# PROCESS BENEFITS OF USING BIOMASS PELLETS IN A BIOREFINERY

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**Title**

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## ABSTRACT

Pelleted biomass can help simplify biomass supply systems and reduce downstream processing costs that are vital for the development of commercial biorefineries. This study is based on comparison of process benefits and economic factors of using loose and pelleted biomass over a range of low to high pretreatment severity and hydrolysis enzyme loadings. Use of pelleted biomass provides flexibility either to reduce pretreatment severity, enzyme loadings, hydrolysis time, or combinations of these. Either enzyme loadings can be reduced by 80% or hydrolysis times reduced by 58% with the use of pelleted biomass. A comparative techno-economic analysis using each form of biomass reveals that using pelleted biomass is economically beneficial. The minimum ethanol selling price for loose biomass was found to be $4.41/gal ethanol and $3.83/gal ethanol for pelleted biomass. The economic study suggests that optimizing conversion processes could lower the final ethanol costs even further.

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# 1. GENERAL INTRODUCTION

With the increasing world population and economic growth of developing countries, the demand of energy is increasing and is expected to double by 2050 compared to what we consumed in 2006 (Ladanai and Vinterbäck, 2009). Current energy supply is heavily dependent on fossil fuels which have negative environmental impacts. Among the economic sectors, the transportation sector solely is responsible for around one-fourth of global greenhouse gas emissions (Taptich et al., 2016). The supply of fossil fuels in the future is also uncertain due to unknown reserves of fossil fuels. From energy security and environmental point of view, several alternative renewable energy sources are needed. Biomass can be an option to reduce the use of fossil fuels and its chemical derivatives. Lignocellulosic biomass is renewable and the most abundant biological material on earth. A recent study indicates that 1.2-1.4 billion tons of biomass can be supplied sustainably on an annual basis in the United States alone (Langholtz et al., 2016). The Energy Independence and Security Act (2007) also envisioned the necessity of producing 16 billion gallons per year of cellulosic biofuels by 2022 (EISA, 2007). This requires both a cost-effective biomass supply and processing technologies.

The processing of lignocellulosic biomass into advanced biofuels and chemicals, however, has challenges. Biomass is bulky and spread out geographically, which translates to a costly supply system. Biomass is also harvested seasonally requiring a huge capital for storage systems to make it available year-round for processing. In addition to difficult and costly supply chain logistics, processing of biomass within a biorefinery could be costly. The recalcitrant nature of biomass, due in part to the presence of lignin around the bundle of cellulose-hemicellulose, makes it difficult to breakdown into simple sugars by enzymatic action. A costly

pretreatment process and large amounts of costly enzymes are needed to overcome the biomass recalcitrance and process it to fermentable sugars (Himmel et al., 2007).

Biomass densification to form pellets can be one option to minimize transportation and processing costs. The primary advantage of pelleting is the simplified supply chain systems with reduced transportation, handling, and storage costs (Balan, 2014). It can also have processing advantages in downstream processes like pretreatment and hydrolysis cost reduction. Use of pelleted biomass enables higher pretreatment solids loading, lower pretreatment severity, and reduced enzyme loadings and hydrolysis time (Nahar and Pryor, 2017; Nahar et al., 2017). Earlier studies overlooked the benefits of using pelleted form of biomass because it was thought that the logistics advantages would be offset by the pelleting cost itself.

This thesis focuses on determining the process and economic benefits of using pelleted biomass compared to loose biomass in a biorefinery. A set of low to high pretreatment conditions following soaking in aqueous ammonia pretreatment and enzyme loadings will be tested to determine the conditions required to achieve 90% glucose yields. Based on these results, a comparative techno-economic analysis of producing ethanol using loose and pelleted biomass will be done. The techno-economic analysis will be done following a National Renewable Energy Laboratory (NREL) dilute acid model (Humbird et al., 2011). The analysis will determine the capital and operating cost requirements for each set of processing conditions for both loose and pelleted biomass, and this information will be used to determine the minimum ethanol selling price.

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## 2. LITERATURE REVIEW

Biomass is defined as any organic matter derived from animals, plants, or micro-organisms. It stores chemical energy originally derived from sunlight through photosynthesis. That stored energy can be used in different ways: thermally by combustion, or chemically in the form of fuels (Agbor et al., 2011; McKendry, 2002). The conversion of biomass energy into different forms (e.g. biofuels, biogas, or heat energy) is based on the end-use purpose. For example, conversion of biomass into biofuels such as ethanol and butanol is needed for using them as motor vehicle fuels.

Biomass energy is considered a renewable energy source because biomass is constantly generated over time. For example, plants and animals are continuously growing on earth and biomass derived from them, such as dead plants and animals, organic wastes, and agricultural crops, can always be used as an energy source. Biomass can be taken as an alternative to fossil fuels, but the carbon emissions from them while harnessing their energy is similar. One benefit of using biomass is that it doesn't build-up carbon dioxide in the atmosphere while the use of fossil fuels does. The carbon dioxide released from biomass energy extraction is constantly taken up by plants in the carbon cycle while the fossil fuels release carbon dioxide trapped inside the land into the atmosphere and hence result in a net accumulation of carbon in the atmosphere. This makes biomass superior to conventional energy sources from an environmental perspective.

Biomass accounts for about 1.5% of the energy used in the United States (U.S. Energy Information Administration, 2016) and around 10% (50 EJ) of world total primary energy supply today (International Energy Agency, 2017). Interest in biomass energy is increasing due to the increasing world energy demand, diminishing conventional energy sources, and increasing

environmental problems. Biomass is readily available around the world and can help reduce the dependence on conventional energy.

## 2.1. Biomass Sources

There is no strict way for classifying biomass. Generally, biomass is classified based on the types (like vegetation, and ecology) of biomass available in nature or on their applications. Broadly, biomass can be classified into the following categories:

### 2.1.1. Dedicated energy crops

These crops are planted explicitly for the purpose of using them as an energy feedstocks. They are planted on marginal lands where other food crops are not suitable. They can be further classified as herbaceous plants or woody biomass. Examples include herbaceous plants like switchgrass, miscanthus, sorghum, and woody plants like bamboo, poplar, and willow.

### 2.1.2. Agricultural crops

Corn, sugarcane, soybean and other oilseeds are the most prevalent examples under this category. Corn and sugarcane have been used widely for producing ethanol while soybean is used for producing biodiesel.

### 2.1.3. Agriculture crop residues

Crop residues are the leftovers of plants from the field production. They include leaves and stalks of the crops. Examples include corn stover, wheat straw, and rice straw.

### 2.1.4. Forestry residues

They include the residues from the forest materials such as leaves, smaller branches, bark, and dead trees. A primary product called round wood is more valuable and used for pulp/paper or lumber.

### 2.1.5. Aquatic plants

The aquatic plants include varieties of sources that originate in fresh water, ponds, lakes or sea water. Examples include water hyacinth, reeds, and rushes.

### 2.1.6. Biomass processing residues

Biomass processing residues originate from the wood industry, paper and pulp industry, and food processors. Sawdust from the wood processing plant and apple pomace from apple processing plants are some of the examples. These sources of biomass are generally inexpensive and are a useful on-site energy source, but are not plentiful enough for conversion to other fuels.

### 2.1.7. Municipal waste

Municipal waste includes a significant amount of biomass that can be further processed to produce energy. Energy can be produced from wastes either by burning the wastes to generate heat or by decomposing them to get methane gas (Li et al., 2011). This organic matter includes sewage sludge and industrial wastes. Using these wastes as an energy source not only reduces their environmental impact but also the overall cost of such processing plants.

### 2.1.8. Animal waste

Animal manure from farms and animal processing plants can be used to produce energy, primarily in the form of biogas via anaerobic digestion.

## 2.2. Uses of Biomass

Biomass can be a valuable energy source to fulfill the increasing energy demands and overcome the dependence on traditional fossil fuels. It can be used in the production of fuels like ethanol, butanol, biodiesel or biogas. Ethanol and biodiesel can be blended with gasoline and petroleum diesel in certain ratios without any major modification in the engine parts. Biomass is also used in the production of biogas which can be used for generating heat and electricity.

Biomass can also be combusted directly (sometimes in a mixture with coal or other fuels) to generate electricity or for heating purposes.

## 2.3. Current Trends

This literature review is focused on biofuel generated from cellulosic biomass. Biofuels can be produced by fermentation of the fermentable sugars extracted from biomass (Badger, 2002) using yeast or several other micro-organisms.

### 2.3.1. Starch and sugar feedstocks – first generation biofuel

The current forms of biofuel are mostly produced by using starch or sugar feedstocks; these fuels are generally called first-generation biofuels. Either the sugars are extracted directly from sugarcane or beets, or they are obtained by hydrolyzing starch from corn, wheat or potatoes. These feedstocks fall into human food chains, so, use of these sources for energy production has led to public concerns. It has also created concerns about competition for using the land for energy and food crops, which might increase food price.

### 2.3.2. Cellulosic feedstocks – second generation biofuel

There are concerns about promoting starch and sugar feedstocks because in future there might be competitions in using lands in planting energy crops rather than the food crops which might lead to food shortages. This fact motivated researchers to look for technologies to convert lignocellulosic biomass to biofuels. Lignocellulosic biomass is more desirable because it is available all around the world. Cellulose and hemicellulose are building blocks for every plant cell wall. They are relatively abundant, widespread, and fall outside of the human food chain. Biofuels produced from these carbohydrate sources are called second generation biofuels.

There are generally two ways to convert biomass to biofuels: the thermochemical platform and the biochemical or sugar platform (Balan, 2014). In the thermochemical platform,

syngas is produced by gasification and that gas is converted to biofuel by chemical or biological conversion. The biochemical platform involves deconstructing longer carbohydrate chains into pentose and hexose monomers (mostly glucose) using enzymes; sugars are then fermented to biofuels or other bioproducts. However, the extraction of fermentable carbohydrate from cellulosic biomass is expensive due to the energy and chemical inputs required to overcome biomass recalcitrance to biodegradation. Recalcitrance is related to the components and structure of the cell wall.

## 2.4. Conversion Processes

Biomass conversion is necessary to convert the biomass to a more useful energy form, according to end-use purpose. Biomass in its raw form is not suitable for all purposes. There are losses during all conversion processes and so it is important to minimize such losses. The biomass characteristics that impact conversion processes include energy density, moisture content, alkali metal content, the proportion of cellulose/hemicellulose/lignin, and ash contents (McKendry, 2002).

### 2.4.1. Combustion

This is the simplest method of conversion of biomass into energy. The biomass is directly burned and energy is used for heating or electricity production.

### 2.4.2. Gasification and pyrolysis

Gasification essentially involves high-temperature processing of biomass in the presence of a low amount of oxygen (not sufficient to start combustion) which deconstructs biomass into syngas (a mixture of primarily hydrogen and carbon monoxide). The gas is then converted into fuel by catalytic or biological conversion (Balan, 2014). Pyrolysis is a similar process, but it is carried out at lower temperatures to produce a condensable product called bio-oil.

### 2.4.3. Biochemical conversion

In biochemical conversion, biomass (cellulose and hemicellulose) is reduced to the simpler form of carbohydrates which is then fermented to desired fuel or chemical. The technology behind the biochemical conversion of lignocellulosic biomass is not fully commercialized and a lot of research is going on to optimize the process and reduce costs.

## 2.5. Biomass Composition

Biomass is composed of cellulose, hemicellulose, lignin and a small percentage of other proteins and extractives (Verardi et al., 2012). The percentage of each component varies with different plants. Cellulose is a homogeneous polymer of glucose (a hexose) while hemicelluloses are heterogeneous in nature and are a mixture of 5-carbon and 6-carbon sugars. Cellulose has a fibrous-like structure arranged in bundles called fibrils and hemicellulose is intertwined with cellulose. Lignin acts as a binding material in the cell wall and consists of phenylpropane units (Verardi et al., 2012). Lignin is particularly resistant to biodegradation and contributes to recalcitrance. Cellulose is of greater importance while it comes to biochemical conversion because it is composed entirely of glucose, which is most readily fermentable to more valuable products. The composition of major lignocellulosic materials are shown in Table 1 (Sun and Cheng, 2002).

**Table 1. Composition of some common biomass (Sun and Cheng, 2002)**

| Lignocellulosic materials | Cellulose (%) | Hemicellulose (%) | Lignin (%) |
|---|---|---|---|
| Hardwood trees | 40-55 | 25-40 | 18-25 |
| Softwood trees | 45-50 | 25-35 | 25-35 |
| Corn cobs | 45 | 35 | 15 |
| Grasses | 25-40 | 35-50 | 10-30 |
| Paper | 85-99 | 0 | 0-15 |
| Wheat straw | 30 | 50 | 15 |
| Leaves | 15-20 | 80-85 | 0 |
| Cotton seed hairs | 80-95 | 5-20 | 0 |
| Newspaper | 40-55 | 25-40 | 18-30 |
| Waste papers from chemical pulps | 60-70 | 10-20 | 5-10 |
| Primary wastewater solids | 8-15 | - | 24-29 |
| Solid cattle manure | 1.6-4.7 | 1.4-3.3 | 2.7-5.7 |
| Coastal Bermuda grass | 25 | 35.7 | 6.4 |
| Switchgrass | 45 | 31.4 | 12 |

### 2.6. Biomass Densification/Size Reduction

Biomass has a lower energy content per unit volume and per unit mass compared to fossil fuels. The lower bulk density and energy density requires higher volumes of biomass to be processed for the same energy output (Clarke and Preto, 2011). These properties also complicate the storage, transportation, and handling of the biomass. So, biomass densification is advantageous before any further processing. The primary reason for biomass size reduction and densification is to increase the density. Densification of biomass is typically done by forming briquettes or pellets.

Briquettes are produced either by using piston press or by screw extrusion. Biomass is first ground to fine particles and is pressed through a die at high pressure (Clarke and Preto, 2011). Generally, briquettes have a diameter equal to or greater than 25 mm. Biomass briquettes are mostly used for combustion to generate heat. They are used in industries in combination with

coal and sometimes as a substitute to heat boilers. Briquettes are also used as a substitute for cooking fuels and heating system in homes.

Biomass pellets are another form of densified biomass which are smaller in size (typically 6 to 8 mm diameter) than the briquettes. They can be advantageous in biochemical conversion of biomass to biofuels (Nahar and Pryor, 2017). Biomass bales from the field can be passed through a hammer mill for initial size reduction. That material can then be fed to a pellet mill where further grinding occurs. Finely ground material passes through extrusion dies to form pellets (Mani et al., 2006). The temperature during the pellet forming process is around 100-130 $^{o}$C which melts the lignin to act as a binder. In some cases, starch, soluble sugars, fat or protein are used as external binders (Kaliyan and Morey, 2010).

### 2.6.1. Advantages of size reduction and densification

Biomass size reduction and densification have several advantages in subsequent processes. Some of the advantages are:

- Easier mechanical handling and storage, and lower transportation cost (Clarke and Preto, 2011).
- Homogeneous shape and structure distribution and minimization of dust formation (Clarke and Preto, 2011).
- Biomass particle size reduction increases total surface area and pore size of the ground particles and also reduces the cellulose crystallinity (Lin et al., 2016).
- Reduction in pretreatment severity and enzyme loadings (Nahar and Pryor, 2014; Rijal et al., 2014; Rijal et al., 2012).
- Reduction in use of water, chemicals, and energy; thus, reducing the net greenhouse gas emissions (Nahar, 2017).

### 2.6.2. Pelletization

Pelletization is a relatively energy-intensive process. Biomass pellets are formed by coarsely grinding the biomass in a hammer mill and then feeding to a pellet mill where fine grinding occurs. The powdered form is densified by passing them through extrusion dies at high pressure. During the compaction phase, the biomass passes through elastic and plastic phases (Balan, 2014) and the temperature during the process reaches 130 ˚C.

Some factors affecting pelletization include: moisture content, temperature, particle size, and pelletizing pressure (Stelte et al., 2012).

#### *2.6.2.1. Moisture content*

The moisture content of the biomass affects the quality of pellets. The optimum moisture content for pelleting corn stover was found to be 10% (wt) (Kaliyan and Morey, 2010). Moisture content above the optimum level adversely affects the mechanical properties and density of the pellets. Nielsen et al. showed the increase in moisture content reduces the energy requirement for pelletization for pine and beech (Nielsen et al., 2009).

#### *2.6.2.2. Temperature*

Heat is generated as a result of friction between the biomass and the die. Increase in temperature during the processes enhances the strength and durability of pellets, reduces friction in the press channel of the mill (Stelte et al., 2012), and hence lowers energy required for the process (Nielsen et al., 2009).

#### *2.6.2.3. Particle size*

Stelte et al. (2012) found that the friction increases with a decrease in biomass particle size due to the increase in particle surface area. Kaliyan et al. (2010) also found the density of the pellets increased with a decrease in particle size, but Serrano et al. (2011) found just the

opposite. The difference in the result is explained to be the use of an industrial pellet mill in the latter one and the laboratory scale single pellet press units in the former (Stelte et al., 2012). A broad variation in particle sizes, in general, is beneficial for good quality pellets. For pellets, particles with a size 0.5 mm diameter should not exceed 10-20% if no external binding agents are used (Stelte et al., 2012).

#### *2.6.2.4. Pelletizing pressure*

The pressure applied during pelleting also influences pellet density, durability, and energy consumption (Stelte et al., 2012). Several studies have been done in this area (Carone et al., 2011; Gilbert et al., 2009; Kaliyan and Morey, 2010). It was found that the correlation between pressure and pellet density follows the saturation curve in which the maximum limit of pellet density to be equal to that of the plant cell wall of that particular biomass. The compressive strength and durability also increases with increase in pressure up to a certain limit, after which any further increase in pressure didn't give better quality pellets. The excess pressure results in heat production and loss (Stelte et al., 2012).

## 2.7. Biomass Pretreatment

Lignocellulosic materials are recalcitrant to cellulase enzyme activity in their raw form. The cellulose fibers are intertwined with hemicellulose and covered by lignin in an outer layer (Haghighi Mood et al., 2013). Lignin and hemicellulose are closely associated with cellulose microfibrils which limit the accessibility of microbial enzymatic hydrolysis (Agbor et al., 2011). The digestibility of biomass depends on factors including feedstock particle size, total lignin content, cellulose crystallinity and degree of polymerization, porosity (accessible surface area of cellulose), cellulose sheathing by lignin and hemicellulose, and cellulose fiber strength (Alvira et al., 2010; Mosier et al., 2005).

Pretreatment is defined as any process which helps to decrease the inherent recalcitrance of biomass and increase the accessibility of enzymes for effective hydrolysis. Therefore, the main principle behind any pretreatment process is to weaken the biomass structure to make cellulose more susceptible to microbial and enzyme actions. This could be done either by removing lignin or hemicellulose. Removing or deconstructing either of those increases porosity, thus, increasing the accessibility to cellulose. This means the pretreatment process would be regarded as effective if it removes lignin preventing unwanted binding of the enzyme with it and thus increases cellulose digestibility. Pretreatment is considered the most expensive unit operation in processing biomass to biofuels, but it is required for effective downstream processing to increase the yield of fermentable sugars in less time.

### 2.7.1. Methods of pretreatment

Pretreatments methods are typically classified into physical, chemical, biological and physicochemical pretreatments. Physical pretreatment reduces the particle size and increases surface area, but are typically not effective enough on their own. Chemical pretreatments are carried out using different chemicals like acids (sulfuric acid, hydrochloric acid or organic acids), bases (sodium hydroxide, ammonia, or lime) or other solvents (organic solvents, ionic liquids, or ozone) (Balan, 2014). Biological pretreatment uses microorganisms like fungi and bacteria to degrade lignin and hemicellulose. Even though the process is inexpensive, it is very slow and introduces the possibility of further cellulose degradation (Balan et al., 2008). Physico-chemical processes include pretreatments like a steam explosion, liquid hot water, ultrasound pretreatment and ammonia fiber expansion (AFEX).

#### *2.7.1.1. Acidic pretreatment*

Acidic pretreatments solubilize hemicellulose into monomeric xylose while disrupting lignin structure to improve enzyme accessibility (Balan, 2014; Modenbach and Nokes, 2012; Yang and Wyman, 2008). Either dilute acids (up to 2.5% wt/wt) are used at a higher temperature (>160 ˚C), or concentrated acids (up to 85% wt/wt) are used at lower temperatures (<160 ˚C) (Alvira et al., 2010; Sun and Cheng, 2002). Concentrated acids are not used much because they produce more inhibitory compounds, are corrosive, toxic, and hazardous, requiring higher costs for operation and maintenance. Moreover, the cost of concentrated acids is high and requires the process to incorporate acid recovery. Dilute acids are more favorable than concentrated acid in that they tend to produce fewer inhibitory degradation compounds. The degradation compounds like furan and HMF (5-hydroxy methyl-furfural) can inhibit hydrolysis or fermentation and further degrades into undesirable products like formic and levulinic acids at high temperatures, resulting in loss of fermentable sugars (Modenbach and Nokes, 2012). Formation of these inhibitory compounds occurs at the expense of sugar yields (Jönsson and Martín, 2016). Jönsson et al. (2016) have studied the formation of different inhibitory products in different pretreatments.

#### *2.7.1.2. Alkaline pretreatment*

Alkaline pretreatments work primarily by solubilizing or modifying lignin and increasing biomass surface area by swelling (Carvalheiro et al., 2008; Modenbach and Nokes, 2012). This helps increase enzyme accessibility to cellulose. The crystallinity and degree of polymerization of cellulose are also reduced. Some of the cellulose and hemicellulose may be solubilized in the process, but much less than with acidic pretreatment (Carvalheiro et al., 2008). Alkaline pretreatments can be done at lower temperatures, but the effectiveness of the pretreatment

depends on a combination of the severity of temperature, time, and concentration. Alkaline pretreatment is more effective on agricultural residues than wood materials because of the higher lignin content in the latter (Kumar et al., 2009). Some inhibitors like phenolic compounds and hydroxyl acids are also produced during the alkaline pretreatment (Hendriks and Zeeman, 2009). Washing can remove these inhibitors, but any solubilized sugars are also washed off decreasing the overall yield. Alkaline pretreatments are less corrosive than the acidic pretreatments.

#### *2.7.1.3. Ultrasound pretreatment*

Ultrasonic pretreatment is based on the principle that when ultrasound waves are introduced in a liquid or slurry medium, acoustic streaming occurs and cavitation is formed generating strong mechanical shear forces that help to disintegrate biomass materials and increase surface area (Bussemaker and Zhang, 2013; Mason and Lorimer, 2002; Yachmenev et al., 2009). Ultrasound has sonochemical and mechnoacoustic effects on lignocellulosic materials (Bussemaker and Zhang, 2013). The sonochemical effect includes the production of oxidizing radicals which causes degradation of carbohydrates, mostly hemicellulose because of its easy accessibility, and the degradation of lignin through breaking of carbon-carbon linkages in lignin structure. The erosion of surface structures is influenced by mechanoacoustic effects. The parameters that can be altered during the process includes solid loadings, ultrasonic frequency, residence time and reactor configuration (Bussemaker and Zhang, 2013). The effectiveness of ultrasound pretreatment in downstream processes has not been explored much compared to other forms of pretreatment. Yachmenev et al. (2009) found that ultrasound could increase hydrolysis yield of corn stover and sugar cane bagasse up to 60%, but yields were not comparable to other pretreatment processes.

### 2.7.2. Pretreatment selection

Selection of a suitable pretreatment is a crucial step in biomass processing, and it influences downstream processes. Pretreatment is necessary for increasing overall sugar yields in the hydrolysis step. An ideal pretreatment process would have the following characteristics (Alvira et al., 2010; Balan, 2014; Yang and Wyman, 2008):

- Highly digestible pre-treated solid
- Minimum or no formation of toxic compounds
- Operation in reasonably-sized and moderate-cost reactors
- Minimization of waste residues
- Moderate operating parameters
- Lignin recovery
- Scaling-up

#### *2.7.2.1. Highly digestible pre-treated solid*

The pretreatment should be able to produce highly digestible cellulose which can give yields of more than 90% in a short time (preferably less than 3 days) with low enzyme requirements. The loss of sugars and their degradation during the pretreatment should be minimum. Higher recovery of xylose can be an additional advantage for the production of sugars.

#### *2.7.2.2. Minimum or no formation of toxic compounds*

The pretreatment process should not produce degradation products which inhibit downstream processes. If such products are formed, it adds additional costs to remove those products, ultimately making the pretreatment more expensive.

#### *2.7.2.3. Operation in reasonably-sized and moderate-cost reactors*

The reactor cost can be minimized by the use of inexpensive construction materials. So, the pretreatment process should not be corrosive to such materials. The size of the reactor should also be minimum for lowering the costs.

#### *2.7.2.4. Minimization of waste residues*

The process should not produce toxic residues. Any wastes produced should be easily disposable or usable for other processes.

#### *2.7.2.5. Moderate operating parameters*

Moderate operating parameters like low temperature, low pressure, and low chemical needs will reduce the processing cost and help for commercialization. If the process requires low temperature and pressure, the reactors cost is reduced as are safety concerns.

#### *2.7.2.6. Lignin recovery*

Lignin can be useful for generating heat and electrical energy required for the biorefineries. If it can be recovered, then it is an additional benefit that helps to minimize the production cost by lowering the external energy input.

#### *2.7.2.7. Scaling-up*

The pretreatment process should be compatible at industrial scale and without significant scale-up challenges.

## 2.8. SAA Pretreatment

Soaking in aqueous ammonia (SAA) pretreatment is an example of an alkaline pretreatment which is effective in removing lignin and disrupting the hemicellulose-cellulose structure to improve enzymatic hydrolysis. Ammonia concentration, pretreatment temperature and pretreatment time can be altered to adjust the severity and effectiveness of pretreatment.

Glucan, xylan, and lignin removal depend upon the severity of the pretreatment. Higher temperatures, higher concentrations of ammonia, and longer pretreatment time lead to higher delignification. SAA pretreatment retains almost all of the glucan, and more than 80% of xylan, but typically removes 60-65 % of lignin (Kim and Lee, 2007).

The composition and yield of SAA pretreated corn stover pellet with different pretreatment severities are shown in Table 2 (Nahar and Pryor, 2017).

**Table 2. Composition and yield (24 h) of SAA pretreated corn stover pellet with different pretreatment severities (Nahar and Pryor, 2017)**

| Pretreatment condition | | | Composition (%) | | | Glucose yield |
|---|---|---|---|---|---|---|
| T (ºC) | A (%) | H (h) | Glucan | Xylan | Lignin | |
| 30 | 8 | 2 | 34.5 | 18.9 | 19.2 | 65.7 |
| 30 | 22 | 4 | 40.8 | 21.3 | 18 | 71.4 |
| 45 | 15 | 4 | 40.8 | 21.2 | 17.6 | 83.3 |
| 45 | 22 | 3 | 36.6 | 19.2 | 17 | 77.8 |
| 60 | 8 | 4 | 36.4 | 20.5 | 15.7 | 86.4 |
| 60 | 15 | 3 | 43.3 | 21.9 | 14.4 | 93.3 |
| 60 | 22 | 4 | 42.7 | 21.4 | 14.2 | 97.8 |

*T – pretreatment temperature
*A – ammonia concentration
*H – pretreatment time

The trend from the above table shows glucan recovery for pretreated biomass and glucose yield from hydrolysis increase with an increase in pretreatment severity.

### 2.9. Enzymatic Hydrolysis

Hydrolysis of lignocellulosic biomass can either be done using acid or enzymes. Enzymatic hydrolysis is preferable because it is cost effective and more environmentally friendly compared to acid hydrolysis. Acid hydrolysis requires high operating temperature, produces degradation compounds like hydroxyl-methyl furfural (HMF), is corrosive and has a lower conversion efficiency than the enzymes (Bon and Ferrara, 2007; Lynd et al., 2005).

Enzymes help to degrade biomass to simple sugars. The degradation products formed during the processes and the lignin present can inhibit the enzyme, increasing the overall enzyme requirement. Hydrolysis effectiveness depends on the effectiveness of pretreatment. The removal of lignin and hemicellulose increases enzyme accessibility. During hydrolysis, some oligosaccharides are also formed which are unproductive during fermentation without further hydrolysis.

Glucose is the main product from cellulose hydrolysis and can be used to produce several other products. Some of the uses of glucose are production of ethanol or butanol through fermentation, biopolymer production, single cell protein production, and in the production of chemicals and drugs such as penicillin, acetone, citric acid, or amino acids (Fan et al., 2012).

### 2.9.1. Principle of hydrolysis

Cellulose enzymatic hydrolysis is separated into three steps catalyzed by three categories of cellulases (Verardi et al., 2012):

- Endoglucanase changes the degree of polymerization chemically by cutting the long chain polymers internally, and the accessible surface area of the cellulose fibers for enzymes.
- Cellobiohydrolase (or exoglucanases) facilitates hydrolysis by breaking the long chain polymers to cellobiose from the ends of the chains. This is a slower process.
- β-glucosidases catalyze the final step incomplete hydrolysis by breaking down the soluble intermediate cellobiose to glucose.

### 2.9.2. Factors affecting enzymatic hydrolysis

Biomass digestibility and sugar yield from hydrolysis are dependent on several parameters which can be broadly classified into substrate and enzyme related factors (Alvira et al., 2010; Leu and Zhu, 2013; Mansfield et al., 1999; Sun and Cheng, 2002).

#### *2.9.2.1. Substrate-related factors*

Enzymatic saccharification of lignocelluloses basically depends on the contact between cellulose and cellulase enzymes, reactivity between them, and reaction conditions (Leu and Zhu, 2013). Lignin acts as a binding agent to keep hemicellulose and cellulose together and is a barrier for cellulose hydrolysis. Removal of lignin provides accessibility to hemicellulose and cellulose which are intertwined between each other. Hemicellulose is more rigid and recalcitrant to enzyme action than cellulose. Deconstruction or removal of hemicellulose increases the porosity of biomass and hence, increases the accessibility for enzymes (Alvira et al., 2010; Leu and Zhu, 2013). Enzyme reactivity with the substrate is related more to the inhibition caused by lignin and hemicellulose (Leu and Zhu, 2013; Mansfield et al., 1999). Non-productive binding of cellulase to lignin reduces the enzyme activity on cellulose. Since SAA pretreatment removes around 65% of lignin, enzyme reactivity is not much of a problem. The main reaction conditions that influences hydrolysis include temperature and pH (Leu and Zhu, 2013). The optimal temperature for most cellulase enzymes is 50ºC and the optimal pH is in the range 4.8-5. However, Leu et al. (2013) suggested to use pH in the range 5.2 to 6.2 for higher cellulose saccharification and to reduce cellulase binding to lignin. pH can influence the surface charge of the biomass and induce hydrophobicity which enables lignin binding to cellulase (Leu and Zhu, 2013). Mansfield et al. (1999) have studied other substrate related factors that influence

hydrolysis. Those factors include degree of polymerization, crystallinity, accessible surface area, particle size, and lignin distribution.

#### *2.9.2.2. Enzyme-related factors*

The enzyme-related factor that affects enzymatic hydrolysis includes end-product inhibition by cellulase complex, thermal inactivation, and irreversible adsorption of enzymes (Mansfield et al., 1999). Cellobiose causes inhibition of cellobiohydrolases. This can be addressed by adding β-glucosidases which helps in cleaving the cellobiose to glucose. The synergism between different enzymes is very important while designing the enzymatic hydrolysis process (Alvira et al., 2010; Mansfield et al., 1999). Different enzymes work synergistically to break down the complex carbohydrate structure into simpler sugar. Cellulase, cellobiase, and xylanase enzymes are needed in appropriate proportion for this, but they are typically added as complex mixtures. Cellulase helps to break down the cellulose structure. It includes endoglucanase that acts on the low crystallinity region of the cellulose, while exo-glucanase acts on the free chain end. Cellobiase (β-glucosidase) enzymes breakdown the cellobiose into glucose monomers while xylanase breaks down the xylan to give monomeric xylose. Cellulase binding is mostly related to the structure of biomass and its crystallinity. Cellulases sometimes irreversibly bind with lignin thus inhibiting the enzymatic action. Similarly, the crystalline structure of biomass also influences the relative binding of enzymes. The crystalline structure can have differing faces and corners that result in differential adsorption (Mansfield et al., 1999).

## 2.10. Effect of Pelletization and Pretreatment on Enzymatic Hydrolysis

Biomass particle size reduction and densification are very important for effective downstream processing. Size reduction occurs in two stages: one by passing through hammer

mills to coarsely grind, and then within the pellet mill to finely grind. The finely ground particles are formed into pellets by passing them through extrusion dies. These processes involve the generation of high shear stress between biomass particles, and generates high temperature and pressure. These factors help to reduce biomass recalcitrance by disrupting the hemicellulose and lignin structure, and increases available surface area for better enzyme accessibility (Rijal et al., 2012; Sun and Cheng, 2002).

Pretreatment of biomass to reduce biomass recalcitrance is an important processing step before enzymatic hydrolysis. Biomass recalcitrance can be caused by structural components like lignin and hemicellulose. The modification of structural components is dependent on the types of pretreatment method used (Alvira et al., 2010). Therefore, it is very important to understand the interaction between the densification and pretreatment methods. Acidic pretreatment is better at removing hemicellulose, while alkaline pretreatments tend to remove lignin.

The interaction between pelletization and pretreatment on enzymatic hydrolysis has been studied for different pretreatment processes. All studies (Guragain et al., 2013; Nahar and Pryor, 2014; Ray et al., 2013; Rijal et al., 2012; Shi et al., 2013; Theerarattananoon et al., 2012a) that considered pelleting showed that the chemical composition of biomass doesn't change with pelletization. Even though there are fundamental differences between different pretreatment methods, the results of enzymatic hydrolysis obtained from the combination of biomass pelleting and pretreatment are worthwhile.

The effect of pelleting conditions on enzymatic hydrolysis of corn stover, big bluestem, wheat straw, and sorghum stalk was studied by Theerarattananoon et al. (2012a). The study used original biomass samples and three different pellet sets; first, with 3.2 mm screen size and 31.8 mm die thickness, second with 3.2mm screen size and 44.5 mm die thickness, and the third one

with 6.5 mm screen size and 44.5 mm die thickness. The study showed that using a thicker die and larger hammer mill screen to form pellets gives pellets with higher durability and bulk density. Theerarattananoon et al. (2012b) also studied the effect of pelleting and dilute acid pretreatment on above feedstocks. The study found an increase in biomass crystallinity after pelleting and dilute acid pretreatment. The increase in crystallinity by pelleting maybe due to change in lignin structure and crystallization of the amorphous phase of cellulose. The increase in crystallinity after dilute acid pretreatment is due to the removal of amorphous hemicellulose and the disruption of hydrogen bonding of cellulose chains. Theerarattananoon et al. (2012b) also did a FTIR spectra analysis and did not find much difference in the pattern of FTIR spectra between loose biomass and different pelleting conditions. This suggests that the pelleting process did not have much effect in the biomass structure. A similar study was done with pretreated samples which showed varying intensities in cellulose signals of biomass samples. This suggests that pretreatment did alter the cellulose structure of biomass.

Ray et al. (2013) studied the effect of pelletization on bioconversion of corn-stover for dilute acid pretreatment. The study was carried out under low (3.3% w/w) and high solids (25% w/w) loading conditions. The low solids dilute acid pretreatment with the pellets showed 59% xylose yield, which is about 50% increase from the ground and source material. The higher yield suggests that pretreatment severity conditions can be reduced to achieve comparable yields to what have been achieved commercially at high severity conditions. The study also showed better results with low-solids pretreatment than with high solids pretreatment. The combination of severity parameters at different conditions can produce similar results which show that different severity conditions have a different effect on sugar release. Even though the specific surface area and pore volume of biomass decrease with pelleting due to densification, the pretreatment

process increases surface area and pore volume once it gets mixed with the chemicals. Large surface area and pore volume are essential for better enzyme accessibility. (Shi et al., 2013) also demonstrated a higher rate of biomass saccharification while using pellets in combination with ionic liquid pretreatment than with loose biomass.

Alkali NaOH pretreatment with different feedstocks viz. corn stover, sorghum stalk, wheat straw, and big bluestem showed higher delignification with the pelleted biomass compared to loose biomass Guragain et al. (2013). This indicates that pelleting help in deconstructing lignocellulosic biomass. Even though there was higher mass loss i.e. low mass recovery in pelleted samples after pretreatment, the final ethanol yield for both pelleted and loose biomass was similar. This suggests that hydrolysis efficiency for the pelleted sample was higher. The study also looked at the delignification values in these feedstocks in unpelleted and pelleted forms and found the pelleting process aided in delignification. This means the pelleting process can be done in biomass in which the selected pretreatment process is less effective in removing lignin. Guragain et al. (2013) also showed higher hydrolysis productivity for pelleted biomass that with unpelleted biomass which means we can achieve similar hydrolysis yield between pelleted samples and loose biomass samples either by reducing enzyme loadings or hydrolysis time or the combination between them.

The combination of switchgrass pelleting with soaking in aqueous ammonia and dilute acid pretreatment was studied by Rijal et al. (2012). They considered loose biomass, biomass pellets, and powdered biomass for the study. Powdered biomass is the biomass obtained from the pellet mill that goes find grinding, but not the extrusion process. Pelleting alone increased the glucose yield by 210% compared to the yield obtained from loose biomass. The 24-hr glucose yield was 7 g/L and 6.5 g/L for powdered biomass and pellets while the original biomass had just

1.5 g/L. This shows that the pelleting process facilitates a better enzymatic hydrolysis. The graph between glucose or xylose concentration and time showed a steep slope for pelleted and powdered biomass compared to loose biomass. The higher slope in yield means we can obtain higher output in less time. This suggests that the pretreatment severity parameters (time, temperature and chemical concentration) and enzyme loadings can be reduced to obtain comparable yields with the loose biomass.

Nahar and Pryor (2014) studied the effect of pelleting on SAA pretreatment and enzymatic hydrolysis. The hydrolysis result of loose switchgrass and pelleted switchgrass without pretreatment showed a 120% increase in glucose yield for pelleted biomass (from 2.3 g/L to 5.1 g/L). The increase in yield suggests that the pretreatment severity and enzyme loadings can be reduced with the pelleted samples. The comparison was also made with two pretreatment conditions at 40 ºC and 60 ºC, and 15% aqueous ammonia for 6 hours. In both cases, the pelleted biomass showed a higher yield (56% in the former and 76% in the latter case). Similarly, the effect of enzyme loadings was also studied. Cellulase loading of 10-18 FPU/g glucan and hemicellulase loading of 300-125 XU/g glucan can be applied to get over 90% yield for pelleted biomass. Previous study by Karki et al. (2011) showed 70% glucose yields with 25 FPU/g glucan loading and similar pretreatment condition. Rijal et al. (2012) tested with the addition of 25 FPU/g glucan and 3500 XU/g glucan which only gave 79% yield. This suggests that pelleting in combination with pretreatment can help in reduction of enzyme loadings.

A study on SAA pretreatment showed 70% greater delignification for corn stover pellets compared to loose corn stover Nahar and Pryor (2017). Higher delignification is beneficial for better enzymatic hydrolysis as it helps to open up biomass structure for enzyme accessibility. The study showed higher lignin removal results in higher sugar yields. The study also showed a

higher solid loading of up to 20% during pretreatment can be done with the pellets without negatively affecting the hydrolysis yields. The study also found the increase in crystallinity after SAA pretreatment which supports previous findings (Theerarattananoon et al., 2012b). The SEM micrographs of non-treated corn stover and pelleted stover showed a distinct difference. The loose stover sample showed an ordered and more rigid fibril while the pellets showed distorted and rough surface. The increase in surface roughness may be attributed to better enzyme accessibility. Similarly, the SEM micrographs of pretreated samples showed shortened, loosened, and exposed fibers. This is due to the disruption and removal of hemicellulose and lignin structure. This effect increased with the increase in pretreatment severity. Response surface model of glucose yields after 24-hour hydrolysis when varying severity parameters was also studied and showed that above 90% yield can be achieved reducing one severity parameter and increasing the other. It can also be achieved by either reducing or increasing hydrolysis time and the opposite with the pretreatment severity. The study showed temperature to be more important severity parameter than other parameters for obtaining higher glucose yields.

### 2.11. Techno-economic Analysis of Producing Ethanol

The first techno-economic analysis of producing ethanol from cellulosic biomass was conducted by NREL in 1987 (Aden and Foust, 2009; Gnansounou and Dauriat, 2010). These studies included both biochemical and thermochemical approaches. The thermochemical approach was based on acid hydrolysis. In a report by Badger Engineers, Inc., a subcontractor for NREL, use of hardwood chips as a feedstock resulted in minimum ethanol selling price (MESP) of $1.32/gal for the base case and $1.63/gal for the design case (1984 dollars). In another study by Stone & Webster Engineering Corp., eucalyptus was considered as the feedstock for a biorefinery located in Hawaii. The feedstock was pretreated with sulfuric acid and steam

explosion and then enzymatic hydrolysis was performed. That economic model estimated the MESP to be $3.5/gal (1984 dollars). Another study in the same year by Chem Systems, Inc., another subcontractor for NREL, studied the use of mixed feedstock (aspen forest hardwoods and maple) for 25 million gallons of ethanol per year biorefinery. The resulting MESP was $2.06/gal (1984 dollars).

Wooley et al. (1999) developed a detailed techno-economic model of producing cellulosic ethanol. The study considered a detailed mass and energy balance, and process flow diagrams using the ASPEN model. Yellow poplar wood was considered as the feedstock with dilute acid pretreatment, and simultaneous saccharification and co-fermentation. The enzyme was produced on-site. The model resulted in ethanol price of $1.44/gal (in 1997 dollars). Feedstock, pretreatment, enzyme production, and boiler/turbogenerator had higher cost contribution. Another similar study was conducted by Aden et al. (2002). In this study, corn stover was considered as the primary feedstock and enzyme were purchased from external vendors. The model resulted in a minimum ethanol selling price of $1.07/gal (in 2000 dollars). Final ethanol price was highly sensitive to hemicellulose sugar conversion yield and stover cost. The systematic approach of techno-economic analysis of different pretreatment technologies for production of ethanol was conducted by the Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) which was funded by the U.S. Department of Energy (Eggeman and Elander, 2005; Elander et al., 2009; Wyman et al., 2013; Wyman et al., 2005). Pretreatment methods studied included dilute acid, hot water, ammonia fiber expansion, ammonia recycle percolation, and lime. When oligomer credit was also considered, the final ethanol price did not have much difference between any pretreatment form. When only monomer sugar yield was considered, dilute acid pretreatment gave the lowest ethanol price and hot water

pretreatment gave highest. After this several other techno-economic studies were conducted for different pretreatment systems and feedstocks (Aden and Foust, 2009; Humbird et al., 2011; Kazi et al., 2010a; Kazi et al., 2010b).

A techno-economic analysis of ethanol production following different pretreatment methods was performed by Kazi et al. (2010a). Dilute acid, two-stage dilute acid, hot water, and AFEX pretreatments were considered with separate hydrolysis and fermentation, and onsite enzyme production. Discounted cash flow analysis was performed to get the final product value. Corn stover was used as the feedstock. Nine different sections were considered for the analysis: feed handling (Area 100), pretreatment and detoxification (Area 200), enzymatic hydrolysis and fermentation area (Area 200), on-site enzyme production (Area 400), product recovery (Area 500), wastewater treatment (Area 600), storage (Area 700), burner/boiler turbo-generator (Area 800), and utilities (Area 900). AspenPlus was used for process modeling. Among the different pretreatment scenarios studied, dilute acid pretreatment resulted in the least product value of $3.40/gal of ethanol (in 2007 dollars). Feedstock and enzyme costs were the most sensitive parameters in the analysis.

Humbird et al. (2011) did a detailed techno-economic analysis of producing fuel ethanol from corn stover following dilute acid pretreatment. The report provided detailed AspenPlus design, process description, mass and energy balances, and process flow diagrams. The report updated the previous reports in the areas of feedstock composition, pretreatment reactor configuration, pH adjustment of the pretreated slurry using ammonia, and a wastewater treatment section that can handle inorganics. The model resulted in a minimum ethanol selling price of $2.15/gal of ethanol (2007 dollars). The study used a discounted cash flow analysis and the plant is 40% equity financed. MESP was calculated based on a 10% internal rate of return. The

process resulted in an ethanol yield of 86.8 gals/MT of feedstock. The 2000 MT/day facility can have an annual ethanol production of 61 million gallons. Sensitivity analysis was also performed to see the sensitivities of input parameters on MESP and ethanol yield. Capital cost and conversion parameters like cellulose to glucose, xylose to ethanol were the most sensitive parameters.

Following NREL dilute-acid report (Humbird et al., 2011), several other studies were done based on their design and calculations (Isci, 2008; Littlewood et al., 2013; Uppugundla et al., 2014; Zhao et al., 2015). Isci (2008) considered the techno-economic analysis of ethanol production using switchgrass following SAA pretreatment. The study showed that the combustion area was the most expensive unit, and pretreatment was the second most expensive. Pretreatment soaking time was taken to be 5 days. The longer soaking time increased the capital costs because a large number of reactors are required. Usually, for a dilute acid pretreatment, the pretreatment soaking time is below 10 minutes. The results showed that SAA pretreatment is generally more expensive than dilute acid pretreatment. The resulting MESP was $2.99/gal of ethanol (2007 dollars) in the base case scenario which can go up to $3.90/gal of ethanol when considered the most likely scenario (higher feedstock cost, lower conversion rates). Feedstock price and enzyme costs were the most sensitive parameters to MESP in that study. Littlewood et al. (2013) also did a techno-economic analysis of producing ethanol from bamboo feedstock following three different pretreatments – SAA, dilute acid, and liquid hot water. SAA pretreatment had higher ethanol yields at higher enzyme loadings than other two pretreatments. Also, the energy requirement is also higher for SAA pretreatment.

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# 3. PROBLEM STATEMENT AND OBJECTIVES

## 3.1. Problem Statement

The use of lignocellulosic biofuel as an alternative to fossil fuels is still lagging due to high production costs. The costs associated with biomass handling, pretreatment, and hydrolysis are particularly challenging. Apart from the economics of production, the environmental impacts of producing lignocellulosic biofuel are also limiting the current use of technologies. Pretreatment and hydrolysis cost reductions with improvement in biomass logistics could help the development of large-scale cellulosic biorefineries while decreasing the use of fossil fuels and their overall environmental footprint.

Biomass conversion to biofuel involves several processes including transporting biomass from remote locations to the biorefineries and subsequent processing. Conventionally, biomass is collected from the field in the form of bales. Because of the low bulk density, biomass transportation, handling, and storage become cumbersome, labor-intensive, and expensive. The direct costs for the above processes are high, as are the indirect costs like road maintenance because of higher road traffic. The higher road traffic is associated with the fact that more truck loads are required because of low biomass bulk density. Due to the bulky nature of the biomass, it is also not suitable for automated handling.

Using loose biomass also leads to high costs for downstream processing like pretreatment and hydrolysis. The pretreatment severity parameters like time, temperature, and chemical concentration are higher which can increase the cost. The lower bulk density of the loose biomass limits solid loadings and hence a larger number of reactors are required (Nahar, 2017) to process the same amount of material. Low pretreatment solid loading for loose biomass also leads to larger requirements of chemicals, water, and energy (Nahar, 2017). Similarly, during the

enzymatic hydrolysis step, the amount of enzyme required for loose biomass is also higher. In order to make lignocellulosic biofuels economically competitive with conventional fuels, these processes have to be optimized to reduce costs and environmental impact. The problems associated with biomass logistics, and the high cost of pretreatment and hydrolysis, have to be addressed.

Biomass densification through pelleting may be a good option to overcome the above problems. The pelleting process includes grinding loose biomass into powdered form and then densifying it into durable pellets. The process generates high temperatures and pressures, and high shear stress between biomass particles (Stelte et al., 2012) which appears to help reduce biomass recalcitrance. Nahar and Pryor (2017) observed the SEM (scanning electron microscopy) micrographs of the loose corn stover and pelleted corn stover. While the loose stover showed ordered and rigid fibrils, the pelleted material showed distorted fibrils and rough surface. The reduction in recalcitrance may be caused by the disruption in cellulose-hemicellulose structure and increase in surface area along with the structural modifications due to high pressure, temperature and shearing force (Rijal et al., 2012; Theerarattananoon et al., 2012).

Using biomass pellets as a biorefinery feedstock has several advantages including easier handling and transportation, and storage cost reduction. Pelleting increases the density of biomass several folds which helps to reduce the cost of biomass transportation due to higher truck-loads and subsequently reduced load numbers. Pellets are also suitable for automated processing due to their uniform shape and size. Conventional grain handling technologies can be incorporated for such tasks.

A potentially greater advantage of biomass pelleting can be seen during the pretreatment and enzymatic hydrolysis steps. Previous studies have indicated the advantages of using pellets instead of loose biomass (Nahar and Pryor, 2014; Rijal et al., 2012; Theerarattananoon et al., 2012). Hydrolysis yields of biomass pellets have shown pretreatment severity can be reduced to achieve comparable results with loose biomass. Lower pretreatment severity can help reduce energy, chemical, capital costs associated with the process. Due to higher bulk and particle density of pellets, pretreatment reactor loading can be increased without any negative effect on hydrolysis yields. Hydrolysis of pretreated pelleted biomass can be done with lower enzyme loadings and shorter residence time. All of these parameters contribute significantly to the final ethanol price.

Although there has been research on loose and pelleted stover with different sets of pretreatment, those are not sufficient for quantifying benefits of using one over the other. The comparison between them and quantification of the benefits of using pelleted stover requires both loose and pelleted biomass studied with the same conditions of pretreatment and enzymatic hydrolysis. This research considers the pretreatment and hydrolysis under the same sets of conditions, apart from solid loadings for both loose and pelleted corn stover. The results obtained from this research can be a valuable source for further work like life cycle assessment and techno-economic analysis.

The yield results depend on the interaction between pretreatment conditions and enzyme loadings. We may achieve similar yield results with low severity pretreatment and high enzyme loadings, or with high severity pretreatment and low enzyme loadings. The optimum choice depends on which conditions are economically more feasible at the time. Achieving a 100% yield (in terms of the total amount of glucose that can be obtained theoretically) is certainly

desirable, but it takes a longer time to reach there. 90% yield is taken as a reasonable value as a trade-off between both hydrolysis time and yield. Previous studies have only considered a fixed time of either 24, 48, or 72 h (Nahar and Pryor, 2014; Nahar et al., 2017; Rijal et al., 2012) to show that 90% yield was achieved. In this study, a follow-up study is conducted to determine the closest theoretical time to achieve 90% glucose yield. Based on the follow-up study results, a comparative techno-economic analysis of using loose and pelleted corn stover in a biorefinery is done.

### 3.2. Hypotheses

1. Loose biomass requires higher pretreatment severity and enzyme loadings, and longer hydrolysis time than pelleted biomass for a comparable sugar yields.
2. MESPs are lower when using pelleted biomass as a feedstock than when using loose biomass
3. Pelleting costs do not have significant influence in the MESP of ethanol.

### 3.3. Objectives

There are two objectives in this study.

**Objective 1:** To quantify the benefits of using biomass (corn stover) pellets compared to loose biomass with low severity pretreatment and reduced enzyme loadings for enzymatic hydrolysis. The objective is achieved through the following tasks:

- Use of loose corn stover to determine the baseline relationship between SAA pretreatment severity parameters (time, temperature, and ammonia concentration) and their respective hydrolysis yields.
- Use of pelleted corn stover to determine the relationship between SAA pretreatment parameters and their respective hydrolysis yields under the same conditions as used for loose stover.

- Determine the theoretical time to achieve 90% glucose yields for both loose and pelleted forms of biomass with the pretreatment and enzyme loadings condition determined from preliminary experiments. Frequent sampling of hydrolysate is done to get the closest time that gives 90% glucose yield.

**Objective 2:** To perform a comparative techno-economic analysis using loose and pelleted forms of biomass as a biorefinery feedstock for the production of cellulosic ethanol. The objective is achieved through the following tasks:

- Determine the capital and operating costs of the processing conditions determined from follow up study in objective 1 for both loose and pelleted forms of biomass.
- Compute a minimum ethanol selling price for each processing conditions following a discounted cash flow analysis.

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# 4. QUANTIFYING REDUCTIONS IN SOAKING IN AQUEOUS AMMONIA PRETREATMENT SEVERITY AND ENZYMATIC HYDROLYSIS CONDITIONS FOR CORN STOVER PELLETS

## 4.1. Abstract

The benefits of using pelleted corn stover compared to loose corn stover with low severity soaking in aqueous ammonia (SAA) pretreatment and reduced enzyme loadings were studied. Loose and pelleted corn stover were treated with the same set of pretreatment and hydrolysis conditions. A range of low to high severity pretreatment conditions and enzyme loadings were tested to determine conditions to achieve 90% glucose yields. Glucose yields from pelleted biomass reached 90% with reduced pretreatment severities, enzyme loadings, hydrolysis time, or various combinations of these. At the highest enzyme loadings, use of pelleted corn stover enabled reductions in hydrolysis time up to 58%. It also allowed 80% reduction in enzyme loading at higher pretreatment conditions. At moderate pretreatment levels, either enzyme loadings can be reduced by 40% or hydrolysis time by up to 48%. Using pelleted biomass as a biorefinery feedstock allows flexibility in production with different processing options which depend on the market cost dynamics and production economics.

## 4.2. Keywords

SAA pretreatment, Biomass pelleting, Hydrolysis, Enzyme, Biomass conversion

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R Pandey, N Nahar, JS Tumuluru, SW Pryor., 2019. Quantifying reductions in soaking in aqueous ammonia pretreatment severity and enzymatic hydrolysis conditions for corn stover pellets. Bioresour. Technol. Reports, 100187. Ramsharan Pandey had primary responsibility of conducting, collecting, and analyzing laboratory data under direct supervision of Dr. Scott W. Pryor. Dr. Nurun Nahar helped in conducting experiments and analyzing results. Dr. JS Tumuluru helped with biomass pelleting.

## 4.3. Introduction

Commercialization of lignocellulosic biofuels requires a consistent supply of low-cost biomass and a low-cost processing system. Cellulosic biomass is bulky in nature and is geographically distributed. For a large-scale biorefinery, the continuous supply of biomass feedstock poses a huge challenge due to the large required volume of biomass and the subsequent costly supply system. In addition, biomass is recalcitrant to microbial decomposition (Zhao et al., 2012) requiring costly pretreatment and expensive enzymes for hydrolysis (Himmel et al., 2007). Simplified biomass logistics systems coupled with pretreatment and hydrolysis cost reductions could facilitate the development of large-scale cellulosic biorefineries while decreasing the use of fossil fuels and the overall environmental carbon footprint of the biorefinery.

Biomass pelleting can be a good option to reduce the supply system cost and also improve feedstock handling and storage properties (Tumuluru et al., 2011). Pelleting increases the density of biomass by approximately three-fold over bales which reduces trucking requirements and associated handling costs (Balan, 2014; Nahar, 2017). Studies also suggest that pelleting can reduce pretreatment and hydrolysis costs because pelleting itself is a mild form of physical pretreatment (Balan, 2014; Nahar and Pryor, 2014; Nahar and Pryor, 2017; Nahar et al., 2017; Rijal et al., 2012). The interaction between pelletization and pretreatment has been studied for different pretreatment processes. Although pelleting is not a sufficient pretreatment on its own, results show that hydrolysis can be effective when coupled with low-severity conventional pretreatment and low enzyme loadings (Guragain et al., 2013; Nahar and Pryor, 2014, 2017; Nahar et al., 2017; Ray et al., 2013; Rijal et al., 2012; Shi et al., 2013; Theerarattananoon et al., 2012b).

Several studies showed that the chemical composition of biomass does not change significantly with pelletization (Guragain et al., 2013; Nahar and Pryor, 2014; Ray et al., 2013; Rijal et al., 2012; Shi et al., 2013; Theerarattananoon et al., 2012a). Ray et al. (2013) studied the effect of pelletization on bioconversion of corn-stover following dilute acid pretreatment. The study showed that pelleting does not have an adverse effect on pretreatment efficacy, and resulted in 24% higher ethanol yields than non-pelleted stover during the SSF (simultaneous saccharification and fermentation) process. Similarly, Theerarattananoon et al. (2012) showed that the enzymatic conversion of cellulose from pelleted wheat straw, corn stover, big bluestem, and sorghum stalks was higher or equivalent to the corresponding unpelleted material following dilute acid pretreatment. The higher yields were attributed to the effect of shear force and frictional heat generated during the pelleting process. Shear force during pelleting reduces biomass crystallinity by continuously removing the softened surfaces surrounding cellulose (Lamsal et al., 2010), thus exposing cellulose to enzymatic action. Rijal et al. (2012) also reported higher sugar yields following soaking in aqueous ammonia (SAA) pretreatment of switchgrass pellets. Higher sugar yields in that study were attributed to the increase in biomass surface area due to size reduction occurring within the pellet mill. Biomass undergoes fine grinding inside the pellet mill prior to densification, and similar sugar yields were reported for both the pellets and the finely ground material taken from the pellet mill. Nahar and Pryor (2017) showed evidence that pelleting modifies biomass macro-structure and disrupts lignin-hemicellulose linkages.

Other studies also considered the use of pelleted biomass with alkali pretreatments. Guragain et al. (2013) showed higher delignification and higher hydrolysis productivity with the pelleted samples following NaOH pretreatment using pelleted biomass from corn, sorghum stalk,

wheat, and big bluestem. Results showed that lower enzyme loadings and hydrolysis time were effective for biomass pellets. Nahar and Pryor (2014; 2017) showed similar trends for switchgrass and corn stover following SAA pretreatment. That pretreatment resulted in 70% higher delignification of corn stover pellets than from loose corn stover (Nahar and Pryor, 2017). Higher delignification improves hydrolysis yields as it opens up biomass structure for enzyme accessibility. The study also showed that solid loadings can be doubled to 20% for pelleted biomass without affecting the hydrolysis yields. This enables a reduction in pretreatment chemicals and a reduction in the required number of reactors in a biorefinery (Nahar, 2017). The effectiveness of lower pretreatment severity parameters like time, temperature, and chemical concentration, as well as lower enzyme loadings and hydrolysis times, results in a reduction in overall energy requirements and greenhouse gases emissions (Nahar, 2017).

Many of the previous studies focused only on optimizing conversion processes either for loose or pelleted biomass forms. Some of those studies identified some potential synergies and benefits of using pelleted biomass over loose biomass, those studies often failed to consider a direct comparison of loose and pelleted biomass under the same conditions. Such results could be used to more accurately quantify the benefits in terms of reduction in pretreatment severity parameters, enzyme loadings, or hydrolysis time. The results obtained from this research can be a valuable source for further work on techno-economic analysis, and life-cycle assessment to further quantify benefits of using biomass pellets as a cellulosic biorefinery feedstock.

The objective of this study is to quantify the benefits of using corn stover pellets with a range of low-severity pretreatments compared to non-pelleted corn stover. Pretreatment and hydrolysis conditions needed to achieve 90% glucose yields for each form of biomass will be identified across a range of conditions. The benefits will be quantified by showing reductions in

pretreatment severity (temperature, time, and ammonia concentration), hydrolysis time, enzyme loadings, or a combination of these.

## 4.4. Materials and Methods

### 4.4.1. Raw material

Corn stover (*Zea mays L.*) was collected from a field in Mandan, ND. Baled corn stover was ground using a hammer mill (Schutte-Buffalo Hammermill, LLC; Model W-6-H; Buffalo, NY, USA) and separated through a ¼ inch screen.

### 4.4.2. Biomass moisture content

The moisture content of the ground corn stover was determined by following the National Renewable Energy Laboratory (NREL) protocol (Sluiter et al., 2008a).

### 4.4.3. Biomass pelleting

Ground corn stover was pelleted at Idaho National Lab using a laboratory-scale ECO-10 flat-die pellet mill (Colorado Mill Equipment; Canon City, CO, USA) which consists of a rotating die and a stationary roller shaft. The die rotation speed was adjusted to 60 Hz (350 rpm) and 6-mm die diameter was used for extrusion of the pellets. The length-to-dimeter ratio of the die used was 2.0. The moisture content of the material was adjusted to 15% (wet basis) prior to pelleting. The adjustment was done by mixing the raw material with the calculated amount of water in a ribbon blender (RB 500, Colorado Mill Equipment). The material was fed into the pellet mill where fine grinding of the material occurs, and the fine powder is compressed through the die to form the pellets. No external binding agent was used. The pellets were dried in an oven at 70°C to reduce the final moisture content to <10% (wet basis).

### 4.4.4. Pellet density

Pellet unit density, bulk density, and tapped density were calculated following the ASABE Standard S269.5 (ASABE, 2012). Unit density is a measure of the density of the individual pellets. The volume of the individual pellet was calculated by measuring the length and diameter using a Vernier caliper. The mass of the same pellet was measured using a balance. Unit density was calculated by dividing mass by the volume. Bulk density is the measure of the density for a larger quantity of pellets in a given volume. Pellets were transferred to a measuring cylinder filling up to the top and the volume of the cylinder and the corresponding mass of the pellets were recorded. The bulk density was then calculated by dividing mass by the volume. The tapped density is calculated as bulk density after tapping the cylinder to encourage settling. The loosely filled cylinder was tapped approximately five times to allow settling and then pellets were again filled up to the top. The density was then calculated as before.

### 4.4.5. Pellet durability

Durability is the measure of the pellet strength to withstand damage, i.e. the ability of the pellets to remain intact during transportation and handling. Pellet durability was calculated following the ASABE Standard S269.5 (ASABE, 2012). Triplicate 500-g samples of pellets were tumbled in a dust-tight enclosure at 50 rpm for 10 min. The material was then sieved through a 5.7 mm sieve and final weight was measured. Durability is the ratio of the final weight of the pellets and the initial 500-g weight of the pellets.

### 4.4.6. Micro-CT imaging

Micro-CT (computed tomography) imaging was used to record the surface structure of the pellets. The images were analyzed for the presence of any voids or other defects (Figure A2).

### 4.4.7. Experimental design

The objective of the study was to quantify the processing benefits of using pellets instead of loose corn stover over a range of pretreatment conditions. Therefore, an experimental design was prepared to test both the loose corn stover and pelleted corn stover. Both materials were tested at the same pretreatment conditions.

A total of five different pretreatment conditions with increasing pretreatment severity parameters (time, temperature, concentration of aqueous ammonia) were used as shown in Table 3. Untreated material (loose and pellets) were used as controls in both cases.

**Table 3. Pretreatment experimental setup**

| Treatment | Pretreatment Conditions | | |
|---|---|---|---|
| | Temperature (°C) | Time (h) | Ammonia Concentration (% as w/v) |
| UT* | - | - | - |
| PT1 | 40 | 4 | 10 |
| PT2 | 45 | 9 | 12 |
| PT3 | 50 | 14 | 14 |
| PT4 | 55 | 19 | 16 |
| PT5 | 60 | 24 | 18 |

*UT refers to Untreated biomass (no pretreatment)

Three different enzyme loadings were tested for enzymatic hydrolysis of all treatments as shown in Table 4.

**Table 4. Hydrolysis experimental setup**

| Enzyme level | Cellulase (FPU/g glucan) | Cellobiase (CBU/g glucan) | Xylanase (XU/g glucan) |
|---|---|---|---|
| Low | 5 | 5 | 100 |
| Medium | 15 | 15 | 300 |
| High | 25 | 25 | 500 |

### 4.4.8. Soaking in aqueous ammonia (SAA) pretreatment

Pretreatment was done in a 2000-ml flask with 80 g (dry weight) of loose or pelleted biomass. The solid loading for pretreatment was 10% (weight by volume basis) for loose biomass and 20% for pelleted biomass. Therefore, 800 ml of aqueous ammonia was used in each of the pretreatment conditions for loose biomass and 400 ml for pelleted biomass. After preheating the ammonia to the desired temperature, biomass was added and incubated in a water bath at the design temperature and time without agitation. Pretreated materials were washed using distilled water to remove ammonia and bring the pH close to 7. Litmus paper was used to check pH. The washed biomass was then filtered through Whatman #41 filter paper (20-25 µm pore size) in a vacuum filtration unit and weighed. A small portion of biomass was dried at room temperature to use for compositional analysis. Pretreated biomass was stored in sealed plastic bags in a freezer until used for enzymatic hydrolysis. The moisture content of the pretreated material was also determined in triplicate to calculate the total biomass recovered after pretreatment.

### 4.4.9. Compositional analysis for carbohydrates

SAA-pretreated and non-pretreated corn stover (both loose and pelleted forms) were analyzed for lignin (acid-soluble and acid insoluble lignin) and carbohydrates. For non-pretreated corn stover, extractive-free biomass was used. The extractives in the corn stover were determined following NREL procedures (Sluiter et al., 2008c). Lignin and carbohydrate in the biomass were also calculated using NREL procedures (Sluiter et al., 2008b). All analyses were done in triplicate.

### 4.4.10. Enzymes

A combination of different enzymes is needed for effective hydrolysis of cellulose and xylose. Cellulase is required to hydrolyze cellulose, xylanase is required to hydrolyze xylose, and β-glucosidase (cellobiase) is required to hydrolyze cellobiose (product of cellulose hydrolysis). The enzymes NS50013 (cellulase), Novozyme 188 (β-glucosidase), and Cellic HTec (xylanase) were provided by Novozymes (Franklinton, NC). The cellulase activity and β-glucosidase activity were 60 filter paper units (FPU) $ml^{-1}$ and 476 cellobiase units (CBU) $ml^{-1}$ as determined by (Ghose, 1987), and xylanase activity was 10,600 xylanase unit (XU) $ml^{-1}$ as determined by Bailey et al. (1992).

### 4.4.11. Enzymatic hydrolysis

Enzymatic hydrolysis of pretreated and non-pretreated corn stover (both loose and pelleted) was done in 125-ml Erlenmeyer flask with 50 ml of working volume. The hydrolysis was done by mixing the biomass samples (mass of samples calculated on the basis of 1 g glucan per 100 ml) in sodium citrate buffer (pH 4.5). Sodium azide (0.04%) was added to prevent microbial contamination. A set of three different enzyme loadings from low to high (Table 2.2) were tested for each sample. All treatments were done in triplicate. Flasks were placed in a water bath shaker (MaxQ 7000, Thermo Scientific; Dubuque, IA, USA) at 50 °C and stirred at the rate of 150 rpm for 48 h. Aliquots (~1 ml) were taken from each flask at 4 h, 24 h, and 48 h and centrifuged at 13,226 x g for 10 min (Galaxy 16 Micro-centrifuge, VWR International; Bristol, CT, USA). The supernatant was filtered through a 0.2-µm nylon filter (Pall Corporation; West Chester, PA, USA). Filtered samples were placed in vials and stored at -20 °C until used for sugar analysis.

### 4.4.12. High performance liquid chromatography (HPLC) analysis

The concentration of monomeric sugars (glucose, and xylose) in the hydrolysis samples was determined by HPLC (Waters Corporation; Milford, MA). The sample injected for analysis was 20 µl and the sugars were eluted using a mobile phase of 18-mΩ nanopure water at a flow rate of 0.6 ml $min^{-1}$. A Bio-Rad Aminex HPX-87P column (Bio-Rad Laboratories; Hercules, CA) and refractive index (RI) detector (model 2414, Waters Corporation) were used for separation and quantification. The column temperature and the detector temperature were set at 50 °C and 85 °C, respectively. Known standards of glucose, cellobiose, and xylose were used to generate standard curves for quantification.

### 4.4.13. Statistical analysis

Data obtained from HPLC was used to determine glucose and xylose yield percentage for each sample. Statistical analysis of the yield results was done to see if the results were significantly different from each other and if there were any interactions between biomass form (loose and pellets) and different conditions of pretreatment, enzyme loadings, and hydrolysis time. SAS® (SAS Institute, Inc., Cary, N.C.), was used for statistical analysis.

### 4.4.14. Follow-up study

After determining the combinations of pretreatment conditions, enzyme loadings, and hydrolysis times that resulted in greater than 90% glucose yields, a follow-up study was carried out to determine the closest hydrolysis time to achieve 90% yield through more frequent hydrolysis sampling. The sampling was done at the following times: 4, 8, 16, 24, 36, and 48 h. All samples were analyzed via HPLC as done for original testing. Interpolation and a linear fit between data points was used to estimate the time required to reach the target yield. Results were then compared based on pretreatment severity parameters, enzyme loadings, and hydrolysis time.

## 4.5. Results and Discussion

### 4.5.1. Properties of loose and pelleted corn stover

The moisture contents (dry basis) of loose and pelleted corn stover were determined to be 7.3% and 5.0%, respectively. The unit density, bulk density, and tapped density of the pellets were determined to be 1189.2, 522.8, and 574.2 kg/m$^3$, respectively. The durability of the pellets was determined to be 91.2%.

The results of compositional analysis of loose and pelleted corn stover with and without pretreatment are summarized in Table 5. The fraction of original biomass recovered after pretreatment tends to decrease with increasing pretreatment severity. Higher severity pretreatment causes more lignin to dissolve in addition to some loss of carbohydrate extractives. The results of the compositional analysis confirmed that pelleting did not have a large effect on the composition. Although there was a slight variation in composition, this could be due to inherent variability in the protocols (Sluiter et al., 2010; Templeton et al., 2010). Similar results were shown by previous studies (Nahar and Pryor, 2017; Rijal et al., 2012; Theerarattananoon et al., 2012a; Wolfrum et al., 2017).

**Table 5. Composition of untreated and pretreated loose and pelleted corn stover**

| Treatments | Solids recovered (%) | Glucan (%) | Xylan (%) | Total lignin (%) | Total delignification (%) |
|---|---|---|---|---|---|
| Loose corn stover | | | | | |
| UT | - | 31.2±1.0 | 20.8±1.8 | 18.9±0.4 | - |
| PT1 | 84.9 | 31.1±0.4 | 23.5±1.0 | 17.0±0.3 | 23.6 |
| PT2 | 74.2 | 35.1±0.1 | 21.9±0.1 | 15.3±0.7 | 40.0 |
| PT3 | 71.7 | 40.9±1.8 | 26.5±0.7 | 12.4±0.2 | 52.9 |
| PT4 | 70.5 | 44.1±2.9 | 27.5±4.4 | 10.8±0.1 | 59.9 |
| PT5 | 65.8 | 47.8±0.0 | 29.1±0.7 | 10.0±0.5 | 65.3 |
| Pelleted corn stover | | | | | |
| UT | - | 32.0±1.6 | 21.0±1.1 | 17.3±0.2 | - |
| PT1 | 75.9 | 39.2±1.3 | 24.1±0.4 | 16.2±0.3 | 28.7 |
| PT2 | 77.3 | 40.6±0.2 | 25.0±0.3 | 12.7±0.9 | 43.1 |
| PT3 | 72.3 | 43.3±1.4 | 24.8±0.9 | 11.9±1.1 | 50.4 |
| PT4 | 68.6 | 47.2±0.7 | 24.3±0.3 | 9.6±0.1 | 50.4 |
| PT5 | 67.0 | 50.2±1.4 | 25.0±0.5 | 9.7±1.9 | 62.4 |

The composition of carbohydrates and lignin followed a similar trend for both loose and pelleted biomass with increasing pretreatment severity. The glucan and xylan composition increased with increasing pretreatment severity because of the loss of lignin and some extractives. The difference in composition before and after pretreatment showed a minor loss in cellulose. Although there was an increase in xylan content after pretreatment, results show that up to 20% of xylan was removed during pretreatment as has been found previously (Kim and Lee, 2007). Increasing pretreatment severity led to the higher loss of xylan from pelleted biomass and similar findings were reported elsewhere (Kumar et al., 2012; Rijal et al., 2012).

### 4.5.2. Glucose and xylose yields from hydrolysis of loose and pelleted corn stover

Enzymatic hydrolysis of loose and pelleted corn stover, at increasing pretreatment severities, were carried out at three different enzyme loadings to see the interaction between pretreatment and corn stover form (loose and pelleted) on hydrolysis yields. Sampling was done

at 4, 24, and 48 h. As expected, for all cases there was an increasing trend in glucose yields with the increasing pretreatment severity. Previous studies also showed that pelleting allows a reduction in pretreatment severity and enzyme loadings (Guragain et al., 2013; Nahar and Pryor, 2014; Nahar and Pryor, 2017; Nahar et al., 2017; Rijal et al., 2012; Theerarattananoon et al., 2012a). This study showed that pelleting biomass improves hydrolysis yields or rates across a range of pretreatment conditions and enzyme loadings. Figure 1 shows glucose yields at 24 and 48 h for both loose and pelleted stover.

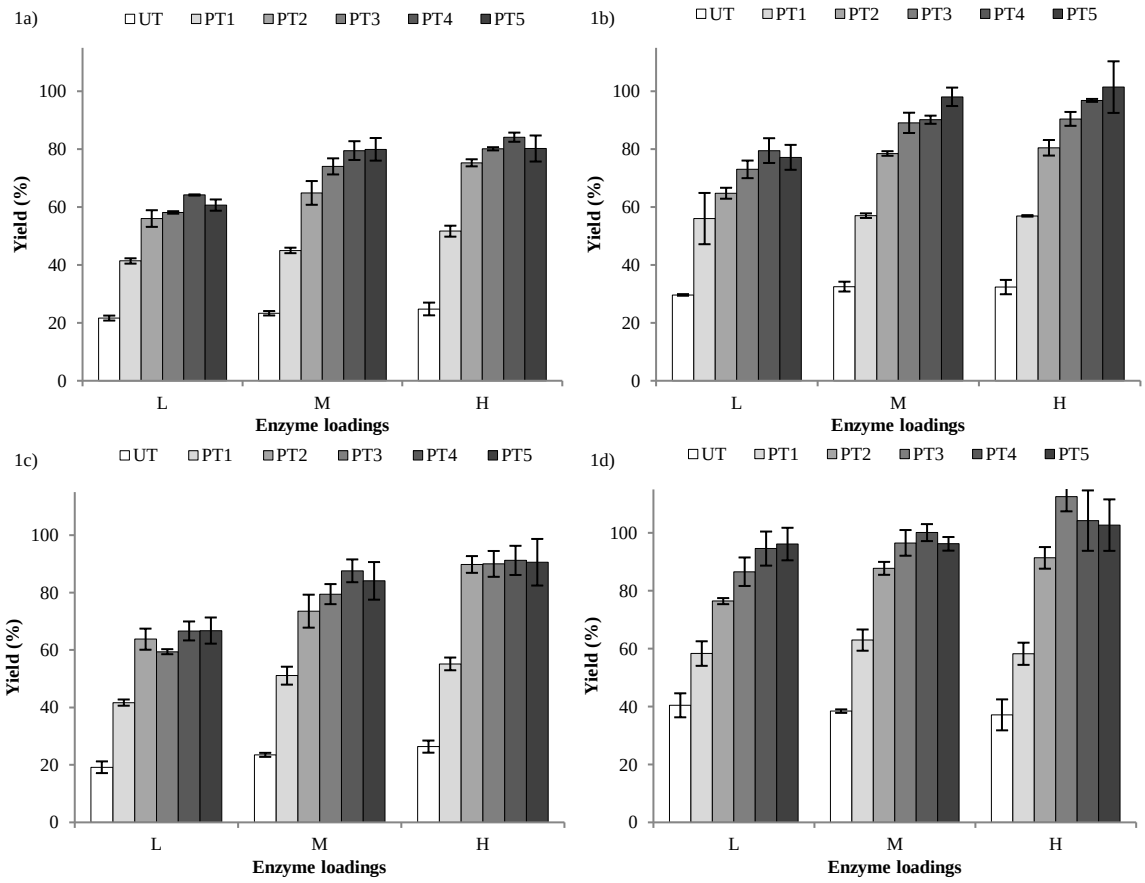


**Figure 1. Hydrolysis glucose yields at 24 h for a) loose and b) pelleted, and 48 h for c) loose and d) pelleted corn stover at different enzyme loadings (low, medium, and high) and pretreatment conditions (UT, PT1, PT2, PT3, PT4, PT5)**

Despite some variability, differences in glucose yields between pelleted and loose corn stover were more noticeable (higher) with lower enzyme loadings at 24 h and 48 h hydrolysis time. Yields from pellets were generally 10-30% higher than from loose stover at low enzyme loadings. Medium enzyme loadings showed a consistent difference of 10-20% across different pretreatment conditions. However, the difference was less consistent for high enzyme loadings. Using high enzyme loadings masks the benefits of pelleting in pretreatment and hydrolysis because those loadings are effective for either biomass form. The yield results at 4 h (data not shown) also showed that yields were consistently 15-20% higher for the pellets indicating that hydrolysis rates are improved in addition to final yields. Similar trends were seen with xylose yields.

### 4.5.3. Quantification of process benefits of using pelleted biomass over loose biomass

Hydrolysis yields using three different enzyme loadings were compared for loose and pelleted corn stover with different SAA pretreatment conditions. The results (Fig. 1) showed that doubling solid loadings for pellets does not have any negative effect on sugar yields, and still allows improved hydrolysis performance compared to loose stover. Doubling solid loadings enables a reduction in the number of reactors, the volume of ammonia, and the energy required (Nahar, 2017).

Hydrolysis glucose yields of at least 90% is a benchmark to make a commercial biorefinery more economical (Yang and Wyman, 2008). Based on this, the pretreatment conditions and enzyme loadings that resulted in at least 90% glucose yields for both loose and pelleted biomass were determined. Table 6 shows the summary of the least severe pretreatment conditions and lowest hydrolysis time required to achieve 90% glucose yields at each enzyme loading. Hydrolysis results showed that none of the loose biomass treatments achieved 90% yield

at 24 h hydrolysis time. At 48 h, the lowest severity pretreatment for loose biomass to cross the 90% threshold was PT3 at highest enzyme loading. None of the pretreatment conditions resulted in 90% glucose yields with lower enzyme loadings for loose stover.

**Table 6. Least severe pretreatment and lowest hydrolysis time required to achieve at least 90% glucose yields for loose and pelleted forms of corn stover**

| Enzyme loadings | Biomass Form | |
|---|---|---|
| | Loose | Pellets |
| L | - | PT4 at 48 h |
| M | - | PT4 at 24 h |
| | - | PT3 at 48 h |
| H | PT3 at 48 h | PT3 at 24 h |
| | - | PT2 at 48 h |

Although loose stover only yielded 90% glucose under the highest enzyme loadings, several combinations of pretreatment conditions and enzyme loadings resulted in at least 90% yield for pellets. At high enzyme loadings, the lowest severity pretreatment to achieve 90% yields was PT2 after 48 h hydrolysis time. At medium enzyme loadings, the lowest severity pretreatment to reach the target yield was PT3 after 48 h hydrolysis time. If hydrolysis time is reduced to 24 h, similar results can be obtained by increasing pretreatment severity. Similarly, for low enzyme loadings, PT4 achieved 90% yield by 48 h hydrolysis time.

The results show that 90% glucose yields can be achieved with pelleted stover using a range of pretreatment and hydrolysis conditions. Different combinations of hydrolysis time, enzyme loadings, and pretreatment severity could be chosen depending on the economic repercussions of those changes. If enzymes are inexpensive, enzyme loadings can be increased; if energy and chemical prices are low and enzymes prices are high, pretreatment severity can be

increased and enzyme loadings can be reduced. Detailed techno-economic and sensitivity analyses are needed to model the effect of changing these parameters on overall processing costs.

Statistical analysis of the hydrolysis results confirmed that pretreatment has a different effect based on biomass form (loose and pelleted). A summary of ANOVA is shown in Table 7.

**Table 7. Summary of the p-value for the least significant difference between different parameters (treatments, biomass form, enzyme loading) and their interactions for glucose yield at 24 and 48 h**

| Source | DF | Pr > F (24 h) | Pr > F (48 h) |
|---|---|---|---|
| Pretreatment | 5 | <.0001 | <.0001 |
| Biomass form | 1 | <.0001 | <.0001 |
| Pretreatment*Biomass form | 5 | 0.0005 | 0.0003 |
| Enzyme loading | 2 | <.0001 | <.0001 |
| Pretreatment*Enzyme loading | 10 | <.0001 | <.0001 |
| Biomass form*Enzyme loading | 2 | 0.1564 | <.0001 |
| Pretreatment*Biomass form*Enzyme loading | 10 | 0.255 | 0.2166 |

Statistical analysis showed that pretreatment was more effective with pellets than loose stover as expected. The statistical analysis showed glucose yield differences were significant across increasing pretreatment conditions and increasing enzyme loadings ($p<0.05$) for both loose and pelleted corn stover at 24 h and 48 h hydrolysis time. However, no significant differences were found between PT4 and PT5 at 24 h for either loose or pelleted stover (not shown in table). The results show that the effect of increasing pretreatment severity beyond PT4 is not significant. Similarly, no significant differences in glucose yields were found between PT3, PT4, and PT5 at 48 h hydrolysis time (not shown in table). This may be because biomass hydrolysis levels had already reached a plateau and much longer times would be required to substantially increase yields.

### 4.5.4. Follow-up study

A follow-up study was done after determining the combinations of pretreatment conditions, enzyme loadings, and hydrolysis times that resulted in at least 90% glucose yields. The conditions with lowest enzyme loadings, least hydrolysis time, and least severe pretreatment that reached the target yield in the initial study were chosen for additional testing with more frequent sampling. Table 8 shows the conditions chosen for the follow-up study. PT2, PT3, and PT4 at high enzyme loadings were chosen for loose biomass. Similarly, PT2 at high enzyme loadings, PT3 at medium and high enzyme loadings, and PT4 at low, medium and high enzyme loadings were chosen for pelleted biomass. In the follow up-study, frequent samplings were done to estimate the time at which the samples reached a 90% yield.

**Table 8. Enzyme loading levels in each pretreatment for follow-up study for each biomass form**

| Biomass form | PT2 | PT3 | PT4 |
|---|---|---|---|
| Loose corn stover | H | H | H |
| Pelleted corn stover | H | M and H | L, M, and H |

The least severe pretreatment condition to achieve a 90% yield for loose biomass was PT3 at high enzyme loadings. Loose biomass did not reach the target yield with lower enzyme loadings. For pellets, the least severe pretreatment condition to achieve 90% glucose yield was PT2. Similarly, PT4 achieved that level with the lowest enzyme loadings by the first hydrolysis sampling. PT3 was used to determine the balance between pretreatment severity and enzyme loadings. PT2 and PT4 were also chosen for follow-up study with loose biomass as a basis of comparison with the corresponding pelleted biomass.

The results of follow-up showing estimated time to reach target yields are summarized in Table 9.

**Table 9. Hydrolysis time to achieve 90% glucose yields for loose and pelleted corn stover at different enzyme loadings from a follow-up study**

| Treatments | Enzyme loadings | Hydrolysis time to get 90% glucose yields (in h) | |
|---|---|---|---|
| | | Loose corn stover | Pelleted corn stover |
| PT2 | H | 48 | 20 |
| PT3 | H | 33 | 17 |
| | M | >48 | 23 |
| PT4 | H | 17 | 17 |
| | M | >48 | 27 |
| | L | >48 | 48 |

Due to inherent variability during experiments, hydrolysis yields determined in follow-up experiments did not completely agree with initial results. However, the overall trend showing reduced requirements for pretreatment severity and enzyme loadings with pelleted biomass is in agreement with initial results and shows several advantages of using pellets compared to loose stover. Using the high level of enzyme loadings following PT2, pelleting biomass allows 58% reduction in hydrolysis time compared to loose corn stover. Hydrolysis time can be reduced almost by half at high enzyme loading following PT3. Use of pelleted biomass with PT3 also allows 90% yields with 30% less time than the loose and with 40% reduction in enzyme loadings (from high to medium). Increasing pretreatment severity conditions from PT3 to PT4 did not increase subsequent hydrolysis time for pelleted corn stover, but it did for loose stover. With the increase in pretreatment severity, the advantages of using pellets decreases because those conditions are already favorable for loose stover processing, leaving little room for improvement. Although the hydrolysis time is same for both loose and pelleted stover at PT4 for high enzyme loadings, enzyme loadings can be reduced for pellets by compromising hydrolysis time. If time is not a limiting factor, the enzyme loadings can be reduced by 80% to achieve the same result. Operating with longer hydrolysis times and lower enzyme loadings and reduced pretreatment severity is associated with higher capital costs but lower operating costs. Similarly, lower

hydrolysis times with higher pretreatment severity and enzyme loadings are associated with lower capital costs but higher operating costs. The conditions that best suits the production process can be obtained through an economic analysis incorporating the above results.

## 4.6. Conclusion

Biomass pelleting is a costly process, but it is advantageous for downstream processing. Use of pelleted biomass allows reductions in enzyme loadings of up to 80% or hydrolysis time by up to 58% while maintaining equivalent hydrolysis sugar yields. Pretreatment time, temperature, and ammonia concentrations can also be adjusted more easily when processing pellets without appreciable changes to hydrolysis rates. Processing improvements using non-pelleted biomass will lead to cost reductions which may offset pelleting costs depending on specific economic conditions. The processing benefits using pelleted biomass are greater at lower pretreatment severities so benefits may not be as apparent when using typical pretreatment conditions. The different options available for processing pellets compared to loose stover provides industry with flexibility in production planning. The results from this study are valuable for a further study on comparative techno-economic and environmental analyses of loose and pelleted biomass.

## 4.7. Acknowledgments

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# 5. COMPARATIVE TECHNO-ECONOMIC ANALYSIS OF BIOETHANOL PRODUCTION FROM LOOSE AND PELLETED CORN STOVER FOLLOWING SOAKING IN AQUEOUS AMMONIA PRETREATMENT

## 5.1. Abstract

A comparative techno-economic analysis of producing ethanol using loose and pelleted forms of biomass was performed. A spreadsheet model was developed adapting from the National Renewable Energy Laboratory (NREL) (Humbird et al., 2011) process model. The pretreatment and hydrolysis input data were based on our lab results and other process assumptions were based on other studies. The biorefinery operates at 2000 metric tons (dry)/day capacity. Analysis showed that using a pelleted form of biomass is more economical than using loose form of biomass. The lowest minimum ethanol selling price (MESP) for loose biomass was $4.41/gal ethanol while it was $3.83/gal ethanol using pelleted biomass. Among all processing conditions analyzed, MESP with loose biomass was always higher than with pelleted biomass. Shorter pretreatment and hydrolysis times, higher pretreatment solids loading, lower ammonia requirement, and reduced enzyme loadings were the main reasons behind lower MESP for pelleted form of biomass.

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[1] This paper will be submitted for publication under the authorship of Ramsharan Pandey, Dr. Ghasideh Pourhashem, Dr. Nurun Nahar, and Dr. Scott W. Pryor. Ramsharan Pandey had primary responsibility of preparing the model, analyzing data, and drafting the paper under the supervision of Dr. Ghasideh Pourhashem and Dr. Scott W. Pryor. Dr. Nurun Nahar helped with the experiments.

## 5.2. Introduction

Corn ethanol has been widely used in blending with gasoline in the US. Although corn ethanol is environmentally a better fuel compared to fossil fuels, lignocellulosic biomass-derived ethanol is considered superior to them. Lignocellulosic biomass is considered the next generation feedstock for producing biofuel because of its potentially wider availability, lower cost, and the fact that it is separate from food and feed chain. However, the production cost of cellulosic ethanol is high relative to corn ethanol and gasoline. In this paper, a comparative techno-economic analysis (TEA) of producing ethanol using either loose or pelleted form of biomass following soaking in aqueous ammonia pretreatment (SAA) is studied.

Biomass is inherently bulky in nature and recalcitrant to microbial degradation making economical production of biofuel still challenging. Biomass densification through pelletization is seen as an option to improve handling, transportation, storage costs as well as processing costs within biorefinery (Balan, 2014). Pelleted biomass has exhibited advantages in pretreatment and hydrolysis (Pandey et al., 2019). Use of pelleted biomass allows reductions in pretreatment severity parameters such as time, temperature, and ammonia concentration. It also allows doubling of solid loadings during pretreatment, which reduces requirements for reactors, energy, and ammonia (Nahar, 2017). Enzyme loadings and hydrolysis time can also be reduced with the use of pelleted biomass (Guragain et al., 2013; Nahar and Pryor, 2014; Nahar and Pryor, 2017; Theerarattananoon et al., 2012b). The synergies between pretreatment and hydrolysis under low to high pretreatment severity and enzyme loadings for pelleted biomass were identified in Pandey et al. (2019). The processing benefits of using pelleted biomass over loose biomass were quantified. Processing conditions to achieve 90% hydrolysis glucose yields were identified for both loose and pelleted biomass based on least severe pretreatment conditions, lowest enzyme

loadings, and least hydrolysis time. Here, we aim to study how the changes in biomass process conditions (pretreatment and hydrolysis) influenced by pelletizing biomass can affect the overall cost of biofuel production.

TEA is a method for determining the technical and economic feasibility of a process. It helps to identify key parameters in a process that influences cost and technology limitation. The Biomass Refining Consortium for Applied Fundamentals and Innovation (CAFI) project used the concept of TEA to determine the minimum ethanol selling price (MESP) for six different pretreatment processes (Wyman et al., 2005). Similarly, NREL used a TEA approach to determine MESP for dilute acid pretreated corn stover (Aden et al., 2002; Humbird et al., 2011). Several other studies have used baseline NREL model to build similar scenarios since (Eggeman and Elander, 2005; Elander et al., 2009; Isci et al., 2008; Kazi et al., 2010; Littlewood et al., 2013; Tao et al., 2011; Uppugundla et al., 2014; Wyman et al., 2013; Zhao et al., 2015) to understand the economic competitiveness of the conversion process of cellulosic biomass with corn ethanol and fossil fuels. However, none of those studies considered the use of pelleted biomass in their analysis.

In this study, the use of both loose and pelleted forms of biomass as a processing feedstock is considered. Process data from Pandey et al. (2019) is used to construct and conduct a comparative TEA. An Excel spreadsheet-based TEA model was prepared based on the original NREL model (Humbird et al., 2011). The objective of this study is to compare the economics of producing ethanol using loose and pelleted forms of biomass over a range of pretreatment and hydrolysis conditions. Here we investigate the hypothesis whether using pelleted biomass can lead to higher economic benefits for a biorefinery. We estimate and compare both biorefinery operating and capital costs for loose and pelleted forms of biomass. We then carry out sensitivity

analyses to identify the effect of variations in selected input parameters on the final MESP of ethanol.

## 5.3. Materials and Methods

The analyses are done for a 2000 MT/day capacity biorefinery. Majority of the process designs in this study are adapted from the NREL study (Humbird et al., 2011). The data on the biomass compositional analysis, pretreatment, and hydrolysis conditions are taken from Pandey et al. (2019). The processes are divided into a total of 8 different areas (Figure 2) including: Feedstock handling (Area 100), Pretreatment (Area 200), Hydrolysis and Fermentation (Area 300), Distillation, Dehydration, and Solids Separation (Area 400), Product Storage (Area 500), Waste Water Treatment (Area 600), Combustor/Turbogenerator (Area 700), and Utilities (Area 800). Each area is briefly described in section 5.3.2.

### 5.3.1. System boundary

The system boundary for the analysis includes all processes within the biorefinery. We consider two scenarios of biofuel production that correspond to using either loose or pelleted biomass as the biorefinery feedstock. In both scenarios' ethanol is the primary output product of the biorefinery. Excess lignin, biogas, and residual biomass are burned to produce steam and electricity. The extra energy remaining after supplying energy to the plant is assumed to be sold to the grid.

### 5.3.2. Process description

#### *5.3.2.1. Feedstock handling*

Corn stover is used as the biorefinery feedstock. The radius for collecting corn stover is assumed similar to Humbird et al. (2011). Depending on the scenario, corn stover can be delivered either in the form of bales or pellets. In the case of bales, fine grinding occurs onsite at

the biorefinery before further processing. For pellets, there is no need for grinding, as pellets can be directly used in the pretreatment processes. The feedstock cost is taken from Sokhansanj et al. (2010). The cost of material handling in the biorefinery (Area 100 in Figure 2) is included with the feedstock price.

#### *5.3.2.2. Pretreatment*

SAA is the model pretreatment method used for both loose and pelleted biomass scenarios (Area 200 in Figure 2). SAA pretreatment is an alkaline pretreatment that can be effective in removing up to 80% of lignin and about 20% hemicellulose (Kim et al., 2003), but retains more than 95% of the cellulose. Aqueous ammonia (typically 12-16%) is used to soak the biomass for a specified time and temperature. Majority of the equipment and costs in area 200 are adapted from NREL and scaled according to the studied process. SAA pretreatment does not require a costly reactor as is needed for dilute acid pretreatment. Stainless steel reactor can be used for SAA pretreatment while dilute acid pretreatment requires expensive Incoloy 825 alloy to withstand acid corrosiveness. The pretreatment reactor used in this study is assumed to be similar to the ethanol storage tank with 40% higher costs to accommodate the modifications for material handling (Isci et al., 2008). Reactor downtime is assumed to be 4 hours in between batches. The number of pretreatment reactors needed was calculated by the sum of pretreatment residence time and reactor downtime multiplied by the daily biomass processing capacity and divided by the biomass capacity of each reactor.

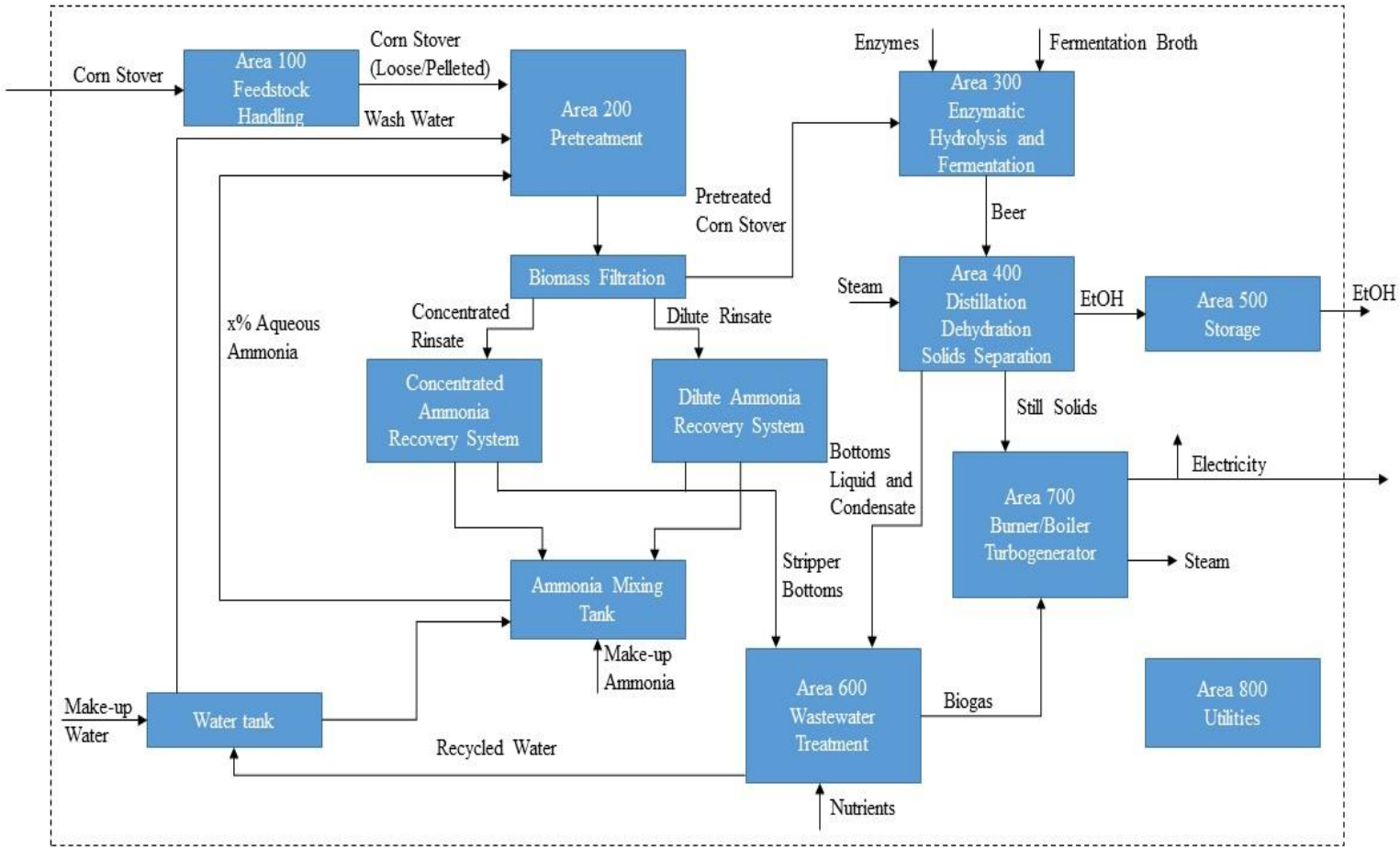


**Figure 2. General Process layout for cellulosic ethanol production from corn stover in a biorefinery**

Ammonia used during pretreatment is recovered using two ammonia recovery systems. After pretreatment, biomass is pressed to drain the concentrated ammonia that goes to ammonia recovery system 1. Process water is used to wash the biomass to bring back its pH close to neutral and then the biomass is pressed again. The ammonia drained from the wash (dilute ammonia) goes to ammonia recovery system 2. In the recovery unit, ammonia is stripped using the steam generated from the boiler in Area 700. It is assumed that 98% of ammonia can be recovered from the pretreatment process. The capital cost of ammonia stripping section is adapted from Isci et al. (2008).

Since there is no commercial facility available to get the exact wash water requirements for SAA pretreatment, it is assumed that the system is similar to a multistage leaching process (Oudenhoven et al., 2016). The volume of water required is taken as 26 liters per kilogram of biomass. The capital cost for installing such a system is not included in this calculation. The solid loadings in the pretreatment reactor is assumed to be 10% for loose biomass and 20% for pelleted biomass (Nahar and Pryor, 2017).

#### *5.3.2.3. Hydrolysis and fermentation*

Pretreated biomass is transferred to Area 300 (Figure 2), which represents hydrolysis and fermentation section. Hydrolysis and fermentation are done in batches with a single reactor being used for both processes. The solids loading is assumed to be 10% for hydrolysis for both biomass forms. Although in the laboratory experiments (Pandey et al., 2019), the solids loading used is 1% glucan loading (w/v) (equivalent to 2.5% solid loading), 10% would be reasonable for a commercial facility. However, the effect of this solids loading needs to be confirmed by further testing (Littlewood et al., 2013). Enzymes are added based on glucan content of the pretreated feedstock. A combination of 3 different enzymes (cellulase, cellobiase, and xylanase) are used.

Enzymes are purchased from commercial vendors and a separate enzyme production unit is not considered as they are in NREL report (Humbird et al., 2011). The price of enzymes is taken from Hong et al. (2013). The equipment and costs are adapted from NREL and scaled accordingly. In the base case scenario, 95% of glucose and 85% of xylose are converted to ethanol, respectively (Humbird et al., 2011). Hydrolysis and fermentation reactor down-time is assumed to be 12 hours for reactor unloading, cleaning, and loading.

#### *5.3.2.4. Distillation, dehydration, and solids separation*

The equipment sizes and costs in the distillation section (Area 400) are adapted from NREL. The distillation of beer is done in two steps to get 190 proof (95%) ethanol. The remaining water is removed by molecular sieve adsorption. The solids remaining in the distillation column are filtered and sent to the combustor in Area 700. The ethanol recovered from this section is sent to the ethanol storage tanks.

#### *5.3.2.5. Storage*

Area 500, which represents the storage section, is also adapted from the NREL study (Humbird et al., 2011). Equipment such as sulfuric acid tank and pump are not required in ammonia pretreatment and are removed from the analysis. The sizing and costs for the rest of the equipment are scaled from the NREL study according to the flow rates.

#### *5.3.2.6. Wastewater treatment*

Wastewater treatment section is represented by Area 600. The equipment sizes and costs are scaled down from the NREL study. The scaling ratio is based on the flow of solids from the pretreatment section to the wastewater treatment area (Isci et al., 2008). Wastewater treatment generates biogas and sludge that goes to the combustor. It is assumed that the water treated in this section is sufficiently clean to be used for all processes within the biorefinery.

#### *5.3.2.7. Combustor*

Area 700 includes a combustor for biogas, residual lignin, slurry from wastewater treatment, and the biomass remaining from the distillation area. The equipment size and costs are adapted from the NREL report and scaled according to the flow rate.

#### *5.3.2.8. Utilities*

Area 800 represents the utilities section. The equipment sizes and costs are scaled from the NREL report based on flow rates.

### 5.3.3. Process conditions for loose and pelleted biomass

Table 10 shows the pretreatment conditions used in this study. Pretreatment parameters that are varied include temperature, time, and ammonia concentration.

**Table 10. Pretreatment conditions chosen for loose and pelleted biomass**

| Treatment | Pretreatment Conditions | | |
|---|---|---|---|
| | Temperature (°C) | Time (h) | Ammonia Concentration (w/v %) |
| UT | - | - | - |
| PT1 | 45 | 9 | 12 |
| PT2 | 50 | 14 | 14 |
| PT3 | 55 | 19 | 16 |

UT: Untreated Biomass PT: Pretreated Biomass

The compositions of corn stover used in calculations are taken from Pandey et al. (2019) (Appendix Table A1). The composition of biomass may vary with the variety, and the season it is harvested. The ash percentage for both forms of biomass is taken as 12% based on the laboratory results.

Table 11 shows the hydrolysis and enzyme loading conditions used in the analysis for both forms of biomass with equivalent xylose yields (Pandey et al., 2019). Low enzyme loadings refer to 5 Filter Paper Unit (FPU)/g glucan and 100 Xylanase Unit (XU)/g glucan. Similarly, medium and high enzyme loadings refer to 15 and 25 FPU/g glucan, and 300 and 500 XU/g

glucan respectively. Cellobiase is added at a 1:1 ratio of FPU and CBU (Cellobiase Unit). These enzyme loadings conditions are chosen based on previous laboratory experiments (Pandey et al., 2019). Under 48 h hydrolysis time, loose biomass achieved 90% yield only with high enzyme loadings while pelleted biomass achieved with low, medium, and high enzyme loadings.

**Table 11. Conditions used in the analysis for loose and pelleted forms of biomass**

| Treatments | Enzyme loadings | Hydrolysis time (h) to achieve 90% glucose yields (Pandey et al., 2019) | | Xylose yields (%) | |
|---|---|---|---|---|---|
| | | Loose corn stover | Pelleted corn stover | Loose corn stover | Pelleted corn stover |
| PT1 | High | 48 | 20 | 63 | 60 |
| PT2 | High | 33 | 17 | 72 | 74 |
| | Medium | >48 | 23 | - | 73 |
| PT3 | High | 17 | 17 | 59 | 66 |
| | Medium | >48 | 27 | - | 66 |
| | Low | >48 | 48 | - | 62 |

### 5.3.4. Process economics and assumptions

Equipment sizing and costs were adapted from the NREL report. Total direct costs include the sum of capital costs for all the areas and costs for site development. Fixed capital cost is the sum of total direct and indirect costs. Similarly, total capital costs include fixed capital costs, working capital, and land costs. The assumptions are summarized in Table 12 (Humbird et al., 2011).

**Table 12. Summary of assumptions made for discounted cash flow analysis**

| Item | Unit |
|---|---|
| Working capital | 5% of Fixed Capital Investment |
| Depreciation Period for General Plant | 7 years |
| Depreciation Period for Steam/Electricity System | 20 years |
| Construction Period | 3 years |
| Start-up Time | 0.25 years |
| Income Tax Rate | 35% |
| Cost Year for Analysis | 2017 |
| Equity | 40% |
| Loan Interest | 8% |
| Loan Term | 10 years |
| Project Life | 30 years |

#### *5.3.4.1. Operating costs*

The summary of variable operating costs in per unit basis is shown in table 13. The costs are adjusted to 2017 dollars. Operating costs varies in each analyses for loose and pelleted biomass based on different flow rates.

**Table 13. Variable operating costs**

| Item | Unit | Unit cost ($) |
|---|---|---|
| Loose corn stover[a] | MT | $95 |
| Pelleted corn stover[a] | MT | $106 |
| Anhydrous ammonia[b] | MT | $567 |
| Corn steep liquor[b] | MT | $72 |
| Enzyme protein cost[c] | Kg | $6 |
| Diammonium phosphate (DAP) | MT | $1,247 |
| Sorbitol[b] | MT | $1,423 |
| Wastewater treatment chemicals[d] | Kg | $529 |
| Caustic[b] | MT | $189 |
| Wastewater treatment polymers[d] | MT | $8,537 |
| Boiler Chemicals[b] | MT | $6,311 |
| Flue-gas desulfurization (FGD) lime[b] | MT | $252 |
| Ash disposal[b] | MT | $40 |
| Cooling water chemicals[b] | MT | $3,782 |
| Make-up water[b] | MT | $0.32 |
| Electricity[b] | kWh | $0.07 |

Sources: [a](Sokhansanj et al., 2010), [b](Humbird et al., 2011), [c](Hong et al., 2013), [d](Isci, 2008)

### 5.3.5. Sensitivity analysis

Sensitivity analysis was done to determine the effect of input parameters on the minimum ethanol selling price (MESP). It helps to determine which parameters have a greater influence on MESP so that future research can be guided to optimize those parameters. For sensitivity analyses, the processing conditions which give the lowest MESP for each loose and pelleted form of biomass were chosen.

## 5.4. Results and Discussion

The volume of ethanol produced from loose and pelleted biomass under different pretreatment and enzyme loading conditions is given in Table 14. Although glucan hydrolysis was considered 90% for all cases, the ethanol production varies due to the differences in xylan hydrolysis and the amount of sugars lost during pretreatment. Ethanol yield rates varied from 57 to 69 gallon of ethanol per metric ton of feedstock (Table 14).

**Table 14. Annual ethanol production for loose and pelleted forms of biomass under different pretreatment and enzyme loadings condition**

| Biomass form | Pretreatment | Enzyme loading | Annual ethanol production (MMgal/year) |
|---|---|---|---|
| Loose | PT1 | H | 39.60 |
| | PT2 | H | 46.88 |
| | PT3 | H | 46.44 |
| Pellets | PT1 | H | 46.93 |
| | PT2 | M | 48.44 |
| | | H | 48.63 |
| | PT3 | L | 46.69 |
| | | M | 47.40 |
| | | H | 47.40 |

### 5.4.1. Capital cost

The capital cost of producing ethanol for each case listed in Table 11 was determined. Figure 3 shows the breakdown of capital cost for each case. There is not a huge difference in capital cost between different pretreatment scenarios. The boiler and generator section is the

costliest installed equipment section followed by wastewater treatment and pretreatment section for all cases. The turbogenerator and wastewater sections alone contribute more than 50% of the installed equipment costs for all cases. Even though they are the costliest systems, they are vital for the biorefinery. Turbogenerator uses the process byproducts to produce heat and electricity that are used to run the plant. The excess electricity can be sold to the grid to generate extra revenue. Similarly, the wastewater section recycles the water required for the processes.

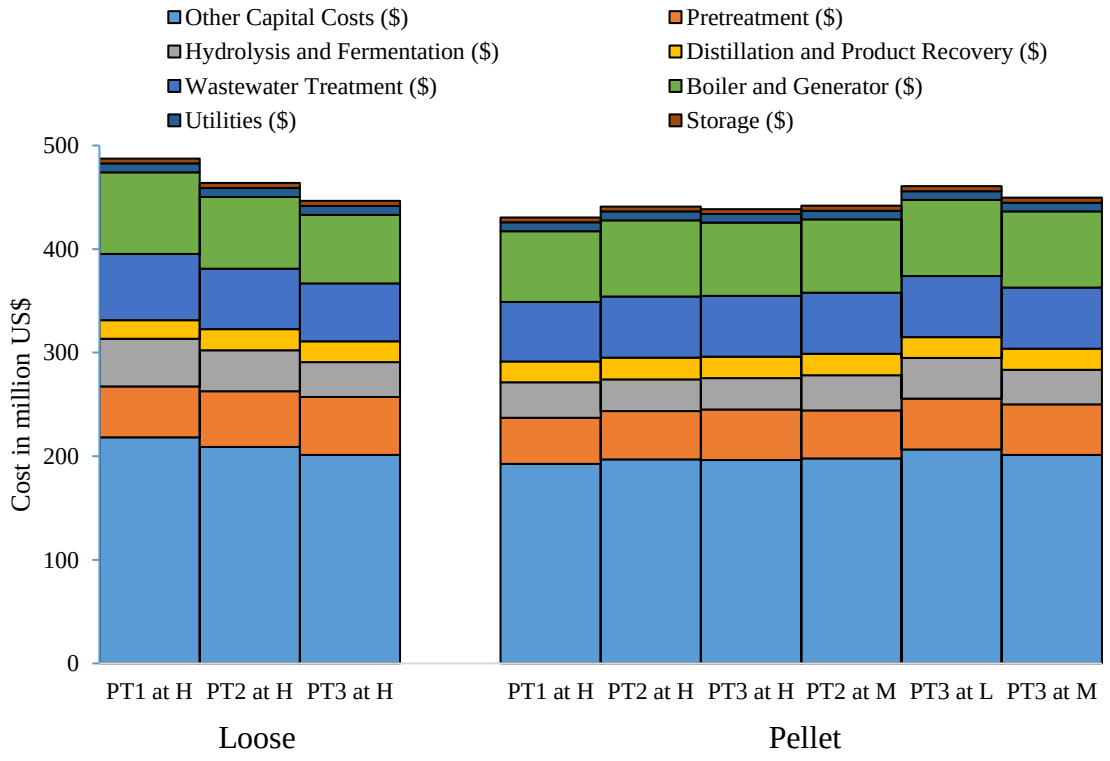


**Figure 3. Capital cost breakdown into each area for loose and pelleted forms of biomass under different pretreatment and enzyme loadings conditions (PT1, PT2, PT3 and low, medium, and high enzyme loadings)**

The capital cost trend shows that with increasing pretreatment severity, the cost goes down for loose biomass as expected. Similarly, for pelleted biomass, the cost is lower at high enzyme loadings but is not apparent as with the loose biomass. Though the use of pelleted biomass allows doubling the pretreatment solid loadings, the cost advantage is small compared to total equipment cost. Among the conditions for pelleted biomass, PT3 at low enzyme loadings

has the highest and PT1 at high enzyme loadings has the lowest capital cost. This is because of PT3 at low enzyme loadings has longer hydrolysis time, which requires more reactors. Hydrolysis cost is directly dependent on pretreatment and enzyme loadings condition chosen. Higher severity pretreatment condition with higher enzyme loadings leads to lower hydrolysis equipment cost. Although the pretreatment cost is lower at lower severity, the hydrolysis cost goes up for lower severity which lowers the cost difference. Other capital costs include warehouse and site development, construction, land, working capital, and other contingencies.

The capital investment required for SAA pretreatment ranges from $9/gal to $12/gal ethanol produced which is very high compared to other pretreatment methods like dilute acid, ammonia fiber expansion, and hot water (Eggeman and Elander, 2005). The capital investment required for other pretreatment technologies varies from $3.05/gal to $5.55/gal ethanol produced (adjusted to 2017 dollars) (Eggeman and Elander, 2005). Although SAA pretreatment does not require high temperature and costlier pretreatment reactors, the cost gain in those areas are negligible compared to the additional cost of ammonia recovery that is required in the process.

### 5.4.2. Operating cost

The breakdown of annual operating costs (both fixed and variable) in terms of per gallon of ethanol for each form of biomass under different pretreatment conditions is shown in Figure 4. The trend shows that for loose biomass the operating cost is higher at less severe pretreatment. However, the by-product credit is also higher at lower pretreatment severity which balances out the higher operating costs. Lower pretreatment severity conditions require less steam. Loose biomass at PT3 requires extra energy from the grid, but the requirement is very negligible and is assumed as zero. For pelleted biomass, the operating costs per gallon of ethanol are lower for lower enzyme loadings because enzyme is costly. Among all conditions modeled, PT3 at low

enzyme loadings for pelleted biomass has the lowest operating cost followed by PT2 at medium enzyme loadings.

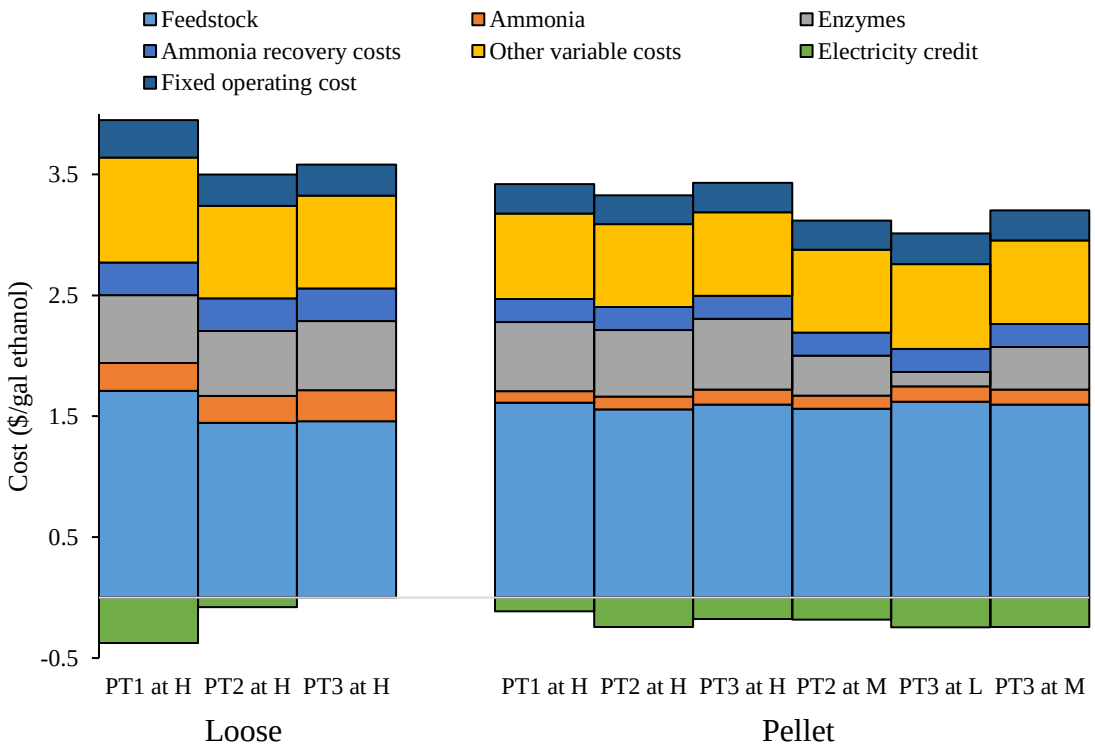


**Figure 4. Breakdown of operating costs (variable and fixed) for loose and pelleted forms of biomass under different pretreatment conditions (PT1, PT2, PT3) and enzyme loadings (high for loose, and low, medium, high for pelleted biomass)**

Among all operating variables, feedstock cost has the highest contribution in the final ethanol price. Feedstock cost contribution on the final ethanol price is higher for pelleted biomass. This is expected because of the additional cost required for pelleting. For loose biomass, ammonia has double the cost contribution compared to pelleted biomass. The cost ranges from 0.22-0.26 $/gal for loose biomass compared to 0.10–0.13 $/gal for pelleted biomass. The reason for lower ammonia cost contribution is because pelleted biomass allows doubling the solids loading, which enables reduction of ammonia by half. Enzyme use is another costly operating variable. In the case of loose biomass, enzyme contribution ranges from 0.54–0.57

$/gal while pelleted biomass contribution ranges from 0.12-0.58 $/gal. Lower enzyme cost contribution for pelleted biomass is because enzyme loading can be reduced by 80% which was not possible with loose biomass. If we look at the ammonia recovery cost, loose biomass processing requires $0.27/gal ethanol produced and pelleted biomass requires $0.19/gal ethanol produced (adjusted to varied ammonia loading and 2017 dollars) (da Costa Sousa et al., 2016). Fixed operating costs are assumed the same as Humbird et al. (2011).

### 5.4.3. Minimum ethanol selling price at different scenarios

The minimum ethanol selling price (MESP) for both loose and pelleted biomass under different pretreatment conditions is shown in Table 15. MESP varies between $3.83/gal and $4.87/gal ethanol. The lowest cost was $3.83/gal for pelleted biomass at PT3 and low enzyme loadings. The highest cost for pellets was $4.29 with PT1 at high enzyme loadings. The cost of producing ethanol using pellets is lower than using loose biomass for all conditions. The minimum cost for loose biomass was $4.41/gal ethanol with PT2 at high enzyme loading.

**Table 15. Minimum ethanol selling price (MESP) for loose and pelleted biomass under different pretreatment and enzyme loadings conditions (PT1, PT2, PT3, and low, medium, high enzyme loadings)**

| Biomass form | Pretreatment | Enzyme loading | Minimum ethanol selling price (MESP) (S/gal) |
|---|---|---|---|
| Loose | PT1 | H | $4.87 |
| | PT2 | H | $4.41 |
| | PT3 | H | $4.54 |
| Pellets | PT1 | H | $4.29 |
| | PT2 | M | $3.91 |
| | | H | $4.05 |
| | PT3 | L | $3.83 |
| | | M | $3.98 |
| | | H | $4.24 |

### 5.4.4. Sensitivity analysis

For sensitivity analysis, PT2 at high enzyme loadings was chosen for loose biomass, and PT3 at low enzyme loadings was chosen for pelleted biomass. The lower and upper limit of input parameters assumed is based on lab experiences and other research studies (Humbird et al., 2011; Isci, 2008). The assumptions for loose and pelleted biomass are given in Table 16.

**Table 16. Sensitivity analysis assumptions for loose (PT2 at high enzyme loadings) and pelleted biomass (PT3 at low enzyme loadings)**

| Parameters | Scenarios | | |
|---|---|---|---|
| | Least favorable | Base case | Favorable |
| Feedstock price (per unit MT) | | | |
| Loose biomass | $110 | $95 | $80 |
| Pelleted biomass | $120 | $106 | $90 |
| Enzyme protein cost ($/kg) | $8 | $6 | $4 |
| Hydrolysis solids ratio | 5% | 10% | 20% |
| Hydrolysis time (h) | | | |
| Loose biomass | 48 | 33 | 24 |
| Pelleted biomass | 60 | 48 | 36 |
| Fermentation time (h) | 48 | 36 | 24 |
| Ammonia loss | 3% | 2% | 1% |
| Glucan hydrolysis | 75% | 90% | 95% |
| Xylan to xylose | | | |
| Loose biomass | 50% | 72% | 90% |
| Pelleted biomass | 50% | 62% | 90% |
| Glucose to ethanol | 85% | 95% | 98% |
| Xylose to ethanol | 75% | 85% | 95% |
| Fixed capital investment | 25% | - | -25% |

Figure 5 shows the variation in MESP due to variation in sensitivity parameter for loose biomass. The results show that MESP is highly sensitive to changes in hydrolysis solids ratio, xylan conversion to xylose, and capital investment. Increasing solids ratio in hydrolysis reduces the required number of hydrolysis reactors along with reduction in energy for distillation due to increase in ethanol concentration. Another sensitive parameter for MESP is the xylan conversion

to xylose. Based on our experimental result, in the base case scenario, we had a lower value (72%) for xylose conversion. Increasing xylan conversion rate also helps in increasing ethanol concentration. In the case of capital cost, the cost calculations are assumed for the $n^{th}$-plant model. So, the capital cost may be higher for initial plants. Reducing capital cost plays an important role in lowering MESP. Feedstock cost has also a higher contribution in MESP. Feedstock cost includes transportation, handling, and storage. Developing a cost-effective supply system is crucial in the development of lignocellulosic biorefineries. Interestingly, fermentation and hydrolysis time had little impact on MESP. Although longer hydrolysis and fermentation time increases the capital cost, the cost increases are minimal when their impact on MESP is considered. MESP was also highly sensitive to changes in Enzyme cost. Reducing the use of enzyme as well as its cost is also important.

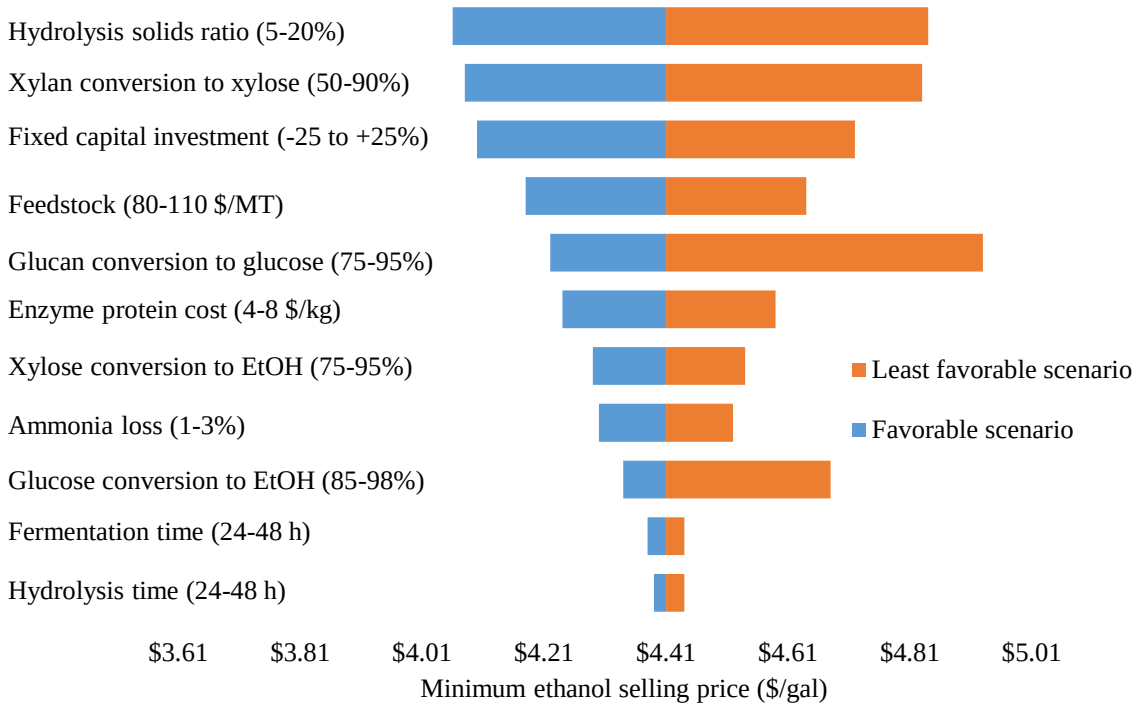


**Figure 5. Sensitivity analysis for PT2 at high enzyme loadings for loose biomass**

For pelleted biomass (Figure 6), most of the sensitivity parameters showed a similar response to what was seen with the loose biomass. MESP was highly sensitive to xylan conversion to xylose. This is because the conversion yield was even lower in the case of pellets (62%) compared to loose biomass (72%). But, enzyme was not a significant factor for pellets because enzyme loading was reduced by 80% compared to loose biomass. Looking at both sensitivity analyses, the biggest opportunity to reduce final ethanol prices could be to focus on the conversion of carbohydrates into sugars and also increasing solids loading in hydrolysis. Use of pelleted biomass may have a higher advantage in solids loading during hydrolysis which we did not conduct a laboratory study. In dilute acid pretreatment, parameters like capital cost, cellulose to glucose, xylose to ethanol were the most sensitive parameters (Humbird et al., 2011). Kazi et al. (2010) found that ethanol price is sensitive to feedstock, enzyme, and installed equipment costs.

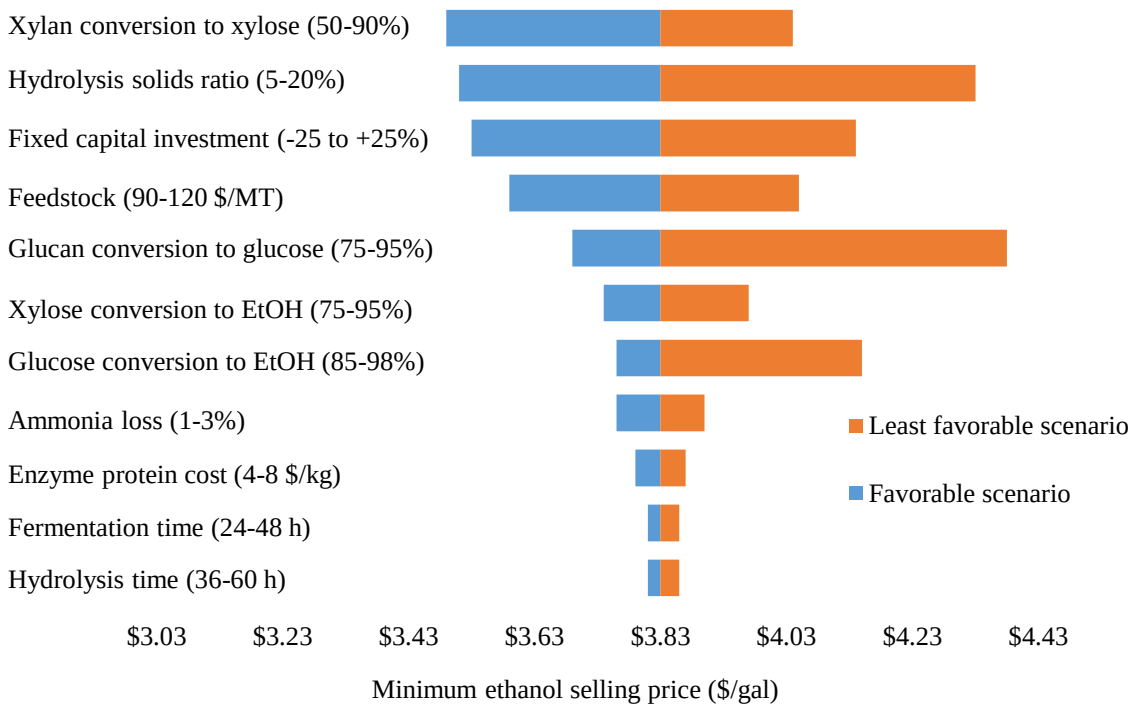


**Figure 6. Sensitivity analysis for PT3 at low enzyme loadings for pelleted biomass**

## 5.5. Conclusion

Use of pelleted biomass as a biorefinery feedstock is a more economic option for a biorefinery than using loose biomass. Though feedstock price is higher for pelleted biomass compared to loose, the downstream process benefits with the use of pelleted biomass outweigh the higher feedstock price. The MESP cost trend showed that lower operating costs and higher ethanol yield results in lower MESP. All the conditions analyzed for pelleted biomass resulted in lower MESP than loose biomass. The lowest MESP for loose biomass was $4.41/gal ethanol and for pelleted biomass was $3.83/gal ethanol. Use of pelleted biomass allows different processing options compared to loose biomass depending on enzyme and energy costs. Increasing the solids loading is the most important parameter to reduce ethanol price.

## 5.6. Acknowledgments

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# 6. CONCLUSION AND RECOMMENDATIONS

## 6.1. Conclusion

Biomass pelleting is advantageous in downstream processing in a biorefinery. Use of pelleted biomass enables doubling of solids loading in pretreatment which reduces the chemical requirement by half and also reduces the number of reactors needed. It also enables reductions in either pretreatment severity, enzyme loadings, hydrolysis time, or a combination of these. Reduction in pretreatment severity helps in reducing energy requirement and required reactor volume. Reduction in hydrolysis time helps to lower the hydrolysis reactor volume. Use of pelleted biomass will provide a biorefinery with the flexibility to have different processing options, the choice of which depends on market costs of energy, enzymes, and chemicals.

The techno-economic analysis of producing ethanol from both loose and pelleted forms of biomass also suggests that using pelleted biomass is economically beneficial for a biorefinery. Despite the additional costs of pelleting, the economic gains in downstream processing outweigh those costs. The variable operating costs such as ammonia and enzyme costs can be greatly reduced with the use of pelleted biomass. For all the conditions studied here, the minimum ethanol selling price is lower when produced with pellets compared to loose biomass.

## 6.2. Recommendations for Future Work

Future research works can continue to quantify the benefits of pelleting with other pretreatment methods such as dilute acid. The effect of pelleting at lower pretreatment severity and enzyme loadings on hydrolysis should be considered to see the effectiveness of pelleting with other pretreatments. The study can also focus on obtaining higher xylose yields by optimizing xylanase supplementation. Hydrolysis time of loose biomass for some pretreatments did not completely agree between initial hydrolysis and follow up study. So, further study can be

done to validate the current results. Based on the follow-up study, it would have been better to test loose biomass at lower enzyme loadings too.

Generally, SAA pretreatment is done at a lower temperature for a longer duration of time. The effect of higher temperature (between 60-100 °C) at lower concentration (below 5% w/v) can be tested for a shorter duration of time.

The economic analysis suggested that further study should be focused on increasing solid loadings and improving xylose yield during hydrolysis. Those factors have significant contribution in the final ethanol price. Our preliminary study on solid loadings in simultaneous saccharification and fermentation showed that use of pelleted biomass enables higher solid loadings than loose biomass without compromising the ethanol yields. The cost of loose and pelleted biomass also needs to be updated with a further study on supply chain logistics and improvements in the pelleting process.

The calculations did not consider minor sugars like mannose, arabinose, and galactose. Those sugars can comprise about 5% of biomass and can have significant influence in final ethanol cost. We did not do the compositional analysis of those minor carbohydrates. It would be better to include those in future works.

The results of this work can also be used for a further study on life cycle assessment. The techno-economic analysis provides economic insight of using loose and pelleted biomass in a biorefinery. Life cycle study can give further information on how pelleted biomass performs overall in environmental perspective and whether it is beneficial to use pelleted biomass.

# APPENDIX

**Table A1. Composition of untreated and pretreated loose and pelleted corn stover**

| Treatments | Solids recovered (%) | Glucan (%) | Xylan (%) | Total lignin (%) |
|---|---|---|---|---|
| Loose corn stover | | | | |
| UT | - | 31.2±1.0 | 20.8±1.8 | 18.9±0.4 |
| PT1 | 74.2 | 35.1±0.1 | 21.9±0.1 | 15.3±0.7 |
| PT2 | 71.7 | 40.9±1.8 | 26.5±0.7 | 12.4±0.2 |
| PT3 | 70.5 | 44.1±2.9 | 27.5±4.4 | 10.8±0.1 |
| Pelleted corn stover | | | | |
| UT | - | 32.0±1.6 | 21.0±1.1 | 17.3±0.2 |
| PT1 | 77.3 | 40.6±0.2 | 25.0±0.3 | 12.7±0.9 |
| PT2 | 72.3 | 43.3±1.4 | 24.8±0.9 | 11.9±1.1 |
| PT3 | 68.6 | 47.2±0.7 | 24.3±0.3 | 9.6±0.1 |

**Table A2. Assumptions for determining total capital costs (Humbird et al., 2011)**

| Item | Cost |
|---|---|
| Warehouse | 4% of ISBL |
| Site Development | 9% of ISBL |
| Additional Piping | 4.5% of ISBL |
| Prorateable Expenses | 10% of Total Direct Costs |
| Field Expenses | 10% of Total Direct Costs |
| Home Office and Construction Fee | 20% of Total Direct Costs |
| Project Contingency | 10% of Total Direct Costs |
| Other costs (Start-up, Permits, etc.) | 10% of Total Direct Costs |

Loose biomass: 100 g Pellets: 100 g

**Figure A1. Comparison between 100 g of loose and pelleted biomass**

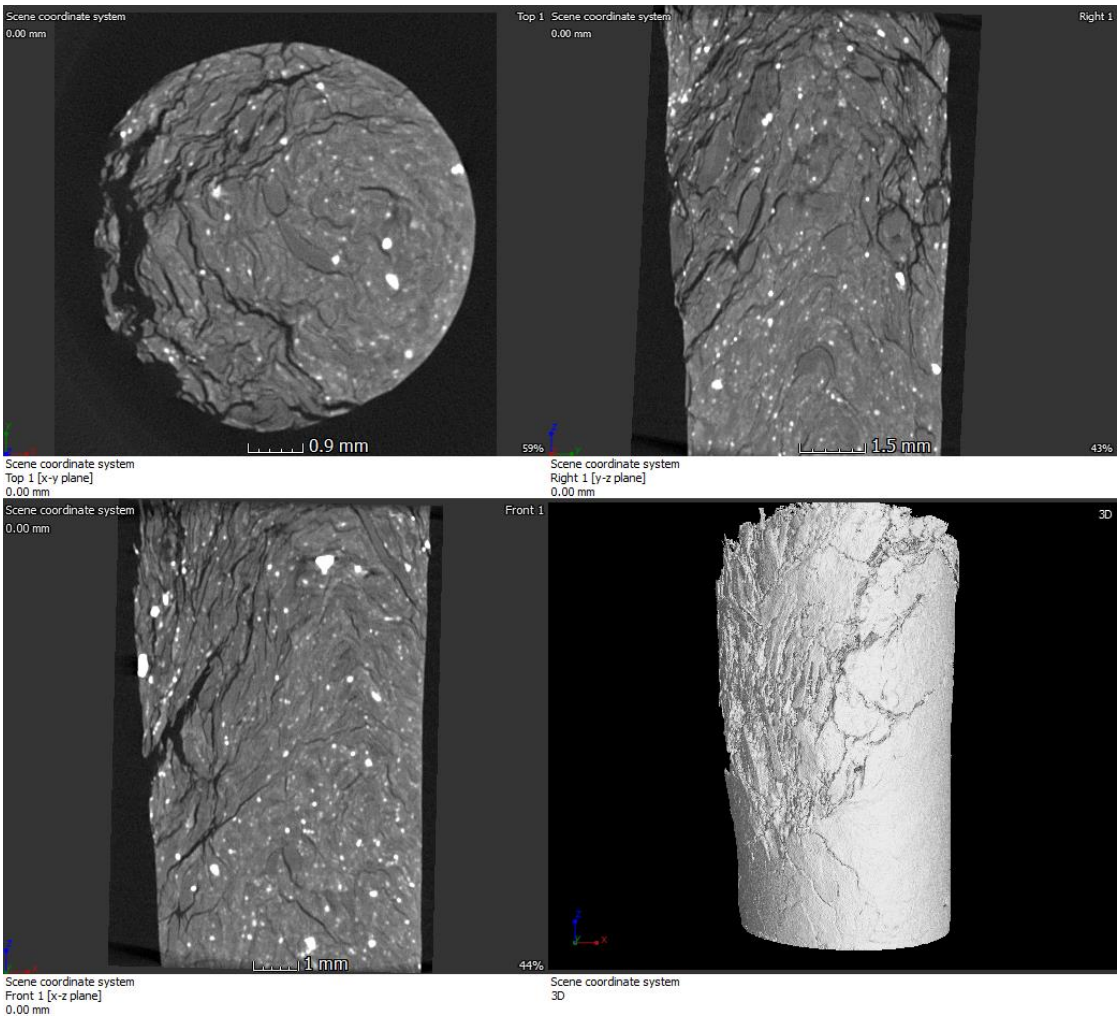


**Figure A2. Micro-CT image of corn stover pellets showing top, side, front, and 3-D views**


**Figure A3. Pretreatment setup of loose and pelleted biomass in a water bath**

**Figure A4. Washing of pretreated loose biomass**

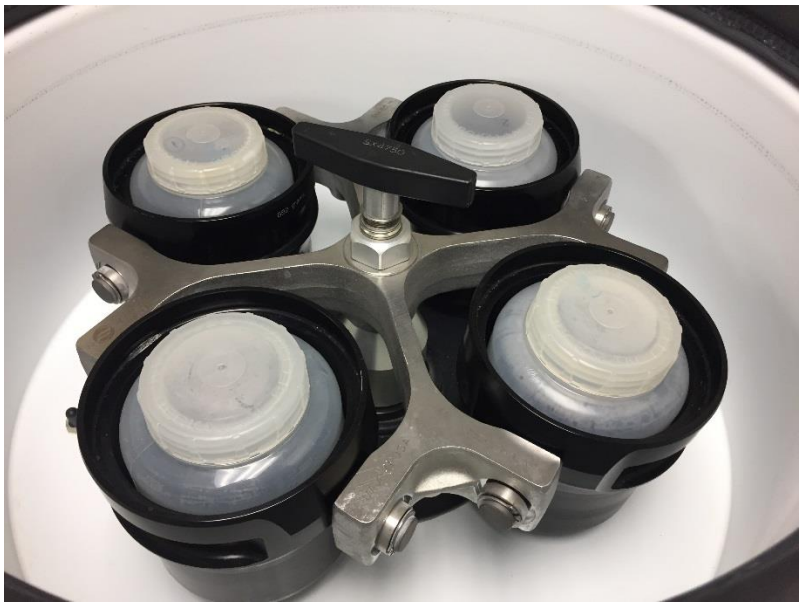

**Figure A5. Washing of pretreated pelleted biomass in centrifuge**

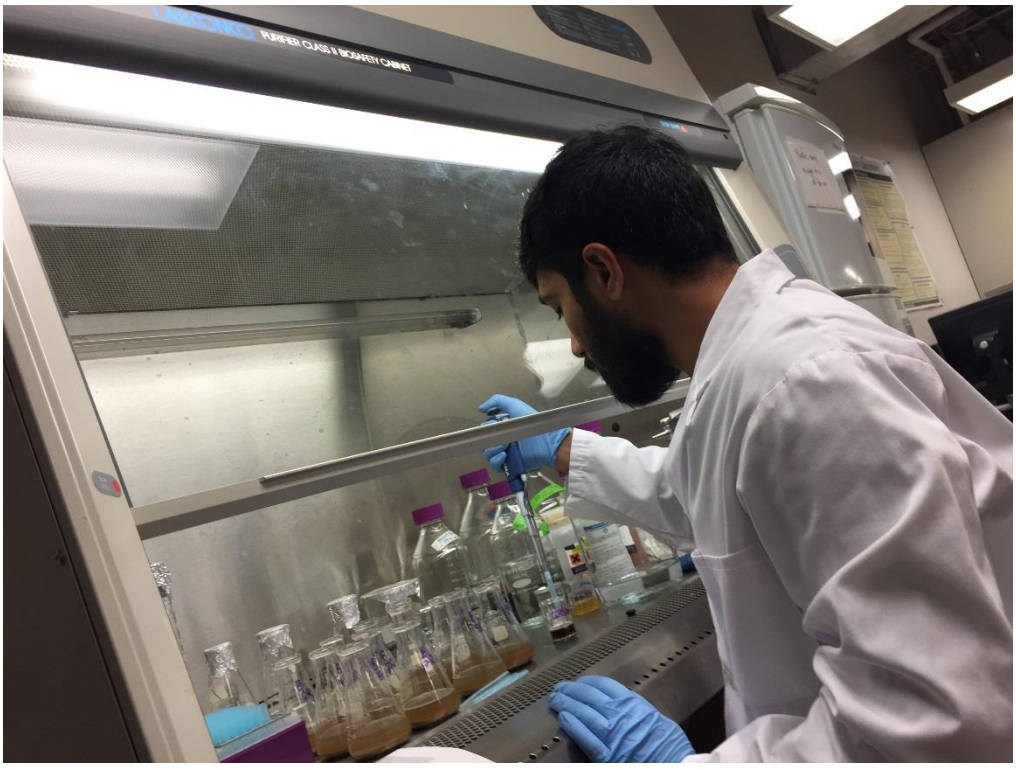

**Figure A6. Pipetting enzymes to hydrolysis flasks**


**Figure A7. Hydrolysis setup in water bath**