Res, 48 (8), 3713-3729.

65. Chornet, E., & Overend, R. P. (1998). Phenomenological Kinetics and Reaction Engineering Aspects of Steam/Aqueous Treatments. Proceedings of the International Workshop on Steam Explosion Techniques. Oct 20-21, Milan Italy. 21-58.

66. Wang, K., Jiang, J. X., Xu, F., & Sun, R.C. (2009). Influence of steaming explosion time on the physico-chemical properties of cellulose from Lespedeza stalks. Bioresoure Technology, 100, 5288-5294.

67. Mohammad, J. T., & Karimi, K. (2008). Pretreatment of lignocellulosic wastes to improve ethanol and biogas production. International Journal of Molecular Sciences, 9, 1621-1651.

68. Sierra, R., Granda, C. B., & Holtzapple, M. T. (2009). Lime pretreatment. Biofuels Methods and Protocols, in: Methods in Molecular Biology. 581, 115-124.

69. Kim, T. H., Gupta, R., & Lee, Y. Y. (2009). Pretreatment of biomass by aqueous ammonia for bioethanol production. Methods Mol Biol, 581, 79-91.

70. Zhaoyang, X., & Fang, H. (2014). Pretreatment Methods for Bioethanol Production. Aplied Biochemistry and Biotechnology, 174(1), 43-62.

71. Teymouri, F., & Laureano-Perez, L. (2005). Optimization of the ammonia fiber explosion (AFEX) treatment parameters for enzymatic hydrolysis of corn stover. Bioresource Technology, 96, 2014- 2018.

72. Alizadeh, H., Teymouri, F., Gilbert, T. I., & Dale, B. E. (2005). Pretreatment of switchgrass by ammonia fibre explosion (AFEX). Applied Biochemistry and Biotechnology, 124, 1133- 1141.

73. Murnen, H. K., Balan, V., Chundawat, S. H. S., Bals, B., Sousa, L. D. C., & Dale, B. E. (2007). Optimization of ammoin fiber expansion (AFEX) pretreatment and enzymatic hydrolysis of Miscanthus x giganteus to fermentable sugars. Biotechnology Process, 23(4), 846-850.

74. Zhao, X., Cheng, K., & Liu, D. (2009). Organosolv pretreatment of lignocellulosic biomass for enzymatic hydrolysis. Applied Microbiology and Biotechnology, 82, 815-827.

75. Sannigrahi, P., Miller, S. J., & Ragauskas, A. J. (2010). Effects of organosolv pretreatment and enzymatic hydrolysis on cellulose structure and crystallinity in Loblolly pine. Carbohydrate Research, 345, 965-970.

76. Chandra, R. P., Au-Yeung, K., Chanis, C., Roos, A. A., Mabee, W., Chung, P. A., et al. (2011). The influence of pretreatment and enzyme loading on the effectiveness of batch and fed-batch hydrolysis of corn stover. Biotechnology Progress, 27(1), 77-85.

77. Ligero, P., Villaverde, J. J., de Vega, A., & Bao, M. (2008). Delignification of Eucalyptus globulus saplings in two organosolv systems (formic and acetic acid): Preliminary analysis of dissolved lignins. Ind Crops Prod, 27, 110–117.

78. Soudham, V. P., Rodriguez, D., Rocha, G. J. M., Taherzadeh, M. J., & Martin, C. (2011). Acetosolv delignifi cation of marabou (Dichrostachys cinerea) wood with and without acid prehydrolysis. Forestry Studies in China, 13(1), 64–70.

79. Harmsen, P. F. H., Huijgen, W. J. J., Bermúdez López, L. M., & Bakker, R. R. C. (2010). Literature Review of Physical and Chemical Pretreatment Processes for Lignocellulosic Biomass. Energy Research Centre of Netherlands (ECN).

80. Klein-Marcuschamer, D., Oleskowicz-Popiel, P., Simmons, B. A., & Blanch, H. W. (2012). The Challenge of Enzyme Cost in the Production of Lignocellulosic Biofuels. Biotechnology and Bioengineering, 109, 1083-1087.

81. Brijwani, K., Oberoi, H. S., & Vadlani, P. V. (2010). Production of a cellulolytic enzyme system in mixed-culture solid-state fermentation of soybean hulls supplemented with wheat bran. Process Biochem, 45, 120-128.

82. Fang, X., Yano, S., Inoue, H., & Sawayama, S. (2009). Strain improvement of Acremonium cellulolyticus for cellulase production by mutation. J Biosci Bioeng, 107, 256-261.

83. Teugjas, H., & Väljamäe, P. (2013). Product inhibition of cellulases studied with ^{14}C-labeled cellulose substrates. Biotechnology for Biofuels, 6(104), 1-14.

84. Tang, H., Hou, J., Shen, Y., Xu, L., Yang, H., Fang, X., & Bao, X. (2013). High β-Glucosidase Secretion in Saccharomyces cerevisiae Improves the Efficiency of Cellulase Hydrolysis and Ethanol Production in Simultaneous Saccharification and Fermentation. J. Microbiol. Biotechnol, 23(11), 1577-1585.

85. Zhang, Y. P., Himmel, & M. E., Mielenz, J. R. (2006). Outlook for cellulase improvement: Screening and selection strategies. Biotechnol Adv, 24, 452-481.

86. Wang, M., Liu, K., Dai, L., Zhang, J., & Fang, X. (2013). The structural and biochemical basis for cellulose biodegradation. J. Chem. Technol. Biotechnol, 88, 491-500.

87. Sørensen, A., Lübeck, M., Lübeck, P. S., & Ahring, B. K. (2013). Fungal Beta-Glucosidases: A Bottleneck in Industrial Use of Lignocellulosic Materials. Biomolecules, 3, 612-631

88. Janick, J., & Whipkey, A. (2002). Ethanol from Cellulose: A General Review. Trends in new crops and new uses. ASHS Press, Alexandria, VA.

89. Gray, K. A., Zhao, L., & Emptage, M. (2006). Bioethanol. Current Opinion in Chemical Biology, 10, 141-146.

90. Casey, G. P., & Ingledew, W. M. (1986). Ethanol tolerance in yeasts. Critical reviews in microbiology, 13 (3), 219-280.

91. Verduyn, C., Postma, E., Scheffers, W. A., & van Dijken, J. P. (1990). Physiology of Saccharomyces Cerevisiae in Anaerobic Glucose-Limited Chemostat Culturesx. Journal of general microbiology, 136 (3), 395-403.

92. Hahn-Hägerdal, B., Jeppsson, H., Olsson, L., & Mohagheghi, A. (1994). An interlaboratory comparison of the performance of ethanol-producing micro-organisms in a xylose-rich acid hydroysate. Appl Microbiol Biotechnol, 41 (1), 62-72.

93. Olsson, L., & Hahn-Hägerdal, B. (1993). Fermentative performance of bacteria and yeasts in lignocellulose hydrolysates. Process Biochemistry, 28 (4), 249-257.

94. Ostergaard, S., Olsson, L., & Nielsen, J. (2000). Metabolic engineering of Saccharomyces cerevisiae. Microbiology and Molecular Biology Reviews, 64 (1), 34-50.

95. Lin, Y., & Tanaka, S. (2006). Ethanol fermentation from biomass resources: current state and prospects. Appl Microbiol Biotechnol, 69, 627-642.

96. Sanchez, N. V., & Karhumaa, K. (2015). Xylose fermentation as a challenge for commercialization of lignocellulosic fuels and chemicals. Biotechnol Lett, 37(4), 761-72.

97. Aristidou, A. & Penttilä, M. (2000). Metabolic engineering applications to renewable resource utilization. Curr Opin Biotechnol, 11(2), 187-98.

98. Jeffries, T. W. (2006). Engineering yeasts for xylose metabolism. Current Opinion in Biotechnology, 17, 320-326.

99. Matsushika, A., Inoue, H., Kodaki, T., & Sawayama, S. (2009). Ethanol production from xylose in engineered Saccharomyces cerevisiae strains: current state and perspectives. Appl Microbiol Biotechnol, 84, 37-53.

100. Nevoigt, E. (2008). Progress in metabolic engineering of Saccharomyces cerevisiae. Microbiology and Molecular Biology Reviews, 72 (3), 379-412.

101. Olsson. L., Soerensen, H. R., Dam, B. P., Christensen, H., Krogh, K. M., & Meyer, A. S. (2006). Separate and simultaneous enzymatic hydrolysis and fermentation of wheat hemicellulose with recombinant xylose utilizing Saccharomyces cerevisiae. Appl. Biochem. Biotechnol, 129-132, 117-29.

102. Saha, B. C., Iten, L. B., Cotta, M. A., & Wu, Y. (2005). Dilute acid pretreatment, enzymatic saccharification and fermentation of wheat straw to fuel ethanol. Process Biochem, 40, 3693-3700.

103. Taherzadeh, M. J., & Karimi, K. (2007). Enzyme-based hydrolysis processes for ethanol from lignocellulosic materials: a review. BioRes, 2(4), 707-738.

104. Galbe, M., & Zacchi, G. (2002). A review of the production of ethanol from softwood. Applied Microbiology and Biotechnology, 59, 618-628.

105. Philippidis, G. P., Smith, T. K., & Wyman, C. E. (1993). Study of the enzymatic hydrolysis of cellulose for production of fuel ethanol by the simultaneous saccharification and fermentation process. Biotechnol. Bioeng, 41(9), 846-853.

106. Philippidis, G. P., & Smith, T. K. (1995). Limiting factors in the simultaneous saccharification and fermentation process for conversion of cellulosic biomass to

fuel ethanol. 16th Symposium on Biotechnology for Fuels and Chemicals Humana Press, 117-124.

107. Alfani, F., Gallifuoco, A., Saporosi, A., Spera, A., & Cantarella, M. (2000). Comparison of SHF and SSF processes for the bioconversion of steam-exploded wheat straw. Journal of Industrial Microbiology and Biotechnolgy, 25, 184-192.

108. Balat, M. (2011). Production of bioethanol from lignocellulosic materials via the biochemical pathway: A review. Energy Conversion and Management, 52, 858-875.

109. Rass-Hansen, J., Falsig, H., Jørgensen, B., & Christensen, C. H. (2007). Prospective Bioethanol: fuel or feedstock?. J Chem Technol Biotechnol, 82, 329-333.

110. Hoyer, K., Galbe, M., & Zacchi, G. (2009). Production of fuel ethanol from softwood by simultaneous saccharification and fermentation at high dry matter content. J ChemTechnol Biotechnol, 84, 570-577.

111. Sanchez, Ó. J., & Cardona, C. A. (2008). Trends in biotechnological production of fuel ethanol from different feedstocks. Bioresour Technol, 99, 5270-5295.

112. Liu, K., Lin, X., Yue, J., Li, X., Fang, X., Zhu, M., Lin, J., Qu, Y., & Xiao, L. (2010). High concentration ethanol production from corncob residues by fed-batch strategy. Bioresour Technol, 101, 4952-4958.

113. Vasquez, M. P., Da Silva, J. N. C., De Souza, M. B., & Pereira, N. (2007). Enzymatic Hydrolysis Optimization to Ethanol Production by Simultaneous Saccharification and Fermentation. Applied Biochemistry and Biotechnology, 136-140, 141-154.

114. Wyman, C. E., & Abelson, P. H. (1996). Handbook on Bioethanol: Production and Utilization, first ed. Taylor and Francis, Washington, DC.

115. Morales-Rodriguez, R., Gernaey, K. V., Meyer, A. S., & Sin, G. A. (2011). Mathematical Model for Simultaneous Saccharification and Cofermentation (SSCF) of C6 and C5 Sugars.Chinese J. Chem. Eng., 19 (2), 185-191.

116. South, C. R., Hogsett, A. L., & Lynd, L. R. (1995). Modeling simultaneous saccharification and fermentation of lignocellulose to ethanol in batch and continuous reactors. Enzyme and Microbial Technology, 17, 797-803.

117. Kroumov, A. D., Módenes, A. N., & Tait, M. C. (2006). Development of new unstructured model for simultaneous saccharification and fermentation of starch to ethanol by recombinant strain. Biochem. Eng. J., 28(3), 243-255.

118. Sasikumar, E., & Viruthagiri, T. (2010). Simultaneous Saccharification and Fermentation (SSF) of Sugarcane Bagasse - Kinetics and Modeling.Int. J. Chem. Biomol. Eng, 3 (2), 57-64.

119. Kadam, K. L., Rydholm, E. C., & McMillan, J. D. (2004). Development and validation of a kinetic model for enzymatic saccharification of lignocellulosic biomass. Biotechnol. Prog., 20, 698-705.

120. Krishnan, M. S., Ho, N.W.Y., & Tsao, G.T. (1999). Fermentation kinetics of ethanol production from glucose and xylose by recombinant Saccharomyces 1400(pLNH33). Appl. Biochem. Biotechnol., 78, 373-388.

121. Sin, G., Meyer, A. S., & Gernaey, K. V. (2010). Assessing reliability of cellulose hydrolysis models to support bioefuel process design-identifiability and uncertainty analysis. Comput. Chem. Eng., 34, 1385-1392.

122. Hodge, D. B., Karim, M. N., Schell, D.J., & McMillan, J.D. (2009). Model-based fed-batch for high-solids enzymatic cellulose hydrolysis. Appl. Biochem. Biotechnol., 152, 88-107.

123. Morales-Rodriguez, R., Capron, M., Huusom, J.K., & Sin, G. (2010). Controlled fed-batch operation for improving cellulose hydrolysis in 2G bioethanol production. Computer-Aided Chemical Engineering, 28, 1497-1502.

124. Zhang, Y. P., & Lynd, L. R. (2004). Towards an aggregated understanding of enzymatic hydrolysis of cellulose: Noncomplex cellulase systems. Biotechnol Bioeng, 88, 788-824.

125. Converse, A. O., & Optekar, J. D. (1993). A synergistic kinetics model for enzymatic cellulose hydrolysis compared to the degree-of-synergism experimental results. Biotechnol Bioeng, 42, 145-148.

126. Philippidis, G. P., Spindler, D. D., & Wyman, C. E. (1992). Mathematical modeling of cellulose conversion to ethanol by the simultaneous saccharification and fermentation process. Applied Biochemistry and Biotechnology, 34-5, 543-556.

127. Philippidis, G. P., & Hatzis, C. (1997). Biochemical engineering analysis of critical process factors in the biomass to ethanol technology. Biotechnology Progress, 13(3), 222-231.

128. Shin, D., Yoo, A., Kim, S. W., & Yang, D. R. (2006). Cybernetic modeling of simultaneous saccharification and fermentation for ethanol production from steam-exploded wood with Brettanomyces custersii. Journal of Microbiology and Biotechnology, 16(9), 1355-1361.

129. van Zyl, J. M., van Rensburg, E., van Zyl, W. H., Harms, T. M., & Lynd, L. R. (2011). A Kinetic Model for Simultaneous Saccharification and Fermentation of Avicel with Saccharomyces cerevisiae. Biotechnol. Bioeng, 108, 924-933.

130. Pettersson, P. O., Eklund, R., & Zacchi, G. (2002). Modeling Simultaneous Saccharification and Fermentation of Softwood. Applied Biochemistry and Biotechnology, 98-100, 733-746.

131. Wahono, S. K., Rosyidaa, V. T., Darsiha, C., Pratiwia, D., Frediansyaha, A., & Hernawana. (2015). Optimization of Simultaneous Saccharification and Fermentation Incubation Time Using Cellulose Enzyme for Sugarcane Bagasse on The Second-Generation Bioethanol Production Technology. Energy Procedia, 65, 331-36.

132. Dasgupta, D., Suman, S. K., Pandey, D., Ghosh, D., Khan, R., Agrawal, D., Jain, R. K., Vadde, V. T., Adhikari, D. K. (2013). Design and optimization of ethanol production from bagasse pith hydrolysate by a thermotolerant yeast Kluyveromyces sp. IIPE453 using response surface methodology. SpringerPlus, 2(159), 1-10.

133. Shanavas, S., Padmaja, G., Moorthy, S. N., Sajeev, M. S., & Sheriff, J. T. (2011). Process optimization for bioethanol production from cassava starch using novel eco-friendly enzymes. Biomass and Bioenergy 35(2), 901-909.

134. Betiku, E., & Taiwo, A. E. (2015). Modeling and optimization of bioethanol production from breadfruit starch hydrolyzate vis-à-vis response surface methodology and artificial neural network. Renewable Energy, 74, 87-94.
135. Triwahyuni, E., Hariyanti, S., Dahnum, D., Nurdin, M., & Abimanyu, H. (2015). Optimization of Saccharification and Fermentation Process in Bioethanol Production from Oil Palm Fronds. Procedia Chemistry, 16, 141-148.
136. Zhang, Q., Weng, C., Huang, H., Achal, V., & Wang, D. (2016). Optimization of Bioethanol Production Using Whole Plant of Water Hyacinth as Substrate in Simultaneous Saccharification and Fermentation Process. Frontiers in Microbiology, 6, 1-9.
137. Bandaru, V. V. R., Somalanka, S. R., Mendu, D. R., Madicherla, N. R., & Chityala, A. (2006). Optimization of fermentation conditions for the production of ethanol from sago starch by co-immobilized amyloglucosidase and cells of Zymomonas mobilis using response surface methodology. Enzyme and Microbial Technology, 38 (1-2), 209-214.
138. Wang, R., Unrean, P., & Franzen, C. J. (2016). Model-based optimization and scale-up of multi-feed simultaneous saccharification and co-fermentation of steam pre-treated lignocellulose enables high gravity ethanol production. Biotechnology for Biofuels, 9(88), 1-13.
139. Unrean, P., Khajeeram, S., & Laoteng, K. (2016). Systematic optimization of fed-batch simultaneous saccharification and fermentation at high-solid loading based on

enzymatic hydrolysis and dynamic metabolic modeling of Saccharomyces cerevisiae. Applied Microbiology and Biotechnology, 100(5), 2459-2470.

140. Deb, K. (2011). Multi-Objective Optimization Using Evolutionary Algorithms: An Introduction. In: Multi-objective Evolutionary Optimisation for Product Design and Manufacturing, 3-34.

141. Deb, K. (2001). Multi-objective optimization using evolutionary algorithms. Chichester, UK, Wiley.

142. Abraham, A., Lakhmi, J., & Goldberg, R. (2005). Evolutionary Multiobjective Optimization: Theoretical Advances and Applications. Springer London Ltd, 1st edition.

143. Konak, A., Coit, D. W., & Smith, A. E. (2006). Multi-objective optimization using genetic algorithms: A tutorial. Reliability Engineering and System Safety, 91, 992-1007.

144. Siinivas, N., & Deb, K. (1995). Muiltiobjective Optimization Using Nondominated Sorting in Genetic Algorithms. Evolutionary Computation, 2(3), 221-248.

145. Bandyopadhyay, S. (2012). Modified NSGA-II for a Bi-Objective Job Sequencing Problem. Intelligent Information Management, 4, 319-329.

146. Zitzler, E., & Thiele, L. (1999). Multiobjective evolutionary algorithms: a comparative case study and the strength pareto approach. IEEE Trans on Evolutionary Computation, 3(4), 257-271.

147. Zitzler, E., Marco, L., & Lothar, T. (2001). SPEA2: improving the strength Pareto evolutionary algorithm. Computer Engineering and Networks Laboratory, Swiss Federal Institute of Technology Technical Report 103.
148. Schaffer, J. D. (1984). Some experiments in machine learning using vector evaluated genetic algorithms. PhD thesis, Vanderbilt University.
149. Horn, J., Nafpliotis, N., & Goldberg, D. E. (1994). A niched Pareto genetic algorithm for multiobjective optimization. Proc of the 1st IEEE Conf on Evolutionary Computation, 82-87.
150. Srinivas, N., & Deb, K. (1995). Multi-objective function optimization usingbnon-dominated sorting genetic algorithms. Evolutionary Computation, 2(3), 221-248.
151. Yijie, S., & Gongzhang, S. (2008). Improved NSGA-II Multi-objective Genetic Algorithm Based on Hybridization-encouraged Mechanism. Chinese Journal of Aeronautics, 21, 540-549.
152. Deb, K., Pratap, A., Agarwal, S., & Meyarivan, T. (2002). A Fast and Elitist Multiobjective Genetic Algorithm: NSGA II. IEEE Transactions on Evolutionary Computations, 6(2), 182–197.
153. Goedkoop, M., Schryver, A.D., Oele, M., Durksz, S., & Roest, D.D. (2010). Introduction to LCA with SimaPro 7. PRé Consultants.
154. Goedkoop, M., Schryver, A.D., Oele M., Roest, D.D., Vieira, M., & Durksz, S. (2010). SimaPro 7 Tutorial. PRé Consultants.
155. ISO. 14040. (2006). Environmental management-life cycle assessment-principles and frameworks International Organization for Standardization, Switzerland.

156. ISO. 14044. (2006). Environmental management-life cycle assessment-requirement and guidelines International Organization for Standardization, Switzerland.
157. Roy P., Tokuyasu K., Orikasa T., Nakamura N., & Shiina T. (2012). Review: a review of life cycle assessment (LCA) of bioethanol from lignocellulosic biomass. Jpn Agric Res Q, 46, 41–57.
158. Von Blottnitz, H., & Curran, M. A. (2007). A review of assessments conducted on bioethanol as a transportation fuel from a net energy, greenhouse gas, and environmental life cycle perspective. Journal of Cleaner Production, 15, 607-619.
159. Hedegaard, K., Thyo, K. A., & Wenzel, H. (2008). Life cycle assessment of an advanced bioethanol technology in the perspective of constrained biomass availability. Environmental Science Technology, 42(21), 7992-7999.
160. Patrizi, N., Caro, D., Pulselli, F., Bjerre, A. B., & Bastianoni, S. (2013). Environmental feasibility of partial substitution of gasoline with ethanol in the Province of Siena (Italy). J Cleaner Prod, 47, 388-95.
161. Morales, M., Quintero, J., Conejeros, R., & Aroca, G. (2015). Life cycle assessment of lignocellulosic bioethanol: Environmental impacts and energy balance. Renewable and Sustainable Energy Reviews, 42, 1349-61.
162. González-García, S., Gasol, C. M., Gabarrel, X., Rieradevall, J., Moreira, M. T., & Feijoo, G. (2010). Environmental profile of ethanol from poplar biomass as transport fuel in Southern Europe. Renewable Energy, 35, 1014–23.

163. Edwards, R., Larivé, J. F., Mahieu, V., & Rouveirolles, P. (2011). Well-to-wheels analysis of future automotive fuels and powertrains in the European context. 3c ed. EU: CONCAWE, EUCAR and JRC.

164. Munoz, I., Flury, K., & Jungbluth, N. (2014). Life cycle assessment of bio-based ethanol produced from different agricultural feedstocks. Int J Life Cycle Assess, 19, 109-119.

165. Davis, S. C., Anderson-Teixeira, K. J., & DeLucia, E. H. (2009). Life-cycle analysis and the ecology of biofuels. Trends in Plant Science, 14, 140–146.

166. Gonzalez-Garcia, S., Moreira, M. T., & Feijoo, G. (2010). Comparative environmental performance of lignocellulosic ethanol from different feedstocks. Renewable and Sustainable Energy Reviews, 14, 2077-2085.

167. Neupane, B., Halog, A., & Dhungel, S. (2011). Attributional life cycle assessment of woodchips for bioethanol production. J Cleaner Prod, 19, 733–41.

168. Dias, M., Junqueira, T., Cavalett, O., Cunha, M., Jesus, C., Rossell, C., Filho, R., & Bonomi, A. (2012). Integrated versus stand-alone second generation ethanol production from sugarcane bagasse and trash. Bioresour Technol, 103, 152–61.

169. Cherubini, F., & Jungmeier, G. (2010). LCA of a biorefinery concept producing bioethanol, bioenergy, and chemicals from switchgrass. Int J Life Cycle Assess, 15, 53-66.

170. González-García, S., Iribarren, D., Susmozas, A., Dufour, J., & Murphy R. (2012). Life cycle assessment of two alternative bioenergy systems involving Salix spp. biomass: bioethanol production and power generation. Appl Energy, 95, 111-122.

171. Menichetti, E., & Otto, M. (2008). Energy balance & greenhouse gas emissions of biofuels from a life cycle perspective. In: Biofuels: environmental consequences & implications of Changing land use. Gummersbach, 81-109.

3 Experimental Analysis of Impacts of Enzyme Loading and Sugars Concentration on SSF Process [1]

Preface

A version of this manuscript has been published in the International Journal of Chemical Engineering and Applications. I am the primary author of this paper. Along with the co-authors, Faisal Khan and Yan Zhang, I developed the concept of interactive influence of enzyme and sugars on SSF process. I conducted the literature review, performed the experiments, and analysed the results. I prepared the first draft of the manuscript and subsequently revised the manuscript based on the co-authors' feedback and also the peer review process. The co-author Yan Zhang helped in providing the experiments' setup, reviewed and corrected the achieved results, and contributed in preparing, reviewing and revising the manuscript. The co-author Faisal Khan contributed through support in conceptual development of the study, research methodology design, analysis and discussion of the results. Faisal Khan also assisted in reviewing and revising the manuscript.

Abstract

Enzyme loading and initial concentration of fermentable sugars are the key parameters in the simultaneous saccharification and fermentation (SSF) process to produce bioethanol.

[1] Shadbahr et al. International Journal of Chemical Engineering and Applications, 2016, 7(6): 383-387.

To study the interactive influence of enzyme loading and initial concentration of sugars on the final ethanol yield and concentration, batch SSF experiments were carried out at three enzyme loadings (10, 15 and 20 FPU/g cellulose) and two levels of initial concentrations of fermentable sugars (glucose and mannose). Results indicated that the maximum ethanol yield and concentration were obtained at high level of sugar concentration with intermediate enzyme loading (15 FPU/g cellulose). Increasing the enzyme loading from intermediate level (15 FPU/g cellulose) to high level (20 FPU/g cellulose) diminished the ethanol yield due to the inhibitory effect of the glucose and insufficient amount of yeast.

Keywords: Bioethanol, Enzyme loading, Ethanol Yield, Simultaneous saccharification and fermentation

3.1 Introduction

Bioethanol produced from lignocellulosic biomass have been considered as one of the most attractive and promising renewable energy sources [1]. The most abundant sources of lignocellulosic materials are forestry and agricultural residues which are considered as renewable, low-priced, noncompetitive to food sources, and available sources for future energy [2]. The chemical composition of the lignocellulosic materials mainly consists of cellulose, hemicellulose, and lignin. Compositions of the lignocellulosic materials are different in cellulose, hemicellulose, and lignin contents as well as in the structure of the materials and how they entangled together. In the complicated created matrix of the lignocellulosic material, cellulose is well protected and surrounded by hemicellulose and

lignin which makes the cellulose recalcitrant for degradation and producing glucose out of it. In order to make the cellulose more accessible for enzymes, pretreatment of the lignocellulosic substrate is unavoidable to have an efficient enzymatic cellulose hydrolysis in next step [3-6].

Numerous research studies have demonstrated that SSF process is capable of improving the biomass conversion by reducing the inhibitory impact of converted sugars [7-10]. Usually, a high ethanol concentration and yield from SSF is prerequisite to make the process economically feasible. Nevertheless, the contribution of enzyme costs to the economics of lignocellulosic biofuel production continues to be a major barrier for the commercial-scale production of bioethanol [11-13]. There is potential for cost reduction by optimizing the operating conditions of SSF process so that maximum ethanol concentration and yield can be achieved at relative lower enzyme loading.

Main factors affecting the final ethanol concentration and yield of SSF process include substrate concentration, enzyme loading, solution pH, and reaction temperature [14 and 15]. Due to the compromise between reaction conditions for hydrolysis and fermentation processes, the optimal pH (5.0) and reaction temperature (37°C) of SSF process turned out to be very restricted [14 and 16]. Dissimilarly, the optimal substrate concentration and enzyme loading are very difficult to be determined [17-19]. To obtain high ethanol concentration and yield, a high substrate concentration and, hence high water insoluble solids (WIS), has to be used in the SSF process [20-22]. However, high substrate concentration leads to substrate inhibition, which substantially lowers the rate of the hydrolysis and metabolism of yeast [21]. For optimal enzyme loading, increasing the

dosage of enzymes, to a certain extent, is able to enhance the yield and rate of the hydrolysis, but also significantly increases the cost of the process [23]. Systematic optimization of the SSF process regarding the substrate concentration and enzyme loading needs to be carried out.

Monomeric sugars released from the pretreatment process are also served as the feedstock of SSF process. The initial concentration of the fermentable sugars varies based on the pretreatment method and the raw biomass materials used. The concentration of fermentable sugars definitely affects the final ethanol concentration and yield of a SSF process because sugar concentrations have significant impacts on the reaction rates of both enzymatic hydrolysis and fermentation. It is therefore important to investigate how the initial concentrations of fermentable sugars influence the SSF process. So far very limited research work has been performed to address this issue [24]. In the current study, the interactive influence of the initial concentrations of fermentable sugars and enzyme loading on the SSF of cellulose to ethanol has been explored to provide the profound insight on the process improvement.

3.2 Materials and Methods

3.2.1 Feedstock

Extra pure microcrystalline cellulose, ACS grade glucose and 99% mannose were used as feedstock for SSF process. Cellulose content was adjusted to 5% (w/v) and initial

fermentable sugar concentration was considered at high and low levels in order to evaluate the impact of sugars concentration on ethanol yield.

3.2.2 Enzymes

To provide the activities of 10, 15, and 20 FPU/g cellulose, cellulose enzyme from Trichoderma reesei (ATCC 2921), was utilized and supplemented with β-Glucosidase with the fixed activity of 30 U/g cellulose.

3.2.3 Yeast preparation

Preparation of the yeast for fermentation process consists of four steps: (1) Propagation of saccharomyces cerevisiae cells purchased from VWR onto the agar plate under the sterile condition and storage in fridge at 4°C; (2) Preparation of YPD solution from YPD broth (HIMEDIA) with the concentrations of yeast extract, peptone, and dextrose being 10, 20, and 20 g/L respectively; (3) Addition of the cells to autoclaved YPD solution and shaking in a rotary shaker at 30°C for 24 hours; (4) Separation of the grown cells by centrifuge, washing the cells with DI water twice and storage in fridge for further use.

3.2.4 SSF experiments

An experimental setup consists of 250 mL jacketed stirred tank reactor and a Jualbo FP 50 heated/refrigerated circulator for temperature control. Experiments were carried out at

37°C and pH of 5.0 for 96 hours. During SSF experiments, solution pH was monitored with accumet AB 15 plus pH meter and adjusted by 1M *NaOH* solution. Agitation was provided by a baffled magnetic stirrer at the speed of 350 rpm. Three chemical components were also added as nutrients supplementary to reactor with the following concentrations: $(NH_4)_2HPO_4$: 0.5 g/L, $MgSO_4.7H_2O$: 0.025 g/L, and Yeast Extract: 1g/L.

The SSF process takes place in a single reactor with a series of the simultaneous reactions presented in equation 1. Produced glucose from the hydrolysis process is then fermented to ethanol by yeast.

Cellulose → Cellobiose → Glucose → Ethanol (3-1)

In order to evaluate the SSF performance, ethanol yield was considered as the determinant parameter. Total amount of sugars in the reaction media includes glucose, mannose, and convertible glucose from cellulose and defined as:

$$\text{Total sugars} = [G]_0 + [M]_0 + 1.111\,[C]_0 \quad (3\text{-}2)$$

Where the $[G]_0$, $[M]_0$, and $[C]_0$ are the initial amount of the glucose, mannose, and cellulose, respectively. The constant 1.111 is the stoichiometry conversion factor of cellulose to glucose. According to total available sugars, the theoretical maximum ethanol that can be calculated as:

$$\text{MaxEthanol} = 0.511*[\text{Total sugars}] \quad (3\text{-}3)$$

The constant 0.511 is the stoichiometry conversion factor of glucose to ethanol. The ethanol yield is defined as the ratio of experimentally produced ethanol to maximum theoretical ethanol by Eq. 4.

$$\text{Yield}\ (\%) = \frac{[E]_f - [E]_0}{0.511([G]_0 + [M]_0 + 1.111[C]_0)} \tag{3-4}$$

3.2.5 Analysis method

The Dionex HPLC system including a binary HPG-3200SD pump, an ACC-3000 autosampler, RefractoMax 521 RI detector, and Chromeleon 7 software were used for the analysis of concentartions of ethanol, glucose, mannose, and cellobiose. All the samples were taken in duplicate, centrifuged, filtered by 0.2 µm sterile filter and finally stored in a freezer for further analysis. Two Agilent columns: Agilent Hi-Plex H and Agilent Hi-Plex Pb columns were implemented to analyze the samples. Temperature for the RI detector was adjusted at 55°C and for the HPLC column was set to 50°C. DI water and 0.005 M sulfuric acid both with the flowrate of 0.7 mL/min, were used as the mobile phases for Agilent Hi-Plex Pb and Agilent Hi-Plex H columns respectively.

3.3 Results and Discussions

In order to investigate the impacts of initial sugars concentration and enzyme loading on the ethanol yield and productivity in SSF process, six experiments were performed at

different conditions of investigated parameters. Table 3.1 shows the detailed conditions of the six experiments.

Table 3.1. Initial sugar concentrations, enzyme and yeast loadings for SSF experiment

Exp.	Glucose Concentration (g/L)	Mannose Concentration (g/L)	Cellulase (FPU/g cellulose)	β-Glucosidase (U/g cellulose)	Yeast g dry cell/L
1	5	4.5	10	30	5
2	10	9			
3	5	4.5	15		
4	10	9			
5	5	4.5	20		
6	10	9			

Note: The amount of cellulose substrate was fixed at 5% (w/v) for all the experiments

It must be noted that the other parameters of the reaction such as pH, temperature, time of the process, sampling, and analysis of the samples were performed in the same condition for all the experiments. Final ethanol concentration after 96 hours of SSF process is presented by $[E]_f$ whereas the initial concentration of ethanol is stated by $[E]_0$ in Eq. 4.

As seen from Figure 3.1, Exp. 4 with the initial concentrations of 10 g/L for glucose and 9 g/L for mannose and enzyme loading of 15 FPU/g cellulose has the highest ethanol yield among all the experiments. The concentration profiles of glucose, mannose, cellobiose and ethanol are presented in Figure 3.2.

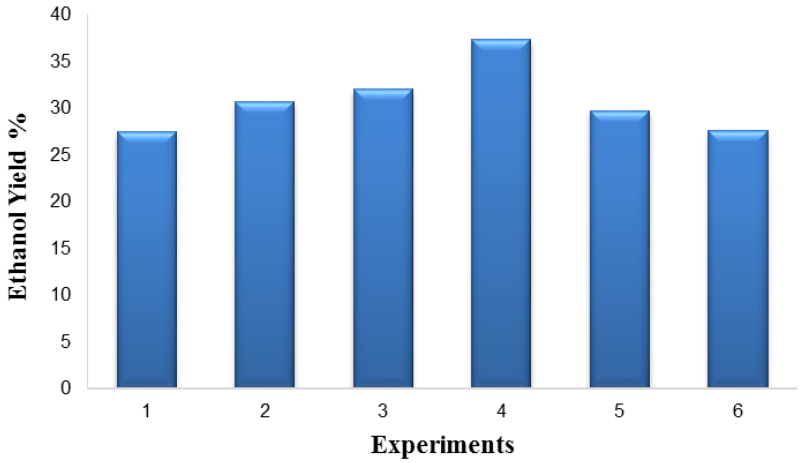


Figure 3.1. Ethanol yield% of the six SSF experiments

In each case, glucose and mannose present in the feedstock were quickly converted to ethanol, accompanied by dramatic changes in the concentrations of glucose, mannose and ethanol within the first 2 hours. After that, the concentrations of glucose and mannose varied very slightly. Concentration of cellobiose, an intermediate product converted from cellulose by means of cellulase enzyme, increased quickly to peak values in the first 2 hours and then declined gradually. In addition, increasing the cellulase loading helps to enhance the conversion of cellulose, which is disclosed by the higher cellobiose concentration obtained from Exps. 3 & 4 shown in Figure 3.2c.

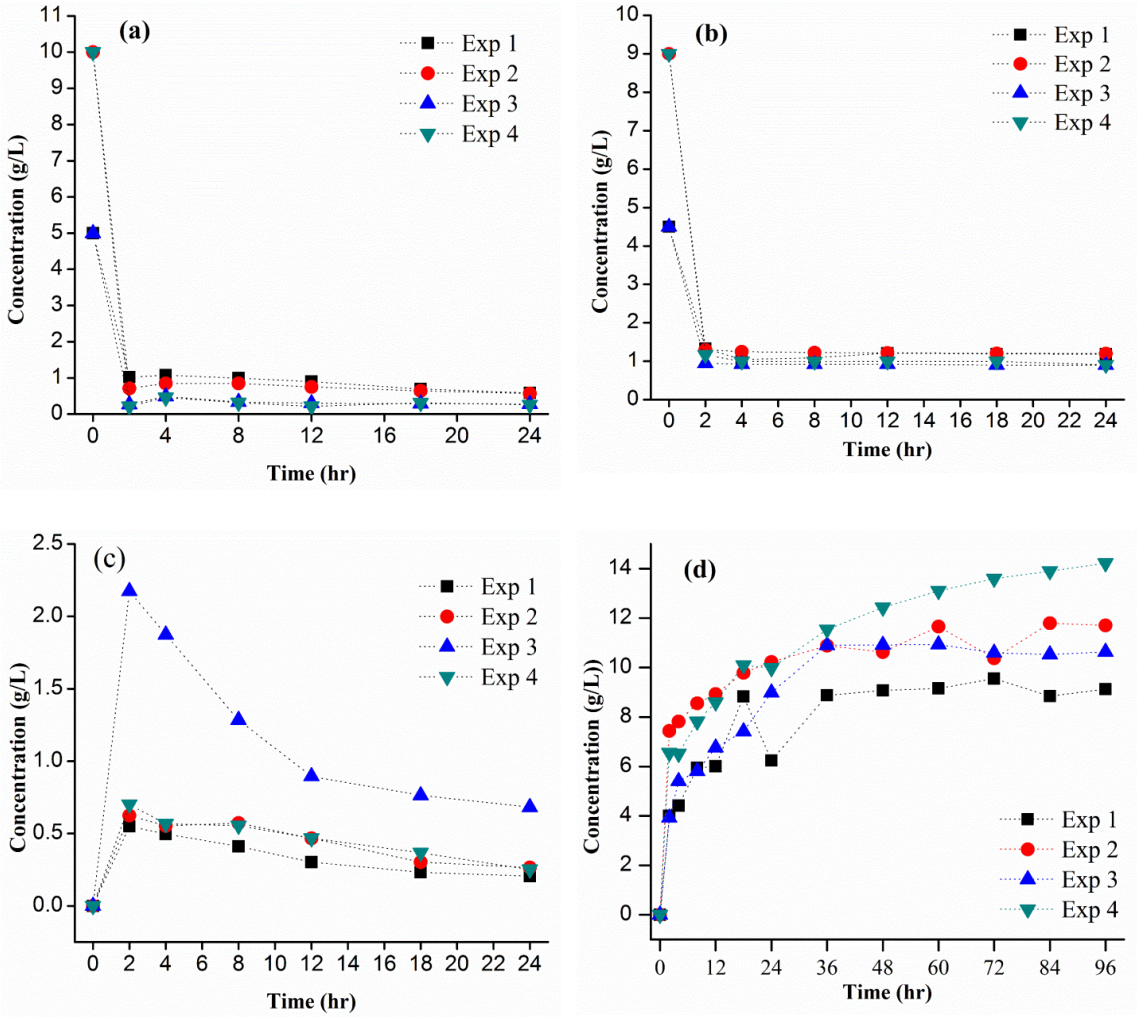


Figure 3.2. Concentration profiles of (a) glucose (b) mannose (c) cellobiose and (d) ethanol for SSF experiments

3.3.1 Impact of initial concentration of fermentable sugars

Initial sugar concentration plays an important role in the SSF reaction. As seen from Figure 3.2d, increasing the glucose concentration from 5 to 10 g/L and mannose from 4.5 to 9 g/L led to the higher ethanol concentration and yield when low and intermediate levels of enzyme loadings were used. Nonetheless, at relative higher enzymatic loading (20 FPU/g

cellulose), increasing the initial concentration of sugars resulted in a decrease in ethanol yield although a slightly higher concentration of ethanol was obtained in case of Exp. 6 (Figure 3.3).

This is reasonable, with a fixed yeast concentration being used in the SSF process, higher concentration of fermentable sugars in the feedstock helps to produce more amount of ethanol, leading to higher ethanol concentration (reaction volume unchanged). But the increase in ethanol production is limited by the yeast loading and performance. As a result, the ethanol yield with respect to the total sugars in the media decreases at high initial concentration of sugars.

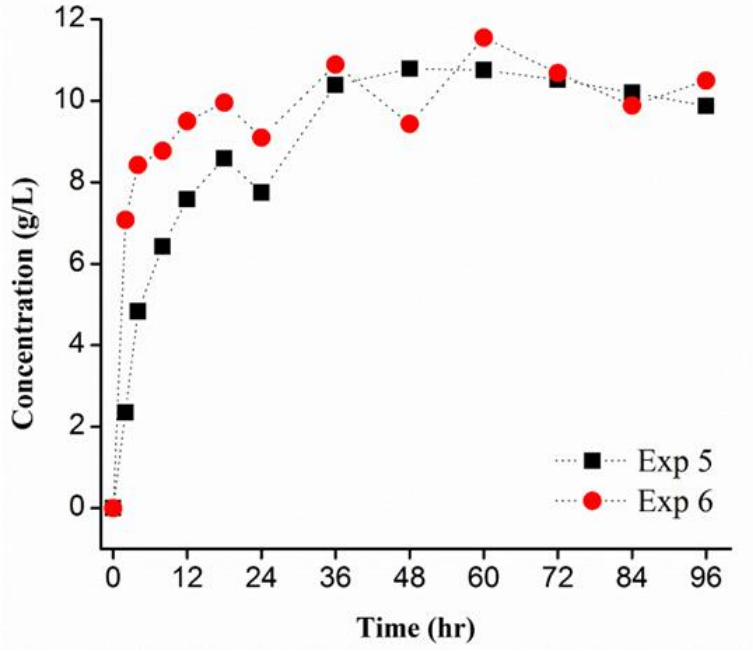


Figure 3.3. Comparison of ethanol concentration for Exps. 5 and 6 with enzyme loading of 20 FPU/g cellulose

3.3.2 Impact of enzyme loading

Impacts of cellulase loading on ethanol yield and concentration were illustrated in Figures 3.1 and 3.4, respectively. For each level of initial concentration of sugars, the highest ethanol yield and concentration were obtained with an enzyme loading of 15 FPU/g cellulose. In spite of the amount of soluble glucose and mannose present at the start of SSF, increasing cellulase loading from 10 FPU/g cellulose to 15 FPU/g cellulose helps to improve both ethanol yield and ethanol concentration as illustrated in Figures 3.1 & 3.2c. However, such an enhancement in ethanol production was not observed when further increasing the cellulase loading to 20 FPU/g cellulose due to the inhibitory effect of the cellobiose and glucose. High enzyme loading in the SSF process accelerates the rate of enzymatic hydrolysis, leading to higher concentrations of cellobiose and glucose, which according to Ishmayana et al. (2011) [25], exposes the yeast to high osmotic stress, influences on fermentation performance of the yeast and reduces the amount of produced ethanol. This means that for certain cellulose and yeast loading, there is an optimum enzyme loading, beyond which ethanol yield and concentration cannot be increased.

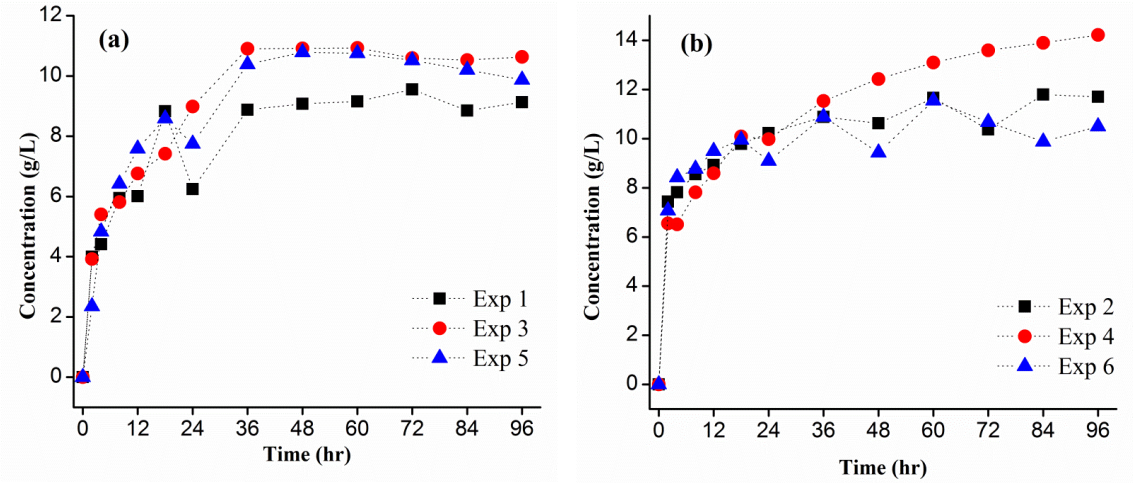


Figure 3.4. Impact of enzyme loading on the ethanol concentration at different initial sugar concentrations (a) 5 g/L glucose, and 4.5 g/L mannose; and (b) 10 g/L glucose, and 9 g/L mannose

3.3.3 Interactive impacts of cellulase loading and initial concentration of sugars

For SSF process with fixed substrate and yeast loading, the interplay between the enzyme loading and initial concentration of fermentable sugars is obvious. With lower initial concentration of sugars, the enhancement of ethanol yield and concentration is easily attainable by employing higher enzyme loading. However, due to the strong inhibitory effect of cellobiose and glucose, high enzyme loading results in a significant decrease in ethanol yield and concentration when the feedstock contains very high concentration of fermentable sugars. This provides useful information with respect to the optimization of SSF process. Depending on the substrate and sugar concentration in the feedstock of SSF, enzyme loading should be selected strategically.

3.4 Conclusion

Influences of enzyme loading and initial concentration of fermentable sugars on the final ethanol concentration and yield of SSF process was studied in this work. Results indicated that there is a saturation of enzyme loading for each level of sugar concentration. With 5% (w/v) cellulose and 5 g dry cell/L yeast loading, ethanol concentration and yield cannot be improved by purely increasing the enzyme loading. Moreover, interactive impact of enzyme loading and initial concentration of fermentable sugars on SSF process was observed. High enzyme loading helped to increase the final ethanol concentration and yield if the initial concentration of fermentable sugars was low. However, high enzyme loading resulted in a decrease in ethanol concentration and yield when feedstock contains high concentration of fermentable sugars. Therefore, enzyme loading of SSF process need to be selected strategically from the process economics perspective.

3.5 References

1. Z. S. Zhu, M. J. Zhu, W. X. Xu, and L. Liang, "Production of Bioethanol from Sugarcane Bagasse Using NH_4OH-H_2O_2 Pretreatment and Simultaneous Saccharification and Co-fermentation," *Biotechnology and Bioprocess Engineering*, vol. 17, pp. 316-325, 2012.

2. R. A. Jones, J. A. Gandier, J. Thibault, and F. H. Tezel, "Enhanced Ethanol Production through Selective Adsorption in Bacterial Fermentation," *Biotechnology and Bioprocess Engineering,* vol.16, pp. 531-541,2011.

3. S. P. S. Chundawat, G. T. Beckham, M. E. Himmel, and B. E. Dale, "Deconstruction of lignocellulosic biomass to fuels and chemicals," *Annu Rev Chem Biomol Eng,* Vol. 2, pp. 121-145, 2011.

4. X. Q. Zhao, L. H. Zi, F. W. Bai, H. L. Lin, X. M. Hao G. J. Yue, and N. W. Y. Ho, "Bioethanol from Lignocellulosic Biomass," *Adv Biochem Engin/Biotechnol,* vol. 128, pp. 25-51, 2012.

5. J. S. Van Dyk, and B. I. Pletschke, "A review of lignocellulose bioconversion using enzymatic hydrolysis and synergistic cooperation between enzymes Factors affecting enzymes, conversion and synergy," *Biotechnology Advances,* vol. 30, no. 6, pp. 1458-1480, 2012.

6. D. H. Cho, D, S. J. Shin, B. I. Sang, M. H. Eom, and Y. H. Kim, "ABE Production from Yellow Poplar through Alkaline Pre-hydrolysis, Enzymatic Saccharification, and Fermentation," *Biotechnology and Bioprocess Engineering,* vol. 18, pp. 965-971, 2013.

7. G. P. Philippidis, T. K. Smith, and C. E. Wyman, "Study of the Enzymatic Hydrolysis of Cellulose for Production of Fuel Ethanol by the Simultaneous Saccharification and Fermentation Process," *Biotechnology and Bioengineering,* vol. 41, pp. 846-853, 1993.

8. J. Rass-Hansen, H. Falsig, B. Jørgensen, and C. H. Christensen, "Prospective Bioethanol: fuel or feedstock?," J *Chem Technol Biotechnol,* vol. 82, pp. 329-333, 2007.

9. K. Hoyer, M. Galbe, and G. Zacchi, “Production of fuel ethanol from softwood by simultaneous saccharification and fermentation at high dry matter content,” *J ChemTechnol Biotechnol,* vol. 84, pp. 570-577, 2009.

10. Ó. J. Sanchez, and C. A. Cardona, “Trends in biotechnological production of fuel ethanol from different feedstocks,” *Bioresour Technol,* Vol. 99, pp. 5270-5295, 2008.

11. D. Klein-Marcuschamer, P. Oleskowicz-Popiel, B. A. Simmons, and H. W. Blanch, “The Challenge of Enzyme Cost in the Production of Lignocellulosic Biofuels,” *Biotechnology and Bioengineering,* Vol. 109, pp. 1083-1087, 2012.

12. K. Brijwani, H. S. Oberoi, and P. V. Vadlani, “Production of a cellulolytic enzyme system in mixed-culture solid-state fermentation of soybean hulls supplemented with wheat bran,” *Process Biochem,* vol. 45, pp. 120-128, 2010.

13. X. Fang, S. Yano, H. Inoue, S. Sawayama, “Strain improvement of Acremonium cellulolyticus for cellulase production by mutation,” *J Biosci Bioeng,* vol. 107, pp. 256-261, 2009.

14. M. P. Vasquez, J. N. C. Da Silva, M. B. De Souza, and N. Pereira, “Enzymatic Hydrolysis Optimization to Ethanol Production by Simultaneous Saccharification and Fermentation,” *Applied Biochemistry and Biotechnology,* vol. 136-140, pp. 141-154, 2007.

15. J. C. Lopez-Linares, I. Romero, C. Cara, E. Ruiz, E. Castro, and M. Moya, "Experimental study on ethanol production from hydrothermal pretreated rapeseed straw by simultaneous saccharification and fermentation," *J Chem Technol Biotechnol,* vol. 89, pp. 104-110, 2014.

16. K. Liu, X. Lin, J. Yue, X. Li, X. Fang, M. Zhu, J. Lin, Y. Qu, and L. Xiao, "High concentration ethanol production from corncob residues by fed-batch strategy," *Bioresour Technol,* vol. 101, pp. 4952-4958, 2010.

17. K. Stenberg, M. Bollok, K. Reczey, M. Galbe, and G. Zacchi, "Effect of Substrate and Cellulase Concentration on Simultaneous Saccharification and Fermentation of Steam-Pretreated Softwood for Ethanol Production," *Biotechnology and Bioengineering,* vol. 68, no. 2, pp. 204-210, 2000.

18. M. Balat, H. Balat, and C. Oz C, "Progress in bioethanol processing," *Prog Energy Combust,* vol. 34, pp. 551-573, 2008.

19. M. A. Neves, T. Kimura, N. Shimizu, and M. Nakajima, "State of the art and future trends of bioethanol production," *Dynamic biochemistry, process biotechnology and molecular biology,* vol. 1, pp. 1-14, 2007.

20. S. W. Cheung, and B. C. Anderson, "Laboratory Investigation of Ethanol Production from Municipal Primary Wastewater Solids," *Bioresource Technology,* vol. 59, pp. 81-96, 1997.

21. X. Huang, and M. H. Penner, “Apparent substrate inhibition of the Trichoderma reesei cellulase system,” *J Agric Food Chem,* vol. 39, pp. 2096-2100, 1991.

22. P. Valjamae, G. Pettersson, and G. Johansson, “Mechanism of substrate inhibition in cellulose synergistic degradation,” *Eur J Biochem,* vol. 268, pp. 4520-4526, 2001.

23. Y. Sun, and J. Cheng, “Hydrolysis of lignocellulosic materials for ethanol production: a review,” *Bioresource Technology,* vol. 83, pp. 1-11, 2002.

24. K. Hoyer, M. Galbe, and G. Zacchi, “Influence of fiber degradation and concentration of fermentable sugars on simultaneous saccharification and fermentation of high-solids spruce slurry to ethanol,” *Biotechnology for Biofuels,* vol. 6, no. 1, pp. 145-153, 2013.

25. S. Ishmayana, R. P. Learmonth, and U. J. Kennedy, “Fermentation performance of the yeast Saccharomyces cerevisiae in media with high sugar concentration,” *Proceedings of the 2nd International Seminar on Chemistry 2011,* pp. 379-385.

4 Kinetic Modeling of Simultaneous Saccharification and Fermentation Process[2]

Preface

A version of this manuscript has been published in the Energy conversion and Management journal. I am the primary author of this paper. Along with the co-authors, Faisal Khan and Yan Zhang, I developed the model. I conducted the literature review, experiments, and applied the results to tune the kinetic parameters to improve the kinetic model. I prepared the first draft of the manuscript and subsequently revised the manuscript based on the co-authors' feedback and also the peer review process. The co-author Yan Zhang helped in choosing the appropriate model, programming and running the simulation codes, reviewing and correcting the achieved results, and contributed in preparing, reviewing and revising the manuscript. The co-author Faisal Khan contributed through support in conceptual development of the study, analysis and discussion of the modeling results, and research methodology design. Faisal Khan also assisted in reviewing and revising the manuscript.

Abstract

Kinetic modeling and dynamic analysis of the simultaneous saccharification and fermentation (SSF) of cellulose to ethanol was carried out in this study to determine the key reaction kinetics parameters and product inhibition features of the process. To obtain

[2] Shadbahr et al. Energy Conversion and Management, 2017, 141: 236-243.

the more reliable kinetic parameters which can be applied for a wide range of operating conditions, batch SSF experiments were carried out at three enzyme loadings (10, 15 and 20 FPU/g cellulose) and two levels of initial concentrations of fermentable sugars (glucose and mannose). Results indicated that the maximum ethanol yield and concentration were achieved at high level of sugar concentrations with intermediate enzyme loading (15 FPU/g cellulose). Dynamic analysis of the acquired experimental results revealed that cellulase inhibition by cellobiose plays the most important role at high level of enzyme loading and low level of initial sugar concentrations. The inhibition of glucose becomes significant when high concentrations of sugars were present in the feedstock. Experimental results of SSF process also reveal that an efficient mixing between the phases helps to improve the ethanol yield significantly.

Keywords: Simultaneous saccharification and fermentation, Enzyme loading, Bioethanol; Glucose; Mannose, Ethanol Yield

4.1 Introduction

Ethanol produced from lignocellulosic biomass, the most abundantly available raw material on Earth has been considered as one of the most attractive and promising renewable energy sources [1]. Lignocellulosic material, obtained as a by-product of the agriculture/forestry industries or energy crops, is mainly composed of cellulose, hemicellulose, and lignin, among which cellulose and hemicellulose are digestible by microorganisms for energy [2]. Due to the complexity of the lignocellulosic

macromolecular structure, biochemical conversion of lignocellulosic biomass consists of four processing steps: (1) pretreatment to liberate cellulose and hemicellulose. The main purpose of this step is to disorganize the crystalline structure of macro- and micro fibrils to release the polymer chains of cellulose and hemi-cellulose [3, 4]; (2) enzymatic hydrolysis of polysaccharides; (3) fermentation of monomeric sugars and (4) ethanol recovery and dehydration [5-7]. The current technology of lignocellulosic ethanol does not support the cost-efficient production, preventing its commercialization [8]. Exploration of cost-reduction strategy is essential for the commercialization of lignocellulosic ethanol.

Cost-competitive technology can be developed by improving the performance of enzymatic hydrolysis and fermentation, the key processing steps in lignocellulosic ethanol. The reasons are twofold. Firstly cellulase, the enzyme used in hydrolysis of cellulose contributes significantly to the cost of the bioethanol production, accounting for 20–30% of the total cost [9, 10]. Secondly, the cost of the downstream ethanol distillation is directly bound up with the ethanol concentration attainable from the fermentation of monomeric sugars. Ethanol concentration higher than 40g/L is prerequisite to make the distillation process economically feasible [11]. Therefore, reducing the cost of lignocellulosic ethanol can be achieved by optimizing the hydrolysis and fermentation processes so that maximum ethanol concentration and yield are attainable at relative lower enzyme consumption.

Extensive research has demonstrated that SSF, the simultaneous saccharification (hydrolysis) of cellulose to fermentable sugars and fermentation of sugars to ethanol, helps to achieve higher ethanol productivity by reducing the inhibitory impact of converted sugars [12-15]. Nonetheless, SSF process is exceptionally complex and its performance

(reaction conversion, final ethanol yield and concentration) is greatly influenced by many factors such as the type of lignocellulosic feedstock, the substrate concentration, the type and amount of cellulolytic enzymes and microorganisms, solution pH and reaction temperature [16, 17]. The optimal pH (≈ 5.0) and reaction temperature (37°C) [18, 19] of SSF process turned out to be quite rigid because a compromise between optimal pH and temperatures of the cellulolytic enzymes and the yeast is needed [16, 20]. Dissimilarly, determination of the optimal substrate concentration and enzyme loading is not straightforward [5, 21, 22]. Usually, a high substrate concentration has to be used in the SSF process to obtain high ethanol concentration and yield [23-25]. However, high substrate concentration causes substrate inhibition, which substantially lowers the rate of the hydrolysis and metabolism of yeast [24]. Increasing the dosage of enzymes, to a certain extent, helps to increase the conversion rate of substrate, and hence improve the final ethanol yield and concentration. But high enzyme consumption significantly increases the operating cost [26]. Finding an optimum combination of substrate concentration and enzyme/microorganism loading for a specific feedstock is challenging in this regard.

Kinetic modeling of cellulose bioconversion is an important tool in predicting the rates of enzymatic hydrolysis and fermentation as well as the dynamic features of the process [27, 28]. Kinetic modeling of SSF process is an influential step toward industrialization of bioethanol production from lignocellulosic biomass due to the fact that establishing the concepts of production process with emphasizing on the experimental data is not sufficient and it costs plenty of time and resources. Moreover, proper kinetic model and reliable model parameters are indispensable for optimizing the performance of SSF process.

Although a number of kinetic models have been developed over the past years for SSF process [29-32], these models were usually tuned for specific substrates over a relatively narrow range of operating conditions. In this study, kinetic model of a batch SSF process was developed to incorporate the variations of substrate composition and enzyme loading. Obviously hydrolysate fraction of the pretreated biomass could be diluted in different ratios, which could lead to varied initial sugars concentration in the SSF feedstock. Evaluating kinetic model and kinetic parameters to variation of sugars concentration helps to obtain the more reliable kinetic parameters which can be applied for a wide range of operating conditions and biomass compositions. Therefore, in the current study, kinetic model parameters were estimated by fitting the models to experimental data obtained from a wide range of operating conditions. Dynamic characteristics and rate limiting causes of the SSF process were analyzed through the interactive influence of the initial concentrations of fermentable sugars and enzyme loading on the bioconversion of cellulose to ethanol.

4.2 Kinetic Modeling

Simultaneous enzymatic hydrolysis and fermentation of cellulose is a complex multistep process and interactions between enzymes with solid substrate as well as the product inhibition mechanism are not fully understood. A modified mathematical model based on those reported by Philippidis et al. [12, 31, 32] and Pettersson et al. [33] were used in this study to quantify the enzymatic hydrolysis and sugar fermentation. The kinetic model assumes that cellulase hydrolyzes cellulose to cellobiose with negligible formation of

glucose through the cooperation of endo- and exoglucanases and β-glucosidase converts one mole of cellobiose (342.29 g/mol) to two moles of glucose (180.16 g/mol). In addition, fermentation of mannose, a C-2 epimer of glucose which is usually present in a pretreated softwood substrate is also taken into account. One mole of glucose or mannose (180.16 g/mol) will be fermented to two moles of ethanol (46.06 g/mol) and two moles of carbon dioxide (44.01 g/mol). The reaction network for the biochemical conversion of cellulose is supposed to follow the route listed below:

$$\text{Cellulose (C)} \xrightarrow{r_1} \text{Cellobiose (B)} \xrightarrow{r_2} \text{Glucose(G)} \xrightarrow{r_X, r_G} \text{Ethanol (E)} \,\&\, \text{Cellmass (X)}$$

$$\text{Mannose(M)} \xrightarrow{r_X, r_M} \text{Ethanol(E)} \,\&\, \text{Cellmass(X)}$$

Figure 4.1. Reaction network of SSF process

The rates of reactions listed in Figure 4.1 are expressed by Eqs. 4-1 to 4-5.

$$r_1 = \frac{k_1'[C]e^{-\lambda t}}{1+[B]/K_{1B}+[G]/K_{1G}}\left(\frac{K_{1E}}{K_{1E}+[E]}\right) \text{ Where } k_1' = \frac{k_1[enzc]}{K_{eq}+[enzc]} \tag{4-1}$$

$$r_2 = \frac{k_2[enzg][B]}{K_M(1+[G]/K_{2G})+[B]}(1-K_L[L]) \tag{4-2}$$

$$r_X = \mu_m[X]\left(\frac{[G]+[M]}{K_G+[G]+[M]}\right)\left(\frac{K_E}{K_E+[E]}\right) \tag{4-3}$$

$$r_G = \frac{[G]}{[G]+[M]}\left(\frac{r_X}{Y_{XG}}+m_s[X]\right) \tag{4-4}$$

$$r_M = \frac{[M]}{[G]+[M]}\left(\frac{r_X}{Y_{XG}}+m_s[X]\right) \tag{4-5}$$

The mass balance for the key components in the SSF process can be described by the equations listed below:

$$\frac{d[C]}{dt} = -r_1 \tag{4-6}$$

$$\frac{d[B]}{dt} = 1.056r_1 - r_2 \tag{4-7}$$

$$\frac{d[G]}{dt} = 1.053r_2 - r_G \tag{4-8}$$

$$\frac{d[M]}{dt} = -r_M \tag{4-9}$$

$$\frac{d[X]}{dt} = r_X \tag{4-10}$$

$$\frac{d[E]}{dt} = 0.511(r_G + r_M) \tag{4-11}$$

In this study, ethanol yield with respect to total sugars in the media, including initial glucose and mannose and potential sugar which is convertible from cellulose by hydrolysis reaction, was used to evaluate the SSF performance. Total amount of sugars can be calculated based on the stoichiometry of the components involved in the process as illustrated below:

Total sugars = $[G]_0 + [M]_0 + 1.111\,[C]_0$ (4-12)

where $[G]_0$, $[M]_0$ and $[C]_0$ are the initial concentrations of glucose, mannose and cellulose respectively. The theoretical maximum ethanol that can be achieved is calculated based on the total sugars in media and is defined as:

Theoretical maximum ethanol = $0.511([G]_0 + [M]_0 + 1.111[C]_0)$ (4-13)

The constant 0.511 is the conversion factor of glucose to ethanol extracted from stoichiometry of the reaction. Based on the theoretical maximum ethanol produced from SSF process, the ethanol yield can be calculated by the following equation:

$$\text{Ethanol yield (\%)} = \frac{[E]_f - [E]_0}{0.511([G]_0 + [M]_0 + 1.111[C]_0)} \quad (4\text{-}14)$$

where $[E]_0$ and $[E]_f$ represent the initial and final concentration of ethanol.

4.3 Materials and Methods

4.3.1 Feedstock

Feedstock for batch SSF process in this research includes extra pure microcrystalline cellulose, ACS grade glucose and 99% mannose purchased from Fisher Scientific. To investigate the influence of the initial sugar concentrations on the final ethanol concentration and yield, two levels of sugar concentration were used in the experiment. At the first level concentrations of 5 g/L and 4.5 g/L were used for glucose and mannose, respectively. For the second level of the experiments, glucose and mannose concentrations were increased to 10 g/L and 9 g/L, respectively. The amount of insoluble cellulose was fixed at 5% (w/v) for all the experiments and fresh ultrapure water was used for all steps of the experiments.

4.3.2 Enzymes

The commercial enzyme cellulase from *Trichoderma reesei* (ATCC 26921) supplemented with β-Glucosidase from almonds was purchased from Sigma-Aldrich. Filter paper units (FPU) were calculated using Santos et al. [34]. The amount of enzymes added to the

reactants provides the activities of 10, 15, and 20 FPU/g cellulose. The activity for the β-Glucosidase was fixed at 30 U/g cellulose for all experiments.

4.3.3 Yeast preparation

Inoculum was prepared on the agar plate from the *saccharomyces cerevisiae* demo plate purchased from the VWR Canada under the sterile condition, and then stored at 4 °C. The YPD broth from the HIMEDIA was used for the preparation of YPD solution with the concentrations of 10, 20, and 20 g/L for yeast extract, peptone, and dextrose, respectively. The YPD solution then sterilized in autoclave for 30 minutes at the pressure of 15 psi and temperature of 121°C. When the temperature of YPD solution reached room temperature, the cells were added to solution and placed in the rotary shaker and incubated at 30 °C with the speed of 200 rpm for growing. After 24 hours, grown cells were centrifuged for 10 minutes at 4500 rpm centrifuge to separate cells from the YPD solution. The separated cells were washed and centrifuged twice by ultrapure water and then stored in fridge at 4 °C for use.

4.3.4 SSF experiments

The SSF experiments were carried out in a 250 mL jacketed flask (Bellco, US) with an active volume of 100 mL. The reaction temperature was controlled by a Julabo FP 50 heated/refrigerated circulator (Allentown, PA, US). Experiments were conducted at 37 °C and pH of 5.0 for 96 hrs. During SSF experiment, solution pH was monitored using an Accumet AB 15 plus pH meter and adjusted by 1M *NaOH* solution. For agitation, a baffled

magnetic stirrer was used to provide the agitation at the speed of 350 rpm. In addition to the reactants, enzymes, and yeast, three chemical components were also added to reactor supplement the reaction as nutrients with the following final concentrations: $(NH_4)_2HPO_4$: 0.5 g/L, $MgSO_4.7H_2O$: 0.025 g/L, and Yeast Extract: 1.0 g/L. The samples were taken at 2, 4, 8, 12, 18, 24, 36, 48, 60, 72, 84, and 96 hours for analysis. The summary of the SSF experimental conditions are given in Table 4.1.

Exp. #3 and Exp. #4 were carried out twice to examine the repeatability of the experimental methodology. It is confirmed that experiment results are duplicable and the ethanol yields under the conditions of Exp. #3 and Exp. #4 were achieved with ±2%.

Table 4.1. Initial sugar concentrations, enzyme and yeast loadings for SSF experiment

Exp. #	Glucose Concentration (g/L)	Mannose Concentration (g/L)	Cellulase (FPU/g cellulose)	β-Glucosidase (U/g cellulose)	Yeast g dry cell/L
1	5	4.5	10	30	5
2	10	9			
3	5	4.5	15		
4	10	9			
5	5	4.5	20		
6	10	9			

Note: The amount of cellulose substrate was fixed at 5% (w/v) for all the experiments

4.3.5 Analytical method

Analysis of the samples was performed by HPLC system for the concentration of ethanol, glucose, cellobiose, and mannose. All the samples were taken in duplicate and after centrifuge and filtration by 0.2 µm sterile filter stored in a freezer for further analysis.

Samples were analyzed using Agilent Hi-Plex H and Agilent Hi-Plex Pb columns by Dionex Ultimate 3000 HPLC system equipped with a Refractive Index detector (RefractorMax 520). Temperatures of the RI detector and HPLC column were set to 55°C and 50°C, respectively. 0.005 M sulfuric acid solution and ultrapure water, with the flowrate of 0.7 mL/min, were used as the mobile phases for Agilent Hi-Plex H and Hi-Plex Pb columns respectively.

4.3.6 Numerical method

The values of inhibition parameters K_m, K_{1B}, K_{1E}, K_{1G} and $K_{2G,}$ the enzyme parameter K_L as well as the microorganism parameters K_E, K_G, m_s, and Y_{XG} were determined through a number of specific experiments by Philippidis et al. and were used directly in this study as the operating conditions of SSF experiment in this work are quite close to those reported by Philippidis et al. [12, 31, 32].

The remaining kinetic parameters (k_1, k_2, K_{eq}, λ and μ_m) were determined by minimizing the differences between experimental data and predicted amount at the same time. An error function $F(p)$, defined as the sum of square deviations of the calculated concentration profiles from the experimentally measured curves, is used as the objective function to obtain the best-fit values of kinetic parameters.

$$F(p) = \min \sum_{i=1}^{n} \sum_{j=1}^{m} [c_{i,j}^{\exp} - c_{i,j}^{\mathrm{mod}}]^2 \qquad (4\text{-}15)$$

Where, $c_{i,j}^{\exp}$ and $c_{i,j}^{\mathrm{mod}}$ are the measured and model predicted concentrations of component *i* at sampling point *j*. The best–fit kinetic parameters were determined by minimizing the scalar function *F*(*p*) using "fmincon", a constraint nonlinear optimization solver from MATLAB 2014b. Model predicted concentrations of sugars and ethanol, $c_{i,j}^{\mathrm{mod}}$ were obtained by solving the initial problem ODEs (listed in Eqs. 6-11) by ode15s, a stiff ODE solver which uses the backward differentiation formula (BDF, also known as Gear's method).

4.4 Results and Discussion

4.4.1 Kinetic parameters

Experimental measurements from Exp. #1- Exp. #4 were used to determine the kinetic parameters and two sets of kinetic parameters were finally obtained with respect to the different initial sugar concentrations. The best-fit values of the kinetic parameters are listed in Table 4.2. Figure 4.2 illustrates the comparison between model predictions and the measured experimental results of ethanol concentration from Exp. #1 - Exp. #4. Results from Table 4.2 indicated that cellulase adsorption saturation constant, K_{eq}, converged to a uniform value regardless of the variation of operating conditions. Higher initial sugar concentration resulted in strong product inhibition of enzymatic hydrolysis, leading to smaller values of k_1, λ and k_2. Meanwhile, higher initial sugar concentration accelerated the growth rate of microorganisms due to the presence of more nutrients, reflected from the convergence of a higher value of μ_{m}.

To verify the validity of the derived kinetic parameters, the kinetic parameter values listed in Table 4.2 were used to simulate the ethanol concentration obtained from the batch SSF process under the operating conditions of Exp. #5 and Exp. #6 with the highest cellulase loading being used.

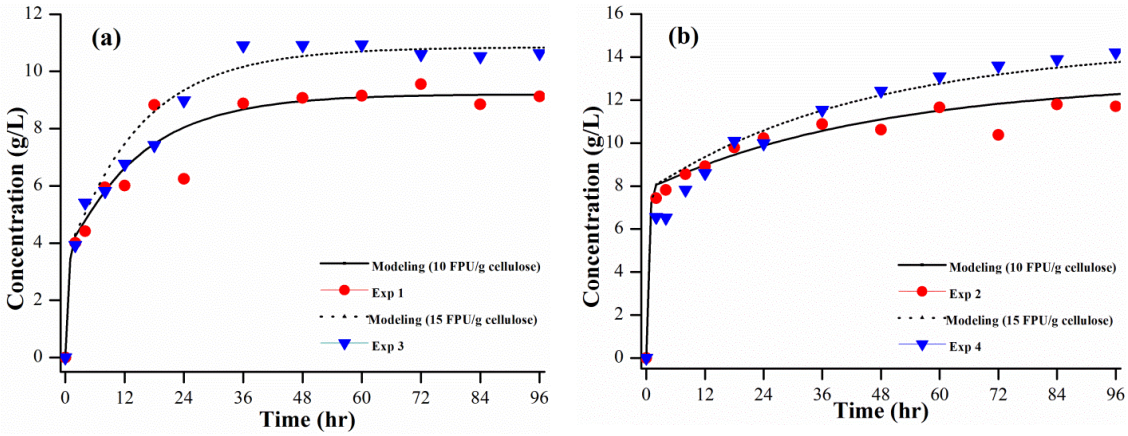


Figure 4.2. Kinetic modeling and experimental ethanol concentration at different initial sugar concentration: (a) Glucose: 5 g/L and Mannose 4.5 g/L; and (b) Glucose: 10 g/L and Mannose 9 g/L

The comparison of the simulation results and the corresponding experimental data is shown in Figure 4.3, from which a very good agreement between the model predictions and experimental results is observed.

The validity of the derived parameters is also proved by comparing them with those determined by other work under similar operating conditions. The values of the parameters listed in Table 4.2 are quite consistent with those reported by Pettersson et al., van Zyl et al., and Philippidis, et al. [30, 32, 33].

Table 4.2. Estimated kinetic parameters at different levels of sugar concentrations

Tuned Parameters		**Low Sugar Level (Exp.#1, 3 &5)**			**High sugar level (Exp. #2, 4 & 6)**
k_1	h^{-1}	0.165-0.256			0.043-0.074
λ	h^{-1}	0.058-0.064			0.019-0.039
K_{eq}	FPU/g	117.90			117.81
k_2	g/U·h	0.24-0.33			0.19-0.20
μ_m	h^{-1}	0.18-0.21			0.39-0.40
*Other parameters**					
K_{1B}	g/L	5.85	K_M	g/L	10.56
K_{1E}	g/L	50.35	K_G	g/L	3.73×10^{-5}
K_{1G}	g/L	53.16	K_L	L/g	0.0053
K_{2G}	g/L	0.62	m_s		0
K_E	g/L	50	Y_{XG}	g/g	0.113

* The values of these parameters come from Pettersson et al., [33].

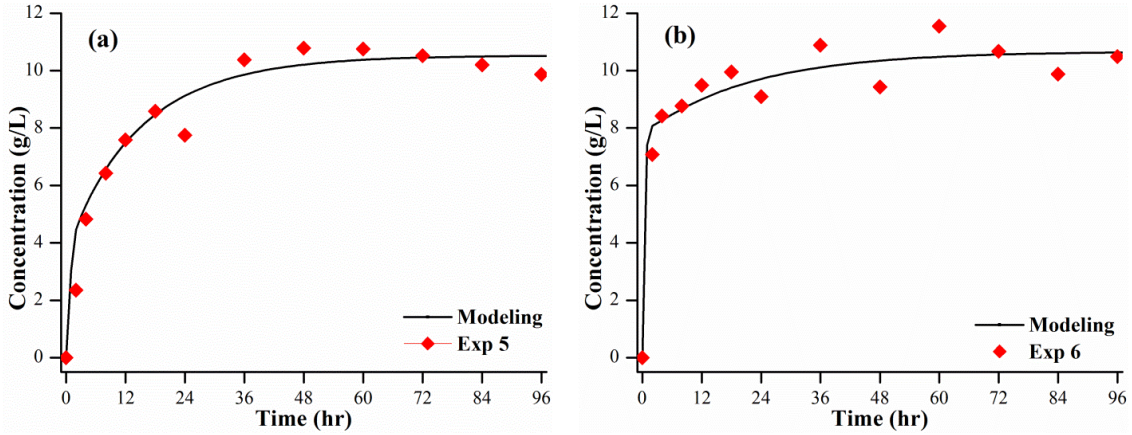


Figure 4.3. Comparison of model prediction and experimental ethanol concentrations at enzyme loading of 20 FPU/g cellulose

4.4.2 Dynamic characteristics of the SSF process

The dynamic features of the SSF process can be analyzed from the measured concentration profiles of glucose, mannose, cellobiose and ethanol illustrated in Figure 4.4. In each case, glucose and mannose present in the feedstock were quickly converted to ethanol,

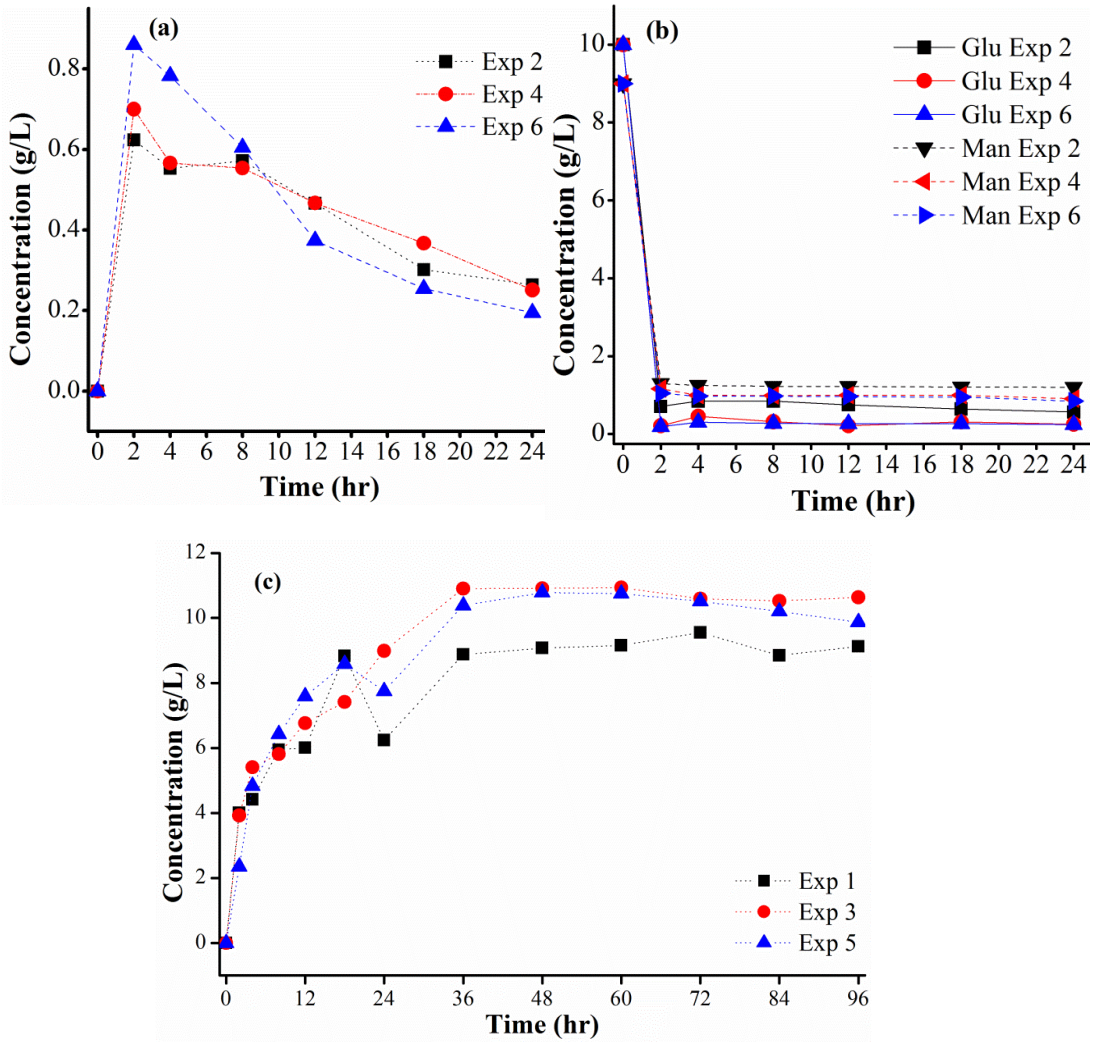
accompanied by dramatic variations in the concentrations of glucose, mannose and ethanol within the first 2 hours. After that, the concentrations of glucose and mannose decreased very slowly whereas the concentration of ethanol ascended gradually. Concentration of cellobiose, an intermediate product converted from cellulose by enzymatic hydrolysis, increased quickly to peak values in the first 2 hours and then declined gradually till the end of the experiments.

Experimental results of the SSF process indicated that initial concentrations of fermentable sugars (glucose and mannose) have great impact on ethanol concentration. As seen from Figures 4.4c & 4.4d, increasing the glucose concentration from 5 to 10 g/L and mannose from 4.5 to 9 g/L in the feedstock led to an escalation of ethanol concentration from 2.5 - 4.1 g/L (Exp. #1, #3 & #5) to about 6.5 – 7.6 g/L (Exp. #2, #4 & #6) after 2 hrs of SSF experiment. The highest ethanol concentration was obtained from Exp. #4 with the high level sugar concentration in the feedstock and intermediate enzyme loading (15 FPU/g cellulose). It is clearly seen from Figure 4.4 that high concentration of sugars in the feedstock led to a strong inhibition effect on hydrolysis and fermentation when high enzyme loading (Exp. #6) was applied, under which a final ethanol concentration of 10.49 g/L was reached.

4.4.3 Product inhibition on enzymatic hydrolysis

Cellulase inhibition by hydrolysis products (cellobiose and glucose) has long been known. It is widely reported that cellobiose was the stronger inhibitor of cellobiose formation (reaction r_1) and glucose inhibition in reaction r_2 should be greater than cellobiose

inhibition [12, 35]. Experimental results from this study proved the strong inhibition of cellobiose and glucose on enzymatic hydrolysis. As seen from Table 4.3, the combination of the highest enzyme loading and high initial



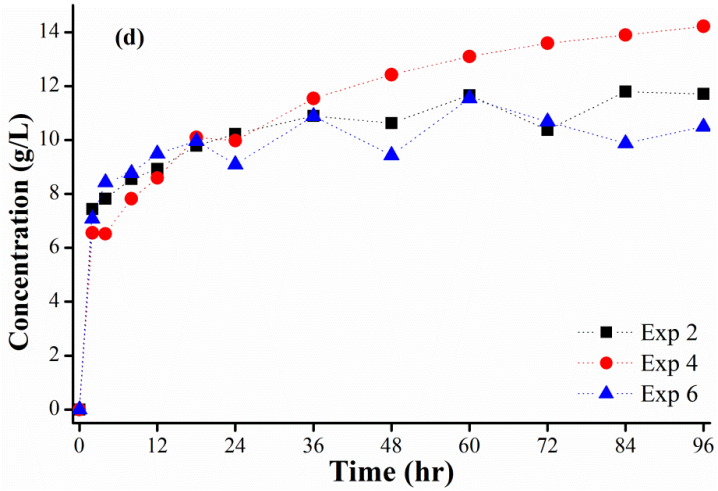


Figure 4.4. Concentration profiles of cellobiose (a), sugars (b), and ethanol (c & d) for SSF experiments

sugar concentration (Exp. #6) provides very low ethanol yield and ethanol concentration. Similar inhibition effect can also be observed from the experiments using low level of initial sugars, both ethanol yield and final ethanol concentration obtained from Exp. #5 are much lower than those from Exp. #3.

Table 4.3. Final ethanol yield and concentration from SSF with different operating conditions

Exp. #	Ethanol Yield, %	Ethanol Concentration, g/L
1	27.45	9.13
2	30.72	11.70
3	31.98	10.63
4	37.32	14.22
5	29.69	9.87
6	27.54	10.49

The product inhibition mechanism can be better understood by investigating the reaction rates of enzymatic hydrolysis (r_1, and r_2) based on different initial concentrations of sugars.

At low level of initial sugars, the highest reaction rate of r_1 and r_2 occurred in Exp. #3 as seen from Figure 4.5a, followed by those from Exp. # 5. The reaction rates of r_1 and r_2 are the lowest from Exp. #1. Reaction rates obtained at different enzyme loadings demonstrate that increasing enzyme loading from 10 to 15 FPU/g cellulose accelerated the conversion rate of cellulose (r_1), and relative higher amount of cellobiose produced from r_1 is the main cause for the attainment of higher r_2 and the subsequent conversion of glucose. However, further increase of enzyme loading to 20 FPU/g cellulose resulted in the accumulation of cellobiose in the substrate, which strongly inhibited the cellulase activity, leading to reduced reaction rates in r_1 and r_2 from Exp. #5. These results clearly indicate that cellobiose inhibition is dominant during the SSF process when low concentrations of sugars were used in the feedstock. In order to overcome the impact of cellobiose inhibition at high level of enzyme loading, changing the mode of the SSF reaction from batch to fed-batch seems to be promising. Gradually adding the enzyme into the reaction media ensures that there is fresh enzyme available any time for hydrolysing the remaining cellulose in reactor. Another solution to resolve the inhibition impact is implementing the continuous design instead of batch mode.

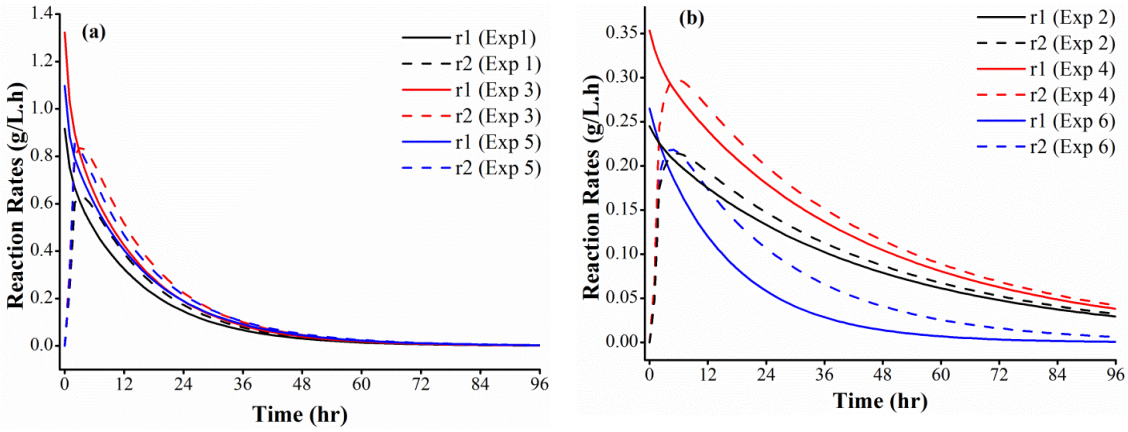


Figure 4.5. Comparison of dynamic reactions rates for simultaneous enzymatic hydrolysis and fermentation

In case of high initial concentrations of sugars being used, inhibition of glucose became significant, which is reflected from the much slower reaction rates (r_1 and r_2) obtained from Exps. #2, #4 and #6 (Figure 4.5b). Likewise, reaction rates of r_1 and r_2 are the highest from Exp. #4 when intermediate level of enzyme loading (15 FPU/g cellulose) was employed, followed by those from Exp. #2 (lowest enzyme loading, 10 FPU/g cellulose) and Exp. #6 (highest enzyme loading, 20FPU/g cellulose). The lowest reaction rates of r_1 and r_2 from Exp. #6 proved the strongest inhibition effects of both cellobiose and glucose. The higher concentrations of cellobiose and glucose in Exp. #6, according to Ishmayana et al. [36], expose the yeast to high osmotic stress, influences on fermentation performance of the yeast and reduces the amount of produced ethanol. These results clearly Figure 4.5 also reveals that in the certain dosage of enzyme loading, glucose inhibition causes the significant decrease in r_1 and r_2. Comparing the reaction rates of Exp. #1 and Exp. #2 for instance highlights the significant inhibition impact of glucose at higher initial sugar level.

For SSF process with fixed substrate and yeast loading, the interplay between the enzyme loading and initial concentration of fermentable sugars is obvious. With lower initial concentration of sugars, the enhancement of ethanol yield and concentration is easily attainable by employing higher enzyme loading. However, due to the strong inhibitory effect of glucose, high enzyme loading results in a significant decrease in ethanol yield and concentration when the feedstock contains very high concentration of fermentable sugars. This provides useful information with respect to the optimization of SSF process. Depending on the substrate and sugar concentration in the feedstock of SSF, enzyme loading should be selected strategically.

The kinetic model and the acquired kinetic parameters are able to help the future studies regarding the optimization of SSF process. The five kinetic parameters were tuned in different conditions of the SSF reaction to evaluate the response of the system to various reaction conditions, therefore optimization of the SSF process in a wider range of sugars concentration and enzyme loading would be possible through this model for further studies.

4.4.4 Impact of Agitation

Inhomogeneity caused by inadequate mixing when working with high water insoluble solid content has been previously addressed in several ways. Several research articles reported that purely increasing the agitation speed does not have significant influence on final ethanol yield. In this work, two agitation modes were used to evaluate the impact of agitation on final ethanol concentration and yield. Two additional batch SSF runs were performed by implementing a magnet stirrer with the speed of 600 rpm instead of the

baffled stirrer with the speed of 350 rpm while keeping other operating conditions the same as Exp. #4 and Exp. #5. The effect of agitation mode on the ethanol concentration is presented in Figure 4.6.

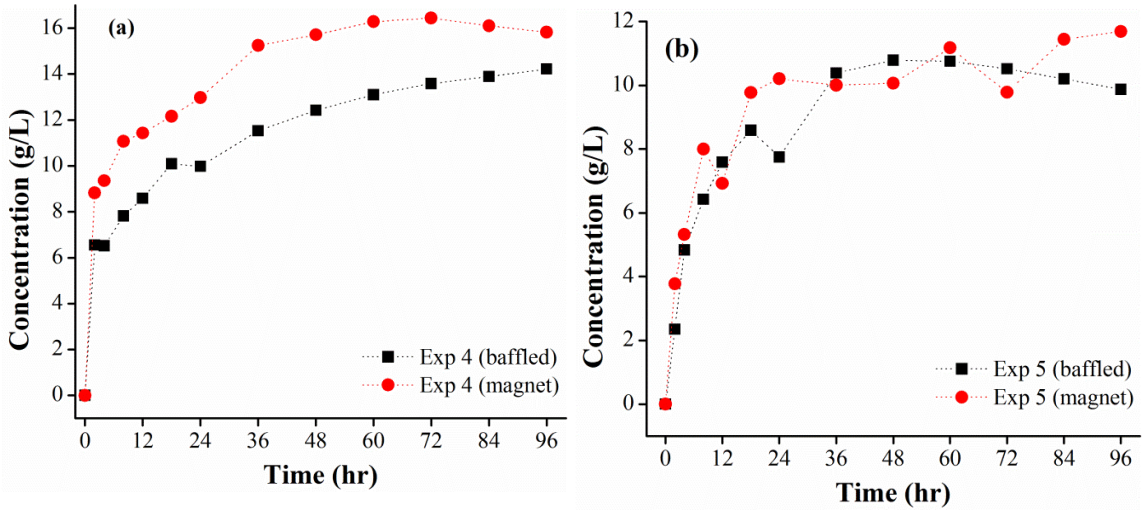


Figure 4.6. Comparison of the ethanol concentration in (a) Exp. #4 and (b) Exp. # 5 by using baffled stirrer and magnet stirrer

Results from Figure 4.6 reveal that magnet stirrer helps to enhance the ethanol yield for Exp. #4 from 37.32% to 41.53% and for Exp. #5 from 29.69% to 35.15%. Comparing the results of Exp. #4 and Exp. #5 reveals that stirring is an influential parameter that must be taken into account. Increasing the agitation rate in case of high solids loading significantly improves the SSF efficiency [37] and as it can be seen from Figure 4.6, in both cases efficient agitation rate enhances the final ethanol concentration. The lower efficiency of the baffled stirrer might be due to incomplete mixing or even the formation of some blind spots in the reactor. These disadvantages led to insufficient interaction between enzyme

and cellulose, as well as yeast and sugars. It causes diminish in efficiency of the system and in general decreases the ethanol yield.

4.5 Conclusion

Variations in the enzyme loading and initial sugar concentration lead to different product inhibition mechanism of batch SSF process. At low sugar concentrations, main cellulase inhibitory is caused by cellobiose. However, at high initial sugar concentrations, inhibition effects from cellobiose and glucose are both important. These inhibition effects are more remarkable in the batch media of process due to the accumulation of the end-products of the hydrolysis process. Moreover, at relative low enzyme loading, adding fermentable sugars to the reaction media diminishes the glucose inhibitory impacts and increases the final ethanol yield and concentration.

Results from this study also demonstrated that initial sugar concentration has significant influence on the reaction rate and rate constants. Higher initial sugar concentration resulted in strong product inhibition of enzymatic hydrolysis, leading to smaller values of k_1, λ and k_2. Meanwhile, higher initial sugar concentration accelerated the growth rate of microorganisms due to the presence of more nutrients, reflected from the convergence of a higher value of μ_m.

Finally, results from batch SSF experiments with two agitation methods reveal that a better mixing between the solid substrate and liquid mixture helps to improve the ethanol yield significantly.

4.6 References

1. Wagner, H., & Kaltschmitt, M. (2013). Biochemical and thermochemical conversion of wood to ethanol – simulation and analysis of difference processes. *Biomass Conversion and Biorefinery,* 3, 87–102.

2. Watanabe, T. E. (2013), in *Lignocellulose Conversion: Enzymatic and Microbial Tools for Bioethanol Production*: Introduction: Potential of Cellulosic Ethanol (Faraco V., ed) Springer, Berlin, pp. 1-20.

3. Conde-Mejia, C., Jimenez-Gutierrez, A., & El-Halwagi, M. (2012). A comparison of pretreatment methods for bioethanol production from lignocellulosic materials. *Process Safety and Environmental Protection*, 90, 189-202.

4. Shadbahr, J., Zhang, Y., & Khan, F. (2015). Life Cycle Assessment of Bioethanol Production from Woodchips with Modifications in the Pretreatment Process. *Applied Biochemistry and Biotechnology*, 175(2). 1080-1091.

5. Balat, M., Balat, H., & Öz, C. (2008). Progress in bioethanol processing. *Progress in Energy and Combustion Science,* 34, 551–573.

6. Van Dyk, J. S., and B. I. Pletschke (2012) A review of lignocellulose bioconversion using enzymatic hydrolysis and synergistic cooperation between enzymes Factors affecting enzymes, conversion and synergy. *Biotechnology Advances,* 30(6):1458-1480.

7. Jönsson, L.J., Alriksson, B., & Nilvebrant, N.-O. (2013). Bioconversion of lignocellulose: inhibitors and detoxification. *Biotechnology for Biofuels,* 6:16.

8. Srirangan, K., Akawi, L., Mon-Young, M., & Chou, C. P. (2012). Towards sustainable production of clean energy carriers from biomass resources. *Applied Energy*, 100, 172-186.

9. Klein-Marcuschamer, D., Oleskowicz-Popiel, P., Simmons, B.A., & Blanch, H.W. (2012). The challenge of enzyme cost in the production of lignocellulosic biofuels. *Biotechnology and Bioengineering*, 109, 1083-1087.

10. Geraili, A., Sharma, P., & Romagnoli, J.A. (2014). Technology analysis of integrated biorefineries through process simulation and hybrid optimization. *Energy*, 73, 145-159.

11. Koppram, R., Tomás-Pejó, E., Xiros, C., & Olsson, L. (2014). Lignocellulosic ethanol production at high-gravity: challenges and perspectives. *Trends in Biotechnology* 32, 46-53.

12. Philippidis, G. P., Smith, T. K., & Wyman, C. E. (1993). Study of the Enzymatic Hydrolysis of Cellulose for Production of Fuel Ethanol by the Simultaneous Saccharification and Fermentation Process. *Biotechnology and Bioengineering*, 41, 846-853.

13. Rass-Hansen, J., Falsig, H., Jørgensen, B., & Christensen, C. H. (2007). Prospective Bioethanol: fuel or feedstock? *Journal of Chemical Technology and Biotechnology*, 82, 329–333.

14. Hoyer, K., Galbe, M., & Zacchi, G. (2009). Production of fuel ethanol from softwood by simultaneous saccharification and fermentation at high dry matter content. *Journal of Chemical Technology and Biotechnology*, 84, 570–577.

15. Sanchez, Ó. J., & Cardona, C. A. (2008). Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresource Technology*, 99, 5270–5295.

16. Vasquez, M. P., Da Silva, J. N. C., De Souza, M. B., & Pereira N. (2007). Enzymatic Hydrolysis Optimization to Ethanol Production by Simultaneous Saccharification and Fermentation. *Applied Biochemistry and Biotechnology,* 136-140, 141-154.

17. Lopez-Linares, J. C., Romero, I., Cara, C., Ruiz E., Castro, E., & Moya, M. (2014). Experimental study on ethanol production from hydrothermal pretreated rapeseed straw by simultaneous saccharification and fermentation. *Journal of Chemical Technology and Biotechnology,* 89, 104–110.

18. Zhu, M., Li, P., Gong, X., & Wang, J. (2012). A comparison of the production of ethanol between simultaneous saccharification and fermentation and separate hydrolysis and fermentation using unpretreated cassava pulp and enzyme cocktail. *Bioscience, Biotechnology, and Biochemistry,* 76(4), 671-678.

19. Hoyer, K., Galbe, M., & Zacchi, G. (2010). Effects of enzyme feeding strategy on ethanol yield in fed-batch simultaneous saccharification and fermentation of spruce at high dry matter. *Biotechnology for Biofuels,* 3(14), 1-11.

20. Liu, K., Lin, X., Yue, J., Li, X., Fang, X., Zhu, M., Lin, J., Qu, Y., & Xiao, L. (2010). High concentration ethanol production from corncob residues by fed-batch strategy. *Bioresource Technology*, 101, 4952–4958.

21. Stenberg, K., Bollok, M., Reczey, K., Galbe, M., & Zacchi, G. (2000). Effect of Substrate and Cellulase Concentration on Simultaneous Saccharification and Fermentation of Steam-Pretreated Softwood for Ethanol Production. *Biotechnology and Bioengineering*, 68(2), 204-210.

22. Neves, M. A., Kimura, T., Shimizu, N., & Nakajima, M. (2007). State of the art and future trends of bioethanol production. *Dynamic biochemistry. Process Biotechnology and Molecular Biology*, 1, 1–14.

23. Cheung, S. W., & Anderson, B. C. (1997). Laboratory Investigation of Ethanol Production from Municipal Primary Wastewater Solids. *Bioresource Technology*, 59, 81-96.

24. Huang, X., & Penner, M. H. (1991). Apparent substrate inhibition of the Trichoderma reesei cellulase system. *Journal of Agricultural and Food Chemistry*, 39, 2096–2100.

25. Valjamae, P., Pettersson, G., & Johansson, G. (2001). Mechanism of substrate inhibition in cellulose synergistic degradation. *European Journal of Biochemistry*, 268, 4520–4526.

26. Sun, Y., & Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Technology*, 83, 1–11.

27. Morales-Rodriguez, R., Gernaey, K. V., Meyer, A. S., & Sin, G. A. (2011). Mathematical model for simultaneous saccharification and cofermentation (SSCF) of C6 and C5 Sugars. *Chinese Journal of Chemical Engineering*, 19 (2), 185-191.

28. Sasikumar, E., & Viruthagiri, T. (2010). Simultaneous Saccharification and Fermentation (SSF) of Sugarcane Bagasse - Kinetics and Modeling. *International Journal of Chemical and Biomolecular Engineering*, 3 (2), 57-64.

29. Shin, D., Yoo, A., Kim, S. W., & Yang, D. R. (2006). Cybernetic modeling of simultaneous saccharification and fermentation for ethanol production from steam-exploded wood with Brettanomyces custersii. *Journal of Microbiology and Biotechnology*, 16(9), 1355-1361.

30. van Zyl, J. M., van Rensburg, E., van Zyl, W. H., Harms, T. M., & Lynd, L. R. (2011). A Kinetic Model for Simultaneous Saccharification and Fermentation of Avicel with Saccharomyces cerevisiae. *Biotechnology and Bioengineering*, 108, 924-933

31. Philippidis, G. P., Spindler, D. D., & Wyman, C. E. (1992). Mathematical modeling of cellulose conversion to ethanol by the simultaneous saccharification and fermentation process. *Applied Biochemistry and Biotechnology*, 34-5, 543-556.

32. Philippidis, G. P., & Hatzis, C. (1997). Biochemical engineering analysis of critical process factors in the biomass to ethanol technology. *Biotechnology Progress*, 13(3), 222-231.

33. Pettersson, P. O., Eklund, R., & Zacchi, G. (2002). Modeling Simultaneous Saccharification and Fermentation of Softwood. *Applied Biochemistry and Biotechnology*, 98-100, 733-746.

34. Santos, A. L. F., Kawase, K. Y. F., & Coelho, G. L. V. (2011). Enzymatic saccharification of lignocellulosic materials after treatment with supercritical carbon dioxide. *The Journal of Supercritical Fluids*, 56, 277–282.

35. Kent, R., Kurašin, M., Teugjas, H., & Väljamäe, P. (2013). Strong cellulase inhibitors from the hydrothermal pretreatment of wheat straw. Biotechnology for Biofuels, 6:135.

36. Ishmayana, S., Learmonth, R. P., & Kennedy, U. J. (2011). Fermentation performance of the yeast Saccharomyces cerevisiae in media with high sugar concentration. *Proceedings of the 2nd International Seminar on Chemistry*, 2011, 379-385.

37. Kadic, A., Palmqvist, B., & Lidén, G. (2014). Effects of agitation on particle-size distribution and enzymatic hydrolysis of pretreated spruce and giant reed. *Biotechnology for Biofuels*, 7(77), 1-10.

5 Multi-Objective Optimization of Simultaneous Saccharification and Fermentation Process

Preface

A version of this manuscript is submitted to the Renewable Energy journal. I am the primary author of this paper. Along with the co-authors, Faisal Khan, Yan Zhang, and Kelly Hawboldt, I developed the multi objective optimization of SSF process. I conducted the optimization cases, literature review, and experiments. I prepared the first draft of the manuscript and subsequently revised the manuscript based on the co-authors' feedback. The co-author Yan Zhang helped in choosing the appropriate optimization method, programming and running the simulation codes, reviewing and correcting the achieved results, and contributed in preparing, reviewing and revising the manuscript. The co-author Faisal Khan contributed through support in the conceptual development and improvement of the optimization results, research methodology design, and reviewing and revising the manuscript. Co-author Kelly Hawboldt also assisted in reviewing and revising the manuscript.

Abstract

A multi-objective optimization of simultaneous saccharification and fermentation process for cellulosic ethanol production was carried out to simultaneously maximize the ethanol yield/cellulose conversion and minimize the enzyme consumption by manipulating the

initial sugar concentrations, and cellulose and enzyme loadings. The study was based on an experimentally verified kinetic model. Several bi-objective optimization problems with different combinations of objectives and constraints were solved by a controlled elitist genetic algorithm, a variant of the non-dominated sorting genetic algorithm II (NSGA-II). The optimal operating conditions attained through optimization were verified by experiments. Significant performance improvement of ethanol yield, cellulose conversion and enzyme loading is achieved by systematic optimization. The optimal operation conditions are highly sensitive to kinetic model and relevant kinetic parameters. Therefore, uttermost care must be paid in choosing the kinetic model and its parameters.

Keywords: Simultaneous saccharification and fermentation, Cellulose, Bioethanol, Multi-objective optimization

5.1 Introduction

Second-generation bioethanol produced from lignocellulosic biomass, i.e., waste plant matter from forestry or agriculture is a potential alternative to fossil fuels due to its renewable nature and availability [1,2]. The bioconversion of nonedible polysaccharides (cellulose and hemicellulose) in agricultural residues to ethanol is commercially viable. However, bioconversion of woody biomass, from forestry residues, to ethanol has not yet been translated from demonstration scale due to high capital and operating costs [3,4]. In addition to the issues associated with removing the lignin, there are several technical challenges, e.g., low depolymerisation efficiency of cellulolytic enzymes, high end-product

inhibition and insufficient mixing at high substrate concentration which need to be overcome to make the cellulosic ethanol more competitive with fossil-based transportation fuels.

Although simultaneous saccharification and fermentation (SSF) helps to mitigate the inhibitory effect of converted sugars by in-situ ethanol fermentation [5,6], the performance (reaction conversion, final ethanol yield and concentration) is highly dependent on the type of lignocellulosic feedstock, the substrate concentration, the type and amount of cellulolytic enzymes and microorganisms, solution pH and reaction temperature among others [7]. Moreover, SSF is usually requires a high substrate loading to achieve a high enough ethanol concentration to make the process economically viable but high substrate loading limits mixing and mass transfer of the hydrolysis fermentation system and subsequently the overall performance of the process. Simultaneously optimizing substrate concentration and enzyme/microorganism loading could potentially minimize these transport phenomena limitations while maximizing ethanol formation [8-10].

Optimization of SSF process based on statistically designed experiments has been widely studied [11-16]. Benjamin et al., (2014) [13] applied a central composite design (CCD) under response surface methodology to maximize the combined sugar yield and ethanol concentration for batch and fed-batch SSF of sugarcane. A three-factor-three-level Box-Behnken design was employed to predict the optimum substrate concentration, enzyme loading, and inoculum size for maximum ethanol yield from cassava peel [14]. Cavalaglio and co-workers (2016) [15] identified the optimal water-insoluble substrate amount, optimal liquid fraction and enzyme loading of a SSF bioconversion of Phragmites Australis

through CCD. A Taguchi orthogonal array design was implemented by Das et al., (2016) [16] to find the optimum operation conditions (cellulose and hemicellulose loading, yeast amount, solution pH and temperature) for ethanol production from Eichhornia crassipes. These studies outline the main impacts and interaction of the key operating parameters of SSF process. However, the accuracy of optimization results is highly dependent on the design of the set of experiments and therefore difficult to compare or draw major trends from. Furthermore, response surfaces are valid only in range of parameters studied and therefore cannot be applied to wider ranges directly. Systematic optimization of SSF process based on mechanistic mathematical model is more attractive as it provides more reliable and accurate predictions of system performance.

In contrast to the many studies using response surface methodology, optimization using mechanistic kinetic and reactor model is less well studied. Wang et al., (2016) [17] assessed the effects of substrate, enzyme and cell feeding strategies on fed-batch simultaneous saccharification and co-fermentation (SSCF) of SO2-catalyzed steam pre-treated wheat straw based on a rigorous kinetic model and developed an optimal multi-feed strategy for maximum ethanol concentration. Unrean et al., (2016) [10] developed a SSF model to quantitatively characterize dynamic response of yeast cell growth, hydrolysis and fermentation kinetics. The model was used to optimize the fed-batch SSF performance to maximize ethanol yield and validated with experiments. Liu et al., (2016) [18] optimized the reaction temperature of a SSF process for ethanol production by incorporating a temperature-dependent kinetic model. All these optimization studies for bioethanol production involved a single objective function (ethanol concentration or ethanol yield)

without considering the costs associated with enzyme consumption. Maximum ethanol yield and minimum enzyme loading can't be achieved simultaneously. Optimization studies incorporating these conflicting objectives would be invaluable to process engineers and decision makers.

In this study, multi-objective optimization (MOO) of SSF to maximize the cellulose conversion/ethanol yield and to minimize enzyme loading was carried out based on enzymatic hydrolysis kinetics and a dynamic metabolic model of yeast cell. To the best of our knowledge, the present work is the first attempt to investigate the improvement of SSF performance by systematic multi-objective optimization using a validated kinetic model. After the careful assessment of the interactions between of substrate concentration and enzyme loading, several bi-objective optimization problems were defined and solved by a controlled elitist genetic algorithm for Pareto optimal solutions. Fast convergence to the true Pareto optimal fronts and well-distributed solutions were obtained in three case studies with different combinations of objectives and constraints. The reliability and accuracy of the bi-objective optimization of SSF were verified by comparing the experimental results with the model predictions. This optimization study not only gives us deeper insight into interactions of key operating parameters of SSF process, but also provides a methodology to balance these interactions into an optimized process.

5.2 Multi-objective Optimization of SSF

5.2.1 Kinetic modeling of SSF process

A model to predict the ethanol production in SSF is prerequisite for the optimization investigation. An integrative model combining the kinetic of enzymatic hydrolysis and dynamic fermentative metabolism model developed by Philippidis et al. [19-21] and Shadbahr et al. (2016) [22] was employed in this study. This model considers the fermentation of glucose and mannose alongside simultaneous enzymatic hydrolysis of cellulose to glucose, and is capable of predicting the dynamic profiles of released sugars and ethanol over wide range of operating conditions [21]. Detailed kinetic and reactor model as well as the methodology used for determination of the reaction kinetic parameters were presented in our previous study [22]. The model and kinetic parameters used here are the same as those reported previously [22].

5.2.2 Formulation of optimization problems

The SSF process involves the interaction between the enzyme which hydrolyses solid substrate to sugars and yeast which utilizes the sugars for growth and fermentation [23]. Optimal operation of SSF relies on balancing the rates of hydrolysis and fermentation, which can be achieved by proper selection of the initial substrate loading, the enzyme dosage and inoculum size. The conversion of cellulose (X), the final ethanol yield (Y) and/or ethanol concentration ($[E]_f$) are the key performance parameters of SSF process. The operating cost of SSF is impacted most dramatically by the enzyme loading. As such, maximization of ethanol yield is the main objective function for the optimization of SSF

process. This objective can be further specified to maximization of cellulose conversion, an important indicator of the depolymerisation efficiency of cellulolytic enzymes. Minimization of enzyme consumption, which is essentially in conflict with the first objective, can be considered as another objective function. Several combinations of the two objective functions are outlined in Table 5.1. Definitions of the objective functions considered in this study are listed below.

Cellulose conversion: $X = \frac{[C]_0 - [C]_f}{[C]_0} \times 100\%$ (5-1)

Ethanol yield: $Y = \frac{[E]_f - [E]_0}{0.511*([G]_0 + [M]_0 + 1.111*[C]_0)} \times 100\%$ (5-2)

Enzyme consumption per 1g/L ethanol produced: $Z = [enz] / [E]_f$ (5-3)

Table 5.1. Optimization problem formulations for SSF of cellulose

Case	Objectives	Constraints	Decision variables
I	Max $I_1(u) = X$ Min $I_2(u) = Z$	$[E]_f \geq 12$ g/L	$5.0 \leq [G]_0 \leq 10.0$ (g/L) $[M]_0 = 0.9[G]_0$ $5.0 \leq [C]_0 \leq 8.0$ % (w/v) $10.0 \leq [enz] \leq 20.0$ (FPU/g cellulose)
II	Max $I_1(u) = Y$ Min $I_2(u) = Z$	$[E]_f \geq 12$ g/L	Same as Case I
III	Max $I_1(u) = Y$ Min $I_2(u) = [enz]$	$Y \geq 30\%$ or $X \geq 20\%$	Same as Case I

Important operating parameters in SSF process include initial concentration of fermentable sugars, cellulose loading, dosage of the enzymes, and the loading of yeast strain. Earlier experiments indicate that SSF performance was not significantly influenced by the dosage of β-Glucosidase and the loading of yeast strain [19,22,24]. In addition, results from many simulations runs of SSF process indicated that cellulose conversion and final ethanol yield/concentration are greatly influenced by initial sugars concentration, cellulose and cellulase loadings. Therefore, in this multi-objective optimization study, three variables (initial concentration of fermentable sugars, cellulose loading, and dosage of cellulase) were used as decision variables due to the significant impact on the performance of SSF. The lower and upper bounds of the decision variables used for the optimization are summarized in Table 5.1 and are based on the experimental stability and process economy of SSF process reported in open literature [20,22,24]. To achieve a better and fast convergence of Pareto optimal solutions, a constraint was defined in each bi-objective optimization problem. The optimization problem formulation is summarized in Table 5.1.

5.2.3 Controlled elitist multi-objective genetic algorithm

The genetic algorithm (GA), an adaptive heuristic search method based on population genetics, has been proved to be one of the most robust optimizers for MOO problems [25-28]. GA mimics the principles of natural genetics and natural selection in solving optimization problems through four basic operators, namely inheritance, cross-over, reproduction and mutation [29,30]. However, GA sometimes fails to address complex high dimensional multi-modal problems where fitness function evaluation becomes

computationally complex [31]. In this study, a controlled elitist GA, which not only improves the chance of finding global optimal solutions but also increases the diversity of the population is employed to solve bi-objective optimization problems. Bi-objective optimization problems listed in Table 5.1 were performed by implementing "gamultiobj" tool (controlled elitist GA) provided by MATLAB R2016b. The computational parameters used in the algorithm are provided in the Table 5.2.

Table 5.2. Computational parameters used by generic algorithm

Parameter	Value
Population size	50
Elite count	0.05 * population size
Mutation function	Constraint dependent
Crossover function	Constraint dependent
Migration fraction	0.2
Generations	100 * variables
Stall generations	50

5.3 Experimental Method

Experimental investigations of batch SSF process were carried out to verify the optimization results. The experimental methods with respect to feedstock and enzyme compositions, yeast preparation, SSF experiments, and analytical method are explained elsewhere [22] and not included here for brevity. The SSF experiments were performed at 37 ºC in 250 mL jacketed flask with 100 mL active volume and the solution pH maintained at 5.0 over the 96 hours reaction time. Other operating conditions are summarized in Table 5.3. Exp. #1 in Table 5.3 was conducted twice to check the repeatability of the experiments and the results of Exp. #1 are the average values from two runs.

Table 5.3. Optimal operating conditions of SSF process for experimental validations

Exp. #	$[G]_0$ (g/L)	$[M]_0$ (g/L)	$[C]_0$ %(w/v)	[*enz*] (FPU/g cellulose)
1	10.0	9.0	8.0	10.0
2	10.0	9.0	5.32	12.23
3	6.88	6.19	5.07	10.0

***** The activity for the β-Glucosidase and yeast loading were fixed at 30 U/g cellulose and 5.0 g dry cell/L for all experiments, respectively.

5.4 Results and Discussion

The solutions of bi-objective problems listed in Table 5.1 give rise to Pareto-optimal sets and the range of trade-offs between the competing objectives. The predicted enhancement of SSF performance by bi-objective optimization was verified by experiment using optimal operating conditions.

5.4.1 Case I: Maximization of cellulose conversion and minimization of enzyme consumption per ethanol produced

Figure 5.1a presents the Pareto optimal solutions for simultaneous maximization of cellulose conversion and minimization of enzyme consumption per 1 g/L ethanol produced (hereinafter referred to unit enzyme consumption). Not surprisingly, the objectives are in conflict, and cellulose conversion increases as the unit enzyme consumption increases. However, there is an upper bound of cellulose conversion (roughly 30%) which cannot be further improved by purely increasing the enzyme loading and/or varying the feedstock conditions.

Each point on the Pareto optimal front corresponds to a set of decision variables, which are plotted in Figures 5.1.b-d. Figure 5.1b illustrates that higher cellulose loading resulted in reduced cellulose conversion even though higher enzyme loading was used. The result is logical and a result of two factors. First, inhibition of cellulose hydrolysis by cellobiose, glucose and ethanol is more severe as cellulose loading increases, leading to a reduced conversion of cellulose. Secondly, cellulase activity is profoundly influenced by the direct physical contact between cellulolytic enzyme and substrate. Higher solid loading limits the cellulose accessibility to cellulase, which limits the effectiveness of cellulase. Therefore, to maximize cellulose conversion, lower cellulose loading with relatively higher enzyme loading would be an option. A similar impact of initial sugar concentrations (glucose and mannose) on cellulose conversion is also observed (Figure 5.1c). At higher initial concentrations of sugars there is a resulting increase in inhibition of hydrolysis by the end product. Figure 5.1d summarizes the optimum enzyme loading as a function of cellulose conversions. In general, increasing enzyme loading (ignoring sugars concentration and cellulose loading) improves the hydrolysis rate, particularly at lower initial sugar concentrations. From Figure 5.1e, it is clear operating conditions leading to highest final ethanol concentration were different with those for maximum cellulose conversion.

In order to confirm that the obtained Pareto solutions of Case I are the true global optimal solutions, optimization was also carried out on divided domains of sugar concentrations and cellulose loading. That is, optimization was performed at low sugar (5.0-7.5 g/L glucose and 4.5-6.75 g/L mannose) and high sugar concentrations (7.5-10.0 g/L glucose and 6.75-9.0 g/L mannose), low cellulose (5.0-6.5% w/v) and high cellulose loading (6.5-

8.0% w/v) separately while keeping the same lower and upper limits for the other two variables. Figure 5.2 compares the results of five different optimization scenarios. The converged Pareto optimal solutions of Case I are the global optimums with respect to the two objectives in the defined searching domain (Figure 5.2).

Batch SSF experiment (Exp. #1) under the calculated optimal operating conditions was carried out to validate the optimization results. The maximum optimized final ethanol concentration was 14.11 g/L and ethanol yield was 25.60%. The experimental average final ethanol concentration and ethanol yield (Exp. #1) are 13.8 g/L and 25.03%, very close to the optimization predictions. This confirms the approach of using systematic multi-objective optimization in SSF analysis.

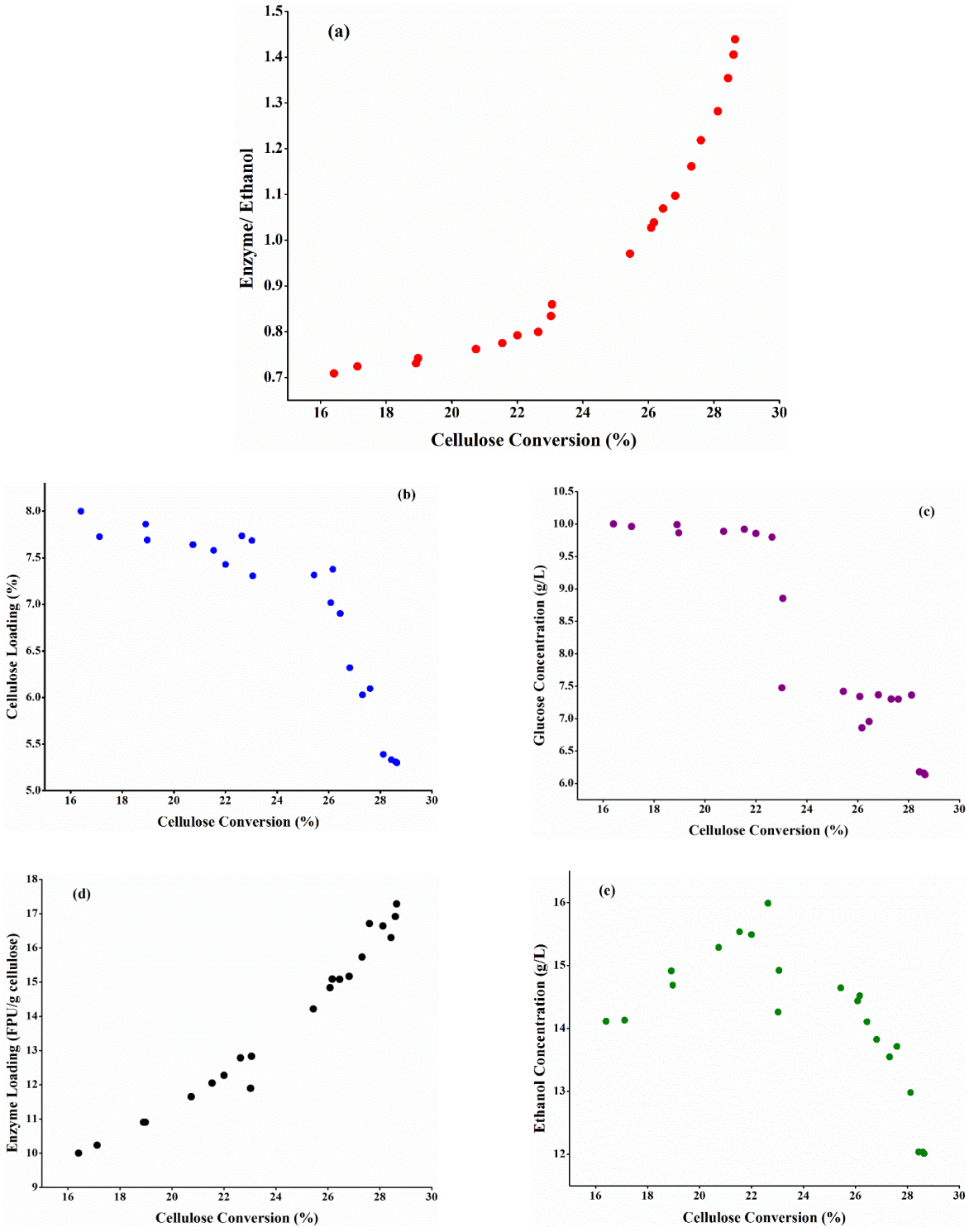


Figure 5.1. Pareto optimal solutions and corresponding decision variables for Case I, (a) objectives trade-offs, cellulose conversion vs (b) cellulose loading, (c) glucose concentration, (d) enzyme loading, and (e) ethanol concentration

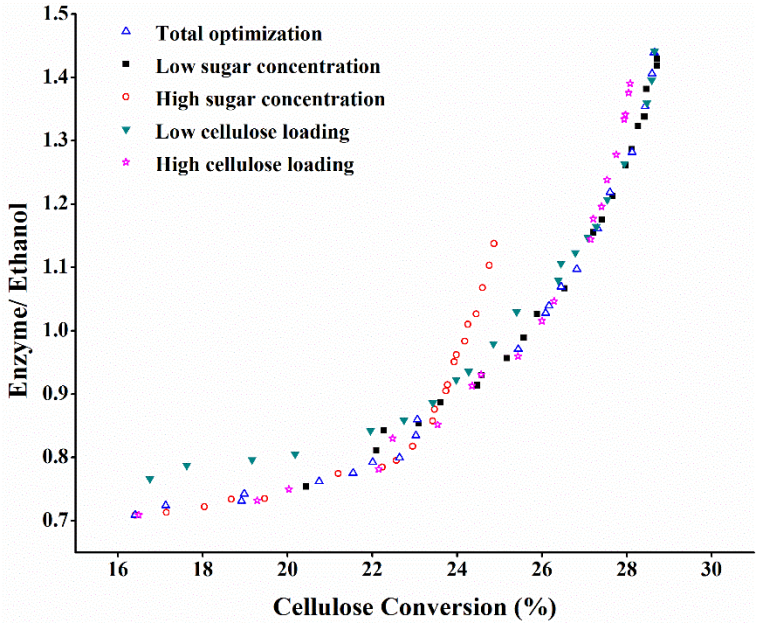


Figure 5.2. Comparison of optimization results at different ranges of sugar and cellulose concentrations

5.4.2 Case II: Maximization of ethanol yield and minimization of enzyme consumption per ethanol produced

With our validated model, the next step is to determine conditions where ethanol yield is maximized and the unit enzyme consumption minimized. The same constraint and ranges of decision variables as those of Case I were used. Well-distributed Pareto optimal solutions and the corresponding optimal sets of decision variables are illustrated in Figure 5.3. A trade-off exists between the two objectives; it is challenging to reduce the unit enzyme consumption without sacrificing the ethanol yield. Similar to Case I, ethanol yield cannot be continuously increased by optimizing the operating conditions and there is an upper limit of ethanol yield for batch SSF.

As seen from Figure 5.3c, optimal solutions for initial glucose concentration converge to the higher bound (logically higher initial sugar concentrations tend to increase ethanol yield). Maximizing ethanol yield as an objective forces the optimizer to restrict the sugar concentration to a narrow range. A significant scatter in other two decision variables, $[C]_0$ and [enz] accompanied the convergence of Pareto solutions. As illustrated from Figures 5.3b and 5.3d, different combinations of cellulose loading and enzyme loading are able to generate the same or very close objective values (points A, B and C in Figure 5.3a), the set of solutions ultimately chosen (by the optimizer) for generating the Pareto optimal points is determined by parameters randomly generated and convergence by the optimization algorithm. Figure 5.3 demonstrates the impact of the variables on the targeted objectives. For example, when low cellulose loading and high concentration of fermentable sugars (glucose and mannose) are selected, increasing enzyme loading results in higher ethanol yield and/or lower unit enzyme consumption. However, if high cellulose loading and sugar concentrations are used, increasing enzyme loading has little impact on ethanol yield due to the severe product inhibition.

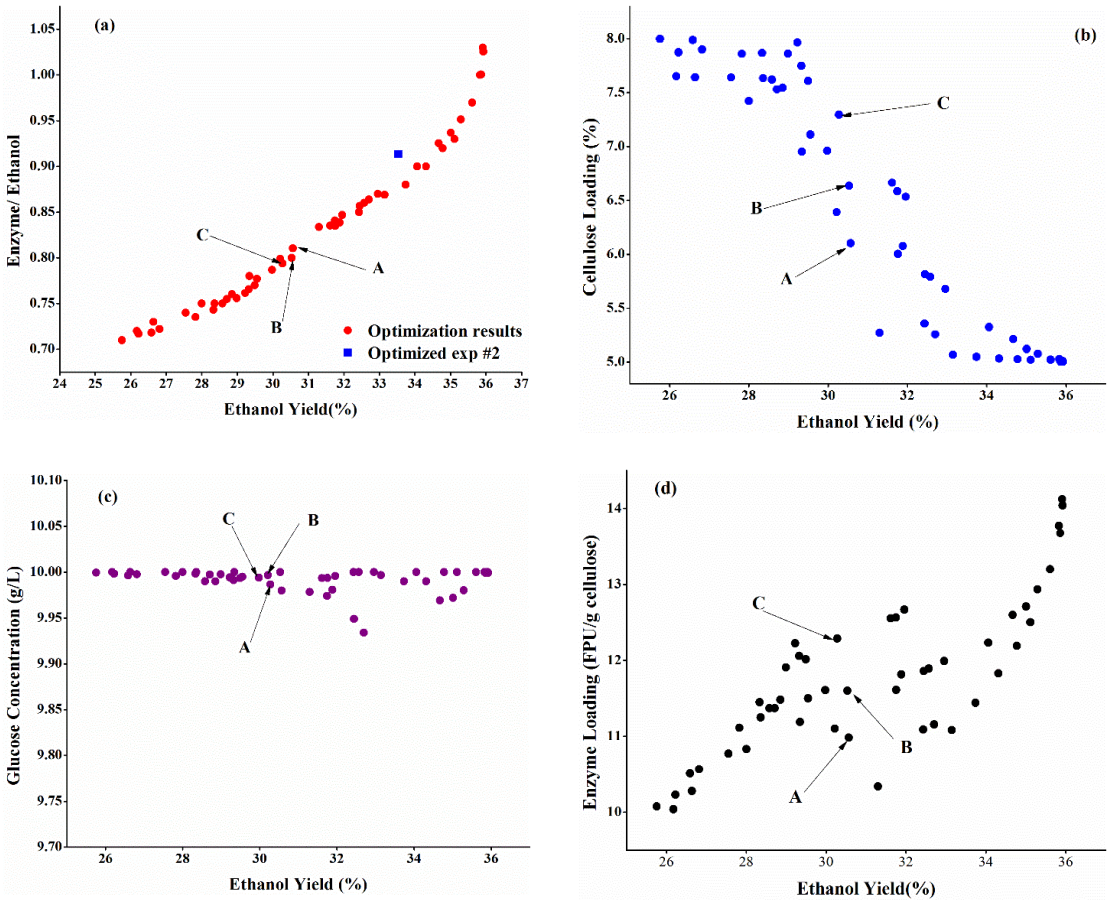


Figure 5.3. Pareto optimal solutions and corresponding decision variables for Case II with experimental validation. (a) objectives trade-offs, ethanol yield vs (b) cellulose loading, (c) glucose concentration, and (d) enzyme loading

The optimized variables from Case II were also verified by batch SSF process under the operating conditions of Exp. #2 (Table 5.3). This time, small deviations from the calculated optimum objective values were obtained. The predicted ethanol yield and unit enzyme consumptions are 34.06% and 0.89 FPU L/g-g, comparable to experimental values of 33.53% and 0.91 FPU L/g-g respectively. The enhancement of SSF performance through optimization (Figure 5.4) was assessed by comparing ethanol yields and unit enzyme

consumptions from three non-optimized experiments [22]. Experimental point D is certainly better than points E and F in terms of the two objectives. However, all the points on the Pareto set are better than the experimental points, leading to increase of ethanol yield or remarkable reduction in unit enzyme consumption.

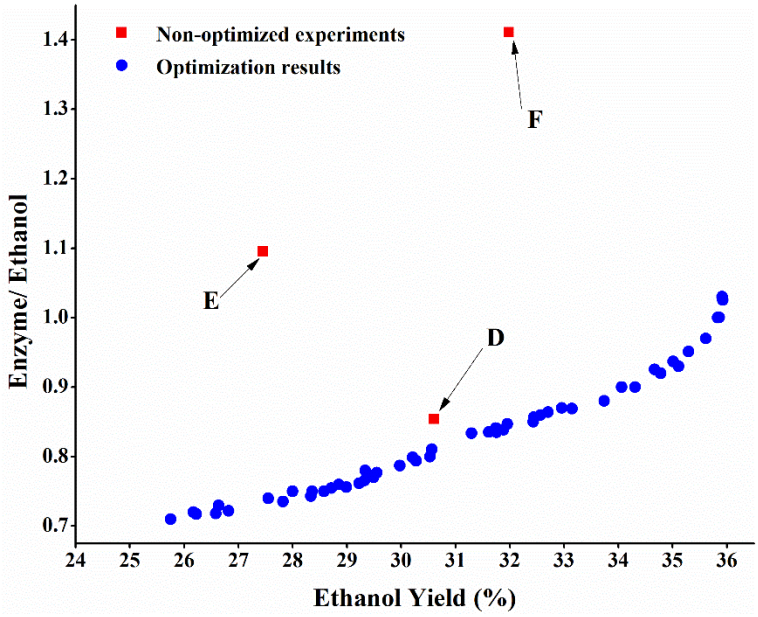


Figure 5.4. Enhancement of SSF performance by optimization in Case II

5.4.3 Case III: Maximization of ethanol yield and minimization of enzyme loading

The total amount of enzyme consumption is one of the key factors impacting the overall cost of bioethanol production by SSF. To study this in the third case, Case III, maximization of ethanol yield and minimization of enzyme loading were investigated under two scenarios with different constraints: (I) ethanol yield not less than 30% ($Y \geq$

30%); and (II) cellulose conversion not lower than 20% ($X \geq 20\%$). Comparison of the optimization results for the two scenarios is presented in Figure 5.5.

Figure 5.5a show the converged Pareto optimal solutions from the two scenarios differ slightly. For constrained optimization problems, constraints can be considered as high-priority (hard) objectives which must be satisfied before the optimization of the remaining soft objectives (ethanol yield and enzyme loading in this case) takes place [32]. As such, as one varies the high priority objectives from $Y \geq 30\%$ to $X \geq 20\%$ the solution changes. For instance, when lower enzyme loadings ($\leq$ 12.0 FPU/g) are selected, the constraint of $Y \geq 30\%$ forces higher sugar concentrations and lower cellulose loading to achieve maximum ethanol yield, whereas $X \geq 20\%$ forces the optimizer to select lower sugar and cellulose concentrations. This reveals that increasing sugar concentrations in the feedstock are favorable to the attainment of maximum ethanol yield at low enzyme loading. However, when enzyme loading is higher than 12.0 FPU/g both scenarios converged to the same Pareto front.

Enzyme loading converged to values lower than 14.5 FPU/g and cellulose loading converged to lower bound in both scenarios, indicating that maximum ethanol yield or cellulose conversion cannot be achieved by purely increasing the enzyme loading. Balanced rates of hydrolysis and fermentation rates as a result of proper combination of feedstock condition and enzyme loading are essential for the optimal operation of batch SSF. This observation is in agreement with the conclusions of experimental investigators [8,33,34].

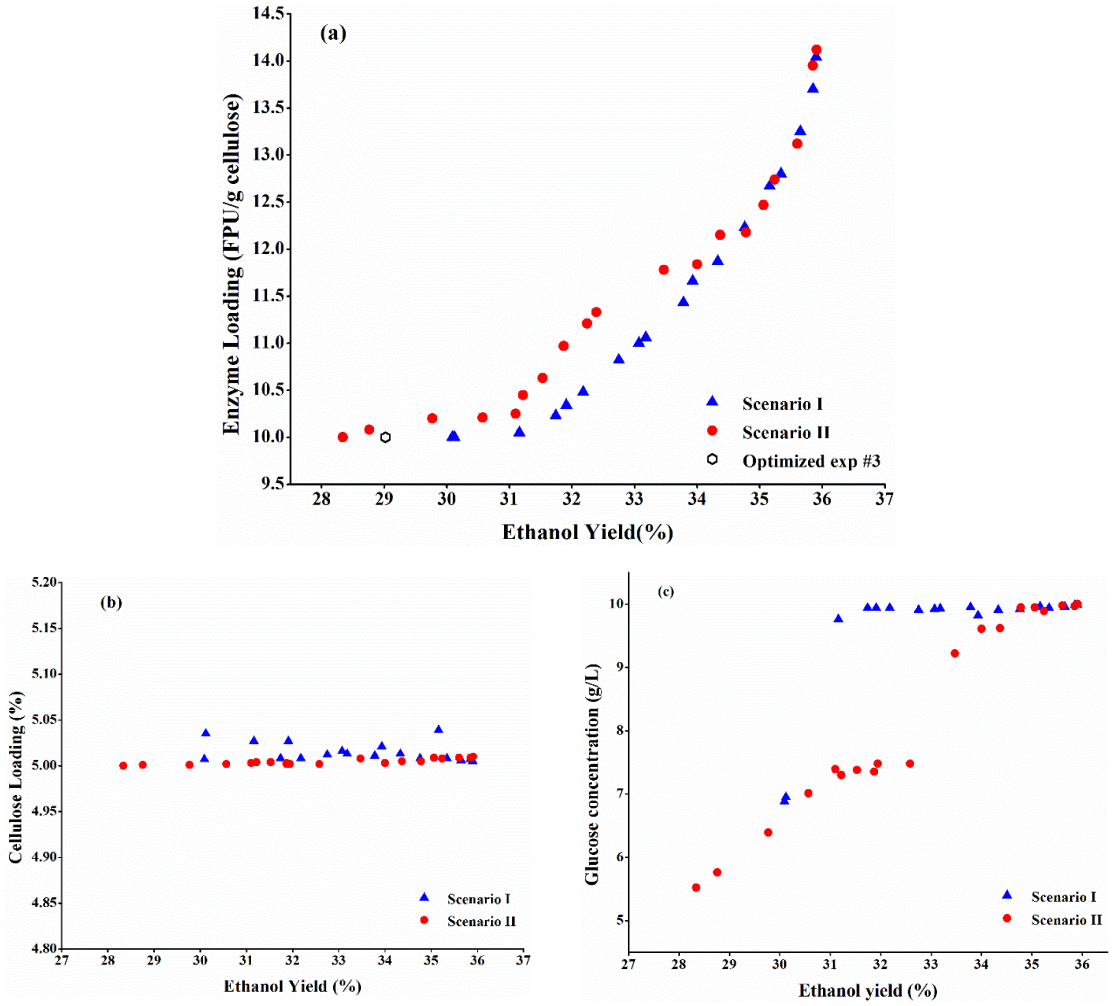


Figure 5.5. Pareto optimal solutions and corresponding decision variables of Case III. (a) objectives trade-offs, ethanol yield vs (b) cellulose loading, and (c) glucose concentration

Experimental validation of optimization results for Case III was also performed. Under the same operating conditions, predicted ethanol concentration by optimization is 10.56 g/L and corresponding to ethanol yield of 30.09%, again in good agreement with experimental values from Exp. #3 of 10.29 g/L and 29.32%. Enhancement of SSF performance with respect to maximizing ethanol yield and minimizing enzyme loading by optimization can be shown by comparison of our previous (non-optimized) experimental results (Figure 5.6)

[22]. To achieve the same ethanol yield, enzyme consumption can be reduced more than 50% by optimization.

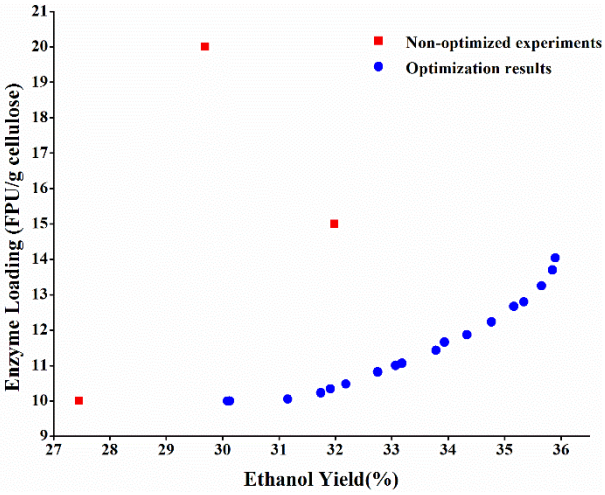


Figure 5.6. Enhancement of SSF performance by optimization in Case III

5.5 Conclusion

Multi-objective optimization of SSF for bioethanol production was investigated in this study by employing the controlled elitist GA. The objective of the study is to maximize ethanol yield/cellulose conversion and minimize enzyme consumption by optimizing the initial sugar concentrations, the cellulose and enzyme loadings. Simultaneous maximization of cellulose conversion and minimization of enzyme consumption per ethanol produced were performed. Results indicated that higher cellulose loading and sugar concentrations in the feedstock resulted in reduced cellulose conversion due to the strong product inhibition. In the second case, maximization of ethanol yield and minimization of enzyme consumption per ethanol produced were used as the objective functions. This time,

optimal solutions for initial glucose concentration converge to the higher bound, whereas cellulose loading and enzyme loading were converged to wider ranges. Therefore, enzyme loading needs to be properly selected based on cellulose loading for the attainment of maximum ethanol yield and minimum unit enzyme consumption. Optimization aimed at maximization ethanol yield and minimization of enzyme loading was finally conducted. Results reveal that high sugar concentrations in the feedstock is beneficial to high ethanol yield when low enzyme loading. In case of higher cellulose conversion is also desired, ethanol yield can be maximized only by properly selecting the enzyme loading for balanced rates of hydrolysis and fermentation.

Batch SSF experiments conducted under the predicted optimal operating conditions were used to verify the optimization results. Good agreement between the experimental measurements and optimization predictions was obtained, indicating that performance enhancement of SSF is attainable by systematic optimization based on reliable and robust kinetic models. The results and findings of this study can further be applied in a pilot plant to evaluate the performance of process in a larger scale.

Nomenclature

$[C]_0$	Initial cellulose loading (w/v)
$[E]_0$	Initial ethanol concentration (g/L)
$[E]_f$	Final ethanol concentration (g/L)
[enz]	Enzyme loading (FPU/g cellulose)
$[G]_0$	Initial glucose concentration (g/L)

$[M]_0$	Initial mannose concentration (g/L)
X	Cellulose conversion (%)
Y	Ethanol yield (%)
Z	Enzyme consumption per 1 g/L ethanol generated

5.6 References

1. M.F. Demirbas, M. Balat, H. Balat, Potential contribution of biomass to the sustainable energy development, Energy Convers Manage. 50(7) (2009), 1746-1760.

2. D. Zilberman, G. Hochman, D. Rajagopal, S. Sexton, G. Timilsina, The impact of biofuels on commodity food prices: Assessment of findings, Am. J. Agr. Econ. 95 (2) (2013), 275-281.

3. J. Larsen, M.Ø. Haven, L. Thirup, Inbicon makes lignocellulosic ethanol a commercial reality, Biomass Bioenerg. 46 (2012), 36-45.

4. L. Viikari, J. Vehmaanpera, A. Koivula, Lignocellulosic ethanol: From science to industry, Biomass Bioenerg. 46 (2012), 13-24.

5. R. Koppram, L. Olsson, Combined substrate, enzyme and yeast feed in simultaneous saccharification and fermentation allow bioethanol production from pretreated spruce biomass at high solids loadings, Biotechnology for Biofuels 7(54), (2014), 1-9.

6. M. Galbe, G. Zacchi, A review of the production of ethanol from softwood, Appl Microbiol Biotechnol. 59 (2002), 618-628.

7. J.C. Lopez-Linares, I. Romero, C. Cara, E. Ruiz, E. Castro, M. Moya, Experimental study on ethanol production from hydrothermal pretreated rapeseed straw by simultaneous saccharification and fermentation, J Chem Technol Biotechnol. 89 (2014), 104-10.

8. R. Koppram, E. Tomás-Pejó, C. Xiros, L. Olsson, Lignocellulosic ethanol production at high-gravity: challenges and perspectives, Trends Biotechnol. 32 (2014), 46-53.

9. K. Olofsson, M. Wiman, G. Lidén, Controlled feeding of cellulases improves conversion of xylose in simultaneous saccharification and co-fermentation for bioethanol production. J Biotechnol. 145 (2010), 168-175.

10. P. Unrean, S. Khajeeram, K. Laoteng, Systematic optimization of fed-batch simultaneous saccharification and fermentation at high-solid loading based on enzymatic hydrolysis and dynamic metabolic modeling of Saccharomyces cerevisiae. Appl Microbiol Biotechnol. 100 (2016), 2459-2470.

11. E. Betiku, A.E. Taiwo, Modeling and optimization of bioethanol production from breadfruit starch hydrolyzate vis-à-vis response surface methodology and artificial neural network, Renewable Energy. 74 (2015), 87-94.

12. S. Shanavas, G. Padmaja, S.N. Moorthy, M.S. Sajeev, J.T. Sheriff, Process optimization for bioethanol production from cassava starch using novel eco-friendly enzymes, Biomass and Bioenergy. 35(2) (2011), 901-909.

13. Y. Benjamin, M.P. García-Aparicio, J.F. Görgens, Impact of cultivar selection and process optimization on ethanol yield from different varieties of sugarcane, Biotechnol. Biofuels. 7 (2014):60.

14. S. Sivamani, R. Baskar, Optimization of bioethanol production from cassava peel using statistical experimental design, Environ. Prog. Sustain. Energy, 34 (2015), 567-574.

15. G. Cavalaglio, M. Gelosia, D. Ingles, E. Pompili, S. D'Antonio, F. Cotana, Response surface methodology for the optimization of cellulosic ethanol production from Phragmites australis through pre-saccharification and simultaneous saccharification and fermentation, Ind. Crops Prod. 83 (2016), 431-437.

16. S.P. Das, A. Gupta, D. Das, A. Goyal, Enhanced bioethanol production from water hyacinth (Eichhornia crassipes) by statistical optimization of fermentation process parameters using Taguchi orthogonal array design, Int. Biodeterior. Biodegradation. 109 (2016), 174-184.

17. R. Wang, P. Unrean, C.J. Franzén, Model-based optimization and scale-up of multi-feed simultaneous saccharification and co-fermentation of steam pre-treated lignocellulose enables high gravity ethanol production, Biotechnol Biofuels. 9 (2016), 88.

18. D. Liu, H. Zhang, C.C. Lin, B. Xu, Optimization of rice wine fermentation process based on the simultaneous saccharification and fermentation kinetic model, Chinese J. Chem. Eng. 24 (2016), 1406-1412.

19. G.P. Philippidis, D.D. Spindler, C.E. Wyman, Mathematical modeling of cellulose conversion to ethanol by the simultaneous saccharification and fermentation process, Appl Biochem Biotechnol. 34 (1992), 543-56.

20. G.P. Philippidis, T.K. Smith, C.E. Wyman, Study of the enzymatic hydrolysis of cellulose for production of fuel ethanol by the simultaneous saccharification and fermentation process, Biotechnol Bioeng. 41 (1993), 846–53.

21. G.P. Philippidis, C. Hatzis, Biochemical engineering analysis of critical process factors in the biomass to ethanol technology, Biotechnol Prog. 13(3) (1997), 222-31.

22. J. Shadbahr, F. Khan, Y. Zhang, Kinetic modeling and dynamic analysis of simultaneous saccharification and fermentation of cellulose to bioethanol, Energy Convers Manage. (141) 2017, 236-243.

23. S. Ostergaard, L. Olsson, J. Nielsen, Metabolic engineering of Saccharomyces cerevisiae, Microbiol. Mol. Biol. Rev. 64 (2000), 34-50.

24. P.O. Pettersson, R. Eklund, G. Zacchi, Modeling simultaneous saccharification and fermentation of softwood, Appl Biochem Biotechnol. 98-100 (2002), 733-46.

25. V. Bhaskar, S.K. Gupta, A.K. Ray, Applications of multi-objective optimization in chemical engineering, Rev. Chem. Eng. 16 (2000) 1-54.

26. K. Deb, A. Pratap, S. Agarwal, T. Meyarivan, Fast and elitist multi-objective genetic algorithm: NSGA-II, IEEE Transactions on Evolutionary Computation. 6 (2002), 182-197.

27. Y. Zhang, K. Hidajat, A.K. Ray, Multi-objective optimization of simulated moving bed and Varicol processes for enantio-separation of racemic pindolol, Sep. Purif. Technol. 65 (2009), 311–321.

28. J.K. Rajesh, S.K. Gupta, G.P. Rangaiah, A.K. Ray, Multi-objective optimization of industrial hydrogen plants, Chem. Eng. Sci. 56(2001), 999-1010.

29. S. Forrest, Genetic algorithms: principles of natural selection applied to computation, Science, 261 (1993), 872-878.

30. C.R. Reeves, J.E. Rowe, Genetic algorithms: Principles and perspectives: A guide to GA theory. New York: Kluwer Academic, 2002.

31. D.E. Goldberg, Genetic algorithms. New York: Pearson Education, 2006.

32. C.M. Fonseca, P.J. Fleming, Multiobjective optimization and multiple constraint handling with evolutionary algorithms–Part I: A unified formulation, IEEE Trans. Syst. Man Cybern. A Syst. Humans. 28 (1998), 26-37.

33. J.B. Kristensen, C. Felby, H. Jørgensen, Yield-determining factors in high-solids enzymatic hydrolysis of lignocellulose, Biotechnology for Biofuels. 2(2009), 1-10.

34. H. Jørgensen, J. Vibe-Pedersen, J. Larsen, C. Felby, Liquefaction of lignocellulose at high solids concentrations, Biotechnol Bioeng. 96 (2007), 862-870.

6 Comparative Life Cycle Analysis of Bioethanol production [3]

Preface

A version of this manuscript has been published in the Journal of Applied Biochemistry and Biotechnology. I am the primary author of this paper. Along with the co-authors, Yan Zhang and Faisal Khan, I developed two case studies for LCA study. I carried out most of the literature review, data collection, life cycle analysis and the comparison of scenarios. I prepared the first draft of the manuscript and subsequently revised the manuscript based on the co-authors' feedback and also the peer review process. The co-author Faisal Khan helped in defining the scenarios, choosing the analysis method, reviewed and corrected the method and results, and reviewed and revised the manuscript. The co-author Yan Zhang contributed in developing the new process design, improving the analysis, preparing, reviewing, and revising the manuscript.

Abstract

Pretreatment as a crucial step in the process of ethanol production has significant influences on the process efficiency and on the environmental performance of the bioethanol production from lignocellulosic biomass. In present life cycle analysis (LCA) study, two cases for pretreatment of woodchips were considered as the focal point of the ethanol plant. One was assumed as base scenario whereas the second is the proposed alternative by

[3] Shadbahr et al. Applied Biochemistry and Biotechnology, 2015, 175:1080–1091

implementation of modifications on the base design. In the first stage, LCA results of pretreatment unit showed lower environmental impacts in respiratory inorganics and land use than in new scenario, while the base scenario revealed better performance in fossil fuels. The results of the second stage of LCA study demonstrated improvement in proposed design in most categories of environmental impacts such as 18.5 % in land use as well as 17 % improvement in ecosystem quality.

Keywords: Bioethanol, Life cycle assessment, Lignocellulosic biomass, Pretreatment, Environmental impacts

6.1 Introduction

Increased effort is being made to improve the economic viability and technological advancement of processes that convert lignocellulosic biomass to bioethanol. Compared with the first generation bioethanol which is derived from sugar and starch produced by food crops [1, 2], the second generation bioethanol, which is produced from non-edible lignocellulosic biomass, offers the potential to provide a significant source of energy sustainably, affordably, and with greater environmental benefits [3–6]. Lignocellulosic biomass mainly consists of cellulose, hemicellulose, and lignin. This type of feedstock is available in abundance in the forms of agricultural residues, forestry residues, yard waste, municipal solid waste, and wood products [7]. However, the high production cost still hinders the production of lignocellulosic ethanol on an industrial scale.

Recently, biochemical conversion of lignocellulosic biomass through saccharification and fermentation has become a major pathway for ethanol production due to the advantages of this technology, such as mild operating conditions, a lower rate of by-product formation, a lower consumption of energy [8], etc. The biochemical process for converting lignocellulose to fuel ethanol involves four steps: (a) delignification to liberate cellulose and hemicellulose from their complex with lignin, (b) hydrolysis (saccharification) of cellulose and hemicellulose to produce fermentable sugars, (c) fermentation of hexose and pentose to ethanol, and (d) product separation and ethanol purification [9]. Among these steps, delignification of lignocellulosic raw material by pretreatment is the rate-limiting and the most challenging task. The efficiency of the pretreatment method to break the lignin structure and disrupt the crystalline structure of biomass determines the accessibility and digestibility of cellulose and, hence, governs the overall process economy of lignocellulosic ethanol [6, 10, 11]. Pretreatment still has great potential for improvements in efficiency and lowering of costs through further research and development [12].

Apart from the long-term economic viability of lignocellulosic ethanol, the environmental impacts of bioethanol production also attract a major concern. The environmental impacts of the bioethanol production system can be evaluated through life cycle assessment (LCA), a proven methodology to quantitatively analyze the environmental burden of a product or process over its entire life. Although many LCA studies have shown environmental benefits associated with lignocellulosic ethanol, most studies have focused on assessing the farming systems/different feedstocks with generic assumption of the ethanol conversion process [13, 14] or comparing the LCA results of bioethanol production system

with those of conventional fossil energy systems [15, 16]. Very few have addressed the specific environmental issues related to the conversion process due to process uncertainties and non-availability of commercial scale plants [17]. Research on how process design and technology improvements in an ethanol plant affect the environmental performances of the system is still required.

In the present study, environmental impacts of the chain process of ethanol production from woody biomass were investigated through life cycle analysis at two levels for two scenarios in pretreatment. Dilute sulfuric acid pretreatment of wood chips reported by National Renewable Energy Laboratory (NREL) [18] was selected in the base scenario. In the new pretreatment scenario, some modifications were applied to the base case. In this study, the effectiveness of different pretreatment designs on the life cycle analysis results of the individual unit as well as of the whole production plant was considered. By comparative LCA study of the two scenarios, this work aims to evaluate the influence of process design on the environmental impacts of cellulose bioethanol production.

6.2 Materials and Methods

6.2.1 Bioethanol Production System

The plant for producing bioethanol from lignocellulosic biomass consists of several units, such as pretreatment, saccharification and fermentation, product recovery, wastewater treatment, and power and steam production. Figure 6.1 shows the process of the bioethanol conversion system with woodchips used as the feedstock [18]. It must be noted that in this

study, two scenarios for the pretreatment unit were investigated from an environmental impact point of view. The influences of different designs of the pretreatment on the life cycle analyses of the individual unit as well as for the whole production plant were studied. Except for the pretreatment unit, other units such as saccharification and fermentation, product recovery, wastewater treatment, and power and steam production are assumed in the same operational conditions for both cases.

After pretreatment, most of the pretreated products are sent to the saccharification and fermentation unit and a small fraction of the products is used in the cellulase production unit to produce the required enzyme for hydrolysis of unconverted polymer sugars (mostly cellulose). Fermentation in this design occurs simultaneously with saccharification in a single reactor where the produced sugars (mainly glucose from cellulose and xylose from xylan) are immediately exposed to the fermentation process for conversion to ethanol. Products of the simultaneous saccharification and co-fermentation (SSCF) unit which contain ethanol, water, and sugars are then fed along with the unconverted lignin to the product recovery unit, where high purity ethanol is obtained by the distillation and dehydration processes. Ethanol is then sent to storage, and the remaining mixture of water and soluble and insoluble solids is sent to the wastewater treatment unit where wastewater is first separated from solids and then treated in anaerobic and aerobic digestion pools. The recovered water is used in the plant as recycled water. Solid phase (mostly lignin) from the wastewater treatment unit and biogas produced from anaerobic digestion are burned in the waste combustion unit to generate steam and electricity [18]. Electricity generated by waste combustion exceeds the demand of the plant and can be exported to the grid. Therefore, it

is usually considered as the co-product of the process, which is counted as a credit to the environmental impacts of the bioethanol production plant.

6.3 Life Cycle Assessment

6.3.1 Goal and Scope

The main goal of this study is to investigate the influence of process design on the environmental impacts of bioethanol production by comparing the LCA results from a basic design of a bioethanol plant with results after implementing some modifications in the pretreatment step of the process. What processes are included in the analysis and what are excluded must be clearly defined for LCA study. As explained later in pretreatment scenarios, two cases of the life cycle assessment for bioethanol production were studied, with each case being evaluated at two levels. At the first level, LCA was carried out on the sub-system boundary which only includes the pretreatment unit for the two cases. The second level was performed on all the units included in a bioethanol production plant which is shown in Figure 6.1. The defined system boundary for the first level is presented in Figures 6.2 and 6.3. It must be emphasized that capital goods and facilities are excluded from analysis in this study as boundary system for each step shows that.

The functional unit plays the role of a reference for calculating the amount of inputs, outputs, and energy demand of the system. In this context, the functional unit for first-level analysis is 1 kg of pretreated woodchips which are the product of pretreatment, and the

functional unit of the second level of analysis is 1 kg of produced ethanol which is the main product of the bioethanol production plant.

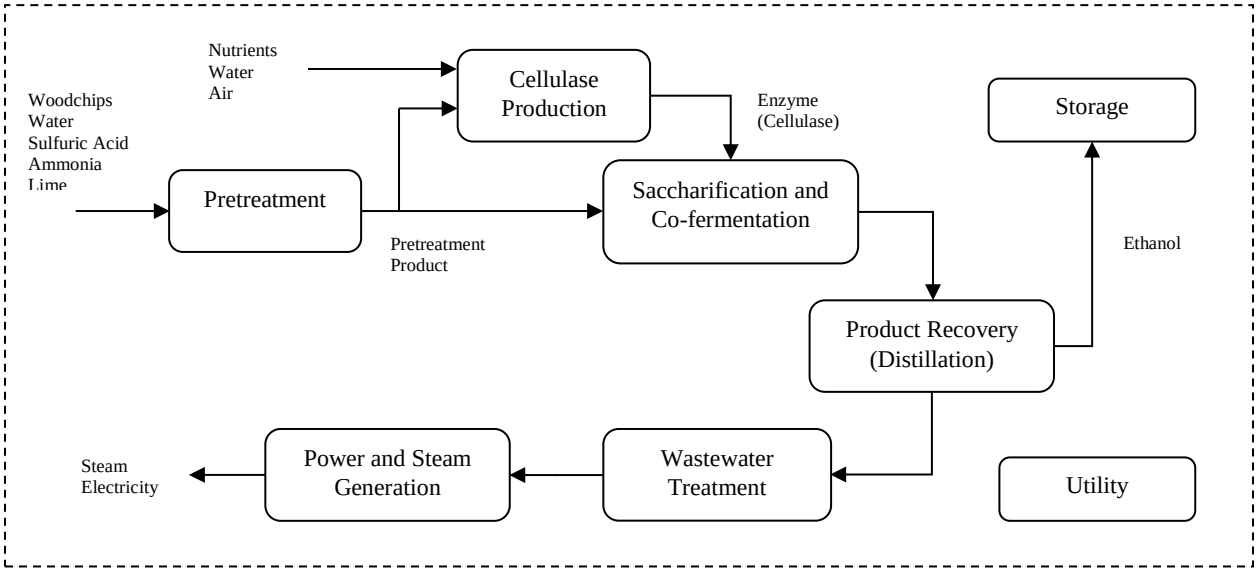


Figure 6.1. Bioethanol production plant and boundary system for all units in life cycle analysis [18]

6.3.1.1 Pretreatment Scenarios

Liberating the sugars (xylose, mannose, arabinose, and glucose) from the cellulose and hemicellulose contents of lignocellulosic biomass requires the pretreatment of the biomass, which is the bottleneck of the biochemical production of bioethanol. The conversion rate of cellulose (the main part of lignocellulosic biomass) to glucose in the hydrolysis step strongly depends on the accessibility to the enzyme and reactivity of cellulose which are obtained from the pretreatment unit. Compared with other types of lignocellulosic biomass materials such as herbaceous plants and agricultural residues, woody biomass has more lignin content [19, 20] and this characteristic causes more recalcitrance of the woody biomass to enzymatic hydrolysis. As a result, more energy is demanded for pretreatment.

However, the lower content of pentose in woody biomass in comparison to agricultural feedstocks is an advantage. This is because the lower rate of conversion of pentose to ethanol in fermentation and lower pentose content lead to a decrease in the amount of degradation products such as furfural and hydroxymethylfurfural (HMF) in pretreatment and hydrolysis which are acting as inhibitors for saccharification and fermentation [21, 22]. The feedstock for this study is yellow poplar chips, which are seen in the design by NREL [18]. The chemical composition of the feedstock is presented in Table 6.1.

Table 6.1. Chemical composition of the analyzed feedstock [18]

Component	% Dry Basis
Cellulose	42.67
Xylan	19.05
Arabinan	0.79
Mannan	3.93
Galactan	0.24
Acetate	4.64
Lignin	27.68
Ash	1
Moisture	47.90

In this study, two scenarios for pretreatment were investigated from the life cycle perspective. The base case was originated from the design of NREL [18]. In this design, dilute sulfuric acid pretreatment of yellow poplar chips was adopted, which is currently the most widely used technology for lignocellulosic biomass pretreatment. In the base case, the process was started with the pretreatment reactor and the pretreated biomass was separated into solid and liquid phases. Then, the liquid phase was detoxified in the lime addition unit, and its pH was readjusted by adding sulfuric acid. The main purpose of the

detoxification in the acid pretreatment method is to reduce the amount of some hydrolyzed products such as acetic acid, furfural, and HMF which play inhibitory roles in next process unit (SSCF). Precipitation of lime and sulfuric acid as gypsum and then separation of gypsum from the detoxified liquid phase led to a loss of significant amount of sugars. The conditioned liquor was then mixed with the separated solid phase, and the mixture was sent to saccharification and fermentation for further reactions. A flow diagram of the base case is shown in Figure 6.2.

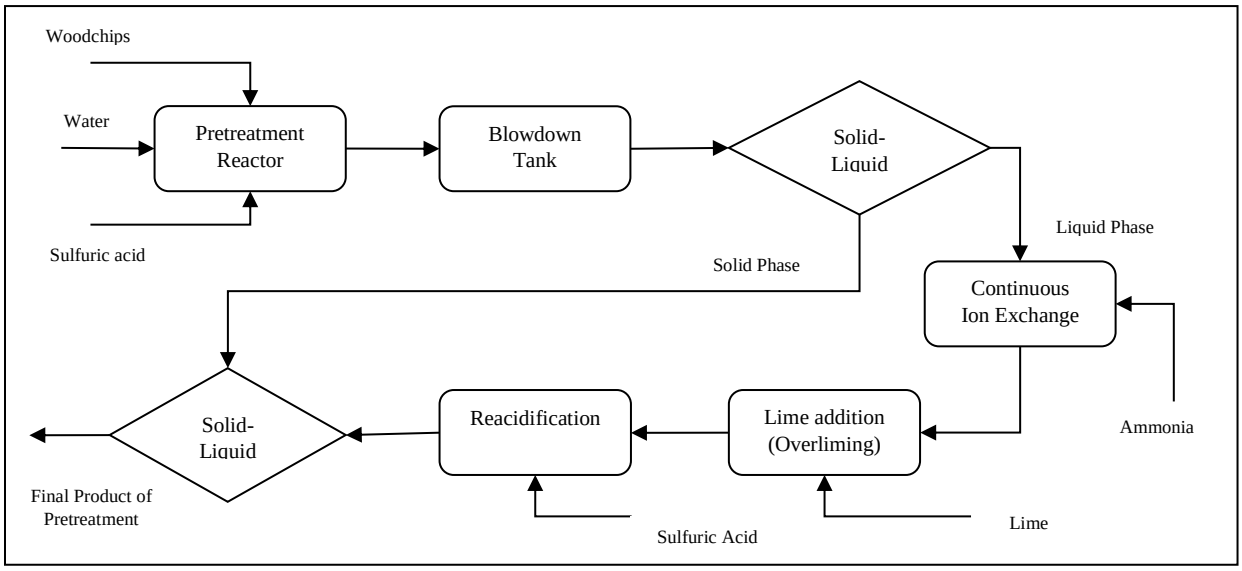


Figure 6.2. Pretreatment unit step in base case scenario

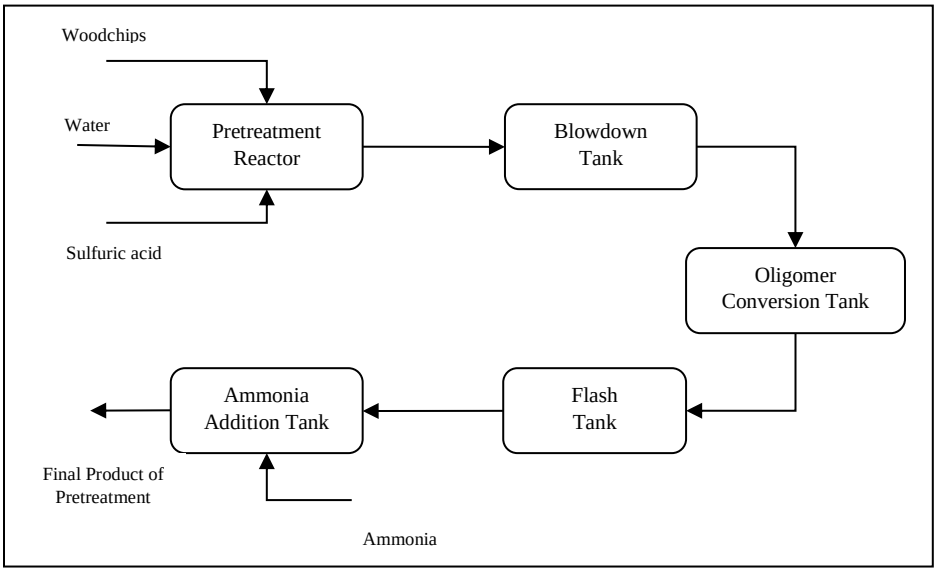


Figure 6.3. Pretreatment unit step in new scenario

The new scenario of pretreatment was developed based on some modifications of the base case. Sulfuric acid concentration in the new scenario is increased from 0.5 [18] to 2 % [23] based on the dry weight of the feedstock. Residence time in the pretreatment reactor is also decreased from 10 [18] to 1.1 min [23]. This decreasing of residence time in the pretreatment reactor led to a significant reduction in the degradation of sugars to inhibitors such as furfural and HMF. Moreover, the method developed by NREL [24] for the pretreatment of corn stover was adopted for wood chips in the new scenario in the current study. Finally, the separation of solid and liquid after pretreatment reactor was removed in the new scenario due to the use of ammonia in place of lime. The high miscibility of ammonia in the mixture of solids and liquids prevents the loss of sugars due to the absence of gypsum. Fermentation studies show that there is no advantage in using lime for

detoxifying the hydrolyzed products of pretreatment [24]. The flow diagram of the new scenario for pretreatment is shown in Figure 6.3.

6.3.2 Life Cycle Inventory

Life cycle inventory is the most time-consuming part of the LCA study. In this step, all input and output materials as well as the consumed and produced energy of the defined system boundary must be considered and must be normalized according to the defined functional unit. One of the most popular softwares for conducting LCA is Simapro 7.3 [25, 26] which includes several inventory databases and different impact assessment methods. Ecoinvent 2.0 [27] was chosen as inventory database, and Ecoindicator 99 [28] was used according to the designed plant by NREL for the production of ethanol from woodchips [18]. In the second scenario, modifications were made to the pretreatment unit based on Wyman et al. [23] and Humbird et al. [24] while other units in the new scenario are assumed to be the same as the base scenario. General process conditions of the pretreatment reactor for both cases are compared in Table 6.2.

Table 6.2. Pretreatment process conditions of both scenarios

Parameter	**Base case**	**New case**
Acid concentration	0.5%	2%
Residence time	10 minutes	1.1 minutes
Temperature	190ºC	190ºC

The required inventory data for the base case of this LCA study was extracted from the NREL report, not only for the pretreatment unit but also for all other units involved in the production process. The major efforts for inventory data in this study were done by generating the required data for new case. Every small change in the parameters of pretreatment affects the performance either directly or indirectly of the following units in the plant. In this regard, all the input and output materials and energy for each unit were calculated based on the quality and quantity of the pretreatment products. Additional amount of hydrolyzed hemicellulose and cellulose as well as the produced inhibitors were considered in order to calculate the required chemicals, air, water, and energy.

Due to the aforementioned modifications on the pretreatment unit, sugar recovery and inhibitor production for the two scenarios are different. Sugar recovery and inhibitor production of new scenario are compared with the base scenario presented by Wooley et al. [18] and results are illustrated in Table 6.3. Usually, degradation of pentose sugars (mainly xylose) and hexose sugars (mainly glucose) to furfural and 5-hydroxymetylfurfural (HMF), respectively, is increased by increasing the residence time in the pretreatment reactor. Due to the short residence time used in the new scenario of pretreatment, a significant increase in sugar recovery from hydrolysis and ethanol yield from fermentation can be obtained. In the new scenario, all produced acetic acid (a kind of inhibitor of sugar fermentation) liberated from the acetyl groups of hemicellulose in the pretreatment unit is neutralized by the addition of ammonia before more processing in the saccharification and fermentation unit [24].

Table 6.3. Comparison of sugar recovery and inhibitor production of two cases

Reaction	Conversion (Base Scenario)	Conversion (New Scenario)
Cellulose to Glucose	Cellulose 8%	Cellulose 23%
Cellulose to HMF	Cellulose 0	Cellulose 0.3%
Xylan to Xylose	Xylan 80%	Xylan 62.4%
Xylan to Furfural	Xylan 10%	Xylan 5%
Mannan to Mannose	Mannan 80%	Mannan 62.4%
Mannan to HMF	Mannan 15%	Mannan 5%
Galactan to Galactose	Galactan 80%	Galactose 62.4%
Galactan to HMF	Galactan 15%	Galactan 5%
Arabinan to Arabinose	Arabinan 80%	Arabinan 62.4%
Arabinan to Furfural	Arabinan 10%	Arabinan 5%
Acetate to Acetic Acid	Acetate 100%	Acetate 100%

6.3.3 Life Cycle Impact Assessment

There are several impact assessment methods for analyzing the life cycle of a product, and these can be divided into two general groups. The first group of methods is called the midpoint approach (problem oriented) such as CML 2001, and the second group is called the endpoint approach (damage oriented) such as Ecoindicator 99 [28]. Ecoindicator 99 was chosen for this study to evaluate and compare the environmental impacts and damages caused by a bioethanol production plant, and it has an advantage of simplicity for interpretation of results for using the single-point indicator scores. The score points of each environmental impact are the environmental burden of materials or processes, and higher score points represent higher environmental impact and damages. The impact categories

which are analyzed in this study based on the Ecoindicator 99 are carcinogens, respiratory inorganic, respiratory organic, climate change, radiation, ozone layer, ecotoxicity, eutrophication and acidification, land use, minerals, and fossil fuels. In Ecoindicator 99, there are three main damage categories, i.e., human health, ecosystem, and resources. Based on the predefined contribution of each impact in each category, the resultant damages in three categories are obtained and compared for both pretreatment cases.

6.3.4 Interpretation

Interpretation is the last stage of the life cycle assessment study. In this step, impact categories and damages caused are discussed, and the most influential reasons for an increase in impacts and damages are investigated. The recommendations for improvement or modification of the situation from the LCA point of view are presented.

6.4 Results and Discussions

In the present study, two levels of LCA are applied. The first level is conducted at the modifications of the pretreatment unit. The second level is conducted at all units in the plants to evaluate the impacts of conventional process. For each level of study, after determining the functional unit and system boundary, the amount of input materials and energy transfer across the system boundary are identified and normalized. Once inventory is established, impacts are assessed using Ecoindicator 99 approach [25]. In the subsequent section, results are discussed briefly.

6.4.1 Comparative Life Cycle Analysis of Pretreatment Unit

In the current study for the first level of analysis, a functional unit is defined as 1 kg of pretreated woodchips for both cases. The required materials and energy for both cases regarding to functional unit are shown in Table 6.4.

Table 6.4. Required materials and energy for pretreatment in both scenarios

Input Materials and Energy	Base Scenario	New Scenario
Woodchips (kg)	1.97	1.90
Water (kg)	4.15	3.57
Sulfuric Acid (kg)	0.023	0.020
Ammonia (kg)	0.014	0.019
Lime (kg)	0.009	-
Energy (kWh)	0.018	0.018

The analyses of the two scenarios are performed based on the Ecoindicator 99 impact assessment methodology. Comparison of the environmental impacts of the two cases is illustrated in Figure 6.4. As can be seen from Figure 6.4, the most dominant environmental impacts in pretreatment unit in both cases are fossil fuels, respiratory inorganics, and land use. Results show that in some environmental impact categories such as climate change, both scenarios have the same performance, and in some categories, the new scenario shows lower environmental impacts for respiratory inorganics and land use. However, the base case shows better environmental efficiency in fossil fuels impact. The main reason for the increase in fossil fuels impact for the new case is the consumption of more ammonia in the

new scenario. It must be noted that the effects of existing furfural and ethanol in recycled water from the wastewater treatment plant are also considered in this study.

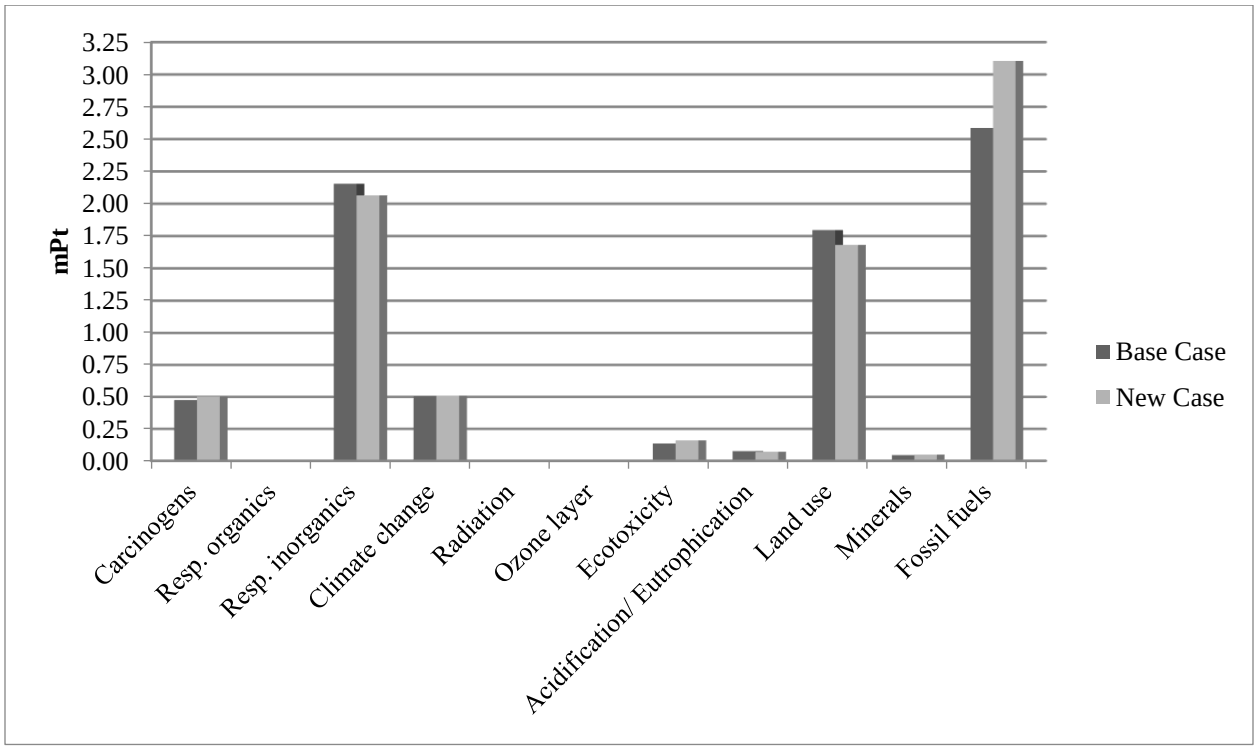


Figure 6.4. Comparison of environmental impacts of two scenarios on pretreatment unit

6.4.2 Comparative Life Cycle Analysis of Bioethanol Plant

The second level of life cycle analysis in the current study was carried out based on all the units of the bioethanol plant. The major objective of this study is to assess the life cycle of all system boundary by considering all the inputs (raw materials, water, chemicals, etc.) and outputs (ethanol, gas emissions, and others). The system boundary for this level of study is presented in Figure 6.1 [18], and the functional unit of this level is defined as 1 kg of produced ethanol for each case. The implemented impact assessment methodology for this level of study is Ecoindicator 99. As mentioned earlier in the units' description, in

addition to the main product, ethanol, electricity generation from unconverted wood components (mainly lignin) is also considered as the co-product of the bioethanol production plant and as a credit for environmental impacts as well as the damage categories.

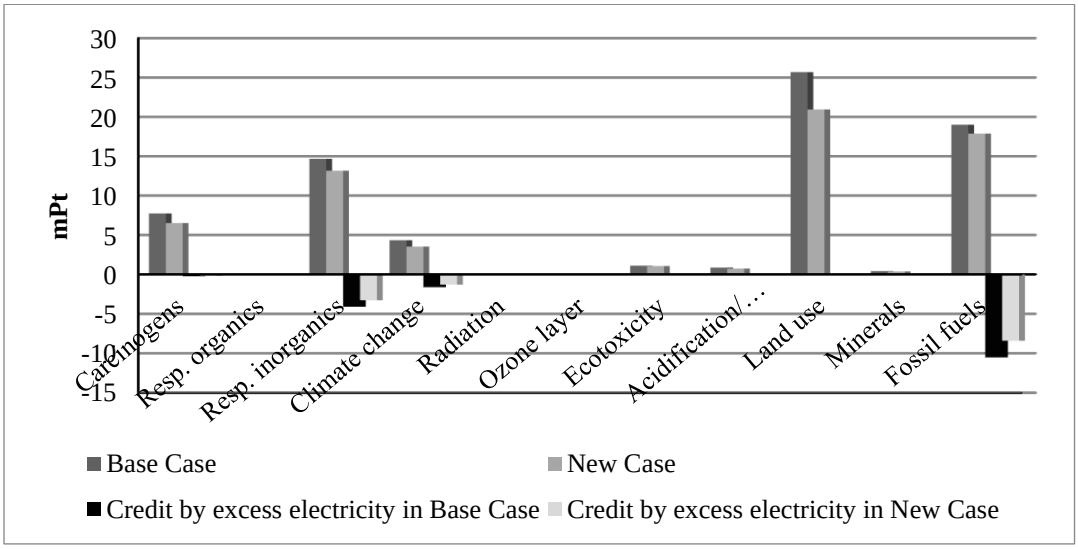


Figure 6.5. Comparison of environmental impacts of both scenarios with considering the excess produced electricity

The excess produced electricity in the waste combustion unit of the plant can be exported to the grid and thus reduces the electricity generation from other resources such as natural gas or coal. As a result, credits for different environmental impact categories and damage categories are shown as the negative amount on the diagrams, which signifies a remarkable reduction in each impact. The comparison between the two scenarios in this situation is demonstrated in Figure 6.5, and the reduction percentage of each impact is presented in Table 6.5. The results of comparison for damage categories are also illustrated in Figure 6.6, and the improvement percentages are given in Table 6.6.

Table 6.5. Improvements in impact categories in new case compared to base case with considering excess produced electricity

Environmental Impact Category	Percentage of Improvement (%)
Carcinogens	15.68
Resp. organics	63.69
Resp. inorganics	6.43
Climate change	17.81
Radiation	17.60
Ozone layer	8.96
Ecotoxicity	4.68
Acidification/ Eutrophication	13.23
Land use	18.50
Minerals	9.80
Fossil fuels	-11.52

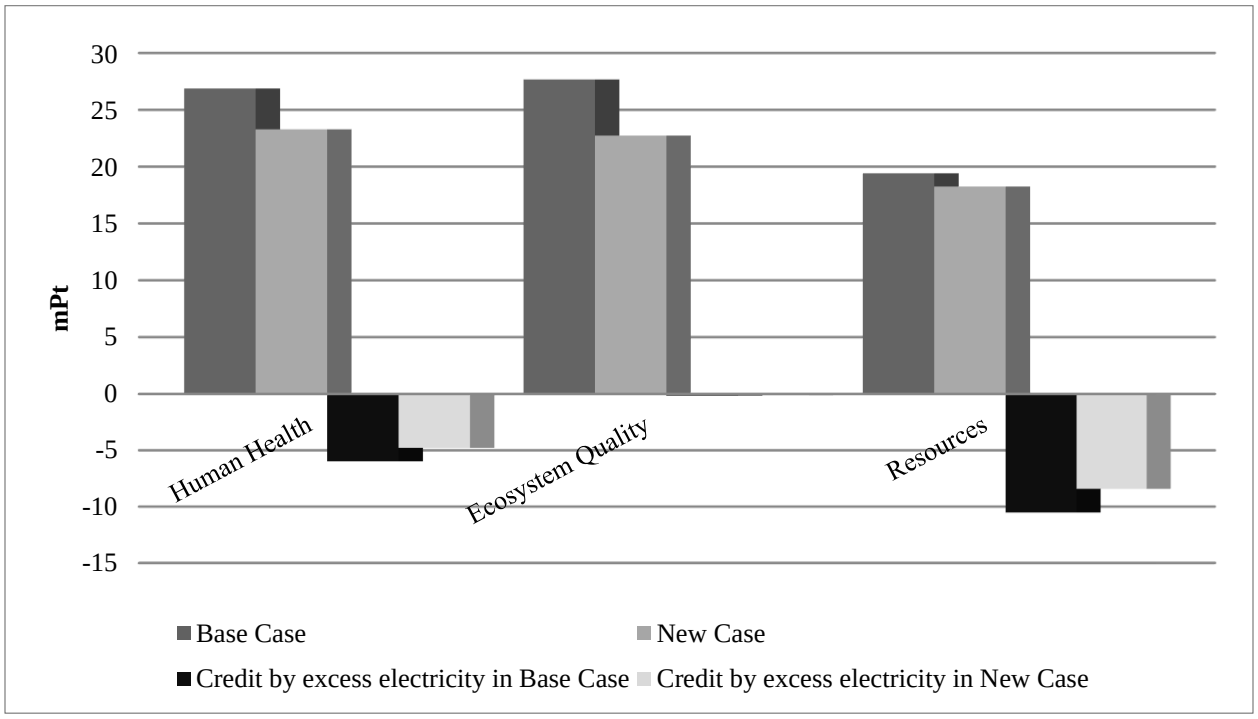


Figure 6.6. Comparison of damage categories of two scenarios with considering the excess produced electricity

The improvements in environmental impacts as well as in the damage categories in the new case in comparison with the base case are due to two main factors. The first reason is the increase in produced ethanol in the new scenario for pretreatment in comparison to the base scenario. Based on the new scenario design, ethanol productivity in the new case is about 28 % higher than that of the base case under the same reaction conditions for saccharification and fermentation. The increase of ethanol productivity in the new scenario leads to lowered environmental impacts from the process regarding to defined functional unit.

Table 6.6. Improvements in damage categories in new case compared to base case with considering excess produced electricity

Environmental Impact Category	Percentage of Improvement (%)
Human Health	11.44
Ecosystem Quality	17.81
Resources	-10.53

The second factor affecting the environmental impacts of bioethanol production is the amount of inhibitors generated. As shown in Table 6.2, residence time in the pretreatment reactor in the new case is much shorter than in the base case, which leads to a decrease in the formation of inhibitors such as furfural and HMF. Lower amounts of furfural and HMF cause a decrease in the inhibitory effect, while improving the liberating of sugars in saccharification and conversion of sugars to ethanol in fermentation. Another inhibitor which is produced in pretreatment is acetic acid, which is almost neutralized in the new case by ammonia before saccharification. However, in the base case, ion exchange and lime addition cannot remove acetic acid completely which results in some inhibitory effect on saccharification and fermentation.

6.5 Conclusion

Environmental performance of the chain process of ethanol production with dilute acid pretreatment for a designed plant by NREL [18] was investigated in this study for two pretreatment scenarios. Results from the first-level (single pretreatment unit) LCA study indicate that the proposed scenario (referred as new scenario) helps to lower the

environmental impacts for respiratory inorganics and land use, while the base scenario has better performance in fossil fuels. This is because more ammonia is consumed in the new scenario, which leads to an increased amount of fossil fuels consumption. A different trend of LCA results is observed when the system boundary is extended to the whole ethanol production plant. Results from the second-level study for the new scenario offer better performance in most environmental impact and damage categories. In this level, when the produced electricity is considered as a coproduct, credits for different environmental impact categories and damage categories can be obtained for both cases. Further analysis of the LCA results shows that an increased ethanol yield is the main factor for the reduced environmental impacts in the new scenario.

6.6 References

1. Broder, J. D., Harris, R. A., & Ranney, J. T. (2001). Using MSW and industrial residues as ethanol feedstocks. Biocycle, 42, 23–26.

2. Luo, L., van der Voet, E., & Huppes, G. (2009). Life cycle assessment and life cycle costing of bioethanol from sugarcane in Brazil. Renewable and Sustainable Energy Reviews, 13, 1613–1619.

3. Crutzen, P. J., Mosier, A. R., Smith, K. A., & Winiwarter, W. (2007). N2O release from agro-biofuel production negates global warming reduction by replacing fossil fuels. Atmospheric Chemistry and Physics, 7, 11191–11205.

4. Searchinger, T., Heimlich, R., Houghton, R., Dong, F., Elobeid, A., Fabiosa, J., Tokgoz, S., Hayes, D., & Yu, T. (2008). Use of U.S. croplands for biofuels increases greenhouse gases through emissions from land use change. Science, 319, 1238–1240.

5. Fargione, J., Hill, J., Tilman, D., Polasky, S., & Hawthorne, P. (2008). Land clearing and the biofuel carbon debt. Science, 319, 1235–1238.

6. Zheng, Y., Zhongli, P., & Zhang, R. (2009). Overview of biomass pretreatment for cellulosic ethanol. International Journal of Agricultural and Biological Engineering, 2, 51–68.

7. Mu, D., Seager, T., Rao, P. S., & Zhao, F. (2010). Comparative life cycle assessment of lignocellulosic ethanol. Environmental Management, 46, 565–578.

8. Wingren, A., Galbe, M., Roslander, C., Rudolf, A., & Zacchi, G. (2005). Effect of reduction in yeast and enzyme concentrations in a simultaneous saccharification and fermentation based bioethanol process. Applied Biochemistry and Biotechnology, 122, 485–499.

9. Alvira, P., Tomás-Pejó, E., Ballesteros, M., & Negro, M. J. (2010). Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresource Technology, 101, 4851– 4861.

10. Mosier, N., Wyman, C. E., Dale, B. D., Elander, R. T., Lee, Y. Y., Holtzapple, M., & Ladisch, C. M. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technology, 96, 673–686.

11. Conde-Mejia, C., Jimenez-Gutierrez, A., & El-Halwagi, M. (2012). A comparison of pretreatment methods for bioethanol production from lignocellulosic materials. Process Safety and Environmental Protection, 90, 189–202.

12. Kumar, S., Singh, S. P., Mishra, I. M., & Adhikari, D. K. (2009). Recent advances in production of bioethanol from lignocellulosic biomass. Chemical Engineering Technology, 32, 517–526.

13. Davis, S. C., Anderson-Teixeira, K. J., & DeLucia, E. H. (2009). Life-cycle analysis and the ecology of biofuels. Trends in Plant Science, 14, 140–146.

14. Gonzalez-Garcia, S., Moreira, M. T., & Feijoo, G. (2010). Comparative environmental performance of lignocellulosic ethanol from different feedstocks. Renewable and Sustainable Energy Reviews, 14, 2077–2085.

15. Von Blottnitz, H., & Curran, M. A. (2007). A review of assessments conducted on bioethanol as a transportation fuel from a net energy, greenhouse gas, and environmental life cycle perspective. Journal of Cleaner Production, 15, 607–619.

16. Hedegaard, K., Thyo, K. A., & Wenzel, H. (2008). Life cycle assessment of an advanced bioethanol technology in the perspective of constrained biomass availability. Environmental Science Technology, 42, 7992–7999.

17. Borrion, A. L., McManus, M. C., & Hammond, G. P. (2012). Environmental life cycle assessment of lignocellulosic conversion to ethanol: a review. Renewable and Sustainable Energy Reviews, 16, 4638–4650.

18. Wooley, R., Ruth, M., Sheehan, J., Ibsen, K., Majdeski, H., & Galvez, A. (1999). NREL/TP-580-26157. Golden: Colorado National Laboratory of the U.S. Department of Energy. Lignocellulosic Biomass to Ethanol Process Design and Economics Utilizing Co-Current Dilute Acid Prehydrolysis and Enzymatic Hydrolysis Current and Futuristic Scenarios..

19. Tian, S., Zhu, W., Gleisner, R., Pan, X. J., & Zhu, J. Y. (2011). Comparisons of SPORL and dilute acid pretreatments for sugar and ethanol productions from aspen. Biotechnology Progress, 27, 419–427.

20. Zhu, J. Y., Pan, X. J., Wang, G. S., & Gleisner, R. (2009). Sulfite pretreatment for robust enzymatic saccharification of spruce and red pine. Bioresource Technology, 100, 2411–2418.

21. Hendriks, A. T. W. M., & Zeeman, G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresource Technology, 100, 10–18.

22. Zhu, J. Y., & Pan, X. J. (2010). Woody biomass pretreatment for cellulosic ethanol production: technology and energy consumption evaluation. Bioresource Technology, 101, 4992–5002.

23. Wyman, C. E., Dale, B. E., Elander, R. T., Holtzapple, M., Ladisch, M. R., Lee, Y. Y., Mitchinson, C., & Saddler, J. N. (2009). Comparative sugar recovery and fermentation data following pretreatment of poplar wood by leading technologies. Biotechnology Progress, 25, 333–339.

24. Humbird, D., Davis, R., Tao, L., Kinchin, C., Hsu, D., Aden, A., Schoen, P., Lukas, J., Olthof, B., Worley, M., Sexton, D., & Dudgeon, D. (2011). NREL/TP-5100-47764. Golden: Colorado National Laboratory of the U.S. Department of Energy. Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol Dilute-Acid Pretreatment and Enzymatic Hydrolysis of Corn Stover.

25. Goedkoop, M., Schryver, A.D., Oele, M., Durksz, S., & Roest, D.D. (2010). Introduction to LCA with SimaPro 7. PRé Consultants.

26. Goedkoop, M., Schryver, A.D., Oele M., Roest, D.D., Vieira, M., & Durksz, S. (2010). SimaPro 7 Tutorial. PRé Consultants

27. Frischknecht, R., Jungbluth, N., Althaus, H. J., Doka, G., Heck, T., Hellweg, S., Hischier, R., Nemecek, T., Rebitzer, G., Spielmann, M., & Wernet, G. (2007). Overview and methodology (ecoinvent report No. 1). Dübendorf: Swiss Centre for Life Cycle Inventories.

28. Goedkoop, M., Oele, M., Schryver, A. D., & Vieira, M. (2008). SimaPro database manual methods library. the Netherlands: PRé Consultants.

7 Summary, Conclusion, and Recommendations

7.1 Summary

Cellulosic ethanol production has been investigated from different points of view. In the first step, experimental studies on simultaneous saccharification and the fermentation (SSF) process were performed to evaluate the influences of sugars concentration and enzyme loading on the amount of produced ethanol and ethanol yield. Experiments were conducted at two different sugars concentration level and three various enzyme loadings.

Results of the experiments on the SSF process were implemented in a proposed kinetic model to adjust five significant kinetic parameters. Tuning the kinetic parameters based on the experimental results enabled the kinetic model to predict the behavior of the system without running unnecessary experiments which cost more money and time. A reliable kinetic model with verified results is a key tool in the optimization approach of ethanol production.

Multi-objective optimization of the SSF process was the next step in this study. The kinetic model was used to optimize the defined objectives regarding decision variables and constraints. Three cases of optimization with different combinations of objectives and constraints were optimized by controlled elitist GA and the results were validated by experiments for each case. Multi objective optimization is a significant step toward industrialization of bioethanol production from cellulosic resources.

In the last chapter, environmental performance of the chained production process was evaluated to highlight the key role of process design on the productivity of a plant as well

as the environmental impacts of the production process. Life cycle assessment (LCA) has been implemented for this study to compare the results of two pretreatment scenarios in two limited and expanded levels.

7.2 Conclusions

This general objectives of this thesis are to present a new perspective on environmental performance of bioethanol production and optimization of the SSF process, towards commercialization of cellulosic ethanol production. The specific conclusions could be categorized as follows:

7.2.1 Interactive Influence of Enzyme Loading and Sugars Concentration on SSF Process

Experimental studies on the SSF reactions in the batch conditions were performed and it was observed that increasing the amount of enzyme loading would not necessarily lead to higher ethanol concentration and ethanol yield. Six batches of SSF experiments revealed that with the fixed amount of cellulose there is a saturation limit for enzyme at each level of fermentable sugars (glucose and mannose) concentration. This means that, beyond the optimum point for enzyme loading, increasing the enzyme amount not only does not help to increase ethanol yield or ethanol concentration, but also raises the cost of the process. Therefore, simulation of the results of experiments with a reliable kinetic model would be beneficial to analyse the performance of the batch SSF process.

7.2.2 Kinetic Modeling of Simultaneous Saccharification and Fermentation of Cellulose to Ethanol

Kinetic modeling of the SSF process clarifies the mechanism of inhibition impacts of monomer (glucose) and dimer (cellobiose) sugars in the batch media. Inhibition effects of hydrolysis products are more notable in a batch reactor, due to the accumulation of glucose and cellobiose in the media. Results indicate that while at high sugars concentration both cellobiose and glucose play inhibitory roles on enzyme, at low sugars level, cellobiose mainly acts as inhibitor for enzyme and prevent it from reaching the cellulose. Moreover, at a low level of enzyme loading, increasing the amount of fermentable sugars enhances the final ethanol concentration as well as the ethanol yield.

Initial sugars influence on reaction rates and rate constants have also been investigated. It is concluded that higher amounts of sugars in media, which means more nutrients for the microorganism, improves the growth rate of the microorganism. Nonetheless, higher product inhibition of hydrolysis due to the higher sugars concentration is reflected in lower values of the hydrolysis rate constants.

A reliable kinetic model which considers the interaction of involved components (sugars, ethanol, and enzyme) and their restrictive roles in the SSF process is a key tool to optimize the SSF process.

7.2.3 Multi-Objective Optimization of SSF Process

Three bi-objective optimization cases for the SSF process were designed and optimized by controlled elitist GA (gamultiobj tool from MATLAB R2016b). Interactions of the

components involved in the SSF process are reflected in tuned kinetic parameters, as well as selected objectives and constraints for each case significantly affecting on the results of the optimization.

The first case was designed to maximize the cellulose conversion and minimize the enzyme consumption per produced ethanol. Optimized operational conditions in this case show that higher cellulose loading and sugars concentration lead to lower cellulose conversion, due to the product inhibition. Maximizing the ethanol yield with the simultaneous minimization of the enzyme consumption per produced ethanol was studied as the objective of the second optimization case. Optimal solutions in this case resulted in converging of the glucose concentration to a higher bond, while enzyme loading and cellulose loading could be chosen in a wider range. In order to satisfy both objectives in this case, proper selection of enzyme loading based on cellulose loading must be considered. Maximum ethanol yield and minimum enzyme loading aimed for optimization in the final case. High sugars concentration is beneficial to high ethanol yield, while cellulose loading converges to the lower defined range. Comparing the results of this case with two different constraints reveals that appropriate selection of a constraint notably influences the optimization results, especially at a lower loading of the enzyme.

Optimization results of each case have been validated by running batch SSF experiments and good agreement among predicted results and experimental measurements has been attained, which proves that the findings of this study can be further expanded to larger scales to improve the performance of the SSF process.

7.2.4 Life Cycle Analysis of Bioethanol Production with Different Pretreatment Designs

Life cycle analysis of bioethanol production with two scenarios for the pretreatment process reveals the significant role of pretreatment in ethanol production. For example, replacing the lime with ammonia improves the removing inhibitors more efficiently, which enhances the hydrolysis process and therefore increases the ethanol yield. Increasing the ethanol yield basically diminishes the most environmental impacts.

Nevertheless, it should be considered that although in the first or second level of LCA study, most of the environmental impacts (such as respiratory inorganics and land use) decreased due to the change in pretreatment design, some other impacts (fossil fuels) increased. In general it can be concluded that applying modifications to the pretreatment unit improved the environmental performance of the ethanol production plant at both levels of study. Damage categories have also shown better results for the new scenario in comparison to the base scenario.

7.3 Recommendations

This presented work highlights the kinetic behavior of the SSF process for optimization of bioethanol production in a batch system and also the significant role of process design in the environmental performance of bioethanol production. This study can be extended in the following suggested areas to overcome the limitations of production of second generation ethanol.

7.3.1 Economic analysis of ethanol production with various process designs

As was shown, process design significantly affects the environmental performance of the bioethanol production process. Currently, although attention to phenomena such as global warming and greenhouse gas emissions has made the life cycle assessment a key factor for decision makers, economic evaluation and comparison of the various process designs still play determining roles in the commercialization of bioethanol production. Therefore, it is highly recommended to perform economic analysis alongside LCA studies of various options for a process such as pretreatment. Allocating a weight for each parameter and comparing the achieved results would be an interesting topic for further studies on bioethanol production.

7.3.2 Multi-objective optimization of fed-batch system for SSF process

Fed-batch design for the SSF process reduces product inhibition impacts of ethanol, glucose, and cellobiose and also enables the system to hydrolyse more cellulose, due to the lower amount of the inhibited enzyme. Optimization of the SSF process in a fed-batch design has been mostly investigated experimentally to achieve the highest ethanol concentration. However, multi-objective optimization of ethanol production in a fed-batch system is recommended for further studies. The desired objectives could be achieved by implementing an appropriate kinetic model with reliable kinetic parameters to optimize the involved operating parameters such as strategies for feeding the fed-batch system by cellulose as well as the rate of enzyme loading and yeast amount in the system. Other decision variables in this regard should also be considered, such as fermentable sugars

concentration. Multi-objective optimization of the SSF process in a fed-batch design would provide decision makers with an invaluable insight into feasible bioethanol production.

Appendix (A) Experiment Apparatus

The reaction system of batch SSF process mainly consists of a 250 mL jacketed flask (Bellco, US) and a Julabo FP 50 heated/refrigerated circulator (Allentown, PA, US) for temperature control.

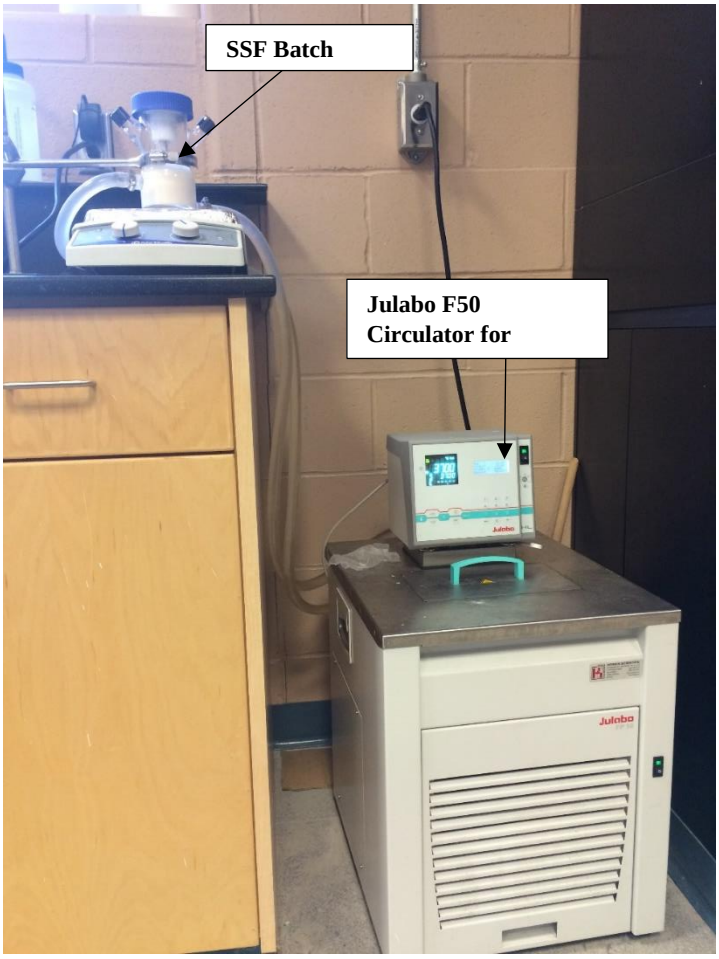


Figure A-1. Reaction system for batch SSF process

Analysis of the samples was performed by Dionex Ultimate 3000 HPLC system for the concentrations of ethanol, glucose, cellobiose, and mannose. Ultimate 3000 HPLC system

was equipped with an online degasser, a binary HPLC pump, an autosampler and a refractive index detector as illustrated in Fig. A-2.

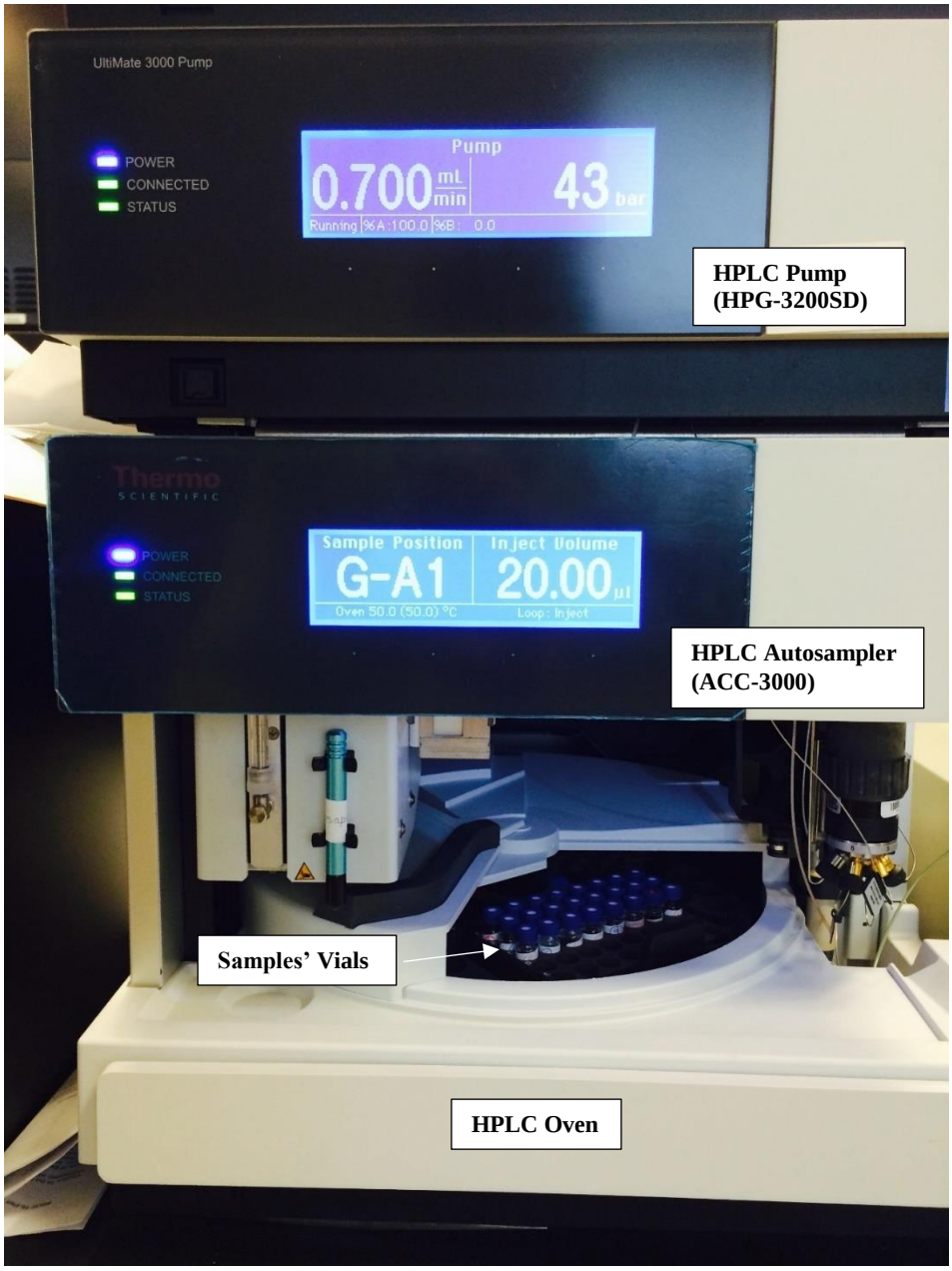


Figure A-2. Dionex Ultimate 3000 HPLC system