2010

Pretreatment And Fractionation Of Biomass For Biological Production Of Transportation Fuels And Value Added Chemicals

Samuel Asomaning Agyemang
North Carolina Agricultural and Technical State University.

PRETREATMENT AND FRACTIONATION OF BIOMASS FOR BIOLOGICAL PRODUCTION OF TRANSPORTATION FUELS AND VALUE ADDED CHEMICALS

by

Samuel Asomaning Agyemang

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

Department: Chemical and Bioengineering
Major: Chemical Engineering
Major Professor: Dr. Lijun Wang

North Carolina A&T State University
Greensboro, North Carolina
2010

School of Graduate Studies
North Carolina Agricultural and Technical State University

This is to certify that the Master's Thesis of

Samuel Asomaning Agyemang

has met the thesis requirements of
North Carolina Agricultural and Technical State University

Greensboro, North Carolina
2010

Approved by:

_____	_____
Dr. Lijun Wang Major Professor	Dr. Abolghasem Shahbazi Committee Member
_____	_____
Dr. Guibing Chen Committee Member	Dr. Leonard Uitenham Department Chairperson

_____
Dr. Alan Letton
Interim Associate Vice Chancellor for Research and Graduate Dean

DEDICATION

I will like to dedicate this thesis to my father, Mr. Darko Agyemang, for his clear vision concerning my life, his undying support throughout my academic life and his life changing "knocks" without which I may be an entirely different person today.

BIOGRAPHICAL SKETCH

Samuel Asomaning Agyemang was born on March 25, 1983, in Greater Accra, Ghana. He received the Bachelor of Science degree in Chemical Engineering from Kwame Nkrumah University of Science and Technology (KNUST) in Kumasi, Ghana in 2006. He received a Waste Management Certificate from the Interdisciplinary Waste Management Institute at North Carolina Agricultural and Technical State University, Greensboro, North Carolina in 2010. Mr. Agyemang has been engaged in biofuel research and development and has presented research findings on "Reactive-screw Extrusion Pretreatment of Lignocellulosic Biomass with Acetic Acid for Ethanol Production" at the American Society of Agricultural and Biological Engineers (ASABE) of which he is a member. He is a candidate for the Master of Science degree in Chemical Engineering.

ACKNOWLEDGMENTS

"My son, attend unto my wisdom, and bow thine ear to my understanding; that thou mayest regard discretion, and that thy lips may keep knowledge" Proverbs 5:1-2, KJV. I thank God almighty for granting me patience, guidance, humility and perseverance which I needed foremost in order to accomplish the daunting task of making new discoveries.

I am grateful to the Department of Chemical and Bioengineering at North Carolina Agricultural and Technical State University under which I was given the opportunity to higher learning and to contribute a quota to the progressive search for alternative fuels and cleaner environments. I am deeply grateful to Dr. Lijun Wang, my major advisor, for his immense academic and practical contribution to the successful completion of this thesis. I am thankful to Dr. Abolghasem Shahbazi, whose constructive critique brought clarity and a deeper meaning to the findings made in this research.

My most fond gratitude goes to Michele R. Mims (Lab Manager, Sockwell Hall, NCA&TSU) who has been a silent stalwart executing all chemical analysis on the HPLC and being on call at all times throughout the research. My regards goes to the other researchers within Sockwell Hall (including students and post-doctorate researchers) who sacrificed space and time, and shared an idea or two with me when most needed.

I'd also like to express my sincere gratitude to my family (Mr. and Mrs. Selby, Pastor Albert Som-Pimpong, Mr. Harry K. Pimpong) who through kind words and gratuitous assistance created a benign atmosphere leading to my successful academic achievement.

TABLE OF CONTENTS

LIST OF FIGURES

FIGURES **PAGE**

LIST OF TABLES

LIST OF NOMENCLATURE

ASE	Accelerated Solvent Extractor
AST	Ambient Storage Tank
CSTR	Continuous Stirred Tank Reactor
RSE	Reactive Screw Extrusion
PLE	Pressurize Liquid Extraction
WC	Woody Biomass
SG	Switchgrass
CS	Corn stover
WS	Wheat straw
SSB	Sweet Sorghum Bagasse
LAP	Laboratory Analytical Procedures
NREL	National Renewable Energy Laboratory
HPLC	High Pressure Liquid Chromatography
RCF	Relative Centrifugal Force
PID	Proportional-Integral-Derivative
M	Molar concentration
N	Normal (concentration)
EG	Engoglucanase
CBH	Cellobiohydrolase
FPU	Filter Paper Unit

CFU	Colony Forming Units
IU	International Unit
FBG	Fungal Beta-glucanase Unit
YM	Yeast Moth
ATCC	American Type Culture Collection
RID	Refractive Index Detector
STDEV	Standard Deviation
$Ca(OH)_2$	Calcium Hydroxide
NH_4OH	Ammonium Hydroxide
CH_3COOH/A.A	Acetic Acid
CH_3CH_2OH/ETOH	Ethanol
H_2O	Water
CO_2	Carbon Dioxide
Al_2O_3	Aluminum Oxide
Co-Mo	Cobalt – Molybdenum
Ni-Mo	Nickle – Molybdenum
Pt/ZrO_3	Platinum/Zirconium Oxide
$Ru/MgO/Al_2O_3$	Rubidium/Magnesium Oxide/Aluminum Oxide
KOH	Potassium Hydroxide
H_2SO_4	Sulfuric Acid
H_3PO_4	Phosphoric Acid
H_2S	Hydrogen Sulfide

NaOH	Sodium Hydroxide
LiOH	Lithium Hydroxide
HCl	Hydrochloric Acid
Hg	Mercury
MnP	Manganese Peroxidase
LiP	Lignin Peroxidase
Lac	Laccase
C=C	Carbon double bond
C-C	Carbon single bond
AFEX	Ammonia Fiber Explosion
ARP	Ammonia Recycle Percolation
SHF	Separate Hydrolysis and Fermentation
SSF	Simultaneous Saccharification and Fermentation
MSW	Municipal Solid Waste
PLPW	Pressurized Low Polarity Water
PAE	Pressurized Aqueous Ethanol
MW-ethanol	Microwave ethanol
MW-water	Microwave water
LDL	Low Density Lipoprotein
HDL	High Density Lipoprotein
DDG	Dry Distillers Grain
GPC	Gel Permeation Chromatography

PC	Policosanol
NO_x	Nitrous Oxides
SO_x	Sulfur Oxides
PAH	Polycyclic Aromatic Hydrocarbons
VOC	Volatile Organic Compound
β	Beta
α	Alpha
pH	Measure of acidity or alkalinity
d.b	Dry bais
μm	micro meter
rpm/RPM	Revolution per minute
wt	Weight
wt/wt	Weight per weight
MJ/m^3	Mega Joules per cubic meter
MJ/kg mole	Mega Joule per kilogram mole
Psi	Pound Square Inch (Pressure Units)
MPa	Mega Pascals
^oC	Degree Centigrade
g	Gram
g/ 100 ml	Gram per one Hundred milliliter
GHz	Giga hertz
Pa.s	Pascal Second

ABSTRACT

Agyemang, Samuel Asomaning. PRETREATMENT AND FRACTIONATION OF BIOMASS FOR BIOLOGICAL PRODUCTION OF TRANSPORTATION FUELS AND VALUE ADDED CHEMICALS. (**Major Advisor: Lijun Wang),** North Carolina Agricultural and Technical State University.

Lignocellulosic biomass is an abundant renewable material from hardwood, softwood, grasses and agricultural residues for ethanol production. Pretreatment of lignocellulosic materials has been a main technical challenge to a cellulosic ethanol production process. The purpose of this thesis is to investigate four pretreatment methods and conduct comparative analyses among pretreatment methods, chemicals used and biomass species to determine the best process in terms of glucan to ethanol conversion efficiencies. The separation of hemicellulose fractions to be used as precursors for the production of high value chemicals after pretreatment of biomass samples is also investigated. Pretreatment methods including Accelerated Solvent Extraction (ASE), Reactive Screw Extrusion (RSE), Continuous Stirred Tank Reactor (CSTR) and Ambient Storage Tank (AST) were used along with abrasive chemicals including 10% acetic acid, 10% ammonium hydroxide (NH_4OH), 10% calcium hydroxide ($Ca(OH)_2$), 30% ethanol solution and deionized water at different conditions for the fractionation of biomass into monomeric sugars for ethanol fermentation in a Simultaneous Saccharification and Fermentation (SSF) process. Separation of hemicellulose fractions after pretreatment was done via a liquid-liquid extraction procedure in a mass ratio of biomass extract to 95.5% ethanol solution at 1:4.

ASE pretreatment of biomass samples with 10% acetic acid solution at 180^{o}C resulted in 100% solvation of hemicellulose fractions into the liquid extract stream for all biomass samples treated. Pretreatment of corn stover with a 10% ammonium hydroxide aqueous solution in the CSTR resulted in the glucose to ethanol conversion efficiency of 85.2%. Alkali ($Ca(OH)_2$ and NH_4OH) pretreatment of biomass in the AST resulted in the highest glucose to ethanol conversion yields of 30.4%, 23.0%, 38.2% and 47.4% for switch grass, corn stover wheat straw and sweet sorghum bagasse pretreated with 10% NH_4OH respectively; 29.2%, 24.1%, 40.3% and 37.6% for switch grass, corn stover wheat straw and sweet sorghum bagasse pretreated with 10% $Ca(OH)_2$ respectively over acetic acid and deionized water used in the AST. RSE pretreatment of switch grass using 10% $Ca(OH)_2$ at 180^{o}C resulted in a 75.5% glucose to ethanol conversion efficiency. Ammonium hydroxide pretreatment of biomass resulted in the highest hemicellulose fractions after liquid-liquid extraction with 95.5% ethanol solution.

CHAPTER 1

Introduction

On March 24, 1989, an oil tanker ran aground on a reef off the Alaskan coast releasing 11 million gallons of crude oil into Alaska's Prince William Sound in an environmental disaster commonly referred to as the Exxon Valdez Oil Spill (1989) [1]. It has been reported that as many as half a million birds perished, several aquatic lives destroyed along with the creation of dead zones which perpetrate aquatic mortality till date [1]. Recently, a similar incident occurred along the coast of Louisiana where a British Petroleum (BP) oil rig exploded and resulted in the immediate loss of eleven human lives with the concomitant spillage of approximately 200 million gallons of crude oil into the Gulf of Mexico [2]. Several billions of dollars have been spent in clean-up programs to clear the coastal beaches, the sea, contaminated birds and also to reimburse businesses and individuals who had incurred losses as a result of the BP oil disaster [2].

Crude Oil price fluctuations as a result of policies churned out by members of the Organization of Petroleum Exporting Countries (OPEC) along with the high demand and consumption of petroleum products by developed (European countries and the United States) and rapidly developing nations such as China and India have thrown the budgets of many non-oil producing and lesser developed nations awry. Crude oil prices have gyrated violently in recent times averaging per barrel $23.19 (1990), $16.75 (1995), $27.39 (2000), $58.30 (2006), $64.20 (2007), $91.48 (2008) and currently stands at $70.67 (partially 2010) [3]. These price variations foment the desire of non-oil producing

countries to attain energy independence as they are held to ransom by the whims of OPEC and the developmental agendas of nations that have a high demand for petroleum products.

Alternative energy sources including coal, nuclear, solar and wind have been suggested and widely demonstrated to be effective replacements to petroleum. However, these energy sources are either unstable in supply (wind and solar), highly toxic (nuclear) or lead to adverse environmental degradation and pollution (coal) by the methods of acquisition such as mining and use such as combustion. Coal is the most abundant fossil fuel in the United States, currently contributing to about 55% of the energy needed for electricity production [4]. However, coal is one of the world's most notorious air pollution sources, contributing up to 78% of its mass as carbon dioxide from a single coal combustion process into the atmosphere. Coal mining and preparation contributes to the most non-methane volatile organic compounds and methane (over 98%) as well as the most dissolved solids to water (over 76%). Furthermore, the disposal of coal combusted products either by landfilling or surface impounding results in the emission of particulate matter into the air and a variety of metals to land [5]. Recently, 25 coal miners perished on duty in a coal mine (Massey Energy Company) in West Virginia as a result of operational mishaps. This tragedy reiterates the need for a much safer and environmentally benign source as well as means of obtaining energy.

In the quest to achieve energy security and reduce our environmental footprints across the globe, many governments and industries are locked in a race to develop new and alternative energy technologies that are green and produce equal amounts of energy

as that obtained from fossil fuels including coal and petroleum. Ethanol production from biomass has received a lot of attention in the last few decades because of the favorable life cycle assessment (cradle-to-grave) it has on the environment. The use of non-food biomass materials including agricultural residues, municipal solid waste and forest wood is highly recommended in biomass to ethanol processes since food grade materials are consumed by both man and livestock and should not be competed for energy production.

Lignocellulosic biomass is an almost inexhaustible renewable source for production of energy and chemical products in a biorefinery process. In the United States, the annual production of agricultural residue is about 355 million metric dry tons including 200 million tons of corn stover and 70 million tons of cereal straw [6]. Lignocellulosic biomass is a complex polymeric combination of hemicellulose, cellulose and lignin. None of the primary components of lignocellulose: cellulose, hemicellulose and lignin are dominant. Ongoing researches to convert biomass to ethanol face the challenge of fractionating and hydrolyzing the complex matrix of lignin, hemicellulose and cellulose into simple sugars that can be fermented into ethanol [6].

Plants contain a wide range of bioactive compounds including lipids, phytochemicals, pharmaceutics, flavors, fragrances and pigments [7, 8]. Extraction is an age old technique for isolating these essential components of plants for commercialization. Hemicellulose makes up approximately one-fourth to one-third of most plant materials and is primarily composed of xylose. Xylan (xylose polymer) has been found to have several industrial and medicinal applications depending on the plant from which it is obtained. The isolation of essential plant components has led to the

production of pharmaceuticals that have antiproliferative activity against cancerous cells including Hep-2 (larynx carcinoma), MCF7 (breast epithelial adenocarcinoma) and vero (African green monkey kidney) [9]. Tazopsine, a morphinan alkaloid, extracted from the stem of *Strychnopsis thouarsii* has been found to fully inhibit the development of *P. falciparum and P. yeolii* hepatic parasites in cultured primary hepatocytes especially at the early developmental stages. Tazopsine is particularly active against the liver stage developmental forms of the malaria parasite [10].

In this thesis, our objectives are to (1) advance the knowledge of alternative pretreatment methods for breaking the interlinkages existing among the components of lignocellulosic biomass including lignin, hemicellulose and cellulose for easier sugar hydrolysis; (2) to convert the monomeric sugars produced as a result of hydrolysis into ethanol via simultaneous saccharification and fermentation (SSF); and (3) isolate and concentrate hemicellulose fractions from the liquid stream of pretreated biomass for the preparation of value added products.

CHAPTER 2

Literature Review

2.1 Biomass Resources

Biomass resources are usually classified into four main categories: agriculture, forestry, municipal solid waste (MSW) and energy crops. Agricultural residues are the wastes associated with the cereal harvest and processing, such as straws, stalk and rice hulls. Forest residues are the wastes associated with the processing of forest products such as prunings, wood sawdust, bark, needles and wood chips. MSW is the residue associated with human activity, such as waste rubber tyre, waste plastic and waste paper. Other biomass resources include fast-growing energy trees, short rotation crops and some kinds of grass species [11]. World production of biomass is estimated at 146 billion metric tons per year, mostly wild plants [12].

2.1.1 Woody Biomass

It is estimated that 30% of the earth's land area or approximately 3870×10^6 ha is covered forests. About 95% of this estimate is natural forests and the remaining 5% is plantations. Tropical and subtropical forests comprise of 56% of the world's forests, while temperate and boreal forests account for another 44%.The world's total above-ground biomass in forests is 420×10^9 tonnes, of which more than 40% is located in South America and about 27% is in Brazil alone [13].

The forests may be divided into five categories: (1) protection forests, (2) timber stands for timber production, (3) economic forests for the production of fruits, edible oils,

soft drinks and ingredients, industrial raw materials, and medicinal materials (4) firewood for the production of fuels, (5) forests for special uses as national defense, environmental protection and scientific experiments. Besides the five categories, there are other kinds of forests such as sparse forests, shrubs and orchards [11].

The worldwide average above-ground woody biomass is 109 tonnes/ha. Estimates by FAO (2000) show that the global production of woodfuel and roundwood reached 3268×10^6 m^3 in 1999. The global use of woodfuel and roundwood is 3271×10^6 m^3 per year. About 55% is used directly as fuel, (e.g. as split firewood) mainly in developing countries. The remaining 45% is used as industrial raw material, about 40% of which becomes primary or secondary processing. These processing residues are suitable for energy use such as production of biofuels. About 70–75% of the global wood harvested is either used or potentially available as a renewable energy.

2.1.2 Agricultural Residues and By-products

In the United States corn is the most widely planted crop (31.9 million ha) and corn stover is the most abundant agricultural residue (USDA, 2002). The land areas cultivated for other agriculture crops are (in millions of ha): soybean 29.6, hay 26.2, wheat 24.3, cotton 5.8, grain sorghum 3.8, oats 2.1, barley 2.0, rice 1.3 and rye 0.6 [14]. Estimates of corn stover availability vary widely depending on what fraction of this agricultural residue can be sustainably collected. Some after-harvest residues are left in the field to protect the soil from water and wind erosion. The amount left on the field is dependent of tillage practice, topography, soil type and crop rotation.

Glassner estimated a corn stover availability of 153 million dry tones/yr assuming a no-till farming technique. USDA guidelines require that 30% of collected stover should be used for soil coverage by all farmers engaged in its programs. By this standard, approximately 40% removal of residue or 82 million dry tones/yr of corn stover is available for other uses including ethanol production [14]. In the United States, 19-26 billion liters of ethanol can be produced yearly from corn stover [14]. Other uses of corn stover include feed for dairy cattle. Corn stover mixed with high moisture hay-crop forage may provide 20-30% of the forage dry matter for dairy cattle. Corn stover after hammer milling can be used directly as a fuel source in a boiler furnace.

Particleboards and building panels are also produced from corn stover residues. Corn stover based pulp is popular in the paper industry because it can be bleached without chlorine. This alternative eliminates the production of dioxins which are environmental pollutants. Corn stover also requires less bleach because of its low lignin content. Corn cobs are now used as a raw material for producing furfural. As a result of the high cellulose and low lignin content of corn cobs they are used to prepare dissolving pulp, which is a prerequisite in producing high cellulose derivatives such as rayon, cellulose nitrates and cellulose acetates.

Wheat straw is an abundant by-product from wheat production with an average yield of 1.3-1.4 kg/kg of wheat grain. The Food and Agriculture Organization (FAO) estimated in 2003 a 63.5 million tonnes of wheat produced in the United States of America and a worldwide production of 556.3 million tonnes [15]. Based on the USDA 2002 guidelines, 30% of harvested crop residues are to be left on the farm for soil

enrichment making approximately 40% of residue to be sustainably harvested for ethanol production and other uses. This assumption makes available 33 million dry t/yr of wheat straw for ethanol production. This estimate is equivalent to 9.6 billion liters of ethanol per year assuming an ethanol yield of 292 L/tone of wheat straw [14].Wheat straw, a lignocellulosic material, contains about 35-40% cellulose, 30-35% hemicellulose, 10-15% lignin, 5-10% mineral and trace amounts of other components [16].

2.1.3 Energy Crops

Sweet sorghum (*Sorghum bicolor* var. *saccharatum*) is a high yielding C_4 grain crop with high photosynthetic activity [17]. The highest recorded yield for the crop is 20.1 tons per hectare. In the United States 8.3 million acres of sweet sorghum was harvested in 2008/2009 with production concentrated within the southern and central plains of five states – Kansas, Texas, Nebraska, Oklahoma and Missouri. Africa leads the global production of sweet sorghum with 21.6 million metric tons per year. Sorghum is one of the most drought tolerant crops under cultivation and it offers farmers very little cost on irrigation and other farm expenses. Sorghum bagasse is reported to contain 34 % cellulose, 25 % hemicellulose and 18 % lignin [17].

Sipos *et al*. 2008 [17] pretreated SO_2 impregnated sweet sorghum bagasse using steam explosion at mild (180 ^oC, 10 min; 190 ^oC, 5 min) and harsh (190 ^oC, 10 min; 200 ^oC, 5 min) pretreatment conditions. Pretreated samples were separated into two parts and enzymatically hydrolyzed. One part was the whole slurry and the other part was washed with hot distilled water to remove solubilized sugars and inhibitors and separated into fibers. Enzymatic hydrolysis of untreated sorghum resulted in only 16 % conversion of

cellulose into glucan after 48 h. Mild pretreated "whole slurry" sorghum resulted in 48 % and 55 % cellulose to glucan conversion while the harsh pretreatment resulted in 83 % and 86 % glucan conversion. However washed fibers from bagasse pretreated at milder conditions saw 45 % and 53 % cellulose to glucan conversion while harsher pretreatments resulted in 89 % and 92 % cellulose to glucan conversion. These results prove the high quality of sweet sorghum for the production of ethanol. Sipos *et al*. also reported 80 – 90 % glucose to ethanol yield after fermentation with *S. cerevisiae.*

Switchgrass (*Panicum virgatum*) is a North American perennial C_4 grass that grows very well in the warm seasons. It occurs naturally from 55^{o}N latitude to central Mexico [18] where it has greater productivity and survival. It is grown mainly as a forage crop or as a ground cover to control erosion. Switchgrass grows very well in moderately-well to well drained soils with average pH of 5.5-7.0 and medium soil fertility. Switchgrass is a seed grown plant and is slow to mature requiring two to three growing seasons to become fully established as a dense and vigorous stand. It appears in several varieties with varying compositions of cellulose, hemicellulose and lignin. Switch grass varieties with their respective percentage compositions of cellulose, hemicellulose and lignin on dry basis (d.b) include: Alamo 33.48, 26.10, 17.35; Blackwell 33.65, 26.29, 17.77; Cave-in-Rock 32.85, 26.96, 18.36 and Trailblazer 32.06, 26.24, 18.14 [18]. Switchgrass is an attractive bio-fuel source because of its rapid growth rate, winter weather hardiness, reduced energy and agrochemical consumption and less intensive agricultural management practices. It produces close to 540% more energy than is required to grow and process it into ethanol [19].

Salix is an energy crop that is capable of absorbing undesirable inorganic substances and heavy metals such as cadmium from the soil. Energy crops such as Salix can be grown in plantations and irrigated with urban waste water. Salix could therefore be used as a municipal waste water purification agent which can be later combusted or converted for energy and the ash recirculated to the Salix plantation [20].

2.2 Properties and Quality

Production of biofuels and biobased products from biomass depends upon the chemical constituent and physical properties of the biomass. As a result of the carbohydrate structure, biomass is highly oxygenated compared to conventional fossil fuels including coal and petroleum. Typically, 30 to 40 wt. % of the dry matter in biomass is oxygen. The main element of biomass is carbon, which is from 30 to 60 wt. % of dry matter depending on the ash content of the biomass. Hydrogen is the third major constituent, comprising typically 5 to 6% dry mater. Nitrogen, sulfur and chlorine can also be found in biomass, usually less than 1% dry matter [21]. Biomass contains about 40-50% cellulose, 20-25% hemicelluloses, 20-25% lignin and 5% extractives [21].

Cellulose in biomass appears both as crystalline and amorphous with the former being the most abundant form. Cellulose consists of D-glucose subunits linked by β-(1,4)-glycosidic bonds. The β-(1,4)-glycosidic bond is resistant to enzymatic hydrolysis. Only few micro-organisms can hydrolyze β-(1,4)-glycosidic bonds of cellulose. Cellulose is a linear molecule composed of repeating cellobiose (2 glucose molecules) units. Bundles of cellulose form microfibrils, which build up to fibrils and finally cellulose

fibers. Efficient cellulose hydrolysis remains one of the major challenges in converting cellulosic biomass into fuels or chemicals. Unlike cellulose, starch is made up of glucose polymer that includes amylase linked by α-(1,4)-glycosidic bonds and branched amylopectin linked by α-(1,6)-glycosidic bonds. Depending on the plant, starch generally contains 20 to 25 % amylose and 75 to 80 % amylopectin. The hydrolysis of starch with (acid or enzyme) produces glucose, maltose and dextrins. The success in enzymatic conversion of starch (mainly corn starch in the U.S) to ethanol has been achieved because it is easy for micro-organisms to break down the α-(1,4) and α-(1,6)-glycosidic bonds of starch into smaller glucose units for fermentation.

Hemicellulose differs from cellulose by virtue of the short lateral chains of different carbohydrate polymers that branch off of the main hemicellulose backbone. Hemicelluloses are branched polymers of low molecular weight with degree of polymerization of 80 – 200. The general formulas are $(C_5H_8O_4)n$ and $(C_6H_{10}O_5)n$ and are generally referred to as pentosans and hexosans [22]. It is made up of pentoses (xylose, rhamnose and arabinose), hexoses (mannose, glucose and galactose) and some sugar acids such as methylglucuronic, D-glucuronic and D-galactouronic acids. The average molecular weight of hemicellulose <30,000. The hemicellulose backbone is either a homopolymer or heteropolymer with short branches linked by β-(1-4)-glycosidic bonds and sometimes β-(1-3)-glycosidic bonds. Hemicellulose serves as a connection between the lignin and the cellulose fibers and gives the whole cellulose-hemicellulose-lignin network more rigidity. Solubility of hemicellulose compounds of mannose, xylose, glucose, arabinose and galactose increases with increase of temperature in descending

order. Hemicellulose is the most thermochemically sensitive component in biomass compared to cellulose and lignin. During thermochemical pretreatment of biomass, the side groups of hemicelluloses react first followed by the back bone.

Lignin is the most copious aromatic compound on earth and is the second only to cellulose in its contribution to living terrestrial biomass. It is the most recalcitrant organic chemical with a biological function to provide rigidity to vascular plants and protect the structural polysaccharides of cellulose and hemicellulose from attacks from other organisms [23]. Lignin is a complex, variable, hydrophobic, cross-linked, three dimensional aromatic polymers of p-hydroxyphenyl propanoid units connected by C-C and C-O-C links. Lignin is made of three precursor alcohols: p-hydroxylinnamyl (coumaryl) alcohol, which gives rise to p-hydroxyphenyl units in the polymer; 4-hydroxy-3-methoxycinnamyl (coniferyl) alcohol, the guaiacyl units and 3,5-dimethoxy-4-hydroxycinnamyl (sinapyl) alcohol, the syringyl units. Free radical copolymerization of these alcohols produces the heterogeneous, optically inactive, cross-linked and highly polydisperse polymer. In the polymerization process, secondary reactions lead to cross-linking between lignin and hemicelluloses. Lignins are extremely resistant to chemical and enzymatic degradation.

Biological degradation is achieved mainly by fungi, most efficiently by white rot basidiomycetes, and also by certain actinomycetes. The main purpose of lignin is to give the plant structural support, impermeability and resistance against microbial attack and oxidative stress. The amorphous heteropolymer is non-water soluble and optically inactive. This makes the degradation of lignin very difficult. Like hemicellulose, lignin

begins to dissolve in water around 180^{o}C under neutral conditions. The solubility of lignin in acid, neutral or alkaline environments depends on the alcohol precursors of the lignin [23].

2.3 Current Utilization

Biomass currently represents approximately 14% of world's final energy consumption. About 25% biomass energy is used in industrialized countries as an investment to meet strict pollutant emission control. The other 75% of primary biomass energy is used in developing countries to generate heat for households and supply process heat for biomass-based industries using their own generated biomass residues. Biomass residues derived from the forest industries normally have alternative uses as chips for pulp production, raw materials for particleboard and fiberboard production, or as fuel. The direct sale of biomass residues for production of densified fuels e.g. pellets or briquettes have also become attractive and lucrative in several developed countries [13].

If grown and utilized on a sustainable basis, biomass will result in a net reduction in greenhouse gas emissions and the replacement of a non-renewable energy source. Biomass fuels have negligible sulfur content and, therefore, do not contribute to sulfur dioxide emissions which cause acid rain. The combustion of biomass produces less ash than coal combustion. The ash produced during biomass combustion can be used as a soil additive on farm [12]. Certain biomass materials are more suitable to be used in a combustion chamber because of their lower ash contents, reduced fouling tendencies and increased efficiency of boilers.

2.4 Challenges in Supply Chain

Biomass is one of the renewable energy sources which governments and environmental protection agencies want to use to reduce the greenhouse gas emissions. One of its main advantages is that biomass is a very flexible energy source, which can be used to produce not only electricity and heat but also biofuels for transportation. It is also one of the few renewable energy sources that may be stored and can generate energy on demand. One of the most important barriers to using biomass as an energy source is the cost of the biomass supply chain and the technology to convert biomass into useful forms of energy. The large fraction of cost in biomass energy supply comes from the logistics processes. A major issue concerning biomass logistics is its storage, especially when it is characterized by seasonal availability.

Rentizelas *et al.* 2008 [24] summarizes the activities required to supply biomass from production point to a power station: (1) Harvesting of biomass in the field or forest. (2) handling of biomass in the field or forest and moving it to a point where road transport vehicles can be used. It may be necessary to process the biomass into forms that can be easily transported e.g. increasing the bulk density or unitizing the biomass into bales. Movement of the biomass may require a variety of transportation equipments including agricultural or forestry equipments and some heavy goods vehicles. These lead to an increase in the operational cost of energy generation. (3) Loading and unloading of the road transportation vehicles. Once the biomass has been moved to the roadside it will need to be loaded to road transportation vehicles for conveyance to the power station. The biomass will need to be unloaded from the vehicle at the power station. (4) Storage

of biomass until they are needed to be used by the power generating plant. Storage is necessary because biomass availability is seasonal and power stations require year round supply of raw materials to operate.

2.5 Conversion of Biomass into Transportation Fuels and High Value Chemicals

2.5.1 Biological Conversion of Biomass

Biological conversion processes include (1) aerobic fermentation of biomass into compost, carbon dioxide and water (2) anaerobic fermentation leading to the production of fertilizer and biogas and (3) alcoholic fermentation of biomass which produces ethanol, carbon dioxide and water. Biological conversion processes employ microorganisms to generate reverse photosynthesis products (including CO_2, H_2O and energy) and other useful products that have found multiple uses in various sectors of the economy. Biological conversion processes are characterized by low energy consumption, non-polluting, environmental sustainability and their ability to maintain the carbon dioxide balance within the atmosphere.

2.5.1.1 Anaerobic Digestion

Anaerobic digestion is a biogasification process to ferment biomass in the absence of oxygen for 2 – 8 weeks at approximately 37^oC. This process generates biogas as an energy source and organic fertilizer (or compost) and meanwhile eliminates the requirement for disposing waste biomass such as animal manure. Biogas consist of methane (65-70% dry gas), carbon dioxide (30-35% dry gas), water vapor and other traceable gases such as hydrogen and hydrogen sulfide H_2S. The heating value of dry

biogas is approximately of 26 MJ/m^3. The biogas is usually used to heat homes, cook and power farm equipments. The anaerobic digestion of biomass into biogas is usually performed by several microorganisms in several stages including hydrolytic, acidogenic, homoacedogenic and methanogenic steps.

The last stage is conducted by methanogenic bacteria which are able to convert organic acids into methane and carbon dioxide. The efficacy of this stage is dependent of temperature, pH, substrate concentration and minerals. Research has shown that pH ranging from 6.6 to 7.6 is the most appropriate for methanogenesis. Naturally occurring anaerobic digestion of biomass can be found within the rumen of ruminants (four chamber stomach animals e.g. cow, goat, horse etc). Rumen microorganisms have been shown to be capable of converting a wide range of lignocellulosic biomass into biogas in a two phase rumen derived process with efficiencies in the range of 50-60% [25].

2.5.1.2 Composting

Landfills use some aerobic (at the early stages) and anaerobic processes to degrade organic materials. The degradation of organic components in landfills is a complex process that is carried out by a succession of microbial population. During the early stages bacteria present in the waste and the soil used as a cover act as the initial inoculum and begin the degradation process. At this stage, the degradation is aerobic to convert carbon sources to carbon dioxide and water in an exothermic reaction which raises the temperature of the waste and increase the activity of critical bacteria and other organisms while depleting the oxygen present.

After some compacting of the waste pile to prevent the ingress of air, anaerobic activities then take over and methanogenic degradation of the biomass starts. Optimum conditions for mesophilic activities are a neutral pH value and a temperature of 35^{o}C. Methane concentrations have been reported to be increased to 50% in gases released from landfill sites. Carbon dioxide and hydrogen concentrations decrease gradually as the degradation of biomass pile transition from aerobic to anaerobic within the landfill waste pile.

2.5.2 Thermo-chemical Conversion of Biomass

Thermo-chemical conversion processes include combustion, gasification, liquefaction, hydrogenation and pyrolysis [26]. The choice of conversion process is dictated by factors such as the type and quantity of biomass feedstock, the desired form of the energy needed at the consumer level, environmental standards, economic conditions and project specific factors.

2.5.2.1 Combustion

In combustion processes, biomass is directly burnt in the presence of sufficient air to convert chemical energy stored in biomass to heat, mechanical power or electricity, etc. Biomass combustion is feasible when the moisture content is less than 50% [26]. The rate at which biomass fuels burn depends on a number of physical phenomena, two predominant factors are the rates of heat transfer and the kinetic rates of reaction. Particle size is the dominant factor affecting heat transfer. Small thin particles can be heated rapidly while coarser, thicker particles are heated more slowly.

Combustion occurs both in the gas phase with the burning of volatile materials released through the pyrolysis of the fuel upon heating and heterogeneously in the solid phase as char oxidation [21]. Combustion of biomass causes pollution. Primary pollutants from biomass combustion are particulate matter, carbon monoxide, hydrocarbons, oxides of nitrogen (NO_x, principally NO and NO_2), and oxides of sulfur (SO_x, principally as SO_2). Acid gases such as HCl may also be emitted as may lead and other heavy metals. Carbon monoxide and hydrocarbons, including volatile organic compounds (VOC) and polycyclic aromatic hydrocarbons (PAH), are the products of incomplete combustion [21]. These species can be largely controlled by stoichiometry and proper fuel moisture control.

Heavy metals can be present in high concentration in certain urban wood fuels and user derived fuels, especially if treated or painted woods are present. Particulate matter includes soot, ash, condensed fumes (tars/oils), and sorbed materials including VOC and PAH. Emissions of oxides of nitrogen and sulfur arise predominantly from nitrogen and sulfur in the fuel. NO_x in combination with hydrocarbon photochemically leads to the formation of ozone, which is an irritant to the lungs and eyes and a major problem in urban environments. Ozone also causes damage to plants. SO_x are respiratory irritants, and their effects are enhanced in the presence of PM due to transport deep within the lungs. Both NO_x and SO_x contribute to acid rain [21].

2.5.2.2 Gasification

Gasification is the conversion of biomass into combustible gas mixture by the partial oxidation of biomass at high temperatures, typically in the range of 800-900^{o}C, in

gasification media such as air, oxygen or steam [27]. Unlike combustion where oxidation is substantially complete in one process, gasification converts the intrinsic chemical energy of the carbon in the biomass into a combustible gas in the first incomplete oxidization stages and the combustible gas can be further completely oxidized in the second stage. The reactions taking place during gasification can be summarized as follows [28]:

$$Partial\ Oxidation\quad C + \tfrac{1}{2}O_2 \leftrightarrows CO\ \Delta H = -268\,{}^{MJ}/_{kgmole} \tag{2.1}$$

$$Complete\ Oxidation\quad C + O_2 \leftrightarrows CO_2\ \Delta H = -406\,{}^{MJ}/_{kgmole} \tag{2.2}$$

$$Water\ gas\ reaction\quad C + H_2O \leftrightarrows CO + H_2\ \Delta H = +118\,{}^{MJ}/_{kgmole} \tag{2.3}$$

$$Water\ gas\ shift\ reaction\quad CO + H_2O \leftrightarrows CO_2 + H_2\ \Delta H = -42\,{}^{MJ}/_{kgmole} \tag{2.4}$$

$$Methane\ formation\quad CO + 3H_2 \leftrightarrows CH_4 + H_2\ \Delta H = -88\,{}^{MJ}/_{kgmole} \tag{2.5}$$

The low calorific value gas produced can be burnt directly or used as a fuel for gas engines and gas turbines. The product gas can be used as a feedstock in the production of chemicals and liquid fuels [21].

There are two main types of gasification processes: fixed bed and fluidized bed gasification with variations within each type. Depending on the direction of air flow, the fixed bed gasifiers can be classified as updraft, downdraft and cross-flow. Fixed bed gasifiers are usually operated around temperatures of 1000^{o}C. In the updraft design, the biomass is fed to the top of the gasifier while air is introduced from the bottom of the

unit. The updraft gasifier has little pressure drop, good thermal efficiency and little tendency towards slag formation. However it is very sensitive to the moisture content of fuel and generates a lot of tar [28].

Biomass feed and air are introduced in the same direction within a downdraft gasifier. The tar content of the gas leaving the downdraft gasifier is much lower than that from the updraft gasifier. However the gas leaves a downdraft gasifier at very high temperatures of 800-900^{o}C which makes the downdraft gasifiers less energy efficient than the updraft gasifiers. The downdraft gasifier has flexible adaptation of gas production to load and is more tolerant to charcoal dust and tar content of fuel. The downdraft design tends to be very tall and is not usually suitable for fuels with small particle sizes [28].

In the cross-flow gasifier, biomass moves downwards while the air is introduced at the side of the gasifier. Product gases are withdrawn from the opposite side of the unit at the same level as the air feeding port. Gases from this configuration have high tar content with temperatures usually between 800-900^{o}C. The energy efficiency is therefore lower than the updraft gasifier. The cross-flow gasifier is short, has very fast response time to load and flexible gas production ability. It is however very sensitive to slag formation and has a high pressure drop [29].

Fluidized bed gasification has an advantage of keeping temperature uniformity within the gasification zone of the unit. The uniformity of temperature is achieved by fluidizing the bed material and biomass with a gasifying agent such as air to ensure intimate mixing of the hot bed material, biomass and gasifying gas. There are two types

of fluidized bed gasification designs in use; bubbling fluidized bed and the circulating fluidized bed gasification. Bubbling fluidized bed gasifiers consist of a vessel with a grate at the bottom through which air is introduced. Above the grate is the moving hot particle bed into which the prepared biomass feed is introduced. It is usually operated at temperatures of 700 – 900^{o}C.

The circulating fluidized bed gasifier is a high capacity unit usually used in the paper industry for the gasification of bark and forestry residues. The bed material is circulated between the reaction vessel and a cyclone separator where the ash is removed and the bed material and char returned to the reaction vessel. Generally gasification has the flexibility in feedstock and product with a near zero pollutant emission and high energy efficiency. However, it is a complex multistage process which is capital intensive. Product gases must be cleaned and purified before used, which makes the process more expensive.

2.5.2.3 Pyrolysis

Pyrolysis is the thermal degradation of biomass in the absence of air or oxygen leading to the production of liquid oils, gases and solid products [26]. It is the fundamental chemical reaction to produce volatile precursors during gasification and combustion of solid fuels [30]. Pyrolysis is classified into three types namely flash, fast and slow depending on the temperature, heating rate and residence time. Flash pyrolysis is an extremely rapid heating process occurring at 400-900^{o}C with small residence time. The heating rate of fast pyrolysis is much lower than that of flash pyrolysis and the

temperature is lower than 600^{o}C. Slow pyrolysis occurs at 450-700^{o}C with even lower heating rates. The main products of pyrolysis are char, bio-oils or pyrolysis oil and gas.

Char can be used for combustion or as activated carbon. It can be used in gasification processes to obtain hydrogen rich gases by thermal cracking. Char is also converted into briquettes and combusted to generate thermal energies for boilers. The gaseous product can be used for heat supply. Bio-oil can be used either directly as a fuel or as a source to produce high value chemical. The principles to obtain high yield of bio-oils include moderate pyrolysis temperature (~500^{o}C), very high heating rates (10^{3}-10^{5} ^{o}C), short residence times (< 2 s) and rapid quenching of pyrolysis vapors [31]. Pyrolysis gas mainly consists of hydrogen, carbon monoxide, carbon dioxide and methane. Other higher carbon gaseous compounds in the pyrolysis gas include propane, propylene, butane, butenes and ethane. Char from pyrolysis processes contain elemental carbon along with hydrogen.

Oils obtained from pyrolysis of biomass contain several organic and inorganic species. Bio-oil consist of two phases, an aqueous phase containing oxygenated organic compounds of lower molecular weight and non aqueous phase containing organic compounds (mainly aromatics). Organic species present in the bio-oil include (1) acids such as; formic, propanoic, hexanoic and benzoic (2) esters such as; methyl formate, methyl propionate, butyrolactone, methyl n-butyrate and velerolactone (3) alcohols such as methanol, ethanol, 2-propene-1-ol and isobutanol (4) ketones such as; acetone, 2-butanone, 2-pentanone, 2-cyclopentanone and 2,3-pentenedione (5) aldehydes such as; formaldehyde, acetaldehyde, 2-butenal, pentanal and ethanedial (6) phenols such as;

phenols and methyl substituted phenols (7) furans such as; 2-methyl furan, 2-furanone, furfural and furfural alcohol (8) guaiacols such as; 2-methoxy phenol, 4-methyl guaiacol and eugenol (9) miscellaneous oxygenates such as; hydroxyacetaldehyde, hydroxyacetone, dimethyl acetal, acetal and methyl cyclopentenolone (10) syringols such as; methyl syringol, 4-ethyl syringol and propyl syringol (11) nitrogen compounds such as; ammonia, methylamine, pyridine and methylpyridine. Other inorganic species found in biomass include; calcium, potassium, iron, sodium, aluminum, chromium, barium, manganese and chlorine [26].

Bio-oils have several industrial applications including: (1) fuel for combustion (2) production of chemicals and resins (3) production of anhydrous-sugars like levoglucosan (4) making of adhesives (5) production of preservatives e.g. wood preservatives and (6) production of binding agents for pelletizing and briquetting of combustible organic waste materials [27]. Bio-oils have a potential to be used as a fuel oil substitute.

Combustion analysis indicates that bio-oils can be burnt effectively in standard or slightly modified boilers and engines with rates comparable to those of commercial fuels. The oils have heating values of only 40-50% of that of hydrocarbon fuels. However some problems occur in combustion systems when bio-oils are burned without upgrading. Bio-oils have high water content that is unfavorable for ignition. The organic acids in the oils are highly corrosive to common construction materials. Solids (char) in the bio-oils can block injectors or erode turbine blades. The thermodynamic instability and high reactivity of some components in the oils leads to the formation of larger molecules that result in high viscosity and in slower combustion.

As a result of the unfavorable properties, bio-oils need to be upgraded before they can effectively replace fossil derived fuels. A few technologies have been deployed to reduce the oxygen content of the biofuels and to make the bio-oils more favorable to be used in combustion chambers. The recent upgrading technologies include; hydrodeoxygenation, steam reforming, emulsification, catalytic cracking and hydrotreating.

Hydrodeoxygenation: This process is performed in hydrogen providing solvents activated by the catalysts of Co-Mo, Ni-Mo and their oxides or loaded on Al_2O_3 under pressurized conditions of hydrogen and/or CO. Oxygen is removed from the biofuel as H_2O and CO_2 while the energy density of the biofuel is elevated [32].

Steam reforming of bio-oils can be described by the following reaction stoichiometry:

$$C_nH_mO_k + (n-k)H_2O \rightarrow nCO + \left(n + \frac{m}{2} - k\right)H_2 \quad (2.6)$$

The water gas shift (WGS) reaction simultaneously follows as;

$$CO + H_2O \leftrightarrows CO_2 + H_2 \quad (2.7)$$

The overall steam reforming process is thus given as;

$$C_nH_mO_k + (2n-k)H_2O \rightarrow nCO_2 + \left(2n + \frac{m}{2} - k\right)H_2 \quad (2.8)$$

Steam reforming is an endothermic process and is thus favored by high temperatures [33].

Steam reforming is aimed at generating hydrogen from the lighter fractions or the water soluble carbohydrate fractions of bio-oil. Acetic acid which makes up about 31 wt% of bio-oil and a major part of the water soluble phase is reformed to generate hydrogen in the reaction;

$$CH_3COOH + 2H_2O \rightarrow 2CO_2 + 4H_2 \quad (2.9)$$

The hydrogen yield from steam reforming of acetic acid in the aqueous fraction over Pt/ZrO_2 catalyst hand pelletized 5% $Ru/MgO/Al_2O_3$ catalyst was reported to be close to 100% [32, 33]. The catalyst of 5% Ru dispersed on 15% MgO/Al_2O_3 has been found to demonstrate high activity and selectivity as well as satisfactory stability in steam over time under conditions of steam reforming of acetic acid, a model compound for pyrolysis oil. During steam reforming of bio-oil, deactivation due to coke/oligomer deposition on catalysts was found to be the major hindrance to the performance and continuous use of the catalyst [34].

Emulsification is another method to upgrade bio-oils. An emulsion is defined as two immiscible liquids wherein droplets of one phase (the dispersed or internal phase) are encapsulated within a layer of another phase (the continuous or external phase). Three conditions which govern the stability of emulsification are (1) mutual insolubility of the two liquids (2) adequate dispersion of one liquid into the other through agitation (3) an emulsifying agent [35]. Upgrading bio-oils through emulsification with biodiesel creates an avenue for us to further reduce the overdependence on petroleum based fuels.

Biodiesel is composed of monoalkyl esters of fatty acids obtained from natural renewable sources such as animal fats and vegetable oils. It is environmentally benign and safe to handle with a relatively high flash point. Its heating value, density and viscosity are characteristics that are comparable to no. 2 diesel from petroleum.

Emulsification of bio-oil/biodiesel was successfully achieved at the optimal conditions of 4:6 bio-oil/biodiesel ratio by volume, stirring intensity of 1200 rpm, 15 min mixing time, 30^{o}C emulsifying temperature and an octanol surfactant dosage of 4% by volume [36]. At these conditions an emulsion with a viscosity of 4.665×10^{-3}Pa.s at 25^{o}C, density of 0.895 g/cm^{3}, acid value of 14.01 mg of KOH/g, average molecular weight of 311 and water content of 0.4558 wt% was obtained. These results compare favorably with no. 2 diesel which has a viscosity of 0.0041Pa.s, molecular weight of approximately 200, density of 0.8867 g/cm^{3} and negligible water content [U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Alternative Fuels Data Center].

2.5.3 Extraction and Separation

Plants contain a wide range of bioactive compounds including lipids, phytochemicals, pharmaceutics, flavors, fragrances and pigments [7, 8]. Extraction is an age old technique for isolating these essential components of plants for commercialization. Hemicellulose makes up approximately one-fourth to one-third of most plant materials and is primarily composed of xylose. Xylan (xylose polymer) has been found to have several industrial and medicinal applications depending on the plant from which it is obtained. Xylan from corn hulls (a byproduct of starch preparation) is used as food gum [36]. Xylan from ramie hemicelluloses have been used as beater

additives in paper making [37]. Cereals containing xylan have been found to lower cholesterol levels in humans by contributing to the decrease of post-prandial glucose and insulin responses.

Xylan from agricultural residues including corn stalks wheat straw, bamboo leaves and Japanese beechwood (i.e. 4-O-methylglucuronoxylan) have been reported to retard the growth rate of cancerous cells including sarcoma-180. Sarcoma-180 is one of the transplantable, non-metastastizing, connective tissue tumors of the mouse. Other anti-tumor drugs have been made from carboxymethylated xylan rich wood hemicelluloses due to their ability to trigger T-lymphocytes and immunocytes [36]. Withanolids have been successfully separated from the leaves of *Lochroma gesnerioides* by Kaufmann *et al.* 2002 [38] under Soxhlet and pressurized solvent extraction. Withanolids have been reported to have pharmacological properties including antibacterial and virostatic activities. They are also reported to act antagonistically to ecdysteroid and possess immunomodulatory properties [38].

Extraction methods used for obtaining these valuable plant compounds include Soxhlet extraction, Sonication-assisted extraction, Microwave-assisted extraction, Supercritical fluid extraction and Accelerated solvent extraction [7]. In the extraction of essential plant compounds, factors that must be carefully considered include the choice of solvent (it must be environmentally friendly and able to dissolve the desired plant component), thermal stability of the plant component to be extracted (the desired component of the plant must not denature at the extraction temperature), extraction time, liquid-solid ratio and matrix characteristics [7].

The Soxhlet extraction method is one of the oldest and standard techniques for obtaining essential plant components. It is used as a reference procedure for evaluating the efficacy of other solid-liquid extraction methods. A suitable solvent choice should be made during the extraction of plant essentials using the Soxhlet extractor since different solvents will yield different extracts and extracts compositions. Suitable solvents used in extraction include hexane, isopropanol, ethanol, hydrocarbons, water and co-solvent mixtures such as isopropanol and hexane. Hexane (*n*-hexane) is an environmentally unfriendly compound and its use is strongly discouraged. Hexane is however very effective at extracting edible oils from plant sources. Extraction solvents are recovered from the Soxhlet extractor via evaporation. The quality of extracts from Soxhlet extraction may be affected by the extraction and evaporation temperatures.

The merits of the Soxhlet extraction include high extraction gradient since the solid matrix is constantly contacted with fresh solvent, nearly constant extraction temperatures as heating is obtained from the distillation flask and no filtration requirement after leaching [7]. The Soxhlet extraction method is also preferred to other novel extraction methods because of its reproducibility and efficiency as well as the stability of the extract compositions. Long extraction times, large solvent volumes, lack of agitation and likely thermal decomposition of essential plant compounds due to high extraction temperatures makes the Soxhlet extraction technique unattractive.

Sonication assisted extraction is the use of the energy in sound waves to disrupt the rigidity of the intermolecular bonds existing amongst the chemical components in plant materials. Sonication is also used to disrupt biological cell walls leading to the easy

discharge of cell contents. Sound waves with frequencies higher than 20 kHz cause mechanical vibrations in solids, liquids and gases [7]. The mechanical effects of ultrasound encourage a higher penetration of solvents into cellular materials and enhance mass transfer. The factors which govern the action of Sonication assisted extraction include the sound frequency, pressure, temperature, Sonication time and plant characteristics such as moisture content and particle size. The choice of solvent is also critical in Sonication extraction. The advantages of the Sonication assisted extraction include simplicity, increased extraction yield and kinetics, reduced operating temperatures and flexibility of solvent choice for the extraction of a wide variety of natural compounds. The apparatus for Sonication assisted extraction is relatively cheaper compared to microwave assisted extraction and its operation is easier. The effect of Sonication during the extraction of lignin from wheat straw has been investigated by Sun *et al.* 2001 [39]. Results from extraction using 0.5 M KOH with ultrasound showed a slight increase in the lignin extracts by 0.9% over extraction without ultrasound. The higher efficiency of the ultrasound assisted extraction is attributed to the mechanical action of ultrasound on the cell walls resulting in increased accessibility and extractability of the of the lignin component.

Electromagnetic radiations with frequencies in the range of 0.3-300 GHz are referred to as microwaves. Microwaves penetrate deep into biomaterials and interact with polar molecules such as water to generate heat [7]. Microwave assisted extraction is possible because water molecules within the biomaterials are able to absorb large amounts of microwave energy as a result of its polarity and high dielectric constant. Cell

disruption is enhanced due to the internal superheating of the plant matrix. During microwave extraction there is an expansion of the plant cell walls with concomitant release of chemicals into the solvent. The choice of solvent for the microwave assisted extraction is dependent on solubility of the essential compound to be extracted, interaction between the solvent and the matrix and the dielectric constant of the solvent. Probable solvents for microwave assisted extraction include water, methanol and ethanol. Other solvents with strong microwave absorption potential may also be used. Non polar solvents such as hexane and toluene with low dielectric constant are unsuitable for microwave extraction [7]. During extraction, the solvent volume must be adequate to fully submerge the solid matrix. Excessive solvent volumes may not necessarily increase the yield of extracts during microwave assisted extraction because of insufficient agitation. High extraction temperatures enhance the yield of extracts but may also be the cause of extract degradation especially for thermolabile compounds. The microwave assisted extraction has several advantages including reduced extraction time, reduced solvent usage and improved extraction yield. Microwave extraction is a relatively cheaper process compared to supercritical fluid extraction and simpler to operate. However microwave assisted extraction must be followed by a filtration and or centrifugation unit to remove solid residues generated during the extraction stage.

Buranov *et al.* 2010 [40] reports the successful extraction of hemicelluloses from flax shives using different methods including pressurized low-polarity water (PLPW), pressurized aqueous ethanol (PAE), microwave-assisted water (MW-water) and microwave assisted ethanol (MW-ethanol). Extraction results show high hemicellulose

fractions of 90 and 80% of total hemicellulose after extraction with PLPW and PAE respectively. Microwave assisted extraction however gave only 18 and 40% of total hemicelluloses for MW-water and MW-ethanol. Increasing microwave irradiation time was found to be detrimental to hemicellulose extraction due to the degradation of macromolecular xylan [40].

Lignin extraction and separation from wheat straw was conducted by Sun *et al.*1996 [41] using different methods for the isolation of different types of lignin including alkali lignins, organosolv lignins, ball-milled and enzyme lignins. Sodium hydroxide, potassium hydroxide and lithium hydroxide were used for separating the alkali soluble lignins. Ball-milled and enzyme lignins were separated via dioxane/water mixture, dissolved in acetic acid and later precipitated into ether. The dioxane extracted residues washed with water were then treated with cellulase enzyme for the extraction of enzyme lignins. Ethanol (240 ml/160 ml v/v ethanol/water solution) impregnated with 0.02N H_2SO_4 as catalyst was used for the isolation of organosolv lignins in a laboratory blender. The average molecular weights of lignin were determined via gel permeation chromatography (GPC) on a PLgel 5μ after extraction using the three methods. Lignin molecular weights were measured in descending order as 2020, 1890, 1640, 1400 for enzyme lignins, ball-milled lignins, organosolv lignins and alkali lignins respectively. Alkali isolation of lignin was determined to be more effective because it led to the production of purer lignin fractions as lignin associated polysaccharides were greatly reduced in the extracts.

The effects of temperature and solvent type on the extraction of policosanols (PC) form wheat straw, germ and bran has been investigated by Dunford *et al.* 2010 [42]. Policosanols have been found to have some effects on lowering low density lipoproteins (LDL) and increasing high density lipoprotein (HDL) cholesterol levels. Pressurized liquid extraction (PLE) or Accelerated Solvent Extraction (ASE) was applied in the isolation of PC's from wheat straw, germ and bran. The solvents used for the extraction included n-hexane, ethanol, petroleum ether and chloroform. The highest amount of extract was collected from the extraction on wheat germ due to the high triacylglycerol content of wheat germ. It was observed that the dielectric constant of the solvent was very instrumental in the quantity of extracts that could be obtained per solvent type. Ethanol having the highest dielectric constant amongst the four solvents extracted the most policosanol from the wheat germ at elevated temperatures yielding 17.3%, 10.3%, 10.1% and 10.3% for ethanol, hexane, chloroform and petroleum ether respectively.

Soxhlet extraction of lipids form grain sorghum DDG (dry distiller's grain) has been investigated by Wang *et al.* 2005 [43]. The extraction of valuable lipids including triacylglycerols, fatty acids, fatty alcohols, fatty aldehydes, free sterols, wax esters and steryl esters were achieved using n-hexane as extraction solvent. Very high quantities of triacylglycerols were extracted and can be refined and used as vegetable oils. Extraction yield was however highest at near solvent boiling point of 68^{o}C with a solvent to solid ratio of 1:3 and extraction time of 4 h. It was also observed that increasing solvent to solid ratio beyond the optimum resulted in no significant extract yield beyond that obtained at 68^{o}C and 4 h of extraction time.

The steam explosion fractionation of wheat straw and subsequent ethanol extraction of hemicelluloses was studied by Hongzhang *et al.* 2006 [44]. Steam explosion at 1.5 MPa, 34.01% moisture content at 4.5 min explosion time generated an extract stream from which 80% hemicellulose was recovered with a 40% ethanol solution at a fiber/liquor ratio of 1:50 (w/v), severity log(R) = 3.657 (180^{o}C for 20 min) and 0.1% NaOH. Subsequent lignin extraction by acid precipitation also yielded 75% of total lignin from the raw wheat straw sample.

2.6 Pretreatment and Fractionation of Biomass

Hemicellulose and lignin content, cellulose crystallinity and available surface area (or porosity) of biomass are major factors that affect the hydrolysis of cellulose and hemicellulose into sugars (glucose) and xylose for fermentation. Pretreatment of biomass alters its chemical composition and structure so that the hydrolysis of the carbohydrate fractions into simple sugars can be enhanced. The purpose of biomass pretreatment in general is to increase the accessibility of biomass to enzymes and chemicals during hydrolysis by reducing the crystallinity of cellulose, removing lignin and hemicellulose and improving the porosity and surface area of biomass. Lignin removal increases the efficiency of enzymes by eliminating nonproductive adsorption sites and increasing accessibility to cellulose and hemicellulose [45].

A good pretreatment method should be able to: (1) improve sugar formation or provide the opportunity to other subsequent processes to produce sugars (2) minimize the formation of inhibiting byproducts that impede the progress of subsequent hydrolysis and

fermentation processes, (3) minimize the loss of carbohydrates. Good pretreatment methods must also minimize energy demand, reduce the cost of size reduction for feedstock, reduce the cost of material for construction of pretreatment reactors, produce fewer residues and consume little or no chemicals [46]. Pretreatment methods are usually classified into physical pretreatment such as milling and grinding, physicochemical pretreatment such as steam explosion/autohydrolysis, wet oxidation and hydrothermolysis; chemical pretreatment such as alkali, acid, oxidizing agents and organic solvents, biological, electrical or a combination of these. Figure 2.1 is a schematic of the effect of pretreatment on biomass structure and on ethanol production.

2.6.1 Physical Pretreatment

Physical pretreatment involves the reduction of particle size and cellulose crystallinity. The reduction in particle size leads to an increase in surface area and porosity of the biomass as well as the degree of polymerization. Reduction of cellulose crystallinity and particle size is achieved by the comminution of the lignocellulosic materials via chipping, grinding and or milling [47]. Particle sizes of 10-30 mm are usually obtained after chipping and 0.2-2 mm after milling or grinding [45, 48]. Depending on the type of biomass a 5-25% improvement in hydrolysis yield and 23-59% reduction in hydrolysis time have been observed in many lignocellulosic materials that were pretreated physically [47].

Without the use of chemicals physical pretreatment does not generate inhibitors such as furfural and hydroxymethylfurfural (HMF) to the downstream enzymatic hydrolysis and ethanol fermentation. However physical pretreatments such as milling,

grinding and chipping are energy intensive processes which can significantly increase the cost of producing ethanol.

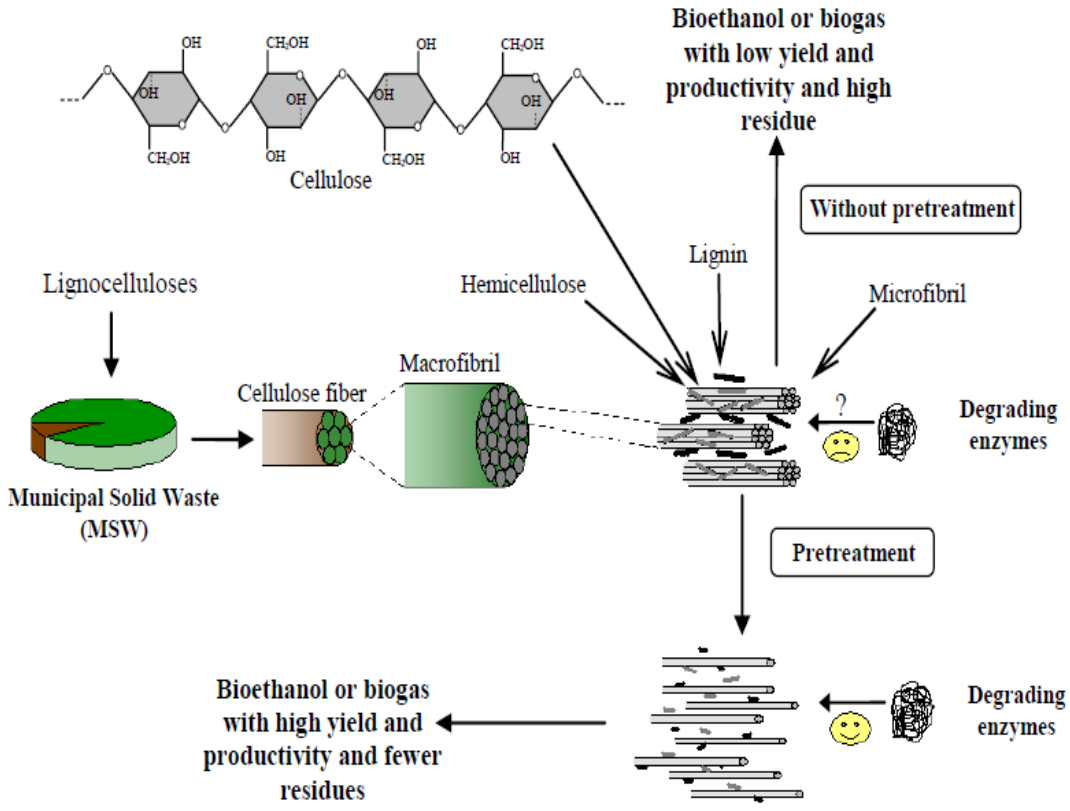


Figure 2.1 Effect of pretreatment on ethanol production (Adapted from Taherzade et al. 2008)

2.6.2 Hot Water Pretreatment

Hot water pretreatment is a type of thermal pretreatment of biomass (also erred to as hydrothermolysis, aquasolv, uncatalyzed solvolysis and aqueous fractionation).The residence time for this process is usually 15 minutes at elevated temperatures of 200 to

230^{o}C. Hot water pretreatment can hydrolyze all hemicellulose and 4-22% cellulose and dissolve 35-60% lignin. During hot water pretreatment approximately 40-60% of the biomass enters into the liquid stream [45]. Beyond solubilization of lignin and hemicellulose within the biomass, the hot water pretreatment is designed to avoid or lessen the formation of inhibitors that affect subsequent downstream ethanol production processes of hydrolysis and fermentation.

The pKa value of water is affected by temperature. For instance the pH value of pure water is nearly 5.0 at 200^{o}C. The high dielectric constant of water at high temperature enables it to dissociate ionic substances. This dielectric property is manifested to increase the ability of water to cleave the hemiacetal linkages in biomass to release acids during hot water pretreatment of biomass. The released acids facilitate the further solubilization of hemicellulose [49]. An average pH range of 4 to 7 during hot water pretreatment was proposed to maximize the formation of monosaccharides which are subsequently converted into degradation products that catalyze the hydrolysis of cellulosic material [47]. Maintaining a 4 to 7 pH range also minimizes the formation of inhibitory byproducts.

Reactors currently used for hot water pretreatment include cocurrent, countercurrent, and flow-through configurations. The biomass and water move in the same direction into the reactor in the cocurrent configuration where the biomass is heated to the desired temperature and held at the pretreatment conditions for a specific time period. In the countercurrent configuration hot water and the lignocellulosic biomass flow

in opposite directions through the pretreatment reactor. In the flow through configuration hot water is made to pass through a bed of biomass material.

Hot water pretreatment differs from steam explosion by concentration of solubilized products within the liquid stream. Hot water pretreatment has higher concentration of xylan and hemicellulose sugars in the liquid stream than steam pretreatment

2.6.3 Steam Explosion

Steam explosion is by far the cheapest and most commonly used pretreatment method for the fractionation of lignocellulosic biomass. In this process, biomass is exposed to saturated high pressure steam usually at temperatures of 160-260^{o}C and pressures of 0.69-4.83 MPa for several seconds to a few minutes. The pressure is then swiftly reduced and the biomass is suddenly exposed to atmospheric pressure. This causes an explosive decompression of the biomass material which leads to hemicellulose degradation and lignin transformation as a result of the high temperature, thereby creating pores with increased surface areas within the lignocellulosic matrix and increasing the potential for cellulose hydrolysis. During steam explosion hemicellulose hydrolysis is catalyzed by the release of organic acids such as acetic acid from the biomass.

Steam explosion is affected by temperature, residence time, moisture content and chip size of biomass. High temperatures and short residence times (e.g. 270^{o}C, 1 min) or low temperatures and long residence times (e.g. 190^{o}C, 10 min) were found to be the most favorable conditions for solvation of hemicellulose during steam explosion pretreatment [48]. At high temperatures, water becomes acidic in its action on biomass.

The temperature and time of steam explosion pretreatment can be drastically decreased by the addition of small amounts (e.g. 0.3 – 3% w/w) of sulfuric acid or CO_2 or SO_2. This approach has been found to improve the hydrolysis of the biomass, decrease the formation of inhibitory compounds and lead to the complete removal of hemicellulose [45].

Steam explosion pretreatment is better than physical pretreatment method such as mechanical comminution because of its environmental friendliness, no recycling and low energy requirement. However the limitation of steam explosion include the degradation of xylan with biomass, incomplete solubilization of lignin to free cellulose for hydrolysis and generation of inhibitory compounds that affect downstream ethanol fermentation. As a result of the formation of inhibitory compounds, steam exploded biomass must be washed before fermentation which leads to the loss of soluble reducing sugars.

2.6.4 Acid Pretreatment

Acids are used to solubilize hemicellulose, degrade the lignin and make cellulose accessible to enzymatic hydrolysis. Acid pretreatments are done with concentrated, dilute and weak organic acids. Strong acids such as sulfuric acid (H_2SO_4) and hydrochloric acid (HCl) in their concentrated and dilute forms have been used in the fractionation of lignocellulosic biomass. Acid hydrolysis of biomass releases oligomers and monosaccharides in a homogeneous reaction where the acid catalyzes the breakdown of cellulose to glucose [49]. Concentrated acid pretreatment was found to be very effective in hydrolyzing biomass for ethanol production. However concentrated acids are toxic, corrosive, hazardous, and require reactors that are resistant to corrosion. The concentrated

acid must also be recovered after hydrolysis. These factors account for the high cost of biomass fractionation using concentrated acids. Two types of dilute acid pretreatment processes are mostly used; (1) a high temperature (>160^{o}C), continuous flow process for low solid loadings (5-10% substrate wt/mixture wt) and (2) a low temperature (<160^{o}C), batch process for high solids loadings (10-40%) [46].

During acid pretreatment, dissolved lignin condensates quickly and precipitates in the acidic environment. Concentrated acid pretreatment causes more dissolution of hemicellulose and precipitation of solubilized lignin than dilute acid pretreatment [19, 47]. During acid pretreatment the sugars may be further degraded to form hydroxymethylfurfural and other degradation products. These by products inhibit the downstream ethanol fermentation. Dilute sulfuric acid with a concentration usually below 4 wt % has been commercially used to manufacture furfural from xylose. In this process, sulfuric acid mixed with biomass hydrolyzes the hemicellulose into xylose and other sugars and then continues to break down xylose to form furfural.

Organic acids such as lactic acid and acetic acid have also been employed in the fractionation of lignocellulosic biomass. Jian *et al.* 2009 [50] pretreated corn stover using lactic acid and acetic acid as catalyst at 195^{o}C and 15 min residence time in a loop autoclave. Enzymatic hydrolysis of lactic acid pretreated corn stover resulted in 73.8% cellulose to glucose conversion. Both lactic and acetic acid pretreated corn stover resulted in 95.66% glucan recovery and acetic acid alone pretreated corn stover led to 88.7% conversion of glucose to ethanol.

2.6.5 Alkaline Pretreatment

The mechanism of alkaline hydrolysis is the solvation and saponification of the ester bonds in cross-linking xylan hemicelluloses and other components such as lignin and other hemicellulose [47, 48]. The alkaline pretreatment can eliminate lignin from biomass, thereby improving the accessibility of the remaining polysaccharides. The lignin content of the biomass pretreated by alkali therefore determines the efficacy of the alkali pretreatment method. Alkali pretreatment also removes acetyl and various uronic acid substitutions in hemicellulose that lower accessibility of enzymes to the hemicellulose and cellulose surface [49]. Alkali pretreatment reagents include sodium hydroxide, potassium hydroxide, calcium hydroxide and ammonium hydroxide. Sodium hydroxide has been the most widely used in research. However calcium hydroxide (or lime) is gaining increasing interests due to its lower cost, safety and ease of recovery as insoluble calcium carbonate in water by reacting with carbon dioxide. The carbonate, in a recycle process, is converted to lime by the lime kiln technology.

Lime pretreatment removes amorphous substances such as lignin and hemicellulose which increase the crystallinity index of cellulose. Enzymatic hydrolysis of lime treated biomass is affected by structural features such as the extent of acetylation, lignification and crystallinity

Dilute sodium hydroxide (NaOH) pretreatment of lignocellulosic biomass has been reported to cause swelling leading to a decrease in cellulosic crystallinity and degree of polymerization, an increase in biomass internal surface area, separation of structural linkages between lignin and carbohydrates and disruption of lignin structure [45,47,48].

The NaOH pretreatment was reported to decrease the lignin content of hardwood from 24-55% to 20% and enzymatic digestibility of the NaOH pretreated hardwood increased from 14% to 55% .Sodium hydroxide has also been found to be effective for pretreating straws with lignin content of 10–18%.

Alkali pretreatment can be carried out at ambient temperatures at long contacts or reaction times in the order of hours or days compared to minutes or seconds for other pretreatment methods. As alkali pretreatment processes employ lower temperatures and pressures compared to acid and steam pretreatment methods, an alkaline process causes less sugar degradation. Unlike acid catalyzed pretreatments, some alkali pretreatments generate irrecoverable salts which are incorporated into the biomass.

2.6.6 Ammonia Explosion

This is a physicochemical process that is similar to steam explosion. In ammonia explosion the lignocellulosic biomass is exposed to liquid ammonia at a high temperature and pressure for a period of time, and then the pressure is abruptly reduced. This process is commonly described as the Ammonia Fiber Explosion (AFEX). The parameters that affect the performance of the AFEX process are ammonia concentration, water loading, temperature, blow down pressure, time and the number of treatment cycles [46].A typical AFEX process uses 1-2 kg of liquid of liquid ammonia to treat 1 kg of dry biomass. The process is run at 90^{o}C with a residence time of 30 minutes. The AFEX method has been widely applied in pretreating various herbaceous crops and grasses such as alfalfa, wheat straw, Bermuda grass, rice straw, corn stover, barley straw and bagasse [45, 48].

During the AFEX pretreatment, the hemicellulose with a biomass material is degraded into oligomeric sugars and deacetylated. The biomass pretreated by AFEX has low hemicellulose content, disrupted structure, increased water holding capacity and higher digestibility. AFEX pretreatment has little effect on the fractionation of biomass with a high lignin content such as woods and nut shells. Hydrolysis yield of AFEX pretreated newspaper and aspen chips (25% lignin) have been reported to be only 40% and below 50% respectively [44]. However an alternative to the AFEX process is the Ammonia Recycle Percolation (ARP) where aqueous ammonia (10–15%) passes through biomass at elevated temperatures (150–180^{o}C). During ARP, aqueous ammonia reacts with lignin to depolymerize lignin and cleave the lignin–carbohydrate linkages. The ammonia is then recovered, separated and recycled [45].

The optimal conditions for AFEX pretreatment of corn stover has been found to be the temperature of 90^{o}C, the mass ratio of ammonia to dry corn stover of 1:1, the moisture content of corn stover of 60% (dry mass basis) and the residence time of 5 min. Under these conditions, the enzymatic hydrolysis of the AFEX pretreated corn stover achieved a 98% glucose yield. The ethanol yield from the AFEX pretreated corn stover was 2.2 times that of the untreated corn stover [51]. The hydrolysis of the AFEX pretreated switchgrass had 93% glucan conversion efficiency compared to 16% for untreated switchgrass. The optimal conditions for AFEX pretreatment of switchgrass has been found to be a mass ratio of ammonia to biomass of 1:1, biomass moisture content of 80% (dry mass basis), temperature of 100^{o}C and residence time of 5 minutes. Under these

conditions, the ethanol yield from the AFEX pretreated switchgrass was 0.2 g/g of dry biomass, which was a 2.5 times increase over untreated switchgrass [45].

Ammonia pretreatment is very effective to pretreat biomass. Another main advantage of the ammonia pretreatment is that it does not produce inhibitors. Therefore, the ammonia pretreated biomass does not need subsequent washing before downstream processes [45]. However, the harmful environmental effects of ammonia, high production and recovery costs make the AFEX and ARP processes still unattractive compared to dilute acid and steam explosion pretreatment methods.

2.6.7 Carbon Dioxide Explosion

Super critical carbon dioxide has been used to pretreat biomass. A supercritical fluid is a fluid that is in a gaseous form but is compressed at temperatures above its critical point to a liquid-like density [45]. Supercritical point of carbon dioxide is the pressure at 7.4 MPa and temperature at 31.1^{o}C. As CO_2 forms carbonic acid when dissolved in water, the acid increases the rate of fractionation of lignocellulosic biomass. The sizes of carbon dioxide molecules are comparable to that of ammonia and water and are therefore capable of penetrating into the small pores of biomass materials. The sudden release of the pressure of the carbon dioxide in the biomass matrix will cause the disruption of cellulosic structure and thus increases the accessible surface area of the biomass for hydrolysis.

Zheng *et al.* 1998 [52] reported on the use of other gases such as helium and nitrogen for explosion of biomass and compared the hydrolysis results to that generated by carbon dioxide. After an explosion with a 3000 psi gas at 35^{o}C and subsequent

enzyme hydrolysis for 24 h, the glucose yields were 72.6% for the carbon dioxide explosion, 65% for nitrogen explosion and 67.2% for helium explosion, compared to 58.95 for the non-pretreated sample, This result showed that other gases are capable of causing disruptions to the cellulosic anatomical structure but CO_2 has a special ability to penetrate into the crystal lattices of crystalline cellulose to cause more disruption upon explosion.

Carbon dioxide explosion pretreatment of biomass is similar to steam and ammonia explosion pretreatments. However, compared to the ammonia pretreatment, supercritical carbon dioxide pretreatment is less expensive. Unlike the steam explosion pretreatment that generates inhibitors to the downstream ethanol fermentation at a very high temperature, supercritical carbon dioxide pretreatment uses very low temperatures, which prevent the formation of inhibitors.

2.6.8 Ozone Pretreatment

Ozone pretreatment is to reduce the lignin content in lignocellulosic biomass. It is effective in pretreating diverse biomass materials such as wheat straw, bagasse, green hay, peanut, pine, cotton straw and poplar sawdust. Ozone is a very strong oxidant, soluble in water and readily available. It is very reactive towards compounds incorporating conjugated double bonds and functional groups with high electron densities. As a result of the high carbon double bond (C=C) content in lignin, it is easily oxidized in an ozonization process. Ozone attacks lignin and releases soluble compounds of small molecular weight, which are usually organic acids such as formic and acetic

acid. The pH value of the ozone solution usually decreases from neutral to 2 due to the release of organic acids [53].

Hemicellulose is slightly affected during ozonization but cellulose is not. The main factors affecting ozonolysis pretreatment are moisture content of the sample, particle size and ozone concentration. The optimum water content of biomass for ozone pretreatment was found to be 30% [46]. Oxalic and formic acids were identified as the most predominant components in the aqueous extract of poplar sawdust pretreated with ozone. Other chemicals such as glycolic, glycoxylic, succinic, glyceric, malonic, p-hydroxybenzoic, fumaric and propanoic acids were also found in the aqueous solution [45].

Unlike other chemical pretreatment methods, ozonolysis seldom produces toxic inhibitors which affect the downstream fermentation processes. Another advantage of ozone pretreatment is that the reaction occurs at an ambient temperature and pressure. As ozone is easily decomposed at elevated temperatures or by a catalytic bed, an ozonization process can minimize the environmental pollution during pretreatment. One main disadvantage to the ozone pretreatment method is that a large amount of ozone is needed.

2.6.9 Biological Pretreatment with Fungi

Biological pretreatment of biomass involves the use of microorganisms in treating lignocellulosics to enhance enzymatic hydrolysis. Fungi and bacteria have been identified to have the ability to degrade lignin and some hemicellulose off the lignocellulosic materials. These microorganisms have very little effect on cellulose since the cellulose has more resistance than the other parts of lignocelluloses to be biologically attacked.

Several fungi species (e.g. brown, white and soft rot fungi) have been used in biomass pretreatment but the white rot fungi has been found the most effective for pretreating lignocelluloses. Brown rots mainly attack cellulose whiles white and soft rot attack both cellulose and lignin [45]. Extensive study of the ligninolytic mechanism of white rot fungi shows that three kinds of extracellular phenoloxidases (i.e. lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac)) are responsible for initiating the depolymerization of lignin. The expression pattern of these enzymes depends on the organism. Some organisms secrete LiP and MnP without Lac while others secrete MnP and Lac without LiP [54].

Examples of white rot fungi include *Pleurotus ostreatus, Phanrochaete sordida, Pycnoporus cinnabarinus, Ceriporiopsis subvermispora, Cyathus stercoreus, Ceriporia lacerata, Stereum hirsutum, Polyporus brumalis* and *Sporotrichum pulverulentum*. *Pleurotus ostreatus* has been reported to convert 35% of wheat straw into reducing sugars within five weeks. *Sporotrichum pulverulentum* has been mutated into a cellulase-less fungi that degrades mainly lignin and leaves cellulose intact. Low energy requirement, no chemical requirement and mild environmental conditions are the main advantages of biological pretreatments [46].

2.7 Enzymatic Hydrolysis

Pretreatment of biomass is used to remove lignin and hydrolyze hemicelluloses in the biomass. Enzymatic hydrolysis follows the pretreatment to break down the cellulose component of the lignocellulose into reducing sugars that can be further fermented to

ethanol using a microorganism. Enzymatic hydrolysis of cellulose is conducted by a myriad of enzymes which act synergistically in a complex fashion, which is not fully understood till date, to reduce the complex crystalline cellulose into fermentable sugars. The enzyme used to breakdown cellulose is called cellulase. Cellulases are mixtures of several enzymes that act in concert to reduce cellulose to glucose for fermentation [55].

Cellulase enzymes are produced by both bacteria and fungi. Three main types of enzymes can be found in cellulases, which are endocellulase (EG, endo-1,4-D-glucanohydrolase), exoglucanase or cellobiohydrolase (CBH, 1,4-β-D-glucan cellobiohydrolase) and β-glucosidase. Cellulase generating bacteria include *Cellulomonas fimi, Thermomonospora fusca, Clostridium thermocellum* and *Bacteroides cellulosolvens.* Examples of fungi that generate cellulase are *Sclerotium rolfsii, Phanerochete chrysosporium, Trichoderma* sp., *Aspergilus* sp., *Schizophyllum* sp. and *Penicillium* sp. Of all the cellulolytic fungi, the various mutants of *Trichoderma reesei* have been most extensively studied for cellulase production [55, 56].

Generally enzymatic hydrolysis is carried out by preparing a broth of cellulase added to a slurry of water-washed pretreated cellulosic material. A small amount of β-glucosidase, which is used to hydrolyze disaccharide cellobiose by acting on β 1->4 bonds linking two glucose molecules, is added to the cellulase broth as β-glucosidase is not produced by many fungi that excrete cellulase. The optimum substrate concentration during enzymatic hydrolysis is 10% (w/v) if rheological problems are to be avoided. Depending on the type of substrate to be hydrolyzed, cellulase enzyme loading from 7 – 33 FPU/g substrate (FPU – Filter Paper Units) may be required to achieve effective

hydrolysis. Hydrolysis is usually carried out at a pH of approximately 4.8 and at a temperature of 45 – 50^{o}C. The hydrolysis slurry must be gently agitated to achieve total mixing and effective mass transfer.

In the scheme of hydrolysis, a simplistic mechanism has been proposed for the sequential conversion of cellulose into glucose. In this theory it is envisioned that endocellulase attacks and cleaves the β-1,4 linkages in the amorphous sections of cellulose. Exocellulase then cleaves cellobiose units from the non-reducing end of the cellulose chains. The exocellulase degrades cellodextrins to cellobiose which are then finally converted into glucose monomers by β-glucosidase. These enzymes are purported to act synergistically to reduce cellulose to glucose. Synergism among these enzymes is however dependent of (1) the nature of the substrate, (2) the affinity of a cellulase component for a substrate, (3) the components stereospecificity, (4) the enzyme concentration and (5) the ratio of enzyme components.

The initial rate of hydrolysis is relatively rapid but declines as the combined effect of end-product inhibition and the loss of enzyme activity become pronounced [56]. For enzymatic hydrolysis conducted in a batch reactor, it usually takes 3 – 4 days to achieve appreciable amount of glucose. Batch hydrolysis with 10% substrate concentration usually has 75% efficiency in conversion of cellulose to glucose. However, batch hydrolysis is limited to laboratory experimentation due to the severe limitation it suffers to end product inhibition. The fed-batch process with sugar removal is more suited for industry and bulk hydrolysis because large amounts of lignocellulose can be digested

using lower enzyme loadings while the generated sugars can be removed by ultrafiltration or simultaneous saccharification and fermentation.

Unlike acid hydrolysis, enzymatic hydrolysis is very specific and yields relatively pure glucose syrups without the generation of glucose degradation products [56]. It requires mild conditions, usually ambient temperature and pressure, making it very inexpensive compared to dilute acid hydrolysis. It is non-toxic and environmentally friendly.

Cellulase enzymes are, however expensive to produce and have lower activity compared to other enzyme reactions. Comparatively amylase degrades starch at a rate of 100 IU/mg while fungal cellulases exhibit specific activity of only 0.6 – 1.0 FPU/mg [57]. Saccharification by enzyme hydrolysis is also limited by the end product inhibition because the activity of the enzyme is severely affected by cellobiose and to some extent by generated glucose. In addition, it has been observed that endo- and exocellulase adsorb tightly unto cellulosic substrate and do not desorb until the substrate is degraded [56]. Large amounts of cellulase enzymes become attached to undegraded lignocellulosic residue creating a deficit of needed enzymes for hydrolyzing more susceptible substrates.

2.8 Fermentation

Fermentation is the biological process where microorganisms such as bacteria and yeast convert reducing sugars (i.e. glucose, xylose, fructose, sucrose) into ethanol and carbon dioxide while obtaining energy for growth and maintenance. Fermentation can be carried out aerobically and anaerobically. Approximately 80% of all ethanol generated in

the world is obtained by biological fermentation and 20% by conversion of petroleum based ethylene to ethanol [58]. In the conversion of lignocellulosic biomass to ethanol, the fractionation of the biomass matrix into monomeric sugars is attained either by acid hydrolysis or enzymatic hydrolysis. Pentose and hexose sugars are produced as a result of either of these hydrolysis procedures. However, many yeast and bacteria have a difficulty in converting pentose sugars into ethanol. This drawback makes the biomass to ethanol process uneconomical since xylose (a pentose), which forms over 50% of fractionated hemicellulose, cannot be converted into ethanol. Theoretically the maximum yields that can be obtained from the conversion of pentose and hexose sugars to ethanol are 0.51 kg ethanol and 0.49 kg ethanol per kg C_6 and C_5 respectively [58].
Stoichiometrically pentose and hexose fermentation can be represented thus:

Pentose fermentation $3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2$ (2.10)

Hexose fermentation $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$ (2.11)

Researches aimed at improving the yield of ethanol generated from microbial fermentation have led to the isolation of yeast species such as *Pichia stipitis, Pachysolen tannophilus* and *Candida shehatae* [56]. These microorganisms have been successfully tested to convert pure xylose solutions to ethanol. The yields of these microorganisms are average to high (0.28 – 0.48 g/g glucose) with reasonable productivities (0.02 – 0.66 g/l h) [59]. Optimal performance of these microbes requires carefully controlled oxygenation. It has been postulated that the inability of these microbes to achieve

theoretical conversion is due to the production of xylitol and the reassimilation of ethanol [59]. The microbes are, however, less effective at fermenting aqueous hemicellulose streams generated by pretreatment processes [56]. This inability may be attributed to the presence of various inhibitors such as acetic acid, furfural, HMF, uronic acids and a variety of aromatic lignin degradation products found within the pretreatment hydrolyzates.

Zymomonas mobilis (a bacterium) has the exceptional ability of fermenting primarily glucose, fructose and sucrose to ethanol with very high yields and productivities but a rather poor activity towards xylose fermentation. Genetic manipulation of *Z. mobilis* by incorporating xylose isomerase, xylulokinase, transketolase and transaldolase enzymes into its genetic structure has resulted in the ability of the organism to simultaneously ferment glucose and xylose at 95% of the theoretical yield [56, 59].

Hexose fermentation has been widely achieved by the use of *Saccharomyces cerevisiae* for thousands of years [58]. This yeast has the advantage of being safe for human consumption and has been employed extensively in breweries in the production of alcoholic beverages. It is also used in bakeries as a bread riser during the production of bread. It has high glucose to ethanol conversion yield and productivity with a remarkable ethanol tolerance. It is reported to be able to generate ethanol at concentrations of as high as 18% of the fermentation broth [58]. *S. cerevisiae* is, however, incapable of fermenting xylose sugars but is favorable towards xylulose (an isomer of xylose). Genetic engineering of *S. cerevisiae* have resulted in strains that are capable of fermenting xylose

to a degree but as yet only low yields have been demonstrated. The limited success has been attributed to limitations in existing pathways and redox imbalances. Some progress has been made however via recombinant strains like *Schizosaccharomyces pombe* which has yielded 0.42 g ethanol/g glucose with productivity of 0.19 g/l h [58]. This recombinant strain is, however, dependent of constant supplementation of nutrients such as malt extract, yeast extract and peptone. Without these nutrients only a meager yield of 0.15 g ethanol/g glucose is attainable [59].

The configurations of bioreactors for fermentation are dictated by the kinetic properties of the fermenting microorganism as well as the process economics. Fermentation can be done in batch, fed-batch, continuous stirred tank or plug flow reactors. Cell productivity during fermentation can be enhanced by restricting the mobility of the cells within the fermenter and also by recycling [59]. Higher cell productivity means smaller fermentation tanks and lower capital cost. In batch fermentation, microorganisms endure a high initial substrate concentration and then a high product concentration at the final process stages. Productivity is low in batch fermentation due to the labor intensive nature of the reactor configuration upon the microorganism. Continuous fermentation configurations are easier to control and less labor intensive but are prone to contaminations as the process has to be stopped, all the equipment cleaned and restarted again with the growth of new inoculum. In the continuous stirred tank reactor (CSTR) microorganisms work at a low substrate concentration and high ethanol concentration all the time. In fed-batch fermentation, the

microorganism works at a low substrate concentration with increasing ethanol concentration during the fermentation process [59].

Two major strategies have been developed for the enzymatic and microbial conversion of polysaccharides to ethanol. Fermentation is accomplished by the conversion of sugars generated from cellulosic hydrolysis into ethanol. The process economics and optimization determines the best strategy for optimum yield of end products. The two common fermentation approaches are separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF).

Under SHF, the lignocellulosic material is hydrolyzed to reducing sugars by cellulase (i.e. endoglucanase, exoglucanase and β-glucosidase) and hemicellulase enzymes operating at their optimum conditions (i.e. 45 – 50^{o}C and pH 5.0) [60]. The sugars in the hydrolyzates after enzymatic hydrolysis are then subjected to fermentation by yeast or other fermenting bacteria in another chamber under different operating conditions. Olsson *et al.*, 2006 [60] reports a deactivation of enzyme activity by boiling the hydrolyzates for 10 min before starting fermentation. Most researchers however carry out fermentation after the activity of the hydrolyzing enzyme is fairly depleted. Fermentation after hydrolysis is carried out at the optimum operating condition of the microorganism to generate ethanol. *Saccharomyces cerevisiae* (baker's yeast), the most popular fermenting yeast, operates optimally between 28 – 35^{o}C.

Separate hydrolysis and fermentation is, however, beset with product inhibition during the hydrolysis stage. The activities of the enzymes are lessened due to the presence of hydrolysis products such as cellobiose and glucose. As a result of the severe

product inhibition of enzyme activity, a batch hydrolysis process of 10% substrate requires an enzyme loading approximately 33 FPU g^{-1} substrate if a 73 – 75% glucose yield is to be attained [56]. However, a fivefold reduction in enzyme loading (i.e. 7 FPU g^{-1} substrate) can be achieved if the product sugars generated by hydrolysis are gradually removed from the hydrolysis reactor [56]. Product removals by ultrafiltration or simultaneous fermentation of produced sugars are viable remedies to alleviate the high enzyme loading and eliminate product inhibition.

Simultaneous saccharification and fermentation (SSF) is a single stage process in which both enzymatic hydrolysis and alcoholic fermentation are carried out within the same vessel. The optimum temperature for SSF, which is a compromise between that of hydrolysis (45 – 50^{o}C) and fermentation (20 – 40^{o}C depending on the microbe), is usually between 35 – 37^{o}C. As a result of the low temperature range under which SSF is conducted, hydrolytic enzymes operate below their optimum and require a longer period of time to fully convert cellulose to glucose for fermentation. Depending on the type and concentration of the substrate used, SSF reactions can be run from 3 to 7 days. The reactants for SSF are pretreated lignocellulose, crude cellulase and ethanologenic microorganisms such as yeast or bacteria.

During the SSF process, the fraction of hydrolyzed sugars that is fermentable by the ethanologenic microorganisms are quickly taken up upon their release and converted to ethanol. Since ethanol is a less potent inhibitor of cellulase compared to cellobiose and glucose, the kinetics of the lignocellulose to ethanol process is greatly improved because the inhibitory compounds of cellulase are readily removed by sugar fermenting microbes

present within the broth. The addition of β-glucosidase (the enzyme which cleaves cellobiose units into monomeric glucose) within the fermenting broth is very important if the accumulation of cellobiose (the most potent inhibitor to cellulase enzyme) is to be prevented. Since most fungal cellulases are deficient of β-glucosidase, it has to be supplemented into the fermenting broth.

Reports by Sheldon *et al.* 1995 [56] indicate that *Brettanomyces clausenii*, a yeast, is capable of fermenting cellobiose directly to ethanol. A co-culture of *S. cerevisiae* and *B. clausenii* resulted in an 88% sugar yield on 10% cellulose solution which was a little higher than that attained by the monoculture of *S. cerevisiae*. This innovation eliminates the need for β-glucosidase during SSF. As a result of these innovations, lesser enzyme loadings can be used to achieve high sugar yields since the activity of the enzyme will no longer be affected by the inhibitor of cellubiose [56].

Although promising, simultaneous saccharification and fermentation have major disadvantages that need to be addressed before it can be applied industrially. One major disadvantage of the SSF process is the compromised temperature at which the yeast and enzymes operate. This snag causes neither the fermentation nor hydrolysis to occur at their most favorable rates, requiring incubation periods of up to 7 days and concomitantly large reaction vessels [56]. The development of thermotolerant bacteria that are capable of fermentation may eliminate the need to operate the SSF process at the compromised temperatures. *Z. mobilis*, a fermenting bacterium, operates optimally at 30^{o}C, however, recombinant strains have been developed that are capable of fermentation at temperatures

up to 45^{o}C [56]. Another disadvantage with the SSF process is the mild denaturing effect that ethanol has on cellulase.

In as much as the inhibitory effect of ethanol is far lesser than that of cellobiose and glucose, the rate of cellulose hydrolysis slows down with the gradual accumulation of ethanol. Microbial contamination is another drawback to the SSF process. In the addition of crude cellulase to the hydrolysis mixture, nutrients and metabolites from the fungal growth medium along with spores and pieces of mycelium are deposited into the SSF mixture. A conducive atmosphere for competitive microbial growth of contaminating microbes is created as the broth is laid to incubate for up to 7 days at 35 – 37^{o}C. Microbial contamination can be alleviated by adding selective inhibitors to the fermentation broth, but this will further increase the cost of the process. Alternatively acid tolerant thermophilic microbes could be used in the fermentation process at conditions (50^{o}C and pH 4.8) where the proliferation of other microbes will be halted [56].

2.9 Ethanol Recovery

Hitherto, proposed technologies for the production of ethanol from lignocellulosic materials have resulted in moderate to low ethanol concentrations within the fermentation broth. There exist several technologies to effectively separate the desired end product (ethanol) from the fermented beer. Traditionally, distillation is able to concentrate ethanol up to 95% after which it is subjected to azeotropic distillation to further dehydrate concentrated ethanol from 95 – 99.9%. Distillation, however, is energy intensive

requiring approximately 7.63 MJ/l of fuel grade ethanol to accomplish both azeotropic and regular distillation. For a fermentation broth containing 9% ethanol, the distillation cost can represent 40 – 60% of the total lignocellulose to ethanol process which makes it very uneconomical [56].

Other technologies that have been employed to recover ethanol from fermentation broths at low ethanol concentrations include vacuum fermentation, a variety of membrane technologies (e.g. pervaporation, perstraction and membrane distillation), extraction with organic solvents, and supercritical CO_2 both *in situ* and in external contactors and bioconversion to a more volatile product [56]. The general goal of these technologies is to preserve the ethanol concentration in the broth at low, non-inhibitory levels, thereby maintaining high glucose-to-ethanol conversion efficiencies in continuous culture.

Reports by Cysewski *et al.* 1977 [61] show that ethanol fermentation could be increased twelve times more than the regular if it was done under vacuum (50 mm Hg). The vacuum configured fermenter boiled away the ethanol as it was formed at the very low temperature of 35^{o}C. This process helped reduce the accumulation of ethanol within the broth and eliminated ethanol inhibition. Vacuum fermentation, however, has the disadvantage of concentrating non-volatile elements within the fermentation broth which could foment new inhibitors.

Membrane separation technologies have far advanced methods that selectively remove ethanol from the fermentation broth. Pervaporation, for instance, is used for separating fluid mixtures that have different diffusivities in a membrane. In an ethanol-water binary system, ethanol selectively diffuses through the membrane and is carried by

a gas stream or by a vacuum created on the other side of the membrane. The vaporized ethanol is then condensed and collected. Perstraction, on another hand, though similar to pervaporation, takes place within an organic solvent where ethanol is partially soluble. The choice of organic solvent is less dependent of the extent of its inhibition to enzymes or its toxicity to yeast but rather on its ethanol extraction coefficient. This is because the membrane barrier is set between the fermentation broth and the organic solvent and this ensures the separation of the aqueous and organic solvent phases without necessarily contacting the two phases. The ethanol crosses the membrane and is dissolved into the organic solvent on the other side. It is later recovered from the organic solvent by flash vaporization or by passing the solvent through a selective packed-bed column for ethanol adsorption [62].

Membrane distillation employs a hydrophobic porous membrane which is placed between two aqueous solutions (i.e. the fermentation broth and water). The broth is kept at a higher temperature than the extraction water on the other side of the membrane. This creates a vapor pressure gradient which encourages the selective exodus of ethanol molecules across the membrane into the cooler water on the other side of the membrane. As a result of the lower temperature of the water, ethanol accumulates to a higher concentration than it is within fermentation broth [53].

One of the oldest and most researched ethanol recovery methods is the extractive fermentation process. In this module, the fermentation is conducted in a two phase system which consists of an aqueous fermentation broth and an immiscible organic solvent. Contacting of the solvent with the dilute ethanol can be done either within the

fermentation vessel (*in situ*) or in an external liquid-liquid contacting device [56]. In the selection of an appropriate solvent a number of factors must be carefully considered: (1) the extractive solvent must be inexpensive, non-volatile and must have a higher boiling point than ethanol, (2) the solvent must be insoluble in water and have high affinity for ethanol solvation so ethanol can be selectively recovered, (3) the solvent must not be toxic to fermenting yeast and must not be inhibitory to the hydrolyzing enzyme if (*in situ)* extraction is being conducted. Recovery of the ethanol is done by running the collected solvent off of the fermentation broth and passing it through a flash vaporization unit.

Ethanol recovery via super critical CO_2 extraction has also been achieved. In this process the fermentation broth is pressurized and run counter-current through a supercritical extraction column and then recycled to the fermenter. The ethanol is then recovered from the supercritical fluid by passing it through an activated carbon bed and recovered with a second CO_2 stream. In supercritical CO_2 extraction, loss of solvent is not a critical concern since CO_2 is a byproduct of fermentation. It is 50% less energy intensive compared to regular distillation.

Ethanol generated within the fermentation broth can be further converted to acetaldehyde by the methylotrophic yeast *Pichia pastoris* in a biological oxidation reaction. Acetaldehyde has a lower boiling point (20.8^{o}C) than ethanol (78.5^{o}C) and does not form azeotropes with water. Therefore acetaldehyde will voluntarily evaporate from mesophilic fermentation broths without any need for temperature rise. Additionally, acetaldehyde has a good market value and can be sold in place of ethanol as an alternative value added chemical. However, when ethanol is the desired end-product, then

acetaldehyde can be readily reduced to ethanol by running it over nickel or copper oxide catalysts [56].

CHAPTER 3

Materials and Methods

3.1 Raw Materials

Biomass materials including corn stover (CS), wheat straw (WS), switch grass (SG), sweet sorghum bagasse (SSB) and woody biomass (WC) were obtained from the farms of North Carolina Agriculture and Technical State University and the Agronomy farms of Purdue University. The collected biomass was air dried to reduce the moisture content to approximately 10%. The dried biomass was then ground to 1 mm mesh size using a Wiley mill.

3.2 Pretreatment by Different Methods

The main objectives of this research were to identify a pretreatment method that was unique and cost effective and optimize pretreatment conditions for the enhancement of enzymatic hydrolysis of cellulose to glucose. Through literature search in biomass pretreatment, four pretreatment methods were investigated for comparative analysis in this research. These methods are (1) pretreatment of biomass with high-pressure and high temperature solvents in an Accelerated Solvent Extractor (ASE 350, Dionex Corporation) (2) reactive screw extrusion pretreatment of lignocellulosic materials in a twin-screw extruder (Twin screw mixer, C.W. Brabender Instruments, Inc.) (3) biomass pretreatment in a high-pressure batch continuous stirred tank reactor (Parr 4570 reactor, Parr

Instrument Company) and (4) pretreatment of biomass at an ambient condition for seven days.

These pretreatment methods were conducted using different abrasive chemicals including ammonium hydroxide (NH_4OH), calcium hydroxide ($Ca(OH)_2$), acetic acid (CH_3COOH), ethanol (CH_3CH_2OH) and water (H_2O). These chemicals were carefully chosen because they are inexpensive, readily available, environmentally friendly and less deleterious to the construction materials of reactor vessels. Water, as part of the named chemicals, was essentially used in control experiments to compare the effect of the least expensive pretreatment procedure. All the biomass samples were subjected to the same pretreatment conditions as per pretreatment method.

3.2.1 Pretreatment of Biomass by Accelerated Solvent Extractor (ASE)

Ten grams of dry biomass at 1 mm particle size was filled into a 66 ml ASE 350 zirconium or stainless steel cell in an accelerated solvent extractor as shown in Figure 3.1. Depending on the resistivity of the cell to wear or corrosion by the chemicals used, an appropriate choice of cell material (zirconium or stainless steel) was made per chemical for the pretreatment. Aqueous solutions of 10% acetic acid, 10% ammonium hydroxide, 30% ethanol and deionized water were used to fractionate the biomass materials during pretreatment. The addition of aqueous solutions to the biomass in the cell was done by pumping the chemicals through tiny tubes laid out within the ASE 350. Calcium hydroxide (or lime) was not used in this pretreatment method because of its low solubility in water (e.g., 0.189 g/100 ml at 0^oC and 0.173 g/100 ml at 20^oC). As a result

of the low solubility of calcium hydroxide in water, undissolved calcium hydroxide will clog the tubing of the extractor.

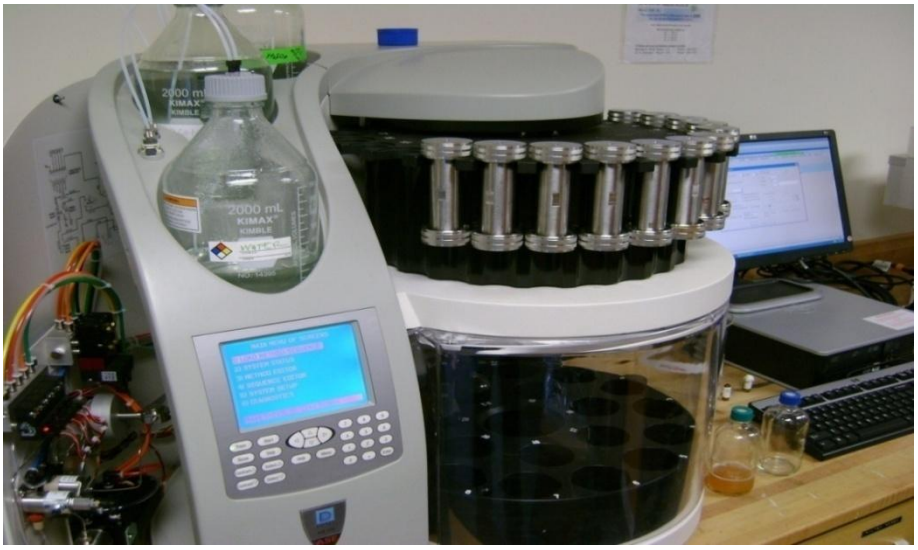
Pretreatment proceeded statically at a temperature of either 90^{o}C or 180^{o}C and a pressure of 10.3 MPa for 10 min after 55 ml of aqueous solution was added to the biomass. About 33 ml of aqueous solution was then used to rinse the biomass sample for all pretreatments. Therefore, the total volume of the aqueous solution used during each pretreatment was between 90-100 ml. The extracts released from the biomass were collected into 250 ml collection bottles.

The moisture content and the mass of the solid residues after pretreatment were measured. The moisture content analysis was conducted on the solid streams according to the National Renewable Energy Laboratory (NREL) Laboratory Analytical Procedure (LAP). Chemical composition of the dried solid stream was analyzed to determine the glucan and xylan contents using the NREL LAP. High Pressure Liquid Chromatography (HPLC) was used to determine the sugar components in the liquid extract stream from pretreatment. The weight and volume of the extracts were measured. The fractions of the biomass in the solid and liquid streams were then calculated.

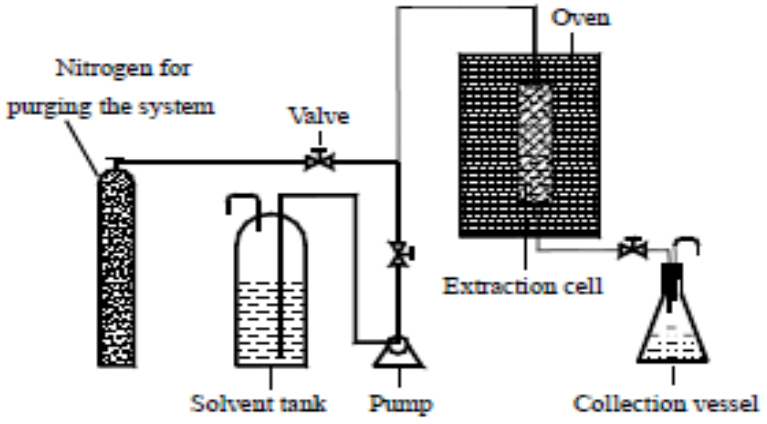
3.2.2 Reactive Screw Extrusion (RSE) Pretreatment of Lignocellulosic Materials

A slurry of 40% biomass and 60% aqueous solution was prepared by stirring 200 g of dry biomass at 1 mm particle size into 300 ml aqueous solutions of 10% acetic acid, 10% calcium hydroxide and 10% ammonium hydroxide. Biomass slurry prepared with distilled water was used as a control. The slurry mixtures were capped and left at room temperature for 24 h. The mixtures were then extruded through a twin screw extruder

(Twin screw mixer, C.W. Brabender Instruments, Inc) as shown in Figure 3.2 at a temperature of 180 ^{o}C, screw speed of 100 rpm, and a feed rate of 10 g/min.



(a)



(b)

Figure 3.1 (a) Accelerated Solvent Extractor (ASE 350) (b) Schematic diagram of an accelerated solvent extraction system (Adapted from Wang et al. 2006)

Figure 3.2 Twin Screw Mixer, (C.W. Brabender Instruments, Inc)

The extruded samples were collected, weighed, washed and centrifuged at 3400 RCF for 15 minutes. The supernatant was decanted and the sugar yields in the liquid and solid fractions were determined using the analytical procedure defined by NREL.

3.2.3 Biomass Pretreatment in a Continuous Stirred Tank Reactor (CSTR)

A slurry of 10% biomass and 90% of aqueous solution was prepared by stirring 75 g of biomass into solutions of 10% calcium hydroxide, 10% ammonium hydroxide and 10% acetic acid. A slurry with 10% biomass and 90% distilled water was prepared to

be as a control. The prepared slurries were pretreated in a high-pressure continuous stirred tank reactor (Model 4570, Parr instrument company, Moline, IL) as shown in Figure 3.3. The batch reactor is rated up to a working pressure of 5,000 psi and working temperature of 500°C. A heavy-duty magnetic drive stirrer associated with the reactor was used for mixing. A type-J thermocouple was inserted into the reactor for the measurements of the temperature of a reaction media. A standard pressure gauge was installed on the reactor head. A PID controller was used to control and indicate the temperature of the reactor. The cylindrical reactor was placed in a tubular electric heater. Nitrogen gas was used to purge the residual air in the reactor three times at the beginning of the experiment. The biomass slurry in the reactor was heated up to the final temperature of 180^{o}C for about half an hour and held at the final temperature for 1 h while the biomass slurry was agitated. The reactor was then cooled down rapidly to a room temperature by using a recycle ice-water cooling coil within half an hour. The gas was then released from the reactor by reducing the pressure in the reactor to the atmospheric pressure.

Sample aliquots from the pretreated slurry were collected for the sugar analysis within the liquid stream. The pretreated biomass samples were then washed twice with distilled water at three times of the volume of the pretreated slurry to remove residual solvents that may be inhibitory to downstream processes. Washed samples were centrifuged at 3400 RCF for 15 min and the supernatants were decanted. The glucan, xylan and moisture content of the solid stream were then determined using the NREL procedures.

(a)

(b)

Figure 3.3 High-pressure Continuous Stirred Tank Reactor (a) closed section (b) opened section (Model 4570, Parr Instrument Company, Moline, IL)

3.2.4 Ambient Storage Tank (AST) Pretreatment of Biomass

Fifty grams of 1 mm dried biomass was stirred into 75 ml of 10% acetic acid, 10% ammonium hydroxide and 10% calcium hydroxide. Distilled water pretreatment was used in a control experiment. The prepared slurries were tightly capped and stored at a room temperature (i.e., 25^{o}C) for seven days. After seven days, the mixture was washed thoroughly to remove all chemicals and centrifuged at 3400 RCF for 15 min. The supernatant was decanted. The glucan, xylan and moisture content analysis were conducted on collected samples using the NREL procedures.

3.3 Simultaneous Saccharification and Fermentation (SSF) of Pretreated Biomass

For the SSF process, 10% pretreated biomass slurry with a pH value adjusted to 4.8 using 1 M citric acid buffer and 1 N sodium hydroxide solution was prepared in a 125 ml capped Wheaton septum glass bottle. Enzymatic hydrolysis was achieved by the addition of cellulase, β-glucosidase and hemicellulase enzymes obtained from Novozyme. Cellulase was added according to the calculated glucan content of the pretreatment hydrolyzate. Essentially Novozyme NS50013 (cellulase complex) with activity of 70 FPU/g and Novozyme NS50010 (β-glucosidase) with activity of 250 CFU/g were added at loadings commensurate with the glucan content of the pretreated hydrolyzate. Hemicellulase enzyme, Novozyme NS22002 (hemicellulase) with activity of 45 FBG/g, was added on to the hydrolysis broth at a rate of 2.5 FGB/g of dry mass of biomass for all hydrolyzates regardless of glucan content. The prepared hydrolysis broth

was then autoclaved at 121^{o}C for 1 h. It was then allowed to cool to a room temperature for yeast inoculation.

Saccharomyces cerevisiae (ATCC 24858) was the yeast used to ferment the enzymatically generated sugars. For the SSF process 50 ml of seed culture was used to inoculate 650 ml yeast moth (YM) medium (Difco 271120) in a 1 liter Erlenmeyer flask. The cultures were incubated in a shaker at 30^{o}C and 150 rpm and grown aerobically overnight. The suspended yeast cultures was transferred into 50 ml capped centrifugation tubes and were harvested by centrifugation at 2600 RCF for 15 min at a room temperature. The supernatant was discarded and the cells were transferred into the 125 ml Wheaton septum glass bottles containing 50 ml of pretreatment hydrolyzate. The bottles were then tightly capped to allow fermentation to occur largely under anaerobic conditions. The cultures were placed in a shaker and incubated at 37^{o}C and 150 rpm. Aliquots of the fermentation broth were collected at designated times of 4, 12, 24, 48, 72, 96, 120, 144 and 168 h. The aliquot samples were analyzed for glucose and ethanol concentrations via a HPLC. Figure 3.4 shows incubated samples under SSF.

3.4 Analyses of Biomass Chemical Compositions

Compositional analysis of biomass was carried out using the Laboratory Analytical procedures (LAPs) developed by the National Renewable Energy Laboratory. The moisture content was determined by LAP #001. Under this procedure a sample of the pretreated biomass was weighed in an aluminum pan and then dried in an air oven at 103-

105^{o}C for 24 h. The dried sample was weighed again and the weight loss accounted for as the percentage of moisture within the pretreated biomass.

Figure 3.4 Environmental Incubator Shaker (New Brunswick Scientific I 26)

The compositions of the treated and untreated biomass were determined by the NREL two-step acid hydrolysis method LAP #002. Under this procedure the first hydrolysis step was done on 0.3 g of dried biomass in a 10 ml test tube with 3.0 ml of 72% H_2SO_4 at 30^{o}C. The hydrolyzing sample was agitated every 15 min using a glass rod. After 120 min hydrolysis, the hydrolyzed biomass was diluted with deionized water to make up the volume of the test tube and then transferred into a 125 ml serum bottle.

The volume of the hydrolyzate in the serum bottle was then increased by making up the volume to 87.0 ml with a commensurate weight of 89.22 g.

The prepared solutions of biomass were then stoppered and crimp sealed using aluminum seals. The biomass solutions were then autoclaved at 121^{o}C for 1 h. After autoclaving, the samples are allowed to cool to room temperature. The hydrolyzates were then filtered by vacuum filtration. Aliquots of the filtrate were then neutralized using calcium carbonate powder to a pH between 5 and 6. The neutralized hydrolyzates were then filtered through 0.2 μm filters into auto-sampler vials and stored in a refrigerator for HPLC analysis. The solid residues after vacuum filtration were dried in an air oven for 24 h. After drying, the weight of the residues was recorded. The content of the dried biomass residues was gravimetrically measured by ashing in a muffle furnace at 575^{o}C. The ash weight was determined as a difference in the weight of the crucible and crucible with ash. Lignin content is however the difference in the weight of the dry biomass residue with crucible and crucible with ash.

3.5 Analyses of sugars and ethanol using HPLC

The amounts of sugar monomers (glucose, arabinose, xylose, galactose and mannose) in all the liquid fractions and the ethanol concentrations were determined by HPLC (Waters, Milford, MA) with a KC-811 ion-exclusion column and a Waters 410 refractive index detector (RID). The mobile phase was a 0.1% H_3PO_4 solution at a flow rate of 1 ml/min. The temperatures of the detector and column were maintained at 35 and

50^{o}C, respectively. Figure 3.5 is the HPLC used for the all compositional analysis in this research.

Figure 3.5 High Pressure Liquid Chromatography HPLC (Waters, Milford, MA)

3.6 Extraction of Hemicellulose Fractions from the Liquid Stream of ASE Pretreated Biomass with Different Chemicals

Four milliliter aliquots of ASE pretreatment biomass extracts were filled into 50 ml centrifuge vials and four volumes of 95% ethanol were added in a 1:4 biomass extract to ethanol ratio as was done by Buranov *et al.* [40]. The solution was agitated for uniform

mixing and allowed to stand at room temperature for 24 h. The resulting colloid was filtered through a 12.5 cm Fisherbrand filter paper 09-790-14D (Fisher Scientific, E.U). The filter paper with the hemicellulose residues were dried at 60^{o}C in an air oven drier for 24 h. The fractionated hemicellulose weight was determined from the difference in the weights of the filter paper with dry hemicellulose and the original filter paper.

CHAPTER 4

Results and Discussion

4.1 Compositions of Raw Biomass

The objective of this thesis was to investigate the pretreatment methods that lead to the fractionation of lignocellulosic biomass into monomeric sugars for ethanol production and hemicellulose extractives. Four different pretreatment methods were investigated to ascertain which gave the best enzymatic digestibility and ethanol yield. The methods tested include (1) pretreatment of biomass with high pressure and high temperature solvents in an Accelerated Solvent Extractor (ASE 350, Dionex Corporation), (2) reactive-screw extrusion pretreatment of lignocellulosic materials in a twin screw extruder (C.W. Brabender Instruments, Inc.), (3) biomass pretreatment in a high-pressure batch continuous stirred tank reactor (Parr 4570 reactor, Parr Instrument Company) and (4) pretreatment of biomass at ambient conditions for seven days. Preliminary compositional analyses were conducted on the biomass raw materials without any pretreatment and the results are shown in Table 4.1.

Table 4.1 Biomass compositions before pretreatment

Raw biomass	Glucan(% by mass)	Xylan(% by mass)	Lignin(% by mass)	Ash(% by mass)	Moisture content (%)
WC	45.3	16.8	31.8	0.1	9.3
SG	34.0	30.9	24.4	0.6	6.4
CS	33.2	24.2	18.6	1.2	9.0
WS	35.1	30.0	26.6	2.5	7.1
SSB	35.8	29.3	24.6	2.4	3.8

4.2 Pretreatment of Biomass with High Pressure and High Temperature Solvents in an Accelerated Solvent Extractor

Table 4.2 shows the results for the compositional analysis of biomass pretreated with different chemicals including deionized water, 10% acetic acid, 10% ammonium hydroxide and 30% ethanol solution at temperatures 90 and 180^{o}C in the Accelerated solvent extractor. Digestibility and fermentation results for the water pretreated biomass are shown in Figure. 4.1. Water at high temperatures has acidic effects on biomass and can dissolve most of hemicellulose sugars within the biomass into the liquid stream [45]. Pretreatment with water at a temperature of 90^{o}C or 180^{o}C and a pressure of 10.3 MPa (the operating pressure of ASE 350) resulted in increased glucan content for the pretreated biomass over the untreated. Pretreatment at 180^{o}C achieved the highest glucan content leading to glucan increments of 74% (woody biomass), 50% (corn stover) and 35% (switchgrass) over the untreated biomass.

Yields of ethanol for all biomass samples pretreated deionized water at 180^{o}C were higher than those of the biomass samples pretreated at 90^{o}C. The corn stover pretreated at 180^{o}C achieved the highest ethanol yield, which was 0.673 g/100 ml of ethanol after 120 h SSF of pretreated corn stover at an initial solid concentration of 10%. Therefore, a higher pretreatment temperature can achieve better disruption of the hemicellulose, lignin and cellulose bonds that make up lignocellulosic materials. Fermentation of woody biomass, however, yielded the least ethanol concentration, which was only 0.340 g/100 ml even after 168 h of SSF of the pretreated woody biomass at an initial solid concentration of 10%.

Table 4.2 Biomass compositions after ASE pretreatment with different chemicals for 10 min static time and single cycle extraction

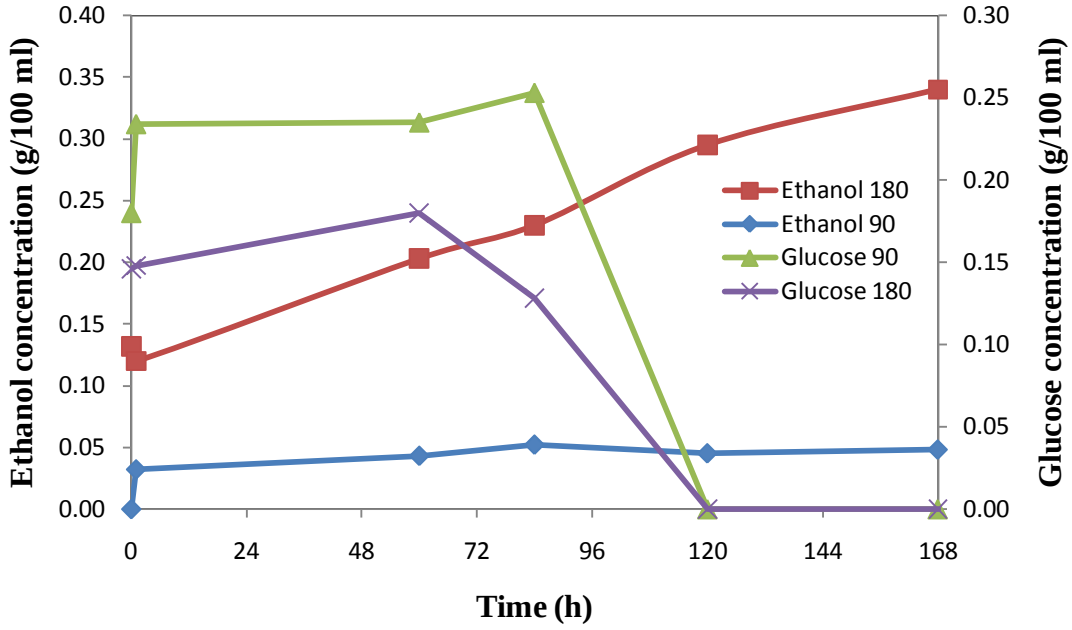
Raw biomass		Glucan(% by mass)	Xylan(% by mass)	Lignin(% by mass)	Ash(% by mass)	Moisture content (%)
WC		45.3	16.8	31.8	0.1	9.3
SG		34.0	30.9	24.4	0.6	6.4
CS		33.2	24.2	18.6	1.2	9.0
WS		35.1	30.0	26.6	2.5	7.1
SSB		35.8	29.3	24.6	2.4	3.8
ASE deionized water pretreatment						
Treated samples	**Temp/^{o}C**	**Glucan(% by mass)**	**Xylan(% by mass)**	**Lignin(% by mass)**	**Ash(% by mass)**	**Solid stream recovery (%)**
WC	90	42.4	19.1	28.2	0.8	~100
WC	180	49.8	17.0	28.6	0.4	86.6
SG	90	36.3	18.5	28.6	2.5	94.0
SG	180	38.3	20.3	29.0	2.5	80.2
CS	90	36.3	20.4	23.2	2.1	91.2
CS	180	41.4	20.0	23.7	1.8	79.1
WS	90	37.0	17.8	29.7	2.3	88.7
WS	180	40.1	17.1	31.8	2.2	72.5
SSB	90	28.6	11.2	29.9	2.9	72.4
SSB	180	43.2	13.6	29.8	3.2	53.4
ASE 10% acetic acid pretreatment						
Treated samples	**Temp/ ^{o}C**	**Glucan(% by mass)**	**Xylan(% by mass)**	**Lignin(% by mass)**	**Ash(% by mass)**	**Solid stream recovery (%)**
WC	90	41.3	17.4	30.5	0.2	~100
WC	180	67.8	0.0	23.4	0.1	67.9
SG	90	36.7	18.3	38.1	2.1	90.1
SG	180	59.0	0.0	26.8	3.5	53.7
CS	90	34.7	19.2	27.9	1.4	93.9
CS	180	68.0	0.0	21.2	2.6	54.1
WS	90	35.8	16.5	34.4	1.7	84.8
WS	180	62.0	0.0	25.5	3.1	51.4
SSB	90	48.2	17.7	21.1	2.4	72.5
SSB	180	59.9	0.0	29.6	4.1	45.9

Note: The biomass compositions do not add up to 100% due to the presence of volatile components such as waxes and proteins which are lost with increasing temperatures

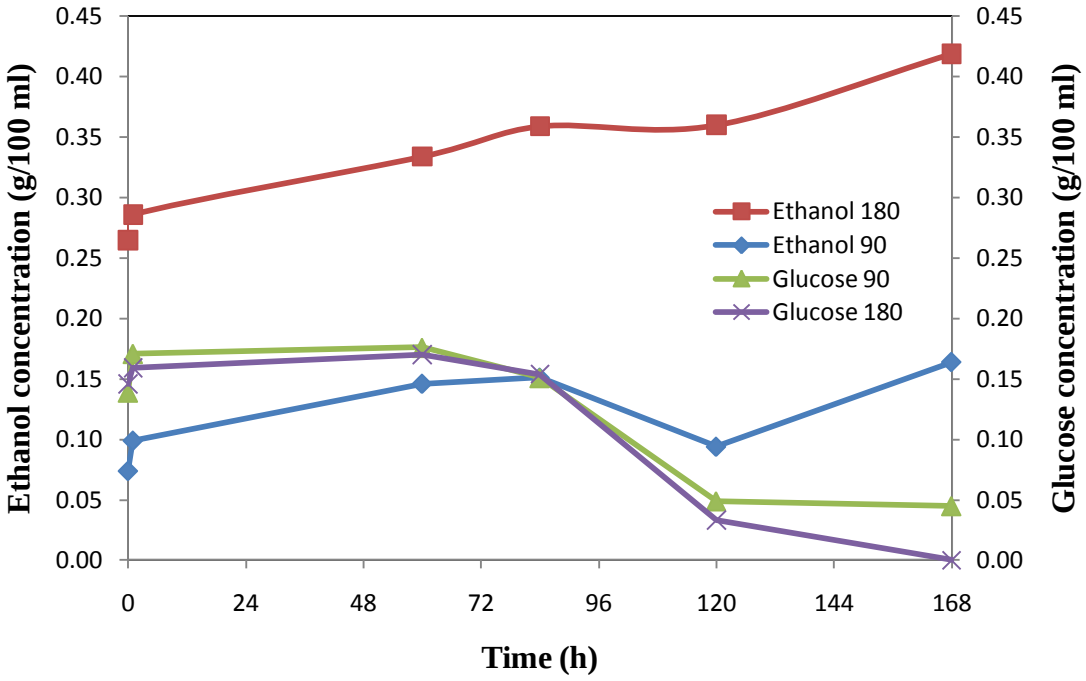
Table 4.2 Biomass compositions after ASE pretreatment with different chemicals for 10 min static time and single cycle extraction (cont.)

ASE 10% NH_4OH pretreatment						
Treated samples	**Temp/ ^oC**	**Glucan(% by mass)**	**Xylan(% by mass)**	**Lignin (% by mass)**	**Ash(% by mass)**	**Solid stream recovery (%)**
WC	90	43.4	14.8	30.2	0.2	97.7
WC	180	47.8	13.5	28.0	0.1	79.4
SG	90	39.6	19.0	27.7	1.1	87.2
SG	180	51.7	15.2	16.7	0.8	58.8
CS	90	40.1	20.6	19.8	0.7	85.1
CS	180	51.5	17.8	14.3	0.9	59.9
WS	90	39.5	19.1	12.4	1.0	82.7
WS	180	51.0	16.7	21.7	1.7	58.0
SSB	90	41.6	17.1	29.1	1.5	67.1
SSB	180	51.5	14.4	23.0	2.5	47.4
ASE 30% ethanol pretreatment						
Treated samples	**Temp/ ^oC**	**Glucan(% by mass)**	**Xylan(% by mass)**	**Lignin(% by mass)**	**Ash(% by mass)**	**Solid stream recovery (%)**
WC	90	42.2	16.7	29.6	0.4	~100
WC	180	45.1	14.6	28.8	0.3	94.3
SG	90	35.9	17.7	27.5	2.4	94.6
SG	180	34.2	17.5	27.0	2.5	81.0
CS	90	36.3	19.1	24.4	2.2	92.3
CS	180	37.6	19.0	21.2	1.9	80.7
WS	90	36.4	17.1	27.9	2.4	92.3
WS	180	38.0	17.9	28.0	2.7	77.0
SSB	90	37.7	15.2	30.0	3.6	75.2
SSB	180	38.4	13.4	29.9	3.4	59.2

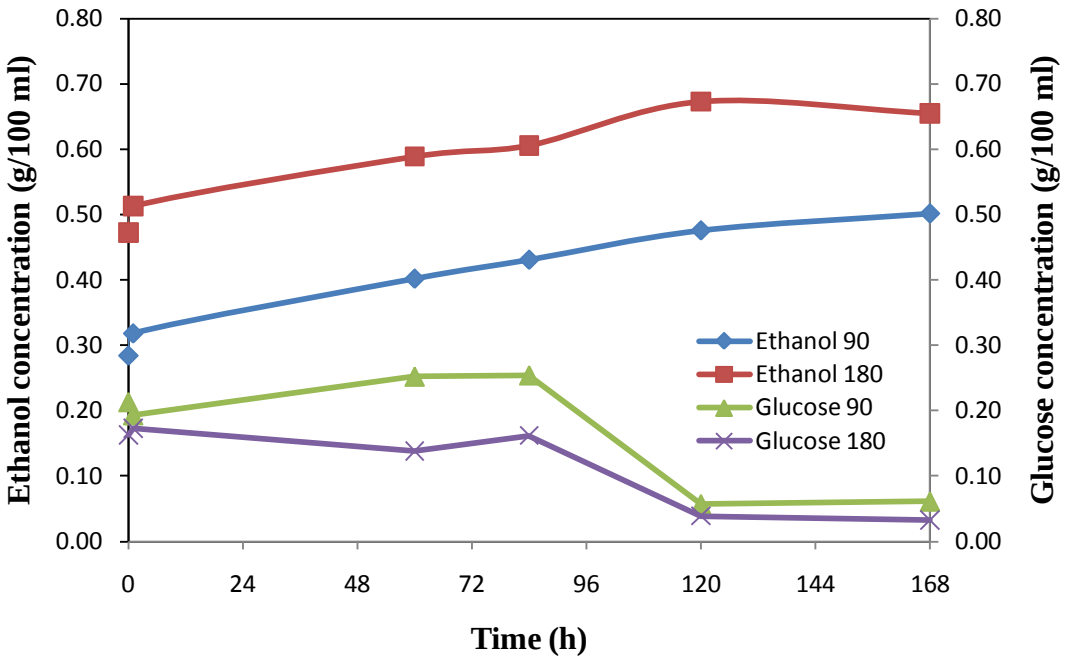
The yields of ethanol after 168 h SSF for other biomass samples pretreated at 180^{o}C were 0.419 g/100 ml for switch grass, 0.387 g/100 ml for wheat straw and 0.497 g/100 ml for sweet sorghum bagasse. Glucose concentrations during SSF generally reduced for all pretreated samples as it was simultaneously converted to ethanol by yeast cells within the broth.



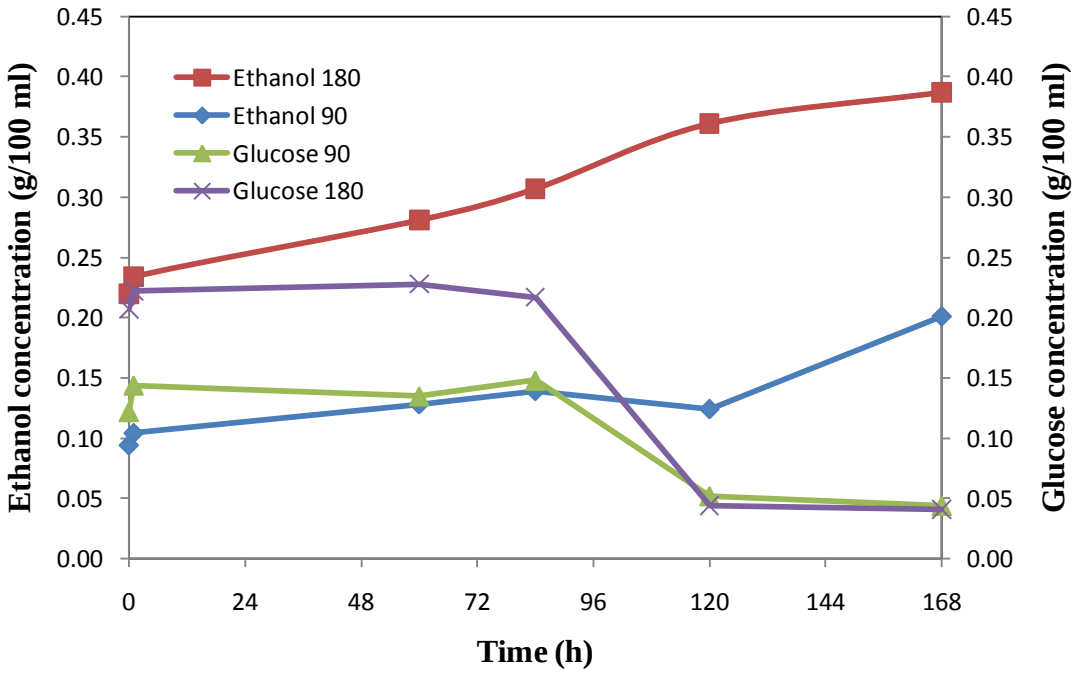
(a) *Woody biomass pretreated by ASE with deionized water*



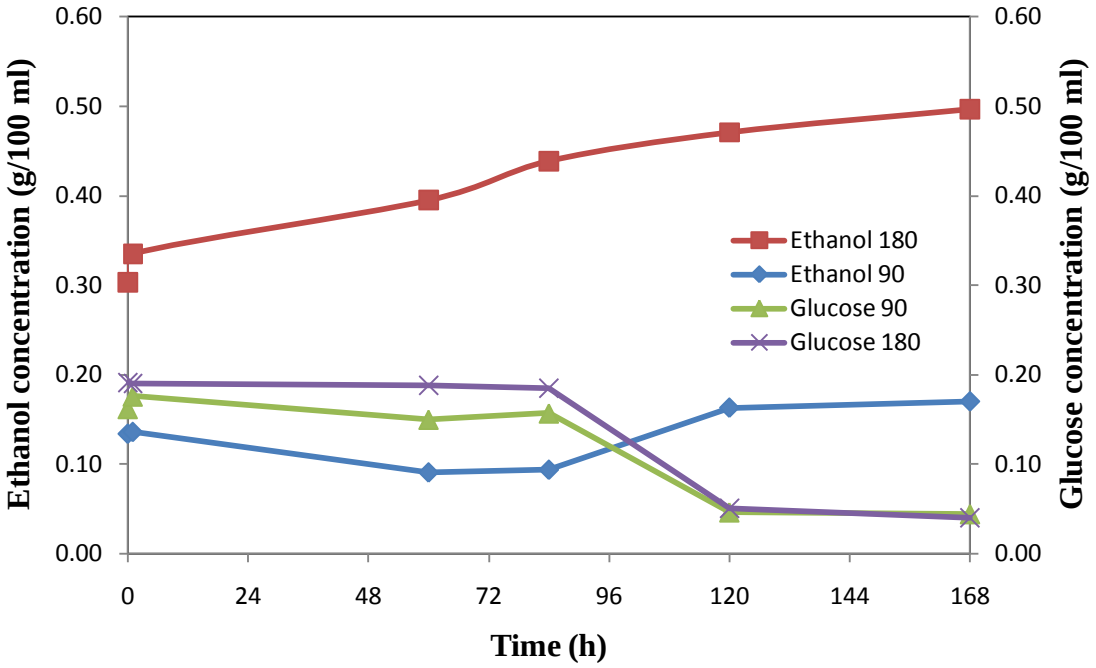
(b) *Switch grass pretreated by ASE with deionized water*



(c) *Corn stover pretreated by ASE with deionized water*



(d) *Wheat straw pretreated by ASE with deionized water*



(e) *Sweet sorghum bagasse pretreated by ASE with deionized water*

Figure 4.1 Glucose and ethanol profiles during Simultaneous Saccharification and Fermentation (SSF) for deionized water pretreated (a) woody biomass (b) switchgrass (c) corn stover (d) wheat straw and (e) sweet sorghum bagasse. (Initial solid concentration: 10%, pH value: 5.0 and temperature: 37^{o}C)

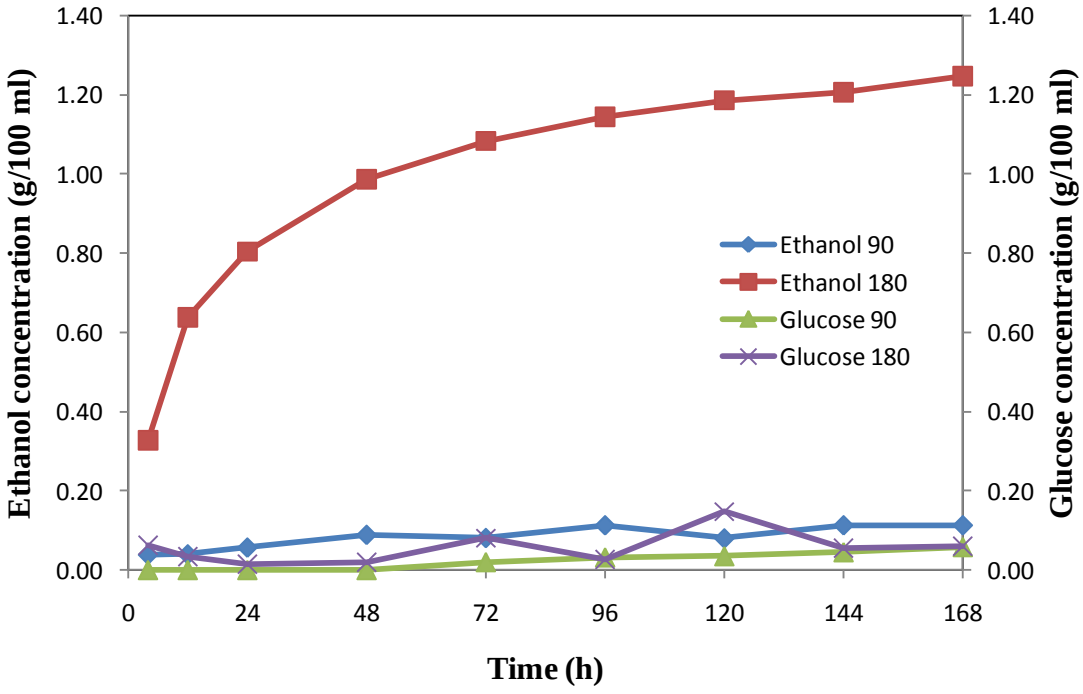
Results for the compositional analysis of the biomass materials pretreated with a 10% acetic acid solution using the ASE 350 at 90 and 180^{o}C is also given in table 4.2. Results for the SSF of the pretreated samples are shown in Figure. 4.2. Like the hot water pretreatment, acid pretreatment leads to the solvation or hydrolysis of hemicellulose fractions in biomass. Pretreatment with dilute strong acid such as sulfuric acid has been found to encourage the formation of compounds such as furfural from xylose degradation and hydroxymethylfurfural (HMF) from glucose degradation [49]. These compounds are inhibitory to the action of cellulase enzymes and yeast during SSF. The effects of these

inhibitors are more pronounced at higher pretreatment temperatures since temperature increases the rate of the glucose and xylan degradation process. However, the use of weak acids such as acetic acid can minimize the degradation of hydrolyzed sugars. From Table 4.2, pretreatment with the acetic acid solution at 180^{o}C resulted in the total solvation of hemicellulose from the biomass into the liquid stream. This is confirmed by the negligible xylose composition for all biomass pretreated with the acetic acid solution at 180^{o}C.

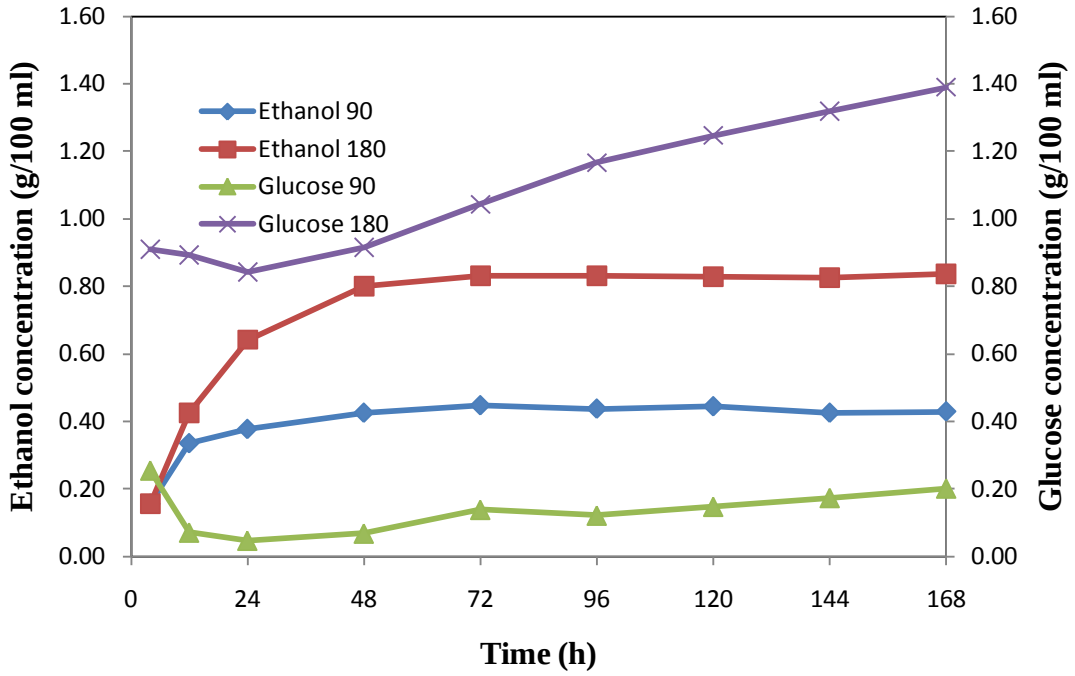
There was a significant increase in the glucan content for all acetic acid pretreated biomass over the biomass that received no pretreatment. At 180^{o}C and 10.3 MPa pretreatment conditions, the glucan concentration of corn stover pretreated with acetic acid solution was the highest at 78% and increased by 144% and 62% compared with untreated corn stover and corn stover pretreated with deionized water respectively. The woody biomass pretreated with acetic acid solution had 132% more glucan than the untreated sample and 34% more glucan than the woody biomass pretreated with deionized water. Glucan contents for the other biomass samples pretreated with acetic acid solution at 180^{o}C were also high. Switchgrass resulted in 85% higher glucan content, 94% for wheat straw and 96% for sweet sorghum bagasse over biomass that received no treatments.

The ethanol yield for the sweet sorghum bagasse pretreated with acetic acid solution at 180^{o}C gave the highest ethanol yield, which was 1.275 g/100 ml after 96 h of SSF as shown in Figure 4.2e and compared to 0.500 g/100 ml for the sweet sorghum bagasse pretreated with deionized water as shown in Figure 4.1e. The ethanol yield for

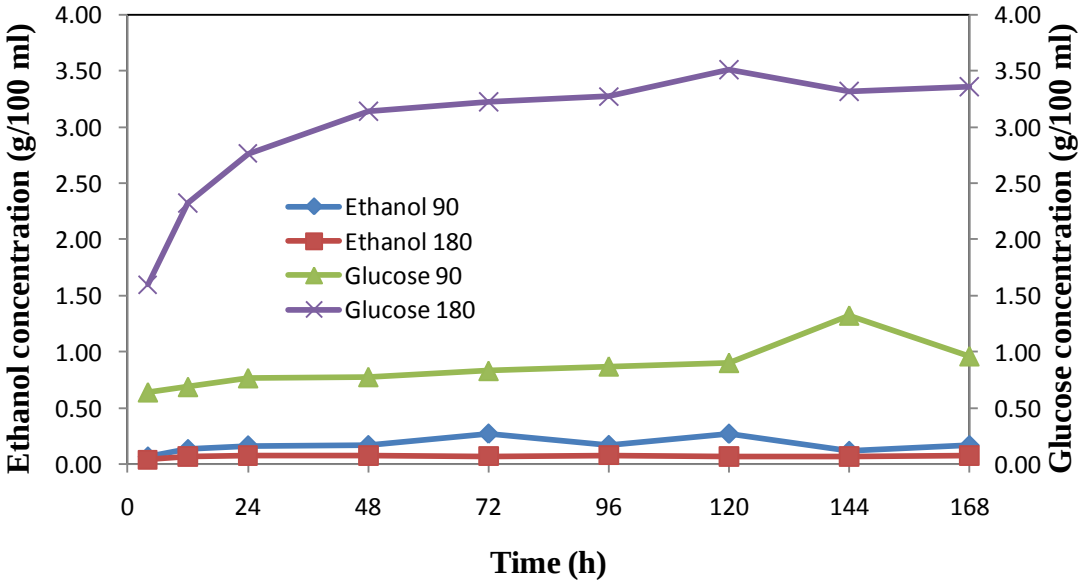
the woody biomass pretreated with acetic acid solution at 180^{o}C was also as high as 1.200 g/100 ml after 96 h of SSF as shown in Figure 4.2a, compared to 0.350 g/ 100 ml for the woody biomass pretreated with deionized water as shown in Figure 4.1a. However, although acetic acid pretreatment can significantly enhance the enzymatic hydrolysis of biomass into glucose it may be inhibitory to the activity of both enzymes and yeast during ethanol fermentation. It can be seen from Figure 4.2 b, and that acetic acid pretreatment significantly enhanced the enzymatic hydrolysis of pretreated biomass, resulting in very high glucose concentrations in the fermentation broths. However, the ethanol yields in the fermentation broths were very low due to the inhibition of residual acetic acid on the yeast used for ethanol fermentation.



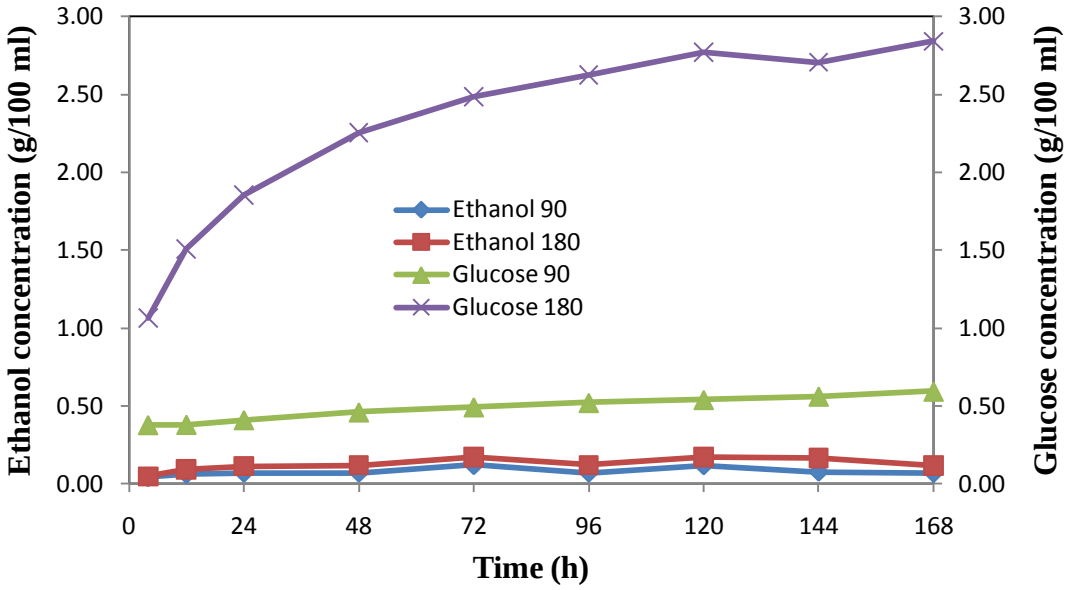
(a) Woody biomass pretreated by ASE with 10% acetic acid



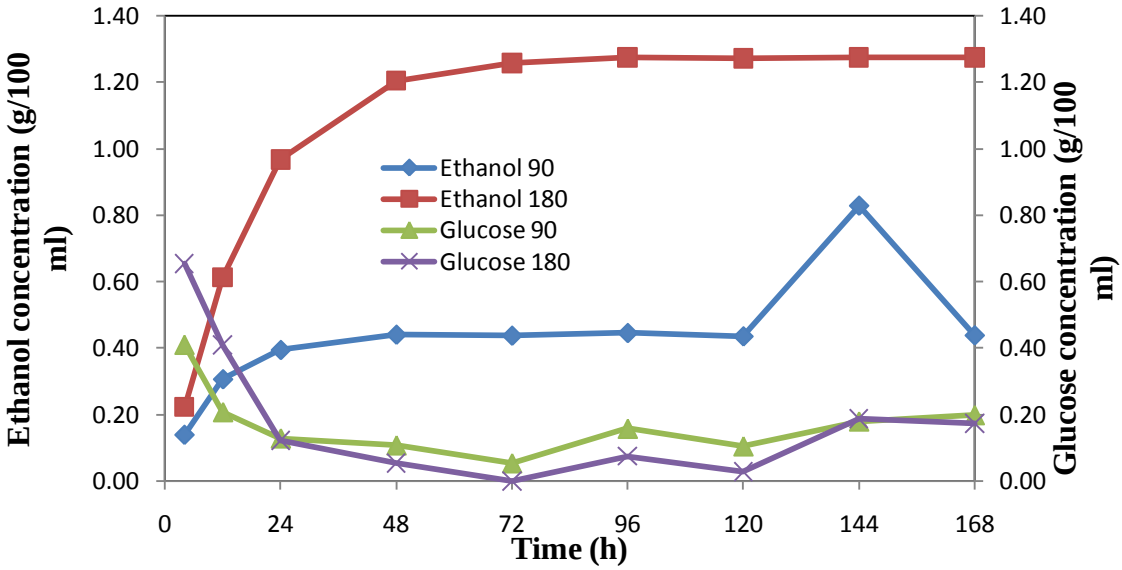
(b) Switchgrass pretreated by ASE with 10% acetic acid



(c) Corn stover pretreated by ASE with 10% acetic acid



(d) Wheat straw pretreated by ASE with 10% acetic acid



(e) Sweet sorghum bagasse pretreated by ASE with 10% acetic acid

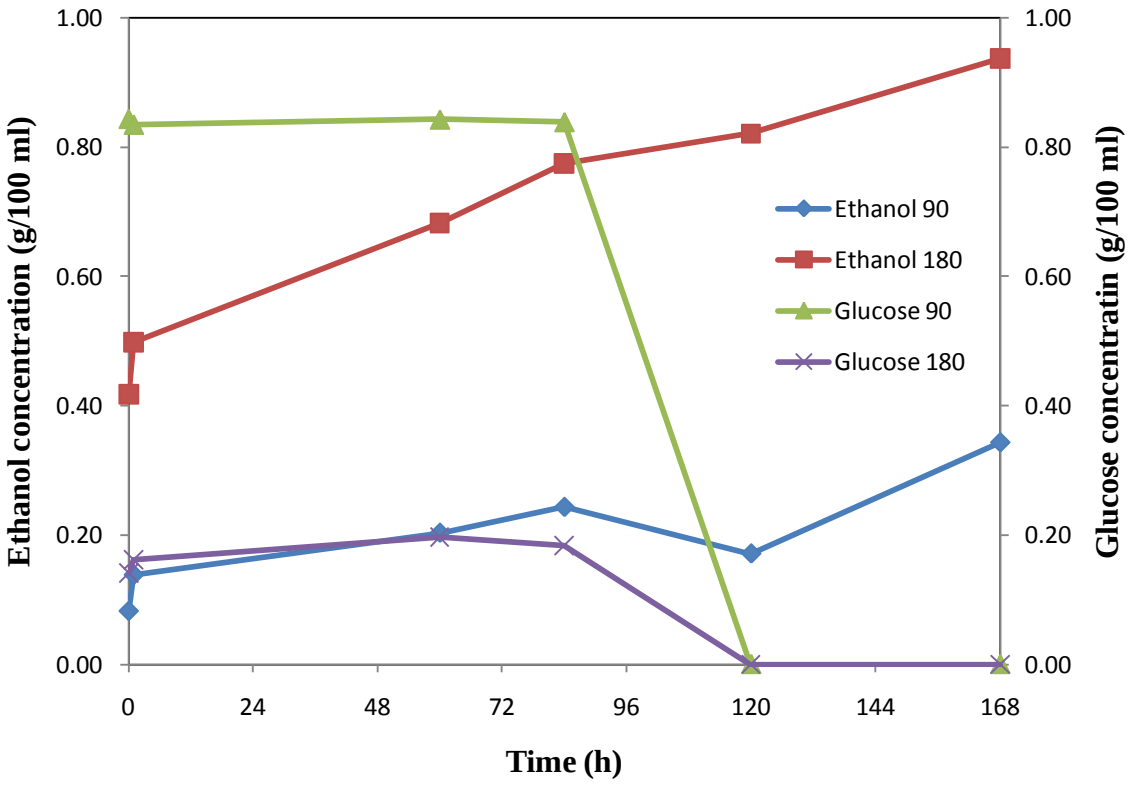
Figure 4.2 Glucose and ethanol profiles during Simultaneous Saccharification and Fermentation (SSF) of (a) woody biomass (b) Switchgrass (c) corn stover (d) wheat straw and (e) sweet sorghum bagasse pretreated by 10% acetic acid solution. (Initial solid concentration: 10%, pH value: 5.0 and temperature: 37°C)

Table 4.2 also shows the composition results for biomass pretreated with 10% ammonium hydroxide (NH_4OH) solution. Figure 4.3 also gives the ethanol and glucose profiles from the SSF process for the NH_4OH pretreated biomass samples. Glucan content, like the acetic acid and deionized water pretreatment, increased above that obtained from the untreated biomass samples with pretreatment at 180^{o}C attaining the highest yields. Alkaline pretreatment of biomass eliminates lignin from the lignocellulosic matrix via solvation into the liquid stream, thereby improving the reactivity of the other polysaccharides [49]. At 180^{o}C, corn stover pretreated with ammonium hydroxide yielded 0.60 g/100 ml of glucan which is 88% more than its untreated complement. Other impressive results were obtained with pretreatment at 180^{o}C yielding for Switchgrass (0.59 g/100 ml, 73%), Woody biomass (0.53 g/100 ml, 64%), Wheat straw (0.63 g/100 ml, 80%) and Sweet Sorghum (0.62 g/100 ml, 73%) over the untreated biomass samples.

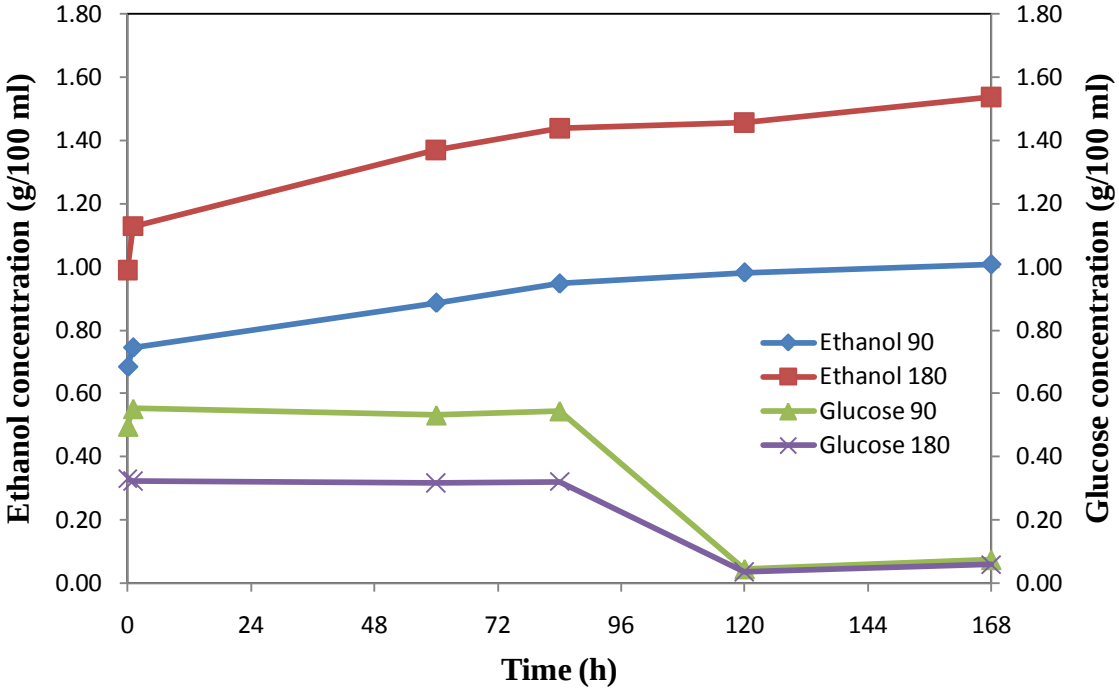
Compared to deionized water pretreatment, ammonium hydroxide pretreated biomass resulted in increased glucan yields of 28%, 25%, 36% and 32% for Switchgrass, Corn stover, Wheat straw and Sweet Sorghum respectively. Ammonium hydroxide pretreatment of woody biomass however generated a lower amount of glucan compared to deionized water pretreatment by 6%. Mosier *et al* .2005 [49] attributes this situation to the higher lignin content of woody biomass. The efficacy of alkaline pretreatment has been found to be dependent on the amount of lignin present in the biomass. However

longer pretreatment times and higher solvent concentrations may be necessary to achieve higher glucan yields from woody biomass.

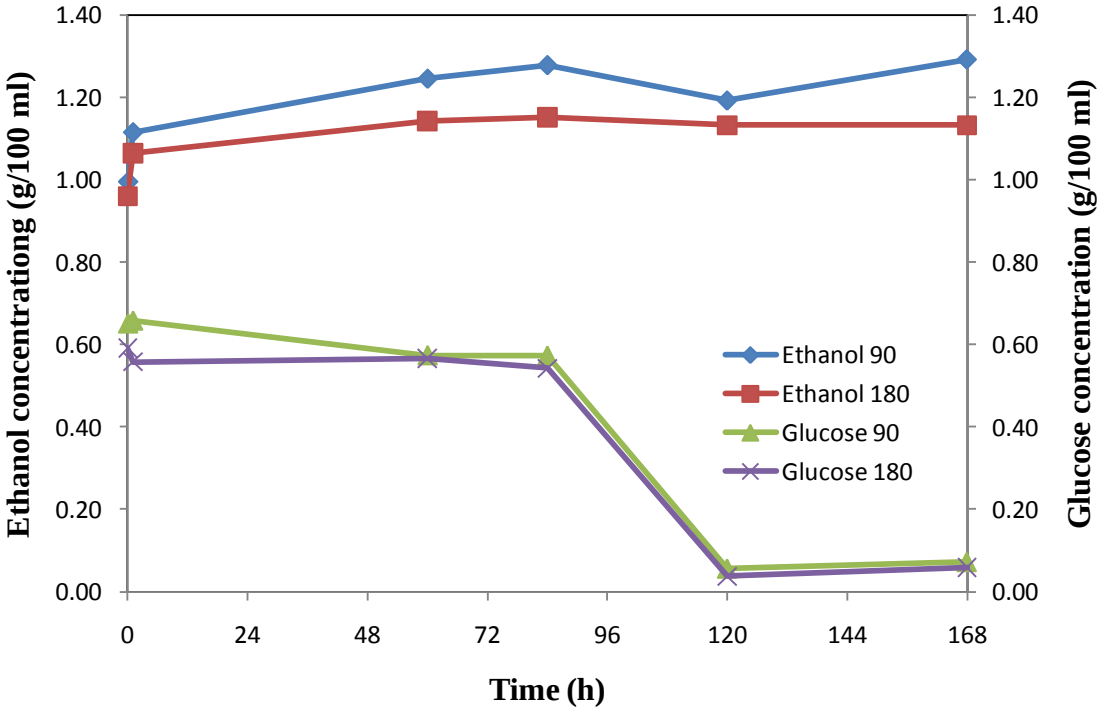
Fermentation results from the SSF process show that the ethanol yield of switch grass after the ammonium hydroxide pretreatment was 1.537 g/100 ml after 168 h SSF, which was the highest of all samples pretreated with (NH_4OH). The highest ethanol yields of pretreated corn stover, wheat straw, sweet sorghum bagasse and woody biomass were 1.152 g/100 ml at 84 h, 1.423 g/100 ml at 168 h, 1.325 g/100 ml at 168 h and 0.937 g/100 ml at 168 h.



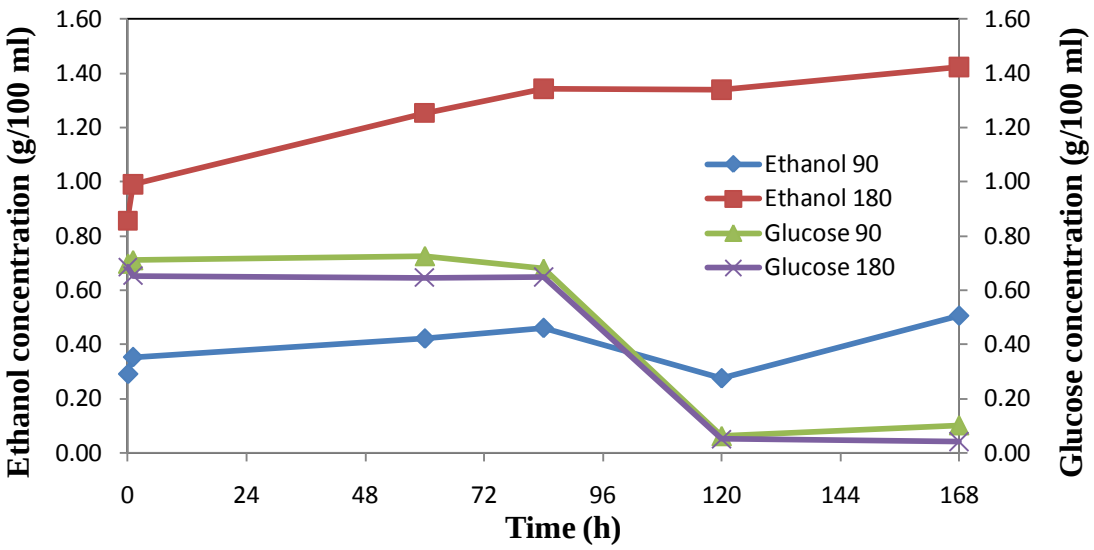
(a) Woody biomass pretreated by ASE with 10% NH_4OH



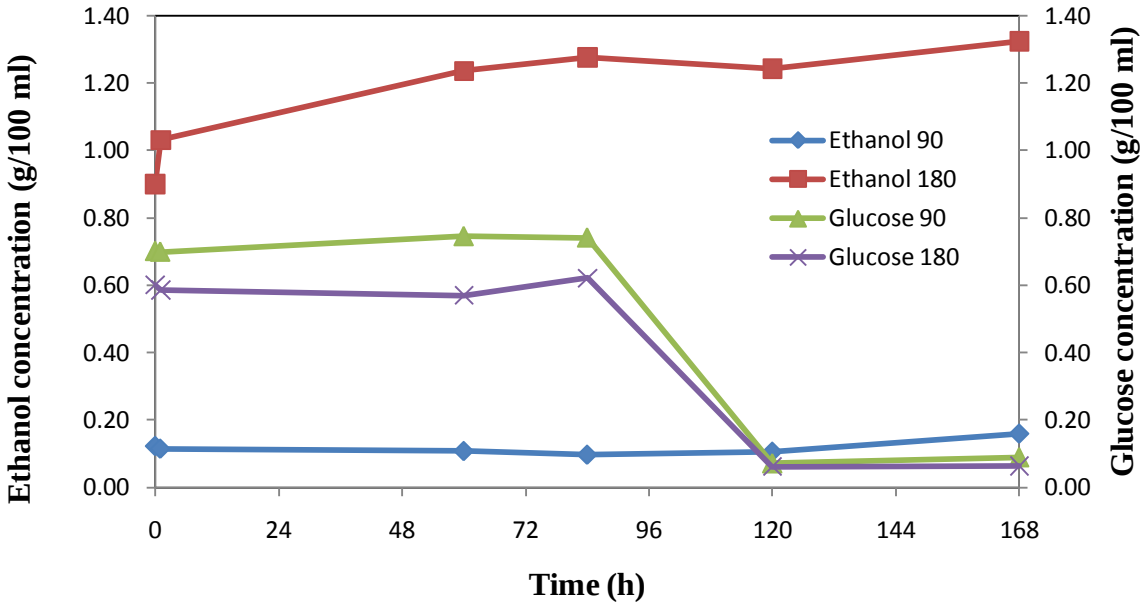
(b) Switch grass pretreated by ASE with 10% NH_4OH solution



(c) Corn stover pretreated by ASE with 10% NH_4OH solution



(d) Wheat straw pretreated by ASE with 10% NH_4OH solution



(e) Sweet sorghum bagasse pretreated by ASE with 10% NH_4OH solution

Figure 4.3 Glucose and ethanol profiles during Simultaneous Saccharification and Fermentation (SSF) of (a) woody biomass (b) switchgrass (c) corn stover (d) wheat straw (e) sweet sorghum bagasse, pretreated by 10% ammonium hydroxide (NH_4OH) in ASE (Initial solid concentration: 10%, pH value: 5.0 and temperature: 37°C)

The lowest ethanol yield for woody biomass may be attributed to its higher lignin content than most of other biomass samples. Table 4.1 shows wheat straw has higher lignin content at 26.6% than the woody biomass at 25.1%. Wheat straw is, however, easily hydrolyzed during pretreatment at slightly elevated temperatures. Wheat straw has very high lignin content because it is a forage crop and requires plenty lignin for sturdiness.

The compositions of different biomass materials pretreated with 30% ethanol in ASE are also given in table 4.2. The subsequent SSF profiles for the glucose and ethanol yields are given in Figure 4.4. Organosolv pretreatment processes employ organic or aqueous organic mixtures with the addition of an acid or alkaline catalyst to disrupt the lignin and hemicellulose bonds that make up lignocellulosic materials [63]. Organic solvents usually used in the organosolv process include acetone, methanol, ethanol, ethylene, glycol, triethylene glycol and tetrahydrofuryl alcohol [56]. High temperature ($180 – 210^{o}C$) organosolv processes do not require catalyst addition as the process is purported to cause the release of organic acids that will autocatalyze the solvation of soluble biomass components. Higher yields have been, however, reported for processes that are catalyzed by the addition of acids [56].

In the ethanol pretreatment process both the hemicellulose and lignin fractions are solubilized while the cellulose remains as a pure crystalline or amorphous solid [56]. Cellulose fractions obtained from organosolv processes are very susceptible to enzymatic hydrolysis and the susceptibility increases with the increased solvation of hemicellulose fractions leading to the creation of pores within the cellulose structure [64]. Glucan

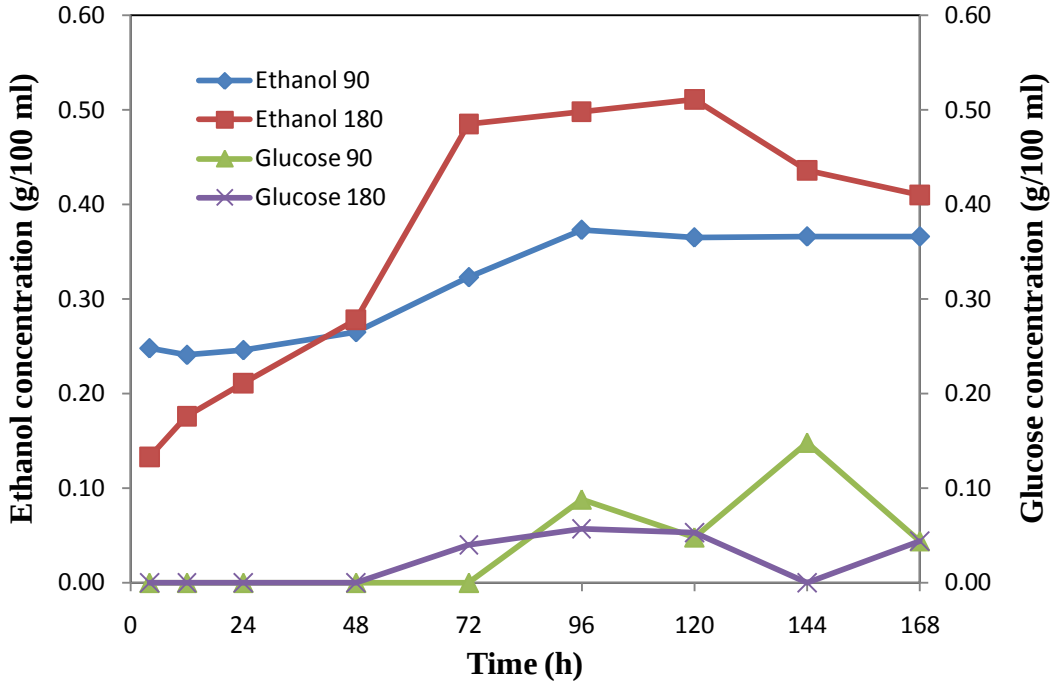
contents after pretreatment with ethanol did not generate very impressive results, compared to the untreated biomass samples as well as the deionized water pretreated biomass.

The glucan contents increased by 53%, 21%, 35%, 20% and 23% for pretreatment at 90^{o}C, and 60%, 3%, 32%, 23% and 23% for pretreatment at 180^{o}C for woody biomass, switch grass, corn stover, wheat straw and sweet sorghum, compared to those of their untreated biomass materials, respectively.

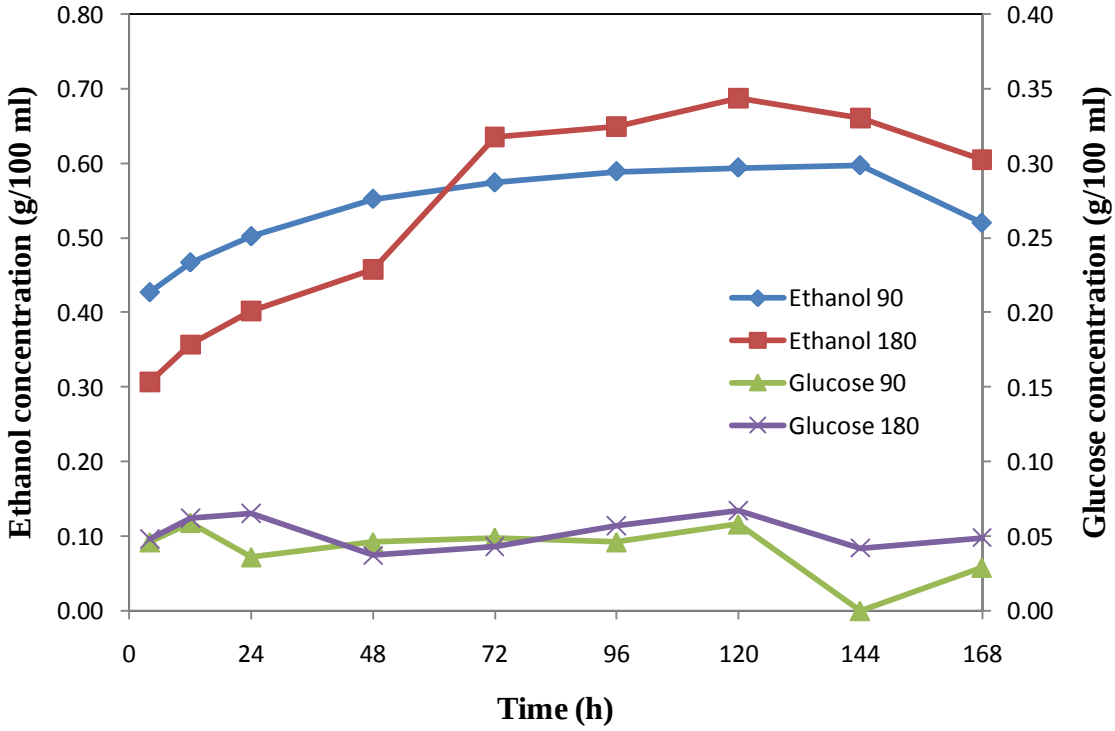
The ethanol yields of corn stover pretreated with ethanol at 180^{o}C and 90^{o}C were 1.207 g/100 ml and 1.079 g/100 ml after 168 h of SSF for the corn stover pretreated with deionized water at 180^{o}C and 90^{o}C, respectively. Overall, ethanol yields increased slightly for all samples pretreated with ethanol as relatively pure cellulose was made available for enzymatic hydrolysis and subsequent fermentation. As shown in Figure 4.4a, the lignin recalcitrance within the matrix of woody biomass resulted in a very low ethanol yield than those of other pretreated biomass samples. Perhaps the addition of catalytic agents such as acids or alkalines and longer pretreatment times will enhance its glucan and concomitant ethanol yield.

4.3 Reactive-screw Extrusion Pretreatment of Lignocellulosic Materials in a Twin Screw Extruder

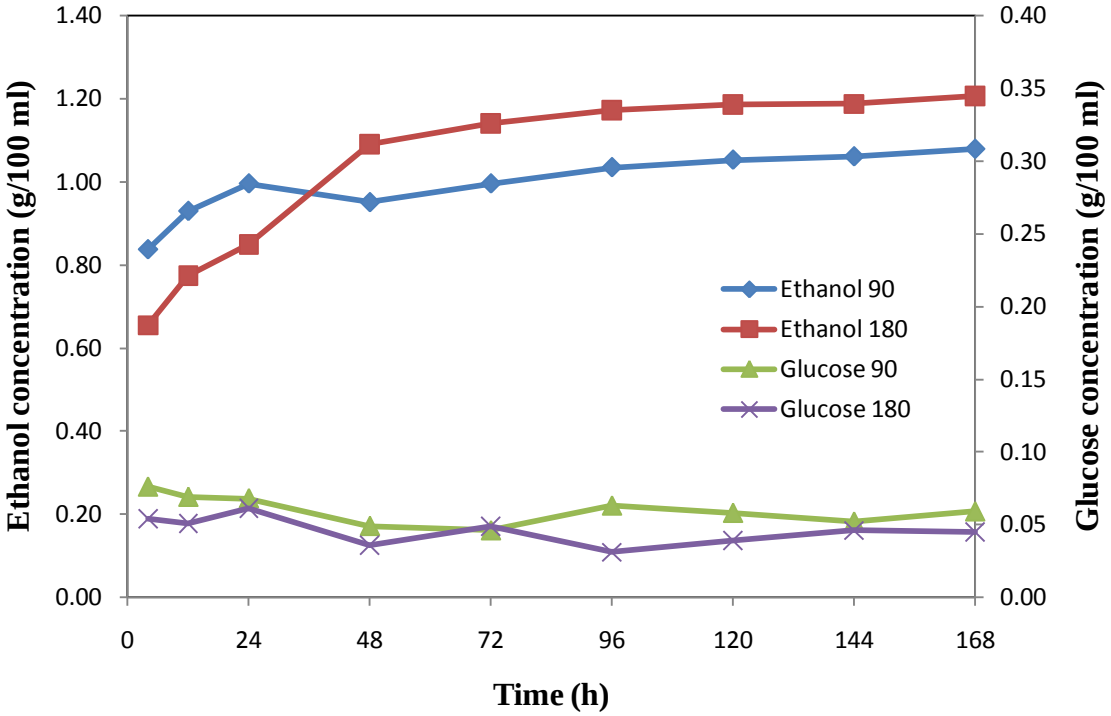
The purpose of the reactive-screw extrusion pretreatment method is to simultaneously (1) hydrolyze sugars from wet biomass, (2) squeeze sugar juice out of the biomass matrix and (3) compress the solid biomass residue into compact fuel pellets.



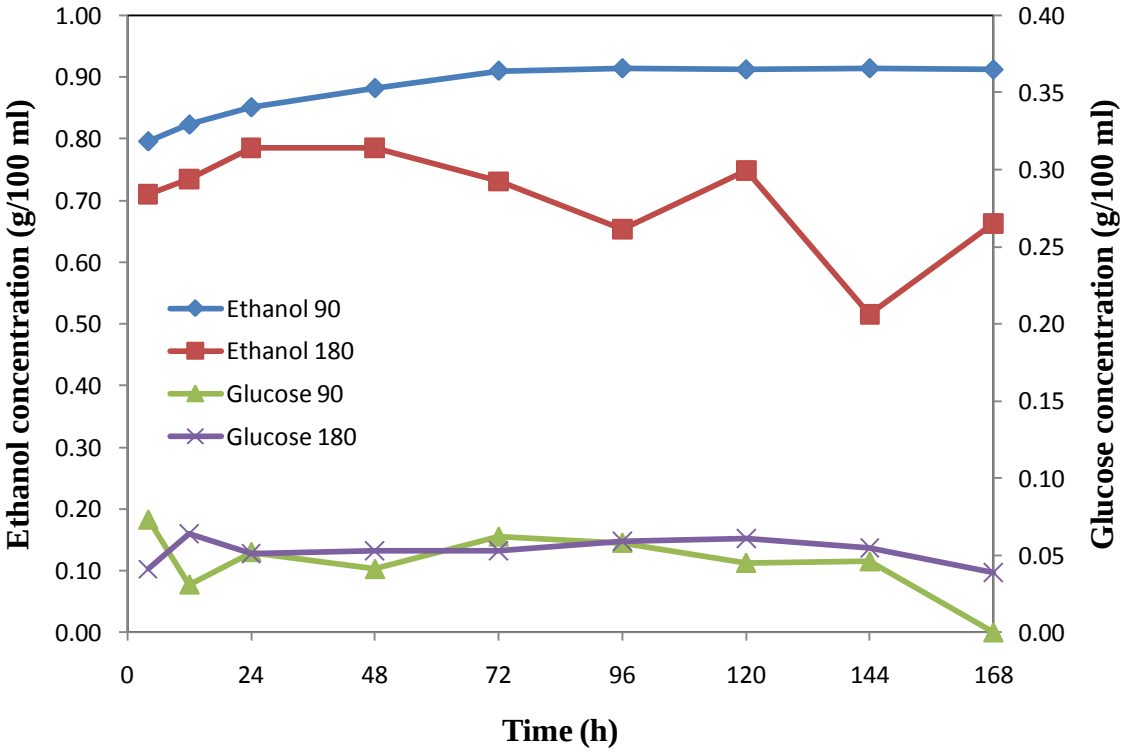
(a) Woody biomass pretreated by ASE with 30% ethanol solution



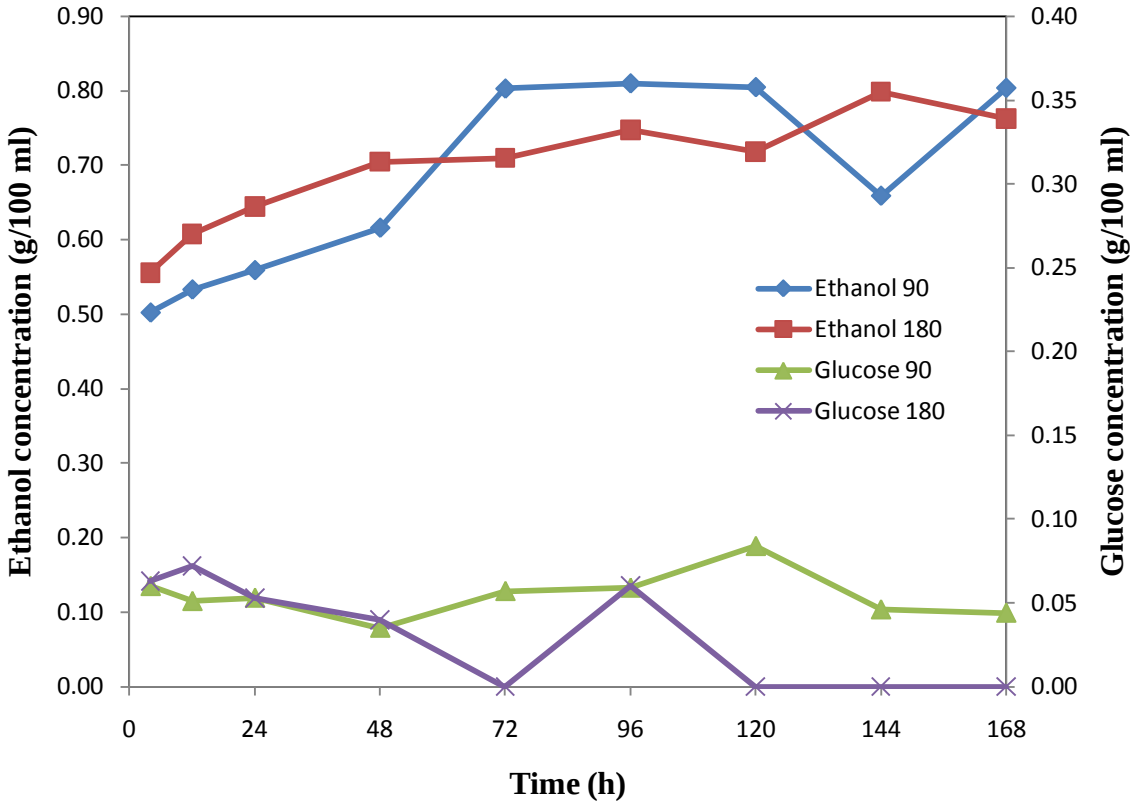
(b) Switch grass pretreated by ASE with 30% ethanol solution



(c) Corn stover pretreated by ASE with 30% ethanol solution



(d) Wheat straw pretreated by ASE with 30% ethanol solution



(e) Sweet sorghum bagasse pretreated by ASE with 30% ethanol solution

Figure 4.4 Glucose and ethanol profiles during Simultaneous Saccharification and Fermentation (SSF) of (a) woody biomass (b) Switchgrass (c) corn stover (d) wheat straw (e) sweet sorghum bagasse, pretreated with 30% ethanol solution (Initial solid concentration: 10%, pH value: 5.0 and temperature: 37^{o}C)

The shear stress offered as a result of the rotary and sliding motion of the twin screw adds some mechanical pretreatment impetus to the fractionation process. Tables 4.3 summarize the compositional results after extrusion pretreatment of Corn stover, Wheat straw and Switchgrass respectively. Figure 4.5 also gives the ethanol and glucose profiles after simultaneous saccharification and fermentation of the pretreated samples.

Table 4.3 Composition of corn stover, wheat straw, switchgrass pretreated by reactive screw extrusion with different chemicals at 180°C and 100 RPM screw rotational speed

Sample	Chemical	Ash content(% by mass	Lignin content(% by mass)	Glucan content(% by mass)	Xylan content(% by mass)
Raw material		1.2	18.6	33.2	24.2
Corn stover	Acetic Acid	8.5	56.2	13.8	3.4
	NH_4OH	2.4	20.6	34.5	19.6
	$Ca(OH)_2$	2.0	15.6	29.7	16.5
	H_2O	2.7	20.3	35.9	19.7
Raw material		2.5	26.6	35.1	30.0
Wheat straw	$Ca(OH)_2$	0.0	20.6	27.8	19.5
	Water	0.6	27.2	35.7	18.1
	Acetic Acid	0.1	22.1	34.2	17.8
Raw material		6.0	24.4	34	30.9
Switchgrass	$Ca(OH)_2$	1.0	20.1	30.1	21.1
	H_2O	1.7	25.3	38.4	24.7
	Acetic Acid	2.1	23.9	37.9	22.5

Extrusion pretreatment of biomass resulted in relatively lower glucan concentrations compared to the pretreatment of the Accelerated Solvent Extraction (ASE). This may be attributed to the more compact conditions operated by the ASE, including higher pressure of 10.3 MPa, higher mass ratio of solvent to biomass, and better heat and mass transfer in closed reactor cells. As shown in Table 4.3, the highest glucan content was achieved by the screw extrusion pretreatment of corn stover using deionized water at 180°C.

For screw extrusion pretreatment with acetic acid, the glucan contents of corn stover and wheat straw decreased by 52% and 2.1% over their untreated raw materials. However, the glucan content of switch grass increased by 11%. A big decrease of the

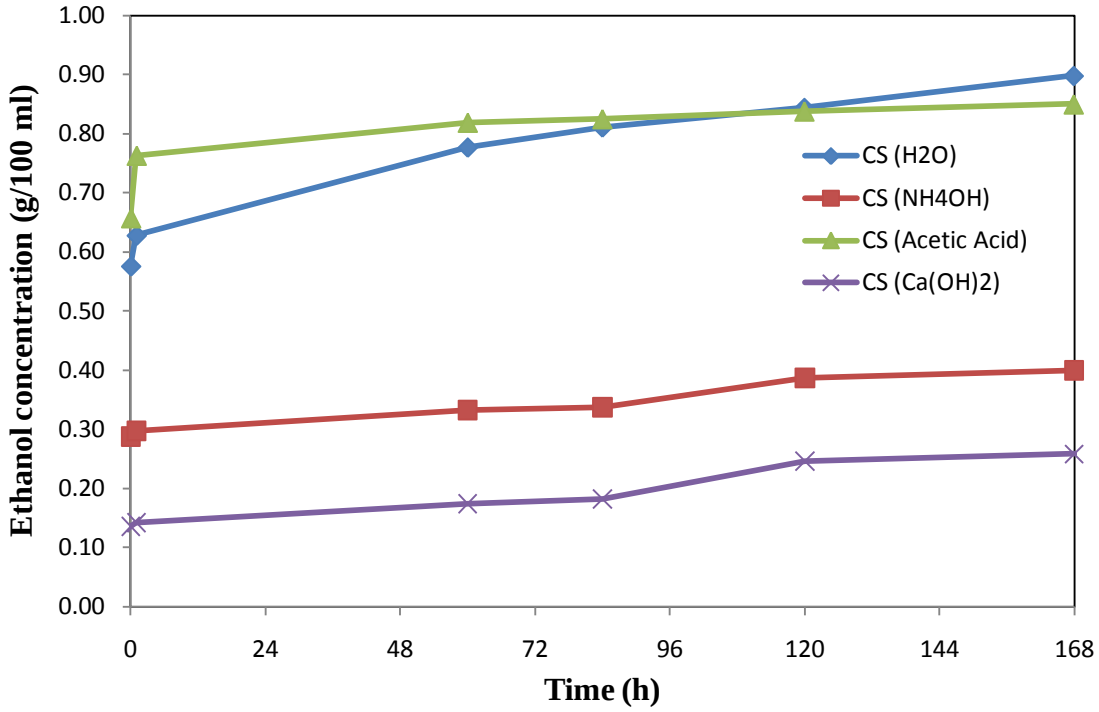
glucan in the corn stover sample may be caused by the hydrolysis of the glucan to glucose during the screw extrusion pretreatment with acetic acid.

For screw extrusion pretreatment with lime ($Ca(OH)_2$), the glucan contents of wheat straw and switch grass decreased by 21% and 11% over their untreated raw materials, respectively. However, the glucan content of pretreated corn stover slightly increased by 3.4%. Hydroxy-carboxylic acids including glucoisosaccharinic and xylosaccharinic acids are generated from the degradation of carbohydrates such as cellulose and hemicellulose in the presence of alkali and oxygen via oxidation reactions. Lime pretreatment at a high temperature results in the formation of low molecular mass fragments such as glycolic and lactic acids, which may contribute to the lowering of glucan content within the pretreated biomass [65].

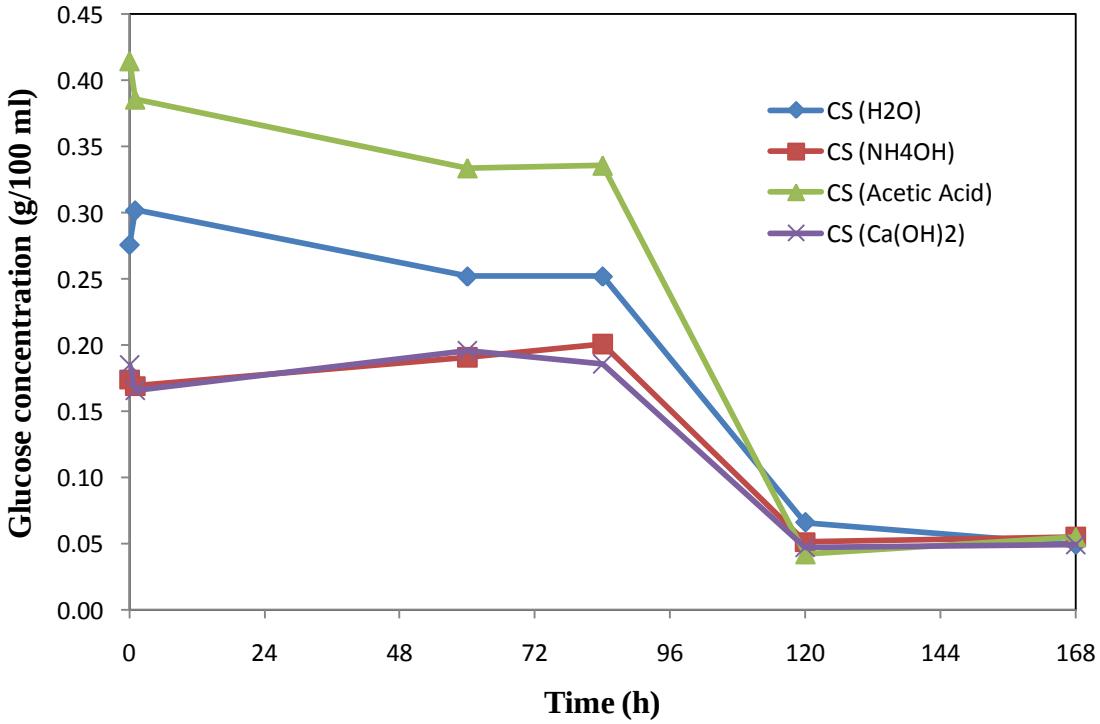
The screw extrusion pretreatment of biomass with deionized water increased the glucan contents of corn stover, wheat straw and switch grass by 25%, 1.7% and 13%, respectively. As hot water pretreatment of biomass generates little to no degradation products, most of the cellulose within the biomass matrix is left intact for enzymatic hydrolysis.

4.4 Biomass Pretreatment in a High Temperature Continuous Stirred Tank Reactor

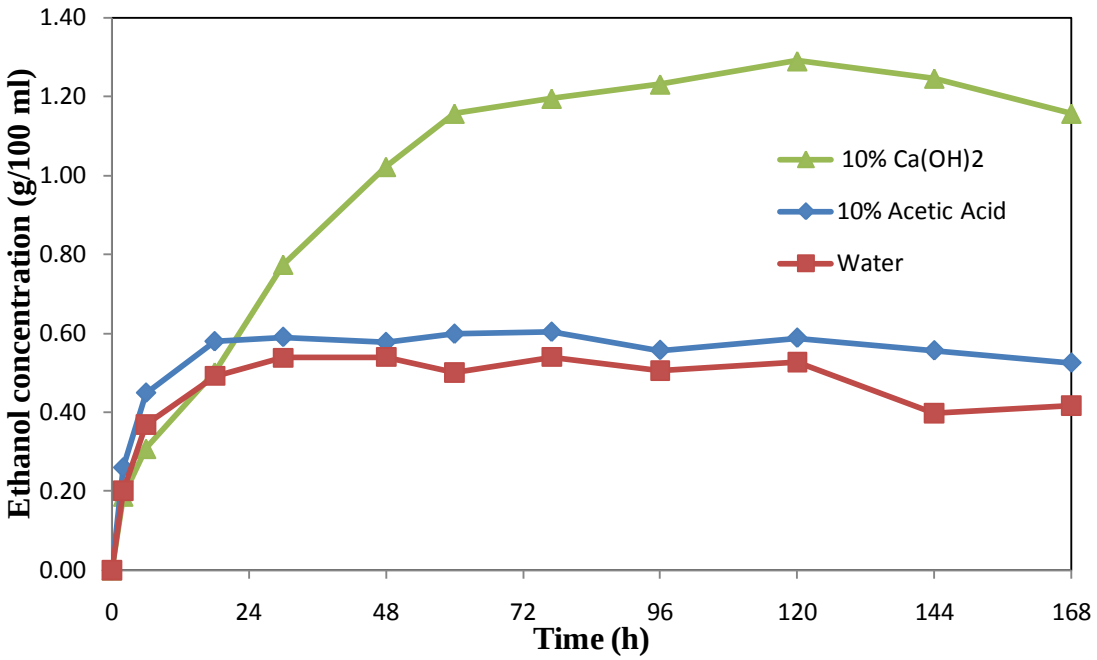
Table 4.4 shows the composition of corn stover samples pretreated with different abrasive chemicals including 10% acetic acid, 10% ammonium hydroxide and 10% calcium hydroxide in a high-pressure continuous stirred tank reactor (CSTR).



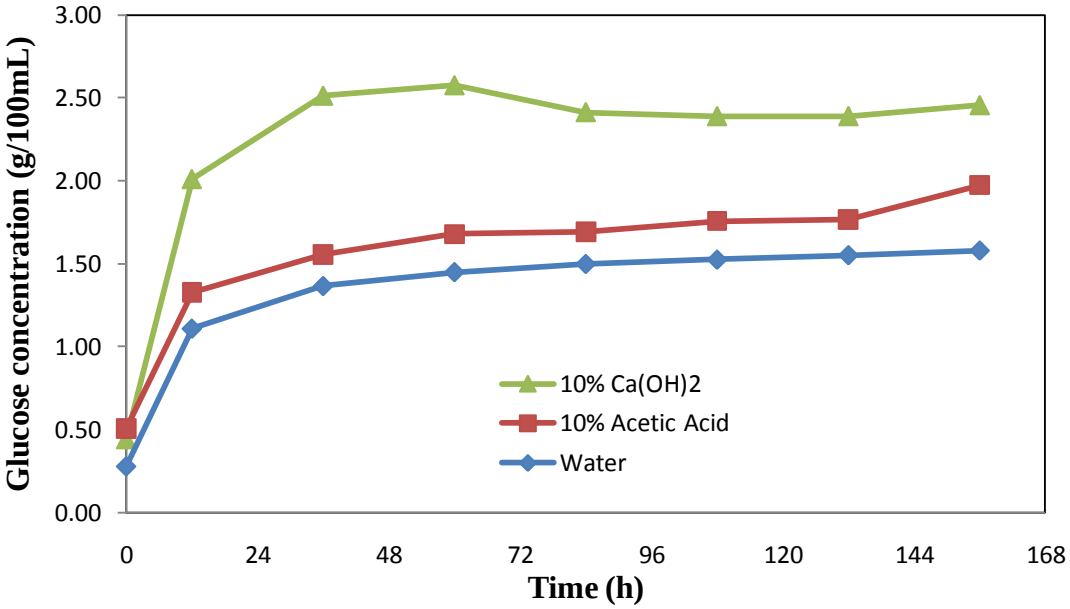
(a) Ethanol yield for reactive screw extrusion pretreatment of corn stover



(b) Glucose yield for reactive screw extrusion pretreatment of corn stover



(c) Ethanol yield for reactive screw extrusion pretreatment of switch grass



(d) Glucose yield for reactive screw extrusion pretreatment o f switch grass

Figure 4.5 Ethanol and glucose profiles during SSF of reactive screw extrusion pretreated biomass: (a) ethanol yield for corn stover (b) glucose yield for corn stover (c) ethanol yield for switchgrass (d) glucose yield for switchgrass. (Initial solid concentration: 10%, pH value: 5.0 and temperature: 37^{o}C)

Deionized water pretreatment was also conducted for comparative analysis. The pretreatment in the CSTR combines the pretreatment at a high pressure and temperature with agitation of the reaction mixture to attain fractionation of biomass into monomeric sugars for ethanol fermentation. Pretreatment was conducted at 180^{o}C at a pressure between 0.965 and 1.103 MPa, which is much lower than the pressure of 10.3 MPa in the Accelerated Solvent Extractor.

Glucan contents of corn stover pretreated with deionized water, 10% ammonium hydroxide and 10% acetic acid at 180^{o}C in a CSTR reactor resulted in 18%, 47% and 73% increases over untreated corn stover, respectively. Lime pretreatment resulted in a 42% decrease in the glucan content over untreated corn stover. The conversion of glucan and other carbohydrate fractions into organic acids such as glycolic acid, lactic acid and some degradation compounds in the presence of lime at a high temperature may contribute to the glucan loss. As shown in Figure 4.6 a, the corn stover samples pretreated with water, ammonium hydroxide and calcium hydroxide at 180^{o}C in a Parr reactor achieved ethanol yields as high as 2.000 g/100 ml. However, the ethanol yield for the corn stover pretreated with an acetic acid solution was very low. As shown in Figure 4.6 b, the corn stover pretreated with the acetic acid solution generated the highest yield of glucose, which could not be converted to ethanol via the fermentation with yeast. The low ethanol yield of acetic acid pretreated corn stover may be attributed to the low activity of the yeast due to high cell mortality. Yeast cell mortality can be induced by unfavorable operating conditions cells including pH, inhibitory compounds, oxygen

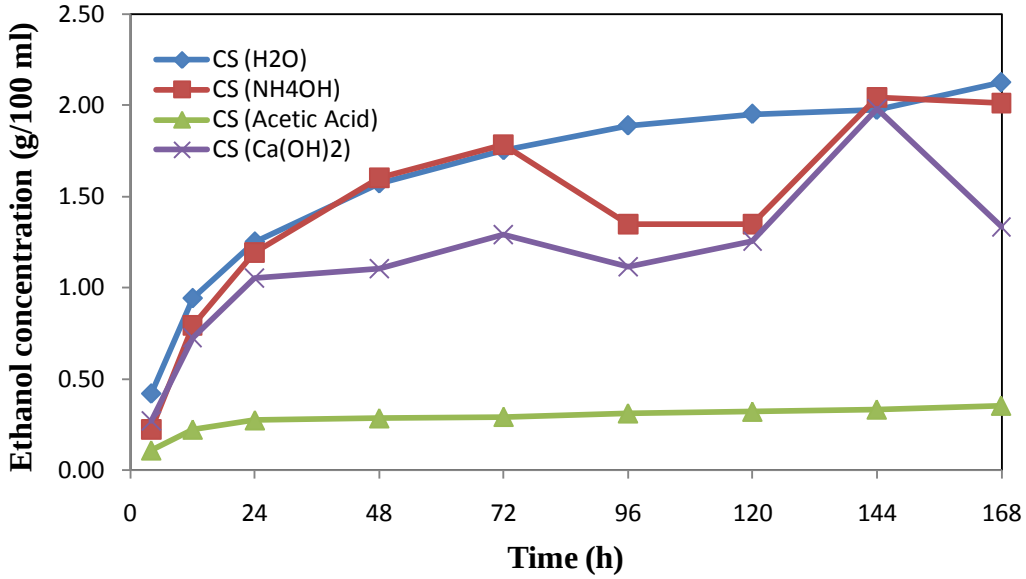
concentration within the broth and the hostile compromised temperatures used in SSF processes.

Table 4.4 The composition of corn stover pretreated with different chemicals at 180^{o}C in a batch continuous stirred tank reactor

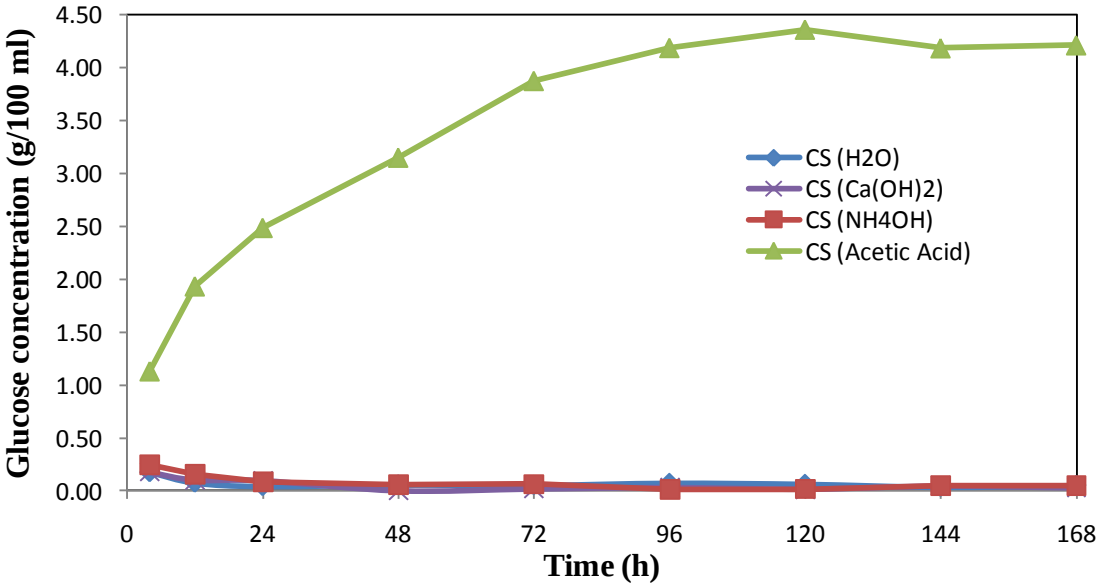
	Chemical	Ash content(% by mass	Lignin content(% by mass)	Glucan content(% by mass)	Xylan content(% by mass)
Corn Stover Parr reactor pretreatment at 180 C	**Raw material**	1.2	18.6	33.2	24.2
	H_2O	3.0	34.8	34.0	4.2
	NH_4OH	2.0	21.5	42.3	13.1
	Acetic Acid	3.4	38.0	49.8	0.0
	$Ca(OH)_2$	2.7	11.4	16.7	4.2

4.5 Pretreatment of Biomass with Different Chemicals at an Ambient Condition

Table 4.5 gives the composition of switchgrass, corn stover, wheat straw and sweet sorghum bagasse pretreated with different chemicals at the ambient condition for seven days. Figure 4.7 gives the ethanol and glucose profiles after SSF of the above pretreated biomass samples. Pretreatment was conducted on all four biomass materials using deionized water, 10% ammonium hydroxide, 10% calcium hydroxide and 10% acetic acid. The glucan contents in the biomass samples pretreated with different chemicals at the ambient temperature were slightly different from those of their corresponding raw materials.



(a) Ethanol yield for continuous stirred tank reactor pretreatment of corn stover



(b) Glucose yield for continuous stirred tank reactor pretreatment of corn stover

Figure 4.6 Saccharification and Fermentation yield profiles for (a) ethanol and (b) glucose after pretreatment of Corn stover with different abrasive chemicals using the high pressure batch continuous stirred tank reactor (Parr) (Initial solid concentration: 10%, pH value: 5.0 and temperature: 37^{o}C)

The glucan contents were 25%, 32%, 14%, and 16% higher for switchgrass pretreated with acetic acid, ammonium hydroxide, calcium hydroxide and deionized water, respectively. Other samples including corn stover and sweet sorghum bagasse pretreated with calcium hydroxide, however, suffered 20% and 3% decrease in glucan concentration after pretreatment as shown in Table 4.5. The glucan contents decreased by 9%, 9%, 7% and 10% for the wheat straw pretreated with acetic acid, ammonium hydroxide, calcium hydroxide and deionized water, respectively.

Table 4.5 Biomass composition after ambient pretreatment with different chemicals for seven days

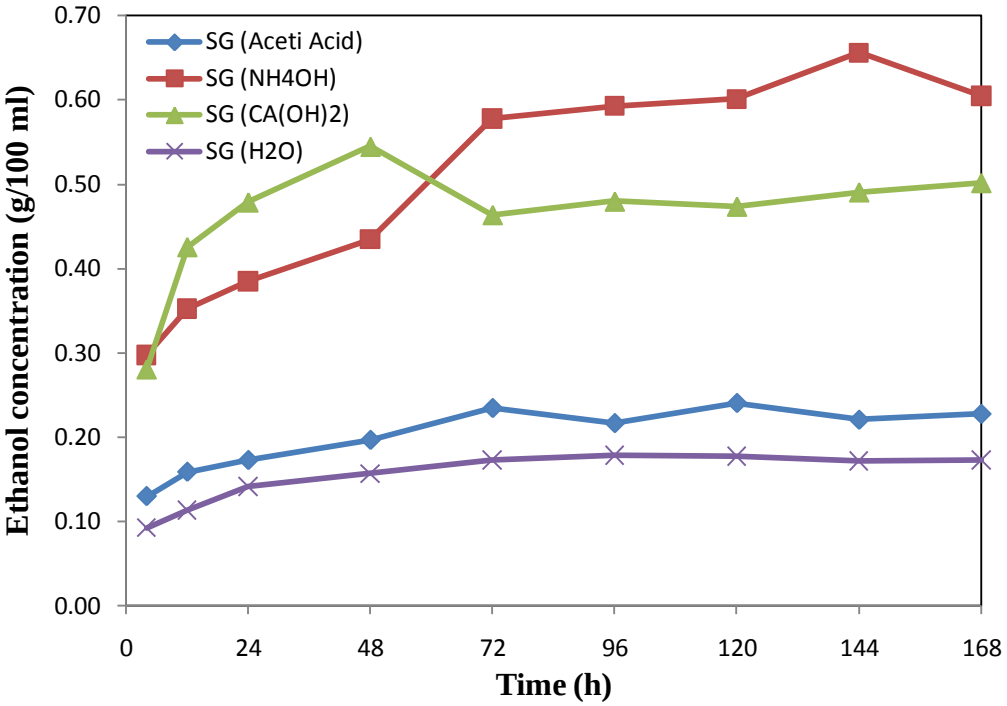
	Chemical	Ash content(% by mass	Lignin content(% by mass)	Glucan content(% by mass)	Xylan content(% by mass)
Switchgrass	**Raw material**	6.0	24.4	34.0	30.9
	Acetic Acid	3.3	21.2	36.1	22.2
	NH_4OH	3.1	30.9	38.1	18.6
	$Ca(OH)_2$	3.7	26.4	33.0	16.0
	H_2O	3.9	25.9	33.7	19.7
Corn stover	**Raw material**	1.2	18.6	33.2	24.2
	Acetic Acid	1.6	28.4	32.6	17.3
	NH_4OH	1.3	25.6	36.4	19.8
	$Ca(OH)_2$	1.1	24.3	34.4	16.8
	H_2O	3.4	21.1	33.5	17.3
Wheat straw	**Raw material**	2.5	26.6	35.1	30.0
	Acetic Acid	2.5	25.5	34.5	17.6
	NH_4OH	1.1	26.7	34.6	15.0
	$Ca(OH)_2$	1.3	21.1	29.3	11.1
	H_2O	2.3	24.0	35.0	17.6
Sweet sorghum bagasse	**Raw material**	2.4	24.6	35.8	29.3
	Acetic Acid	1.9	27.8	33.9	13.3
	NH_4OH	1.2	29.2	36.1	16.6
	$Ca(OH)_2$	2.2	19.3	31.1	13.1
	H_2O	2.9	26.8	34.4	15.6

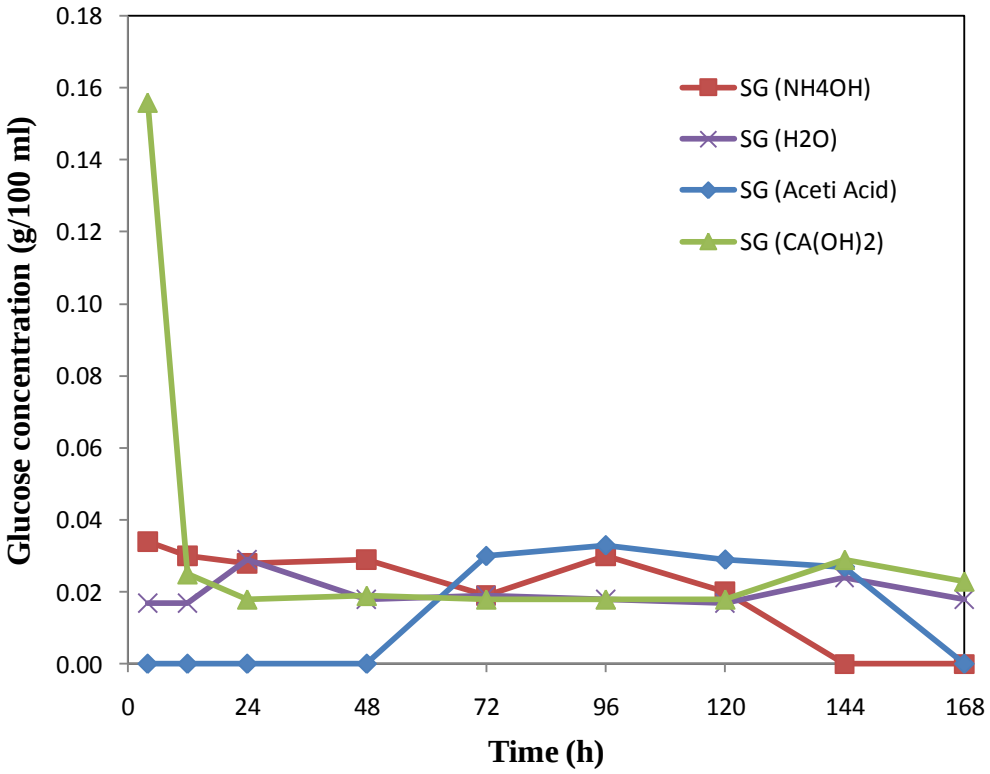
As seen from Figure 4.7, the pretreatment of biomass with base solutions including ammonium hydroxide and calcium hydroxide at the ambient temperature significantly enhanced the enzymatic hydrolysis and ethanol fermentation with yeast. The biomass samples pretreated with ammonium hydroxide at the ambient temperature achieved the highest ethanol yields. The ethanol yields for the switchgrass, corn stover, wheat straw and sweet sorghum bagasse pretreated with ammonium hydroxide were 0.656 g/100 ml, 0.474 g/100 ml, 0.748 g/100 ml and 0.968 g/100 ml, respectively. This confirms that alkali pretreatment of biomass at low temperatures is effective [49]. The pretreatment of biomass using calcium hydroxide (lime) may not be able to achieve the same high ethanol yield compared to using ammonium hydroxide [66]. As lime dissolves sparingly in water, it required that the pretreated sample be washed many times to remove the residual lime before other downstream processes were conducted. The washing of residual lime after pretreatment may result in high glucan losses and thus low ethanol yields.

Acetic acid pretreatment at the ambient temperature was the third in ethanol yield for all pretreated biomass samples. The lower ethanol yield for the biomass samples pretreated with acetic acid at the ambient temperature indicated that acetic acid pretreatment of biomass at a low temperatures is ineffective.

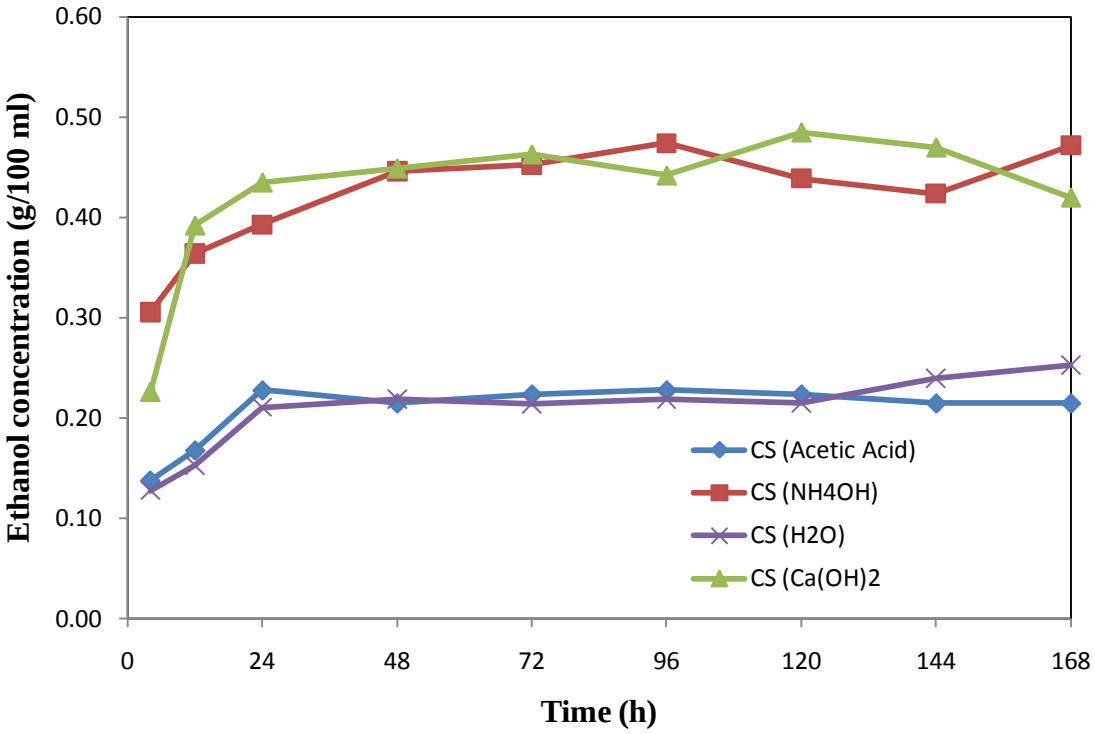
4.6 Statistical Analysis

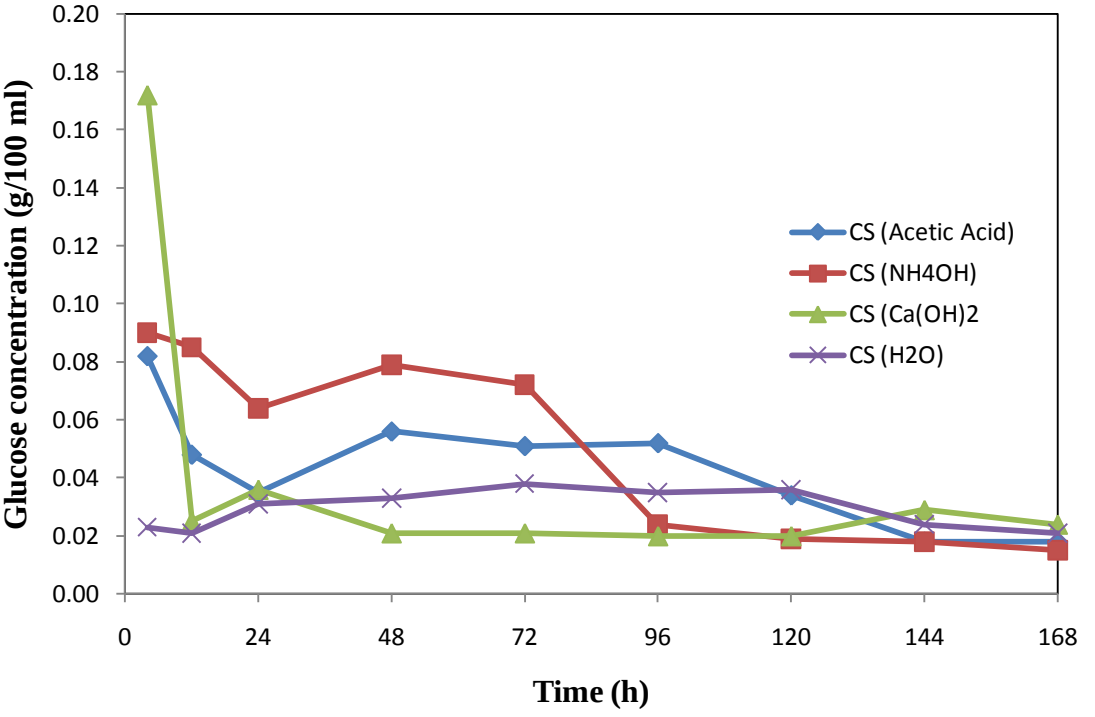
Experiments were duplicated under the same conditions for the ASE, CSTR and RSE pretreatment methods.



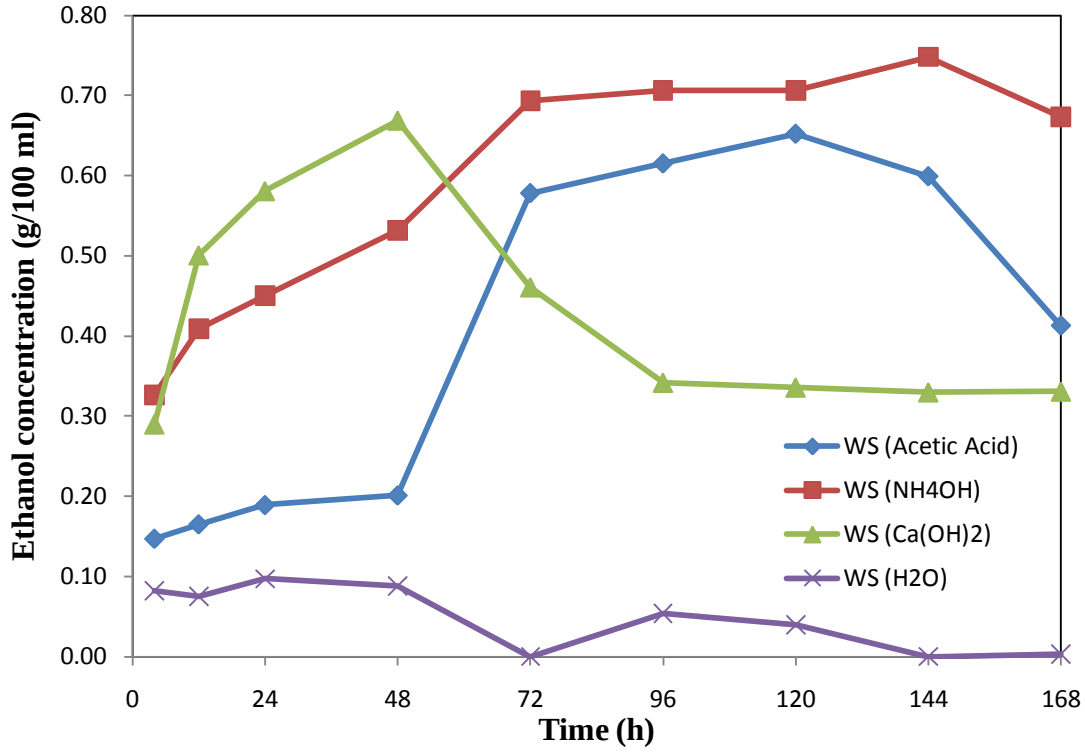


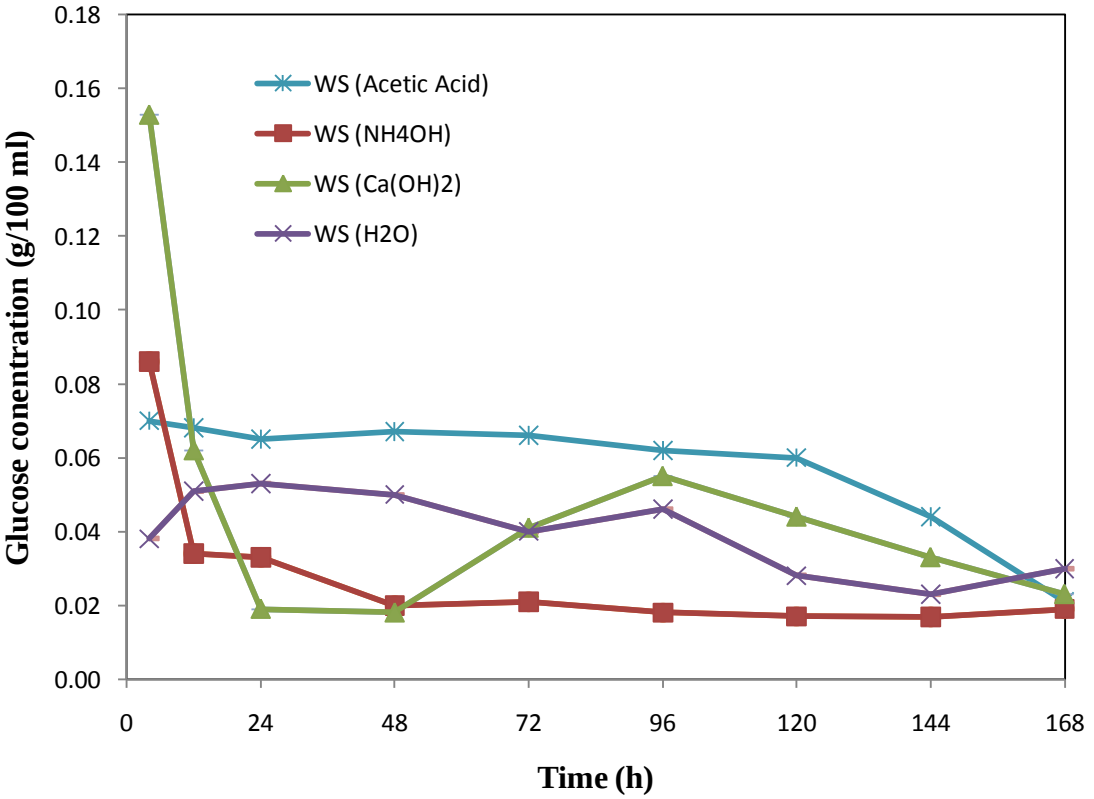
(a) switch grass



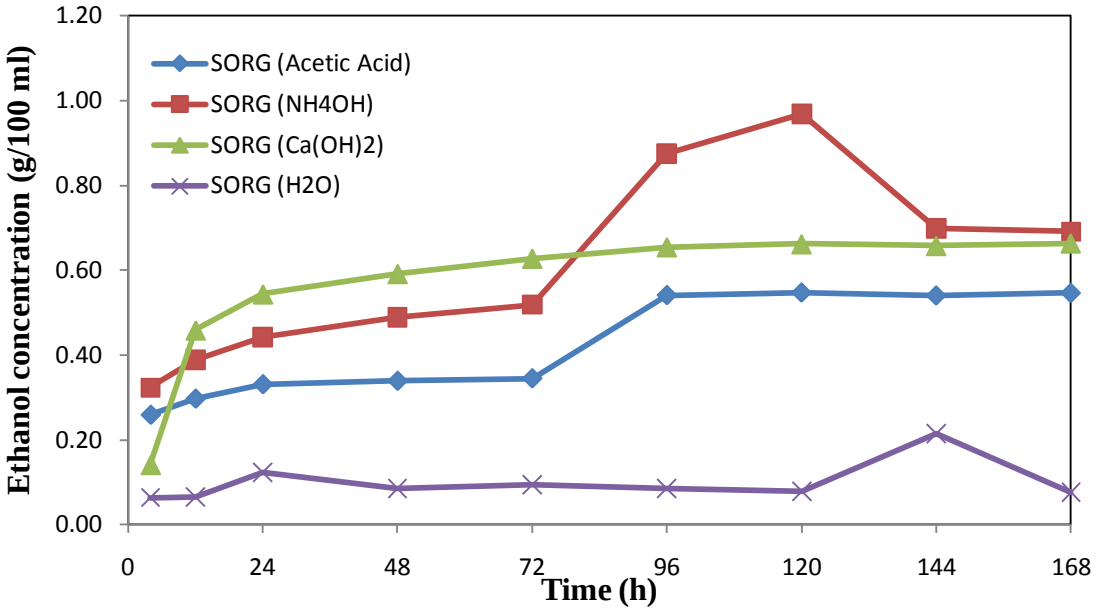


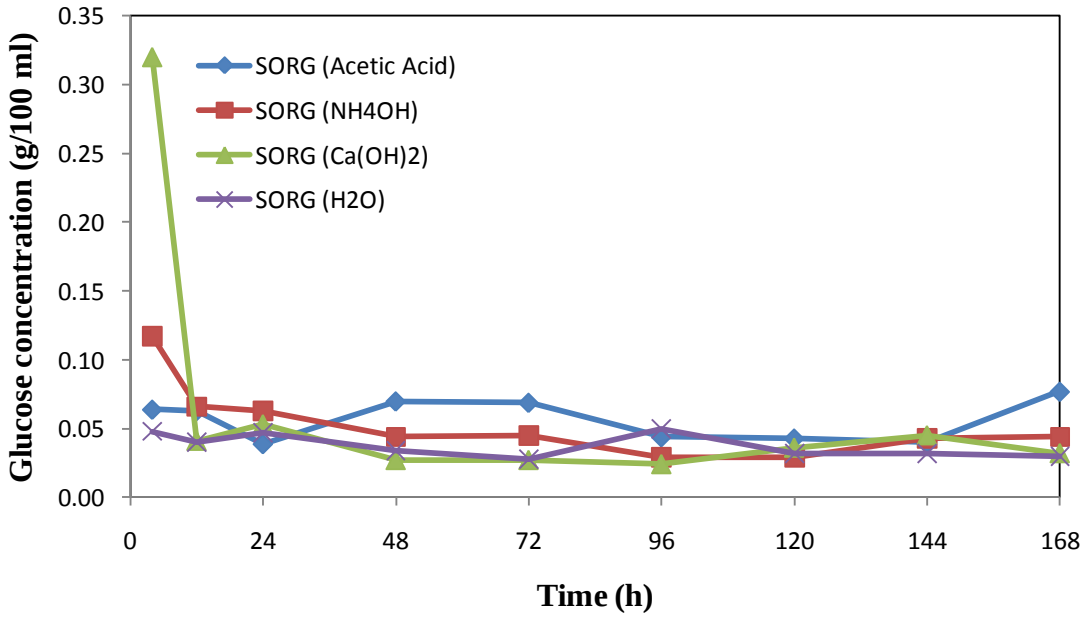
(b) corn stover





(c) wheat straw





(d) sweet sorghum bagasse

Figure 4.7 Ethanol and glucose profiles from SSF of (a) switchgrass (b) corn stover (c) wheat straw (d) sweet sorghum bagasse pretreated with different chemicals at an ambient condition (Initial solid concentration: 10%, pH value: 5.0 and temperature 37^{o}C)

Standard deviation analyses were conducted to determine the accuracy of collated data. T-test statistical analyses were also conducted to ascertain the yield significance of each pretreatment method over the untreated biomass samples as well as the effectiveness of pretreatment variations for similar pretreatment methods. The statistical analyses were conducted using built-in Microsoft Office Excel formulae for determining the mean, standard deviation and T-test.

4.7 T-test Analysis

The T-test was used to compare two different pretreatment methods either from the same equipment or from different pretreatment equipments. T-test comparisons were made for glucan, xylan, lignin and ash contents of biomass materials pretreated with different chemicals, different pretreatment equipments and different pretreatment conditions. The T-test results give the P-value for comparative analysis. A P-value below 0.05 is generally considered statistically significant, while one of 0.05 or greater indicates no difference between the method groups compared. T-test analysis can be conducted using Microsoft Excel by the following procedure.

1. Enter your data in columns
2. Click on an empty cell
3. Hit the = sign in the bar at the top of the spreadsheet
4. Hit the down arrow to the left of the = sign. Now some options will appear
5. If TTEST is not on the list, click “more functions”, choose “statistical”, then “TTEST”.

6. A dialog box will appear. Click in the box next to “Array 1”
7. Drag the dialog box out of the way, then highlight your first column of numbers
8. Click in the box next to “Array 2” and highlight your second column of numbers
9. If you predict group A would be lower than group B, pick 1 for the “tails” query and likewise if group B is predicted lower than group A.
10. If you are not sure which group of data is higher than the other then pick 2 for the “tails” query
11. Excel is capable of three types of T-tests. Pick 1 for the “Type” query if the T-test analyses of the data are “paired” or “dependent”. This is called “Type 1” test.
12. Pick 2 for the “Type” query if the data are “unpaired” or “independent” and the standard deviations are similar for both groups of data.
13. Pick 3 for the “Type” query if the data are “unpaired” or “independent” and the data groups have unequal variances [67].

4.8 Mean and Standard Deviation

Statistically, the degree of error of collated data is determined by the standard deviation, standard error or variance. The mean (average) is a measure of the central tendency of the collated data. The mean and standard deviations of replicated data were determined using built-in Microsoft Excel formulae. The procedure for determining these quantities is as follows:

1. Enter your data in columns
2. Click on the empty cell after your last number in a column

3. Hit the = sign in the bar at the top of the spreadsheet
4. Hit the down arrow to the left of the = sign. Now some options will appear
5. Click on “average”
6. If it is not on the list, click “more functions”, choose “statistical”, the “average”.
7. Do the same thing (using the next empty cell) to get “STDEV”. This gives you the standard deviation [67].

Tables 4.6 and 4.7 compare the glucan, xylan, lignin and ash contents of untreated biomass with biomass pretreated by ASE with deionized water, 10% acetic acid, 10% ammonium hydroxide and 30% ethanol solutions at 90^{o}C and 180^{o}C. Comparison was done based the same pretreatment temperatures. The P-values of the T-test analyses is given in the row after the designated pretreatment chemical used. Tables 4.8 and 4.9 show the T-test analyses comparing the different pretreatment methods including ASE, RSE, CSTR and AST used in this research. Glucan, xylan, lignin and ash components of pretreated corn stover using varied chemicals including deionized water, 10% acetic acid, 10% ammonium hydroxide and 10% calcium hydroxide were juxtaposed for comparative analysis. Again the P-values as a result of the T-test analysis are flushed in the bottom row of the table. The mean and standard deviation of sample replicated experiments (for deionized water pretreatment of biomass) are given in table 4.1l. Data for other replicated experiments are not shown however all experiments were repeated and the average values and standard deviations determined for correctness of procedure and reproducibility. The sample data given in table 4.11 show very low deviation of measured values from the mean hence the consistency of experimental procedure.

Table 4.12 summarizes the ethanol conversion efficiencies of biomass pretreated in the ASE using different chemicals and temperatures. Conversion efficiencies for AST pretreated biomass using different chemicals are also given in table 4.13 while ethanol conversion efficiencies for CSTR and RSE pretreated biomass using different chemicals is shown in table 4.14.

T-test analysis for ASE pretreated biomass at 90^{o}C using chemicals including deionized water, 10% acetic acid, 10% ammonium hydroxide and 30% ethanol solutions had relatively little significance on the total fractionation of core biomass matrix components. P-values lower than 0.05 indicate significant differences between two processes on the basis of comparison. It can be observed however that all the pretreatment chemicals had good effects on the raw biomass by solvating the hemicellulose fractions. This result is buttressed by the P-values of 0.021, 0.013, 0.02, and 0.010 for deionized water, 10% acetic acid, 10% ammonium hydroxide and 30% ethanol solution respectively. This result shows the ease in solubilizing hemicellulose fractions from lignocellulosic materials and confirms the high temperature sensitivity of hemicellulose to fractionation as was reported in literature. Ninety degree pretreatment of biomass using all chemicals showed very little effect on lignin and glucan fractionation compared to the biomass complement that received no treatments. This may be attributed to the recalcitrance of both lignin and cellulose to fractionation. P-values for glucan comparison (0.859, 0.443, 0.110 and 0.696 for deionized water, acetic acid, ammonium hydroxide and ethanol respectively) show a slightly improved fractionation capability of ammonium hydroxide for cellulose.

Pretreatment at a higher temperature (180^{o}C) resulted in much lower P-values for the fractionation of hemicellulose from the biomass samples using all chemicals. Acetic acid pretreatment resulted in high xylan solubilization for all biomass samples which can be confirmed by the negligible xylan content in the solid stream of hydrolyzed biomass from table 4.2. The T-test results in table 4.7 also show significant cellulose fractionation of the treated biomass over the untreated complement with acetic acid being the most effective with a p-value of 1.7E-05. Solvation of lignin was not significantly achieved by either chemicals but ammonium hydroxide indicated a relatively higher solubilization of lignin. This can be confirmed in the relatively lower P-value 0.144, compared to all other pretreatment chemical. This confirms the theory of alkali's being capable of solvating lignin fractions from lignocellulosic materials. Acetic acid however had the least effect on lignin solvation (P-value 0.968).

Table 4.8 compares the different pretreatment processes used in this thesis. P-values from the T-test analysis are shown comparing CSTR, RSE and AST to ASE. All results show P-values much higher than 0.05. This shows that none of the other pretreatment methods including CSTR, RSE and AST had the fractionation capabilities of the Accelerated Solvent Extractor. The high efficiency of the ASE may be attributed to the compact nature by which it operates and the effect that pressure (10.3 MPa) has on biomass fractionation. T-test analysis comparing AST and RSE to CSTR pretreatment of biomass (Table 4.9) and AST to RSE (Table 4.10) showed no significant improvements and differences in the pretreatment methods.

4.9 Ethanol Conversion Efficiency

Ethanol conversion efficiencies for ASE pretreated biomass using different chemicals are given in Table 4.12. Again ethanol conversion efficiencies for AST pretreated and CSTR with RSE pretreatments are also given in Table 13 and 14 respectively. Conversion results obtained as a result of ASE pretreatment of biomass shows higher glucose to ethanol conversions at 180^{o}C than pretreatments at 90^{o}C. It stands to reason that higher temperatures catalyze the fractionation of lignocellulosics creating pores within the biomass matrix for enzymatic hydrolysis and subsequent fermentation. Glucose to ethanol conversions after AST pretreatment show that ammonium hydroxide and calcium hydroxide were better solvents for fractionating the biomass materials for ethanol production. High ethanol conversion rates of NH_4OH-30.4%, $Ca(OH)_2$- 29.2% over acetic acid-11.8% and deionized water-9.4% for switchgrass; NH_4OH-38.2%, $Ca(OH)_2$-40.3% over acetic acid-33.4% and deionized water-4.9 for wheat straw; NH_4OH-23.0%, $Ca(OH)_2$-24.1% over acetic acid-12.3% and deionized water-13.3% for corn stover; NH_4OH-47.4%, $Ca(OH)_2$-37.7% over acetic acid-28.4% and deionized water-11.0% for sweet sorghum bagasse shows the efficacy of alkaline pretreatment of biomass at lower temperatures.

Again glucose to ethanol conversions (Table 4.14) for corn stover pretreated with CSTR using different chemicals shows remarkable conversion rates with the corn stover samples pretreated with alkaline solvents. High conversion rates were achieved at 85.2% for NH_4OH and 35.3% for $Ca(OH)_2$ over 3.9% and 12.5% for deionized water and acetic acid respectively. The high alkaline conversion rates can be attributed to greater

delignification of the biomass during pretreatment as well as the production of very little inhibitors during the pretreatment stages of the biomass to ethanol process.

4.10 Extraction of Hemicellulose Fractions from the Liquid Stream of ASE Pretreated Biomass.

Hemicellulose fractions were extracted from the liquid stream of Accelerated Solvent Extractor pretreated biomass samples. This study was done to ascertain which solvent dissolved the most hemicellulose from the biomass samples. Results given in Table 4.15 show that a greater percentage of hemicellulose fractions were obtained after fractionation with ammonium hydroxide solution. Also higher temperatures resulted in higher hemicellulose weights for all solvents used in the fractionation. The higher masses of hemicelluloses collected after ammonium hydroxide fractionation could also be attributed to the solvation of non-polar lignin fractions within the biomass matrix.

Table 4.6 Compositions of different biomass after ASE pretreatment with different chemicals at 90°C/T-test evaluation

Sample		Glucan(%by mass)	Xylan(%by mass)	Lignin(%by mass)	Ash(%by mass)
Raw biomass compositions	WC	45.3	16.8	31.8	0.1
	SG	34.0	30.9	24.4	0.6
	CS	33.2	24.2	18.6	1.2
	WS	35.1	30.0	26.6	2.5
	SSB	35.8	29.3	24.6	2.4
Deionized H_2O	WC	42.4	19.1	28.2	0.8
	SG	36.3	18.5	28.6	2.5
	CS	36.3	20.4	23.2	2.1
	WS	37.0	17.8	29.7	2.3
	SSB	28.6	11.2	29.9	2.9
T-test		**0.859**	**0.021**	**0.298**	**0.237**
10% Acetic acid	WC	41.3	17.4	30.5	0.2
	SG	36.7	18.3	38.1	2.1
	CS	34.7	19.2	27.9	1.4
	WS	35.8	16.5	34.4	1.7
	SSB	48.2	17.7	21.1	2.4
T-test		**0.443**	**0.013**	**0.187**	**0.782**
10% NH_4OH	WC	43.4	14.8	30.2	0.2
	SG	39.6	19.0	27.7	1.1
	CS	40.1	20.6	19.8	0.7
	WS	39.5	19.1	12.4	1.0
	SSB	41.6	17.1	29.1	1.5
T-test		**0.110**	**0.020**	**0.746**	**0.404**
30% Ethanol	WC	42.2	16.7	29.6	0.4
	SG	35.9	17.7	27.5	2.4
	CS	36.3	19.1	24.4	2.2
	WS	36.4	17.1	27.9	2.4
	SSB	37.7	15.2	30.0	3.6
T-test		**0.696**	**0.010**	**0.282**	**0.265**

Table 4.7 Compositions of different biomass after ASE pretreatment with different chemicals at 180°C/T-test evaluation

Sample		Glucan(%by mass)	Xylan(%by mass)	Lignin(%by mass)	Ash(%by mass)
Raw biomass compositions	WC	45.3	16.8	31.8	0.1
	SG	34.0	30.9	24.4	0.6
	CS	33.2	24.2	18.6	1.2
	WS	35.1	30.0	26.6	2.5
	SSB	35.8	29.3	24.6	2.4
Deionized H_2O	WC	49.8	17.0	28.6	0.4
	SG	38.3	20.3	29.0	2.5
	CS	41.4	20.0	23.7	1.8
	WS	40.1	17.1	31.8	2.2
	SSB	43.2	13.6	29.8	3.2
T-test		**0.082**	**0.017**	**0.212**	**0.361**
10% Acetic Acid	WC	67.8	0.0	23.4	0.1
	SG	59.0	0.0	26.8	3.5
	CS	68.0	0.0	21.2	2.6
	WS	62.0	0.0	25.5	3.1
	SSB	59.9	0.0	29.6	4.1
T-test		**1.7E-05**	**8.4E-06**	**0.968**	**0.153**
10% NH_4OH	WC	47.8	13.5	28.0	0.1
	SG	51.7	15.2	16.7	0.8
	CS	51.5	17.8	14.3	0.9
	WS	51.0	16.7	21.7	1.7
	SSB	51.5	14.4	23.0	2.5
T-test		**0.00029**	**0.004**	**0.204**	**0.799**
30% Ethanol	WC	45.1	14.6	28.8	0.3
	SG	34.2	17.5	27.0	2.5
	CS	37.6	19.0	21.2	1.9
	WS	38.0	17.9	28.0	2.7
	SSB	38.4	13.4	29.9	3.4
T-test		**0.502**	**0.009**	**0.510**	**0.299**

Table 4.8 Compositions of corn stover pretreated with different chemicals and different pretreatment methods/T-test evaluation comparing CSTR, RSE and AST to ASE

Chemical		Glucan(%by mass)	Xylan(%by mass)	Lignin(%by mass)	Ash(%by mass)
ASE pretreatment at 180^{o}C	H_2O	41.4	20.0	23.7	1.8
	10% A.A	68.0	0.0	21.2	2.6
	10% NH_4OH	51.5	17.8	14.3	0.9
	30% Ethanol	37.6	19.0	21.2	1.9
CSTR pretreatment at 180^{o}C	H_2O	34.0	4.2	34.8	3.0
	10% A.A	49.8	0.0	38.0	3.4
	10% NH_4OH	42.3	13.1	21.5	2.0
	10% $Ca(OH)_2$	16.7	4.2	11.4	2.7
T-test		**0.267**	**0.410**	**0.113**	**0.188**
RSE pretreatment at 180^{o}C	H_2O	35.9	19.7	20.3	2.7
	10% A.A	13.8	3.4	56.2	8.5
	10% NH_4OH	34.5	19.6	20.6	2.4
	10% $Ca(OH)_2$	29.7	16.5	15.6	2.0
T-test		**0.072**	**0.854**	**0.360**	**0.245**
AST pretreatment	H_2O	33.5	17.3	21.1	3.4
	10% A.A	32.6	17.3	28.4	1.6
	10% NH_4OH	36.4	19.8	25.6	1.3
	10% $Ca(OH)_2$	34.4	16.8	24.3	1.1
T-test		**0.068**	**0.433**	**0.208**	**0.705**

Table 4.9 Compositions of corn stover pretreated with different chemicals and different pretreatment methods/T-test evaluation comparing RSE and AST to CSTR

Chemical		Glucan(%by mass)	Xylan(%by mass)	Lignin(%by mass)	Ash(%by mass)
CSTR pretreatment at 180^{o}C	H_2O	34.0	4.2	34.8	3.0
	10% A.A	49.8	0.0	38.0	3.4
	10% NH_4OH	42.3	13.1	21.5	2.0
	10% $Ca(OH)_2$	16.7	4.2	11.4	2.7
RSE pretreatment at 180^{o}C	H_2O	35.9	19.7	20.3	2.7
	10% A.A	13.8	3.4	56.2	8.5
	10% NH_4OH	34.5	19.6	20.6	2.4
	10% $Ca(OH)_2$	29.7	16.5	15.6	2.0
T-test		**0.440**	**0.095**	**0.881**	**0.502**
AST pretreatment	H_2O	33.5	17.3	21.1	3.4
	10% A.A	32.6	17.3	28.4	1.6
	10% NH_4OH	36.4	19.8	25.6	1.3
	10% $Ca(OH)_2$	34.4	16.8	24.3	1.1
T-test		**0.848**	**0.005**	**0.812**	**0.177**

Table 4.10 Compositions of corn stover pretreated with different chemicals and different pretreatment methods/T-test evaluation comparing AST to RSE

Chemical		Glucan(%by mass)	Xylan(%by mass)	Lignin(%by mass)	Ash(%by mass)
RSE pretreatment at 180^{o}C	H_2O	35.9	19.7	20.3	2.7
	10% A.A	13.8	3.4	56.2	8.5
	10% NH_4OH	34.5	19.6	20.6	2.4
	10% $Ca(OH)_2$	29.7	16.5	15.6	2.0
AST pretreatment	H_2O	33.5	17.3	21.1	3.4
	10% A.A	32.6	17.3	28.4	1.6
	10% NH_4OH	36.4	19.8	25.6	1.3
	10% $Ca(OH)_2$	34.4	16.8	24.3	1.1
T-test		**0.302**	**0.468**	**0.739**	**0.255**

Table 4.11 Mean and standard deviation analysis for glucose, xylose, lignin and ash contents for biomass pretreated with deionized water

Sample	Temp/ ^{o}C	Glucose				Xylose			
		1	2	mean	Std. Dev	1	2	mean	Std. Dev
WC	90	0.176	0.156	0.166	0.014	0.089	0.066	0.078	0.016
WC	180	0.196	0.189	0.193	0.005	0.081	0.055	0.068	0.018
SG	90	0.142	0.141	0.142	0.001	0.078	0.071	0.075	0.005
SG	180	0.159	0.138	0.149	0.015	0.085	0.078	0.082	0.005
CS	90	0.143	0.134	0.139	0.006	0.078	0.083	0.081	0.004
CS	180	0.168	0.157	0.163	0.008	0.077	0.085	0.081	0.006
WS	90	0.148	0.142	0.145	0.004	0.072	0.072	0.072	0.000
WS	180	0.161	0.151	0.156	0.007	0.072	0.066	0.069	0.004
SSB	90	0.080	0.142	0.111	0.044	0.031	0.059	0.045	0.020
SSB	180	0.163	0.172	0.168	0.006	0.062	0.047	0.055	0.011

Table 4.11 Mean and standard deviation analysis for glucose, xylose, lignin and ash contents for biomass pretreated with deionized water (cont.)

Sample	Temp/°C	Lignin				Ash			
		1	2	mean	Std. Dev	1	2	mean	Std. Dev
WC	90	0.226	0.338	0.282	0.080	0.011	0.006	0.008	0.004
WC	180	0.258	0.314	0.286	0.039	0.006	0.002	0.004	0.002
SG	90	0.243	0.328	0.286	0.060	0.031	0.019	0.025	0.009
SG	180	0.259	0.321	0.290	0.044	0.027	0.022	0.025	0.004
CS	90	0.205	0.258	0.232	0.038	0.026	0.015	0.021	0.007
CS	180	0.198	0.276	0.237	0.055	0.025	0.011	0.018	0.010
WS	90	0.269	0.325	0.297	0.040	0.018	0.027	0.023	0.006
WS	180	0.286	0.350	0.318	0.046	0.020	0.024	0.022	0.003
SSB	90	0.264	0.334	0.299	0.050	0.031	0.027	0.029	0.003
SSB	180	0.277	0.319	0.298	0.029	0.029	0.035	0.032	0.004

Table 4.12 Ethanol conversion efficiencies of biomass pretreated by different chemicals in the ASE

Pretreatment method	Sample	Temp/ °C	Total glucose in ferm. Broth (g/50ml)	Theoretical ETOH yield (g/50 ml)	Exp. ETOH yield (g/100 ml)	Conversion Efficiency (%)
ASE deionized water	WC	90	2.473	1.261	0.052	2.1
	WC	180	2.817	1.436	0.340	11.8
	SG	90	2.000	1.020	0.164	8.0
	SG	180	2.277	1.161	0.419	18.0
	CS	90	2.115	1.079	0.502	23.3
	CS	180	2.384	1.216	0.673	27.7
	WS	90	2.116	1.079	0.201	9.3
	WS	180	2.318	1.182	0.387	16.4
	SSB	90	1.145	0.584	0.170	14.5
	SSB	180	2.339	1.193	0.497	20.8
ASE 10% acetic acid	WC	90	2.333	1.190	0.113	4.7
	WC	180	3.734	1.904	1.247	32.7
	SG	90	2.132	1.087	0.447	20.6
	SG	180	3.164	1.614	0.836	25.9
	CS	90	1.953	0.996	0.271	13.6
	CS	180	3.896	1.987	0.081	2.0
	WS	90	1.981	1.010	0.121	6.0
	WS	180	3.397	1.732	0.172	5.0
	SSB	90	2.158	1.101	0.829	37.7
	SSB	180	3.522	1.796	1.275	35.5

Table 4.12 Ethanol conversion efficiencies of biomass pretreated by different chemicals in the ASE (cont.)

ASE 10% NH_4OH	WC	90	2.504	1.277	0.343	13.4
	WC	180	2.667	1.360	0.937	34.4
	SG	90	2.368	1.207	1.008	41.7
	SG	180	2.969	1.514	1.537	50.8
	CS	90	2.360	1.204	1.292	53.7
	CS	180	2.994	1.527	1.152	37.7
	WS	90	2.279	1.162	0.506	21.8
	WS	180	3.139	1.601	1.423	44.4
	SSB	90	2.515	1.283	0.159	6.2
	SSB	180	3.090	1.576	1.325	42.0
ASE 30% Ethanol	WC	90	2.475	1.262	0.373	14.8
	WC	180	2.571	1.311	0.511	19.5
	SG	90	2.056	1.048	0.597	28.5
	SG	180	1.767	0.901	0.687	38.1
	CS	90	2.128	1.085	1.079	49.7
	CS	180	2.088	1.065	1.207	56.7
	WS	90	2.082	1.062	0.914	43.0
	WS	180	2.164	1.103	0.785	35.6
	SSB	90	2.169	1.106	0.810	36.6
	SSB	180	2.211	1.128	0.799	35.4

Table 4.13 Ethanol conversion efficiencies for biomass pretreated by AST using different chemicals

Pretreatment method	Chemical	Total glucose in ferm. Broth(g/50ml)	Theoretical ETOH yield(g/100 ml)	Exp. ETOH yield(g/100 ml)	Conversion Efficiency(%)
Switchgrass	10%A. A	2.005	1.023	0.241	11.8
	10% NH_4OH	2.118	1.080	0.656	30.4
	10% $Ca(OH)_2$	1.833	0.935	0.545	29.2
	H_2O	1.874	0.956	0.179	9.4
Corn stover	10%A. A	1.814	0.925	0.228	12.3
	10% NH_4OH	2.024	1.032	0.474	23.0
	10% $Ca(OH)_2$	1.912	0.975	0.470	24.1
	H_2O	1.863	0.950	0.253	13.3
Wheat straw	10%A. A	1.915	0.977	0.652	33.4
	10% NH_4OH	1.921	0.980	0.748	38.2
	10% $Ca(OH)_2$	1.628	0.830	0.669	40.3
	H_2O	1.944	0.991	0.097	4.9
Sweet sorghum bagasse	10%A. A	1.885	0.961	0.547	28.4
	10% NH_4OH	2.003	1.022	0.968	47.4
	10% $Ca(OH)_2$	1.729	0.882	0.664	37.6
	H_2O	1.909	0.974	0.214	11.0

Table 4.14 Ethanol conversion efficiencies for biomass pretreated by CSTR and RSE using different chemicals

Pretreatment method	Chemical	Total glucose in ferm. Broth(g/50ml)	Theoretical ETOH yield(g/50 ml)	Exp. ETOH yield(g/100 ml)	Conversion Efficiency(%)
CSTR for corn stover at 180°C	H_2O	1.887	0.962	0.076	3.9
	10% A.A	2.767	1.411	0.354	12.5
	10% NH_4OH	2.351	1.199	2.044	85.2
	10% $Ca(OH)_2$	0.926	0.472	0.333	35.3
RSE for corn stover at 180°C	NH_4OH	1.915	0.977	0.400	20.5
	$Ca(OH)_2$	1.649	0.841	0.258	15.3
	H_2O	1.994	1.017	0.898	44.1
RSE for switchgrass at 180°C	$Ca(OH)_2$	1.672	0.853	1.291	75.7
	H_2O	2.133	1.088	0.540	24.8
	Acetic Acid	2.106	1.074	0.603	28.1

Table 4.15 Hemicellulose extractives from the liquid stream of ASE pretreated biomass using different chemicals at 90°C and 180°C

Sample		Temp/°C	Hemicellulose weight
Deionized water	WC	90	0.000
	WC	180	0.019
	SG	90	0.019
	SG	180	0.065
	CS	90	0.025
	CS	180	0.097
	WS	90	0.068
	WS	180	0.069
	SSB	90	0.044
	SSB	180	0.108
Acetic Acid	WC	90	0.006
	WC	180	0.016
	SG	90	0.016
	SG	180	0.053
	CS	90	0.033
	CS	180	0.093
	WS	90	0.031
	WS	180	0.044
	SSB	90	0.047
	SSB	180	0.082
NH_4OH	WC	90	0.006
	WC	180	0.060
	SG	90	0.038
	SG	180	0.180
	CS	90	0.060
	CS	180	0.245
	WS	90	0.042
	WS	180	0.160
	SSB	90	0.204
	SSB	180	0.392

Table 4.15 Hemicellulose extractives from the liquid stream of ASE pretreated biomass using different chemicals at 90°C and 180°C (cont.)

Ethanol	WC	90	0.002
	WC	180	0.022
	SG	90	0.018
	SG	180	0.032
	CS	90	0.032
	CS	180	0.062
	WS	90	0.030
	WS	180	0.036
	SSB	90	0.050
	SSB	180	0.083

CHAPTER 5

Conclusion

The production of ethanol from non-edible biomass resources is largely proven to be possible after the recalcitrance of the binding components in lignocellulosics is eased. Several pretreatment methods including accelerated solvent extraction (ASE), continuous stirred tank reactor (CSTR), reactive screw extrusion (RSE) pretreatment and ambient storage tank (AST) pretreatment with different chemicals including deionized water and aqueous solutions of acetic acid, ammonia hydroxide and lime were investigated to treat biomass materials for enhancing the cellulosic ethanol production.

The pretreatment of different biomass materials including corn stover, wheat straw, woody biomass, sweet sorghum bagasse and switch grass using the ASE with a 10% acetic acid aqueous solution at 180^{o}C resulted in total solubilization of hemicellulose fractions in all biomass samples into the liquid extract stream. The biomass samples pretreated with the acetic acid solution at 180^{o}C have higher glucan content than the samples pretreated with other aqueous solutions at the same condition. Temperature had a significant effect on the solubilization of hemicellulose and the glucan content of the pretreated biomass for the acetic acid aqueous pretreatment. It was found that an acetic acid solution at 90^{o}C could not hydrolyze all hemicellulose from the biomass samples. The glucan content of the pretreated biomass increased significantly for the acetic acid pretreatment when the temperature increased from 90^{o}C to 180^{o}C. However, the increase of pretreatment temperature from 90^{o}C to 180^{o}C had no significant effect on

the glucan content of all biomass samples pretreated with 30% ethanol aqueous solution and deionized water. The ASE pretreatment with a 10% ammonium hydroxide aqueous solution at 180^{o}C also significantly increased the glucan content of all biomass samples while the ammonium hydroxide pretreatment at the temperature of 90^{o}C generated much lower glucan contents.

For the ASE pretreatment with different aqueous solutions, the biomass samples pretreated with deionized water at both 90^{o}C and 180^{o}C gave the lowest glucan to ethanol conversion efficiencies. This confirms that the pretreatment with deionized water is an effective method to prepare biomass materials for the further enzymatic hydrolysis and ethanol fermentation. Although the glucan contents of the biomass pretreated by ASE with a 10% acetic acid aqueous solution were high, the glucan to ethanol conversion efficiencies of the acetic acid pretreated biomass samples during SSF were moderate. The formation of inhibitors as a result of the acetic acid pretreatment may be attributed to the low conversion efficiency.

Relatively moderate to high glucan to ethanol conversion efficiencies were obtained for the biomass pretreated with 10% NH_4OH aqueous solution. The conversion efficiencies increased with the increase of pretreatment temperature. The higher glucan to ethanol conversion for the ammonium hydroxide pretreatment could be attributed to the effective delignification of biomass after alkali pretreatment, exposing the cellulose fractions to effective enzymatic hydrolysis and fermentation in the SSF process.

The ASE pretreatment with an ethanol solution resulted in relatively moderate to high glucan to ethanol conversion ratios. The organosolv process with the ethanol solution

generates relatively purer cellulose fractions after pretreatment. The less formation of inhibitors during ethanol pretreatment probably contributed to the relatively high conversion efficiency as compared to biomass pretreated with the deionized water.

Statistical analyses showed that the ASE pretreatment with all aqueous solutions resulted in significant hemicellulose fractionation into the liquid stream. These results can be ascertained by the higher xylan and glucan contents of the biomass samples obtained after pretreatment compared to those of the untreated complements. Hemicellulose solvation however varied depending on the chemicals used. T-test analysis shows that for the ASE pretreatment with ethanol, acetic acid, ammonium hydroxide and deionized water at 90^{o}C the corresponding P-values were 0.010, 0.013, 0.020 and 0.021 which indicates the declining significance of the pretreating solvents for hemicellulose fractionation at the pretreatment temperature of 90^{o}C. Biomass pretreatment at 90^{o}C in the ASE had no significant effect on cellulose and lignin fractionation for all chemicals used in the pretreatment methods.

ASE pretreatment of biomass at 180^{o}C resulted in significant solvation of hemicellulose fractions for all chemicals used in the pretreatment process. The fractionation of cellulose was significantly achieved by all chemicals except deionized water and aqueous ethanol solution. T-test analyses gave a P-value of 0.082>0.05 for the deionized water and 0.502>0.05 for aqueous ethanol pretreatment which means that the efficacy of both the deionized water and ethanol solution for cellulose fractionation at 180^{o}C is insignificant. Acetic acid pretreatment resulted in the highest glucan fractionation followed by 10% ammonium hydroxide and then 30% ethanol solution. P-values after T-test analysis of

pretreatment significance for all biomass pretreated by the ASE at 180^{o}C showed that hemicellulose fractionation was best achieved by 10% acetic acid (P-value: 8.4×10^{-6}) followed by 10% ammonium hydroxide (P-value: 0.004), 30% ethanol solution (P-value: 0.009) and then deionized water (P-value: 0.017). T-test analysis for the pretreatment methods on lignin fractionation at 180^{o} showed no significant results for all chemicals used in the ASE. However the P-value for 10% ammonium hydroxide pretreatment was lower than all other pretreatment chemical, confirming the superior ability of alkali's for lignin solvation.

Conversion analyses for AST pretreatment of biomass using different chemicals showed that higher glucan to ethanol conversion efficiencies were obtained for alkali pretreated biomass samples over acidic and deionized water pretreatments. The conversion efficiencies for both $Ca(OH)_2$ and NH_4OH pretreated biomass samples were high. The high conversion efficiency of alkali pretreated biomass at room temperature is due to the ability of alkaline solvents to fractionate lignin effectively and decrease the cellulose crystallinity of biomass at low temperatures. Alkali pretreatment also leads to the reduction of various uronic acid substitutions in cellulose that may be inhibitory to downstream processes.

Pretreatment of corn stover in the CSTR with 10% aqueous ammonium hydroxide solution resulted in a very high glucan-ethanol conversion efficiency of 85.2%. The pretreatment with 10% aqueous calcium hydroxide solution resulted in 35.3% conversion efficiency while the conversion efficiencies were only 12.5% and 3.9% for the samples pretreated with acetic acid and deionized water respectively.

RSE pretreatment of biomass is an ideal industrial process since it can be operated in a continuous mode. However the ethanol yields form the SSF of biomass materials pretreated by RSE at a solid concentration og 10 g/100 ml were relatively low compared to the biomass pretreated by ASE and CSTR. Ethanol yields from the SSF of corn stover pretreated by RSE were 0.898 g/100 ml for the pretreatment with H_2O, 0.400 g/100 ml for the pretreatment with NH_4OH, 0.850 g/100 ml for the pretreatment with acetic acid and 0.258 g/100 ml for the pretreatment with $Ca(OH)_2$.

The biomass pretreatment with a 10% NH_4OH aqueous solution resulted in the highest mass fraction of separated hemicellulose in the liquid stream after liquid-liquid extraction using 95.5% ethanol solution. Since alkali's are powerful to dissolve lignins, the isolation of pure hemicellulose from the extracts after the liquid-liquid extraction process needs to be investigated and optimized to eliminate contaminating lignin fractions.

REFERENCES

[1] P.A. Miller, "Exxon Valdez oil spill: Ten years later. Technical background paper for Alaska Wilderness League," *Artic Connection*, 1999. *Available at* http://arcticcircle.uconn.edu/SEEJ/Alaska/miller2.htm

[2] S. Pappas, "Gulf oil spill update: Just the facts," Science on MSNBC.com, *Available at* http://www.msnbc.msn.com/id/38063264/ns/technology_and_science-science

[3] Bureau of Labor Statistics. Historical Crude Oil prices table. *Available at* http://www.inflationdata.com/inflation/inflation_rate/historical_oil_prices_table.asp 2010.

[4] L.A. Ruth, The U.S. Department of Energy's combustion 2000 program, "Clean, efficient electricity from coal," *Energy Covers. Mgmt,* vol. 38, pp. 1249 – 1257, 1997.

[5] C.W. Babbit and A.S. Linder, "A life cycle inventory of coal used for electricity production in Florida," *Journal of Cleaner Production,* vol.13, pp. 903 – 912, 2005.

[6] J. Hettenhaus, "Biomass commercialization and agricultural residue collection," in *Biorefineries-Industrial Processes and Products, Vol. 1,* ed. B. Kamm, P, R. Gruber, and M. Kamm. Weinheim: Wiley-VCH Verlag GmbH & Co. 2006.

[7] L. Wang and C.L. Weller, "Recent advances in nutraceuticals from plants," *Trends in Food Science and Technology*, vol. 17, pp. 300 – 312, 2006

[8] L. Wang, A. Kumar, C.L. Weller, D.D. Jones, and M.A. Hanna, "Co-production of chemical and energy products from distillers grains using supercritical fluid extraction and thermochemical conversion technologies," *American Society of Agricultural and Biological Engineers.* 2007, Paper number: 076064

[9] W. H. Talib and A.M. Mahasneh, "Antiproliferative activity of plant extracts used against cancer in traditional medicine," *Scientia Pharmaceutica* vol. 78, pp. 33-45, 2010.

[10] M.Carraz, A. Jossang, J.F. Franetich, A. Siau, L. Ciceron, L. Hannoun, R. Sauerwein, F. Frappier, P. Rasoanaivo, G. Snounou, and D. Mazier, "A plant-derived morphinan as a novel lead compound active against malaria liver stages," *PLoS Medicine,* vol. 3, no. 12, pp. 2392-2402, 2006.

[11] L. Cuiping, Yanyongjie, W. Chuangzhi, and H. Haitao, "Study on the distribution and quality of biomass residues resource in China," *Biomass and Bioenergy*, vol. 27, pp. 111 – 117, 2004

[12] A. Demirbas, "Biomass resources facilities and biomass conversion processing for fuels and chemicals," *Energy Conversion and Management.*, vol. 42, pp. 1357 – 1378, 2001.

[13] M. Parikka, "Global biomass fuel resources," *Biomass and Bioenergy*, vol. 27, pp. 613 –620, 2004.

[14] K.L. Kadam and J.D. McMillan, "Availability of corn stover as a sustainable feedstock for bioethanol production," *Bioresource Technology*, vol. 88, pp. 17 – 25, 2003.

[15] R.Gupta and Y.Y. Lee, "Investigation of biomass degradation mechanism in pretreatment of Switchgrass by aqueous ammonia and sodium hydroxide," *Bioresource Technology*. 2010, in press.

[16] X. Pan and Y. Sano, "Fractionation of wheat straw by atmospheric acetic acid process," *Bioresource Technology*, vol. 96, pp. 1256 – 1263, 2005.

[17] B. Sipos, J. Reczey, Z. Somorai, Z. Kadar, D. Dienes, and K. Reczey, "Sweet sorghum as feedstock for ethanol production: enzymatic hydrolysis of steam-pretreated bagasse," *Appl. Biochem. Biotechnol*, vol. 153, pp. 151 – 162, 2009.

[18] R. Deepak, Keshwani, and J.J. Cheng, "Switchgrass for bioethanol and other value-added applications: a review," *Bioresource Technology*, vol. 100, pp. 1515 – 1523, 2009.

[19] R. Gupta and Y.Y. Lee, "Investigation of biomass degradation mechanism in pretreatment of Switchgrass by aqueous ammonia and sodium hydroxide," *Bioresource Technology*, doi: 10.1016/j. biortech.2010.05.039, 2010.

[20] P. Borjesson, L. Gustavsson, L. Christersson and S. Linder, "Future production and utilization of biomass in Sweden: Potentials and CO_2 mitigation," *Biomass and Bioenergy*, vol. 13, pp. 399 – 412, 1997.

[21] B.M Jenkins, L.L. Baxter, T.R. Miles Jr., and T.R. Miles, "Combustion properties of biomass," *Fuel Processing Technology*, vol. 54, pp. 17 – 46, 1998.

[22] J.M. Fang, R.C. Sun, D. Salisbury, P.F. Fowler, and J. Tomkinson, "Comparative study of hemicellulose from wheat straw by alkali and hydrogen peroxide extractions," *Polymer Degradation and Stability*, vol. 66, pp. 423 – 432, 1999.

[23] K. E Hammel, "Fungal degradation of lignin," in Driven by Nature: Plant Litter Quality and Decomposition (eds. G. Cadisch and K.E. Giller). CAB INTERNATIONAL, 1997.

[24] E. Minami and S. Saka, "Biomass resources in Japan – annual quantities grown, unused and wasted," *Biomass and Bioenergy*, vol 29, pp. 310 – 320, 2005.

[25] M.M. Kucuk and A. Dermibas, "Biomass conversion processes," *Energy Convers. Mgmt*, vol. 38, no. 2, pp. 151 – 165, 1997.

[26] H.B. Goyal, D. Seal, and R.C. Saxena. "Bio-fuels from thermochemical conversion of renewable resources: A review," *Renewable and Sustainable Energy Reviews*, vol. 12, pp. 504 – 517, 2008.

[27] P. McKendry, "Energy production from biomass (part 2): conversion technologies," *Bioresource Technology*, vol. 83, pp. 47 – 54, 2002.

[28] P. McKendry, "Energy production from biomass (part 3): gasification technologies," *Bioresource Techology*, vol. 83, pp. 55 – 63, 2002.

[29] A. K. Rajvanshi, "Biomass gasification," in *Alternative Energy in Agriculture* (ed. Y. Goswami). CRC Press 1986.

[30] J. Singh and S. Gu, "Biomass conversion to energy in India – A critique," *Renewable and Sustainable Energy Reviews*, vol. 14, pp. 1367 – 1378, 2010.

[31] L. Qiang, L.Wen-Zhi, and Z. Xi-Feng, "Overview of fuel properties of biomass fast pyrolysis oils," *Energy conversion and management* , pp. 1376-1383, 2009.

[32] Z. Qi, C. Jie, W. Tiejun, and X. Ying, "Review of biomass pyrolysis oil properties and upgrading research," *Energy Conservation and Management*, vol. 48, pp. 87 – 92, 2007.

[33] A. C. Basagiannis and X. E. Verykios, "Steam reforming of the aqueous fraction of bio-oil over structured $Ru/MgO/Al_2O_3$ catalyst," *Catalysis Today*, vol. 127, pp. 256 – 264, 2007.

[34] K. Takanabe and K. Aika, "Sustainable hydrogen from bio-oil catalytic steam reforming of acetic acid as a model oxygenate," *Prepr. Pap.-Am. Chem. Soc., Div Fuel Chem*, vol. 49, no. 2, pp. 807, 2004.

[35] X. Jiang and N. Ellis, "Upgrading bio-oil through emulsification with biodiesel: mixture production," *Energy fuels*, vol. 24, pp. 1358 – 1364, 2010.

[36] R.C. Sun and J. Tomkinson, "Characterization and ultrasonically assisted extractions from wheat straw," *Carbohydrate Polymers*, vol. 50, pp. 263 – 271, 2002.

[37] S.K. Bhaduri, L.N. Ghosh, and N.L. Deb Sarkar, "Ramie hemicellulose as beater additive in paper making from jute-stick kraft pulp," *Industrial crops and products*, vol. 4, pp. 79 – 84, 1995.

[38] L. Wang, C. Weller and K.T. Hwang, "Extraction of lipids from grain sorghum," *American Society of Agricultural and Biological Engineers*, Paper number: 057054, 2005.

[39] R.C. Sun and J. Tomkinson, "Comparative study of lignins isolated by alkali and ultrasound-assisted alkali extractions from wheat straw," *Ultrasonics Sonochemistry* ,vol. 9, pp. 85 – 93, 2002.

[40] A. U. Buranov and G. Mazza, "Extraction and characterization of hemicelluloses from flax shives by different methods," *Carbohydrate Polymers*, vol. 79, pp. 17 – 25, 2010.

[41] R.C. Sun, J.M. Lawther, W.B. Banks and B. Xiao, "Effect of extraction procedure on the molecular weight of wheat straw lignins," *Industrial Crops and Products*, vol. 6, pp. 97 – 106, 1997.

[42] N.T. Dunford, S. Irmak, and R. Jonnala, "Pressurize solvent extraction of policosanol from wheat straw, germ and bran," *Food Chemistry*, vol. 119, pp. 1246 – 1249, 2010.

[43] L. Wang, C. Weller, and K.T. Hwang, "Extraction of lipids from grain sorghum," *American Society of Agricultural and Biological Engineers*, Paper number: 057054, 2005.

[44] C. Hongzhang and L. Liying, "Unpolluted fractionation of wheat straw by steam explosion and ethanol extraction," *Bioresource Technology*, vol. 98, pp. 666 – 676, 2007.

[45] P. Kumar, D.M. Barrett. M.J. Delwiche and P. Stroeve, "Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production," *Ing. Eng. Chem. Res*, vol.48, pp. 3713-3729, 2009.

[46] M.J. Taherzadeh and K. Karimi, "Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review," *Int. J. Mol. Sci*, vol. 9, pp. 1621 – 1651, 2008.

[47] A.T.W.M. Hendriks and G. Zeeman, "Pretreatments to enhance the digestibility of lignocellulosic biomass," *Bioresource Technology*, vol. 100, pp. 10 – 18, 2009.

[48] Y. Sun and J. Cheng, "Hydrolysis of lignocellulosic materials for ethanol production: a review," *Bioresource Technology*, vol. 83, pp. 1 – 11, 2002.

[49] N. Mosier, C. Wyman, B. Dale, R. Elander, Y.Y. Lee, M. Holtzapple, and M. Ladisch, "Features of promising technologies for pretreatment of lignocellulosic biomass," *Bioresource Technology*, vol. 96, pp. 673 – 686, 2005.

[50] J. Xu, M.H. Thomsen, and A.B. Thomsen, "Enzymatic hydrolysis and fermentability of corn stover pretreated by lactic acid and/or acetic acid," *Journal of Biotechnology*, vol. 139, pp. 300 – 395, 2009.

[51] F. Teymouri, L. Laureano-Perez, H. Alizadeh and B.E. Dale, "Ammonia fiber explosion treatment of corn stover," *Applied Biochemistry and Biotechnology*, vol. 113 – 116, pp. 951-953, 2004.

[52] Y. Zheng, H.M. Lin and G. T. Tsao, "Pretreatment for cellulose hydrolysis by carbon dioxide explosion," *Biotechnol. Prog*, vol. 14, pp. 890 – 896, 1998.

[53] M.T. Garcia-Cubero, G. Gonzalez-Benito, I. Indacoechea, M. Coca, and S. Bolado, "Effect of ozonolysis pretreatment on enzymatic digestibility of wheat and rye straw," *Bioresource Technology*, vol. 100, pp. 1608 – 1613, 2009.

[54] M. Ohkuma, Y. Maeda, T. Johjima and T. Kudo, "Lignin degradation and roles of white rot fungi: study on an efficient symbiotic system in fungus-growing termites and its application to bioremediation," *RIKEN Review No. 42*. 2002.

[55] H.J. Gilbert and G.P. Hazelwood, "Bacterial cellulases and xylanases," *J. Gen. Microbial*, vol. 139, pp. 187 – 194, 1993.

[56] S.J.B. Duff and W. D. Murray, "Bioconversion of Forest products industry waste cellulosics to fuel ethanol: a review," *Bioresource Technology*, vol. 55, pp. 1 – 33, 1996.

[57] J.D Wright, "Ethanol from lignocellulosic biomass," *Biores. Technol*, vol. 50, pp. 3 – 16, 1988.

[58] W. Qin, "High consistency enzymatic hydrolysis of lignocellulose," Unpublished masters research thesis. *Available at* http://www.swst.org/wfs/PDF%20Files/ubc_2010_fall_qin_wenjuan.pdf

[59] L. Olsson and B. Hahn-Hagerdal, "Fermentation of lignocellulosic hydrolyzates for ethanol production," *Enzyme and Microbial Technology*, vol. 18, pp, 312 – 331, 1996.

[60] L. Olsson, H.R. Soerensen, B.P. Dam, H. Christensen, K. M. Krogh and A. S. Meyer, "Separate and simultaneous enzymatic hydrolysis and fermentation of wheat hemicellulose with recombinant xylose utilizing *Saccharomyces cerevisiae*," *Applied Biochemistry and Biotechnology*, vol. 129-132, pp. 117 – 129, 2006.

[61] G.R. Cysewski and C.R. Wilke, "Rapid ethanol fermentation using vacuum and cell recycle," *Biotechnology and Bioengineering*, vol. xix, pp. 1125 – 1143, 1977.

[62] M. Matsumura and H. Markl, "Elimination of ethanol inhibition by perstraction," *Biotechnology and Bioengineering*, vol xxviii, pp. 534 – 541, 1986.

[63] M. Oliet, J. Garcia, F. Rodriguez, and M.A. Gilarrranz, "Solvent effects in autocatalyzed alcohol-water pulping," Comparative study between ethanol and methanol as delignifying agents. *Chemical Engineering Journal*, vol 87, pp. 157 – 162, 2002.

[64] H.L. Chum, D.K. Johnson and S.K. Black, "Organosolv pretreatment for enzymatic hydrolysis of poplars 2. Catalyst effects and the combined severity parameter," *Ind Eng Chem Res*, vol. 29, No. 2, 1990.

[65] S. Kim and M. T. Holtzapple, "Lime pretreatment of corn stover," *Bioresource Technology*, vol. 96, pp. 1994 – 2006, 2005.

[66] J. Xu, J.J. Cheng, R.R. Sharma-Shivappa, and J.C. Burns, "Lime pretreatment of Switchgrass at mild temperatures for ethanol production," *Bioresource Technology*, vol. 101, pp. 2900 – 2903, 2010.

[67] R. Burton. Dept. of Biology, Alverno College, "Using Microsoft Excel to do basic statistical test," *Available at* http://depts.alverno.edu/nsmt/stats.htm

[68] P. McKendry, "Energy production from biomass (part 1): overview of biomass," *Bioresource Technology*, vol. 83, pp. 37 – 46, 2002.