

Supercritical Carbon Dioxide Pretreatment of Various Lignocellulosic Biomasses

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Naveen Narayana Swamy

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This thesis titled
Supercritical Carbon Dioxide Pretreatment of Various Lignocellulosic Biomasses

by
NAVEEN NARAYANA SWAMY

has been approved for
the Department of Chemical and Biomolecular Engineering
and the Russ College of Engineering and Technology by

Tingyue Gu
Associate Professor of Chemical and Biomolecular Engineering

Dennis Irwin
Dean, Russ College of Engineering and Technology

ABSTRACT

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Supercritical Carbon Dioxide Pretreatment of Various Lignocellulosic Biomasses

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In the production of cellulosic ethanol, the pretreatment of biomass step is considered the most expensive and difficult part of the process. In Supercritical CO₂ (SC-CO₂) pretreatment method, CO₂, which is considered a green solvent is used to treat the biomass. In this work corn stover, switchgrass and rye straw were pretreated using the SC-CO₂ at various temperatures and pressures and subsequently enzyme hydrolyzed using the cellulase enzyme. The samples were analyzed for the presence of glucose. A typical CO₂ to biomass ratio of 5/50 (g/g) was used in tests. Biomass samples were wetted with water prior to the SC-CO₂ treatment. CO₂ pressure was released as quickly as possible by opening a quick release valve. For all pretreatments glucose yields from corn stover was higher than untreated samples (12mg/100mg biomass) and the maximum glucose yield (30mg/100mg biomass) was found at 3500psi and 150°C. These conditions were chosen for the pretreatment of various other biomasses. The maximum glucose yield for untreated switchgrass and rye straw were found to be 12mg/100mg biomass and 7.6mg/100mg biomass, respectively. The pretreated switchgrass (14mg/100mg of biomass) showed no improvement in the glucose yield as compared to an untreated sample. However, the pretreated corn stover (30mg/100mg biomass) and rye straw (13.5mg/100mg biomass) showed threefold and twofold increase, respectively. The SC-

CO₂ pretreatment with addition of catalysts such as H₂SO₄ and HCl was studied on rye straw. The addition of H₂SO₄ and HCl to the SC-CO₂ pretreatment both improved the glucose yield as compared with the SC-CO₂ pretreatment. The X-Ray diffraction result showed that there was no change in crystallinity of the SC-CO₂ treated corn stover when compared to the untreated. SEM results showed the changes in surface morphology of the SC-CO₂ treated corn stover when compared with untreated corn stover. This shows that the increase in glucose yield from enzyme hydrolysis for the SC-CO₂ treated corn stover is due to increase in surface area. Carbonic acid (a weak acid) from dissolved CO₂ in water phase may also contribute.

Approved: _____

Tingyue Gu

Associate Professor of Chemical and Biomolecular Engineering

DEDICATION

My Parents

&

Jamie and Rob Klinger

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STATEMENT OF PURPOSE

The pretreatment of biomass has received much attention due to the fact that it accounts for 20% of the total cost of the cellulosic ethanol production process. Several pretreatment methods have been investigated in the effort to create an effective and environment friendly method. The predominantly used methods at a pilot plant scale are acid hydrolysis and ammonia treatment. The acid hydrolysis method uses sulfuric acid to treat the biomass and therefore needs corrosion resistant reactors and accessories. The biomass hemicellulose sugars in the biomass dissolve in the acid solution during the pretreatment, and thereby, some sugars are transferred to hydrozylate. These sugars need to be recovered for the process to be efficient which requires acids in hydrozylate to be neutralized by adding lime which results in the formation of gypsum. This accounts for an additional process step and additional cost for gypsum disposal. In the Ammonia fiber explosion (AFEX) pretreatment method ammonia is used to treat the biomass. The used ammonia cannot be vented into the atmosphere after pretreatment as it pollutes the air and endangers equipment operators. Both pretreatment methods are not environment friendly and the requirement of special reactors increases the capital costs. Hence there is a need to find an alternate pretreatment method.

Supercritical carbon dioxide (SC-CO₂) pretreatment is one such method in which carbon dioxide (CO₂) is used to treat the biomass. CO₂ is considered as a green solvent because it is non-toxic. The previous work carried out using the SC-CO₂ used bagasse and recycled paper as raw materials for the pretreatment. There are only a few studies which report the SC-CO₂ method on lignocellulosic biomass. Therefore, the effectiveness of this method on lignocellulosic biomass needs to be explored. In the present work lignocellulosic biomass such as corn stover, rye straw and switchgrass were subjected to the SC-CO₂ pretreatment method. The objective of the present study was to study the effectiveness of the SC-CO₂ method on lignocellulosic biomass by measuring fermentable sugar yields after cellulase enzyme digestion.

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CHAPTER 1: INTRODUCTION

The world's gasoline consumption in the year 2006 was 600 billion gallons and in the U.S.A., the transportation sector consumed approximately 140 billion gallons per year [1], which makes up 40% of the country's annual energy requirements [2]. The U.S.A. annual hydrocarbon consumption exceeds its production and to compensate for this, the country imports large quantities of oil from many countries. The largest exporters of hydrocarbons to the U.S.A. are Canada, Saudi Arabia, Mexico and Venezuela [3]. Petroleum products are increasingly in demand because China's and India's oil consumptions have climbed exponentially over the past few years. The oil dependency of the U.S.A. costs them \$233 billion dollars yearly [4]. The gasoline price in the year 2000 was \$1.51 per gallon and in the year 2007 was \$2.80 per gallon, which results in a price increase of 80% in a span of seven years [3]. The total carbon dioxide (CO₂) emission from hydrocarbon usage in 2006 around the world was 29195.42 million metric tons, of which the U.S.A. contributed 5902.75 million metric tons [5; 6].

The planet's petroleum reserves are estimated to last for another 50 years, taking into account the present rate of consumption [7]. In 1998, Campbell and Laherrere predicted a decline in oil production from 25 billion barrels per year to 5 billion barrels by the year 2050 [8]. Also, the political turmoil in the Middle East and other parts of the world has created an uneven production and supply of oil to countries that rely upon their crude oil supply, which causes an impact on the economies of those dependent countries. To overcome dependency on foreign oil imports, achieve economic stability, and reduce environment pollution, we must find new alternative transportation fuels that are produced locally and are environment friendly. We cannot count on one form of alternative energy to supply the world's energy needs as the demand is too high. Alternative fuels for gasoline should provide superior environmental benefits, economical incentives, and positive net energy values (NEV) when compared with gasoline [9]. It is especially beneficial to create energy from renewable sources such as the sun, wind, and biomass, all of which are readily available.

The primary goal of the present work is to investigate the effectiveness of supercritical carbon dioxide (SC-CO₂) pretreatment method on various biomasses. A brief introduction of the present biofuel consumption, the drawbacks of corn and sugar ethanol, and a note on various pretreatment methods for cellulosic biomass are discussed, followed by experimental methods. These topics give us a picture of the present state of cellulosic ethanol.

1.1 Biofuels

Biofuels are fuels which are derived from biomass and bio products. The various biofuels are ethanol, syn-oil, biodiesel and syn-gas. Liquid biofuels such as ethanol can be readily used in present day internal combustion engine vehicles (ICEVS) with a few modifications [10].

The advantages of biofuels are [6; 7]:

- i. Sustainability – The long term production and supply of biofuels can be achieved as the raw materials for biofuel production are abundantly available and locally produced.
- ii. Reduction in green house gases – The raw material for biofuels is produced from photosynthesis process which uses the atmospheric CO₂. Thus the net CO₂ emissions from biofuels are less when compared to those of the fossil fuels. Bioethanol is an oxygenated fuel composed of 35% of oxygen and reduces the particulates nitrogen dioxide (NO₂) [11], carbon monoxide and particulate emissions [12].
- iii. Regional development – The production of biofuels at the local level increases employment opportunities.

1.1.1 Syn-gas

The syn-gas is produced from biomass by the gasification process. The gasification process is carried out using Fischer Tropsch catalyzed reactions at high temperatures of 775 K to 1025 K (500°C to 750°C) with pressurization at about 20 bars to produce gas mixtures of hydrogen and carbon-monoxide [11]. These gases have high hydrogen content and high calorific value [7]. The reaction catalysts are prone to poisoning by alkali metals, halides, nitrogen and sulfur gases, which are sufficiently abundant in biomass. The tar formation during the process makes syn-gas less efficient, which is one of the drawbacks of this fuel [11].

1.1.2 Syn-oil

Syn-oil is a biofuel produced from biomass through the pyrolysis process. The biomass samples are treated at temperatures over 750 K (480°C) to form vapors with the residence time of 5 to 30 minutes. Cellulose in the biomass decomposes to produce gaseous products, which are later condensed to form syn-oil [8; 11]. Syn-oil contains more than 200 constituents including phenols, hydro-aldehydes, and carboxylic acids. Its complex nature, instability, corrosiveness, and high water content are the drawbacks of this fuel [11].

1.1.3 Bioethanol

Bioethanol, however, is the most dominant biofuel today [10]. It is derived from bio-materials such as corn and sugarcane. Bioethanol can be used as a fuel additive or as a fuel in the present day ICEVS. Gasoline with up to 10% bioethanol is already in use in the U.S.A.. The bioethanol concentration in the blend can be increased up to 85% with slight modifications to ICEVS. In Brazil, E-22 fuel (22% of ethanol blended gasoline) is widely used and makes up 70% of its total gasoline consumption [10]. Some automakers are working on cars which are capable of using bioethanol as fuel and in the near future it is possible that cars can run on bioethanol fuel (ethanol – 95% and gasoline – 5%).

1.2 Bioethanol from Corn and Sugar

The present-day bioethanol is produced from sugar, starch and sweet beet crops. Sugar and starch ethanol are both produced by the fermentation process and are called first generation biofuels. Sugar ethanol accounts for 60% of the world's annual production, with starch based ethanol accounting for 33%, and synthetic ethanol accounting for 7%. The bulk production and consumption of ethanol is by the U.S.A. and Brazil, where Brazil produces the ethanol from sugar crops and the U.S.A. produces it from corn starch. In 2005, 45 billion liters of ethanol was produced worldwide. Brazil's contribution was 17 billion liters from sugarcane, while the U.S.A. produced 18 billion liters from corn and France produced 629 million liters, followed by Spain with 520 million liters. Canada produced 245 million liters [10]. The current ethanol production in the U.S.A. is 5.12 billion gallons and its capacity is expected to increase by another 3.84 billion gallons. In 2005, 4 billion gallons of ethanol were produced using 1.43 billion bushels of corn, which is 13% of the total corn produced in the U.S.A. for that year [11; 13].

Around 2 trillion liters of bioethanol is required to replace the current worldwide gasoline consumption [6]. In the U.S.A., bioethanol production accounts for 4% of the country's annual gasoline consumption and is presently used as a gasoline additive. It can also be considered a partial replacement for gasoline. Bioethanol can act as a buffer energy supply considering its current production rates [14].

1.2.1 Limitations of Corn and Sugar Ethanol

In the present rates of production, bioethanol can act as a buffer energy source for a limited period of time. There has been an increase in the production of biofuels using corn, sugar, and oil seed crops [14]. Corn and sugarcane crops have high food value and their usage in ethanol production can increase the food prices [12]. In the U.S.A., corn is used as food grain and livestock feed, and also for the production of starch, corn oil, and alcoholic beverages. Besides, it accounts for 90% of feed grains produced. The U.S.A. produces 40% of the world's corn and accounts for 60% of the world's total exports [14]. The corn production in the U.S.A. and its usage for fuel ethanol are shown in Table 1. In the U.S.A., corn usage for ethanol production has increased by 400% from 2001 to 2008. The corn prices have reached a record high in the past 3 years and the price per bushel of corn reached \$3.40/bushel in 2007, compared to \$2/bushel in 2005 [15]. The increase in corn usage for ethanol production could be the reason for the sharp increase in prices.

The increase in demand and competitive pricing in the corn market have forced many farmers to turn to corn crops [6]. The producers of corn for ethanol compete for land with those wishing to grow the plants for food and feed products [8]. This leads to a decrease in the production of soybeans, cotton and other crops [16]. In the U.S.A., soybean planting area has in recent years decreased from 310,000 km² to 260,000 km², which accounts for 6% of the world's arable land, and resulting in an increase of soy prices [17]. However, increased use of corn for ethanol production drives up food prices and puts a heavy burden on soil nutrients.

There are around 850 million undernourished people worldwide. If food prices continue to increase and more food grains are diverted to ethanol production, the likelihood of starvation will increase [14]. Hence the increase in food prices will have a devastating effect on underdeveloped countries.

Table 1*. Total corn production and its consumption over various years in the U.S.A.
[15]

Market year	Total corn production	Corn used for beverage alcohol	Corn used for fuel alcohol
2001	2046.37	131.00	705.95
2002	2340.22	131.00	995.50
2003	2537.11	132.00	1167.55
2004	2687.00	132.80	1323.21
2005	2981.72	135.00	1603.32
2006	3490.25	135.82	2119.49
2007	4363.42	135.40	3026.13
2008	4900.00	134.00	3600.0

*Values in million bushels and the rest of corn was used as food, feed, and for starch production.

1.2.2 New Ethanol Source

Although there is a need to generate higher volumes of ethanol, the diversion of corn and sugarcane away from the food supply is not advisable for mass production [11]. It is predicted that the total corn grain potential for ethanol production is 13 to 20 billion gallons per year [18]. In the U.S.A., ethanol from corn gets a subsidy of \$0.54 per gallon in the form of tax incentives from the Federal government. Cumulative funding in the form of tax incentives for corn ethanol program reached \$2.5 billion by end of the year 2006 [10] and these incentives are unsustainable in the long term [6]. For a viable long term solution, there is a need for finding an alternative raw material that would replace the usage of corn. One such raw material is cellulosic biomass, which includes various bio sources such as wood, grass, agricultural waste, sugarcane bagasse, corn stover, forest residue, and municipal waste.

1.3 Cellulosic Ethanol

Cellulosic biomass is a promising replacement for corn and sugarcane in bioethanol production. The sources of cellulosic biomass, also called lignocellulosic biomass, are abundant and widely available on a large scale. The main benefit here is that it is not used for human consumption, and only a small portion of it is used for animal consumption. Therefore, the use of cellulosic biomass reduces the diversion of food crops for energy creation, thereby stabilizing food prices, while also reducing the pressure on soil nutrients [14]. In the U.S.A., 1.24 billion tons of cellulosic biomass can be produced every year: 910 million tons from the agriculture sector and 330 million tons from the lumber sector [10;10 11]. Furthermore, cellulosic biomass can be used to produce cellulosic ethanol in the same way as corn ethanol with a few changes in the production method. Ultimately, cellulosic ethanol is a promising potential substitute for gasoline.

The advantages of cellulosic ethanol are [10; 11]:

- i. Renewable source of energy – Biomass for cellulosic ethanol production is abundant and can be obtained from many sources such as agriculture residue, forest residue and municipal solid waste (MSW).
- ii. Local availability of biomass – The U.S.A. and many other countries has a large supply of agricultural residue and MSW for cellulosic ethanol production.
- iii. Increase in farm income – Presently agricultural residue is considered a waste; however, utilizing it for cellulosic ethanol will provide the farmers with extra income.
- iv. Reduction of green house gases – Cellulosic ethanol is an oxygenated fuel and the biomass used for cellulosic ethanol uses CO₂ from the atmosphere to produce carbohydrate sugars. Thus, the net CO₂ emissions are low when compared to fossil fuels.

1.3.1 Cellulosic Ethanol Production

Cellulosic ethanol is produced by the fermentation of carbohydrates sugars obtained from the hydrolysis process from the biomass. The production process of cellulosic ethanol is complex when compared to corn ethanol [19]. A detailed explanation is given in section 1.4. The pathway for ethanol production from corn and cellulosic biomass is shown in Figure 1. The cellulosic ethanol production process involves four steps (i) Pretreatment, (ii) Hydrolysis, (iii) Fermentation and (iv) Separation. In the pretreatment step the biomass is softened to fasten the hydrolysis process. During the hydrolysis process sugars are formed from the pretreated biomass, which are then fermented, and finally ethanol is separated from the fermentation mixture. The hydrolysis of cellulosic polysaccharides is difficult when compared to the hydrolysis of starch. The kind of technology and enzymes used in corn ethanol cannot be used for cellulosic ethanol production because the hydrolysis and fermentation of sugars from cellulosic biomass is complex. Further the fermentation, enzyme technologies and reaction conditions are well established for corn ethanol; however, research of cellulosic ethanol is still in the initial stage.

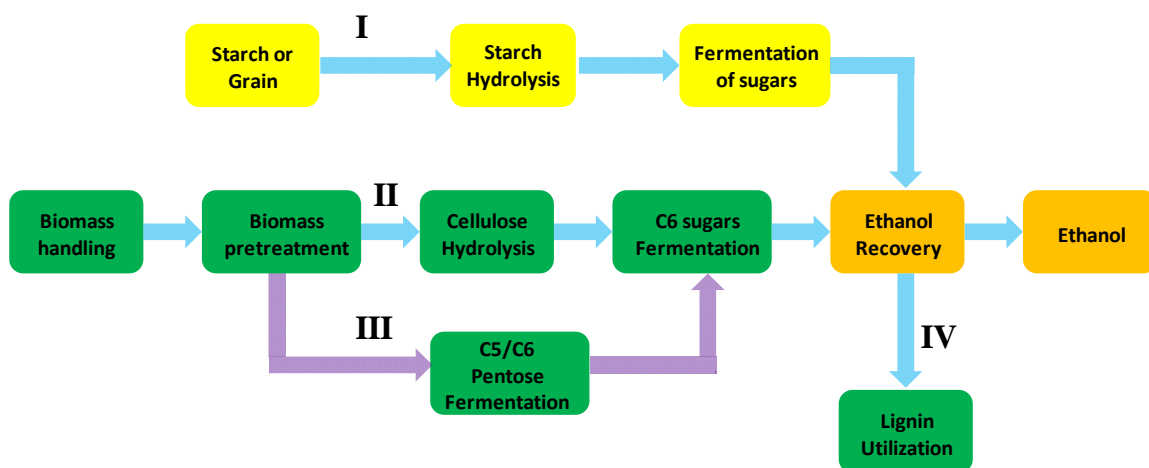


Figure 1. Pathway for ethanol production from both corn starch and cellulosic biomass. Pathway I describes the corn ethanol production process and Pathway II describes the cellulosic ethanol production process, whereas Pathway III and Pathway IV describes recovery of hemicelluloses and lignin from cellulosic ethanol process [19]. [Adapted from NREL, Department of Energy, U.S.A.]

1.3.2 Raw Materials for Cellulosic Ethanol

The raw materials for cellulosic ethanol should come from renewable sources and available locally. The understanding of biomass composition and biomass availability plays an important role in cellulosic ethanol production process. The following sections will discuss the composition of biomass, and later sections will overview the availability.

1.3.3 Biomass Composition and Cell Wall Architecture

Identifying the various components in the plant cell wall is an important part in determining ethanol yield. The plant cell wall is made up of polysaccharides (cellulose and hemicelluloses), lignin and small amounts of glycoproteins. The polysaccharide composition varies from species to species and also within tissues of the same plant. The carbohydrate polymers are composed of many monomer sugars called monosaccharides and there are about 11 different types of monosaccharides present in the plant cell wall like mannose (Man), D-galactose (Gal), xylose (Xly), arabinose (Ara) [12; 20].

1.3.3.1 Cellulose

Cellulose is the most abundant (1.5×10^{12} tons per year) organic compound produced on earth and is considered a renewable raw material. It is a structural material of a plant's cell wall that provides strength and shape for the cell. The primary cell wall of plant contains 15 to 30% dry mass of cellulose and the secondary cell wall contains around 40-55% dry mass of cellulose, 20-40% hemicelluloses and 15-35% lignin. The secondary cell wall has more cellulose when compared to primary cell wall in plants [20]. The general formula of cellulose is $(C_6H_{10}O_5)_n$.

Cellulose chain is made of 1-4- β linkage of D-glucoses which are 6-carbon sugar monosaccharide. The glucose residues are linked to each other by covalent glycosidic bonds and form long chains as shown in Figure 2 [21]. These glucose chains form microfibril bundles and on an average cross-section thickness of each microfibril bundle is 36 individual chains thick. The chain lengths are expressed in degree of polymerization (DP) and they range from a few hundred to a few thousands. Each chain in the microfibril bundle is bonded with another chain through hydrogen bonds forming crystalline structure [20; 22].

Corn starch is a polymer formed of α -linked glucoses which can be easily broken down to glucose residues [19]; whereas cellulose is a beta-linked glucose polymer and is much more difficult to break down. Cellulose in plants predominantly occurs in the crystalline form, however, a small percent of amorphous nature is also observed. The crystalline structure is highly resistant to enzyme attack because its inaccessibility, and forms a barrier for hydrolysis, making ethanol production difficult [23].

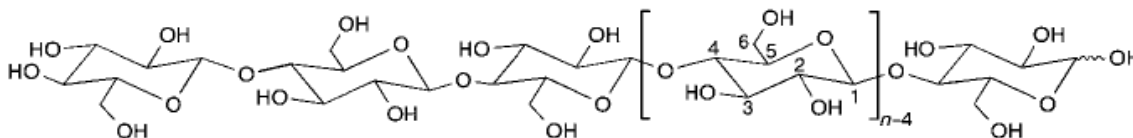


Figure 2. Structure of cellulose chain which is made up of glucose monomers linked with β (1,4) linkages [21] (“Copyrights Wiley InterScience”)

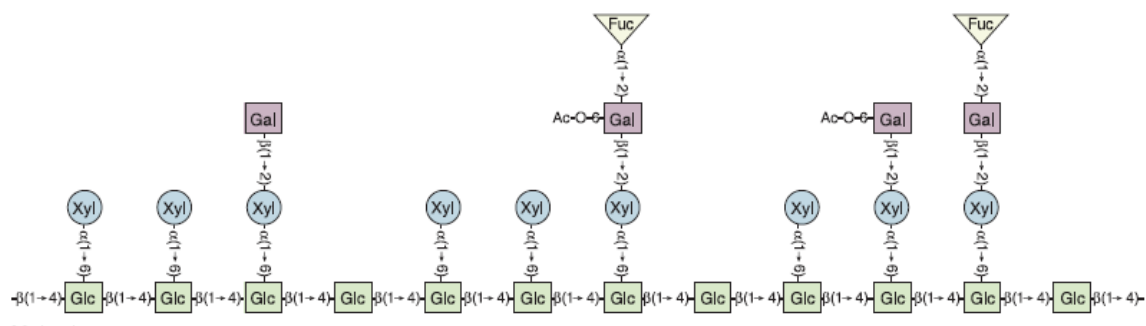
1.3.3.2 Hemicelluloses

Hemicelluloses are short, branched polymers made of various monosaccharides and are amorphous in nature, which can be easily hydrolyzed. Hemicelluloses are also called cross-linking glycans. Hemicelluloses account for 20-40% of dry mass in plants primary cell walls. The various monosaccharides found in hemicelluloses are xylose, arabinose, galactose, fucose. Glucose and xylose are the major hemicellulose components, xylose a pentose, accounts for 5% and 20% of cell wall of dicots and grasses respectively [20; 23; 24].

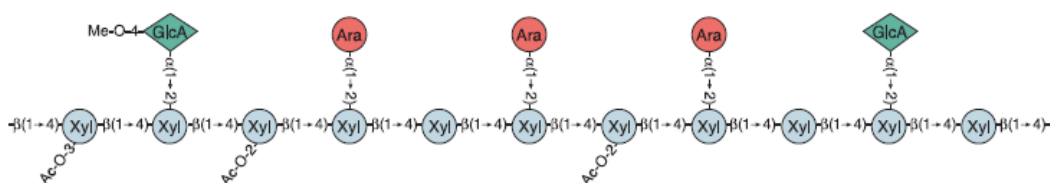
The major hemicelluloses polysaccharides are xyloglucans (XyGs), glucuronoarabinoxylans (GAXs), mixed-linkage glucan (MLG), mannans and callose. The structure of XyGs polysaccharides consists of linear chain of (1-4) β -D-glucan with numerous α -D-xylose units linked to the chain, also other sugar monomers like α -L-arabinose or β -D-galactose which are substituted to the chain at regular sites on the glucose units. The XyGs cross link the cellulose microfibrils in the cell wall of all the dicots and about one-half of monocots including grasses. GAXs are the major cross-linking hemicellulose polysaccharides in cell wall of “commelinids” lines of monocots. GAXs have a xylan backbone branched at various degrees with arabinose and glucuronic acid (GlcA) [20]. The different hemicelluloses structures are shown in Figure 3. Some hemicelluloses bind microfibrils bundles together, providing linkage between lignin and cellulose and also coat the cellulose microfibrils, resulting in a reduction of the surface area available for hydrolysis [25].

1.3.3.3 Lignin

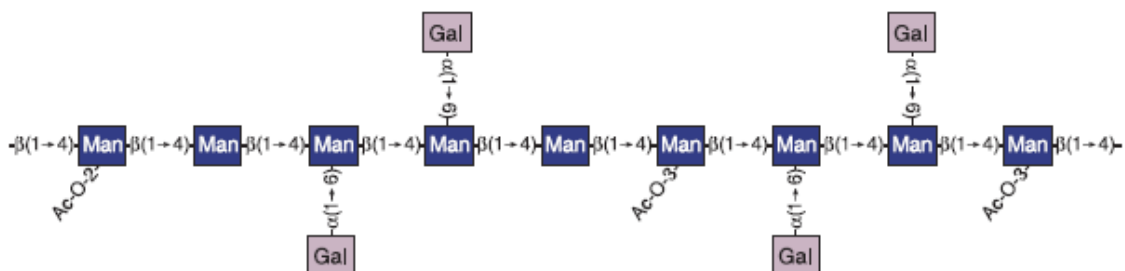
Lignin is a phenolic polymer compound present in the secondary walls of the dead cells such as xylem. Lignin accounts for 15-35% in the cell wall and it's one of the most available organic polymers on earth after cellulose. The polymer is made up of three types of phenylpropanoids units, p-coumaryl, coniferyl and sinapyl alcohols [11]. Lignin units are held together by carbon-carbon bonds. Softwood lignin is composed of mainly coniferyl and hardwood lignin is composed of coniferyl and sinapyl [11]. Lignin forms a linkage with sugars to form the lignin-carbohydrate complexes (LCC). These linkages are formed by covalent bonds between lignin and carbohydrates are also known as glycosidic linkages.



Xyloglucans (XyGs)



Glucuronoarabinoxylans (GAXs)



Galactomannan

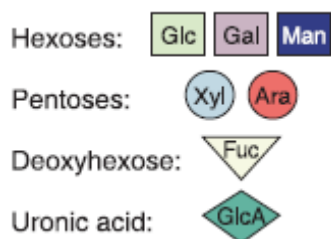


Figure 3. Chemical structures of different hemicelluloses polysaccharides, including monomer sugars and their linkages [26] (“Copyrights from The Plant Journal, Wiley Publications”)

Lignin is a hydrophobic polymer where nature has designed it in such a way that it protects the plant tissue from extreme climatic conditions and also from pathogens [25]. Lignin encrusts the cell wall and cements the cells together thereby reducing the surface area available for cellulose hydrolysis [12]. Lignin not only acts as a barrier to hydrolysis by associating with cellulase enzymes, which are non-productive when attached to the lignin sites but also by limiting the accessibility of the enzymes to cellulose microfibrils [25]. Lignin is degradable only by few organisms and it can be used for producing valuable fuel products. Presently, it is used in boilers to burn and produce steam, used for power generation. The compositions of various biomasses are listed in the Table 2.

Table 2*. Dry weight percentage composition of various biomass feedstocks [24]

Biomass	Cellulose	Hemicelluloses	Lignin
Corn stover	37.5	22.4	17.6
Pine wood	46.4	8.8	29.4
Wheat straw	38.2	21.2	23.4
Switchgrass	31.0	20.4	17.6
Office paper	68.6	12.4	11.3

*Note: The sum does not total to 100% as minor components like glycoproteins are not listed

1.3.3.4 Cell Wall Architecture and Types

The plant primary cell wall can be classified into two distinct types: Type I and Type II. The cell wall components like polysaccharides, phenolics and glycoproteins interact with each other in the cell wall of the plant and the kind of interaction distinguishes the type of plant cell wall. The models for Type I and Type II plant primary cell walls are shown in Figure 4. Type I cell wall contains equal amount of cellulose and XyGs. XyGs in Type I cell wall binds microfibrils to the adjacent microfibrils through hydrogen bonding, also XyGs hydrogen bond with other XyGs present in the cell wall. Figure 4 gives a view of various component interactions in the cell wall [20]. The Type I cell wall is observed in most of dicots and the noncommelinoid monocots. The Type II wall on the other hand, is present in commelinoid monocots, such as the grass. They differ from Type I by their chemical components, as XyGs are replaced by glucuronoarabinoxylans (GAXs) as shown in Figure 4. The GAXs interlock the cellulose microfibrils in the Type II cell wall [20].

The secondary plant cell wall is composed of cellulose, hemicelluloses and lignin. The primary cell wall differs from secondary cell wall by the lignin presence. The secondary cell wall in wood is differentiated into three regions S1, S2 and S3 lamella they differ from each other by the way cellulose microfibrils interact within the layers. The secondary cell wall is more organized than the primary cell wall. The secondary cell wall components are embedded in lignin matrix, whereas the primary cell wall components are embedded in pectin matrix.

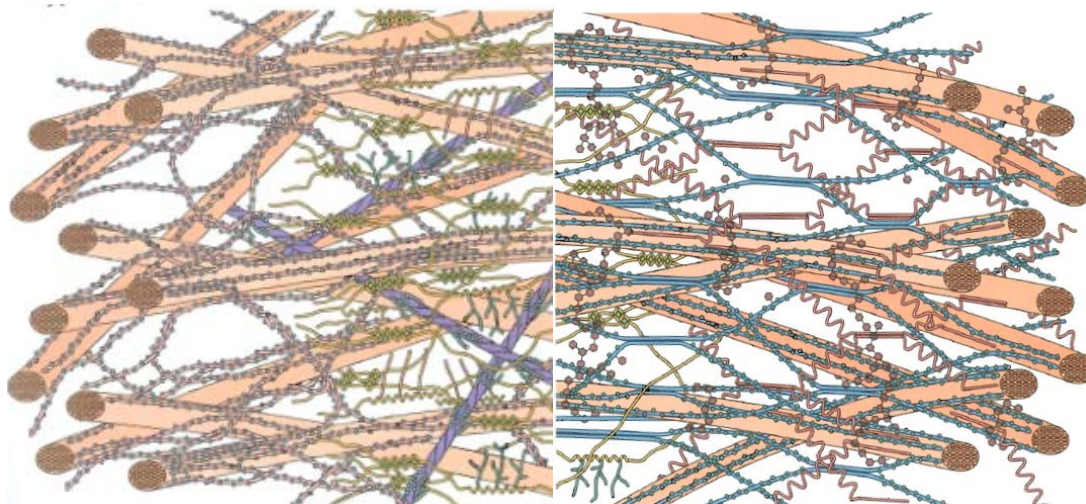
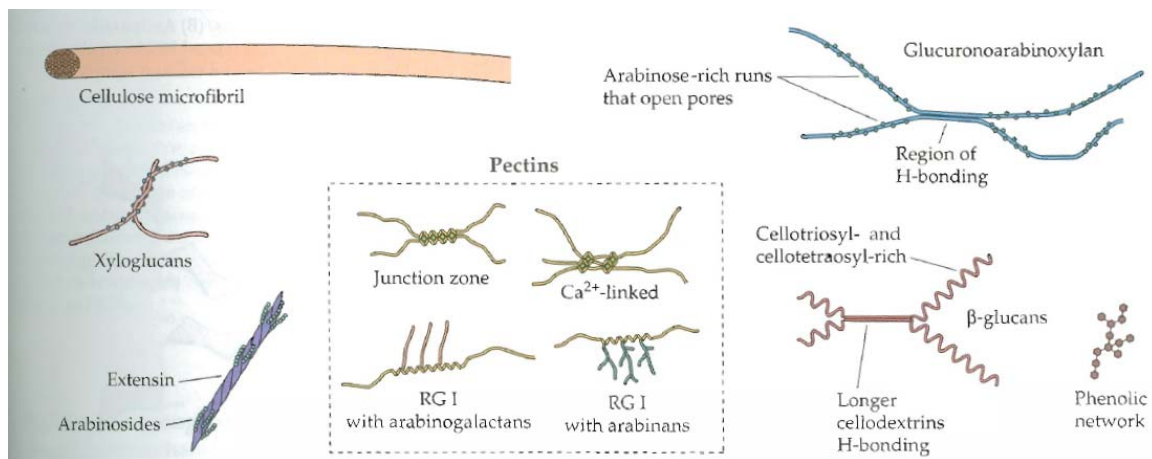
*Type I cell wall**Type II cell wall**Components of cell wall*

Figure 4. Type I and Type II models of cell walls in plants and the cell wall component interactions [20] (Biochemistry & Molecular Biology of Plants “Copyright American Society of Plant Biologists.”)

1.3.4 Biomass Feedstock – Source and Availability

The key to making cellulosic ethanol a competitive alternative fuel is the ability to produce it from low cost feedstocks. The choice of feedstock and biomass composition plays an important role in the cellulosic ethanol production process, as they influence the ethanol yield via their carbohydrate content. The cellulosic feedstock can be of two types: waste materials and energy crops. The various waste materials which can be used as a source of cellulose are agricultural residue, municipal solid waste (MSW), and forest residue. Various energy crops include short rotation woody crops such as poplar and willow, and herbaceous crops like switchgrass, for example. The U.S.A. has more than 1 billion tons of biomass capacity per year [10; 11], and the annual worldwide production of cellulose is about 1.5×10^{12} tons [21]. Many possibilities, such as agriculture residue and municipal solid waste, exist for extraction of energy from biomass, and these will be discussed intricately in the following sections. A promising feedstock source for energy production should satisfy the following criteria:

- i. Easily available – The biomass should be easily available, and has a year round supply.
- ii. Low costs – The raw material should be available at low costs to make the cellulosic ethanol cost effective and not to compete with food crops.
- iii. High cellulose yield – The carbohydrate content of the biomass should be high and the lignin content should be low.

1.3.4.1 Agricultural Residue

Agricultural residue includes crop waste from harvests such as corn stover, sugar cane bagasse, rice husk, and beet pulp. Only a fraction of the agricultural residue is used as food for livestock while the rest is left in the field, which can be a major source of cellulose for cellulosic ethanol production. The agricultural residues like corn stover, rice straw, wheat straw, barley straw, oat straw and sugarcane bagasse are widely available throughout the world, as Table 3 illustrates.

The wide global availability of agriculture residues translates to a local supply for energy producers. Bruce E. Dale of Michigan State University, U.S.A., a leading cellulosic ethanol research scientist, carried out a study on global bioethanol potential. According to his study around 1.5×10^9 tons/year of agriculture residue is available from the above crops, and is capable of producing 442 billion liters of bioethanol per year worldwide. This amount of bioethanol can replace approximately 32% of global gasoline consumption [27]. In the study carried out by him it is noted that 60% of the straw is left behind in the field excluding rice, which requires all the straw to be removed, and sugarcane bagasse, which is collected after sugar is extracted. The study also recognizes rice straw as the most favorable residue for ethanol production as it is available in large quantities, followed by wheat straw, corn stover, and sugarcane bagasse [27].

Table 3. World wide availability of various agriculture residues and their potential ethanol production [27]

Agriculture residue	Residue/Crop ratio (w/w)	Residue generated, Million metric dry tons/year	Ethanol yield, liters/ kg of dry mass	Potential ethanol production, Billion liters
Corn stover	1	203.6	0.29	58.6
Barley Straw	1.2	124	0.31	18
Oats Straw	1.3	11	0.26	2.8
Rice Straw	1.4	731	0.28	205
Wheat Straw	1.3	354	0.29	104
Sugarcane Bagasse	0.6 kg/kg sugar	180	0.50	51
Total		1603.6		439.4

1.3.4 .1.1 Corn Stover

Corn stover is the remnant of the corn plant after corn is harvested and can be used as raw material for the production of cellulosic ethanol. With a ratio of corn grain production to corn stover at 1:1 (w/w), for every ton of grain produced, one ton of corn stover is produced. In the U.S.A. the annual corn stover availability is 153 million dry tons per year [28]. However, the total corn stover produced cannot be completely harvested due to constraints of soil moisture, soil fertility and soil erosion. Even if 50% of the stover is

collected, an estimated amount of 80 million dry tons is available per year for ethanol production. It is estimated that 70 gallons of ethanol can be produced per ton of corn stover (50% of the calculated theoretical yield from National Renewable Energy Laboratory, U.S.A.), netting around 4.8 billion gallons of ethanol from the available 80 million tons of corn stover [23].

The cost of corn stover is determined by the following factors [29]:

- i. Cost of baling and staging of corn stover in the field.
- ii. Transportation cost of corn stover from field to ethanol production plant.

1.3.4.2 Municipal Solid Waste (MSW)

Municipal solid waste, another possibility of biomass, is generated from the waste disposed of by humans. Sources of MSW are residential and commercial buildings. Residential waste contributes around 55 to 65% while commercial waste (includes school, offices and factories) contributes around 35 to 45% of total MSW [30]. The various elements of MSW are paper and paperboard, food scraps, grass clippings, clothing and wood. Paper board alone contributes to 32.7% of annual MSW produced; yard trimmings are 12.8%, and food scraps are around 12.5%. The total MSW generation in the year 2007 was 254 million tons, out of which 63.3 million tons were recycled which accounts for 25% of the total generation, 21.7 million tons were used for composting and 54% of total MSW went to landfill [30]. The annual MSW generation in the U.S.A. for various years is given in Table 4, while the various products collected as MSW are in Table 5. The municipal solid wastes which are good sources for cellulose are paper and paperboard, wood and garden wastes.

Table 4*. The annual generation of MSW for various years in the U.S.A. [30]

Year	1960	1970	1980	1990	2000	2005	2007
Generation	88.1	121.1	151.6	205.2	239.1	250.4	254.1
Total Recovery**	5.6	8.0	14.5	33.2	69.4	79.4	85.0
Combustion for energy	0.0	0.4	2.7	29.7	33.7	31.6	31.9
Discard to landfill	82.5	112.7	134.4	142.3	136.0	139.4	137.2

*Values in million tons. **Recovery includes composting

Table 5. The various materials collected as MSW, their quantities and recoveries for the year 2007 in the U.S.A. [30]

Materials	Weight generated, Million tons	Weight recovered, Million tons	Recovery (%)
Paper and paperboard	83.0	45.2	54.5
Plastics	30.7	2.09	6.8
Rubber and leather	7.48	1.10	14.7
Wood	14.2	1.32	9.3
Food Scraps	31.7	0.81	2.6

1.3.4.3 Energy Crops

The energy crops include short rotation grasses like switchgrass and woody crops like poplar. Farming on large scales for production of energy crops is called energy farming. The best solution would be to grow energy crops on barren lands and the temperate grasslands which are abundant in the U.S.A.

1.3.4.3.1 Switchgrass

Switchgrass is a perennial crop and is a promising feedstock as it contains high cellulose and hemicelluloses contents [12]. Many research groups are working on the switchgrass and its potential use as a raw material for cellulosic ethanol. Kenneth P. Vogel of University of Nebraska, a leading switchgrass research scientist, states that 320 gal of ethanol can be produced from one acre of the switchgrass crop (reference to 0.38 gal/kg of switchgrass) [31]. A study conducted on the fields of Nebraska shows that switchgrass can produce 540% more renewable energy than used to grow it [9; 31] and this study was conducted on 10 different fields of 23 acres of land. Average yields of 5.2 to 11.1 metric ton per hectare were reported in the fields. Cellulosic ethanol from switchgrass emits 94% fewer greenhouse gases when compared with gasoline [9]. Net energy requirements to grow and harvest switchgrass are less than that required for corn [9]. The farm gate price for switchgrass is \$44 per dry ton. Growing switchgrass also helps to reduce soil erosion and improve the water quality [4].

In the U.S.A., 377 million tons of perennial crops are available per year. They can grow on low quality land and impact the environment less. Switchgrass and other perennial crops can be grown on lands which are placed or enrolled in the Conservation Reserve Program (CRP), and also on the lands where annual crop rotation needs to be carried out.

The advantages of growing switchgrass are:

- i. Its cellulose (31%) and hemicelluloses (20.4%) contents are higher.

- ii. It grows faster.
- iii. Less use of fertilizers when compared with corn.
- iv. Can grow on lands where erosion problems occur.

1.4 Production of Cellulosic Ethanol

The production process of cellulosic ethanol from biomass involves four steps [22; 24]:

- i. Pretreatment of biomass.
- ii. Hydrolysis of biomass.
- iii. Fermentation of released sugars.
- iv. Separation of ethanol.

Production of cellulosic ethanol from biomass is a complex process. The barriers for hydrolysis of carbohydrates from biomass are [22; 24]:

- i. Crystalline structure of cellulose is difficult to hydrolyze.
- ii. Protective sheath of lignin and hemicellulose are around the cellulose.
- iii. Lignin binds the cells together reducing the surface area.
- iv. Pentose hydrolysis is more complex than hexose.

These barriers need to be overcome for the efficient production of cellulosic ethanol from biomass. The following sections explain the production steps in detail.

1.4.1 Pretreatment

For efficient hydrolysis of the biomass, the above mentioned barriers need to be addressed, and this is done in the pretreatment step. During this stage the crystalline nature of cellulose is altered, lignin is removed and more surface area is made available for enzyme action. The pretreatment stage involves softening of the biomass, breaking down the cell structure, and enhancing the enzymatic digestibility of biomass. Pretreatment influences and helps in optimizing the sugar yield from feedstock and in the succeeding process [24]. A more detailed explanation of pretreatment with various methods is discussed in section 1.5.

1.4.2 Hydrolysis

Because cellulose is a polymer it cannot be directly fermented to produce ethanol. The long chain of cellulose needs to be broken down to yield glucose; this process is termed as “hydrolysis” or “saccharification” process. Hydrolysis converts the cellulose in biomass to glucose residues by breaking the long chain polymer. Hydrolysis can be carried out in two ways:

- i. Acid hydrolysis.
- ii. Enzyme hydrolysis.

Hydrolysis using strong acids like sulfuric acid (H_2SO_4) has been carried out for many decades and this procedure yields more sugar molecules in less time. Enzymatic hydrolysis is a new method which uses enzymes like cellulase to break down the cellulose. For any process to be economical in the industrial scale the saccharification and fermentation process should be faster [1].

1.4.3 Fermentation

The monosaccharides formed by the hydrolysis process are fermented to produce ethanol. There are some methods like simultaneous saccharification and fermentation (SSF) are carried out in the same step where the saccharification part is for hydrolysis of cellulose polymer to monosaccharides. Several attempts were reported on this method of fermentation, wherein the process of hydrolysis and fermentation can be completed in one step [32].

A new method of simultaneous saccharification and co-fermentation is under study where the 6-carbon monosaccharides such as glucose are fermented and then 5-carbon monosaccharides such as xylose and arabinose are also fermented in the same step. In this process the enzymes first ferment 6-carbon monosaccharides and then 5-carbon monosaccharides. There remains a great deal of research to be done regarding this particular method as 5-carbon monosaccharides (also called pentose) are difficult to ferment.

1.4.4 Separation

Fermentation is then followed by the separation stage, which has been widely studied and well-established. The ethanol from the fermentation broth is extracted using various separation technologies.

1.5 Pretreatment: Key Process in Cellulosic Ethanol

Pretreatment plays a major role in the cost-effectiveness and the overall yield during the production of cellulosic ethanol. After feedstock price, pretreatment is the most expensive unit operation process [1]. Major research is focused on pretreatment of biomass as it is one of the most expensive processing steps [24; 33]. The pretreatment of biomass has received much attention due to the fact that it accounts for 20% of the total cost of the cellulosic ethanol production process [34]. Pretreatment governs the enzyme loading for hydrolysis and substrate related factors and makes the biomass more digestible by enzymes [25].

Efficiency in the pretreatment step can greatly reduce the cellulosic ethanol cost. Hydrolysis of biomass without pretreatment has yields less than 20% of total sugars, whereas after pretreatment the sugar yields have increased to 90% of the theoretical value, as mentioned in some pretreatment methods [33]. Thus, an ideal pretreatment should reduce the lignin content and the crystallinity of the cellulose, while increasing the surface area for enzyme hydrolysis [23]. The methods for increasing the efficiency of pretreatment can have several drawbacks. Therefore, a pretreatment method should be carefully selected by considering the following factors [8; 12; 24]:

- i. Fewer chemical usages – should use fewer chemicals as they are toxic to the environment.
- ii. Milder temperatures – the pretreatment temperatures should be low, as high temperatures around 200°C degrade hemicelluloses and lignin to form unwanted products like furan derivatives and phenolics thus minimizing the ethanol yield.
- iii. Environment friendly – pretreatment should not produce any wastes that are harmful to the environment.
- iv. Recovery of byproducts – the byproducts like hemicelluloses and lignin should be recovered to improve the economics of the process.

- v. Moderate cost for reactors – the reactor cost should be low, as the capital cost for cellulosic ethanol will increase with increase in reactor costs.

Numerous pretreatment methods are available and they have their own advantages and disadvantages. Because of the differences in cell wall structure of plants some pretreatment methods work well on wood, whereas some on grasses. There is no single pretreatment method that works on all kind of biomass. The four different pretreatment methods and their pros and cons are discussed below.

The pretreatment methods can be classified into four categories:

- i. Physical methods
- ii. Chemical methods
- iii. Biological methods
- iv. Combination of the above

1.5.1 Physical Pretreatment

Physical pretreatment can be conducted in a variety of ways. Three are discussed below: comminution (size reduction); steam explosion; and hydrothermolysis.

1.5.1.1 Comminution: Size reduction of biomass

This type of pretreatment includes ball milling, chipping and grinding. The ball milling is carried out either in dry or wet form of biomass. Essentially, this type of size reduction helps to increase the surface area available for enzyme hydrolysis and also reduces cellulose crystallinity. However, this process is time-consuming and not cost effective [8; 12].

1.5.1.2 Steam Explosion Pretreatment

Steam explosion, used for the past two decades, is a pretreatment technique to treat biomass and one of the most promising methods. A lot of work has been done on this method and tried at pilot plant scale. There are no chemicals used in this method. The temperature varies from 210-290°C and the pressure varies from 20-50 bars. High hemicelluloses yield and low lignin solubility are achieved by this kind of pretreatment. The disadvantages of this method are its need for extremely high temperatures, which degrade biomolecules like hemicelluloses and lignin, and leads to unwanted chemical formations like 5-Hydroxymethylfurfural (HMF) and furfural [8; 12].

1.5.1.3 Hydrothermolysis

In this method, the biomass is cooked in hot compressed water at very high temperatures around 200°C for around 30 minutes. The hydrothermolysis method has been practiced for a long time. This method is very expensive for a large scale production [35].

1.5.2 Chemical Pretreatment

Chemical pretreatment methods differ from physical pretreatment methods, and have received more attention than any of the other pretreatment methods. Different chemicals like sulfuric acid (H₂SO₄), hydrochloric acid (HCl), nitric acid (HNO₃), alkaline solvents, and ammonia (NH₃) are used for the pretreatment of biomass. Chemical pretreatment methods can be classified into different types based on the type of chemical used such as:

- i. Acid pretreatment
- ii. Alkaline pretreatment
- iii. Ammonia pretreatment

These three chemical pretreatments are explained below:

1.5.2.1 Acid pretreatment

Sulfuric acid (H_2SO_4) treatment is extensively studied because it is inexpensive and effective. The acid concentration varies from dilute concentration of 0.5 to 1.5% w/v to very high concentration (4 M). Various biomasses like agricultural waste, food waste, sugarcane, and wood are treated by the acid hydrolysis method. Acid pretreatment completely hydrolyzes hemicelluloses and leaves cellulose and lignin intact [1]. Higher xylose yields are obtained using this method [12; 36]. A pretreatment process at pilot plant scale is under testing at Williams Bioenergy's lab at Pekin, Illinois plant. The trial uses 72,000 lbs of biomass/day to test the efficiency of pretreatment process [37]. Although acid pretreatment can be used with various biomasses yielding high xylose sugars, there are few disadvantages which are [8; 12]:

- i. Needs a corrosion resistant construction material.
- ii. Acids are toxic.
- iii. Acid must be neutralized with lime, which results in gypsum formation. Disposal of gypsum is an extra cost on the process.
- iv. Sugar decomposition takes place, resulting in lower ethanol yield and the creation of extraneous substances.

1.5.2.2 Alkaline pretreatment

Alkaline pretreatment uses sodium hydroxide (NaOH), calcium hydroxide ($\text{Ca}(\text{OH})_2$) and potassium hydroxide (KOH) for pretreatment of biomass. It is primarily used for delignification and is more effective on agricultural waste. Most of the lignin and small quantities of hemicellulose are removed during pretreatment [12]. This pretreatment disrupts lignin's interaction with cellulose [1; 8].

The disadvantages of alkaline pretreatment are:

- i. Cost of chemicals is higher. For instance, NaOH costs more than H_2SO_4 .

- ii. Concentration of alkali used is higher than that of acids.
- iii. Recycling problems – the chemicals used should be recycled which needs an additional process.
- iv. Biomass needs to be washed after pretreatment and is an extra cost on waste water treatment.

1.5.2.3 Ammonia Pretreatment

Ammonia (NH₃) pretreatment is usually carried out for delignification of biomass. Ammonia is used in ammonia-recycled percolation (ARP) and ammonia fiber explosion (AFEX) pretreatment methods. The ARP pretreatment method uses liquid ammonia to treat biomass wherein the temperature ranges between 100°C to 180°C. This method swells the biomass, and removes lignin from the biomass. About half of the xylan is dissolved into liquid ammonia and more than 92% of cellulose remains in the biomass. This process increases the enzymatic digestibility of cellulose in biomass and improves saccharification rates of the herbaceous crops and grasses. A high yield of sugars is achieved from enzyme hydrolysis through this pretreatment method [38].

The AFEX pretreatment method uses ammonia fluid at a high pressure to treat the biomass. Biomass is subjected to very high pressure for a short period of time, followed by a quick release of pressure is carried out through a nozzle attached to the reactor. At high pressures the fluid enters the pores of the biomass and swells it, and sudden releases of pressure forces fluid rapidly flow through the pores, thus disrupting the fibers and creating more surface area for hydrolysis. Thus, this process increases enzyme digestibility of the biomass [38; 39]. Also, there are studies which show the impact on cellulose structure and lignin bondage. AFEX method is used by Dale et al., at Michigan State University to treat biomass like switchgrass and corn stover [33].

The ammonia method is effective on grasses such as switchgrass, and less effective on high lignin feed such as wood. This method is expensive and the ammonia should be recycled for the process to be economical [8; 33; 40].

The disadvantages of AFEX treatment are:

- i. Ammonia is expensive.
- ii. High temperatures cause xylose molecules to degrade.
- iii. Ammonia needs to be removed completely from biomass before hydrolysis as the cellulase enzymes can't function in presence of ammonia.
- iv. Ammonia emissions cause pollution and health hazard.

1.5.3 Biological Pretreatment

Biological pretreatment technique uses enzymes such as cellulase to pretreat cellulosic biomass. The enzyme acts on biomass to produce the fermentable glucose molecules or the desired products [12; 23; 41]. Biological pretreatment includes a number of advantages and disadvantages influencing whether or not it is a primary option.

The advantages of biological pretreatment are:

- i. Fewer chemical usages – chemical usage is much less in this method.
- ii. Lower energy requirements – the temperatures used are around 45°C, hence the energy requirements are low.
- iii. Environment friendly – unwanted waste formation is very less.

The disadvantages of biological pretreatment are:

- i. The process is very slow and time-consuming for complete hydrolysis of cellulose.

- ii. Enzyme inhibition – some sugars formed by hydrolysis can inhibit the cellulase enzymes and make them unproductive.
- iii. Enzymes are expensive
- iv. A great deal of care should be taken to maintain the optimal conditions.

1.5.4 Combination of Pretreatments

Another way of addressing the needs for the pretreatment step involves combining two or more pretreatments (physical, chemical, or biological), and applying them either in a sequence or simultaneously (for instance, chemical pretreatment followed by biological treatment, or two physical treatments applied in a sequence.) One such method is the acid catalyzed steam explosion pretreatment, in which diluted acid is used as a catalyst [12].

1.5.5 Common Pretreatment Methods

Pretreatments are generally categorized into physical, chemical or biological methods, with another option being combination, as the previous sections have discussed above. Additionally, acid hydrolysis and ammonia methods are two preferred and promising methods of pretreatment, often for their efficient production of high yields of sugars.

Acid hydrolysis treatment is one of the most preferred pretreatment methods wherein the hemicelluloses sugars of biomass dissolves in the hydrozylate, and are recovered for high ethanol yield. This process requires product recovery system such as ion exchange, which is an extra cost on the overall process associated with yield losses. Also this pretreatment method needs acid resistant constructive materials which are expensive [8; 12; 36].

Ammonia method is also one of the most promising pretreatment methods. In ARP the hemicelluloses sugars dissolve in liquid ammonia and the sugars have to be recovered from liquid ammonia to achieve high yields of ethanol. Ammonia from ARP and AFEX processes must be recycled for the process to be economical. Ammonia from the biomass needs to be removed before enzyme hydrolysis, because it inhibits enzyme activity. Furthermore, ammonia cannot be vented into atmosphere because it causes environmental hazard [8; 33; 40].

The drawbacks of the above pretreatment methods drive us to find even better pretreatment methods. As established, such methods must be effective, environment friendly, and relatively cost-effective. Also as discussed previously, ideal methods should be available locally and provide a degree of ease of handling. Supercritical carbon dioxide pretreatment is one promising method worth exploring.

CHAPTER 2: MOBILE PRETREATMENT USING GREEN CHEMISTRY

The biomass collection and transportation is an expensive operation. As biomass is typically bulky, huge storage units are required to store biomass. The end products of cellulosic ethanol are ash and other biomass waste that needs to be disposed of, and this adds extra expenses to the process. An alternative approach is to bring the pretreatment process to the farm site in the form of a mobile unit or tactical unit which is a small scale process unit mounted on a truck. This unit can be taken to the farm site to extract the sugars from biomass. The biomass is pretreated in the mobile unit followed by hydrolysis process to extract the sugars. These sugars can later be transported to the plant site where fermentation can be carried out. The leftover biomass, which has lignin, can be used for electricity production or used as fertilizer. The ash produced from burning the biomass can be spread on the farms as a fertilizer for the plants. This approach reduces material handling, transportation and waste disposal costs. The sugar removal reduces CO₂ emission from the leftover biomass.

The advantages of mobile pretreatment unit are:

- i. Reduce the biomass handling and transportation costs.
- ii. The final product is hydrozylate (concentrated sugar solution) which can be easily transported to refinery.
- iii. The end product of the process (biomass waste) can be used as fertilizer in the fields.
- iv. The soil nutrients like potassium and nitrogen are retained in the soil.

The pretreatment method used in the mobile/tactical pretreatment unit should be convenient to use. Also, pretreatment should not generate large amount of waste or toxic materials. Acid hydrolysis and ammonia pretreatment methods produce harmful contaminants which cannot be dumped directly into the environment. Ammonia and acid hydrolysis pretreatments can also cause contamination and safety problems for environment and people working with the mobile unit. The steam explosion pretreatment method needs a boiler to produce steam. Boiler installation on a mobile pretreatment unit is not practical due to its need for a huge amount of power supply for operation, as well as the certification required to operate a boiler.

The SC-CO₂ is one pretreatment method that is highly convenient to use in a tactical pretreatment unit. Carbon dioxide can be stored in solid (dry ice), liquid, or gaseous form. The solid or liquid form of CO₂ is easily carried to the agricultural sites. CO₂ is inexpensive, environment friendly, and can be vented into the atmosphere upon use. The pretreated biomass can be hydrolyzed using enzymes to extract sugars. The hydrozylate (sugar concentrate) can then be transferred to the production plant for further fermentation. A flow chart for the SC-CO₂ mobile pretreatment unit for biomass pretreatment at the agricultural site is shown in Figure 5.

The Laboratory of Renewable and Resources Engineering at Purdue University is testing a tactical biorefinery which is shown in Figure 6. The refinery uses 2,500 lbs of biomass/day and converts it to 150 amps (55 kW) electricity out of which 13 amps are used to run the components on board [42]. The ash produced is 1/30th volume of the biomass used, which can be dumped in the farm or can be used as fertilizer for plants. Thus, tactical refinery reduces transportation, eliminates waste disposal problem, and reduces the amount of material handled.

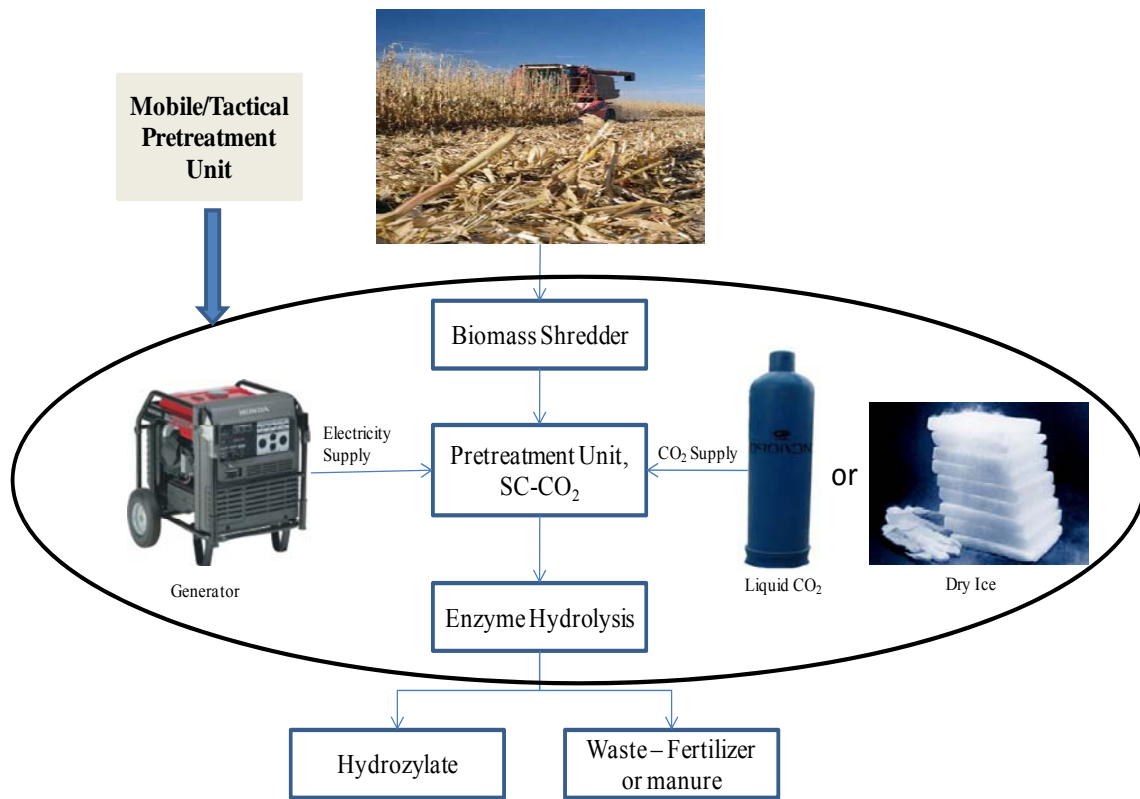


Figure 5. Flow chart for pretreatment of biomass using SC-CO₂ method at a farm site using a tactical/mobile pretreatment unit

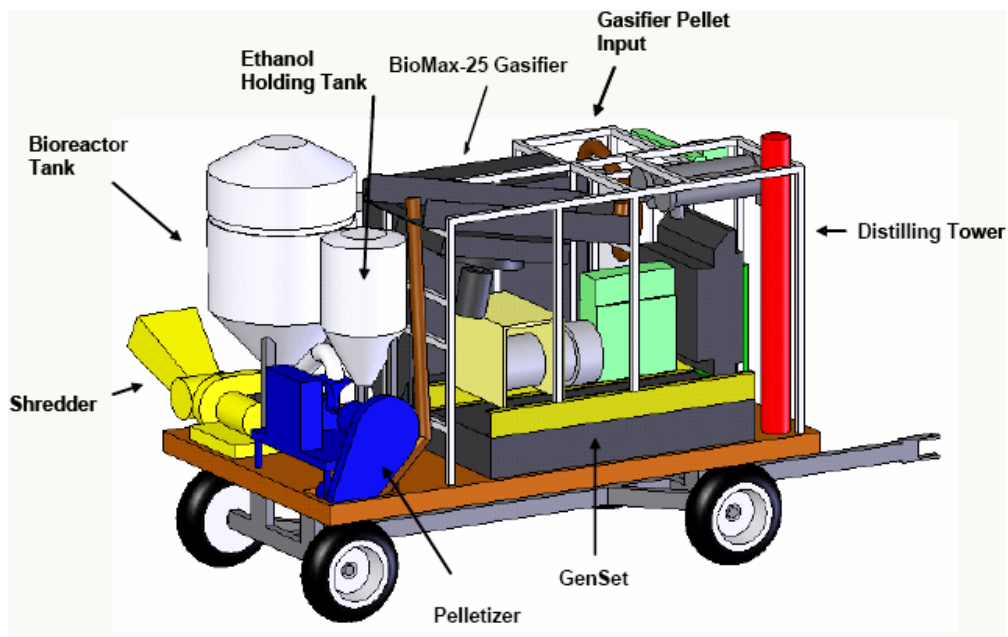


Figure 6. Tactical/mobile refinery design and refinery under construction at Purdue University lab [42] (“Copy Rights Permission from Dr. Nathan Mosier, Purdue University”)

2.1 Supercritical Carbon Dioxide Pretreatment

Supercritical fluids have properties of both gas and liquid appearing simultaneously above their critical temperature and critical pressure points. Figure 7 shows the pressure-temperature phase diagram of fluid which shows the critical phase region appearing between liquid and gas phases. Above the critical point, the fluid exhibits **gas-like viscosity and liquid-like density** which makes it a special solvent [43]. Because of its gas-like viscosity and diffusivity, it can penetrate into the small pores of biomass substance and with its liquid like density it can hold more fluid. For these reasons, supercritical fluids are used in extraction applications. There are many substances like CO₂, H₂O and methanol, which are used as supercritical fluids and their critical points are listed in Table 6. CO₂ is considered a good solvent for the following reasons:

- i. It is non-toxic, and non-flammable.
- ii. It is inexpensive.
- iii. Its supercritical conditions (31.1°C temperature and 73 atm pressure) can be achieved easily compared with H₂O and NH₃.
- iv. It is easily available as a byproduct of many industrial products like ethanol.
- v. CO₂ can be stored in all three forms: solid (dry ice), liquid and gas.

Only a few studies have used the SC-CO₂ for pretreatment of biomass. Zheng et al., (1998) and his team used the SC-CO₂ to treat Avicel, recycled paper and sugarcane bagasse [44]. The pretreatment effect observed had high yields of glucose; however, Zheng et al. used samples like recycled paper and bagasse. The recycled paper is subjected to pulping treatment before paper production. The majority of the lignin content in the paper is removed before the SC-CO₂ pretreatment phase. Kim and his co-workers [45] used the SC-CO₂ pretreatment to treat aspen and southern yellow pine wood.

There are only a few studies which reportedly used the SC-CO₂ method on lignocellulosic biomass and the effectiveness of this method on lignocellulosic biomass needs to be explored. The objective of this study was to examine the effectiveness of SC-CO₂ method on lignocellulosic biomasses such as corn stover and switchgrass under various pretreatment conditions including temperature, pressure and time.

Table 6. The critical temperature and pressure of various fluids [44; 46]

Fluid	Critical Temperature, T _c °C	Critical pressure, P _c atm
Carbon dioxide	31.1	72.8
Methane	-83.0	45.4
Water	374.3	218.3
Ammonia	132	272
Ethanol	234	63

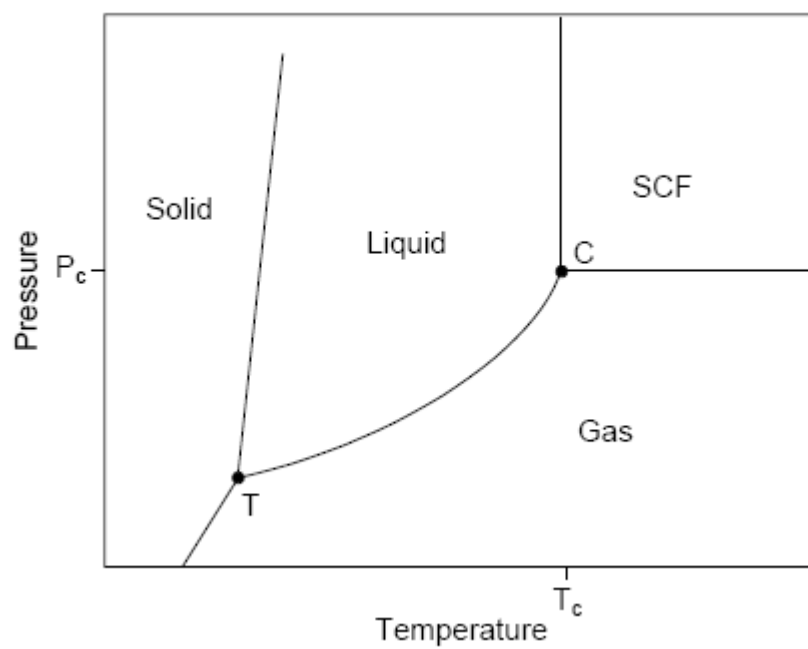


Figure 7. Pressure-temperature phase diagram of fluid: i) T-triple point, ii) C-critical point. Above critical temperature (T_c) and critical pressure (P_c), the fluid falls in supercritical region where both liquid and gaseous properties occur simultaneously [47]

CHAPTER 3: METHODOLOGY

3.1 Materials and Chemicals

The objective of this work was to study the effectiveness of the SC-CO₂ method on lignocellulosic biomasses under various pretreatment conditions. Materials and chemicals utilized for the present work, as well as information on physical setup of the process are detailed below.

3.1.1 Biomass

Corn stover (corn stalk) was collected from a local farm in Athens, Ohio. The switchgrass was supplied by Bob Hendershot (Ohio NRCS Grassland Management Fairfield Soil and Water District). Rye straw was collected from a local farm in Athens, Ohio. These feedstocks were chosen because they are abundantly available in the U.S.A. for cellulosic ethanol production. The corn stover and switchgrass were cut using a rotating knife cutting mill. The samples were sieved using a U.S.A. Standard Testing Sieve No. 16 and then dried at 45°C.

3.1.2 Chemicals

Liquid CO₂ (in a siphon tube cylinder) was purchased from Airgas in Parkersburg, West Virginia. Cellulase enzyme (EC No. 232-734-4, from *Trichoderma reesei* ATCC 26921) was purchased from Sigma Aldrich, U.S.A.. The activity of the cellulase was 700 units/g and density 1.2 g/ml (one unit of cellulase enzyme, as defined by the manufacturer, corresponding to the amount of enzyme which liberates 1.0 μmole of glucose from cellulose in 1 hr at pH 5.0 and 50°C). β-glucosidase (EC No. 232-589-7) was purchased from Sigma Aldrich, U.S.A.. The activity of the β-glucosidase is ≥6 units/mg (one unit of β-glucosidase enzyme defined by manufacture corresponds to the amount of enzyme which liberates 1 μmol of glucose per minute at pH 5.0 and 35°C). The glucose assay kit (Product No.GAHK20-1KT) was purchased from Sigma Aldrich, U.S.A..

3.1.3 Reactor Design

The reactor was built using stainless steel components purchased from Scioto Valve & Fitting Co., Westerville, Ohio. The specifications of different components in the reactor are listed in Table 7 and the reactor is shown in Figure 8.

Table 7*. Items required for the reactor

Item No.	Items	Specifications	Quantity
1	Tubing	1" Outer dia	2 ft
2	Cap	1" dia	1
3	Compression Fitting	1" dia to 1/4" dia	1
4	4-Way Union Cross	1/4" Tubing dia	1
5	Female Connector	1/4" Tubing dia to 1/4" Pipe dia	1
6	2-Way Ball Valves	1/4" Tubing dia	1
7	Metering Valve	1/4" Tubing dia	2
8	Tubing	1/4" dia	6 ft
9	Ferrule and Nuts Set	1/4" dia set	6
10	Pressure Gauge	0-4000 psi range	1

*Note: All the above materials are rated for a pressure of 4000 psi.

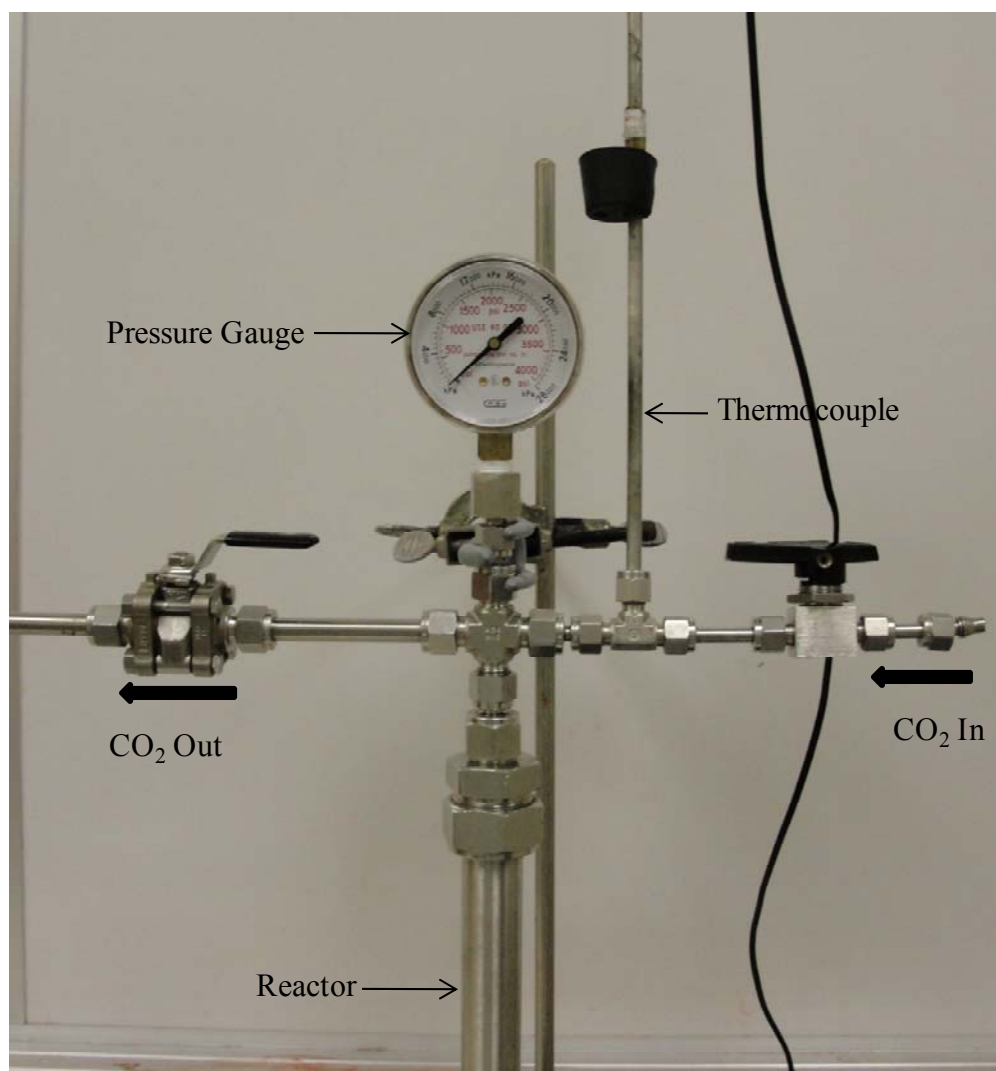


Figure 8. Reactor used for the SC-CO₂ pretreatment (batch process)

3.2 Experimental Method

Figure 9 provides a flow chart of the experimental procedure used in this work. A step-by-step explanation of the experimental procedure follows in sections 3.2.1 – 3.2.6.

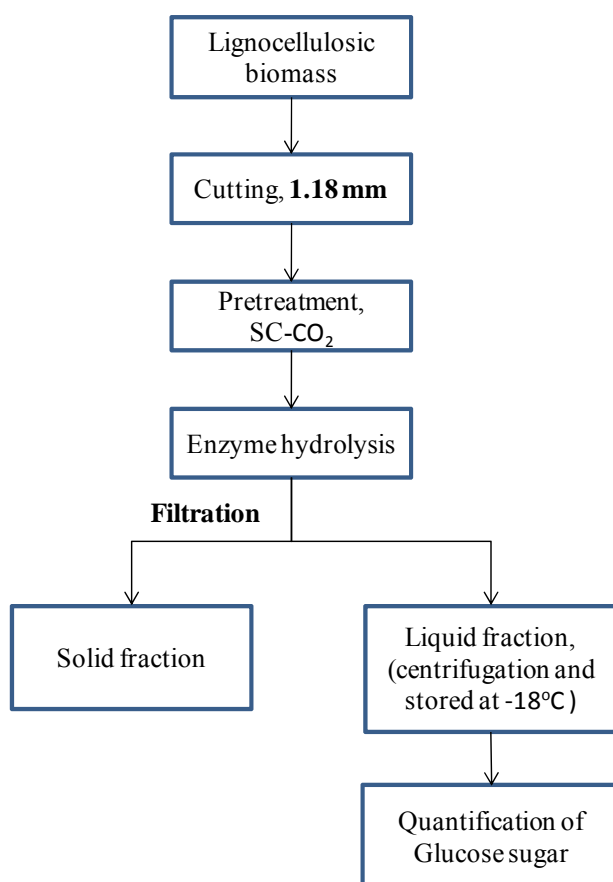


Figure 9. Flow chart of the experimental procedure used for pretreatment of the biomass.

3.2.1 Lignocellulosic Biomass

The biomasses (corn stover, switchgrass and rye straw) were cut using a rotating knife cutting mill and sieved by a U.S.A. Standard Testing Sieve No.16. They are pretreated using the SC-CO₂ in a reactor.

3.2.2 Supercritical Carbon Dioxide Pretreatment of Biomass

A 5g of biomass sample with known amount moisture content was placed in the reactor. The reactor was then vacuumed to remove the air inside the reactor. The reactor was connected to the liquid CO₂ tank and CO₂ was pumped into the reactor. When the pressure in the reactor equals the CO₂ tank, the reactor was placed in an ice bath. The ice bath temperature was maintained around 2°C, which compresses the gaseous CO₂ into liquid CO₂ through a temperature reduction. Again, the liquid CO₂ was pumped into the reactor until the desired amount of CO₂ is achieved. The desired temperature was reached using heating tape or by immersing the reactor in a water bath. The reaction temperature was regulated using a thermocouple inserted into the reactor and Econo temperature controller.

Repeated mixing of samples inside the reactor was carried out manually as the reactor size was small to provide space for a magnetic stirrer. The sample was subjected to high pressure SC-CO₂ for a specific time period and then the pressure was released instantaneously using a quick release (explosive release) ball valve. Thereafter, the pretreated biomass sample was taken out of the reactor and dried at 45°C for 24 hours. The dried samples were then subjected to enzyme hydrolysis.

3.2.3 Enzyme Hydrolysis of Pretreated Biomass

To conduct the enzymatic hydrolysis of pretreated and untreated cellulosic (i.e. control) biomass samples (100 mg of dried biomass samples), 50 Units of cellulase (*Trichoderma*) enzyme and 20 units of β-glucocidase enzyme (β-glucocidase enzyme cleaves disaccharides and trisaccharides to glucose monomers), were added to 30 ml of citrate

buffer solution (0.05 M, pH 4.8) in conical flasks. The buffer solution and conical flask were autoclaved before use to eliminate contaminants. The conical flasks were incubated in a shaking water bath maintained at 47°C and 150 rpm for 24 hours. The reaction mixture samples were collected into test tubes at known time intervals and placed in a water bath maintained at 100°C for 10 minutes to denature the enzymes. Then the samples were centrifuged using a lab scale centrifuge (Model: Marathon micro A from Fisher Scientific). The supernatants obtained were then frozen at -18°C until further analysis. The supernatants were analyzed for glucose sugars using a glucose kit. The enzymatic hydrolysis of each pretreated and untreated cellulosic biomass samples were carried out in two duplicates.

3.2.4 Quantification of Glucose Content

The glucose content from enzyme hydrolysis of untreated and SC-CO₂ treated biomass were analyzed using a glucose kit (Product no.GAHK20-1KT) purchased from Sigma diagnostics. The glucose kit consists of a glucose assay reagent and a standard glucose solution. The above reagent was mixed with 20 ml of deionized H₂O. The 96 plate chamber UV-VIS spectrophotometer (Model: SpectraMax Plus 384, from Molecular Devices) was used to test the samples for glucose content at a 340 nm wavelength. The concentration of glucose present in solution was determined by the standard glucose curve obtained from the known concentration of standard glucose solution.

3.2.5 X-Ray Diffraction (XRD)

An X-ray diffractometer (Make: Rigaku) was used to determine the crystallinity of untreated and the SC-CO₂ treated corn stover. The biomass less than 0.425mm dia was used for X-ray diffraction analysis. A scan type of theta-2-theta with a step size of 0.05 degree was carried out at speed of 0.05 degree/minute.

3.2.6 Scanning Electron Microscope (SEM)

The corn stover (corn stalk) samples used for SEM analysis were cut into small pieces using scissors to make sure the outer surface was not damaged. The SC-CO₂ pretreatment was carried out on these samples to observe any changes in surface morphology. The samples were sent to an independent lab for SEM analysis and the technician was unaware of which samples were treated and untreated. He was asked to take the SEM images of the samples at different magnifications. The surface morphology of untreated and treated biomass was determined by using Hitachi S-4700 SEM - FEGSEM. Samples were prepared by mounting them on sample holder using double-coated tape, and the sample was sputter coated with platinum to make the surface conducive for charge.

3.2.7 Thermodynamic Calculations and Soft Pump

In this work, a pump was not used to pump CO₂ into the reactor. To achieve specified CO₂ pressure and temperature, the CO₂ amount in the reactor was obtained by using a balance to measure the increase in mass in the reactor. Additionally, the thermodynamic properties of CO₂ need to be calculated to understand the reactor conditions and the amount of CO₂ required for the pretreatment. The equations of states (EOS) were used to calculate the thermodynamic properties of CO₂. There is no standard equation available for calculating the supercritical properties of CO₂, as the liquid and gas in critical phase are not in equilibrium. Supercritical CO₂ follows van der Waals equation of state at low densities [48]. Peng Robinson equation of states was also used to calculate the thermodynamic properties of CO₂. The van der Waals equation, Peng Robinson equation and modified van der Waals equation are shown in Equation 1, Equation 2 and Equation 3 respectively. The theoretical and experimental calculations are discussed in the results section in detail.

Equation 1
$$P = \frac{nRT}{V-nb} - a \frac{n^2}{V^2}$$

where P, V, T and n are pressure, volume, temperature and number of moles, respectively. R is the gas constant, a is measure of the attraction between particles, $a = 27R^2T_c^2 / 64P_c$ and b is volume excluded by a mole of particle, $b = RT_c / 8P_c$

Equation 2
$$P = \frac{RT}{V_m - b} - \frac{a(\alpha)}{V_m^2 + 2bV_m - b^2}$$

where, P, V_m and T are pressure, molar volume, and temperature, respectively. R is the gas constant.

$$a = 0.45724R^2T_c^2 / P_c$$

$$b = 0.0778RT_c / P_c$$

$$\alpha = (1 + (0.37464 + 1.54226w - 0.26992w^2)(1 - T_r^{0.5}))^2$$

$$T_r = T/T_c$$

w is the acentric factor of the species, and T_r is reduced temperature, T_c and P_c are critical temperature and critical pressure of CO₂

Equation 3
$$P = \frac{nRT}{V - m^n b} - a \frac{n^2}{V^2}$$

where, m= the correction factor for the volume (constant b) of the molecules. m gets progressively smaller for larger molar or mass density, which means CO₂ molecules shrink more because they are packed more densely.

CHAPTER 4: RESULTS AND DISCUSSIONS

In this study, various pretreatment parameters like temperature, pressure, moisture and time were examined to identify their correlations to the effectiveness of the SC-CO₂ method on lignocellulosic biomasses. The results are expressed in terms of a percentage (% w/w) of biomass glucose yield. A 10% w/w of glucose yield means 10 mg of glucose was produced per 100 mg of biomass from enzyme hydrolysis. The cellulosic biomass analyzed were corn stover, switchgrass and rye straw.

4.1 Corn Stover

The effects of moisture content, temperature, pressure and time during pretreatment of corn stover on glucose yield are presented in this section.

4.1.1 Effect of Moisture on SC-CO₂ Pretreatment

Two samples with different moisture contents were pretreated using the SC-CO₂, whereas temperature, pressure and time were maintained constant for both the samples. The effect of moisture content of biomass in the SC-CO₂ pretreatment of corn stover is shown in Figure 10. The glucose yield for 75% moisture content, 0% moisture content and untreated biomass are 24%, 13% and 12% (w/w) of biomass respectively. The glucose yield of 0% moisture content biomass is similar to that of untreated corn stover and for 75% moisture content biomass it is two times of untreated corn stover at 24 hrs. A similar increase in glucose yield for different moisture content was observed on Avicel by Zheng et al., (1998) [44] and on wood by Kim et al., (2001) [45]. Moisture content above a particular level is not favorable for the SC-CO₂ pretreatment, as noted by Kim et al. [45].

The presence of water in the form of moisture had a positive influence on the glucose yield. It's reported in the literature that, water and high pressure supercritical CO₂ forms weak carbonic acid [49] and the pH of pure water and CO₂ for high pressures is shown Figure 11. Carbonic acid can partially hydrolyze the hemicellulose fraction in biomass at low temperatures [45; 50]. Carbonic acid can dissociate cellulose-hemicelluloses-pectin network which can help removal of hemicelluloses from biomass, which in turn increases cellulose hydrolysis [50]. In addition, water is capable of swelling the biomass, which can open the pores for the SC-CO₂ to penetrate deeper into the biomass, causing an explosive release of pressure that disrupts the biomass fibers and creates more surface area for enzyme action. So far, water phase pH at higher temperatures in the CO₂-water system is still unavailable in the literature. The exact pH in the CO₂-water systems in this work is still unknown.

The solid biomass (dry matter)-to-liquid ratio in the SC-CO₂ pretreatment (25% w/w) is high compared aqueous ammonia (1:10) and acid hydrolysis (1:10) pretreatment methods. In aqueous ammonia pretreatment method, 40-60% of hemicelluloses and 70-85% of lignin is removed from the solids. The hemicelluloses dissolves into the liquid ammonia and must be recovered for higher yields of ethanol; there are yield losses during this process, thus the loss of ethanol yield. In the SC-CO₂ pretreatment method, hemicelluloses and lignin are retained in the solids and can be recovered - thereby increasing the sugar yields.

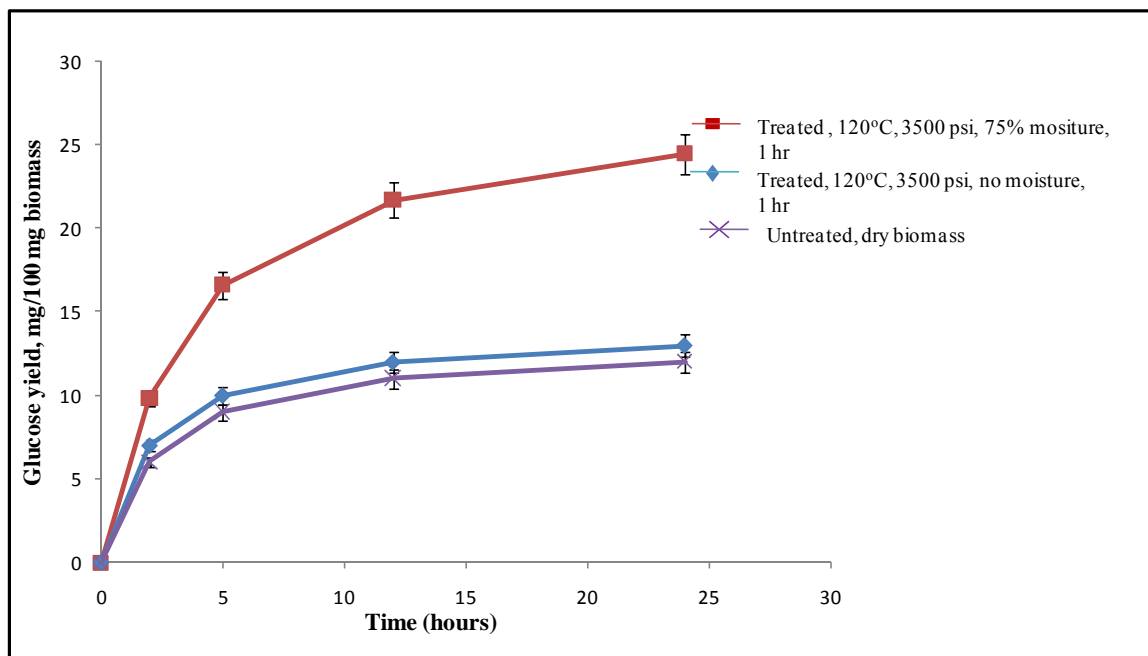


Figure 10. Effect of SC-CO₂ pretreatment on the glucose yield from enzyme hydrolysis of corn stover biomass with 75% moisture content and no moisture when treated at 120°C and 3500 psi for 1 hr

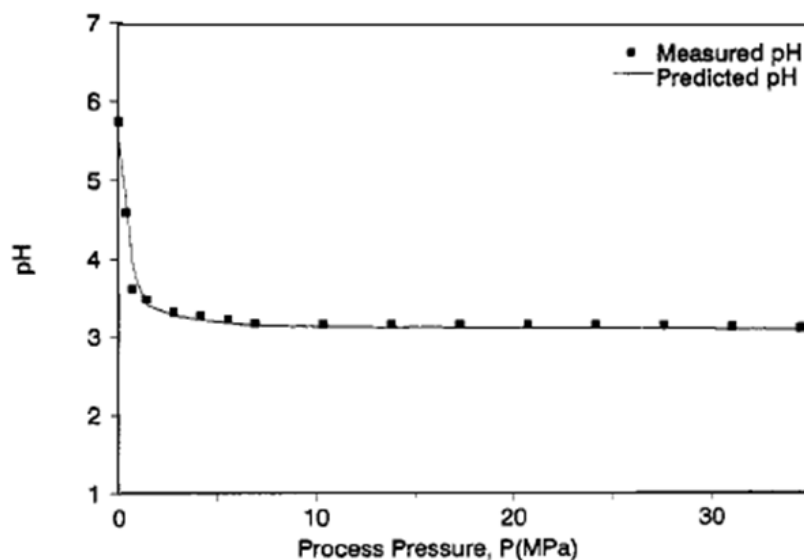


Figure 11. Measured and predicted pH of pure water-CO₂ simulation system at 37°C temperature and pressure upto 34 MPa by Behrouz Meyssami et al., 1992 [49]. [Note: This figure is reproduced from Meyssami et al.]

4.1.2 Effect of SC-CO₂ Pretreatment Temperature

The effect of the SC-CO₂ pretreatment temperature on corn stover is shown in Figure 12. Pretreatment was carried out for various temperatures, whereas the pressure (3500psi), time (1 hr) and the moisture content (75%) were maintained constant. The glucose yields for 80°C, 120°C and 150°C are 13%, 24% and 30% (w/w) of biomass, respectively, and for untreated biomass, it is 12% w/w of biomass. At 80°C, the glucose yield is not significantly different when compared to the untreated corn stover yield. The low yield at 80°C is likely due to the inferior diffusivity of the SC-CO₂ at low temperatures, since the increase in temperature results in high diffusivity, and ultimately, in an increased surface area for enzyme action, as discussed earlier. Therefore, a higher pretreatment temperature correlates with an increase in the glucose yield.

With an increase in pretreatment temperature to 120°C, the glucose yield doubled when compared with the untreated corn stover. A further increase to 150°C results in a glucose yield 2.5 times of that from the untreated corn stover, and a 25% increase when compared with corn stover sample pretreated to 120°C. Thus, with an increase in pretreatment temperature, glucose yields also are increased in the corn stover biomass. A similar effect with an increase in temperature was observed by Zheng et al., (1998) [44] on Avicel and Kim et al., (2001) [45] on wood using a similar pretreatment method. The pretreatment temperatures (150°C and below) used in this method is low when compared with that of the steam explosion pretreatment (200°C), as the high temperatures of about 180°C results in the hemicellulose degradation.

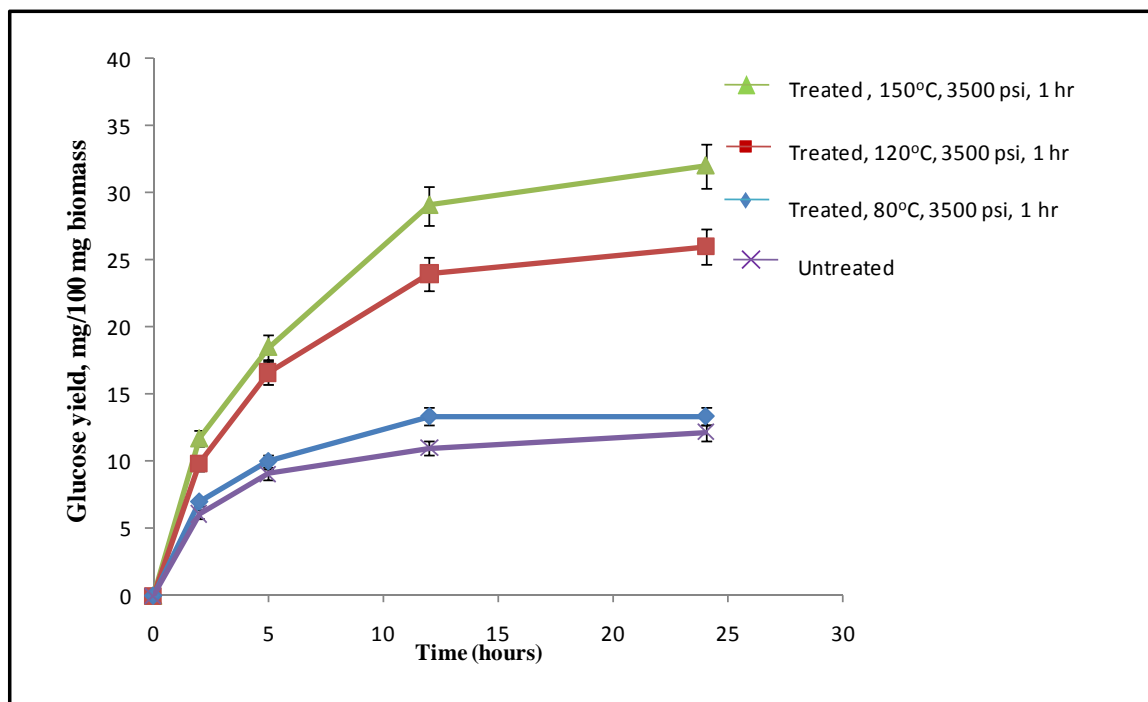


Figure 12. Effect of SC-CO₂ pretreatment temperature on the glucose yield from enzyme hydrolysis of corn stover biomass when the biomass was treated for different temperatures at 3500 psi for 1 hr, with 75% moisture content in biomass

4.1.3 Effect of Pretreatment Pressure

The effect of pretreatment pressure on the SC-CO₂ pretreatment of corn stover is shown in Figure 13. Pretreatment was carried out at various pressures whereas a constant temperature (150°C), time (1 hr) and moisture content (75%) were maintained. The glucose yield for 2500 psi and 3500 psi are 20% and 30% (w/w) of biomass respectively, and for the untreated biomass is 12% w/w of the biomass. At 2500 psi the glucose yield doubled compared with untreated biomass and at 3500 psi, the yield is 2.5X. The results indicate that with an increase in pressure the glucose yield of biomass also increases.

The higher pressure can result in a faster and deeper penetration of the SC-CO₂ into the biomass pores, thus, increasing the surface area for the enzyme action, upon sudden release of pressure. A similar effect of increase in glucose yield was observed by Zheng et al., (1998) [44] on Avicel, using the SC-CO₂ pretreatment method.

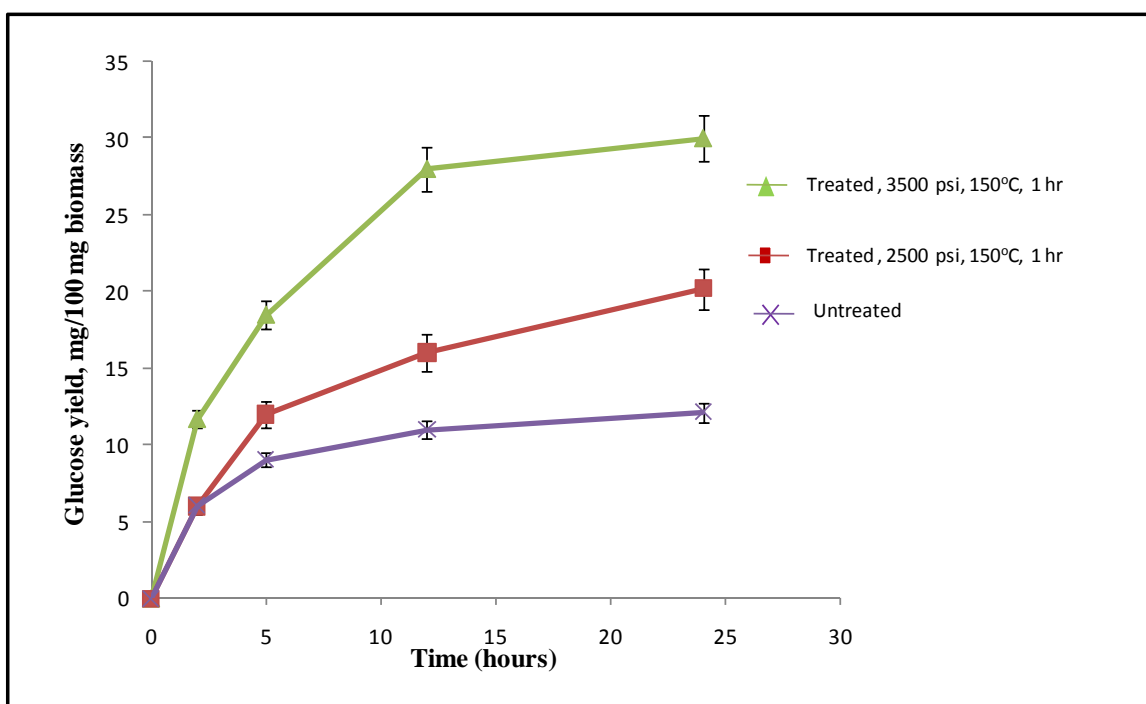


Figure 13. Effect of SC-CO₂ pretreatment pressure on the glucose yield from enzyme hydrolysis of corn stover biomass when the biomass was pretreated for pressures at 150°C, for 1 hr with 75% moisture content in biomass

4.1.4 Effect of Pretreatment Time

The effect of pretreatment time on the SC-CO₂ pretreatment of corn stover is shown in Figure 14. Pretreatment was carried out for various times whereas temperature (150°C), pressure (3500psi) and moisture content (75%) were maintained constant. The glucose yields for 10, 30, and 60 mins are 14%, 18% and 30% (w/w) of biomass, respectively and for the untreated biomass it is 12% w/w of biomass.

The glucose yield for 10 mins pretreatment time is similar to that of the untreated glucose yield. With an increase in the pretreatment duration to 30 mins, the glucose yield increased 1.5X when compared with untreated corn stover. With a further increase in time to 60 mins, the glucose yield increased to 2.5X compared to untreated corn stover. Thus, with an increase in the pretreatment time, the glucose yield also increased for corn stover. A similar pretreatment method used by Kim et al., (2001) [45] on wood for 30 mins of pretreatment time, had a similar effect as that of 60 mins, whereas, in our case, the 60 mins pretreatment time had a higher glucose yield than the 30 mins pretreatment time. This indicates that a pretreatment time of 1 hour or more is certainly preferable. The pretreatment for 1 hour is an affordable time in the industries, when compared with ammonia recycle percolation or the lime pretreatment methods, that takes more than 24 hours.

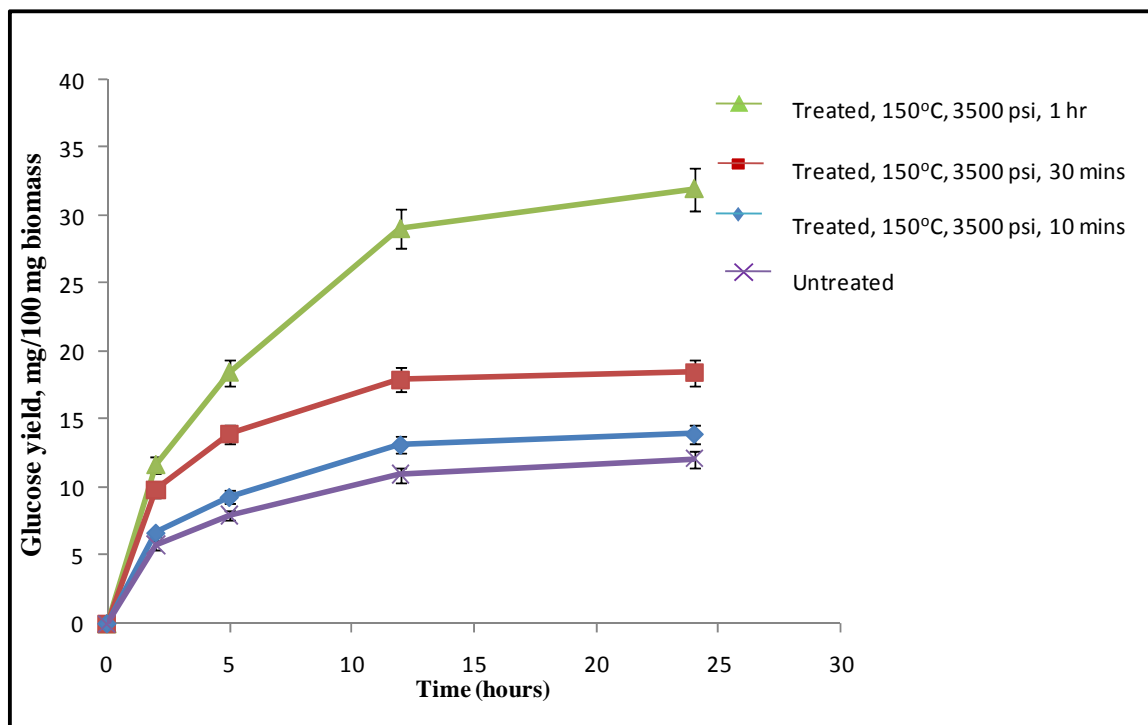


Figure 14. Effect of SC-CO₂ pretreatment time on the glucose yield from enzyme hydrolysis of corn stover biomass when the biomass was treated for different time durations at 3500 psi and 150°C with 75% moisture content in biomass

4.1.5 Pretreatment Reduces Enzyme Usage

The effect of enzyme loading for both the untreated and the SC-CO₂ treated corn stover (pressure 3500 psi; temperature 150°C) is shown in Figure 15. Two untreated samples were loaded with 50 units and 84 units of cellulase enzyme, whereas the pretreated samples were loaded with 50 units of cellulase enzyme. The samples were collected at fixed time intervals, and were analyzed for the glucose yield during the hydrolysis.

The pretreatment conditions are (150°C, 3500 psi, 1 hour and 75% moisture content) glucose yield is 30% (w/w). The glucose yield obtained for the untreated-84 units of enzyme loading, 14% (w/w) and for 50 units of enzyme loading, 12% (w/w). This result shows that with a 66% increase in enzyme loading for untreated samples, the yield obtained was only 16% more. On the other hand, with a 66% increase in enzyme loading for an untreated sample, the yield obtained was only 47% of the pretreated sample glucose yield.

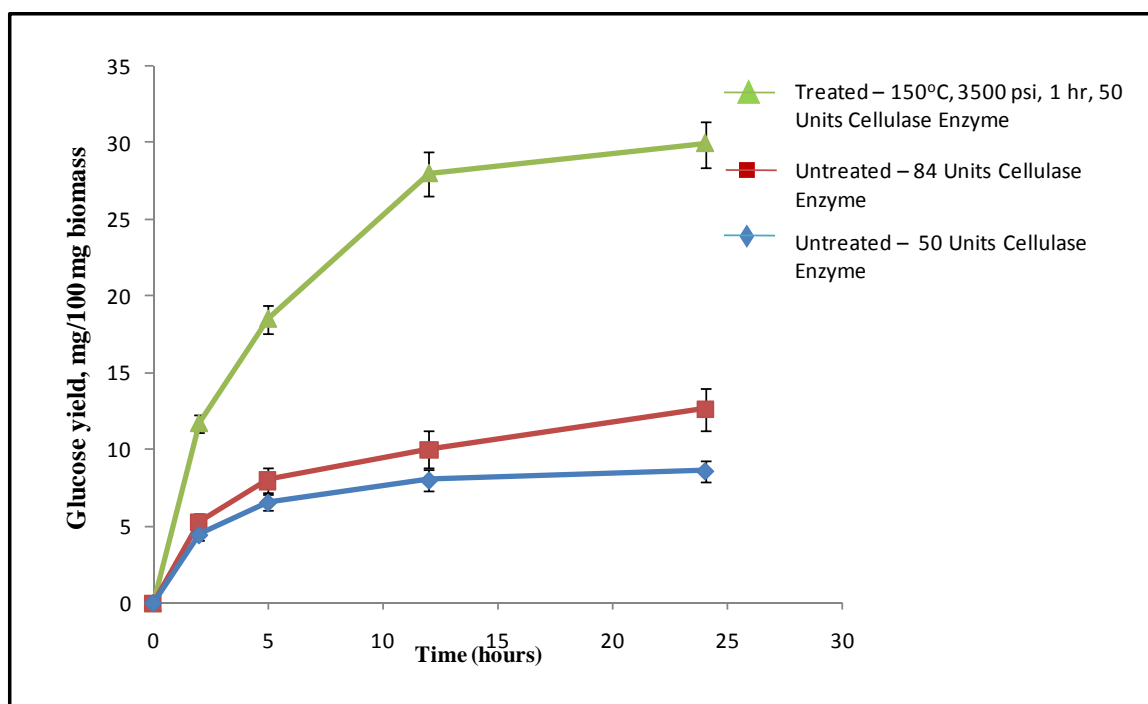


Figure 15. Glucose yields of pretreated and untreated corn stover by enzyme hydrolysis for different enzyme loadings. The pretreated sample was loaded with 50 units of cellulase enzyme and the untreated samples were loaded with 50 units and 84 units of cellulase enzyme, respectively

4.2. Pretreatment of Switchgrass

The effect of the SC-CO₂ pretreatment on switchgrass and pretreatment conditions are shown in Figure 16. The glucose yields for 100°C and 150°C are 13% and 14% w/w, respectively, and for the untreated biomass, 12% w/w of biomass. The glucose yield of the SC-CO₂ pretreated biomass shows no significant difference when compared with untreated biomass. The results indicate that the SC-CO₂ pretreatment had no effect on switchgrass.

The reason for the ineffectiveness of the SC-CO₂ pretreatment on switchgrass and corn stover could be due to the differences between the cell wall structures of the switchgrass and the corn stover. The cell wall structures and cell wall compositions differ between species, which could be true between switchgrass and corn stover, as shown in Table 8. Also the switchgrass used for the study was a long standing and more mature crop.

Table 8. Biomass compositions (% dry weight) of corn stover and switchgrass analyzed at the National Renewable Energy Laboratory [51]

Feedstock	Glucan	Xylan	Galactan	Arabinan	Lignin	Extractives	Ash
Corn Stover	40.9	21.5	1.0	1.8	16.7		6.3
Corn Stover*	36.4	18.0	1.0	3.0	16.6	7.3	9.7
Switchgrass	31.0	20.4	0.9	2.8	17.6	17.9	5.8
Rye Straw	33.1	19.46	0.31	2.47	19.8		6.1

Note: *Corn Stover includes corn stalks and cobs as they come out of the combine. The Mannan composition is not mentioned in the table

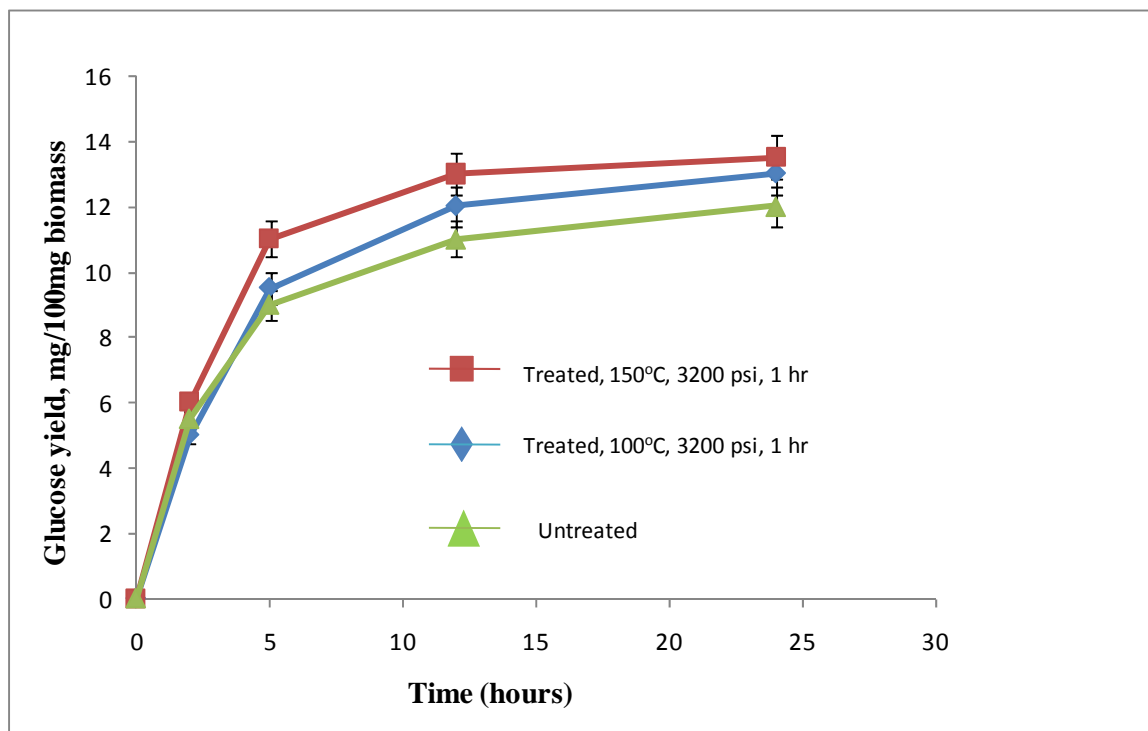


Figure 16. Effect of SC-CO₂ pretreatment on glucose yield of treated and untreated switchgrass by enzyme hydrolysis

4.3 Pretreatment of Rye Straw

The effect of the SC-CO₂ pretreatment on rye straw is shown in Figure 17. Rye straw was treated for different pretreatment conditions as shown. The pretreatment conditions were selected considering the results of corn stover experiments (150°C, 3500 psi, 1 hr and 75% moisture content). The pretreated rye straw glucose yield was 13% (w/w) and for untreated rye straw 7% (w/w). The pretreatment had significant impact on the sugar yield through hydrolysis. The glucose yield obtained for the treated samples are significant compared to untreated rye straw, however they are not of appreciable when compared with the of total glucan (glucose) of rye straw as shown in Table 8. Rye straw is a kind of grass which may have similar cell wall structure as switchgrass, hence the ineffectiveness of the SC-CO₂ pretreatment.

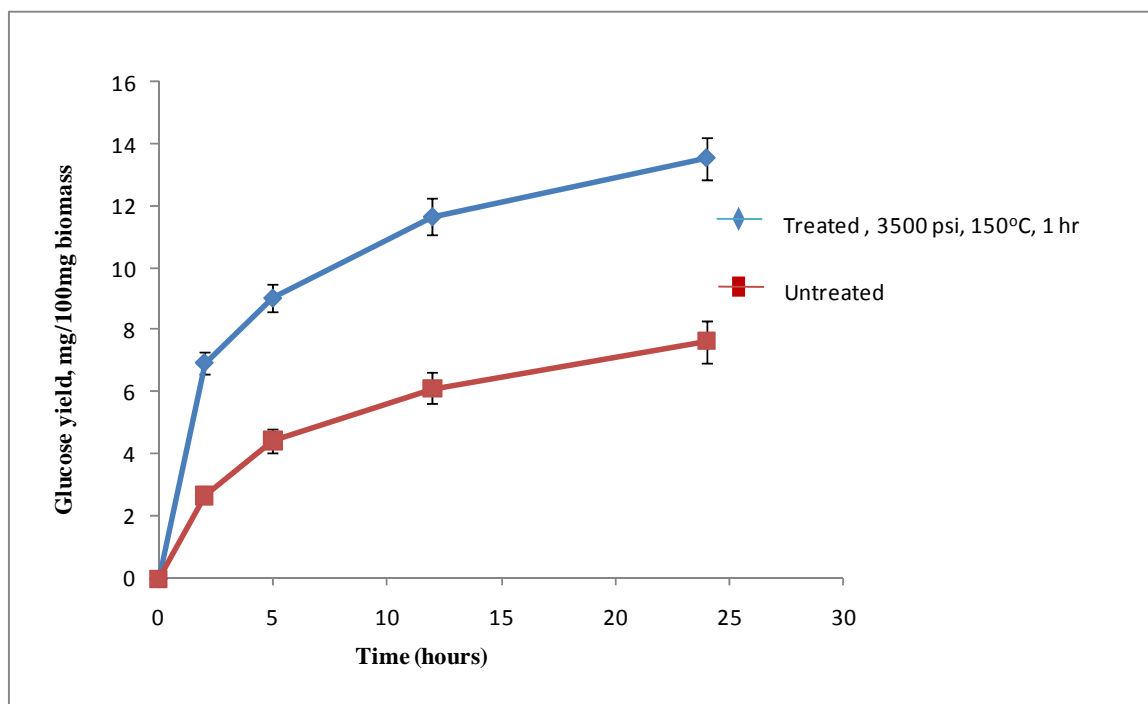


Figure 17. Effect of SC-CO₂ pretreatment on glucose yield of treated and untreated rye straw by enzyme hydrolysis. The rye straw was treated at 150°C, 3500psi for 1 hour with 75% moisture.

4.4 Combinations of Pretreatment

The slight addition of sulfuric acid as a catalyst in the steam explosion treatment helped in achieving the high sugar yields in wood [12]. Also, the single-step conversion of cellulose to HMF (5-hydroxymethylfurfural) using metal chlorides as a catalyst in the ionic liquids was attempted by S. Yu et al. [52]. A similar approach was attempted using the SC-CO₂ and a catalyst. The SC-CO₂ was used as a carrier agent to deliver acids and alkaline chemicals into the pores of biomass. Supercritical CO₂ has liquid-like wetting properties and gas-like diffusivity. Acids such as H₂SO₄ and HCl were used as catalysts in the SC-CO₂ pretreatment. The catalyst in small quantities, 10g of H₂SO₄ or HCl at pH 1.0 was added to the biomass and then subjected to SC-CO₂ treatment. Rye straw was chosen for this study in order to observe the effect of catalysts.

4.4.1 H₂SO₄ as Catalyst

The effect of the SC-CO₂ pretreatment and the SC-CO₂ treatment with H₂SO₄ as a catalyst on rye straw are shown in Figure 18. The pretreatment conditions are temperature-150°C, pressure-3500 psi and time-1 hr. The glucose yields for the SC-CO₂ pretreatment and the SC-CO₂ pretreatment with H₂SO₄ as catalyst are 13% and 17% (w/w) respectively and for untreated biomass, 7% (w/w). The addition of the catalyst increased the glucose yield by 0.3 times when compared to the SC-CO₂ with no catalyst pretreatment.

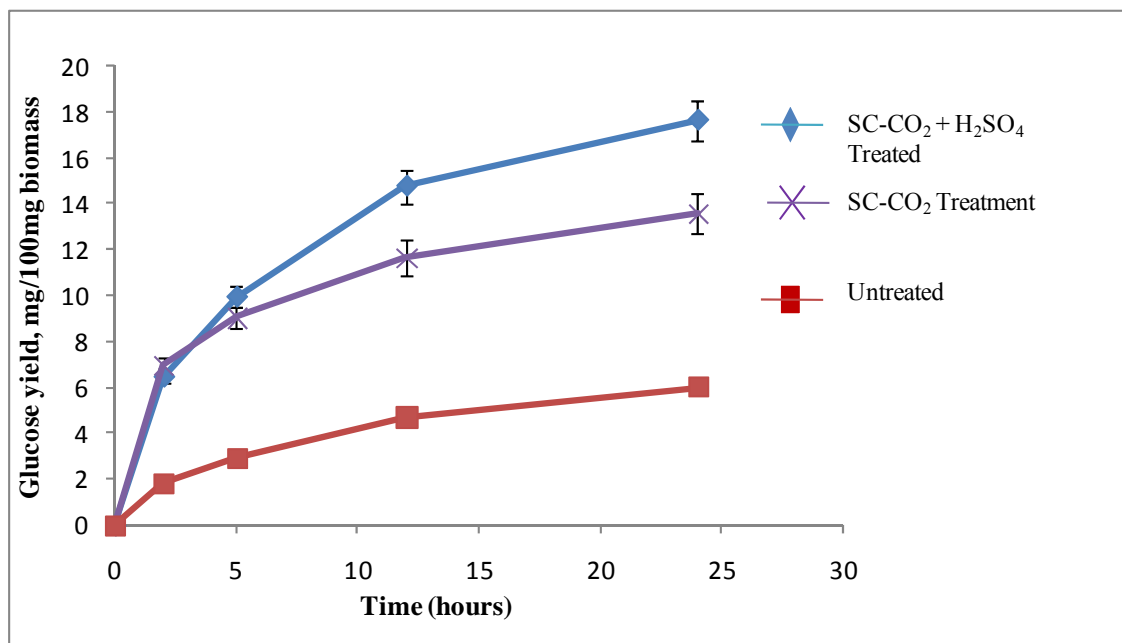


Figure 18. Effect of H₂SO₄ as a catalyst in SC-CO₂ pretreatment on rye straw hydrolysis

4.4.2 HCl as Catalyst

The effect of the SC-CO₂ pretreatment and the SC-CO₂ treatment with HCl as a catalyst on rye straw are shown in Figure 19. The glucose yield for the SC-CO₂ pretreatment and the SC-CO₂ pretreatment with HCl as catalyst are 13% and 22% (w/w) respectively, and for untreated biomass, 7% (w/w). The increase in yield is appreciable and the addition of HCl as a catalyst can be considered to have a significant effect on the SC-CO₂ pretreatment on rye straw.

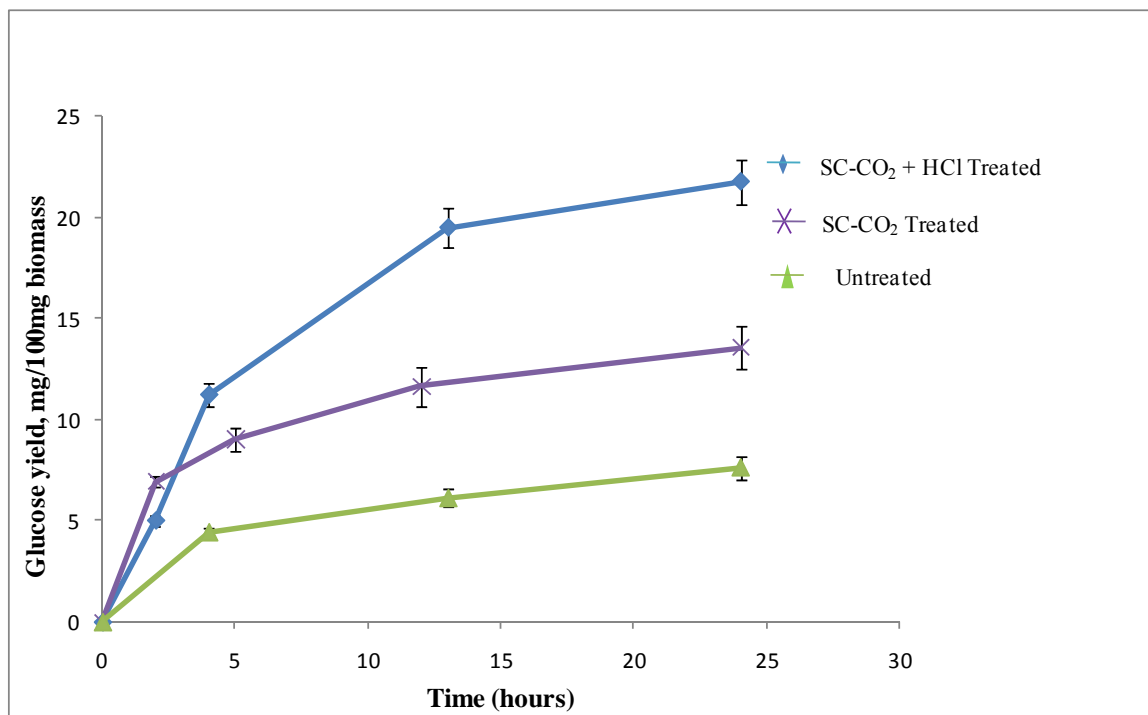


Figure 19. Effect of HCl as a catalyst in SC-CO₂ pretreatment on rye straw hydrolysis

4.5 X-Ray Diffraction Analysis

The X-ray powder diffraction analysis of corn stover (< dia 0.42 mm) was determined using an X-ray diffractometer. The X-Ray diffraction pattern of the SC-CO₂ pretreated and untreated corn stover is shown in Figure 20. Zheng et al., [44] observed reduction of 50% in crystallinity of the SC-CO₂ pretreated Avicel when compared with untreated Avicel. However, in our case the SC-CO₂ pretreated corn stover showed no change in crystallinity when compared with untreated corn stover. The reason could be that Avicel is pure cellulose and not associated with other cell wall molecules, whereas the corn stover is a plant cell wall, which is a complex mixture of cellulose, hemicelluloses, lignin and proteins. The presence of lignin and hemicelluloses in corn stover makes biomass more resistance for change in crystalline structure of the cellulose by the SC-CO₂

pretreatment. Thus, SC-CO₂ pretreatment had no effect on crystallinity of lignocellulosic biomass. It is noted that change in crystallinity is not the only factor which influences the enzymatic hydrolysis of biomass.

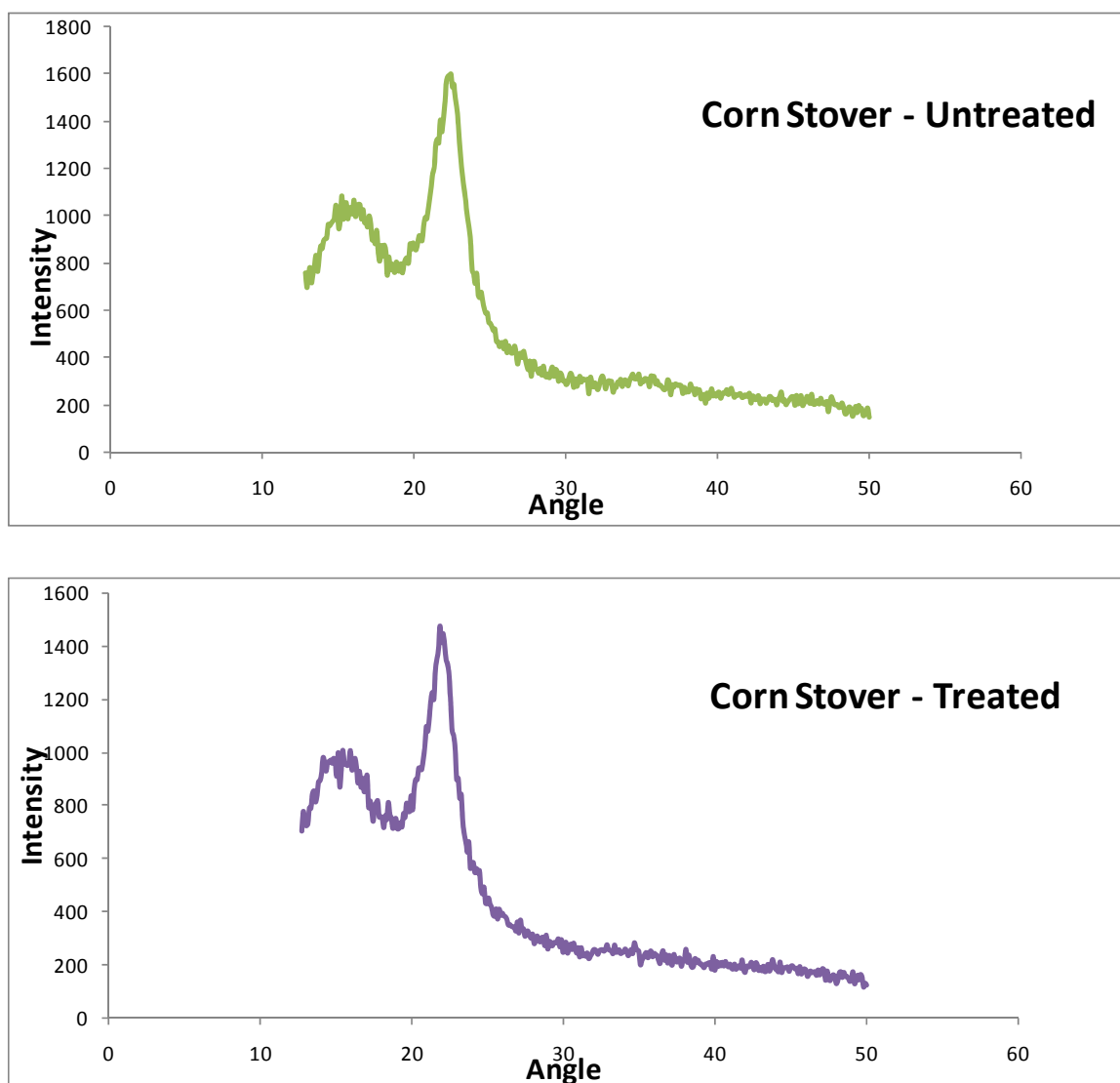


Figure 20. X-Ray diffraction of untreated and SC-CO₂ pretreated corn stover samples using theta-2-theta angle

4.6 Scanning Electron Microscope Analysis

The surface morphologies of untreated and the SC-CO₂ treated (150°C, 3500 psi, 1 hr, 75% moisture) corn stover were determined using SEM. The untreated corn stover samples are shown on the Panel A of Figure 21 and Figure 22 and the SC-CO₂ treated corn stover samples are shown on the Panel B. The samples were analyzed for different magnifications to see any differences in the surface morphology. The higher magnification images in Figure 23 show the inner surface was exposed by the SC-CO₂ pretreatment.

The untreated corn stover surface shows that the fibers are rigid, closely packed together. Thus much of the surface of the untreated corn stover is not accessible for enzyme action. The SC-CO₂ treated corn stover samples shows cracks on the surface of the biomass, exposing the inner surface for enzyme action. The SC-CO₂ treated corn stover surface area increased when compared with untreated corn stover. Increase in surface area leads to more active sites for enzyme action, thereby increasing the sugar yield, as surface area is one of the factors which determine the sugar yield.

A possible explanation for the increase in surface area for the SC-CO₂ treated corn stover is that at high pressure the SC-CO₂ enters the pores of the biomass, followed by a rapid release of pressure, causing the CO₂ to rush out of the pores quickly, thereby rupturing the pores and creating more surface area. The SC-CO₂ pretreatment creates more surface area for enzyme action, thereby increasing the glucose formation when compared with untreated corn stover. The more the surface area, the higher the sugar yields from enzyme hydrolysis. SC-CO₂ treated biomass should be pre-wetted and swollen with moisture. Figure 24 shows a comparison between untreated (Panel A) and SC-CO₂ treated (Panel B) corn stover without moisture. The SEM images indicate that the SC-CO₂ treatment did not open pores, unlike the effect shown in Figure 21. Without moisture, the dry biomass

is compacted such that CO₂ penetration of pores is difficult. It is possible that after the biomass is swollen with moisture, the water inside the pores allows dissolution of CO₂ under a high pressure during SC-CO₂ treatment. This allows CO₂ penetration of the pores, which is required for the rupturing of the pores upon the rapid release of CO₂ pressure. Carbonic acid in the pores may also help weaken the pores.

SEM results reveal only the changes in surface morphology of biomass. It cannot give insight on the total volume available for enzyme action. A further study on pore size change and pore volume distribution using mercury intrusion porosimetry test or solute exclusion porosity method is necessary to understand the effects of the SC-CO₂ pretreatment on biomass.

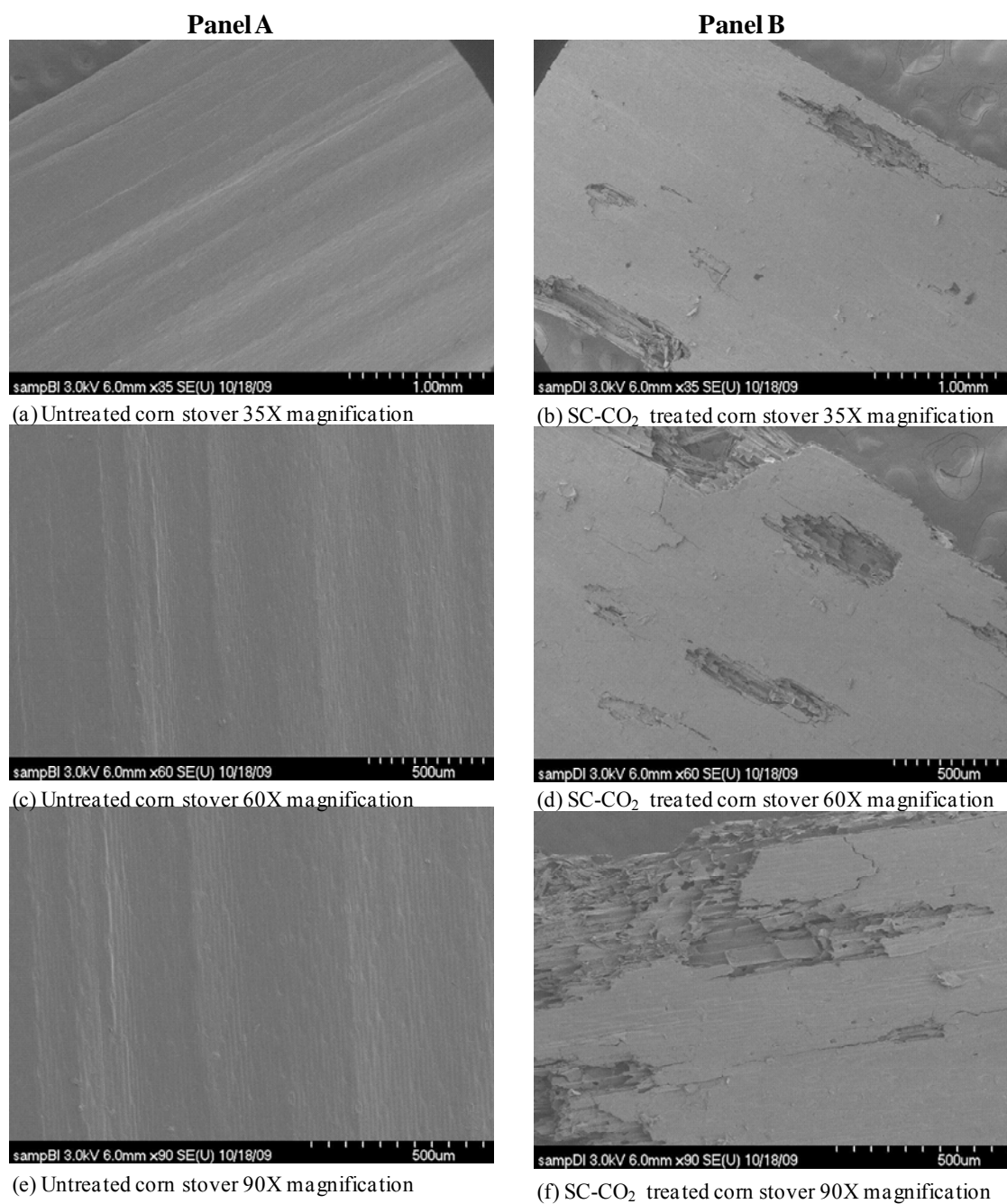


Figure 21. SEM pictures of untreated and SC-CO₂ treated corn stover samples at 35X, 60X and 90X magnifications

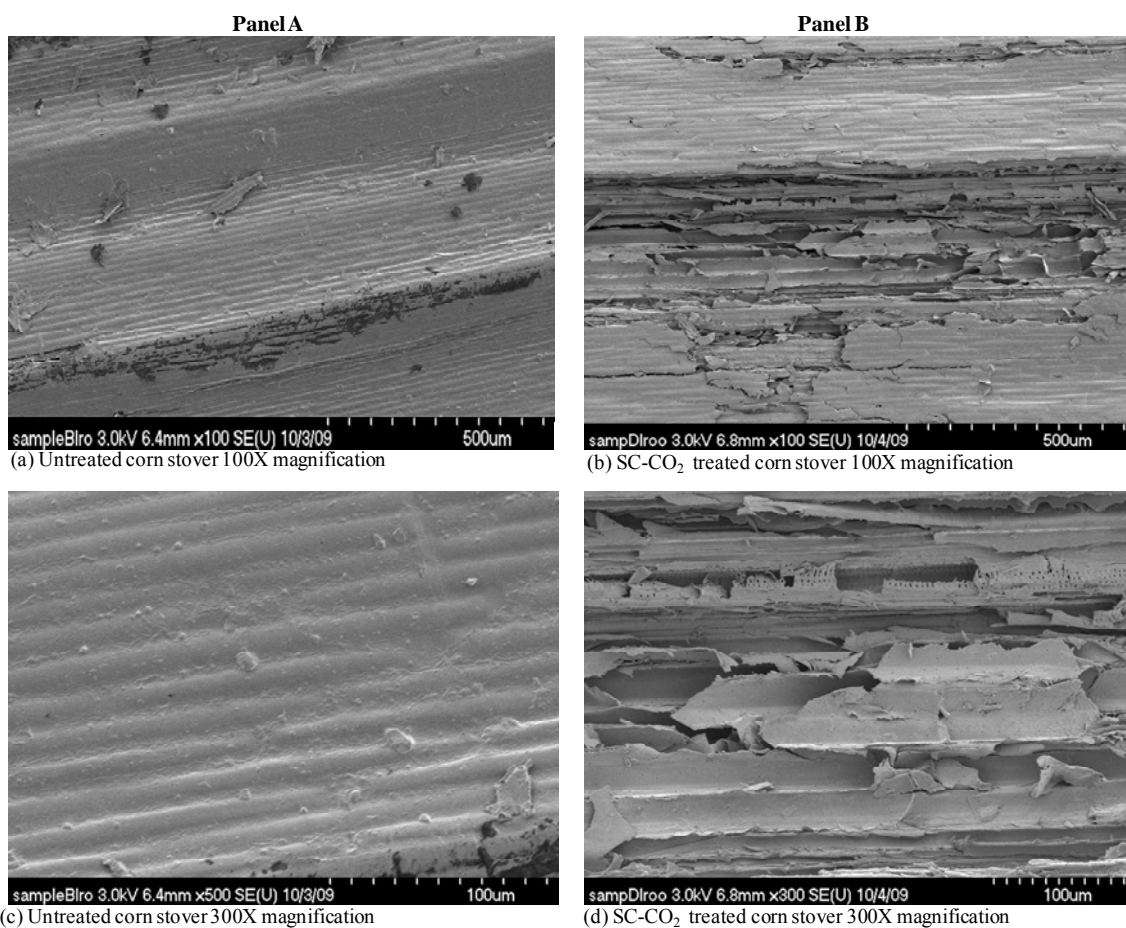


Figure 22. SEM pictures of untreated and SC-CO₂ treated corn stover samples at 100X and 300X magnifications

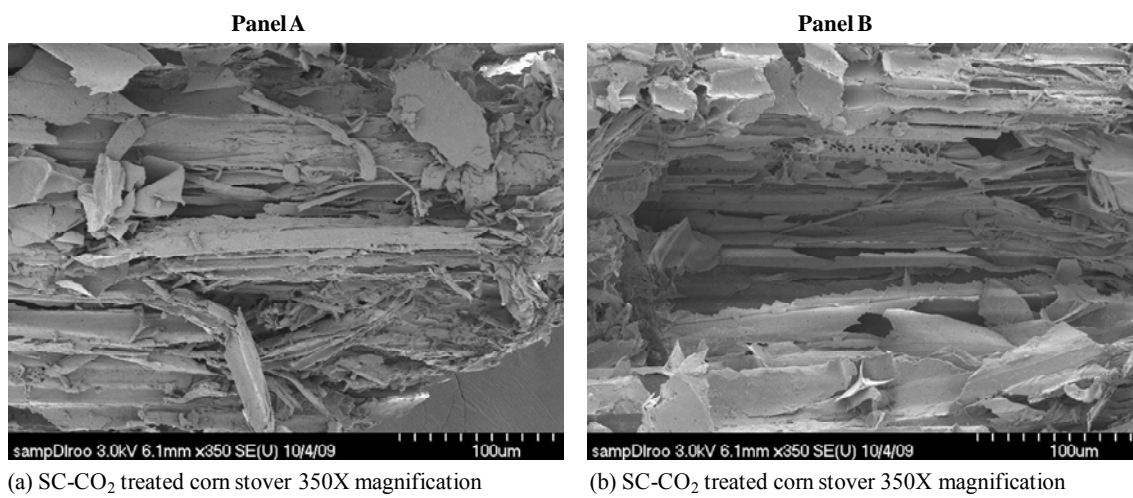


Figure 23. SEM pictures of SC-CO₂ treated corn stover at 350X magnifications

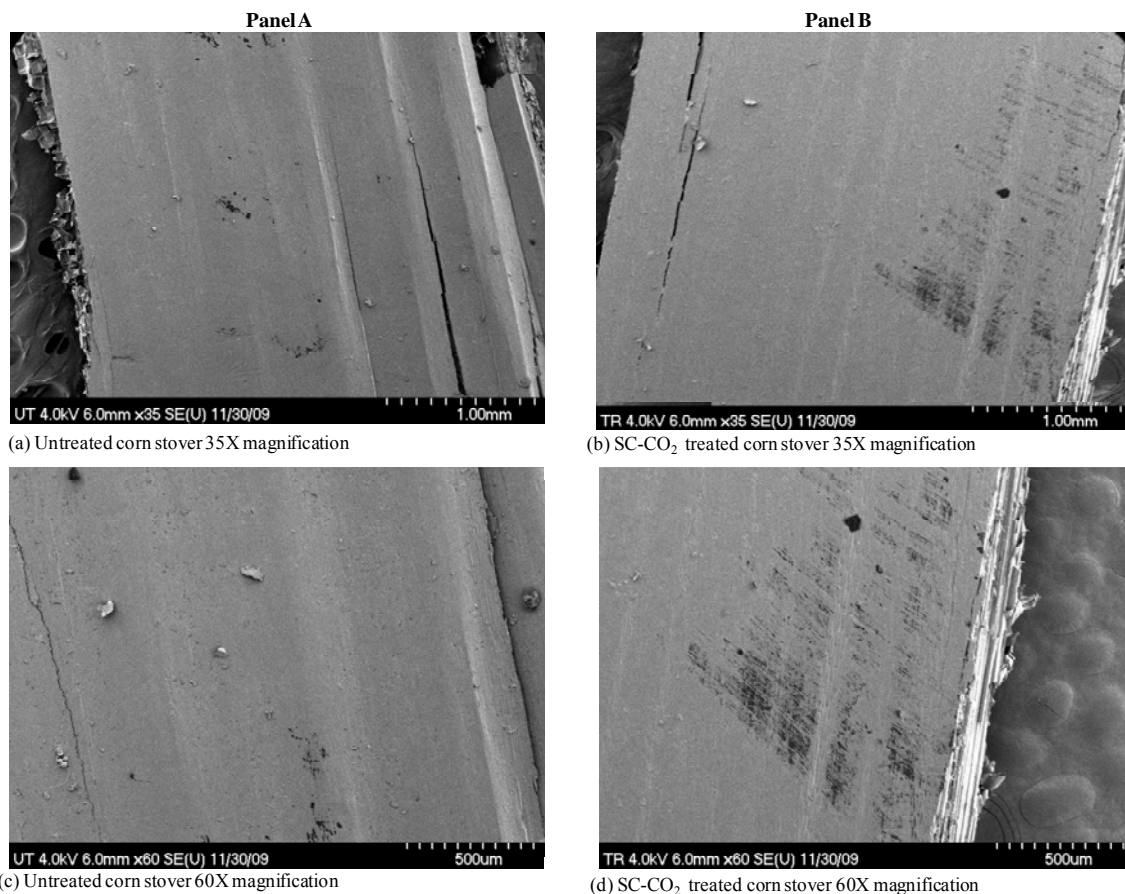


Figure 24. SEM pictures of untreated and SC-CO₂ treated corn stover samples with no moisture content at 35X, 60X and 90X magnifications

4.7 Thermodynamic Calculations of Supercritical CO₂

The amount of CO₂ required to attain supercritical temperature and pressure at a fixed volume can be calculated using EOS. The volume of the reactor was 94.7 ml. The pressure for various temperatures at fixed volume for carbon dioxide was theoretically calculated using van der Waals and Peng Robinson equations of state, compared with the experimental values. The results obtained are shown in Tables 9, 10 and 11.

The calculated pressure results at low densities ($<0.50\text{g/cm}^3$) from van der Waals equation are close to experimental values. However, the experimental values do not match the values obtained from the Peng-Robinson equation as shown in Table 9. The Peng-Robinson equation is applicable for real gases where gas and liquid are in equilibrium with each other. In supercritical phase the liquid and gas phases are not in equilibrium and they merge to form a single phase. This might explain the difference between experimental and Peng Robinson equation results.

With an increase in CO_2 density ($>0.50\text{g/cm}^3$) in the reactor, the pressures values calculated theoretically by van der Waals equation are far apart from experimental results as shown in Table 10. These results indicate that at higher densities the Supercritical CO_2 doesn't act as van der Waals fluids, as observed by Keiko Nishikawa et al., [48]. When the CO_2 density is high, CO_2 molecules are compressed more than the parameter b in the van der Waals equation describe. By using parameter m to reduce the b value in Equation 3, the calculated values from modified van der Waals equation are close to experimental values. If more data are available, a correlation between CO_2 density and m can be obtained. The modified van der Waals equation can be used to calculate pressure based on temperature and moles of CO_2 used. This "soft pump" method does not require a high pressure pump to achieve a certain operating CO_2 pressure in a SC- CO_2 setup since the pressure can be achieved by using the calculated temperature or CO_2 amount. A siphon-type of CO_2 gas cylinder or dry ice can be used to deliver CO_2 to the reactor.

The presence of water in the form of moisture in biomass can impact the thermodynamic properties of CO_2 . A visual observation was noted in critical point drier (CPD), which is equipped with a glass window for visual observation of phase change in fluids. The visual observation revealed that supercritical phase could still be achieved in the presence of water in biomass. Figure 25 shows the CO_2 phase changes in the presence of biomass. Also Raoult's law (mixing rule) was applied to calculate the deviation in pressure for CO_2 and water mixture in the presence of water. The results are shown in Table 9. The results show that the presence of water had less deviation of pressure in the mixture.

Table 9. Thermodynamic values of CO₂ from theoretical calculations (using van der Waals and Peng Robinson EOS) and experimental values at low densities

Temperature, K	No. of moles	Density, g/cm ³	Pressure, Peng Robinson, bars	Pressure, van der Waals, bars	Pressure - Experimental Values, bars	Difference between van der Waals EOS and experimental
325	1	0.46	253	113	103	+10
334	1	0.46	262	128	117	+11
341	1	0.46	269	139	131	+8
349	1	0.46	277	152	137	+15

Table 10. Thermodynamic values of CO₂ from theoretical calculations (using van der Waals and Peng Robinson EOS) and experimental values at high densities

Temperature, K	No. of moles	Density, g/cm ³	Pressure, van der Waals, bars	Pressure, Experimental Values, bars
333	1.6	0.75	716	193
340	1.6	0.75	752	217
334	1.7	0.79	997	220

Table 11. Thermodynamic values of CO₂ from modified van der Waals EOS and experimental values

Temperature, K	No. of moles	Density, g/cm ³	m	Pressure, Modified van der Waals, bars	Pressure, Experimental Values, bars
325	1	0.46	1	113	103
334	1	0.46	1	128	117
341	1	0.46	1	139	131
349	1	0.46	1	152	137
333	1.6	0.75	0.86	192	193
340	1.6	0.75	0.86	218	217
334	1.7	0.79	0.84	225	220

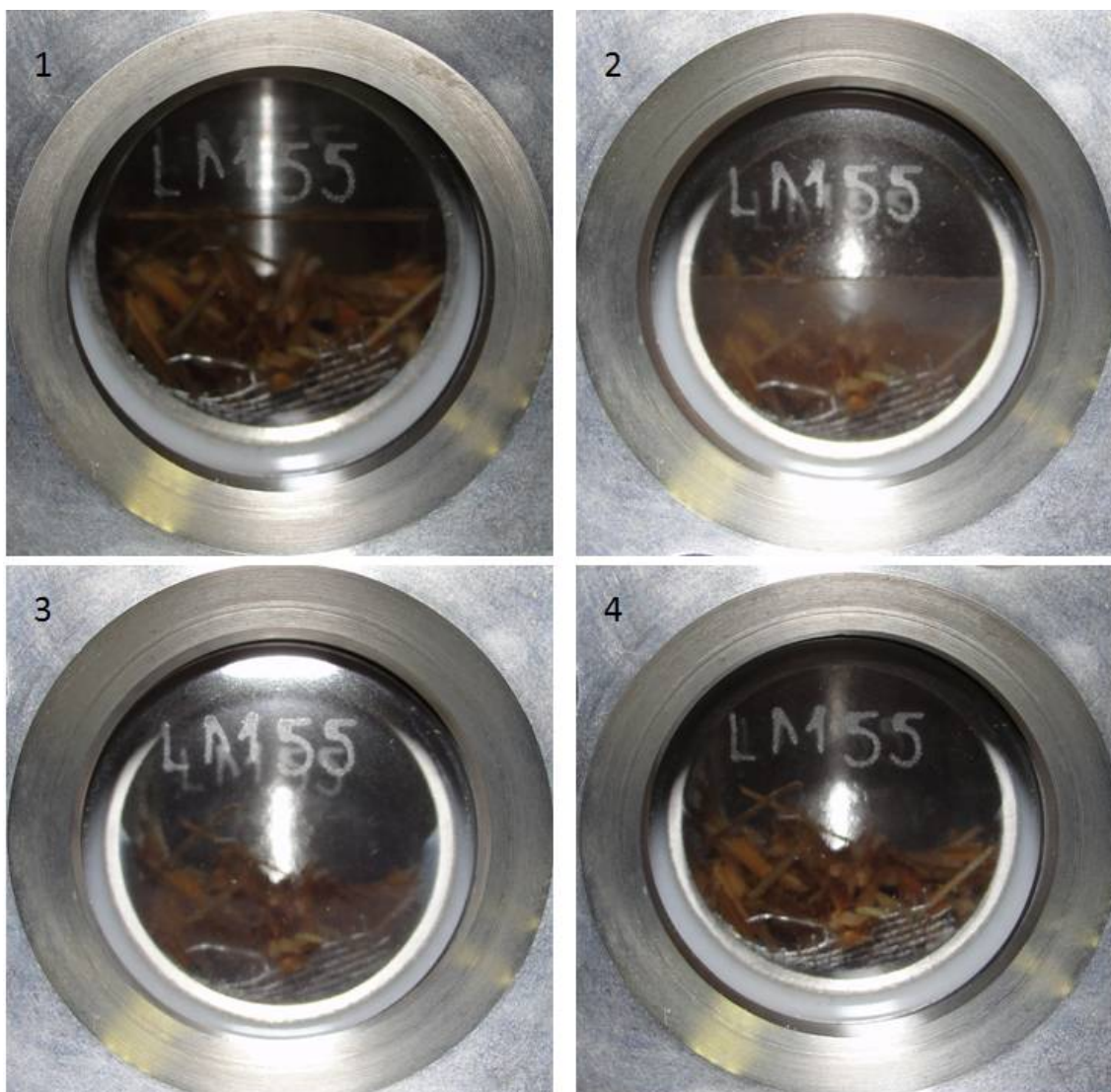


Figure 25. The CO₂ phase changes in the presence of biomass observed in critical point drier (CPD). The images are shown in the sequence of phase change when temperature increased from 12°C to 40°C

CHAPTER 5: CONCLUSIONS

The SC-CO₂ pretreatment on corn stover enhanced the glucose yield by enzyme hydrolysis when compared with untreated corn stover. The temperature and pressure of pretreatment played an important role in the glucose yield. Glucose yield was increased when pressure and temperature increased. The maximum glucose yield of 30% (w/w) was found at 3500 psi at 150°C when treated for 1 hr with 75% moisture content. The maximum glucose yield for untreated corn stover was found out to be 12% (w/w). There was no change in glucose yield of the SC-CO₂ pretreated switchgrass, 14% (w/w) when compared with untreated switchgrass 12% (w/w). The pretreated rye straw, 13% (w/w) showed twofold increase in glucose yield when compared with untreated 7% (w/w). This shows that SC-CO₂ pretreatment had almost no effect on switchgrass and had significant impact on rye straw. However the glucose yield of pretreated rye straw is not appreciable when compared with the total glucose of rye straw. The reason that SC-CO₂ pretreatment has an influence on corn stover, some impact on rye straw and no impact on switchgrass may be due to many factors. As discussed earlier in the Section 1.3.3, although all the plants cell walls have similar structures, the interaction of these components within the cell wall differs from species to species. Also, the cell wall composition is different from each other. The interaction of cell wall components in the cell wall is complex and the study in this field is still in its early stages.

The addition of H₂SO₄ to the SC-CO₂ pretreatment on rye straw showed little improvement in glucose yield as compared to only the SC-CO₂ pretreatment and addition of HCl to SC-CO₂ pretreatment on rye straw showed significant improvement in glucose yield as compared to only the SC-CO₂ pretreatment. XRD results shows that SC-CO₂ pretreatment had no effect on crystallinity of corn stover. The raw material used is lignocellulosic biomass, a complex mixture of cellulose, hemicelluloses, lignin and proteins that, when interact form a network that can be difficult to break the crystalline structure of cellulose. The SEM results show surface changes in the SC-CO₂ treated corn stover when compared with the untreated sample. The increase in surface area might have

increased the glucose yield of pretreated corn stover when compared with untreated corn stover. The SEM images of the SC-CO₂ pretreated corn stover with no moisture content show no changes in the surface morphologies when compared with the untreated corn stover. The results show that water in the form of moisture plays an important role in the SC-CO₂ pretreatment. Water might help to open up the pores in the biomass.

In the SC-CO₂ pretreatment method, the solid residual amount is high compared with acid hydrolysis and aqueous ammonia pretreatment methods. Thus, a higher sugar yield can be achieved in this kind of pretreatment method. Overall, the SC-CO₂ method is an effective pretreatment on corn stover biomass.

The thermodynamic calculations showed that supercritical condition can be easily achieved by using liquid CO₂, eliminating the need for a pump. Dry ice could be one more alternative. The presence of small amount of water in biomass had no influence on CO₂ reaching the supercritical state. The visual observation noted in critical point drier (CPD), which is equipped with glass window for visual observation of phase change in fluids revealed that supercritical phase was achieved in the presence of biomass and moisture.

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