PROCESSING TRADE-OFFS IN A CELLULOSIC BIOREFINERY

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

Commercial scale cellulosic biorefineries face significant challenges to produce low-cost fermentable sugar from biomass. Biorefinery processing steps are interrelated and trade-offs between process parameters impact the cost and efficiency of the overall system. Although densified biomass as a biorefinery feedstock would improve biomass supply logistics, it has not been considered viable due to high energy and emissions associated with the densification process. However, the potential synergies of biomass densification with downstream processing steps are critical. An energy-efficient system with improved supply logistics, reduced severity pretreatment, and improved hydrolysis efficiency will lower the cost of sugar production from cellulosic biomass.

The objective of this research was to increase overall process efficiency of biorefineries by understanding how different process parameters affect the hydrolysis efficiency. Processing trade-offs in pretreatment and enzymatic hydrolysis for densified and non-densified biomass for economical sugar production were evaluated. A life cycle perspective was taken to compare fossil energy and greenhouse gas (GHG) emissions from pelleted and non-pelleted corn stover during transportation and soaking in aqueous ammonia (SAA) pretreatment.

A model developed to demonstrate the interaction of enzymatic hydrolysis factors to improve hydrolysis efficiency showed that enzyme loadings had a more significant effect on hydrolysis rates than pH or temperature. Economical optimal enzyme loadings were lower than loadings to maximize yield, loadings can be adjusted to maximize profit based on enzyme costs, ethanol price, and process temperature.

Pelleted corn stover allowed reduction in SAA-pretreatment severity with different combinations of temperature, time, and ammonia concentration to produce 90% or higher glucose yields. This suggests possible economic and environmental benefits of using pelleted biomass as a biorefinery feedstock. Use of pelleted biomass reduced transportation fossil energy and GHG emissions by 25%. A significant reduction of energy (89%) for SAA-pretreatment was achieved with pelleted biomass due to lower pretreatment time and higher solid loadings. Use of pelleted biomass allowed doubling of pretreatment solid loadings, which lowered pretreatment reactors from 59 to 9, in addition to associated water and chemical savings. This study demonstrated that SAA pretreatment is not feasible for non-pelleted biomass, but process synergies make SAA pretreatment possible for pelleted biomass.

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DEDICATION

To my mother, who wanted to see me as Dr. Nahar

& my kids, Raeef and Ramin for bringing joy in my life

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GENERAL INTRODUCTION

Availability of affordable energy is a critical component for economic development of any country. Society, especially in industrialized countries, is heavily dependent on fossil fuels for energy. As of 2015, 82% of the total primary energy used globally came from fossil fuels (EIA, 2016). The International Energy Outlook 2016 (IEO, 2016) projected a 48% increase in world energy consumption by 2040. Increased energy demand in recent years is due in part to emerging economies in heavily populated countries like China and India as their populations and oil consumption per capita are increasing (EIA, 2016). There are growing concerns for energy security, depletion of fossil fuels, and contribution of climate change through the emissions of greenhouse gases (GHG). New renewable sources for energy and chemicals are therefore a focus of research and development activities to increase environmental sustainability.

There has been an increasing interest in alternate energy sources from renewable resources such as solar, wind, and biomass due to growing concerns around energy security and climate change. In the U.S., the electric power and transportation sectors of the economy account for around 60% of GHG emissions. Currently, the U.S. consumes oil at a rate of approximately 3 $hm^3\ day^{-1}$. This accounts for over 25% of global production, and consumption is expected to grow to 4 $hm^3\ day^{-1}$ by 2025. Around 75% of this is used in the transportation sector as gasoline, diesel, and jet and marine fuel. While electrical generation can use diverse energy sources such as coal, natural gas, and wind, transportation relies almost exclusively on petroleum derived fuels. Since liquid fuels make up a large share of energy demand, a replacement for petroleum is essential. The Energy Independence and Security (EISA) of 2007, established a target that the U.S. must produce 60 hm^3 (16 billion gallons) of cellulosic biofuels per year by 2022. Biofuels provide the U.S. an opportunity to diversify its transportation fuel mix, decreasing dependence on foreign resources, and decreasing the environmental impact associated with it.

Use of cellulosic biomass can help meet the demand for liquid transportation fuels. Biofuels, especially those derived from cellulosic feedstocks such as agricultural residues and low maintenance energy crops, have the potential to reduce GHG emissions significantly (Baral et al., 2012). Although there are tremendous opportunities in the U.S. to use the abundant and diverse agricultural and forest resources to augment domestic energy supply and reduce environmental burdens, there are still many challenges to overcome to make the cellulosic biorefineries economically feasible.

Several challenges must be faced to make cellulosic biorefineries cost competitive. There is still a need to develop cost effective technologies for the two most expensive steps in a cellulosic biorefinery: pretreatment and enzymatic hydrolysis. Cellulosic biomass typically requires a high-severity pretreatment process to enhance the effectiveness of enzymatic hydrolysis. Mild pretreatment typically results in lower enzymatic digestibility. The development of an economical but effective pretreatment is still a significant barrier.

Another biorefinery challenge is dealing with the low bulk density of cellulosic biomass. Use of conventional baled biomass as a biorefinery feedstock poses enormous challenges with loading, unloading, stacking, and processing at industrial-scale facilities (284 – 378 dam^3 yr^{-1}) (75–100 MGY), which may be required to meet cellulosic biofuel production goals in the EISA (2007). Transportation and handling logistics are hindering large-scale cellulosic biofuel production. Densifying biomass through pelleting is one way to improve logistics, but it has received relatively little attention because of the pelleting costs (Sokhansanj and Fenton, 2006; Sultana et al., 2010). However, studies documenting pelleting costs have not accounted for processing synergies of densification and pretreatment. In order to accomplish cellulosic biofuel targets, improvements in the overall process efficiency by reassessing interaction between densification technologies and efficacy of pretreatment and hydrolysis is necessary. One of the aims of this study is to understand how pelleting can make conventional pretreatments less expensive and how it can contribute to reducing GHGs. A life cycle approach will be used for understanding the environmental impact of the carbon footprint of biofuel production for baled and pelleted forms of corn stover using soaking in aqueous ammonia (SAA) pretreatment to determine which processing technology has better environmental performance.

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LITERATURE REVIEW

Our strong dependence on fossil fuels results from the intensive use and consumption of petroleum derivatives and leads to environmental concerns. There is clear scientific evidence that emissions of greenhouse gases (GHG), such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), arising from fossil fuel combustion contributes to the earth's climate change (IPCC, 2007). To combat the potential environmental impact, government regulatory agencies worldwide are considering GHG reduction policies such as the Kyoto Protocol (UN, 1997). The protocol is an international treaty, which calls for the reduction of GHG emission by individual countries of 10% below 1990 levels. However, this climate change treaty failed to stop the rise of carbon pollution in large part because of a deadlock between China and the U.S., which emit more than 40% of the world's carbon dioxide (EIA, 2014). A series of scientific and economic reports have concluded that in order to avoid a 2 °C global temperature rise, the world's largest economies will have to drastically cut the expected increase of carbon emissions (IPCC, 2007). Providentially, there was a historic announcement in November 2014 by President Obama and President Xi Jinping of China to deal with carbon emissions in preparation for the international climate change agreement at a United Nations summit meeting in 2015 in Paris. As a part of the climate agreement, the U.S. pledged to China to cut emissions by at least 26% from 2005 level by 2025 and China promised to peak CO_2 emissions by 2030.

According to the U.S. Energy Information Administration (EIA), fossil fuels meet around 82% of U.S. energy demand (EIA, 2016). Since the fossil fuel reserves are limited, our dependence on these fuels must be reduced. The most challenging part of the fossil fuel reduction is transportation, which is almost solely dependent on petroleum, accounts for 93% of the total energy use, and consumes 2 to 3 times as much petroleum as any other sector. The U.S.

produced an average 1.3 hm^3 day^{-1} (8.2 million barrels day^{-1}) of crude oil and imported an additional 1.1 hm^3 day^{-1} (7.3 million barrels day^{-1}) to meet its oil needs (EIA, 2016). If the U.S. could meet EISA's 136 hm^3 (36 billion gallons yr^{-1}) annual target for total biofuels by 2022, this would displace 16 to 17% of U.S. crude oil.

There has been an increase in lignocellulosic biomass processing research for fuel production, focusing particularly on agricultural and forestry residues, since these are comparatively low in cost, abundant, readily available, and renewable. Biofuels derived from plant biomass can decrease dependence on petroleum and reduce associated greenhouse gas emissions (Schneider and McCarl, 2003; Spatari et al., 2010). The U.S. is taking initiatives to reduce its dependence on foreign oil by setting national biofuels targets and to provide incentives to accelerate bioenergy industry growth. For example, the Renewable Fuel Standard (RFS2) policy, part of the Energy Independence and Security Act (EISA) of 2007, sets production target of 136 hm^3 (36 billion gallons) per year of renewable transportation fuels by 2022 (EISA, 2007). The Environmental Protection Agency (EPA) also has specific thresholds for all categories of the life cycle of GHG emissions of the renewable fuels used to meet the 79 hm^3 (21 billion gallons) per year mandate. In particular, cellulosic biofuels are required to achieve a 60% reduction in life cycle emissions compared with the 2005 gasoline baseline. This policy will help in reducing the GHG emissions from the transportation sector, which currently accounts for 27% of the total 6.5 billion Mg of GHG emissions in the U.S. (Figure 1). However, the production of biofuels continues to fall short of yearly standards set by EPA. The decrease volume of biofuels were set due to lack of production. Technological advancement in the feedstock-to-fuel conversion process, a competitive year-round supply of biomass feedstock is a major constraint in the commercial advancement of biofuel production.

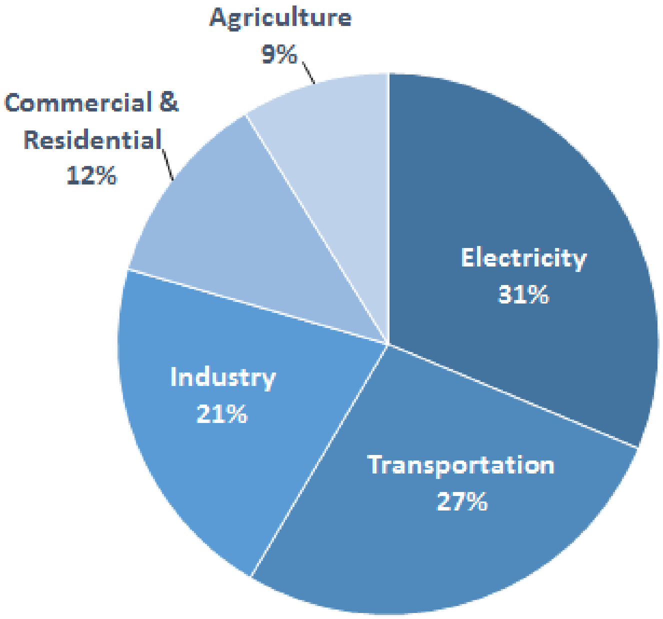

Figure 1. Total U.S. greenhouse gas (GHG) emissions by economic sector in 2013 (Source: Inventory of U.S. Greenhouse Gas Emissions and Sinks 1990–2013 (EPA, 2015 Report).

The key drivers for development of alternative energy sources are diversification and security of fuel supply, as well as rising climate change concerns from GHG emissions of conventional fuels. In the short term, biofuels are the only renewable resources that can address the transportation sector's heavy dependence on foreign oil without replacing the vehicle fleet (US-DOE, 2011). Ethanol–gasoline mixtures are increasingly being used as alternative motor fuels as these can reduce air pollution by decreasing the amount of particulates and NO_x emissions and have the lowest cost. It is foreseeable that bioindustry will likely undergo a rapid expansion in the coming decades. To accelerate the transition towards large-scale, sustainable production and use of biofuels and bioenergy products, we need to critically look at biomass to bioenergy supply chains to design or redesign the biorefinery processes for lowering the costs and environmental impacts.

Biomass

In the context of biomass energy, the term “biomass” refers to crops, residues, and other biological materials that can be used as a substitute for fossil fuels in the production of energy and other products. This includes agricultural and forest residues, animal wastes, etc. Processed organics such as municipal solid waste, sewage, manure, and milling waste are also included. A significant advantage of using biomass for biofuel is that biomass is a form of stored energy, as opposed to other renewable sources such as solar and wind, which are intermittent and can not be used on demand without additional storage capability.

One key route of producing cellulosic biofuels and bioproducts is through sugar intermediates (Agler et al., 2011). Identifying abundant amounts of biomass, which could produce a group of sugar-derived chemicals and materials are of utmost importance to serve as an economic driver for a biorefinery.

Availability and types of biomass

Various types of abundantly available biomass can be used as a feedstock for biochemical or biofuel production. Terrestrial biomass can be generally categorized into two groups. The first group is made of traditional agricultural crops such as corn grain (*Zea mays* L.), sugarcane (*Saccharum officinarum* L.), soybean (*Glycine max*, L.) and other oilseeds. These feedstocks are rich in carbohydrates or lipids, and have high yields after converting into bioethanol or biodiesel. First generation biofuels were produced from starches of corn, wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), cassava (*Manihot esculenta* L.) etc. or sugars from sugarbeet (*Beeta vulgaris* L.), sugarcane (*Saccharum officinarum* L.) or sweet sorghum (*Sorghum bicolor* L.). Sugar can be easily extracted from the sugar crops for subsequent fermentation to ethanol, butanol, or other biobased chemicals. Starches need to be hydrolyzed enzymatically to get glucose monomers from starch chains, which can subsequently be fermented and processed into biofuels and/or biobased chemicals. Corn is currently the dominant biofuel feedstock in the U.S. and competes with food and feed, which directly or indirectly affects the price of other food or food commodities. Availability of corn or other grains as feedstock is limited by the amount of cropland and yield production.

Table 1. Annual total megagrams of potential biomass for biofuel in the U.S.

Biomass	Million Mg yr^{-1}
Agricultural residues	388
Forest resources	335
Energy crops	342
Grains and corn	79
Municipal and industrial wastes	53
Others (e.g. oilseeds)	44
Total	1241

(Source: U.S. Department of Energy Biomass Program, 2009)

The second group of terrestrial biomass feedstock is the cellulosic biomass. The greatest potential for biofuel production in the long run lies in the use of cellulosic feedstock. Second generation biofuels use lignocellulosic biomass such as agricultural residues (i.e. corn stover, wheat straw, rice (*Oryza sativa* L.) straw, forest residues, dedicated herbaceous and woody energy crops, municipal solid wastes, and paper mill sludge wastes. These have distinct advantages over the first generation feedstock since these biomass are relatively less expensive than conventional agricultural feedstocks. The resource of these feedstocks can support a huge amount of biofuel production (Table 1). The 2011 report released by the U.S. Department of Energy (DOE) and Oak Ridge National Laboratory (ORNL) titled “U.S. Billion-Ton Update: Biomass Supply for a Bioenergy and Bioproducts Industry” details biomass feedstock potential in U.S. (DOE, 2011). This report exhibits the U.S. capacity to sustainably produce over 1.6

billion Mg of terrestrial biomass annually for conversion to bioenergy and bioproducts, while continuing to meet existing demand for food, feed, and fiber. The report also estimates that the U.S. could potentially produce approximately 321 hm^3 (85 billion gallons) of biofuels annually—enough to replace approximately 30% of the nation's total current petroleum consumption. The abundance and comparatively lower cost of cellulosic feedstocks make them more attractive as a source of biofuel (Sun and Cheng, 2002).

The main sources of cellulosic feedstocks are described below:

Woody dedicated energy crops

Woody dedicated energy crops refer to biomass material specifically from trees and shrubs for energy purpose, such as hybrid poplar (*Populus*) and willow (*Salix*). Woody energy crops can be an excellent solution for marginal lands, where it is not possible to cultivate any other crop. Woody energy crops are high in lignin, which has a high heating value and low in ash content, so most often it is used for energy by direct combustion or co-fired with coal. Woody crops need to be located near a biorefinery for efficient and economical transportation. There is also a need to develop economically competitive technologies to convert this biomass to liquid fuel.

Herbaceous dedicated energy crops

Herbaceous dedicated energy crops such as switchgrass (*Panicum virgatum* L.), big bluestem (*Andropogon gerardii* L.), and elephant grass (*Pennisetum purpureum* L.) are considered an attractive feedstock for bioenergy production mainly due to high yields and biomass composition (Keshwani and Cheng, 2009). These energy crops have high biomass yield potential on land, which is not suitable for annual crops. Energy crops also tend to have high nutrient and water use efficiency than annual crops, reduced fertilizer and pesticide use, and therefore exhibit positive environmental impacts of soil, water and wildlife habitat. Perennial energy crops have the advantage of not requiring annual tillage or planting; initial costs for establishing the crops such as irrigation, fertilization, disease, and pest control may be required, but the environmental impacts are spread throughout the whole lifetime of the species. However, extensive cropland and pasture would need to be converted to increase production of dedicated energy crops. Challenges also exist for transport and storage infrastructure as well as finding suitable conversion processes.

Agricultural residues

Agricultural residues are the leftover materials in the field usually after the grains are harvested. These feedstocks such as corn stover, wheat straw, rice straw, etc. can be obtained from existing farmland. There are also by-products during processing of food, fiber or feed crops such as husk, bagasse, and molasses. These biomass are traditionally left in the field or burned. Removal of these materials for biofuel production may have additional benefits above the economic potential of product sale, as it is associated with no-till cultivation practices, which might save money on the field. However, some amount of agricultural residues also needs to be left on the field for increasing soil organic matter and reducing soil erosion. These positive environmental impacts reduce soil quality degradation and minimize fertilizer application rates. Therefore, agricultural residue removal rates must maintain soil quality and future productivity. Wilhelm’s (2004) review by looking at the potential effects of corn stover removal varied from a requirement to put additional stover back onto the field after the grain is harvested to the acceptability of removing 80% of the stover from the field. Many studies have indicated an allowable removal of 30% to 50% through reduced tillage practices (Aden et al., 2002; Sheehan et al., 2002; Wilhelm et al., 2004).

Additional infrastructure is needed for collection and baling of these residues (Blanco-Canqui, 2010). The distribution cost is high due to their low density. However, these feedstocks are easier to convert compared with the woody biomass due to their lower lignin content. Some desirable characteristics of feedstock and feedstock supply chains are listed in Table 2. Unfortunately, not all these characteristics are demonstrated by any cellulosic feedstocks. Typically, the feedstock and logistics cost constitutes about 35% to 50% of the total production cost of ethanol (Hess et al., 2007; Sokhansanj and Fenton, 2006). The actual percentage depends upon biomass species, yield, location, climate, local economy, and the type of systems used for harvesting, processing, storing, and transporting of biomass as a feedstock.

Table 2. Desirable properties of cellulosic feedstock and supply chains.

Desirable cellulosic feedstock properties	Desirable supply chain properties
Lower cost	Lower transportation cost
Price stability	Multiple markets available
Consistent composition	Uniform feedstock
Easily stored	Provides local economic opportunities
Dense or easily densified	Satisfies local and global environmental criteria
Not competitive with food crops	
Potential for coproduct generation	

(Source: Eranki et al., 2011)

Corn stover as a biomass feedstock

Corn stover accounts for more than half of available agricultural residues in the U.S. Corn stover is seen as a potential feedstock for a cellulosic ethanol industry as it is readily available, has a lower cost, and can be located in a region within an existing ethanol industry. Corn stover is defined here as the above ground portions of the corn plant including stalks, leaves, and husks but excluding the corn cobs and kernels. The biomass ratio of corn kernels to corn stover is typically 1:1 on a dry basis (Sheehan et al., 2002). As the productivity of corn stover is based on corn grain production, stover yields can be estimated by future corn grain projections. Using this ratio, approximately 359 millions Mg of corn stover was produced in 2014. In spite of large quantities potentially available corn produced, only 6% of stover is collected for animal feeding and bedding, but mostly left in the field (Sokhansanj et al., 2002). Although significant quantities of residue are produced, how much of this residue can be sustainably collected has been subject to debate.

The utilization of available feedstock is subdued by the difficulties of collection and distribution of the biomass. Currently, a single combine pass is used to collect the entire corn plant harvesting the corn kernels and returning the stover back onto the field. Farmers try to minimize the number of passes to preserve topsoil. For stover collection, another piece of equipment would have to be used in the field, which might disrupt the topsoil. A better option would be development of machinery that could collect corn grain and stover, simultaneously. After collection, stover can be baled and stored or transported to ethanol facility.

Biomass composition

Lignocellulosic biomass is composed of a mixture of primarily carbohydrate polymers (cellulose and hemicellulose) and lignin. This chemical composition of the lignocellulosic biomass and oxygenated hydrocarbons is somewhat similar to fossil fuels. This similarity enables the conversion of biomass into transportation fuels, and chemicals. Cellulose and hemicellulose are the most abundant organic sources of food, fuel, and chemicals (Ingram and Doran, 1995). They have a high potential to be converted to valuable fuels and chemicals, but are much more difficult to convert than sugars and starches. In order to efficiently convert the carbohydrate polymers into the desired products, it is important to understand the composition, nature, structure and interactions of these cell wall components and how they affect the degradability of lignocellulosic biomass.

Biomass composition varies with type of feedstock, the growth conditions, soil, climate and fertilizer used as well as harvest time (Pordesimo et al., 2005; Sun and Cheng, 2002). Table 3 shows the average composition of potential lignocellulosic feedstock and their composition (Limayem and Ricke, 2012; Sun and Cheng, 2002).

Table 3. Potential lignocellulosic biomass source and typical composition (% of dry weight).

Feedstock	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Agricultural residues	37–50	25–50	5–15
Hardwoods	40–55	24–40	18–25
Softwood	45–50	25–35	25–35
Grasses	25–40	35–50	10–30
Waste papers	60–70	10–20	5–10
Newspaper	40–55	25–40	18–30

(Source: Sun and Cheng, 2002)

Cellulose

Cellulose is a polymer of glucose molecules $(C_6H_{10}O_5)n$, and is the main component of plant cell walls. In nature, cellulose is usually associated with other polysaccharides such as xylan or lignin. It is the skeletal basis of plant cell walls (Holtzapple, 1993a).

The main advantage of cellulose as a feedstock is its abundance and the competitive price of this feedstock. Cellulosic biomass includes forestry residues (sawdust, forest thinnings, and mill wastes), agricultural residues (corn stover, wheat straw, sugarcane bagasse), waste paper, grasses, and woody crops. In corn stover, cellulose makes up approximately 36% to 42% of the dry weight (Öhgren et al., 2007; Wyman, 1996). Cellulose is a linear polysaccharide of glucose residues connected with β-1,4 linkages. Cellulose molecules have a strong tendency to form intramolecular and intermolecular hydrogen bonds. Bundles of cellulose molecules are thus aggregated together in the form of microfibrils, in which highly ordered (crystalline) regions alternate with less ordered (amorphous) regions (Nishiyama, 2009). The average crystallinity of cellulose in biomass ranges from 50% for corn stover to 80% for cotton (Gupta and Lee, 2009), measured using X-Ray diffractometer (Segal et al., 1959). Native crystalline cellulose is insoluble and occurs as fibers of densely packed, hydrogen-bonded, anhydro glucose chains up to 10,000 glucose units. Its density and complexity resists hydrolysis without any chemical or mechanical pretreatment. Cellulose can be converted to glucose through chemical or enzymatic hydrolysis. Cellulase enzyme can break cellulose to cellobiose unit, and ultimately to glucose, which can be used as a building block in the biorefinery to produce biofuel or biobased chemicals.

Hemicellulose

Hemicellulose is the second most abundant material from plants, which consists of highly branched chains of heterogenous sugars. The branched structure of hemicellulose makes it non-crystalline, amorphous, and relatively easy to hydrolyze to its constituent sugars. Hemicellulose is somewhat easily hydrolyzed at high temperatures (160−180 °C) and under mild acidic conditions. It contains mainly five-carbon sugars (pentoses: xylose, arabinose), a small amount of six-carbon sugars (hexoses: galactose, glucose, and mannose), and uronic acid (Holtzapple, 1993b). Softwood hemicelluloses have higher mannose content while hardwoods and agricultural and herbaceous hemicelluloses contain mainly xylose. The amount of hemicellulose in corn stover is usually in the range of between 20% and 30% (Öhgren et al., 2007; Wyman, 1996). Xylose is not readily utilized as glucose for ethanol production using *Saccharomyces* *cerevisiae*. Yeasts such as *Pichia*, *Klyveromyces*, *Pachysolen* are able to ferment xylose to ethanol.

Lignin

Lignin is the major non-carbohydrate and non-fermentable part of plant material. Lignin acts as a net or glue around fibers and in spaces between cell walls. Therefore, the structural units are highly cross-linked which leads to an amorphous structure (Holtzapple, 1993c). Lignin forms a hydrophobic sheath to make it more resistant to microbial and chemical attack. The presence of lignin directly hinders enzymatic hydrolysis of polysaccharides as it fills spaces in the plant cell wall, thus preventing the enzymes from accessing the polysaccharides. Lignin is very recalcitrant to biochemical conversion and also binds and inactivates the enzymes used to process the molecules. Lignin can be hydrolyzed with alkaline reagents such as NaOH, $Ca(OH)_2$, and NH_3. Alkyl-ether linkages, are the most dominant in the lignin structure, the second most prevalent linkages are alkyl-alkyl (C–C). Lignin has a higher heating value than carbohydrates, so it can be used to produce heat and electricity to support the overall biomass-to-fuel conversion process. The utilization of the lignin as a heat and power source also reduces the fossil energy consumption and GHG emissions compared with corn grain ethanol, which typically uses natural gas for energy source.

Feasibility and impact of the new renewable fuels bill

The Energy Independence and Security Act (EISA) was passed in 2007 established GHG emission thresholds for each renewable fuel (i) any renewable fuel: 20% reduction;(ii) advanced biofuel: 50% reduction; (iii) cellulosic biofuel: 60% reduction. A new renewable fuels standard (RFS2) was passed in 2007 increasing the alternative fuels production target from 28 hm^3 (7.5 billion gallons) to 136 hm^3 (36 billion gallons) annually by 2022 (EISA, 2007). Specifically, the law mandates that the U.S. must produce 60 hm^3 (16 billion gallons) of cellulosic biofuels, along with 57 hm^3 (15 billion gallons) of conventional corn-based ethanol, 4 hm^3 (1 billion gallons) of biodiesel, and 15 hm^3 (4 billion gallons) of advanced biofuels annually by 2022. However, EPA is not aggressively enforcing the mandates. Since 2010, the production of biofuels continues to fall short of yearly standards set by EPA (Irwin and Good, 2016).

Currently, commercial scale production of cellulosic ethanol is still not economical. It is expected that an agricultural residue, like corn stover, will be utilized as an initial cellulosic feedstock. This is because stover is already centrally located within the Corn Belt, near current existing ethanol production and distribution infrastructure. Utilizing an agriculture residue also lowers the risk for cellulosic ethanol producers, as that feedstock is already available. This bill is seen as a way to promote the additional development of biofuels, and specifically second-generation cellulosic-based biofuels, as a way to decrease the nation's petroleum consumption and greenhouse gas emissions. However, some constraints such as E10 market saturation and infrastructure development would affect ethanol's potential scale of production. Therefore, additional markets for increased levels of ethanol need to be established in the future, such as flex-fuel vehicles.

The potential for achieving cellulosic biofuel targets mainly depends upon feedstock availability and cellulosic conversion efficiency. Other key challenges are improving cellulosic ethanol conversion rates through superior enzymes and yeast, improving economic constraints for pretreatment and synergizing different steps to enable the scale-up production.

Logistics

The effectiveness and feasibility of cellulosic biomass as an energy source for ethanol production is limited by the current harvesting/processing equipment, transportation system, and storage systems that we currently have available for on-the-farm use. Hess et al. (2007) stated that feedstock production and logistics constitute 35% to 50% of the total production costs of cellulosic ethanol. The actual percentage depends upon geographical factors such as biomass species, yield, location, climate, local economy, and the types of systems used for harvesting, collection, processing and transportation. The low bulk densities that bales and ground biomass have made it difficult to handle and transport the large quantities required for commercial biofuel production. By increasing the bulk densities of cellulosic biomass, it positively influences the flow characteristics and allows for easier handling. This also facilitates maximizing the payload that can be hauled by tractor/trailers or railcars.

The physical properties of ground biomass prevent it from flowing properly during the unloading, storage, and transfer operations at a biorefinery. These flow characteristics would require biorefineries to install specialized equipment and would make retrofitting existing corn based ethanol plants into cellulosic ethanol plants almost impossible. Conveying equipment is capable of moving specific volumes of material. If a low-bulk density product was introduced into the flow, the performance of all downstream equipment would be affected and plant throughput would be drastically lowered. Other costs associated with handling a low-bulk density feedstock include the additional conveyor capacity and storage facilities in order to handle light material. The added cost of the new equipment could be reduced or eliminated if the bulk density of the feedstock could be increased prior to delivery (Mani et al., 2006).

Another limitation of biomass transportation is the inability to maximize payload due to the bulk density. Tractor/trailers are regulated based on volume and weight. Biomass will take the volume, but will not get the weight in order to haul the most material possible. Increasing the bulk density through additional processing can have a significant impact on the bottom line and increase the distances we can afford to transport product.

A pilot study (Hess et al., 2006) of a straw-based ethanol plant, which will consume 800,000 Mg yr^{-1} of wheat and barley straw reported that 80% of the feedstocks available within a 160 km radius of the plant must be delivered to the plant. To reduce transportation costs, 76% must come from within 80 km of the plant, 17% from 80–120 km of the plant, 5% from 120–160 km of the plant, and 12% from greater than 160 km of the plant to supply 110% of demand to make sure no shortage of supply. If transportation costs can be reduced by increasing feedstock density, it is possible that these percentages can be reduced and the radius that feedstocks are drawn from can be increased to ease the pressure on fields near the biorefinery to supply feedstocks.

Cushman et al. (1995) observed several goals, limitations and research needs in order for biomass-based feedstock's to be feasible. Most limitations were associated with harvesting, preprocessing, transporting, and handling of the feedstock's since the current forage technology is not capable of producing and efficiently transporting the 800,000 to 1,000,000 Mg of feedstock's annually of the industrial scale biorefinery. The strategic goals of increasing efficiency by utilizing existing transportation infrastructure, demonstrating cost effective storage systems for megagram quantities, and increasing biomass value at every stage of the feedstock chain can be addressed with pelleting biomass. If biomass is pelleted, it can be handled and transported with grain handling equipment on the road and at the biorefinery.

Factors of corn stover ethanol scale of production

Biomass availability, technological feasibility, economic viability, development, and synergy of industries, the environmental impact will need to be considered to overcome some barriers for biofuel production.

Preprocessing of lignocellulosic biomass

The collected biomass is transported to a preprocessing depot for storage or preprocessing. Preprocessing of biomass may include one of some or combination of size reduction, fractionation, sorting, and densification (Sokhansanj and Fenton, 2006).

Densification

Densification is one way to increase energy density and overcome handling difficulties of loose biomass. Through various densification technologies, raw biomass is compressed to densities on the order of 7 to 10 times of its original bulk density (Demirbas and Sahin-Demirbas, 2009). Conventional processes for biomass densification can be classified into baling, pelletization, extrusion, and briquetting (Tumuluru et al., 2010). Pelletization and briquetting are the most common processes used for biomass densification for solid fuel applications (Tumuluru et al., 2011).

Pelleting is a traditional form of densification; pelletization process transforms finely ground biomass into dense and durable pellets. A pellet mill comprises of a perforated hard steel die with up to three rollers in industrial scale. A pellet die is used for pressing and can be either a flat die or a ring die. The feedstock is forced through the perforations by the stationary die and rotating rollers to form free-flowing densified pellets. Pellets consist of uniform product characteristics in terms of size, shape and densities (Tumuluru et al., 2010). Pellets are easier to handle than other densified biomass, since the infrastructure for grain handling can be used for pellets. However, pelletization requires high capital investment, as well as high energy inputs. A pellet mill of 400 hp motor could process about 4 to 5 Mg h^{-1} of biomass (Rijal et al., 2012). The most densification literature focuses on understanding the mechanism and quality attributes of the process (Adapa et al., 2002; Li and Liu, 2000). However, the effects of densification and feedstock variables on the biochemical conversion need to be explored to find the synergistic effect.

Processing of the biomass: pretreatment

Effective pretreatment is fundamental for successful hydrolysis and downstream operations (Wyman, 1994). Pretreatment operations include mainly physical, (i.e., biomass size reduction) and thermochemical processes that involve the disruption of the recalcitrant structure of the biomass. Therefore, it increases exposure of cellulases to cellulose surface area to increase hydrolysis with minimal energy consumption and a maximal sugar recovery (Yang and Wyman, 2008; Zheng et al., 2009).

For biofuel production, lignocellulosic biomass poses challenges for suitable economical pretreatment processes, as it requires costly pretreatment process with acid or base and then enzymatic hydrolysis to depolymerize the carbohydrate to sugars. Numerous pretreatment strategies have been developed to enhance the reactivity of cellulose and hemicellulose to efficiently hydrolyze them to fermentable sugars. The physical, chemical, and morphological characteristics of lignocellulose are important to the digestibility of the substrate. Pretreatment changes these characteristics of biomass, leading to enhanced enzyme accessibility (McMillan, 1994). Different pretreatments rely on different mechanisms for lowering the recalcitrance of the substrate. Physical pretreatments reduce the biomass particle size and cellulose crystallinity in order to increase the specific surface area and reduce the degree of polymerization. Dilute acid pretreatments leads to removal of hemicellulose by partially hydrolyzing and solubilizing hemicelluloses while alkaline pretreatments like ammonia are more effective for lignin solubilization or redistribution. Both approaches have been proven to be effective in increasing cellulose hydrolysis (Chang and Holtzapple, 2000; Öhgren et al., 2007).

The primary objective of pretreatment is to increase enzyme efficacy. Pretreatments disrupt this protective lignin and hemicellulose layer of plant cell wall and allow the enzymes access to cellulose for hydrolysis (Sun and Cheng, 2002). Pretreatment plays a central role affecting the design and economic viability of a lignocellulosic biorefinery as the choice of pretreatment affects all biorefinery operations as shown in Figure 2. Thus, pretreatment is considered one of the most crucial steps in bioethanol production (da Costa Sousa et al., 2009). Pretreatment processes are generally capital intensive and are estimated to represent about 18% to 20% of the total cost of a biorefinery (Yang and Wyman, 2008). Pretreatment costs are estimated at approximately \$0.08 L^{-1} ethanol for cellulosic ethanol production (Mosier et al., 2005b). Pretreatment technology is therefore an active area of research to drive down pretreatment costs and energy consumption by identifying and applying new techniques (Banerjee et al., 2010; Tao et al., 2013).

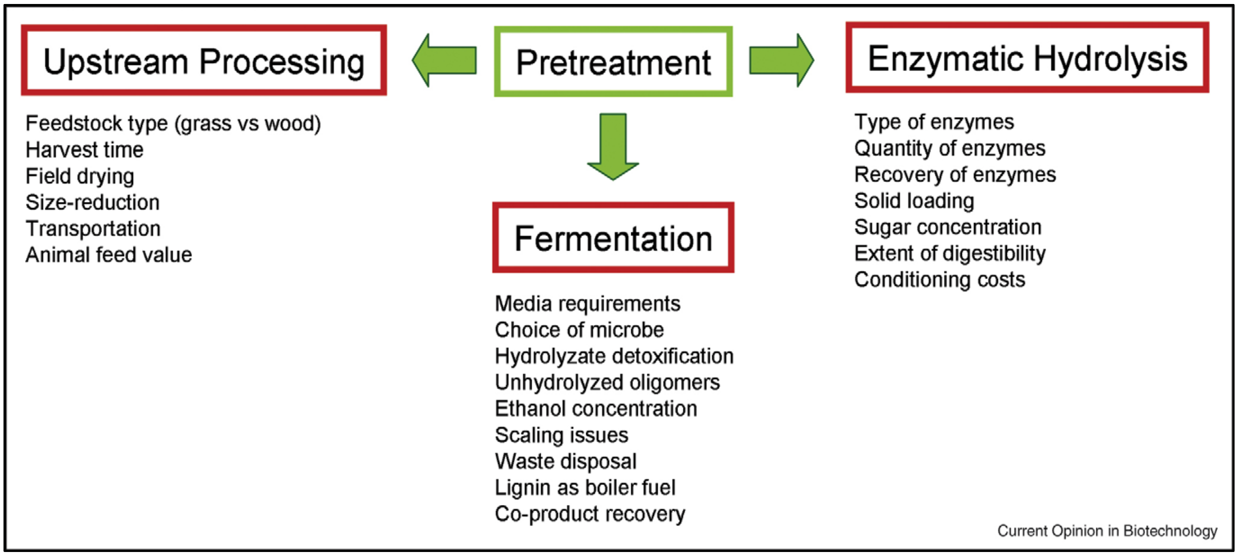

Figure 2. Choice of pretreatment affects all biorefinery related operations (Source: DA Costa Sousa et al., 2009).

In general, any pretreatment must have the following attributes (Alvira et al., 2010; Yang and Wyman, 2008):

- Efficient utilization of sugars from cellulose and hemicellulose in the process with no or little generations of inhibitors.
- Efficient enzymatic hydrolysis and fermentation to increase biofuel yield from hexoses and pentoses at low enzyme cost.
- Integrated process to minimize the use of water, chemical reagents, and energy.
- Efficient use of lignin or other byproducts. Lignin can be used for heat and hemicellulose can be used for production of value-added products.

Pretreatment affects many characteristics of the plant material that impede digestion including cellulose crystallinity, lignin content, acetyl linkages, and the complex hemicellulose-lignin shield that surrounds cellulose in the plant cell wall. For industrial applications, a pretreatment must be effective, economical, safe, environmentally acceptable, and easy to use.

All pretreatments cause one or more of the following changes in biomass (Yang and Wyman, 2008):

- Size reduction
- Degradation of one or more of the main components of biomass– cellulose, hemicellulose, or lignin
- Increase in surface area and porosity of biomass
- Change in crystallinity of cellulose

Often, pretreatment is a balancing act of improving digestibility while minimizing hemicellulose loss and inhibitor formation. Pretreatment technology that use less severe conditions in terms of time and temperature and/or allow the utilization of lignin co-products are likely to have the lowest net pretreatment costs.

Methods of pretreatment

Many different pretreatment technologies have been suggested during the last decades. The pretreatment methods can be classified into different categories according to various criteria (Alvira et al., 2010; Hendriks and Zeeman, 2009). They can be classified according to the different forces or energy consumed in the pretreatment process such as biological, physical, chemical, and physico-chemical pretreatments or even combinations of these methods. Another way to differentiate pretreatment methods is based on high, low, or neutral pH (Xu and Huang, 2014). Principally, low pH methods require addition of acids to increase the hydrolytic capacity while higher pH methods need pH-adjusting agents such as sodium hydroxide or ammonia. The neutral pH methods, mainly liquid hot water (LHW) pretreatment, simply need water in the process. However, the substrate medium even in the neutral methods is weakly acidic due to the release of organic acids from the biomass during the pretreatments. Table 4 summarizes major typical process conditions of pretreatment methods based on pH and how it affects the structure.

Table 4. Typical process conditions of selective pretreatment.

Conditions	DA	LHW	SAA
Temperature (°C)	160–220	160–240	30–75
Pressure (MPa)	Saturated vapor	Up to 5	-
Reagent concentration (%)	0.05–5	-	15–30
Residence time	1–60 min	12–60 min	hour to weeks
Effects on the structure:			
Increases accessible surface area	High	High	High
Hemicellulose solubilization	High	High	Low
Lignin removal	Medium	Low	Medium
Lignin structure alterations	High	Medium	High

DA: dilute acid; LHW: liquid hot water; SAA: soaking in aqueous ammonia

Physical methods

Physical changes of biomass can be done by chipping, grinding, or milling depending on the final particle size (10–30 mm after chipping, 0.2–2 mm after grinding and milling) (Sun and Cheng, 2002). Mechanical comminution reduces the particle size and cellulose crystallinity of lignocellulosic biomass in order to increase the specific surface area. Dasari and Berson (2007) studied the effect of varying initial particle size on the enzymatic hydrolysis rate of saw dust. The study showed that particle sizes in the 33 µm to 75 µm range compared with 590 µm to 850 µm range had 50% higher sugar yields. Yeh et al. (2010) found that the production of cellobiose was increased 5-fold during enzymatic hydrolysis when microcrystalline cotton cellulose was reduced to submicron scale. Reduction in the particle size will increase the biomass surface to volume ratio (Mansfield et al., 1999) or reduce the cellulose crystallinity (Chang and Holtzapple, 2000) thus improving the enzyme accessibility to cellulosic fibers.

Acidic pretreatment

The acidic pretreatments include dilute acid pretreatment (DAP), steam explosion pretreatment (SEP), and organosolv pretreatment. Figure 3 illustrates the process diagram of acidic pretreatment. Acid treatments are more effective for hemicellulose solubilization. Diluted or concentrated acids are used for the process, but the concentrated acid is less preferred because of high operational and maintenance costs and production of fermentation inhibitors (Wyman, 1996). Acid pretreatment has been successful at acid concentrations below 4%, performed over a temperature range of 120 to 210 °C, with a residence time from several minutes to an hour using a wide variety of lignocellulosic biomass (Kim et al., 2011; Taherzadeh and Karimi, 2007; Wyman et al., 2011). The most widely used DAP is based on dilute sulfuric acid since it is inexpensive and effective (Kim et al., 2011). However, nitric acid, phosphoric acid, hydrochloric acid have also been used (Israilides et al., 1978; Mosier et al., 2005a). Wheat straw pretreated with 0.75% v/v of H_2SO_4 at 121 °C for 1 h resulted in a saccharification yield of 74% (Saha et al., 2005). Similar results of 76.5% hydrolysis yield were seen in olive (*Olea europaea* L.) tree biomass at 1.4% H_2SO_4 and 210 °C for 10 min (Cara et al., 2008).

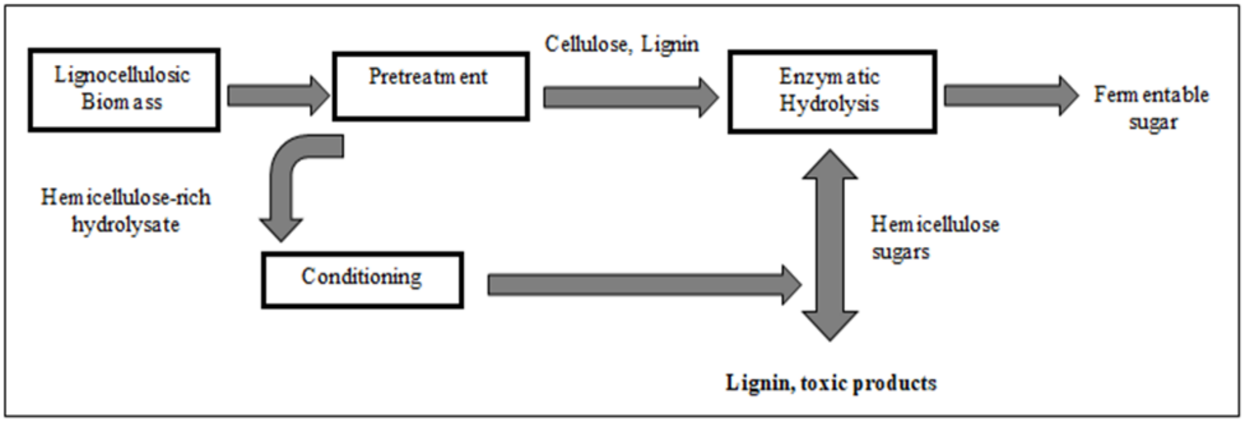

Figure 3. Process diagram of dilute acid pretreatment of biomass for sugar production.

In the DAP, the combined severity factor (CSF) which relates to the experimental effects of temperature, residence time, and acid concentration is used for an easy comparison of pretreatment conditions and for facilitation of process control (Hu and Ragauskas, 2012; Lloyd and Wyman, 2005). Combine Severity Factor was defined as equation (1) according to Lloyd and Wyman (2005):

$$CSF = log\{t \times exp[(TH - TR)/14.75]\} - pH \tag{1}$$

where, *t* is reaction time in minutes, *TH* is the pretreatment temperature in °C, and *TR* is the reference temperature in °C.

The pH is the acidity of the diluted acid solutions, determined by acid concentration. Lower CSF is beneficial for the hemicellulose to hydrolyze to oligomers and monomers while higher CSF could further convert these monomers to furfurals and 5-hydroxymethylfurfural (HMF), which are inhibitors for the subsequent enzymatic hydrolysis (Liu et al., 2005).

During DAP pretreatments, an increase of cellulose crystallinity in biomass was reported (Foston and Ragauskas, 2010; Kumar et al., 2009; Xu and Huang, 2014). Foston and Ragauskas (2010) suggested that the majority of the increase of cellulose crystallinity is primarily due to localized hydrolyzation and removal of cellulose from the amorphous regions. Dilute acid pretreatment not only alters the lignocellulosic biomass chemical structures but also changes the anatomical structure of plant cell wall, especially the pore structures (Yang and Wyman, 2006). Several studies have indicated that the breakdown and loosening of the lignocellulosic structure by DAP increases the specific surface area, pore volume, and pore size of the biomass (Foston and Ragauskas, 2010; Yang and Wyman, 2006).

Steam explosion is another common pretreatment for lignocellulosic biomass. It is a hydrothermal pretreatment where biomass is subjected to pressurized steam for a shorter period, and then suddenly depressurized which causes hemicellulose solubilization and lignin redistribution (Alvira et al., 2010). The mechanical effects of a sudden decompression aid separation the fibers in the biomass. Addition of an acid catalyst such as SO_2 or H_2SO_4 to steam explosion significantly increase its hemicellulose sugar yields (Wyman et al., 2005).

In the organosolv pretreatment, numerous organic or aqueous solvent mixtures such as methanol, ethanol, acetone, ethylene glycol, and tetrahydrofurfuryl alcohol can be used in order to solubilize lignin and hemicellulose, providing treated cellulose suitable for enzymatic hydrolysis (Zhao et al., 2009). The preferred conditions of the organosolv process are generally in the temperature of 180 to 195 °C, for 30 to 90 min. The pH of the liquor ranges from 2 to 4. These mixtures are combined with acid catalysts (HCl, H_2SO_4) to break hemicellulose bonds. Generally, in the organosolv pretreatment, high lignin removal (>70 %) and minimum cellulose loss (less than 2%) can be achieved (Alvira et al., 2010).

Neutral pretreatment

Liquid hot water (LHW) is another hydrothermal pretreatment, which is generally regarded as a neutral pretreatment since the water (pH neutral) is used as pretreatment media. However, water at high temperature acts as an acid because dissociation constant of water changes which increases $[H^+]$ and lower the pH. Liquid hot water does not require rapid decompression and does not require any chemicals or catalyst. Liquid hot water treatment is basically cooking of biomass with the temperature in the range of 160 to 240 °C and pressure higher than 5 MPa (Sánchez and Cardona, 2008; Yu et al., 2010). Pressure is applied to maintain water in the liquid state at elevated temperatures and provoke alterations in the structure of the lignocellulose. This pretreatment enlarges the accessible surface area of the biomass by solubilizing the hemicellulose (Zeng et al., 2007). Liquid hot water has been shown to remove up to 80% of the hemicellulose and to enhance the enzymatic digestibility of corn stover (Mosier et al., 2005a).

During LHW, water acts as a weak acid and releases the hydronium ion, which causes depolymerization of hemicellulose. Similar to the DAP, studies have reported that the crystallinity index of cellulose increased after LHW pretreatment because the amorphous cellulose is more reactive than crystalline cellulose (Yu and Wu, 2010). The degradation product of furfural and HMF may form during LHW pretreatment but quantities are lower than DAP and would not significantly inhibit the fermentation process if LHW pretreatment is performed under 220 °C (Xu and Huang, 2014).

Alkaline pretreatment

Alkaline pretreatment is another major chemical pretreatment technology besides acidic pretreatment. Alkaline pretreatment usually refers to the application of alkaline solutions to remove lignin and various uronic acid substitutions on hemicellulose (Chang and Holtzapple, 2000). Alkaline pretreatments are more effective for lignin solubilization or redistribution with minimal losses of cellulose and hemicellulose. Alkaline pretreatment efficiencies are mainly affected by reaction temperature, pretreatment time, and alkali loading (Hu and Ragauskas, 2012). Figure 4 illustrates the process diagram of alkaline pretreatment of biomass for sugar production. Generally, alkaline pretreatment is more effective on agricultural residues and herbaceous crops than on woody biomass (Hsu, 1996). These pretreatments are often operated under more moderate conditions of temperature and pressure, but the pretreatment time may span from several hours to days (Kumar and Wyman, 2009; Mosier et al., 2005b). Hydroxides of Na, K, Ca, and NH_4 (ammonium) are the most commonly used reagents for alkaline pretreatments. This form of pretreatment causes less sugar degradation than acidic pretreatments (Kumar and

Wyman, 2009). Alkaline pretreatments disrupt the cellulose structure of biomass by swelling. The swelling and delignification together increases the surface area of cellulose and decreases the degree of crystallinity and polymerization (Taherzadeh and Karimi, 2008). Many researchers have found that the crystallinity of cellulose increased after alkaline pretreatment (Kumar et al., 2009; Wu et al., 2011), which is probably due to the removal of the amorphous components by alkali.

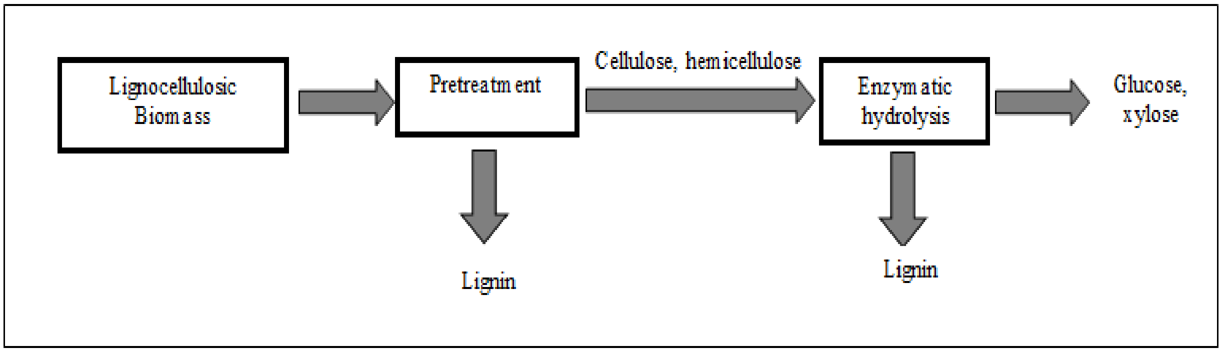

Figure 4. Process diagram of alkaline pretreatment of biomass for sugar production.

Sodium hydroxide and lime pretreatments have been shown to effectively enhance cellulose digestibility. Sodium hydroxide and lime pretreatments are basically a delignification process in which a significant amount of hemicellulose is solubilized as well. The major effect is the removal of lignin from the biomass, thus improving the reactivity of the remaining polysaccharides (Xu and Huang, 2014). In comparison with other pretreatment technologies, sodium hydroxide and lime pretreatments usually use lower temperatures and pressures. However, pretreatment time is much longer (weeks) than other pretreatment processes (Alvira et al., 2010).

Ammonia fiber explosion (AFEX) is another process where biomass is treated with liquid anhydrous ammonia at temperatures between 60 and 100 °C and high pressure for a variable period. The pressure is then released, resulting in a rapid expansion of the ammonia gas that causes swelling and physical disruption of biomass fibers and partial decrystallization of cellulose. Ammonia fiber explosion has been reported to decrease cellulose crystallinity and disrupt lignin–carbohydrates linkages (Alvira et al., 2010). While steam explosion and LHW produces slurry that require separation in a solid and a liquid fraction, AFEX produces only a pretreated solid material since the ammonia is released as a gas.

Soaking in aqueous ammonia (SAA) is an attractive pretreatment technique for agricultural residues (Kim et al., 2008; Ko et al., 2009). Soaking in aqueous ammonia is an interesting alternative among alkaline pretreatment since it is performed at near ambient temperature and pressure. The main purpose of SAA-pretreatment is the removal of lignin (Ko et al., 2009). It is regarded as a valuable pretreatment methodology due to the retention of the hemicellulose and removal of lignin (Kim and Lee, 2007). Soaking corn stover in aqueous ammonia pretreatment of at room temperature for 10 to 60 days resulted in 55% to 74% delignification, retaining 100% of the glucan (polysaccharide of D-glucose monomers) and 85% of the xylan (polysaccharide, that hydrolyzes to xylose) (Kim and Lee, 2005).

Cellulose molecules are made of long chains of glucose molecules. In hydrolysis, these long chains of polysaccharides are cleaved to release individual sugar monomers, before they are fermented for fuel or chemical production. Cellulose is completely hydrolyzed to glucose while hemicellulose hydrolysis results in the formation of a mixture of pentoses and hexoses. Although acid hydrolysis is possible and requires no pretreatment, pretreatment followed by enzymatic hydrolysis is a common approach because of cost projections. Enzymatic hydrolysis has advantages of producing relatively non-toxic hydrolyzates with higher sugar yields.

Enzymatic hydrolysis

Enzymatic hydrolysis of cellulose is done with specific cellulase and hemicellulase enzymes under mild conditions of temperature (40–50 °C) and pH (4–4.8) (Béguin and Aubert, 1994). Cellulase is a group of enzymes that synergistically hydrolyzes cellulose (Reczey et al., 1996). Cellulases are classified into three major classes: endoglucanases (endocellulase), exoglucanases (exocellulase), and β-glucosidases (cellobiase). Endoglucanase randomly attack the amorphous region of cellulose and breaks cellulose chains in internal, amorphous regions, or on the surface of microfibrils to give shorter chains with more chain ends. Endoglucanases reduce the degree of polymerization to increase the number of free chain ends. Exoglucanase cut these chains from ends to yield glucose dimers (cellobiose). Finally, β-glucosidases hydrolyse cellobiose to two glucose units. All these enzymes work synergistically to hydrolyse cellulose by creating new accessible sites for each other, removing obstacles and relieving product inhibition (Eriksson et al., 2002).

The enzymatic hydrolysis of cellulose is known to be affected by both enzyme and substrate-related factors (Chandra et al., 2007; Mansfield et al., 1999). Therefore, efforts to reduce the cost of enzymatic hydrolysis have focused on both factors (Mansfield et al., 1999; Sheehan and Himmel, 1999). The U.S. Dept. of Energy has funded recent research to decrease the cost of the enzymes, increase their specific activities and broaden the substrate range on which the enzymes are being evaluated. It has been reported that an approximate three-fold enzyme cost reduction (from \$0.08 to \$0.03 L^{-1} ethanol) is necessary to reach cost targets for the eventual commercialization of the bioconversion of pretreated corn stover to ethanol (Aden, 2008).

In addition to enzyme-related factors, substrate-related factors also affect enzymatic hydrolysis. Hydrolysis rates and yields are higher in low substrate concentration (Cheung and Anderson, 1997). However, the rate of hydrolysis is inhibited at high substrate concentration, and the level of inhibition depends on the ratio of substrate to the total enzyme (Huang and Penner, 1991). Increasing the ratio of enzyme loading to substrate concentration could enhance the rate of hydrolysis, but significantly increases the production costs.

It has been reported that the efficient enzymatic hydrolysis of cellulose is determined by several factors such as the specific surface area, pore size, crystallinity, and degree of polymerization of the cellulose (Chandra et al., 2007; Mooney et al., 1999; Shevchenko et al., 2000). Some earlier work using relatively pure cellulosic substrates showed that there was some correlation between crystallinity and the rate of hydrolysis (Fan et al., 1980). However, subsequent work using lignocellulosic substrates, the contribution of other substrate characteristics and components, such as lignin, were found to be just as important (Chandra et al., 2007). Several researchers have found that when all other substrate factors are maintained at a similar level, changes in the crystallinity of lignocellulosic substrates do not have a significant effect on the rate or extent of hydrolysis (Puri, 1984; Ramos et al., 1993).

Other factors influencing the enzymatic hydrolysis are quality of substrate and its concentration, applied pretreatment method, and the operating conditions like temperature, pH, and mixing (Alvira et al., 2010). The structure and location of hemicellulose and lignin can affect the hydrolysis efficiency (Chandra et al., 2007). As hemicellulose can cover the surface of cellulose and prevent the access of cellulases to the cellulose, the removal of hemicellulose may be essential to facilitate the complete cellulose hydrolysis. Hemicellulose removal increases the mean pore size of the substrate and therefore increases the enzyme accessibility.

One of the main restrictions to achieving efficient enzymatic hydrolysis is inhibition by lignin, which limits access to the cellulose, irreversibly binds cellulases, and generally inhibits the action of the cellulase complex (Chandra et al., 2007). However, the content and structure of lignin differs according to the type of lignocellulose and the pretreatment method. It is likely that no matter the type of lignocellulosic substrate or pretreatment process are used in the bioconversion of biomass, the resulting cellulosic substrate will always contain some amount of lignin with varying structures.

Enzymatic hydrolysis of biomass could obtain almost 100% cellulose hydrolysis with relatively non-toxic hydrolyzates. However, some disadvantages of enzymatic hydrolysis are longer period of hydrolysis time, high prices of the enzymes, low specific activity and inhibition of enzymes from released sugars.

Life cycle assessment (LCA)

To assess the feasibility and environmental impact of biofuels production from cellulosic biomass, the bioethanol life-cycle assessment (LCA) can be used to identify main areas where advances would have the greatest impact on the environment.

Life cycle assessment is an analytical tool, which allows users to assess the overall impact of a process or product on the environment. The first LCA study looked at the resource use and environmental impacts related to the packaging of Coca-Cola products in 1969 in terms of the environmental consequences of packaging manufacture, and alternative packaging materials (Heiskanen, 2000). Oil crisis of 1970’s and the environmental debate on waste disposal and packaging are considered the potential drivers behind the LCA (Baumann and Tillman, 2004). In the early 1990s, the United Nations Earth Summit recognized LCA as an environmental tool. International Organization for Standardization (ISO, 14040:1996) defined

LCA as the compilation and evaluation of inputs, outputs, and potential environmental impacts of a product system. The term ‘product’ includes both goods and services (ISO, 2006a). Life cycle assessment helps to quantify the total resource use and environmental effects associated with products throughout their entire life cycle, from raw materials acquisition, through production, transportation, use, reuse, recycle and end of life of the product (ISO, 2006a, Figure 5).

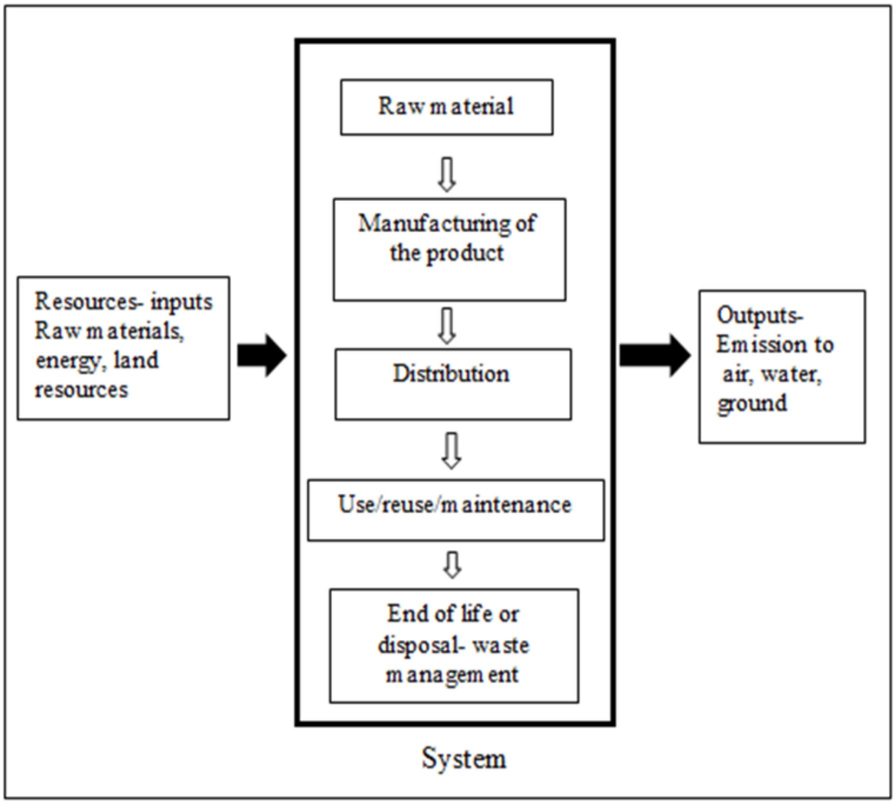

Figure 5. Life cycle stages (Source: EPA, 2006).

According to ISO standard 14040 (ISO, 2006a), LCA can assist in

- identifying opportunities to improve the environmental performance of products at various points in their life cycle,

- informing decision-makers in industry, government or non-government organizations for strategic planning, priority setting, product or process design or redesign,
- selecting relevant indicators of environmental performance, and
- marketing (e.g. making an environmental claim)

Even though LCA allows assessing all the environmental impacts associated with a product or service, it is too complex for direct communication for final consumers. It also needs to be remembered that LCA has a subjective character, so the results are limited to the boundaries used as a basis for decisions. There is an ISO standard for LCA (ISO, 14040:2006 and ISO, 14044:2006), but the variations for conducting a LCA remain significant. The LCA concept is based on the system boundaries and requires a substantial set of data, most of which is highly uncertain. The choice of system boundaries can vary between the technological system and nature, geographical area, time horizon, and especially boundaries among the life cycle of the product studied and related life cycles of other products (Tillman et al., 1994). Depending upon how thorough an LCA should be, gathering the data can be problematic, and the availability of data can greatly influence the accuracy of the results. By doing the uncertainty analysis, the effect of these uncertainties can be minimized. Life cycle assessment does not determine which product or process is the most cost effective or works the best. Therefore, the information developed in an LCA needs to be used as one component of a more comprehensive decision process assessing the trade-offs with cost and environmental performance. Despite the limitations, LCA is one of the most comprehensive tools available now to assess the environmental performance of products or services.

Types of LCA

There are different types of LCA depending on the consideration of life cycle phases (boundary) and intended application (Baumann and Tillman, 2004). Based on the life cycle phases included in the LCA study, the most common LCA are cradle-to-grave and cradle-to-gate. The cradle-to-grave study includes resource extraction (cradle) to use phase and disposal phase (grave) of the product. On the other hand, the cradle-to-gate LCA includes resource extraction (cradle) to the factory gate (i.e., before it is transported to the consumer). GREET (Greenhouse Gases, Regulated Emissions, and Energy Use in Transportation) is a full life cycle model developed by Argonne National Laboratory (GREET 2016), which provide well-to-wheels (WTW) analysis with two stages: well-to-pump (WTP) and pump-to-wheels (PTW). Well-to-pump stages start with fuel feedstock recovery and end with fuels available at refueling stations. Pump-to-wheels stages cover vehicle operation activities.

Depending on the scope of the LCA, there are three general types of LCA studies (Baumann and Tillman, 2004):

1. The accounting type LCA is used to estimate a product's environmental impact, but also to compare different products.
2. The change-oriented type LCA is used for evaluating the best option among different possible scenarios.
3. The stand-alone LCA is the most common type and is conducted before any more detailed studies of a product/system are decided upon. It is an exploratory way to get familiar with environmental characteristics of a product.

A stand-alone, full LCA aims to describe all important environmental effects (e.g., global warming, eutrophication, acidification, human toxicity, resource consumption, biodiversity impacts). However, some studies and regulations concern only the life cycle inventory of GHG emissions associated with a product or process, typically expressed in terms of CO_2 equivalents (CO_2eq), and generally including only emissions of CO_2, CH_4, and N_2O (Delucchi, 2010). These GHG-only analyses are called “carbon footprint” analyses. To calculate a carbon footprint, GHG emission quantities of individual gases are converted to the measurement of CO_2eq using the Intergovernmental Panel on Climate Change (IPCC) 100-year Global Warming Potential (GWP) factors. This allows the potential effect on climate change from different activities to be evaluated on a common basis.

LCA Process

The LCA process is a systematic, phased approach. According to ISO 14044, there are four phases in an LCA study (Figure 6):

1. Goal and scope definition: Under this, life cycle stages, definition of unit processes, identification of the product system boundaries, and environmental effects will be reviewed. This phase defines the “product” (e.g. bioethanol) of the LCA, its functional unit, system boundaries, data collection methods, and the intended audience of the results.

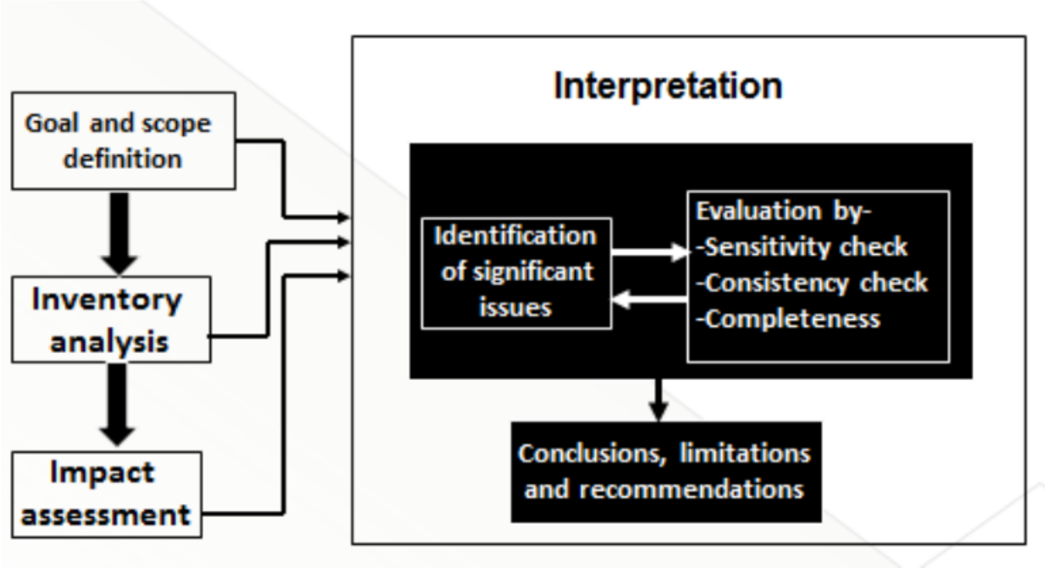

Figure 6. Life cycle assessment framework (Source: ISO, 14040/14044:2006).

2. Inventory analysis: This phase includes: 1) data collection for the background processes (upstream) as defined in the system boundary and, 2) calculation of the amount of resource (input e.g. energy, chemical, water, enzyme etc.) use and pollutant emissions (output) of the system in relation to the functional unit.
3. Impact assessment: This phase assesses potential impact using indicators. The impact assessment phase provides further interpretation of the inventory data. The inventory data are multiplied by characterization factors to give indicators for the environmental impact categories. This phase translates the LCI results (environmental loads) into environmental impacts (Figure 7). The characterization factors are specific for each of the impact category. For example, in global warming potential (GWP) for 100 years, the characterization factor for CO_2 is 1, while for methane the factor is 23, and for N_2O the factor is 298 (EPA, 2015).

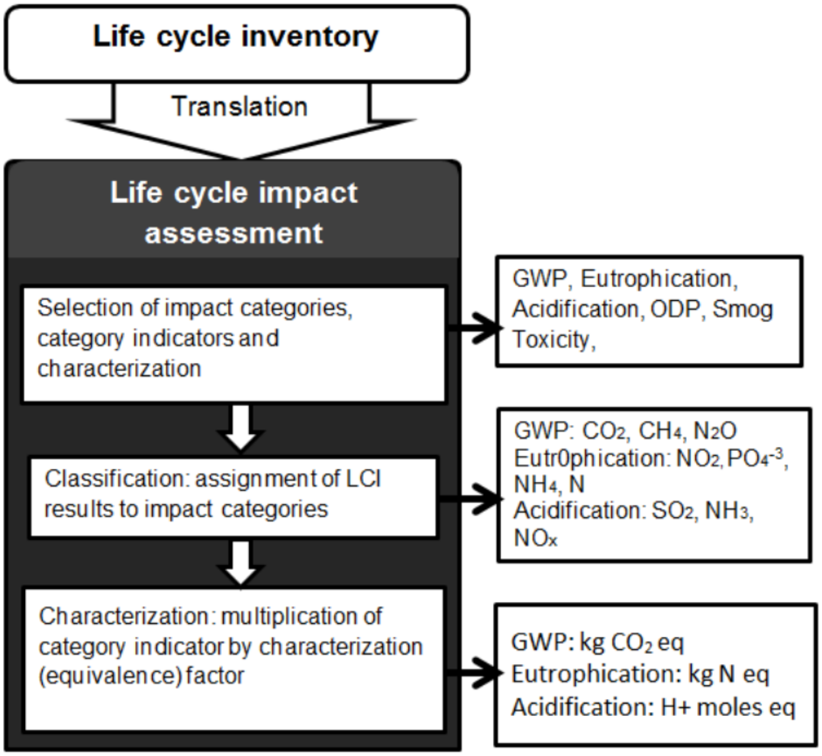

Figure 7. Relationship between inventory analysis and different phases of life cycle impact assessment (Source: ISO 14040/14044:2006).

4. Interpretation: This phase evaluates the results of the inventory analysis and impact assessment to select the product or process with sensitivity analysis and quality check.

Life cycle assessment studies have been conducted on different crops around the world to understand the environmental impacts of bioethanol. A few studies were conducted using a cradle-to-use phase boundary for switchgrass-based biofuels (Bai et al., 2010; Spatari et al., 2005). Bai et al. (2010) found that switchgrass-based bioethanol (E85) performed better in terms of GWP compared with gasoline; but no significant reduction was observed in other impact categories (acidification, eutrophication, and toxicity). Automobiles fueled with switchgrass derived ethanol (E85) showed 57% lower GHG emissions than gasoline (Spatari et al., 2005).

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PROBLEM STATEMENT AND OBJECTIVES

Problem statement

Despite extensive technical advancement of using cellulosic biomass for industrial sugar production, technologies are still under continual evaluation for making the cellulosic conversion process economical. One key route of producing fermentable sugars, which can be used to produce biofuels or biobased products is through biochemical conversion. Cellulosic biomass goes through a pretreatment process to reduce its recalcitrance, and then enzymes are used to hydrolyze structural carbohydrates to monomers. Monomoers can be used as a building block for biofuel or biochemical production. Productivity and efficiency of a biorefinery needs to be increased through optimizing processing operations that lower the overall energy intensity and costs of the biorefinery's unit operations.

Biorefinery processing steps are interconnected and trade-offs of process parameters impact the costs and efficiency of the overall system. The majority of projected production cost of cellulosic ethanol is related to sugars release from cellulose and hemicellulose by the combined effect of pretreatment and enzymatic hydrolysis. Pretreatment is a key cost that accounts for 20% to 30% of total process costs, with enzyme costs typically considered the other significant economic bottleneck (Wooley et al., 1999; Yang and Wyman, 2008). The effectiveness of pretreatment impacts rates and yields from enzymatic hydrolysis, which is a critical factor for the establishment of cellulosic biorefineries. However, enzymatic hydrolysis of biomass for producing sugars is more of an economic challenge. Several studies have focused on optimizing enzyme mixtures for enzymatic hydrolysis following by pretreatment (Pryor et al., 2012; Zhang et al., 2010). Though enzymes play a critical role, other factors, such as pH and temperature, also affects enzymatic hydrolysis rates and yields. Understanding how hydrolysis proceeds across the range of temperature and pH values will allow optimizing enzyme loadings to make biofuels production cost effective.

Establishing a consistent supply of low-cost biomass feedstock is also vital for establishing cellulose-based biorefineries. Due to low biomass bulk densities, transportation energy requirements to deliver baled lignocellulosic biomass to a biorefinery is projected to be about 7 to 26% of total process energy needs compared with just 3% to 5% for transportation of grains and oilseeds (Laser et al., 2009; Richard et al., 2010). The use of baled biomass not only burdens transportation systems, but also leads to enormous challenges for unloading, stacking, storing, and moving massive amounts of biomass at an industrial scale (75–100 MGY) biorefinery. The high transportation, handling, and storage costs for feedstock at the industrial scale may contribute to the delayed development of cellulosic biofuel industries.

Densification of biomass into pellets could be beneficial for both supply systems and the biorefinery itself. Pelleting increases biomass density by about 7 to 10 times and may improve the economics of the cellulosic biofuel production by facilitating improvements for storage, transportation, and handling systems. Previous studies suggest that transportation cost savings for pelleted biomass might be offset by pelleting cost (Sokhansanj and Fenton, 2006; Sultana et al., 2010). However, those studies only considered the cost of transportation and did not account for potential processing synergies of pelleting and pretreatment for biochemical conversion. Even though biomass densification is not a new practice, there is a research gap to understand the interaction between densification and pretreatment efficacy for biochemical conversion.

Pelleting increases biomass bulk density through mechanical and thermal processing. Pelleted biomass will have different processing characteristics than loose biomass as the high temperature and mechanical shearing in pelleting process modifies the feedstock properties, thus affects the key downstream processes such as pretreatment and subsequent enzymatic hydrolysis. Therefore, it is important to understand the inherent link and potential synergies of biomass densification and downstream processing. Limited studies examined the impact of pelleting on biomass pretreatment and bioconversion and indicated that pelleting did not have any negative impact on hydrothermal or dilute acid (DA) pretreatment (Li et al., 2014; Ray et al., 2013; Rijal et al., 2012). For alkali pretreated pelleted biomass, better hydrolysis yields were reported without affecting the quality of sugars for fermentation (Guragain et al., 2013). Moreover, Rijal et al. (2012) reported that pelleting improved the efficacy of soaking in aqueous ammonia (SAA) pretreated switchgrass and therefore suggested that pelleted biomass might allow reduction in pretreatment severity and/or enzyme loadings. Assessment of pretreatment severity reductions when processing pellet is a critical step to identify the economic and environmental benefit of using pelleted biomass for biorefinery.

Literature on the impact of pelleting on different pretreatment process conditions and enzymatic hydrolysis yields is still limited, and reduced severity pretreatments and reduced enzyme loadings in conjunction with pelleting have received little attention (Li et al., 2014; Rijal et al., 2012). Understanding the interaction or trade-offs between densified biomass and downstream processing steps (pretreatment and enzymatic hydrolysis) will help to assess the feasibility of using densified biomass as feedstock in biorefinery.

The goal of this research is to increase economical and environmental sustainability of cellulosic biorefineries by understanding how different process parameters impact the hydrolysis efficiency, quantify the trade-offs between densification and post processing steps, such as pretreatment and hydrolysis to achieve economical sugar production from corn stover. Energy use and greenhouse gas emissions of processing technologies are also dependent on the form of feedstock. Therefore, a life cycle approach is necessary to understand the environmental impact of transporting and processing non-pelleted and pelleted biomass.

Objectives

The objectives for the proposed research are:

Objective 1

To quantify the effect of pH, temperature, and enzyme loadings on enzymatic hydrolysis of soaking in aqueous ammonia (SAA) pretreated non-pelleted corn stover to achieve greater than 90% theoretical yields. An economic analysis will be undertaken to model the most economically efficient processing conditions to compare with conditions based on yield optimization.

Objective 2

To quantify the reduction in SAA pretreatment severity parameters to achieve greater than 90% of theoretical hydrolysis yields from pelleted biomass with reduced enzyme loadings.

Sub-objectives for SAA pretreatment are:

i) To determine the effect of higher pretreatment solid loadings on sugar yields from enzymatic hydrolysis of pelleted corn stover.

ii) Determining the effect of SAA severity [time, temperature, and ammonia concentration] on ultrastructural changes and enzymatic hydrolysis yields of pelleted corn stover with reduced cellulase and hemicellulase loadings.

Objective 3

To quantify differences in energy requirements and GHG emissions for transportation and use of pelleted biomass with low-severity SAA pretreatment in cellulosic biorefinery, compared with transportation and use of baled biomass and conventional SAA pretreatment conditions.

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PAPER 1: PROCESS YIELD AND ECONOMIC TRADE-OFFS FOR ENZYMATIC HYDROLYSIS OF ALKALINE PRETREATED CORN STOVER[1]

Abstract

Response surface methodology was used to investigate the interaction of pH, temperature, and enzyme loadings on corn stover hydrolysis yields following soaking in aqueous ammonia pretreatment. Economic tradeoffs were estimated for cellulase and hemicellulase loadings under different hydrolysis conditions. Enzyme loadings had a more significant effect on yield than did pH or temperature. The effect of hydrolysis pH was independent of temperature and enzyme loadings, and the optimal pH for glucose and xylose yields were 4.5 and 4.3, respectively. Conducting hydrolysis at 50 °C rather than 37 °C enables either a 10% glucose yield increase, or a comparable yield with 40% and 65% reduction in cellulase and hemicellulase loadings, respectively. Although yield models showed that hydrolysis rates increase with higher enzyme loadings, economic models showed that optimal cellulase and hemicellulase loadings were as much as 47% and 23% lower, respectively, than the maximum loadings tested. Optimal enzyme loadings change with fluctuations in enzyme costs and ethanol price, but cellulase loadings were more sensitive to these changes than hemicellulase loadings. Enzyme loadings were also more sensitive to enzyme price at lower processing temperatures. Enzyme loadings can be adjusted to increase return based on enzyme costs, ethanol price, and process temperature.

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Introduction

The enzymatic hydrolysis of cellulosic biomass for producing industrial sugars is a promising strategy for efficient utilization of renewable resources. Cellulase enzymes are the most important for effective enzymatic hydrolysis. Other supplementary enzymes may also be required to aid the hydrolysis depending on the pretreatment technology (Gibson et al., 2011; van Dyk et al., 2012). However, cost reduction by lowering enzyme loading will be critical to make bioindustries more competitive with petrochemical industries (Pryor and Nahar, 2015). Though enzymes play a key role in the hydrolysis process, other factors such as pH, temperature also affect the overall yields. Therefore, developing a model to demonstrate the interactions of enzymatic hydrolysis factors to improve hydrolysis efficiency will have significant economic benefits.

Factors that affect enzymatic hydrolysis of cellulosic biomass includes the amount and type of substrate, enzyme loadings, and reaction conditions (e.g. pH and temperature). The range of pH for commercially available enzymes varies from 2.5 to 6.5, but enzymatic hydrolysis is typically carried out at a pH of 4.8 to 5.0 (Sun and Cheng, 2002). Optimal hydrolysis temperature varies from 45 to 70 °C depending on the enzyme mixture, but the most commonly used temperature is near 50 °C (Sun and Cheng, 2002; Zheng et al., 2013). Enzymatic hydrolysis conditions vary with process configurations such as separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). The temperature for enzymatic hydrolysis can be optimized independently from the fermentation temperature in SHF, whereas compromise is needed for an optimal process temperature in SSF. The use of 37 °C is a favorable condition for SSF, since the yeast (*Saccharomyces cerevisiae*) usually has an optimal temperature around 30 °C and enzymes from *T. reesei* around 45 to 50 °C

(López-Linares et al., 2014). However, there have been few reports of SSF using other strains or organisms with temperature and pH optima closer to those of commercial cellulases. SSF at elevated temperatures were reported for ethanol production using *Candida acidothermophilium* and *Saccharomyces uvarum* (Spindler et al., 1988). Knowing how hydrolysis proceeds across the range of temperatures and pHs can allow mapping of those yields to microbial growth conditions for SSF processes with new organisms.

The primary objective of this study is to model how optimal cellulase and hemicellulase loadings change under a range of hydrolysis conditions (temperature and pH) after alkaline pretreatment. However, processes designed to optimize yield may not generate the greatest economic return. Consequently, we include an economic analysis that combines research-based yield models with select input and output market prices to estimate the most economically efficient processing conditions to compare with conditions based on yield optimization alone. The secondary objective was to determine potential economic tradeoffs between cellulase and hemicellulase enzyme loadings at different hydrolysis conditions.

Materials and methods

Raw material

Corn (*Zea mays* L.) stover (stalks and leaves) was collected from a USDA-ARS research field (46° 48′ 38.51″ N, 100° 54′ 52.53″ W) in Mandan, ND, USA. Corn stover was air dried (10% moisture content, dry basis) and ground in a Wiley Mill with a 6 mm sieve. Sieved corn stover was stored in a sealed plastic bag at room temperature until use.

Soaking in aqueous ammonia (SAA) pretreatment

Biomass was pretreated by soaking in aqueous ammonia with 15% ammonium hydroxide mass fraction at 40 °C for 24 h. Ground corn stover was pretreated at a solid loading of 0.1 g L^{-1}

in a 2-L, screw-capped Pyrex bottle. The pretreatment bottle was placed in a preheated incubator at 40 °C for 24 h after the mixture reached the desired temperature. The pretreated solids were separated by filtering through Whatman # 41 filter paper (20–25 µm pore size) using a vacuum filtration unit. The solids were washed with distilled water (~4 L), weighed, and stored in sealed plastic bags at 4 °C to use for subsequent enzymatic hydrolysis experiments. The moisture content and solid recovery of pretreated biomass were determined in triplicate by drying a small portion of wet solids (~2 g) overnight in a convection oven at 105 °C. A portion of pretreated wet solids (~20 g) was also dried at room temperature for compositional analysis.

Compositional analysis for carbohydrate and lignin determination

Total solids content, structural carbohydrate, and lignin content of non-pretreated raw corn stover and SAA-pretreated corn stover were determined using the standard National Renewable Energy Laboratory Analytical Procedures (Sluiter et al., 2011). It is necessary to remove the non-structural carbohydrates such as nitrites/nitrates, proteins, chlorophyll and waxes from the biomass prior to structural carbohydrate analysis to prevent interference with downstream processing. The extractives were removed from the non-pretreated biomass following the National Renewable Energy Laboratory (NREL) Chemical Analysis and Testing Procedures (Sluiter et al., 2005). All compositional analysis was done in duplicate.

Enzymes

In order to investigate the influence of enzyme concentration on the enzymatic hydrolysis of SAA-pretreated corn stover, commercial enzyme solutions, NS50013 (cellulase complex), and Cellic HTec (hemicellulase) were used. A supplementary cellobiase (β-glucosidase) enzyme, Novozyme 188, was also used as cellulase from *T. reesei* is deficient in cellobiase, restricting the conversion of cellobiose to glucose. All enzymes were provided by Novozymes North America, Inc. (Franklinton, NC, USA). According to the manufacturer information sheet, the optimum temperature for cellulase complex NS50013 is in the range of (45–50 °C), β-glucosidase Novozyme 188 in the range of 45–70 °C and xylanase Cellic HTec operating temperature in the range of 45–50 °C. Regarding operating pH, the range for NS50013 is from 4.5 to 6.5, Novozyme 188 is from 2.5–6.5, and Cellic HTec operating pH is from 4.5–6.0. The cellulase activity of NS50013 and β-glucosidase activity of Novozyme 188, were 77 filter paper units (FPU) mL^{-1} and 500 cellobiase units (CBU) mL^{-1}, respectively, as determined by Ghose (1987). Xylanase activity of Cellic HTec, as determined by Bailey et al. (1992) was 10,600 xylanase units (XU) mL^{-1}.

Enzymatic hydrolysis

Enzymatic hydrolysis was carried out with SAA-pretreated corn stover in 125 mL Erlenmeyer flasks (working volume of 50 mL) with a 1% (w/v) glucan loading. Hydrolysis pH was adjusted between 4 and 5.4 with sodium citrate buffer (50 mM) according to the experimental design. Cellulase and hemicellulase were added on glucan mass basis according to the experimental design. β-glucosidase was added at a constant ratio of cellulase (FPU): β-glucosidase (CBU) at 1:1 across all treatments. Sodium azide was added to the mixture at a concentration of 0.04% to prevent microbial contamination during enzymatic hydrolysis. Flasks were placed in a water bath shaker (MaxQ 7000, Thermo Scientific, Dubuque, IA, USA) and temperature was varied (37–50 °C) based on the experimental design. All flasks were continuously agitated at 130 rpm for 72 h. Aliquots (1 mL) were taken at 24 h intervals from each flask and immediately centrifuged at 13,226 × g for 5 min (Galaxy 16 Micro-centrifuge, VWR International, Bristol, CT, USA). After centrifugation, the supernatant was filtered through a 0.2 µm nylon filter (Pall Corporation; West Chester, PA, USA) and stored at −20 °C until sugar analysis.

High performance liquid chromatography (HPLC) analysis

Hydrolysis samples were analyzed by HPLC (Waters Corporation; Milford, MA) equipped with an autosampler, an isocratic pump, and a refractive index (RI) detector (model 2414, Waters Corporation). The sugars were analyzed using a Bio-Rad Aminex HPX-87P (300 × 7.8 mm) carbohydrate column (Bio-Rad Laboratories; Hercules, CA) and quantified with column and detector temperatures of 85 °C and 50 °C, respectively. The sugars from the injected sample (20 µL) were eluted with 18 mΩ NANO pure water at a flow rate of 0.6 mL min^{-1}. Glucose and xylose were quantified using 4-point external standard curves with mixtures of cellobiose, glucose, xylose, galactose, and arabinose to quantify sugar concentrations in the sample.

Experimental design and statistical analysis

Response surface modeling (RSM) was used to model the effect of pH, temperature, cellulase and hemicellulase enzyme loadings on SAA-pretreated (15% ammonia at 40 °C for 24 h) corn stover hydrolysis yield using a Box-Behnken design of four variables and three levels as defined in Table 5. The ranges of independent variables for cellulase and hemicellulase enzyme were selected based on the results of preliminary experiments (not shown). The lower level of temperature was chosen at 37 °C, typical of the SSF process while the highest level at 50 °C, representing typical optimal for SHF. pH range was selected between 4 and 5.4 as this range was reported to be optimal for all three enzymes (Farinas et al., 2010). The complete design consisted of 27 treatments, including three repetitions at the center point (Table 6).

Table 5. Independent process variables and their levels used in the Box-Behnken experimental design for the SAA-pretreated (15% ammonia at 40 °C, 24 h) corn stover hydrolysis.

Independent variables	Symbol	Factor level		
		Low (-1)	Middle (0)	High (+1)
pH	P	4	4.7	5.4
Temperature (°C)	T	37	43.5	50
Cellulase (FPU g^{-1} glucan)	C	10	20	30
Hemicellulase (XU g^{-1} glucan)	H	0	500	1000

Glucose and xylose yields (% of theoretical) at 24 h of hydrolysis were chosen as representation of hydrolysis rate. The experimental data were analyzed by the response surface regression procedure using the following quadratic polynomial model (equation 2).

$$R = \beta_0 + \beta_P P + \beta_T T + \beta_C C + \beta_H H + \beta_{PP} P^2 + \beta_{TT} T^2 + \beta_{CC} C^2 + \beta_{HH} H^2 + \beta_{PT} PT + \beta_{PC} PC + \beta_{PH} PH + \beta_{TC} TC + \beta_{TH} TH + \beta_{CH} CH \quad (2)$$

R is the response variable (glucose or xylose yield, % of theoretical). P, T, C, and H represent the uncoded independent variables for pH, temperature (°C), cellulase (FPU g^{-1} glucan) and hemicellulase (XU g^{-1} glucan), respectively. The terms β_0, β_i, β_{ii}, and β_{ij} are the constant, linear, quadratic and interaction model coefficients, respectively. Statistically insignificant ($p > 0.05$) terms were identified and removed sequentially until all remaining terms were significant to develop quadratic polynomial regression equations and resulting models. Their significance was evaluated by analysis of variance (ANOVA). The fit of the model was evaluated by the determination of R-squared coefficient, adjusted R-squared coefficient, and lack-of-fit test. The final relationship is represented as contour plots in order to visualize the relationship between the response and experimental levels of each factor. MINITAB 17 statistical software (Minitab Inc.; State College, PA) was used to design the experiment, to develop the empirical model, and to generate the response surface plots.

Table 6. Response surface Box-Behnken design and resulting glucose and xylose yields (% of theoretical) after 24 h enzymatic hydrolysis of SAA-pretreated corn stover with 15% ammonia at 40 °C for 24 h.

	Design uncoded factors (actual levels)				Yield (% theoretical) at 24 h of hydrolysis			
Run	pH	Temp.	Cellulase	Hemicellulase	Glucose		Xylose	
		(°C)	(FPU g^{-1} glucan)	(XU g^{-1} glucan)	Actual	Predicted	Actual	Predicted
1	4	37	20	500	61.0	59.9	58.4	57.2
2	5.4	37	20	500	58.7	57.6	51.0	50.2
3	4	50	20	500	59.9	62.1	58.2	60.3
4	5.4	50	20	500	61.3	59.8	54.5	53.3
5	4.7	43.5	10	0	41.5	40.4	41.5	41.5
6	4.7	43.5	30	0	58.1	56.5	58.8	57.3
7	4.7	43.5	10	1000	57.4	57.9	54.8	55.6
8	4.7	43.5	30	1000	66.0	65.8	64.3	63.6
9	4	43.5	20	0	49.4	49.5	51.8	51.6
10	5.4	43.5	20	0	46.3	47.3	43.9	44.6
11	4	43.5	20	1000	63.8	63.0	62.2	61.8
12	5.4	43.5	20	1000	59.3	60.7	53.5	54.8
13	4.7	37	10	500	52.7	54.4	48.0	49.0
14	4.7	50	10	500	53.4	53.4	52.2	52.1
15	4.7	37	30	500	62.1	63.3	59.2	60.9
16	4.7	50	30	500	69.0	68.6	64.8	64.0
17	4	43.5	10	500	53.6	53.4	53.2	52.8
18	5.4	43.5	10	500	51.0	51.1	46.5	45.8
19	4	43.5	30	500	64.5	65.4	64.1	64.8
20	5.4	43.5	30	500	62.0	63.2	56.5	57.8
21	4.7	37	20	0	49.2	50.3	48.0	47.8
22	4.7	50	20	0	48.2	49.8	48.4	50.9
23	4.7	37	20	1000	60.9	61.0	58.2	58.0
24	4.7	50	20	1000	65.3	65.9	62.6	61.1
25	4.7	43.5	20	500	61.0	61.5	59.5	60.4
26	4.7	43.5	20	500	62.2	61.5	61.3	60.4
27	4.7	43.5	20	500	61.6	61.5	60.0	60.4

Determining the economically optimal loadings of cellulase and hemicellulase

Finding the optimal loadings of enzymes for producing fermentable sugars (glucose and xylose) by SSF and SHF is important to increase the economic efficiency. We use a common profit maximization framework from the field of microeconomic analysis to compare the economic impacts of enzyme loadings of the two processes (Mas-Colell et al., 1995). The analysis compares the economic tradeoffs for cellulase and hemicellulase since a range of loadings can result in equivalent hydrolysis rates. Results from bench scale experiments in this study are used to estimate the economic optimal loadings of cellulase and hemicellulase.

We assume the biorefinery is a profit maximizer and price taker in the input and product markets. Consequently, the biorefinery chooses levels of inputs (X_j), and outputs (Y_i) that maximize profits (π) given the prices of inputs (P_j) and outputs (P_i):

$$MAX\ \pi = \sum_{i=1}^{n} P_i Y_i - \sum_{j=1}^{m} P_j X_j \tag{3}$$

The biorefinery is assumed to be operating in the short run, so the use of at least one input, capital, and its associated costs are fixed. Specifically, we assume that the biorefinery is optimizing enzyme loadings for a facility that is already outfitted with required preprocessing and other capital equipment. Our economic analysis focuses on cellulase (c) and hemicellulase (h) enzymes. We do not include β-glucosidase explicitly but it could be modeled as an integral part of cellulase costs. Glucose and xylose are intermediate products that are fermented into ethanol, representing the biorefinery's primary product.

The biorefineries short-run profit maximizing equation is

$$MAX\ \pi = P_e Q_e\big(glucose(h,c), xylose(h,c)\big) - P_c Q_c - P_h Q_h \tag{4}$$

Optimal use of variable inputs can be found by solving the first order conditions:

$$P_c = P_e\left(\frac{\partial Q_e}{\partial glucose}\frac{\partial glucose(c,h)}{\partial c} + \frac{\partial Q_e}{\partial xylose}\frac{\partial xylose(c,h)}{\partial c}\right) \tag{5}$$

$$P_h = P_e\left(\frac{\partial Q_e}{\partial glucose}\frac{\partial glucose(c,h)}{\partial h} + \frac{\partial Q_e}{\partial xylose}\frac{\partial xylose(c,h)}{\partial h}\right) \tag{6}$$

where, P_c, P_h, and P_e represent prices of cellulase, hemicellulase and ethanol, and Q_c, Q_h, and Q_e represent the quantities of cellulase, hemicellulase and ethanol, respectively. These equations are the variable input profit maximizing conditions: marginal revenue product equals marginal input cost for cellulase and hemicellulase. Conversion efficiencies of glucose to ethanol (95%), xylose to ethanol (85%), ethanol stoichiometric yield (51%), and ethanol price of \$0.634 L^{-1} are taken from Humbird et al. (2011). The common enzyme price is assumed at \$4.70 kg^{-1} protein according to Hong et al. (2013).

Results and discussion

Composition of non-pretreated and SAA- pretreated corn stover and hydrolysis yields

The chemical composition of non-pretreated corn stover and corn stover pretreated by SAA are summarized in Table 7. Nearly 70% of solid was recovered after SAA pretreatment. The majority of glucan (82%) and xylan (72%) were preserved in the pretreated solids after SAA pretreatment. Solids loss during this pretreatment was mainly attributed to lignin removal (55%). The solid retention, carbohydrate retention and lignin removal are in agreement with data in previous reports obtained for SAA pretreated switchgrass (Pryor et al., 2012; Rijal et al., 2012), and corn stover (Kim and Lee, 2007).

Table 7. Chemical composition (dry basis) of non-pretreated and soaking in aqueous ammonia (SAA) pretreated corn stover.

	S.R.[1] (%)	Glucan (%)	Xylan (%)	Lignin[2] (%)
Non-pretreated corn stover	-	41.2 ± 3.83	21.3 ± 2.23	20.2 ± 0.220
SAA-pretreated corn stover	69.7	48.2 ± 0.820	21.7 ± 2.55	12.9 ± 2.23

[1]S.R.: Solid remaining after pretreatment; [2] Sum of acid insoluble and soluble lignin

Table 6 shows the experimental conditions and 24 h hydrolysis yields, according to the design. Glucose and xylose yields at 24 h, expressed as a percent of the theoretical, were chosen for the response surface modeling because the intermediate yield is more representative of initial hydrolysis rates than ultimate hydrolysis yields at 48 h or 72 h. By applying multiple regression analysis on the experimental data, the response variable and the test variables were related by a second-order polynomial equation.

The final models of glucose and xylose yields at 24 h (% of theoretical), after removal of nonsignificant terms, are represented by equations (7) and (8), respectively.

Glucose model: $R = -14.8 + 29.3(P) - 0.524(T) + 0.392(C) + 2.25\times10^{-2}(H) - 3.29(P)^2 - 1.57\times10^{-2}(C)^2 - 0.019\times10^{-3}(H)^2 + 2.40\times10^{-2}(TC) + 4.15\times10^{-4}(TH) - 4.08\times10^{-4}(CH)$ (7)

Xylose model: $R = -190 + 56.1(P) + 4.29(T) + 1.56(C) + 3.39\times10^{-2}(H) - 6.50(P)^2 - 4.66\times10^{-2}(T)^2 - 1.92\times10^{-2}(C)^2 - 0.16\times10^{-4}(H)^2 - 3.90\times10^{-4}(CH)$ (8)

where P, T, C, and H represent the uncoded independent variables for pH, temperature (°C), cellulase (FPU g^{-1} glucan) and hemicellulase (XU g^{-1} glucan), respectively The results were analyzed using analysis of variance (ANOVA) and response plots. The statistical significance of the ratio of the mean square variation due to regression and mean square residual error was tested using ANOVA. Corresponding ANOVA tables for both models are shown in Table 8. For glucose yield, the model fit the data very well with an R^2, adjusted R^2, and predicted R^2 value of 0.977, 0.963, and 0.924, respectively. The lack of fit of the model was not significant (*p*-value = 0.169), further indicating model adequacy. For xylose yield, the model fit the data very well with an R^2, adjusted R^2, and predicted R^2 value of 0.972, 0.957 and 0.923, respectively. A lack-of-fit value of 0.353 also supports the fitness of the model. Figure 8 shows the relationship of both glucose and xylose yield calculated by the model plotted against the corresponding experimental data, which further illustrates the model adequacy over the full range of the data.

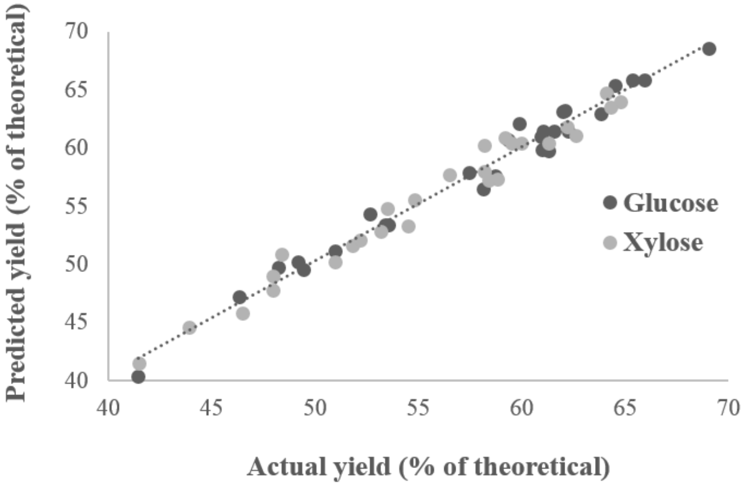

Figure 8. Scatter diagram of predicted response versus actual response of hydrolysis yields after 24 h hydrolysis of SAA-pretreated corn stover.

The ANOVA shows the percent contribution of linear, quadratic and interaction terms of four independent variables, namely pH, temperature, cellulase, and hemicellulase (Table 8). For glucose yield, linear terms account for the majority of the variation (83%), whereas quadratic and interaction terms account for 12% and 3%, respectively. As can be seen from the ANOVA table, among the independent variables cellulase and hemicellulase enzymes had a higher significant effect ($p < 0.001$) than the pH and temperature ($p < 0.05$).

For the xylose model, linear terms accounted most of the variability (86%) followed by around 10% variability from quadratic terms and only 1.4% contribution from the interaction between variables. Among all the variables in xylose model, the cellulase and hemicellulase enzyme together contributed approximately 80% of the variation.

Table 8. ANOVA for response surface models of glucose and xylose yields (% of theoretical) after 24 h hydrolysis when varying hydrolysis pH, temperature and cellulase and hemicellulase enzyme loadings of SAA-pretreated corn stover with 15% ammonia at 40 °C for 24 h.

Source of variation	df	Seq SS	Adj SS	Adj MS	F-value	*p*-value
Glucose Model						
Regression	10	1168.50	1168.50	116.85	68.93	0.001
Linear	4	996.88	996.88	249.22	147.01	0.001
pH (P)	1	15.41	15.41	15.41	9.09	0.008
Temperature (T)	1	13.44	13.44	13.44	7.93	0.012
Cellulase (C)	1	434.16	434.16	434.16	256.11	0.001
Hemicellulase (H)	1	533.87	533.87	533.86	314.93	0.001
Square	3	138.00	138.00	46.00	27.14	0.001
$P\times P$	1	0.79	15.57	15.57	9.19	0.008
$C\times C$	1	0.92	14.81	14.81	8.74	0.009
$H\times H$	1	136.29	136.29	136.29	80.40	0.001
2-Way Interaction	3	33.61	33.61	11.20	6.61	0.004
$T\times C$	1	9.70	9.70	9.70	5.72	0.029
$T\times H$	1	7.26	7.26	7.26	4.28	0.055
$C\times H$	1	16.65	16.65	16.64	9.82	0.006
Residual Error	16	27.12	27.12	1.69		
Lack of fit	14	26.41	26.41	1.88	5.31	0.169
Pure error	2	0.71	0.71	0.35		
Total	26	1195.62				
			$R^2 = 0.977$	$R^2_{adj} = 0.963$	$R^2_{pred} = 0.924$	
Xylose Model						
Regression	9	1050.80	1050.80	116.75	64.92	0.001
Linear	4	932.57	932.58	233.14	129.63	0.001
pH (P)	1	147.00	147.00	147.00	81.74	0.001
Temperature (T)	1	26.70	26.70	26.70	14.85	0.001
Cellulase (C)	1	426.02	426.02	426.02	236.88	0.001
Hemicellulase (H)	1	332.85	332.85	332.85	185.07	0.001
Square	4	103.01	103.01	25.75	14.32	0.001
$P\times P$	1	17.57	54.05	54.04	30.05	0.001
$T\times T$	1	1.69	20.72	20.71	11.52	0.003
$C\times C$	1	2.29	19.68	19.67	10.94	0.004
$H\times H$	1	81.47	81.47	81.46	45.30	0.001
2-Way Interaction	1	15.21	15.21	15.21	8.46	0.01
$C\times H$	1	15.21	15.21	15.21	8.46	0.01
Error	17	30.57	30.57	1.79		
Lack of fit	15	28.85	28.85	1.92	2.23	0.353
Pure error	2	1.73	1.73	0.86		
Total	26	1081.37				
			$R^2 = 0.972$	$R^2_{adj} = 0.957$	$R^2_{pred} = 0.923$	

The variance inflation factor (VIF) is used to check how much multicollinearity (correlation between predictors) exists in the regression as this could be problematic making the regression coefficient unstable and difficult to interpret. When significant multicollinearity issues exist, the VIF will be very large (5 to 10) for the variables involved. In terms of VIF for both glucose and xylose model, all the linear and interaction terms resulted in a VIF of 1 while the quadratic terms had a VIF of 1.11 and 1.25, respectively for glucose and xylose model indicating no correlation among the predictors. Figure 9 shows the profiles for predicting (a) glucose and (b) xylose 24-h yields and the variation of each main variables as there is not much interaction among the variables. Our result shows that variation in enzyme loading is more important than pH or temperature fluctuation, especially for glucose yields.

The predicted optimal pH for glucose yield was 4.5, which is lower than most commonly used pH values (4.8) for commercial cellulase use. Using lower optimal pH for commercial use of cellulase might get benefit to reduce microbial contamination at lower pH values. The highest glucose yield was achieved at the highest temperature tested, 50 °C, and temperature had greater impact than pH (Figure 9a). This finding is contrary to another study which found that the change in temperature was less important than the change in pH (Singh et al., 2009) when optimizing parameters for thermostable cellulase activity from *Aspergillus heteromorphus*, indicating difference in response based on the enzyme-producing organisms.

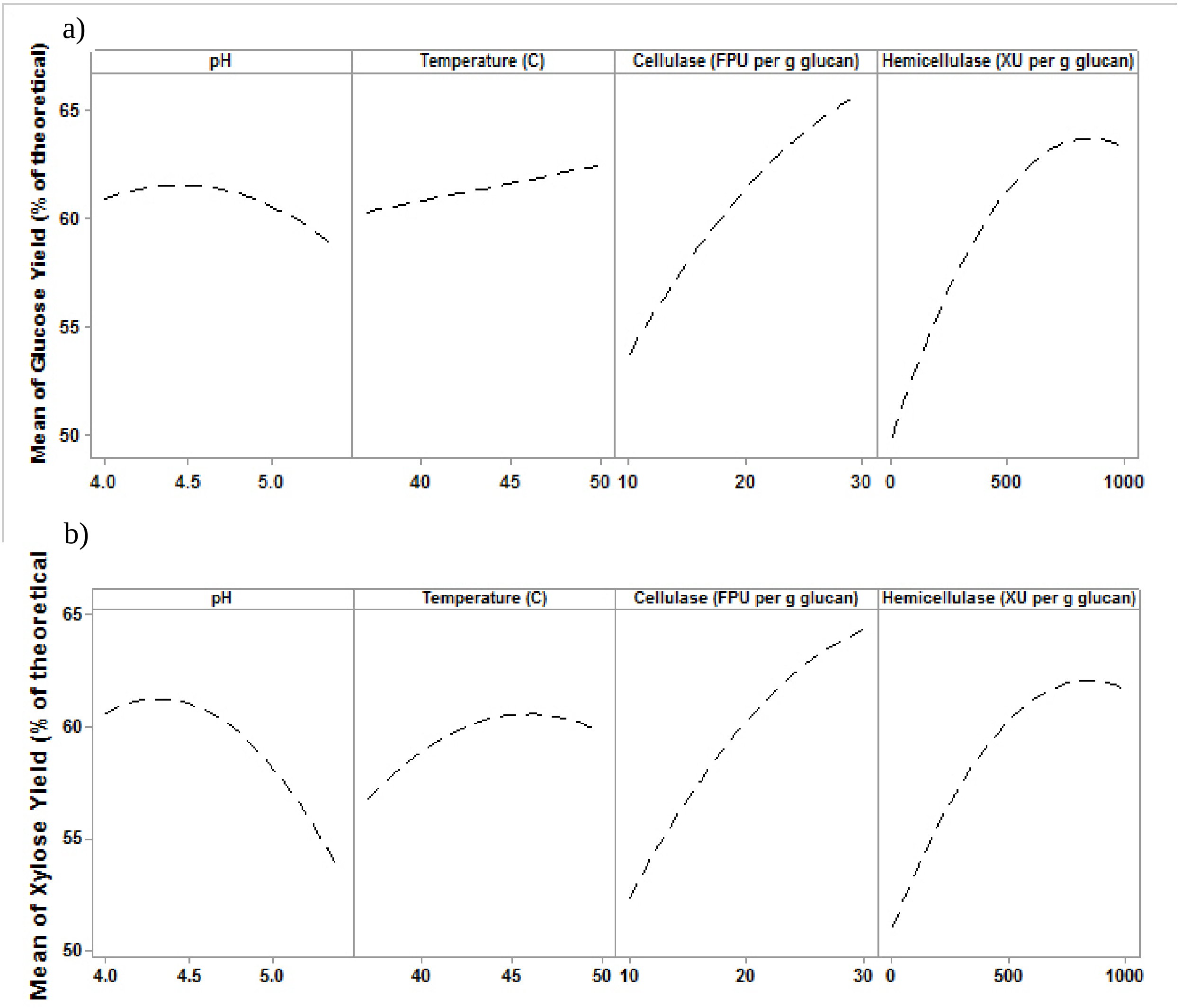

Figure 9. Profiles for predicting a) glucose and b) xylose yields for 24 h hydrolysis for main effects of pH, temperature, cellulase, and hemicellulase loadings for SAA-pretreated corn stover. Each line represents the mean response for the given main factor across all modeled conditions for the remaining factors.

The optimal pH and temperature for xylose yields are lower than for optimal glucose yields. The predicted optimal pH for xylose yield was 4.3 and the optimal temperature was found 46 °C. Figure 9 (b) shows that xylose yields decrease more rapidly than glucose yields with increasing pH, indicating that the hemicellulase enzymes used are more sensitive to pH changes.

Optimal pH values for both glucose and xylose yields in this study were lower than reported in other studies. Our study shows no interaction between pH and temperature for glucose or xylose yields of SAA-pretreated corn stover. The absence of interaction between these variables indicates an additive effect of these variables on the response. Pandiyan et al. (2014) reported a minimum effect of pH from 4 to 5 on the saccharification yield of alkali pretreated *Parthenium* sp. but also found no significant interaction between pH and temperature. On the contrary, Wang et al. (2013) reported pH values greater than 5.5 enhanced saccharification of SPORL (Sulfite Pretreatment to Overcome Recalcitrance of Lignocelluloses) pretreated lodgepole pine when enzymatic hydrolysis was conducted at 50 °C with Celluclast 1.5 L. Differences in the best enzymatic hydrolysis condition is attributed to the variation in the chemical composition of the different biomass, type of pretreatment, as well as the enzyme formulation used. The lack of interaction between temperature and pH is more likely to hold across substrates and pretreatments.

Figure 9 shows that the temperature relationship is nearly linear for glucose yields. Increasing both glucose and xylose yields at higher temperature shows the practicality of developing other microorganism strains that can tolerate higher temperatures than *Saccharomyces cerevissiae*. Several studies have focused on development of thermotolerent microbes for SSF conditions for producing ethanol, butanol or other biobased chemicals. Using an engineered strain of *Thermoanaerobacterium saccharolyticum* ALK2 for SSF at 50 °C, Shaw et al. (2008) showed 40% enzyme loading reduction over SSF using *S. cerevisiae* at 37 °C. Kadam and Schmidt (1997) found a thermotolerant yeast, *Candida acidothermophilum*, produced 80% of the theoretical ethanol yield at 40 °C using dilute acid pretreated poplar as substrate. Another strain, *Kluyveromyces marxians* identified by Ballesteros et al. (1991) have been reported as more thermotolerant (42 °C) than *Candida* and *Saccharomyces* strains. These strains need to be tested to find efficiency in SSF conditions with different pretreated biomass.

Any improvement in strain robustness at higher temperatures will have a direct impact on hydrolysis rates. Even though this information is not new, most studies only show relationship with one enzyme and temperature. This study demonstrates the effect with combined enzymes with any pH between 4.0 and 5.5.

Contour plots were developed to illustrate the interactive effects of different combinations of enzyme loadings, where pH and temperature were kept constant. Such plots can be developed for any combination of pH and temperature based on the desired conditions, but we illustrated the differences using typical SSF and SHF temperatures with optimal pH values (Figure 10). The plots of glucose yield shows that temperature has a somewhat larger impact at higher enzyme loadings than lower loadings. This differential impact is due to the significant interaction terms for temperature and each enzyme loadings (Table 8). These interactions are not present for xylose models. As we chose 24-h yields for modeling, these yields are more representative of initial hydrolysis rates rather than ultimate yields.

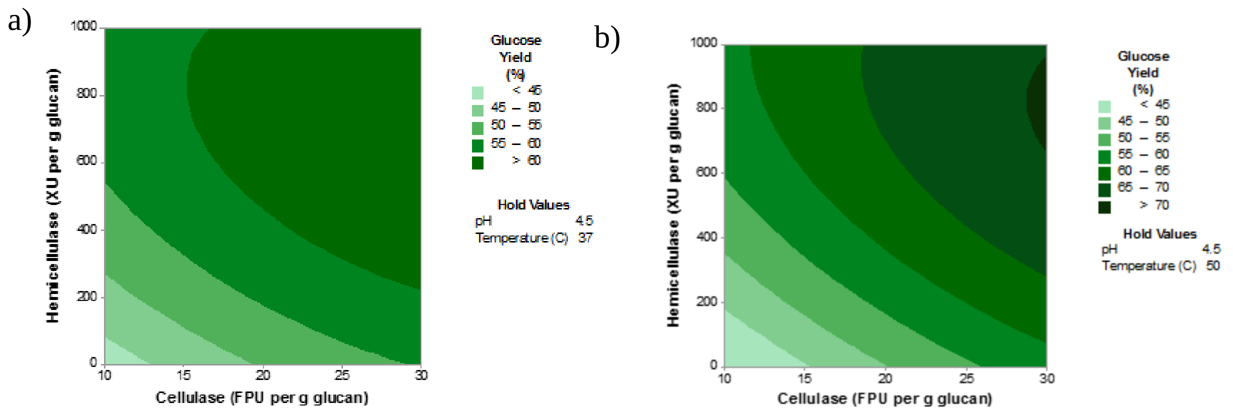

Figure 10. Contour plots of the combined effects of cellulase and hemicellulase on glucose yields at a) SSF (37 °C) and b) SHF condition (50 °C) using SAA-pretreated corn stover with 15% aqueous ammonia for 24 h at 40 °C.

Although it is expected that higher enzyme loadings result in higher hydrolysis rates, this work demonstrate that different combinations of enzymes can be used to achieve similar results. The law of diminishing returns suggests that economically optimal loadings may not be loadings that result in the absolute highest rates or yields. To demonstrate those trade-offs within the design space, we looked at how much each enzyme loading could change under typical SSF (37 °C) and SHF (50 °C) conditions while still predicting 24-h glucose yield of 95% of the predicted optimal value (Figure 11).

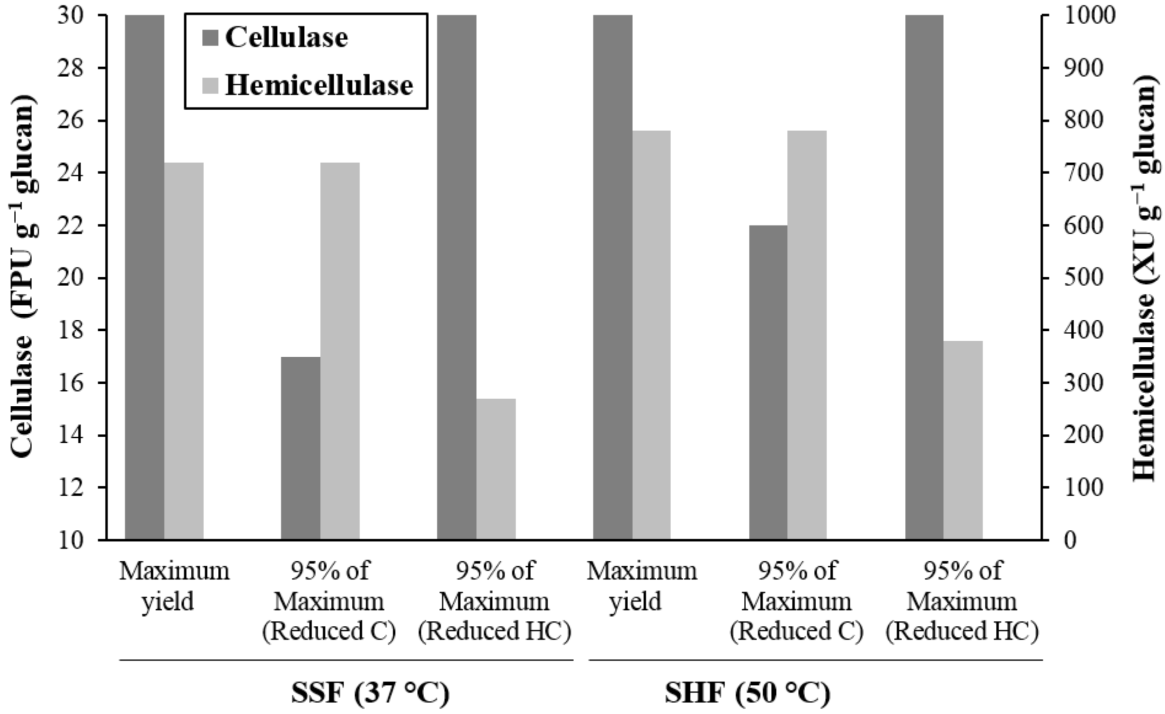

Figure 11. Cellulase and hemicelluase tradeoffs for predicting glucose yield of 95% of the predicted maximum for SSF (simultaneous saccharification and fermentation) and SHF (separate hydrolysis and fermentation) conditions while pH held constant at 4.5.

In SSF, the predicted optimal loadings of cellulase and hemicellulase at 30 FPU and 720 XU g^{-1} glucan respectively, resulted in a predicted glucose yield of 64% at 24 h. Our glucose model predicts that cellulase loadings can be reduced as much as 43%, equivalent to

17 FPU g^{-1} glucan, and still maintain 95% of the predicted optimal values with hemicellulase loadings of 720 XU g^{-1} glucan. On the contrary, hemicellulase loadings can be reduced as much as 62% from the predicted optimum and maintain 95% of the predicted optimal values with cellulase loadings of 30 FPU g^{-1} glucan (Figure 11).

For SHF conditions, the predicted optimal loadings of cellulase and hemicellulase were 30 FPU and 780 XU g^{-1} glucan for maximum glucose yield (around 71%). To achieve 95% of optimal prediction, which is 67% of glucose yield at 24 h, cellulase loadings can be reduced to 26% (22 FPU g^{-1} glucan) with the highest hemicellulase loadings. In contrast, hemicellulase loadings can be reduced by 51% to 380 XU g^{-1} glucan, with the cellulase loadings of 30 FPU g^{-1} glucan. The graphs of xylose yields for varying cellulase and hemicellulase enzymes at SSF and SHF temperatures are not shown as they followed a similar trend to glucose yield.

Enzyme loadings have been reported as the most important factor after effective pretreatment for increasing the rate or yield of enzymatic hydrolysis (Chen et al., 2007; Karki et al., 2011). Our models show that glucose and xylose are similarly sensitive to cellulase and hemicellulase for SAA pretreated corn stover. Gupta et al. (2008) previously explained that hemicellulase supplementation not only increases the xylan digestibility of treated biomass but also the glucan digestibility by reducing the hindrance caused by a resilient hemicellulose layer on the cellulose microfibrils. As cellulase loading increases, the effect of hemicellulase becomes less important but never negligible (Figure 10). This study highlights the need for both enzymes after SAA pretreatment and shows how they can be used in a complementary way. Further, based on market costs of each enzyme, loadings can be adjusted to minimize cost while maintaining higher hydrolysis rates.

Economic analysis

An economic analysis was conducted for SAA-pretreated corn stover assuming typical pH and temperature for SSF and SHF processes. Optimal enzyme loadings to maximize hydrolysis rates or yields may not coincide with optimal loadings to maximize economic return. Table 9 shows the economically optimal enzyme loadings based on enzyme costs and ethanol revenues.

Table 9. Economic optimal loadings of cellulase (C) and hemicellulase (HC) at base case and with 20% changes from baseline for enzyme cost, and ethanol selling price (high + and low -) for typical SSF (simultaneous saccharification and fermentation) and SHF (separate hydrolysis and fermentation) process temperatures. Values in the right section of table represent percent change from the baseline scenario for each adjustment in enzyme or ethanol price.

Conditions	SSF		SHF		SSF		SHF	
Enzyme	C (FPU)	HC (XU)	C (FPU)	HC (XU)	C (FPU)	HC (XU)	C (FPU)	HC (XU)
Baseline	16	771	22	807	-	-	-	-
High hemicellulase cost	17	756	22	793	6.3%	-2.0%	0%	-1.7%
Low hemicellulase cost	16	786	22	822	0%	2.0%	0%	1.9%
High cellulase cost	13	810	19	846	-19%	5.1%	-14%	4.8%
Low cellulase cost	20	732	26	769	25%	-5.1%	18%	-4.7%
High ethanol price	19	750	25	790	19%	-2.7%	14%	-2.1%
Low etahnol price	12	800	18	840	-25%	3.8%	-18%	4.1%

The baseline economically optimal loadings of cellulase and hemicellulase were 38% and 5% higher, respectively, for SHF than for SSF. The higher optimal loadings for SHF are somewhat counterintuitive. We would expect that the SHF process would require less enzyme than SSF because hydrolysis rates are higher under SHF conditions. The yield model predicted glucose yields of 60.5% for SSF, and 67.1% for SHF, for the optimal enzyme loadings, whereas maximum yield in the design space were 64% and 71%, respectively. Although higher yields would be expected for longer hydrolysis times, differences between treatments would be more difficult to distinguish and the 24 h yields are more representative of initial hydrolysis rates. The basic economic model suggests that the higher ethanol revenue that comes with SHF outweighs the associated cost of higher enzyme loadings. The models show that there is a point of diminishing returns on enzyme loadings for process temperatures, but that point is reached at lower cellulase and hemicellulase loadings for the lower temperatures typically required for SSF.

Enzyme prices directly impact the economically optimal loadings, but estimating realistic enzyme prices is challenging. Publicly available information is limited due to the proprietary nature of data from enzyme manufacturers (MacLean and Spatari, 2009). Enzyme cost estimates also vary based on ongoing enzyme development, different enzyme source microorganisms, and mixtures of enzymes activities within a commercial product (Hong et al., 2013; Humbird et al., 2011). We addressed the situation by conducting a sensitivity analysis on each enzyme price. Economic optimum enzyme loadings were estimated while adjusting enzyme prices 20% higher or lower than the assumed baseline prices, and these results are also shown in Table 9.

In comparing SHF and SSF processing temperatures, optimal enzyme loadings are more sensitive to enzyme price fluctuations for lower temperature SSF processes than higher temperature SHF processes. Optimal cellulase and hemicellulase loadings for both processes are more sensitive to cellulase cost than hemicellulase cost (Table 9). Hemicellulase cost changes of 20% had little impact (<1% to 6%) on optimal loadings for either enzyme. However, 20% variation in cellulase price caused changes of up to 25% in cellulase loadings.

Ethanol selling price also varies considerably due to market fluctuations. An ethanol price of \$0.634 L^{-1} (\$2.20 gal^{-1}) was selected for the baseline scenario (Humbird et al., 2011). Given price uncertainty, we also looked at how sensitive the optimum enzyme loadings are by varying ethanol price by 20%. Fluctuations in ethanol price have a greater impact on economically optimal cellulase loadings than on hemicellulase loadings. Effects of ethanol price changes are similar in magnitude to the effects of cellulase cost changes. However, increasing cellulase costs decreases optimal enzyme loadings, while ethanol price increases allow the biorefinery to further increase both yields and profits by adding more enzyme. Similarly, when ethanol prices drop, so does the point of diminishing returns on enzyme loadings and the lower revenue requires a reduction in enzyme loadings.

Conclusions

The effect of hydrolysis pH on yields is independent of temperature or enzyme loadings. Models show that glucose and xylose yields were optimum at pH values of 4.5, and 4.3, respectively, lower than those typically reported in the literature. Changes in temperature and pH have a much lower effect on glucose yields than on xylose yields. Use of higher temperature SHF conditions leads to higher yields but necessitates higher enzyme loadings. However, similar yields in SHF is possible with lower enzyme loadings when compared to the lower temperature SSF conditions. This work shows the interaction between cellulase and hemicellulase loadings on hydrolysis rates and demonstrates that higher loadings of one enzyme can compensate for lower loadings of the other. Therefore, many combinations of enzyme loadings can be used to achieve similar hydrolysis rates. Optimal enzyme loadings to maximize economic return are lower than loadings to maximize rates. Cellulase costs and ethanol price have a larger effect than hemicellulse costs on loadings optimized for economic return.

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PAPER 2: EFFECTS OF REDUCED SEVERITY AMMONIA PRETREATMENT ON PELLETED CORN STOVER[2]

Abstract

Biomass densification impacts pretreatment efficacy and subsequent biochemical conversion to biofuels and other biobased chemicals. Pelleted corn stover was used to evaluate the soaking in aqueous ammonia (SAA) pretreatment efficacy at high solid loadings with reductions ammonia concentration, temperature, and pretreatment time. Hydrolysis of pelleted corn stover resulted in 49% higher glucose yields compared with loose stover at 10% pretreatment solid loadings. Glucose yields from pelleted material did not change with a two fold increase in pretreatment solid loadings. Hydrolysis yields were modeled as a function of pretreatment conditions and the developed model predicted maximum 24 h hydrolysis glucose yields of 96% at a pretreatment temperature of 60 °C, for 4 h, with 18% ammonia. Pretreatment severity can still be reduced while maintaining 90% or higher yields with different combinations of temperature, time, and ammonia concentration. Temperature was the most important pretreatment parameter within the design space for achieving high glucose yields. FTIR and SEM analysis of SAA-pretreated corn stover pellets illustrated that the pelleting process increases pretreatment efficacy by modifying the biomass structure, disrupting the lignin-hemicellulose linkages, and partially removing lignin. Using pelleted biomass can reduce the required severity of SAA-pretreatment while still producing glucose yields above 90%. Using

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[2] This paper was published in September 2017 as Nahar, N., & Pryor, S.W. (2017). Effects of reduced severity ammonia pretreatment with pelleted corn stover. *Ind. Crop Prod.*, 109: 163−172. Nurun Nahar had primary responsibility for collecting and analyzing laboratory data. Nurun Nahar also drafted and revised all versions of this paper.

pelleted corn stover as a biorefinery feedstock has potential to lower pretreatment costs in addition to the improved handling and transportation.

Introduction

Pretreatment of cellulosic biomass is a required step to reduce its natural recalcitrance for biological processing to produce biofuels or biobased chemicals. However, pretreatment adds significant cost constraints due to pretreatment process conditions such as high temperatures, long pretreatment times, and high chemical loadings. Low biomass bulk density also limits pretreatment solid loadings. Obtaining high yields with the reduction of pretreatment severity will reduce the economic input to achieve low-cost conversion of cellulosic biomass and increase the viability of cellulosic bioindustries.

Densification such as pelleting increases biomass bulk density and therefore facilitates cost reductions for handling, storage, and transportation for cellulosic biorefineries (Eranki et al., 2011; Tumuluru et al., 2011). Moreover, biomass form and structure impacts subsequent pretreatment and hydrolysis processes. Therefore, understanding the inherent link and potential synergies of densification and pretreatment processes are crucial. Despite pellet durability, biomass pelleting does not have any negative effect on hydrolysis yields following dilute acid or ionic liquid pretreatments (Ray et al., 2013; Rijal et al., 2012; Shi et al., 2013). Further, pelleting has been shown to improve sugar conversion using hydrothermal and alkaline pretreatments (Li et al., 2014). Guragain et al. (2013) showed significantly higher sugar yield and productivity of enzymatic hydrolysis with alkali-pretreated pelleted biomass than with unpelleted biomass. Pelleting has also been shown to increase sugar yields with switchgrass pretreated by soaking in aqueous ammonia (SAA) (Nahar and Pryor, 2014; Rijal et al., 2012). Nahar and Pryor (2014) found that a less severe SAA-pretreatment (40 °C for 6 h) and lower enzyme loadings

(10 FPU g^{-1} glucan) were still effective for maintaining high hydrolysis yields of pelleted switchgrass. Although the lower-severity pretreatment in that study showed promising results, there are different ways to reduce pretreatment severity and cost. For SAA pretreatment, alkali concentration, temperature, and pretreatment time could be further reduced with densified biomass.

Improving process economics by a combination of a high-solid pretreatment followed by high-solid hydrolysis will increase sugar yield while decreasing capital costs (Roche et al., 2009). Although high-solids enzymatic hydrolysis has been addressed in the literature (Hodge et al., 2008; Jorgensen et al., 2007; Roche et al., 2009), data for high-solid pretreatment is more limited (Modenbach and Nokes, 2012). However, there is potential for increasing pretreatment solid loadings with pelleted biomass, which would have significant impacts on reactor sizing and associated energy and chemical costs. Therefore, it is important to consider the reduced severity conditions for effective SAA pretreatment technologies along with higher solid loadings pretreatment and incorporate modified technologies into the economic and environmental models to quantify the proper benefits of pellets beyond improved logistical handling of biomass.

The objectives of this study were to examine the effect of SAA pretreatment parameters (solid loading, temperature, time, and ammonia concentration) on the digestibility of pelleted biomass, and to identify which of the pretreatment parameters have the greatest impact on hydrolysis yield. Pelleted corn stover was selected as model feedstock and a series of SAA pretreatments were conducted based on a face-centered central composite design involving three process variables: temperature, time, and ammonia concentration. Fourier Transform Infrared (FTIR) spectroscopy, X-ray diffraction (XRD) and Scanning Electron Microscopic (SEM) analysis were used to compare the structure of the constituents that occur during the pelleting and pretreatment process. The efficacy of pretreatment severity was evaluated by measuring 24 h glucose yields from enzymatic hydrolysis of the pretreated pelleted corn stover.

Materials and methods

Raw material

Corn (*Zea mays* L.) stover (stalks and leaves) was collected from a USDA-ARS research field (46°48′ 38.51″N 100°54′ 52.53″W) in Mandan, North Dakota, USA. Corn stover was air dried (10% moisture content, dry basis) and ground in a Wiley Mill with a 6 mm sieve. Sieved corn stover was stored in a sealed plastic bag at room temperature until use.

Pellet production

Dried biomass was ground using a hammer mill and pellets were prepared using a Buskirk Engineering pellet mill (PM 810; North Ossian, IN) in the NDSU Biomass Feedstock Processing Laboratory at the USDA-ARS Northern Great Plains Research Laboratory in Mandan, ND. The biomass was mixed with distilled water to 12% moisture before feeding into the pellet mill. No external binder was added for making pellets. The original corn stover is ground to a fine powder within the pellet mill before entering the plate die (200-mm diameter × 38-mm thickness with 6.3 mm holes) to produce standard 1/4″ pellets. The pellets were stored in sealed plastic bags at room temperature.

Pretreatment

Corn stover pellets were pretreated by soaking in aqueous ammonia. Two sets of SAA pretreatment studies were conducted to identify: 1) the impact of solid loading on pretreatment efficacy and 2) the effect of low-severity pretreatment on hydrolysis yield using different temperatures, times, and ammonium hydroxide concentrations during pretreatment.

Pretreatments were performed in 2-L screw-capped Pyrex bottles. The pretreatment bottles with aqueous ammonia were preheated in a water bath, and corn stover pellets were mixed with the liquid to achieve the chosen solid loadings. Pretreatment temperature was monitored with a thermometer inserted into the bottle and incubated without agitation for a time period according to the experimental design. No grinding of pelleted material is required as preliminary work showed that pellets deteriorate during SAA pretreatment.

The SAA pretreated slurry was filtered through Whatman # 41 filter paper (20–25 µm pore size) using a vacuum filtration unit. SAA-pretreated solids were washed with distilled water to neutralize the pH, weighed, and stored in sealed plastic bags at 4 °C for subsequent enzymatic hydrolysis experiments. Samples were taken to determine ultra-structural changes due to pretreatment. The moisture content and solid recovery of pretreated biomass were determined in triplicate by drying a small portion of wet solids (~ 2 g) overnight in a convection oven at 105 °C. A portion of pretreated wet solids (~ 40 g) was also dried at room temperature for physical and chemical characterization.

Enzymes

Pretreated solids were used for enzymatic hydrolysis to determine the impact of pelleting and low severity pretreatment on glucose yields. Cellulase (NS50013), β-glucosidase (Novo188), and hemicellulase (Cellic HTec) enzymes were used for hydrolysis. All enzymes were provided by Novozymes North America, Inc. (Franklinton, NC, USA). The cellulase activity of NS50013 and β-glucosidase activity of Novo 188, as determined by Ghose (1987), were 77.0 filter paper units (FPU) mL^{-1} and 500 cellobiase units (CBU) mL^{-1}, respectively. Xylanase activity of Cellic HTec was 10,600 xylanase units (XU) mL^{-1} as determined by Bailey et al. (1992).

Enzymatic hydrolysis

Enzymatic hydrolysis of pretreated solids was performed to measure the digestibility of the pretreated substrate. The glucose concentration in the pretreated solid residues was used to determine yields (% of theoretical) after enzymatic hydrolysis for 24 h. All hydrolysis experiments were carried out in triplicate at 1% (w/v) glucan loading in 125-mL Erlenmeyer flasks. Pretreated biomass was mixed with 50-mM sodium citrate buffer (pH 4.8) supplemented with enzymes according to the experimental design. Sodium azide was also added at 0.04% to prevent microbial contamination during hydrolysis. Samples were incubated at 50 °C and 130 rpm for 72 h in a water bath shaker (MaxQ 7000, Thermo Scientific; Dubuque, IA, USA). Aliquots (1 mL) were taken at 24 h intervals and immediately centrifuged at 13,226 × g for 5 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) after centrifugation, and stored at −20 °C until sugar analysis by HPLC.

High performance liquid chromatography (HPLC) analysis

Hydrolysis samples were analyzed by HPLC (Waters Corporation; Milford, MA), equipped with a Bio-Rad Aminex HPX-87P column (Bio-Rad Laboratories; Hercules, CA), and a refractive index (RI) detector (model 2414, Waters Corporation). The sugars from the injected sample (20 µL) were eluted with 18 mΩ NANOpure water at a flow rate of 0.6 mL min^{-1}. The sugars were quantified with column and detector temperatures of 85 °C and 50 °C, respectively. Glucose and xylose were quantified using 4-point external standard curves.

Experimental design and statistical analysis

Testing solid loadings for pretreatment

The effects of higher solid loadings for corn stover pellets was tested using SAA pretreatment with 15% aqueous ammonia at 60 °C for 4 h at 10, 15, and 20% solid loadings. Loose corn stover and corn stover pellets at a 10% solid loadings were used as controls. Low bulk density of loose stover prevented pretreatment at higher solid loadings. Biomass was hydrolyzed by cellulase and hemicellulase at 25 FPU g^{-1} glucan and 500 XU g^{-1} glucan, respectively. β-glucosidase was added using a 1:1 ratio of cellulase (FPU): β-glucosidase (CBU).

Response surface model (RSM) for pretreatment

Response surface methodology (RSM) was used to investigate the effects of pretreatment temperature, time, and ammonia concentration on hydrolysis yields. A face-centered design was used for the three factors with three levels per factor and five replicates at the center point. The levels of each variable in the experimental design are presented in Table 10.

Table 10. Factor levels for face-centered design for pretreatment of pelleted corn stover.

Levels	Factors		
	Temperature (°C)	Time (h)	Ammonia (%)
-1	30	2	8
0	45	3	15
+1	60	4	22

All experiments were carried out in randomized order. Enzymatic hydrolysis yield (24 h) of glucose of pretreated material was used as response variable and measured as a percentage of theoretical yield. Cellulase, β-glucosidase, and hemicellulase were added at 15 FPU g^{-1} glucan, 15 CBU g^{-1} glucan, and 300 XU g^{-1} glucan, respectively.

The experimental data for glucose yield were modeled by the response surface regression procedure using the following quadratic polynomial model (equation 9):

$$R = \beta_0 + \beta_T T + \beta_H H + \beta_A A + \beta_{TT} T^2 + \beta_{HH} H^2 + \beta_{AA} A^2 + \beta_{TA} TA + \beta_{TH} TH + \beta_{HA} HA \quad (9)$$

where, R represents the response variable (glucose yield, % of theoretical), and T, H and A represent the uncoded independent variables for temperature (°C), and pretreatment time (h), and ammonia concentration (%), respectively. The terms β_0, β_i, β_{ii}, and β_{ij} are the constant, linear, quadratic and interaction model coefficients, respectively.

Analysis of variance (ANOVA) was used to estimate the statistical parameters with a 95% confidence level. The second order polynomial equation was used to fit the experimental data. The quality of the fit of the polynomial model was evaluated by the coefficient of determination, R^2, adjusted R^2, and lack-of-fit test. The final fitted polynomial equation was expressed as contour plots in order to visualize the relationship between the response and experimental levels of each factor used in the design. MINITAB 17 statistical software (Minitab Inc.; State College, PA) was used to design the experiment, to develop the empirical model, and to generate the response surface plots within the design space.

Characterization of SAA-pretreated solids

Chemical composition after the pretreatment

Compositional analysis was determined before and after pretreatment of corn stover pellets to determine glucan, xylan, and lignin contents to enable accurate calculation of theoretical sugar yields after enzymatic hydrolysis. Carbohydrate and lignin (acid-soluble and acid-insoluble) contents were determined using NREL Chemical Analysis and Testing Standard Procedures (Sluiter et al., 2012). All compositional analysis was done in triplicate.

Physical Characterizations

Untreated corn stover pellets and pretreated solids were dried at 40 °C for several days and then ground using a kitchen coffee grinder. Ground samples were sieved less to than 180 µm and collected for further characterization, such as crystallinity (%), FTIR and SEM microscopy to assess the physical and chemical impact of pretreatment on the composition of pretreated corn stover pellets.

X-ray diffraction analysis

Cellulose crystallinity of untreated and pretreated samples was determined by X-ray diffractometer (Philips X'pert MPD; PANalytical B.V., the Netherlands). The system is equipped with a vertically mounted goniometer and Cu X-ray source. Samples of particle size less than 180 µm were scanned at a speed of 1° min^{-1}, range from $2\theta = 0$–$40°$, with a step size of 0.04° at room temperature by positioning the samples on a quartz sample holder. Biomass crystallinity (%) of the sample was defined by the intensity ratio of the diffraction peaks and of the sum of all measured intensity as follows:

$$Crystallinity\ [\%] = 100 \times \Sigma I_{net} / (\Sigma I_{tot} - \Sigma I_b) \quad (10)$$

where, I refers to the intensity of diffraction peaks. The percentage of crystallinity was calculated automatically by X'Pert HighScore software by separating crystalline peaks or Inet from an amorphous hump (I_{tot}) and a constant background (I_b).

FTIR spectroscopic analysis

Fourier Transform Infrared (FTIR) spectroscopic analysis was carried out to detect changes in the functional groups that may have been caused by pretreatment. The infrared spectrum between 4000 and 400 cm^{-1} was measured using a Nicolet 6700 Fourier Transform Infrared Spectrometer (ThermoFisher Scientific; Madison, WI, USA) with a universal

Attenuated Total Reflectance (ATR). Samples were pressed against a single reflection diamond crystal with a torque knob to apply similar pressure to all samples. All spectra were measured using 32 scans from 4000 to 400 cm^{-1} at 2 cm^{-1} resolution. Baseline and ATR corrections were applied using OMNIC software supplied with the equipment.

Scanning electron microscopy (SEM)

Physical changes in the untreated and pretreated pelleted biomass were observed by scanning electron microscopy (SEM) to get additional perspective on densification and pretreatment. SEM pictures were taken (particle size <180 μm) at different magnifications using a JEOL JSM-6490 LV scanning electron microscope (JEOL USA, Inc.; Peabody, MA, USA). Prior to taking pictures, dried samples were mounted on aluminum stubs and sputter-coated with a thin layer of gold.

Results and discussion

Effects of solid loadings on SAA pretreatment of loose and pelleted corn stover

The composition of untreated and SAA-pretreated loose corn stover and corn stover pellets are summarized in Table 11. Our results showed that the xylan and lignin content of pelleted corn stover was slightly lower than for loose biomass without any pretreatment. However, there was no significant difference in glucan content for untreated loose corn stover and pellets. Previous research also suggested that pelletization does not have a large change on biomass cell wall composition, but slightly reduces hemicellulose (Kumar et al., 2012; Rijal et al., 2012).

Enzymatic hydrolysis of unpelleted loose corn stover and pelleted corn stover without any pretreatment was carried out to see the effect of pelleting alone on glucose and xylose yields. After 24 h of enzymatic hydrolysis, glucose and xylose yields for pelleted corn stover increased from 18% to 38% and from 6% to 22%, respectively, compared to unpelleted corn stover without any pretreatment (Figure 12). Increased yields for pelleted switchgrass (Rijal et al., 2012), corn stover (Nahar and Pryor, 2014), and several types of herbaceous biomass (Theerarattananoon et al., 2012) has been reported without any pretreatment. Yield improvements from pelleting biomass without any pretreatment suggest that the severity of conventional pretreatments can be reduced.

Table 11. Composition of untreated and SAA-pretreated (60 °C, 6 h, 15% ammonia) loose corn stover and corn stover pellets at different solid loadings.

Pretreatment conditions	SR (%)	Glucan (%)	Xylan (%)	Lignin[*] (%)	Delignification (%)
Untreated loose CS	100.0	35.9±1.1	23.0±0.3	22.0±0.5	-
Untreated CS pellet	100.0	34.7±2.2	20.0±1.2	20.8±0.0	-
Pretreated loose CS (10% solid loadings)	81.8	54.7±1.0	26.0±0.3	17.9±0.9	33.3
Pretreated CS pellet (10% solid loadings)	67.8	50.5±3.1	20.9±1.5	13.2±0.0	56.9
Pretreated CS pellet (15% solid loadings)	70.7	51.3±2.7	21.4±1.4	14.3±0.3	51.4
Pretreated CS pellet (20% solid loadings)	70.3	48.4±0.1	20.2±0.2	14.5±0.1	51.0

[*] Sum of acid insoluble and acid soluble lignin

The impacts of pretreatment solid loadings on hydrolysis glucose and xylose yields are shown in Figure 12. Delignification increased from 33% for loose corn stover to over 50% for pellets pretreated at any solid loadings (Table 11). At 10% solid loadings, glucose yields were 49% higher for pelleted stover than for loose corn stover. Increasing solid loadings from 10% to 20% for pelleted biomass did not decrease glucose or xylose yields (Figure 12).

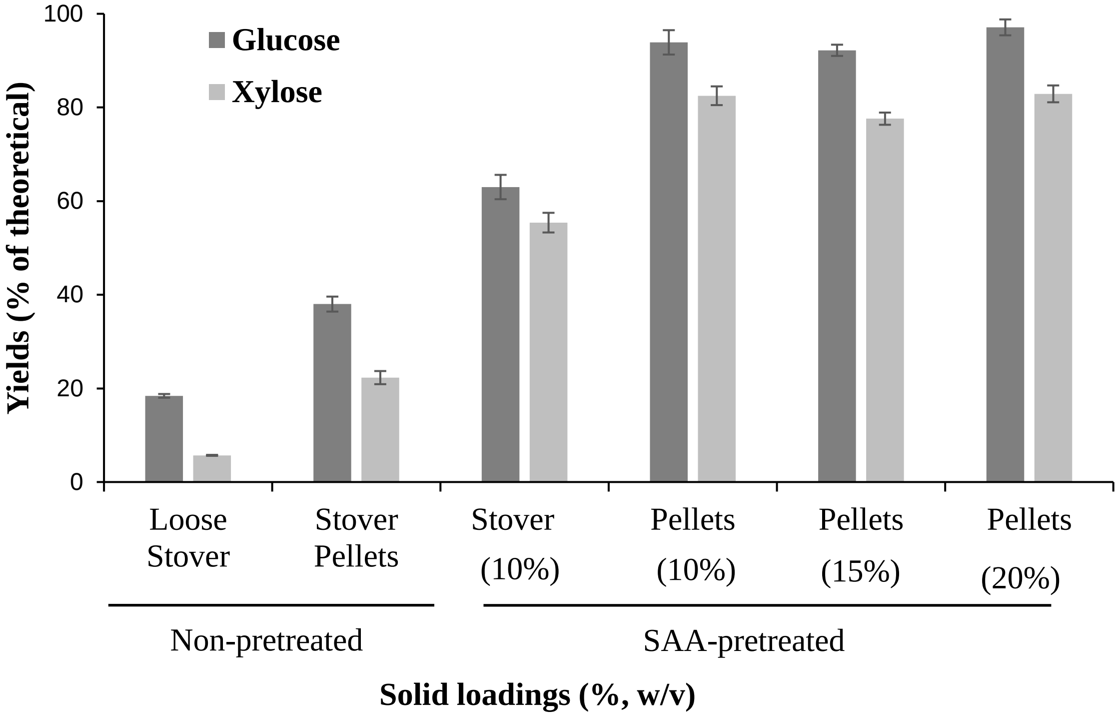

Figure 12. Effects of pretreatment solid loadings on hydrolysis yields at 24 h.

The higher bulk density of pellets allowed higher pretreatment solid loadings without reducing subsequent hydrolysis yields (Figure 12). As pellets rapidly break apart without stirring or using additional mixing equipment, higher solid loadings of pelleted biomass may also simplify pretreatment reactor design. Use of pelleted biomass increased SAA-pretreatment efficacy to achieve 24-h glucose yields over 90% for the tested solid loadings. Xylose yield trends were similar with maximum values of 77% to 83% of theoretical. Therefore, 20% pretreatment solid loadings was chosen for subsequent SAA-pretreatment severity reduction studies with corn stover pellets.

Composition changes of corn stover pellets after SAA-pretreatment

Pretreatment of corn stover pellets was conducted at 30 to 60 °C for 2 to 4 h using 8% to 22% ammonia according to the experimental design outlined in Table 10. The 20 pretreatment conditions were tested, enabling the measured outputs to be modeled across the design space (Table 12). Mild pretreatment conditions, particularly shorter pretreatment times (2 to 4 h) were chosen to examine whether reduced severity pretreatment would still be effective. A previous study (Nahar and Pryor, 2014) showed that a shorter pretreatment time (6 h) was effective for pelleted switchgrass, but further reduction of time and temperature was chosen in this study to test the effectiveness of even milder SAA-pretreatment for pelleted corn stover. The solid recoveries through the pretreatment as well as the compositional changes of the pretreated corn stover pellets at different severity are summarized in Table 12.

The solid remaining (SR), based on the initial biomass weight varied from 72% to 86%, according to the severity of the SAA pretreatment condition. Runs 1 (30 ºC, 2 h, 8% ammonia) and 8 (60 ºC, 4 h, 22% ammonia), were identified as low and high severity pretreatment conditions (Table 12). Solid remaining following SAA pretreatment was 16% lower for the high-severity pretreatment than for the low-severity treatment.

Table 12. Experimental design and results for corn stover pellets pretreated with SAA under different conditions.

Run (Std Order)	Uncoded factors			S.R[1] (%)	Composition (%)					Recovery (%)		Removal (%)	Glucose yield (% theoretical)	
	T (°C)	*H* (h)	*A* (%)		Glucan	Xylan	Lignin[2]	Ash	Cr[3]	Glucan	Xylan	Lignin	Exp[4]	Pred[5]
Untreated corn stover pellet				100	34.7	20.0	21.0	6.5	43.5					
1	30	2	8	85.6	34.5	18.9	19.2	4.0	46.5	85.0	81.0	20.9	60.1	57.5
2	30	2	22	83.3	36.6	20.5	18.9	4.1	50.1	87.7	85.5	24.6	65.7	65.1
3	60	2	8	83.4	38.2	20.6	18.6	3.2	49.9	91.7	86.0	25.5	82.5	82.7
4	60	2	22	79.5	41.4	21.3	14.8	3.9	48.7	94.8	84.6	43.4	88.5	90.3
5	30	4	8	84.9	37.1	20.7	19.1	3.8	47.7	90.6	87.9	22.2	61.4	62.6
6	30	4	22	84.2	40.8	21.3	18.0	3.9	48.9	99.0	90.0	27.3	71.4	70.2
7	60	4	8	75.3	36.4	20.5	15.7	3.6	50.3	78.8	77.2	41.7	86.4	87.7
8	60	4	22	72.0	42.7	21.4	14.2	3.6	49.9	88.6	77.1	50.8	97.8	95.3
9	45	3	8	81.1	35.4	19.8	18.8	4.1	48.0	82.8	80.4	26.8	72.7	72.6
10	45	3	22	78.1	36.6	19.2	17.0	4.2	48.1	82.3	75.3	32.4	77.8	80.2
11	30	3	15	82.3	38.2	21.3	18.4	4.1	49.1	90.6	87.9	27.4	64.3	67.9
12	60	3	15	74.7	43.3	21.9	14.4	3.7	47.3	93.2	81.8	48.5	93.3	93.0
13	45	2	15	83.1	36.1	19.9	17.6	4.1	48.9	86.5	82.7	29.7	78.2	77.9
14	45	4	15	80.8	40.8	21.2	17.6	3.9	46.3	94.8	85.7	31.9	83.3	83.0
15	45	3	15	82.7	37.5	20.1	17.3	3.9	46.8	89.3	83	31.2	81.2	80.5
16	45	3	15	82.4	36.9	21.1	17.5	3.8	47.2	87.6	86.9	30.6	80.4	80.5
17	45	3	15	78.5	37.9	20.7	16.7	4	47.9	85.7	81.1	37.2	83.7	80.5
18	45	3	15	82.6	35.1	20.2	16.7	3.7	49.1	83.5	83.4	33.9	76.6	80.5
19	45	3	15	79.3	35.9	21.1	16.7	4.0	49.3	81.9	83.8	36.3	82.0	80.5
20	45	3	15	82.0	37.7	21.3	16.7	3.8	49.1	89.1	87.4	34.3	81.8	80.5

T: temperature (°C), H: time (h), A: ammonia concentration (%)

[1] S.R: Solid remaining after pretreatment

[2] Lignin: Sum of acid soluble and insoluble lignin

[3] Cr: Crystallinity

[4] Exp: Experimental

[5] Pred: Predicted

Varying pretreatment conditions resulted in a range of glucan and xylan recovery from corn stover pellets. The majority of glucan (81% to 99%) and xylan (77% to 90%) was recovered in the pretreated solids for all pretreatment conditions. The lignin content of pellets decreased from 21% to between 19% and 14% of the initial content of untreated pellets, corresponding to 21% to 51% lignin removal (Table 12). Generally, alkaline pretreatments are more effective for lignin removal without much decrease in carbohydrate content (Kim and Lee, 2007; Sharama et al., 2002). Our results showed the highest delignification of 51% was found for the most severe pretreatment condition (Run 8: 60 ºC, 4 h, 22% ammonia), while the lowest delignification level (21%) occurred with the least severe conditions tested (Run 1: 30 ºC, 2 h, 8% ammonia). The highest lignin removal also corresponded with the lowest recovery of solid material (72%), while highest solid remaining (86%) was found with the least severe pretreatment and lowest lignin removal.

Lignin acts as a physical and chemical barrier that inhibits accessibility of enzymes to the cellulose substrate (Ko et al., 2009), and removal of lignin improves enzymatic digestibility. However, both high solids recovery and effective lignin removal are important to achieve high sugar yields following alkaline pretreatment of cellulosic biomass (Kang et al., 2012). Glucose yields from pelleted corn stover in this study was highly dependent on lignin removal. The lower lignin removal (21%) in the low-severity pretreatment only resulted in 60% glucose yield, while the higher lignin removal (51%) from the high-severity pretreatment resulted in higher glucose yields (98%) from pelleted corn stover. However, even though low-severity pretreatment led to lower delignification of pelleted material than loose stover with, glucose yields were still higher from the pelleted material. Rollin et al. (2011) showed that increasing cellulose accessibility can be more important than delignification in glucan digestibility.

Effects of SAA-pretreatment severity on glucose yield of pelleted corn stover

Enzymatic hydrolysis glucose yields (24 h) for all pretreatment conditions are shown in Table 12. Glucose yield at 24 h, expressed as a percent of the theoretical, was chosen for response surface modeling. Although yields for most or all treatments would be higher at later time points, 24-h hydrolysis yields are more representative of hydrolysis rates. The model was first developed by incorporating all the linear, quadratic, and interaction terms in Eq. (9). None of the model interaction terms, and only one quadratic term (ammonia), were statistically significant ($p > 0.05$). The lack of factor interaction signifies that the effect of each factor in the glucose conversion is independent of the other factors within the design space. Therefore, a reduced model, describing the glucose yield (% of theoretical), R, as a function of ammonia concentration (A), temperature (T), and reaction time (H) was developed as presented in equation (11).

$$\textbf{Glucose model: } R = 8.45 + 0.837(T) + 2.53(H) + 3.02\,(A) - 0.083(A)^2 \qquad (11)$$

The ANOVA table for glucose model is shown in Table 13. All the final model parameters showed a high level of significance ($p \leq 0.002$). The fit of the model was expressed with the coefficient of determination. A strong coefficient of determination ($R^2 = 0.963$ and $R^2_{adj} = 0.954$) was observed indicating model strength in representing the data. This model also passed the lack of fit test ($p = 0.693$) further indicating model adequacy. Furthermore, the experimental values versus the predicted values show a similar quality of fit throughout the design space (Table 12).

Table 13. ANOVA for response surface model of glucose yields (% of theoretical) after 24 h hydrolysis when varying temperature (°C), time (h), and ammonia concentration (%) for SAA-pretreatment of corn stover pellets.

Source of variation	df	Seq SS	Contribution	Adj SS	Adj MS	F	*p*
Glucose Yield							
Model	4	1868.72	96.39%	1868.72	467.18	100.12	0.000
Linear	3	1786.71	92.16%	1786.71	595.57	127.64	0.000
Temperature (*T*)	1	1577.54	81.37%	1577.54	1577.54	338.09	0.000
Time (*H*)	1	64.01	3.30%	64.01	64.01	13.72	0.002
Ammonia (*A*)	1	145.16	7.49%	145.16	145.16	31.11	0.000
Square $A \times A$	1	82.01	4.23%	82.01	82.01	17.58	0.001
Residual Error	15	69.99	3.61%	69.99	4.67		
Lack of fit	10	41.32	2.13%	41.32	4.13	0.72	0.693
Pure error	5	28.68	1.48%	28.68	5.74		
Total	19	1938.71	100%				
			$R^2 = 0.963$	$R^2_{adj} = 0.954$	$R^2_{pred} = 0.935$		

Temperature, time and ammonia concentration for SAA-pretreatment are all important factors in predicting glucose yield. The ANOVA table shows the percent contribution of linear, quadratic terms of the independent variables (Table 13). The correlation between predictors was also checked with the variance inflation factor (VIF). All linear and quadratic terms resulted in VIF of 1 indicating no correlation among the predictors. Linear terms account for the majority of the variation (92%), whereas the quadratic term only accounted for 4% of the variation. Although all the independent variables had a significant effect on glucose yield, temperature had the highest contribution (81%). The variation of each main variable for predicting glucose yield at 24 h also showed that increasing the pretreatment temperature increases glucose conversion. The model shows that at 30 ºC, 24-h average glucose yield is less than 70%, but average yields reach 94% at 60 ºC (Figure 13). The concentration of ammonia was a relatively important source of variation (approximately 7.5%). Because no factor interaction was detected, the effect of each factor could be illustrated individually by plotting the response against each individual factor while holding the remaining factors constant at their midpoint (Figure 13) .The model predicts that 24-h average glucose yields of 73% can be achieved with 8% ammonia, but average yields increase to 82% with 18% ammonia. Pretreatment reaction time between 2 and 4 h has a lower impact on results (3.3% of variation) than temperature or ammonia concentration, although the effect was still significant.

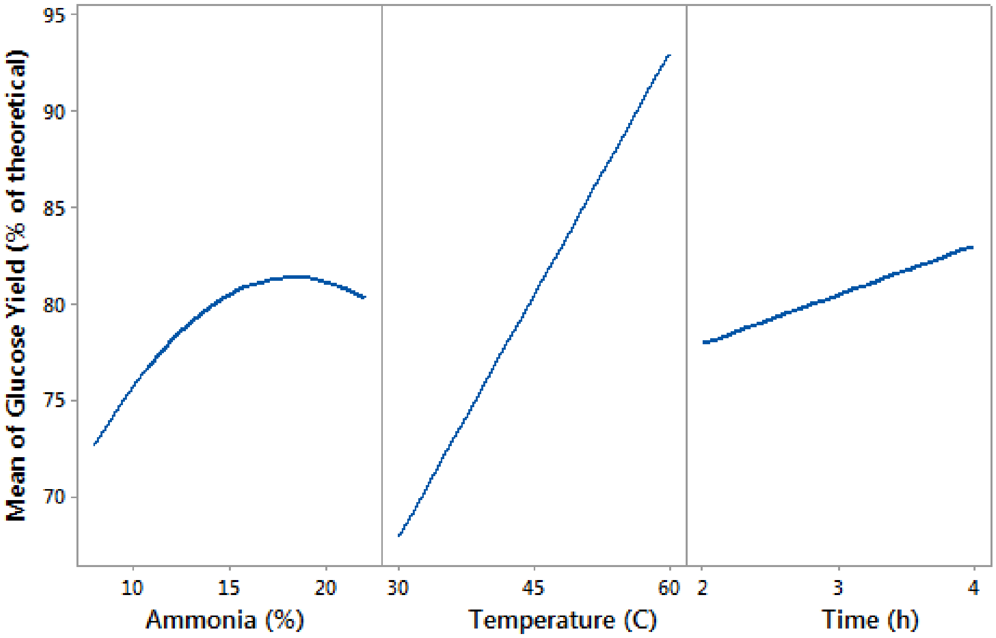

Figure 13. Average responses for each main variable across range of all other conditions.

Response surfaces were drawn as contour plots of the second order polynomial model (Eq. 11) to illustrate the combined effects of the three variables. Glucose yield was plotted as a function of pretreatment temperature, time and ammonia concentration (Figure 14). Although time was statistically significant in the model, the actual effect was much less than that of either temperature or ammonia concentration.

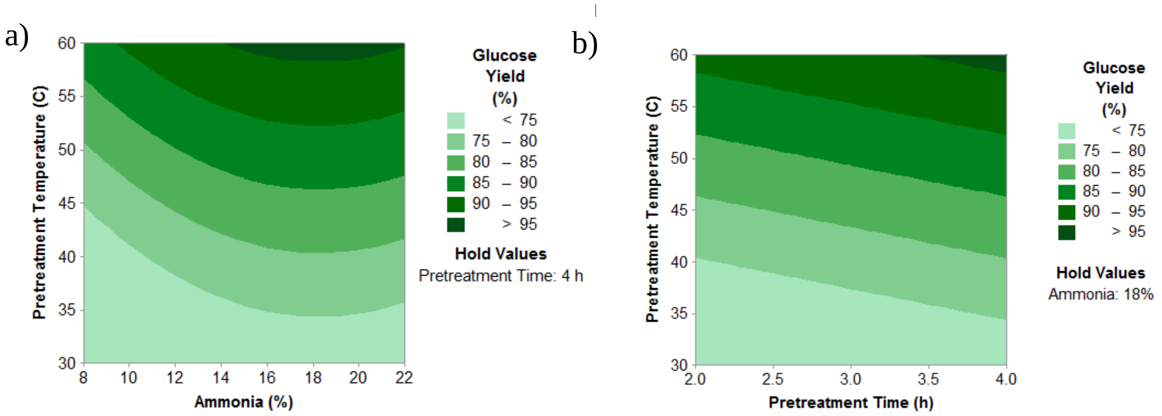

Figure 14. Contour plots of combined effects of a) ammonia and temperature b) pretreatment temperature and time, on 24-h hydrolysis glucose yields from SAA-pretreated pelleted corn stover.

Figure 14a shows that ammonia concentrations of 18% reduce the temperature required to achieve a given hydrolysis yield. The response to changes in temperature and time while holding ammonia concentration at 18% (Figure 14b) shows that there is less variation in response as pretreatment time increases within the design space. The model predicts a maximum 24-h glucose yield within the design space of 96.4%, with a temperature of 60 ºC for 4 h with an 18% ammonia concentration. However, lower yields may be more cost effective based on the greater incremental cost expected to achieve yields approaching 100%. Yields of 90% could be targeted either by reducing pretreatment severity as shown in this study or by reducing hydrolysis times below 24 h.

Model validation

Several combinations of pretreatment parameter levels for which the model predicts 24-h glucose yields of 90% were tested experimentally for model validation. Ammonia concentrations were held constant at 18% and the model was used to determine treatment conditions for validation. The contour plot shows that there are many scenarios of temperature and time that will yield 90% glucose yields at the optimum ammonia concentration. The three combinations selected for validation are shown in Table 14. The differences between the predicted and the observed glucose yields were within 3% for all validation treatments (Table 14).

Table 14. Three experimental runs to validate model results.

Validation set	Factors			Glucose yield at 24 h (% theoretical)	
	Temperature (°C)	Time (h)	Ammonia (%)	Predicted	Experimental
1	57	2.5	18	90	89
2	56	3	18	90	92
3	54	3.5	18	90	87

Characterization of SAA- pretreated corn stover pellet at different severity

FTIR

Infrared spectrum analysis for the untreated loose and pelleted corn stover, and SAA-pretreated stover pellets at increasing pretreatment severities is shown in Figure 15. The FTIR spectra of untreated loose stover showed a relatively narrow band at 2925 cm^{-1}, and two CH bands at 2850 and 2925 cm^{-1}. These results indicate that the pelleting process affects plant cuticular waxes, which have been linked to these bands (Merk et al., 1998) (Figure 15a). Compared to loose stover, the spectra for pellets also showed reduced intensity at 1730 cm^{-1}. This band is attributed to C=O bonds and lower intensity indicates a disruption of acetyl and uronic ester groups in hemicellulose and/or lignin (Li et al., 2014; Xu et al., 2015) during pelleting (Figure 15a).

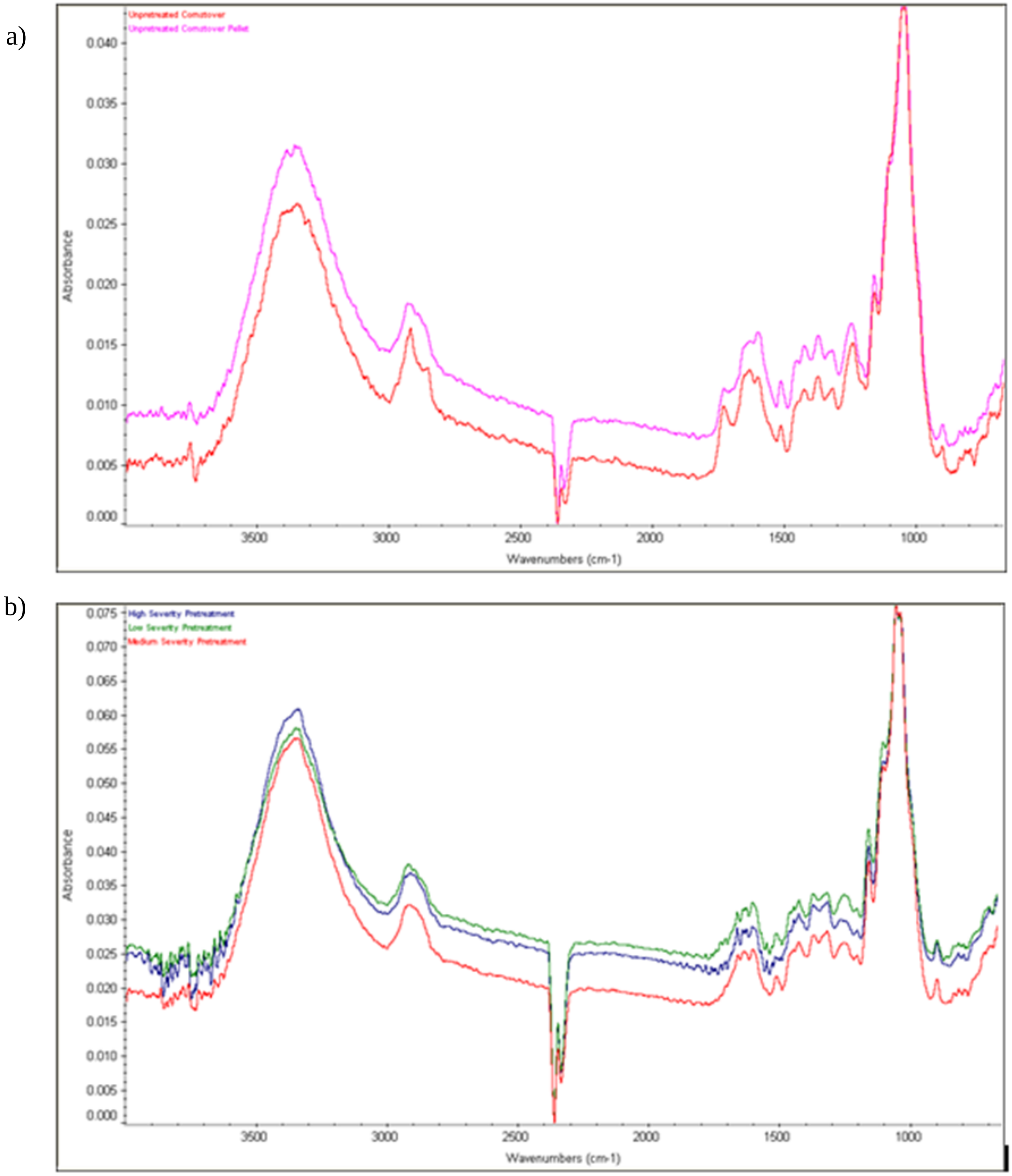

Figure 15. The FTIR spectra of a) untreated corn stover and pellets, and b) SAA-pretreated corn stover pellets. Pretreatment conditions: low severity (30 °C, 2 h, 8% ammonia); medium severity (45 °C, 3 h, 15% ammonia); high severity (60 °C, 4 h, 22% ammonia).

Removing the linkages between cellulose, hemicellulose, and lignin is a crucial step to improve enzyme accessibility and cellulose degradation (Faulon et al., 1994). The characteristic absorption bands for lignin and hemicellulose linkages (C−O−C) at 1462, 1425 and 1320 cm^{-1} for the pretreated pellets were considerably less predominant than those for untreated pellets (Figure 15b). Another linkage through ether bonds between lignin and hemicellulose at band1250 cm^{-1} (ar-C−O−C-al) is lower for all pretreated samples than for untreated pellets. These result suggests that lignin and hemicellulose linkages are partially cleaved by SAA-pretreatment.

FTIR spectral profiles of the SAA-pretreated samples at different pretreatment severities are not considerably different, indicating similar structure of hemicelluloses and lignin following the tested conditions. However, the relative intensities of some bands diminished with increasing severity indicates partial or full cleavage of certain bonds of hemicellulose and lignin. Ether bonds between lignin and hemicellulose at 1250 cm^{-1} were lower for pretreated pellets, but the weakest bands was seen at the high severity pretreatment. This suggest that the severity of pretreatment determines the degree of disruption and affects hydrolysis yields. Although there was a strong reduction of the 1730 cm^{-1} band (C=O) for all pretreated pellets, no differences were noted in the intensity with different pretreatment severities (Figure 15b). The intensity of the band at 1510 cm^{-1}, associated with C=C stretch in lignin (Xiao et al., 2001; Xu et al., 2015), decreased for pretreated pellets. The lowest peak intensity was noted at the most severe pretreatment condition and correlates with the higher lignin removal for this pretreatment. This analysis agrees with coefficient of determination ($R^2 = 0.78$), that 78% of the variability can be explained by lignin removal.

Crystallinity

Crystallinity of untreated corn stover pellets increased from 43% to as much as 50% after SAA pretreatment (Table 12). Low-severity pretreatment (Run 1) had a lower percentage of crystallinity (46.5%) than the high severity pretreatment (Run 8; 50%). However, there was no clear trend in crystallinity change depending on the severity of the pretreatment in terms of temperature, time, or ammonia concentration (Table 12). This result agrees with the FTIR spectra of crystalline cellulose at different severity (Figure 15b). The bands at 1030 cm^{-1} are related to C−O and C−C stretching, structural features of the crystalline cellulose component, and are identical and strong for all pretreated samples suggesting no structural changes to cellulose by SAA-pretreatment. The increased crystallinity of pretreated corn stover pellets in our study is likely due to solubilization of amorphous components. Increased crystallinity after pretreatment has also been observed in many previous studies with both acid and alkali pretreatments (Chang and Holtzapple, 2000; Kim et al., 2003; Kim and Holtzapple, 2006; Liu et al., 2009). Although cellulose crystallinity has been considered an important factor for enzymatic hydrolysis (Van Dyk and Pletschke, 2012), our results did not show any correlation between crystallinity and glucose yield ($r = 0.15$). As seen in FTIR results, non-crystalline, amorphous components such as hemicellulose and lignin are selectively removed by SAA-pretreatment, increasing the relative amount of cellulose in the pretreated solid fraction. The relative increase in crystallinity did not have any negative impact on hydrolysis yield.

Effects of pretreatment of the surface structure of corn stover pellet

The SEM micrographs of untreated corn stover and stover pellets are shown in Figure 16 (a, b). The surface structure of the untreated corn stover exhibited more rigid and ordered fibrils than corn stover pellets. The fiber was slightly distorted and surfaces appear rough for pelleted material due to disruption of semi-macro structure. The rougher surfaces and increased surface area may contribute to the significant glucose yield improvements for pelleted corn stover compared with the loose material. Although no specific mechanism has been identified for increased yields for pelleted biomass, high shearing force along with heat development in the pelleting process has been accredited to partial deconstruction of biomass structure, which may increase enzyme accessibility to cellulose (Theerarattananoon et al., 2012).

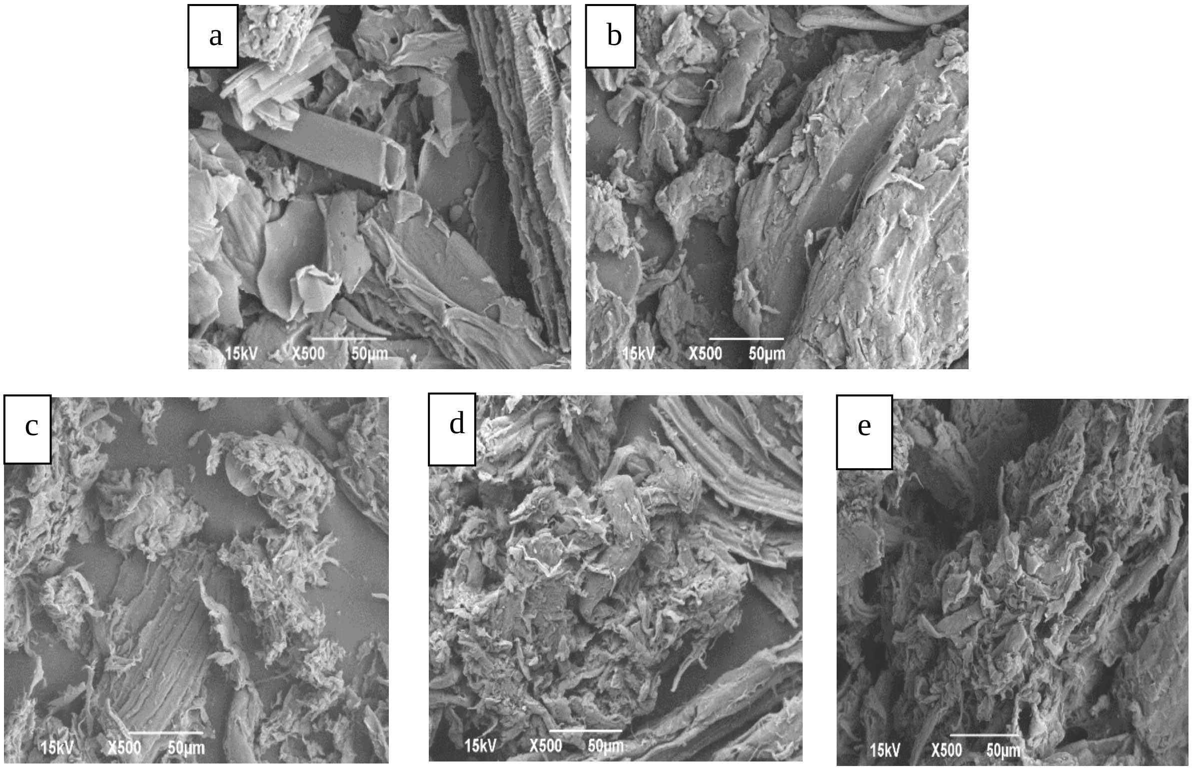

Figure 16. SEM images of untreated a) loose corn stover, and b) corn stover pellets, and pretreated corn stover pellets with c) low severity (30 °C, 2 h, 8% ammonia), d) medium severity (45 °C, 3 h, 15% ammonia), and e) high severity (60 °C, 4 h, 22% ammonia).

Changes in the biomass structure within corn stover pellets with increasing pretreatment severity is shown in Figure 16 (c–e). SEM observations showed that the SAA-pretreatment induced physical changes in the pelleted biomass. Cellulose fibers were peeled off and separated from the initial connected structure after pretreatment. The fibers of SAA-pretreated samples were shortened, loosened, and exposed due to the disruption or removal of hemicelluloses and lignin and this effect increased with higher pretreatment severity (Figure 16e). Cellulose hydrolysis yield is closely related to the removal of hemicellulose and lignin (Yu et al., 2010). Changes in the SEM photos of the pretreated pellets corresponded with glucose yield. The disruption of the fibers in the SAA-pretreated samples increase surface area, which improve enzyme accessibility and hydrolysis yield (Kang et al., 2012).

Conclusions

This study showed that corn stover pelleting allows SAA pretreatment to be effective at lower temperatures, with lower ammonia concentrations, and for shorter pretreatment times. High temperatures and pressures in the pelleting process modify the chemical structure of pelleted biomass to impact pretreatment efficacy. Using pelleted corn stover allowed an increase in pretreatment solid loadings by a factor of two without reducing glucose yields. Within the design space, temperature was more important than pretreatment time or ammonia concentration for achieving high glucose yields with pelleted biomass. The model showed that with 4–h pretreatment, 24–h hydrolysis glucose yields of 90% can be achieved with temperatures as low as 53 °C or ammonia concentrations as low as 10%. High glucose yields from pelleted corn stover using low-severity pretreatment suggests economic and environmental benefits of biomass pelletization beyond those associated with transportation and handling.

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PAPER 3: LIFE CYCLE PERSPECTIVES FOR USE OF CORN STOVER PELLETS IN A CELLULOSIC BIOREFINERY [3]

Abstract

Cellulosic biorefineries have attracted interest due to growing energy security concerns, and environmental concerns related to the current energy and chemical production. Using pelleted biomass as a biorefinery feedstock can reduce processing inputs while improving biomass handling and transportation. However, it is still questionable whether energy and emissions savings from transportation and low severity processing can offset additional emissions and energy use during pellet production. A life cycle assessment approach was used to compare primary fossil energy use and greenhouse gas (GHG) emissions from using pelleted and non-pelleted corn stover as a biorefinery feedstock. Operations considered were densification, transportation, and soaking in aqueous ammonia (SAA) pretreatment. Transportation energy use and GHG emissions were 25% lower with pelleted corn stover than with non-pelleted material. Substantial GHG emissions were associated with the pelleting process. Emissions from pelleting were up to 25 times as much as those from transportation of either non-pelleted or pelleted biomass. In spite of significant GHG emission from the pelleting process, the model showed the opportunity to offset and even reduce overall GHG emissions considering pretreatment process changes. Our process energy analysis showed that SAA pretreatment was unrealistic for non-pelleted material because of high energy requirements for maintaining reactor temperature for an extended time (24 h). However, SAA-pretreatment of pelleted biomass required significantly lower energy inputs (89%) due to the effectiveness of lower severity pretreatment. Higher

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[3] This paper will be submitted for publication under the authorship of Nurun Nahar and Scott Pryor. Nurun Nahar had primary responsibility for collecting and analyzing laboratory data, drafting the paper and primary developer of the conclusions.

pretreatment solid loadings possible with pelleted biomass also reduced the amount of chemicals and water used in pretreatment by 56% and 49%, respectively. This study demonstrated that although SAA-pretreatment of non-pelleted cellulosic biomass may be unrealistic, it may be a feasible option when using pelleted biomass as the biorefinery feedstock.

Introduction

Biobased energy development has potential to contribute to energy independence, rural economic development, and environmental benefits. However, the large quantities of baled or loose biomass required to supply an industrial scale biorefinery causes significant challenges for biomass transportation, handling and storage due to low feedstock bulk density. The economic and technical aspects related to cellulosic biomass conversion, such as cost-effective pretreatment and biomass transportation, are crucial to development of large-scale biorefineries.

Biomass densification has been receiving significant attention because densified biomass could provide a uniform feedstock beneficial for large-scale biorefineries. Several studies investigating logistics systems for transporting baled or ground/chopped biomass from a field to a conversion facility showed that higher biomass bulk density improves biomass handling and reduces transportation cost (Hess et al., 2007; Petrolia, 2008; Sokhansanj and Fenton, 2006). Biomass densification, including pelleting, results in biomass bulk densities that are 4−7 times higher than baled biomass (Sultana et al., 2010) leading to storage, handling and transportation benefits. Feedstock costs are 35% to 50% of the total production cost of cellulosic ethanol, and transportation logistics constitutes 50% to 75% of those feedstock costs (Hess et al., 2007; Sokhansanj and Fenton, 2006).

Densified biomass has not been considered as feedstock for biorefineries despite the interest on uniform feedstock and better supply logistics because transportation cost savings were thought to be largely offset by densification costs (Sokhansanj and Fenton, 2006; Sultana et al., 2010). However, the interaction or tradeoff between densification and conversion processing within the system has not been considered in these technoeconomic analyses. Process synergies between densification and pretreatment efficacy for biochemical conversion reported in recent studies documented that pelleted biomass has the potential to lower pretreatment cost (Nahar and Pryor, 2017; 2014).

Pretreatment, a crucial processing step to reduce the natural recalcitrance of biomass for biological processing, still poses a significant economic challenge due to costs and environmental effects associated with severe pretreatment process conditions: high temperature, time, and chemical inputs. Though pretreatment accounts for almost 20% to 30% of the total cost of biofuel production (Yang and Wyman, 2008), very few studies have looked at the key influence of energy consumption during different pretreatment processes. Reductions in pretreatment temperature, time, and ammonia concentrations for soaking in aqueous ammonia (SAA) pretreatment of pelleted corn stover were reported by Nahar and Pryor (2017). However, energy consumption reductions associated with such reduced severity pretreatments of densified biomass need to be quantified to assess the economic and environmental benefits of processing densified biomass.

Life cycle assessment (LCA) is a quantitative method to quantify specific environmental and energy use impacts of biomass energy chains as well as other products and processes. Life cycle carbon footprints are increasingly used to identify the contribution of a product or process to climate change. Biomass production, particularly fertilization-related processes and soil emissions, contribute significantly to the overall impact of cellulosic biofuels (Biswas et al., 2008; Clair et al., 2008; Kim and Dale, 2004, 2005; Pelletier et al., 2008).

Biomass pelleting has also been studied to model pellet physical characteristics, as well as the energy consumption economics, and environmental impacts such as global warming, acidification, eutrophication, and human toxicity. (Adapa et al., 2009, 2010; Jannasch et al., 2001; Li et al., 2012; Mani et al., 2005, 2006a, 2006b; Pastre, 2002; Sokhansanj and Fenton, 2006). Although several studies (Guragain et al., 2013; Li et al., 2014; Nahar and Pryor; 2014, 2017; Rijal et al., 2012) reported higher hydrolysis yields due to pelleting, no studies have quantified pelleting impact on pretreatment in terms of energy and greenhouse gas (GHG) savings. Documenting the process synergies of pelleted and non-pelleted forms of biomass will provide insight on how to improve system efficiency and reduce the environmental impact for an industrial scale biorefinery.

The objective of this study was to analyze the energy requirement and GHG emission trade-offs for the production, transportation, and processing of pelleted and non-pelleted biomass in a cellulosic biorefinery using soaking in aqueous ammonia (SAA) pretreatment. Corn stover (*Zea mays L.*) is used as a model feedstock because it is considered a likely large-scale biorefinery feedstock in the U.S. due to high corn production (Aden et al., 2002; Perlack et al., 2005).

Materials and methods

An LCA approach was used to assess the fossil energy input and greenhouse gas emissions impacts of transporting and processing pelleted biomass in comparison to non-pelleted biomass delivered as bales (ISO, 2006 a,b). Stages included goal and scope definition, inventory analysis, impact assessment, and interpretation of results. Inventory data were compiled from laboratory results, relevant literature, and GREET (Greenhouse Gases, Regulated Emissions, and

Energy Use in Transportation) databases. Life-cycle primary energy inputs, and GHG emissions were calculated in a Microsoft Excel spreadsheet.

Goal, system boundaries, and functional unit

The goal of this study was to investigate the variations in primary energy inputs and GHG emissions for a comparative LCA by using non-pelleted (base case scenario) and pelleted (densification scenario) forms of corn stover in an industrial scale biorefinery. The system boundaries include all feedstock transportation from the field edge to the biorefinery, densification (as per scenario), and pretreatment sufficient to reach 90% theoretical glucose yields with moderate enzyme loadings. The functional unit of the assessment is 1 Mg of biomass.

The production chain of cellulosic biofuel includes biomass production, harvest, collection and transportation to the biorefinery. Biomass is ground with a hammer mill before pretreatment and subsequent enzymatic hydrolysis to produce fermentable sugars, which can be fermented to biofuel or other chemicals. The system configurations for production through processing of non-pelleted and pelleted biomass are illustrated in Figure 17. Only the processing stages or steps inside a box (as shown in Figure 17) were included in this study. The boundary for the base case scenario (Figure 17a) includes transportation of baled biomass from field edge to the biorefinery, and pretreatment sufficient to reach 90% glucose yields. The system boundary for densification scenario (Figure 17b) includes transportation of baled biomass to the nearest depot for preprocessing and pelleting, delivery of the pelleted biomass to the biorefinery, and pretreatment to reach 90% glucose yields. Cellulase, β-glucosidase, and hemicellulase were assumed to be 15 FPU g^{-1} glucan, 15 CBU g^{-1} glucan, and 300 XU g^{-1} glucan, respectively for enzymatic hydrolysis. Because the amount of sugar generated from each feedstock is same for both scenarios, results can be compared on a feedstock mass basis.

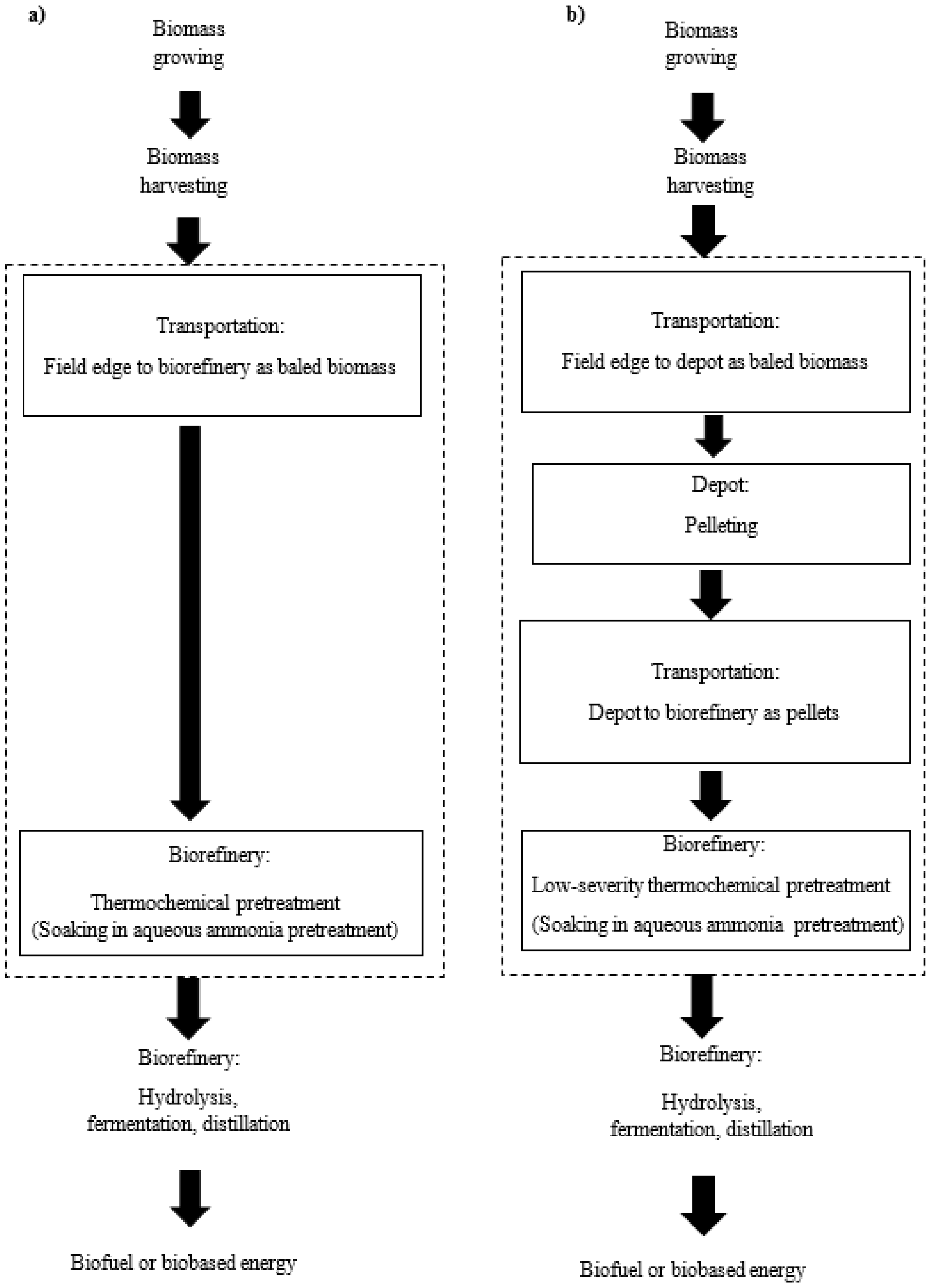

Figure 17. Supply and processing systems for a) base case scenario and b) densification scenario. Only process stages in a box were included in this study.

The primary goal is to quantify differences in energy inputs and carbon emissions associated with transportation, densification, and biomass processing using SAA as an example pretreatment. Pretreatment is the most energy intensive processing step and primary processing differences based on feedstock form. Comparison of the different process conditions for pretreatment of both non-pelleted and pelleted biomass was included. Primary fossil energy use and GHG emissions were accounted for within the system boundaries. Life-cycle GHG emissions related to the transportation, pelleting and pretreatment were calculated as kg of CO_2 equivalent Mg^{-1} of delivered feedstock using the 100-year global warming potential (GWP). The term CO_{2eq} is the weighted sum of CO_2, CH_4 and N_2O emissions weighted with the accepted global warming potential factors of 1, 23 and 298, respectively (EPA, 2009).

The feedstock production system was excluded from the system boundary in this study because agricultural inputs will be identical for both scenarios. Likewise, other processing steps, including fermentation, distillation and fuel distribution were not included because inputs for those processes are not expected to be significantly different for the compared scenarios. While GHG fluxes associated with indirect land use change (ILUC) impacts are recognized as an important to the overall lifecycle, it will not affect the comparative LCA of our study because agricultural production and hydrolysis yields are assumed identical.

Impact categories included in this study were fossil energy and GHG emissions. Impacts were assessed for input diesel fuel and electricity production, as well as for pretreatment chemical inputs. Fossil energy and GHG emission were collected from the Greenhouse Gases, Regulated Emissions, and Energy Use in Transportation (GREET) model developed by the Argonne National Laboratory (GREET, 2016) and listed in Table 15. Fossil energy use and emissions were calculated based on a functional unit of 1 Mg. Embodied energy for the facility structure and equipment including pellet mill, reactors, and transportation vehicles were assumed to have a minor contribution over the project term and were ignored in this study.

Table 15. Fossil energy and GHG emission inputs from GREET (Greenhouse Gases, Regulated Emissions, and Energy Use in Transportation).

Parameter	Fossil fuel (MJ)	GHG emission (kg CO_2eq)
Electricity production (MJ) (Distributed U.S. mix)	1.817	0.15
Conventional diesel (L) (Conventional diesel from crude oil for U.S. refineries)	43	0.67
Sulfuric acid production (kg)	0.608	0.049
Ammonium hydroxide production (kg)	20	1.31

Life cycle inventory (LCI)

Life cycle inventory data required for the studied system are listed in Table 16. Data was collected from the Greenhouse Gases, Regulated Emissions, and Energy Use in Transportation (GREET) model developed by the Argonne National Laboratory (GREET, 2016), peer-reviewed literature, and laboratory experiments (Table 16). The data for pretreatment conditions required for 90% glucose yield production were taken from our laboratory studies. Process differences between feedstock form (non-pelleted and pelleted) included feedstock transportation, densification, and pretreatment conditions.

Table 16. Data required for the studied system.

Unit process	Data required	Data source
Biomass transportation	Fuel economy of trucks, emissions and energy use for transporting baled biomass to a preprocessing depot or biorefinery	GREET, 2016
	Feedstock collection and supply radius	Ebadian et al., 2017; Perlack and Turhollow, 2003
Densification and transportation of pelleted biomass	Pelletization energy	Sokhansanj and Fenton, 2006
	Emissions from electricity for pelletization	GREET, 2016
	Fuel economy of trucks, emissions and energy use for transporting pelleted biomass	Wilson et al., 2014; GREET, 2016
Pretreatment	Production scale	Humbird et al., 2011
	Processing campaign length	Humbird et al., 2011
	Water requirements	Laboratory results
	Chemical requirements	Laboratory results
	Pretreatment reactor (size and materials)	Humbird et al., 2011

Feedstock availability

Corn stover is one of the most abundant lignocellulosic resource available and is widely considered a primary feedstock for cellulosic biofuels (Aden et al., 2002; Perlack et al., 2005). Corn stover consists of the stalks, cobs, and husks and leaves left in the field to dry following grain harvest. We assume corn stover is field-dried to 15% moisture content (w.b.) when collecting bales from field edge during transportation.

An annual biomass supply of 1,000,000 Mg yr^{-1} (dry basis) was assumed for both scenarios. All assumptions made for corn stover availability and collection are given in Table 17. Supply radius for a biorefinery depends on corn stover availability; which depends on many factors including: grain yield, stover to grain ratio, available stover collection fraction based on soil conservation requirements, percent of acreage growing corn, and the proportion of farmers participating. All of these factors may vary significantly across regions. We assumed that corn stover was uniformly distributed and collected from a circular area around the biorefinery. We also assumed that the biomass was uniformly distributed within the circular area. Based on assumptions in Table 17, a 87 km (54 mile) radius is necessary to collect 1,000,000 Mg biomass for the base case. Collection area, transportation distance, and other aspects of each scenario were calculated based on the required supply of 1,000,000 Mg yr^{-1} baled or pelleted corn stover delivered to a biorefinery.

Table 17. Assumptions and calculated values used for a commercial scale cellulosic biorefinery.

Assumptions/Variables	Value	Source
Annual corn stover demand	1,000,000 Mg	
Road tortuosity factor	1.3	Humbird et al., 2011; Perlack and Turhollow, 2002
Biorefinery annual operating days	350 days	Humbird et al., 2011; Wu et al., 2006
Corn grain yield	1176 Mg km^{-2} (4.76 tons ac^{-1})	USDA, 2017
Stover:grain yield	1:1	Perlack and Turhollow, 2003
Corn stover harvest rate	35%	Perlack and Turhollow, 2003
Average harvested corn stover	311 Mg km^{-2} (1.26 tons ac^{-1})	
Percent of acreage growing corn	45%	Ebadian et al., 2017
Farm participation rate	30%	Rosburg et al., 2017

Transportation

Baled corn stover from the field edge is loaded on trucks and transported either directly to the biorefinery in the base case scenario or to a processing depot for pelleting in the densification scenario.

For the base case scenario, a heavy-duty diesel truck with a capacity of 16 Mg (Krishnakumar and Ilelji, 2010) was assumed to deliver baled biomass from the field edge to the biorefinery. The average distance between the center of a circle and any other point within the circle is 2/3rd of the full radius (Morey et al., 2010). Therefore, the average delivery distance (field edge to biorefinery) for all biomass within the collection area is 58 km (36 miles).

In the densification scenario, most corn stover is assumed to be transported from field edge to a pelleting depot within 16 km (10 mile) draw radius as baled biomass and then from pelleting depot to biorefinery as pelleted biomass. The biomass produced within 22 km of the biorefinery (7% of total biomass) was assumed to be delivered directly to the biorefinery as bales, where biomass can be pelleted on site before pretreatment. Pellet mills are distributed at distances of 38 km and 70 km from the biorefinery, resulting in two collection rings around the biorefinery (Figure 18). The stover produced more than 22 km from the biorefinery (93% of total biomass) was only transported an average of 10 km from field edge to the pelleting depot as bales; the stover is transported in pelleted form from the pellet mill to the biorefinery is thus either 38 km (33% of total biomass) or 70 km (60% of total biomass) (Figure 18).

A first set of 10 pellet mills was assumed to be located 38 km away from the biorefinery to collect all biomass produced between 22 and 54 km away from the biorefinery (Figure 18). A second set of 19 pellet mills was located 70 km away from the biorefinery to collect biomass between 54 and 87 km (Figure 18). Considering other assumptions listed in Table 19, each pelleting depot would have a capacity of 34,000 Mg yr^{-1}. This size of pelleting depot would need to process biomass at 4.1 Mg h^{-1}, assuming 24-h pelleting operation in 350 days a year.

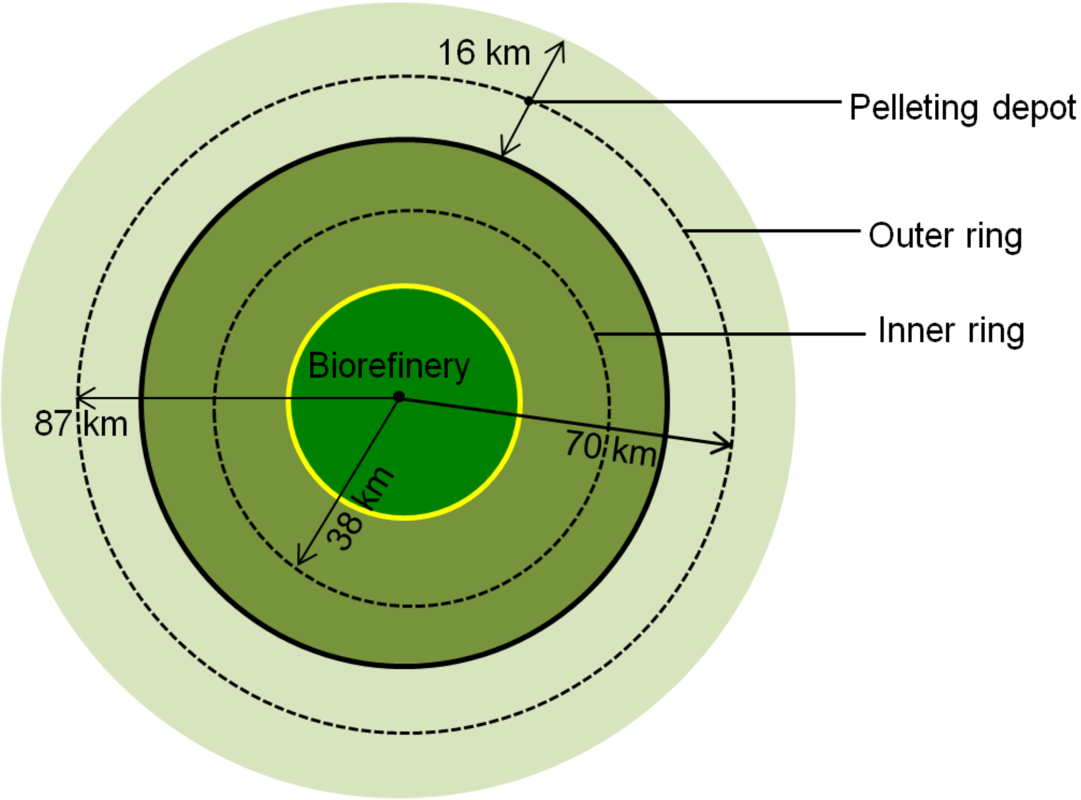

Figure 18. Schematic of pelleting depot and transportation in densification scenario (not to scale).

In the densification scenario, pelleted biomass was assumed to be transported to the biorefinery via a heavy-duty diesel truck with a capacity of 27.3 Mg as used for corn grain (Wilson et al., 2014). A tortuosity factor of 1.3 was used for both scenarios to account for nonlinear paths from the depot to the biorefinery (Humbird et al., 2011; Perlack and Turhollow, 2002). An average fuel efficiency of 2.76 km L^{-1} was considered in this study, which was average of high and low efficiency (3.4 km L^{-1} and 2.13 km L^{-1}) of a commercial semi-trucks, assuming low efficiency when the truck is loaded and high efficiency on the empty return trip (Wilson et al., 2014). The assumptions made for transportation were listed in Table 18.

Table 18. LCA system assumptions for transportation.

Parameter	Non-pelleted	Pelleted
Biorefinery processing capacity (Mg yr^{-1})	1,000,000	1,000,000
Pelleting depot processing capacity (Mg yr^{-1})	--	34,000
Truckload capacity (Mg)	16[a]	27.3[b]

Source: [a] Krishnakumar and Ileleji, 2010; [b] Wilson et al., 2014.

Pelletization

Pellet production includes a combination of sequential operations: grinding, pelleting, cooling, and screening. Dried corn stover (~15% moisture) is milled in a hammer mill (6-mm screen) before feeding to the pellet mill. Ground feedstock is then fed into a pelleting chamber where additional grinding occurs and rollers force the material through holes on the inside face of a 6.3 mm die. High pressures and temperatures generated in the pellet mill soften lignin and bind the biomass particles together to make uniform and durable pellets. Finally, the pellets are cooled before further handling and transportation.

The biomass pelleting requires electricity for milling, pelleting, cooling, and screening. Pelleting process energy was taken from Sokhansanj and Fenton (2006) and only included energy for the pellet mill, cooling, and screening. Drying and grinding were not modeled in the pellet production process because: i) stover was assumed to be field-dried to a moisture content of 15% (w.b.) for both scenarios, and ii) biomass grinding using a hammer mill with a 6-mm screen was assumed to be required prior to pelleting in the densification scenario or as the first step of bale processing at the biorefinery in the base case scenario.

The pelleting process requires only energy (electricity) and corn stover as inputs and produces pellets as the only output. Small amounts of liquid water or steam are sometimes added to improve the pelleting process, but this input was not included in this study. Pelletization does not create any solid waste stream. All residues from the process are recycled, and airborne particulate emissions (dust) were assumed to be insignificant and not included in the analysis.

Pretreatment

In soaking in aqueous ammonia (SAA) pretreatment, biomass is submerged in a diluted ammonia solution for a period of hours at a relatively low temperature. Our previous study (Nahar and Pryor, 2017) quantified the pretreatment parameters (pretreatment solid loadings, temperature, time and ammonia concentration) required to reach 90% glucose yields at 24 h of enzymatic hydrolysis for pelleted biomass. Pretreatment parameters to reach 90% glucose yields at 24 h for non-pelleted biomass were taken from preliminary lab experiments. The enzymes cellulase, β-glucosidase, and hemicellulase were added at rates of 15 FPU g^{-1} glucan, 15 CBU g^{-1} glucan, and 300 XU g^{-1} glucan, respectively for both non-pelleted and pelleted biomass. The pretreatment parameters for base case and densification scenarios are listed in Table 19.

Table 19. LCA system assumptions for SAA-pretreatment.

Parameter	Base case scenario (Non-pelleted)	Densification scenario (Pelleted)
Solid loadings (%)	10	20
Temperature (°C)	60	56
Residence time (h)	24	4
Ammonia (%)	15	12

Soaking in aqueous ammonia pretreatment requires a simple reactor configuration for holding the biomass slurry at a moderate temperature. A cylindrical steel reactor, as described for hydrolysis by Humbird et al. (2011) was assumed as SAA-pretreatment reactor in this study. Reactor specifications are shown in Table 20.

Table 20. SAA-pretreatment reactor specifications (Humbird et al., 2011).

Items	Specifications
Material	Stainless steel
Capacity	950 m^3
Working volume of reactor	618 m^3
Diameter	5.79 m
Height	36.57 m
Insulation	Glass wool (0.05 m)

Biomass is first mixed with aqueous ammonia to obtain 10% solid loading for non-pelleted biomass, and 20% solid loading for pelleted biomass (Nahar and Pryor, 2017). The mixture is heated with steam to the desired pretreatment time and temperature as outlined in Table 19. Once the pretreatment is completed, pretreated biomass is discharged into a flash tank for cooling and neutralization with sulfuric acid. After neutralizing, the mixture is transferred to another reactor for enzymatic hydrolysis. Intermediate time for reactor set-up was assumed to be 4 h for both scenarios.

The energy requirement for pretreatment is assumed to be supplied from steam generated by burning biomass lignin residue. Energy from lignin combustion exceeding heating needs would be used to produce electricity. Pretreatment energy requirements were calculated using the energy necessary to increase the temperature from 25 °C to the assumed pretreatment temperature, and the energy necessary to maintain that temperature throughout pretreatment based on reactor contents, geometry, and insulation (Holman, 1992).

The supply of steam to the pretreatment reactor to reach the desired temperature with non-pelleted or pelleted corn stover was calculated using equation (12):

$$Q_1 = m \times C_p \times \Delta T \tag{12}$$

where,

Q_1 = Energy to increase the pretreatment temperature, MJ Mg^{-1}

m = mass of the reactor contents, Kg (biomass and added liquid)

C_p = Specific heat capacity for ammonia solution and biomass mixture,

C_p (ammonia) = 4.86 KJ $Kg^{-1}.K^{-1}$ (Engineering Toolbox)

C_p (biomass) = 1.03 KJ $Kg^{-1}.K^{-1}$ (Mafe et al., 2015)

C_p (ammonia solution and biomass mixture) = 4.495 KJ $Kg^{-1}.K^{-1}$ (base case scenario)

C_p (ammonia solution and biomass mixture) = 4.194 KJ $Kg^{-1}.K^{-1}$ (densification scenario)

ΔT = Pretreatment temperature increase depending on the biomass forms (environmental temperature was set at 25 ºC)

For the pretreatment reactor, we assumed heat to maintain the temperature is equal to heat loss during the process. Heat loss was assumed to be constant through the side wall as well as through the top and bottom of the cylindrical reactor. The total heat necessary to maintain the temperature during the pretreatment process was calculated considering the pretreatment residence time using equation 13.

$$Q_2 = U \times A \times \Delta T \times t \tag{13}$$

where,

Q_2 = Energy to maintain the pretreatment temperature, MJ Mg^{-1}

U = Overall heat transfer coefficient, (W m^{-2}.K)

A = Area of the reactor, m^2

ΔT = Pretreatment temperature increase depending on the biomass forms (environmental temperature was set at 25 ºC)

t = Pretreatment time (h), 24 h (base case scenario) and 4 h (densification scenario)

U is the overall heat transfer coefficient for the insulated reactor. To calculate overall heat transfer coefficient, i) conduction for stainless steel reactor, ii) conduction for insulation and iii) convection of outside of the reactor were included. Convective heat transfer inside the reactor was not included. The overall heat transfer coefficient for the cylindrical wall and top and bottom of the reactor was calculated using equation (14):

$$U = \frac{1}{\left[\frac{ln\left(\frac{r_2}{r_1}\right)}{(k_1\times 2\times\pi\times L)}+\frac{ln\left(\frac{r_3}{r_2}\right)}{(k_2\times 2\times\pi\times L)}+\frac{1}{(h\times 2\times\pi\times r_3\times L)}\right]} + \frac{1}{\left[\frac{(r_2-r_1)}{k_1}+\frac{\left(r_3-r_2\right)}{k_2}+\frac{1}{h}\right]} \tag{14}$$

where,

U = Overall heat transfer coefficient (W m^{-2}.K)

r_1 = Inside radius of the reactor = 2.9 m

r_2 =   Outside radius of the reactor = 2.906 m

r_3 =   Outside radius of the reactor with insulation = 2.956 m

k_1 = Thermal conductivity of stainless steel = 16 W m^{-1}.K (Engineering Toolbox)

k_2 = Thermal conductivity of glass wool = 0.038 W m^{-1}.K (Woiciechowski et al., 2002)

L = Reactor height = 36.57 m

h = Convective heat transfer coefficient of the outside insulation surface = 50 W m^{-2} K^{-1} (Mafe et al., 2015)

Results and discussion (Impact assessment and interpretation)

Primary fossil energy use

Primary fossil energy use for pelleting and transportation for the base case and densification scenarios are shown in Table 21. Although bale transportation requires 34% more energy than pellet transportation, primary fossil energy inputs for the pelleting process was 3.5 times more than the energy required for transporting bales. However, the amount of energy used to produce pellets is only 3% of the total energy content of the biomass (17,000 MJ Mg^{-1} (Sokhansanj et al., 2010)). Electricity is the main energy source for the pellet mill. The pelleting energy inputs were taken from Sokhansanj and Fenton (2006), as detailed pelleting unit processes and corresponding energy requirements were specified in this study. Pelleting energy requirement of 287 MJ Mg^{-1} was assumed as electricity which included energy consumption for the pellet mill, in addition to cooling, and screening (Sokhansanj and Fenton, 2006). Drying and grinding energy requirements were not included in the total pelleting process energy as biomass was assumed to be field-dried and the grinding energy would be same for both scenarios.

Table 21. Primary fossil energies required for base case and densification scenarios within the system boundary.

Operation	Primary fossil energy inputs (MJ Mg^{-1})	
	Base case scenario	Densification scenario
Pelleting	0	521
Transportation	147	110

Although trucks can carry 70% more biomass as pellets than in bale form, the transportation energy requirement is only 25% less for pelleted biomass. Total trucking distance for transporting 1,000,000 Mg of biomass in the base case scenario (4,714,916 km yr^{-1}, one way) is 75% higher than for the densification scenario; lower trucking capacity for baled biomass leads to more required trips to transport the same total mass. Transportation energy savings in densification scenario is moderated by the fact that biomass still needs to be transported in bale form to the pellet mill before the transportation efficiencies can be realized. Truck transportation was considered because that is used almost exclusively for biomass transportation in North America. However, dependence on truck delivery may be challenging for a large-scale biorefinery due to higher transportation costs and traffic congestion (Kumar et al., 2005 a,b).

Pretreatment input parameters (temperature, time, and ammonia concentration) required to achieve 90% of theoretical glucose yields for the densification scenario were assumed to be lower than for the base case scenario based on previous studies (Table 19). In addition, total pretreatment solid loading was twice as high for pelleted biomass in the densification scenario than for non-pelleted biomass in the base case (Table 19). Higher solid loading resulted in the ability to pretreat 87% more pelleted biomass in the same size of reactor. Assuming a biorefinery with 350 operating days per year and a 4-h interval time for reactor unloading and loading, only 9 pretreatment reactors would be needed to pretreat 1,000,000 Mg of pelleted biomass. Pretreating that amount of non-pelleted biomass at the given solid loading and with the same reactor downtime would require 59 reactors of the same size. From a processor's perspective, the huge number of pretreatment reactors required for processing non-pelleted biomass processing is non-economical and unrealistic.

The doubling of the solid loading enabled by the use of biomass pellets not only significantly lowers the heating energy needs and the number of required reactors, but significantly reduces primary fossil energy and GHG emissions associated with pretreatment chemical use. Figure 19 shows the comparison of water and chemicals for pretreating pelleted and non-pelleted biomass. The gross water requirement for pretreating pelleted biomass is reduced by 49% compared with what would be needed for non-pelleted biomass. Requirements for ammonium hydroxide, the main chemical for SAA-pretreatment, is reduced by 60% when pretreating pelleted biomass while the sulfuric acid for neutralization is reduced by 37%.

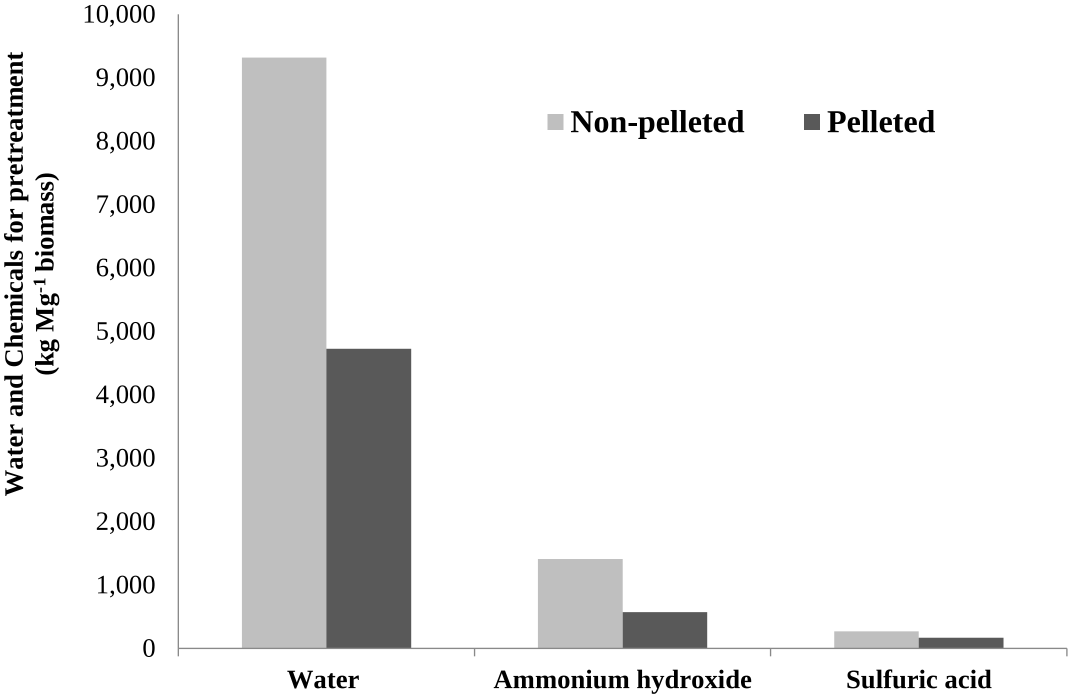

Figure 19. Required chemicals (kg Mg^{-1} biomass) for pretreatment of non-pelleted and pelleted biomass.

The total energy required for pretreatment reactor heating is shown in Figure 20. The energy required for the initial temperature increase was only 8% and 34% of total heating energy for pretreating non-pelleted and pelleted biomass, respectively. The relatively high energy requirements for maintaining reactor temperature compared with the initial temperature increase illustrates the dominant effect pretreatment time has on total heating energy requirements. Energy for maintaining the reactor temperature was around 11 times higher than the energy required for increasing the reactor temperature with non-pelleted biomass; there was only a twofold difference for pelleted biomass where reactor residence times were only 4 h instead of

24 h. The dramatic energy reductions for maintaining the reactor temperature for pelleted biomass were due to a combination of higher solid loadings and significantly lower pretreatment time for pelleted biomass. Models showed that increasing solid loadings from 10% to 20% resulted in 47% decrease in energy requirements, whereas only reducing pretreatment time from 24 h to 4 h would reduce energy demand by 77%. Together, use of pelleted biomass reduced the overall pretreatment heating energy requirements by about 89% compared with the base case of using non-pelleted biomass (Figure 20).

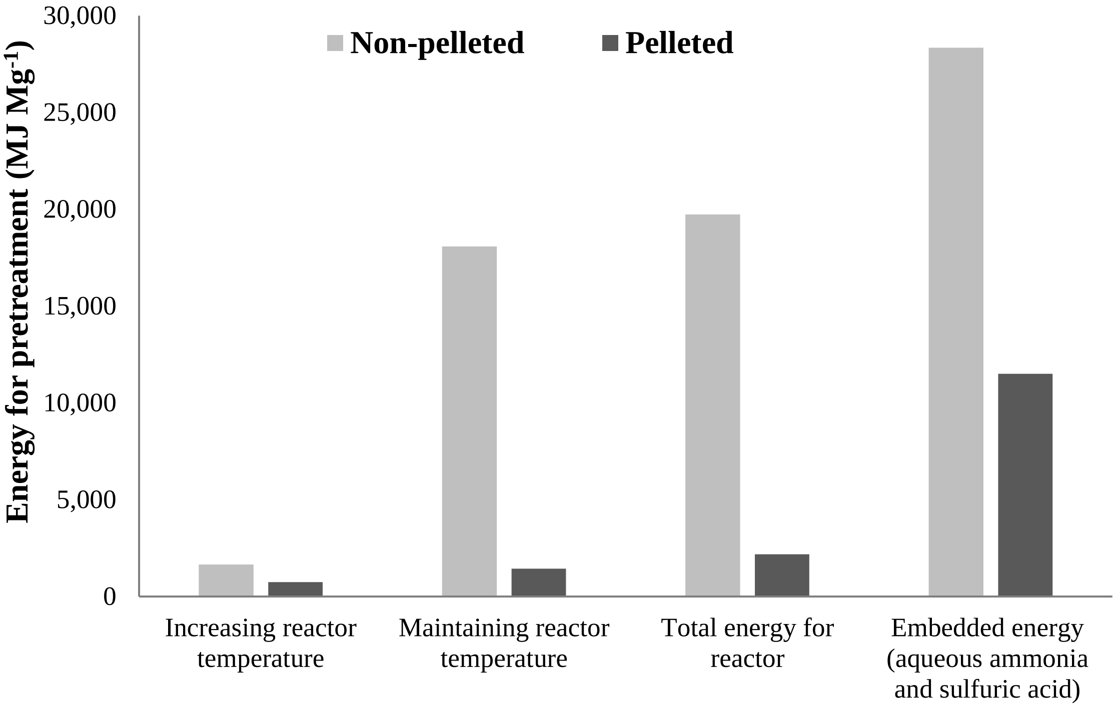

Figure 20. Energy required for pretreatment of non-pelleted and pelleted corn stover.

In cellulosic biorefinery models, lignin residue is assumed to generate sufficient steam and electricity for all on-site energy needs (Humbird et al., 2011). The energy content of corn stover is around 17,000 MJ Mg^{-1} (Sokhansanj et al., 2010), and we assume roughly 30% of that energy (5,000 MJ Mg^{-1}) would remain in lignin and other solid residues such as undigested cellulose, hemicellulose, protein etc. to generate steam and electricity. The modeled pretreatment energy requirement for non-pelleted biomass in the base case scenario is even higher (19,737 MJ Mg^{-1}) than the total energy content of the original biomass. The high energy requirement and the large number of required pretreatment reactors both indicate that SAA pretreatment is not a feasible option under the modeled base case conditions. However, SAA-pretreatment of pelleted biomass required significantly less heating energy (2,178 MJ Mg^{-1}). Steam and heat produced from combusting lignin residues from pelleted biomass in the densification scenario should be sufficient to cover pretreatment reactor heating, and probably other heating needs in downstream processing.

The embedded energy from production of chemicals required for pretreatment and neutralization of pelleted and non-pelleted biomass are shown in Figure 20. Embedded energy from aqueous ammonia production accounted for 99% of the total. Lower concentrations of aqueous ammonia and sulfuric acid for pretreatment of pelleted biomass reduced the embedded energy by 60% and 37%, respectively, compared with the base case scenario. Higher solid loadings for pellet pretreatment assisted in lowering the amount of aqueous ammonia required. This will have significant impact on net GHG emissions. However, if a biorefinery is not required to meet a low-carbon standard, reductions in embedded energy do not have much importance from the processor's perspective. Nonetheless, the amount of ammonia recovered and recycled through the system will affect GHG emissions, economics, and thus the overall process feasibility. Information on ammonia recovery from SAA has not been reported in the literature, although recovery of up to 93% of liquid ammonia (NH_3) from AFEX pretreatment has been suggested by Tao et al. (2011). However, processes and equipment required to recover pretreatment chemicals will also contribute to the overall pretreatment system energy and capital costs (Tao et al., 2011).

Greenhouse gas emissions

The GHG emissions data considered in this study were those associated with pelletization (electricity), transportation (diesel fuel), and pretreatment inputs (chemicals). Because electricity is the main energy source for pelleting, the source of the fuel used for electricity generation is important in determining the environmental impacts of those processes. Electricity inputs for this study were taken from GREET (2016) as outlined in Table 15. Electricity production in GREET identified the major electricity generation sources as coal (47%), natural gas (34%), petroleum (2%), nuclear (10%) and renewable energy (7%) in the U.S. As the primary fuel sources for electricity generation in the U.S. were fossil fuels (83%), these inputs significantly influence GHG emissions. Total GHG emissions for pellet production in this study were 43 kg CO_2eq Mg^{-1} biomass (Table 22).

The emissions from transportation fuel consumption is significantly lower (25%) for pelleted biomass than non-pelleted biomass due to fewer truck trips necessary for pellet transportation in the densification scenario (Table 22). This correlates directly with transportation energy reductions. However, Table 22 shows that the transportation-related GHG emissions for both scenarios are a minor component compared to the emissions associated with other processing steps within the system boundaries. This result agrees with Daystar et al. (2014), who also found transportation to be a minor contributor (~1.4%) in the overall GHG emissions.

Table 22. Greenhouse gas (GHG) emissions for non-pelleted and pelleted corn stover

Operation	GHG emission (kg CO_2eq Mg^{-1})	
	Base case scenario	Densification scenario
Pelleting	0	43
Transportation	2.27	1.70
Chemical production	1,859	755
Total	1,861	800

Greenhouse gas emissions from pelleting were 19 to 25 times higher than the emissions from transporting biomass. However, significantly more emissions were associated with pretreatment chemical production (Table 22). Emissions from chemical production were 43 times and 18 times higher than emissions from pelleting in the base case and densification scenario, respectively. However, recycling and reuse of chemicals were not accounted for in this estimation. The higher magnitude of GHG emissions related to chemical production shows the importance of recycling and reuse of ammonia for SAA pretreatment. Pretreatment heating energy is not included because we are assuming that energy would come from lignin residues rather than an external energy source.

Interpretation

One of the primary goals of current renewable energy policy is the reduction of GHG emissions. Previous research using SAA-pretreatment for cellulosic biomass showed high yields with this pretreatment (Kim and Lee, 2005, 2007; Ko et al., 2009; Nahar and Pryor 2017). Other aspects of the practical feasibility of SAA and many other experimental pretreatments have not been considered extensively. The results indicate that even with the high yields from SAA-pretreatment of non-pelleted corn stover, this pretreatment is not a realistic option under the modeled conditions. Selection of the appropriate pretreatment techniques for a biorefinery not only depends on yields, but also economic feasibility and environmental indicators. In this aspect, pelleted biomass shows more realistic potential as a biorefinery feedstock. In particular, the higher solid loadings and significantly lower pretreatment residence times that are only possible with pelleted biomass make SAA-pretreatment a feasible option.

The results of this study emphasize the importance of reducing pretreatment residence times for SAA and other pretreatments. If pretreatment residence time could be reduced from 24 h to 4 h for non-pelleted corn stover, total pretreatment heating energy would be reduced by 83%. Lowering the pretreatment temperature from 60 ºC to 56 ºC, as used for pelleted biomass, would only reduce energy needs by 11%. However, further reductions are still possible by varying temperature, residence time, and ammonia concentrations while maintaining pretreatment effectiveness. The pretreatment energy and emissions are much lower with the conditions tested using pelleted biomass, but those are not the only conditions possible. Conversely, additional benefits could be gained for pelleted biomass by increasing the temperature beyond 60 °C while reducing pretreatment residence time. Pretreatment residence time had the largest influence on energy requirements, hence pretreating at a higher temperature might allow lowering pretreatment residence time well below the 4 h modeled here, which would further reduce both the number of required reactors and the pretreatment energy requirements.

There are no other manuscripts that have conducted an LCA using SAA or other alkaline pretreatments with pelleted biomass. Kumar and Murthy (2012) are the only authors who conducted a comparative LCA of dilute acid, steam explosion, hot water, and alkali pretreatment. That study showed that alkali pretreatment had the highest fossil energy use and GHG emissions. Their study simulated alkali (NaOH) pretreatment at an operating temperature of 180 ºC with a 15-min residence time, whereas the base case scenario in this study considered pretreatment at 60 ºC for 24 h. Variation in process technologies, system boundaries, and functional units between studies makes the comparison of life cycle studies challenging. Kumar and Murthy (2012) reported approximately 0.37 MJ MJ^{-1} fossil energy use for ethanol production including alkali pretreatment. System boundaries for ethanol production in Kumar and Murthy (2012) included biomass pre-processing, alkali pretreatment, simultaneous saccharification and co-fermentation, and ethanol recovery. However, they did not identify fossil energy use for individual processing steps. Therefore, it is difficult to determine what percentage of total energy was used specifically for the pretreatment. A number of assumptions were made for estimating pretreatment energy in this study using the same functional unit (MJ MJ^{-1}): 36% glucan content in biomass, 90% hydrolysis efficiency, 95% fermentation efficiency for glucose. Pretreatment heating energy requirement for non-pelleted and pelleted biomass of our study was 5.17 MJ MJ^{-1} and 0.68MJ MJ^{-1}, respectively.

Life cycle assessments of cellulosic biorefineries based on actual process data are not available since industrial scale biorefineries have not been established yet. The literature on pretreatment technologies consists mainly of lab-scale experiments and techno-economic models. Although techno-economic models provide valuable information, these are also based on a wide variety of assumptions. Currently, the model developed by NREL is the most widely used techno-economic model (Humbird et al., 2011) to predict the performance of cellulosic ethanol based on dilute acid (DA) pretreatment. However, NREL and other techno-economic studies identify system costs but do not include the environmental indicators like energy use and GHG emissions.

Differences of DA and SAA pretreatment processes will strongly influence the overall cost. From an economic standpoint, cost of construction materials for DA reactors are a large portion of capital costs. Lower severity SAA pretreatment requires less costly pretreatment reactor materials. However, lower SAA pretreatment temperatures require longer residence times than higher temperature pretreatments, which will increase required reactor volume and energy requirements. Inhibitory product separation with DA pretreatment also needs to be accounted for in cost comparison between DA and SAA pretreatments. Therefore, a comparative LCA along with techno-economic analysis for different types of pretreatment with densified and non-densified biomass would provide valuable insights on the potential environmental and economical performance of cellulosic biorefineries.

Conclusions

This study shows remarkable differences in fossil energy requirements, GHG emissions, and water and chemical use for SAA-pretreatment of pelleted and non-pelleted biomass. Soaking in aqueous ammonia pretreatment appears unrealistic for use with non-pelleted biomass, but potentially feasible with biomass pellets despite high energy requirements in the pelleting process. Energy inputs and total reactor volume for SAA pretreatment of non-pelleted biomass are the largest problem areas. The majority of system wide energy reductions from processing pelleted biomass is due to lower pretreatment severity, specifically temperature, time, and higher pretreatment solid loadings. Shorter pretreatment residence times for pelleted biomass lowers the total energy requirements for pretreatment by 89%. Higher pretreatment solid loadings for pelleted biomass not only reduces the amount of chemicals, water, and energy inputs for the pretreatment, but the required pretreatment reactor volume is reduced by 85%. Use of pelleted biomass may allow effective, but otherwise unrealistic pretreatment technologies to be realistically considered for a cellulosic biorefinery.

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GENERAL CONCLUSIONS

The major challenges of cellulosic biorefinery development are associated with supply logistics, techno-economic viability, and GHG emissions reduction. Though technologies for fermentable sugar production from cellulosic biomass are known, productivity improvements are still required to reduce the capital and operating costs. Densification of low density cellulosic biomass improves supply logistics and allows the uniform feedstock supply in the biorefinery. However, understanding the inherent links, potential synergies, and trade-offs between densification and post processing steps, such as pretreatment and hydrolysis, are critical to achieve economical sugar production from biomass.

Biorefinery processing steps are inter-related and trade-offs of different process parameters impact the cost and efficiency of the overall system. The effectiveness of enzymatic hydrolysis of cellulosic biomass is directly dependent on the effectiveness of pretreatment. As pretreatment severity increases, required enzyme loadings and hydrolysis time decrease. There are also trade-offs among different components of pretreatment severity, such as temperature, time, chemical concentration for effective pretreatment. More accessible cellulose after effective pretreatment improves hydrolysis yields with lower enzyme cost. The work of this dissertation demonstrates the significance and importance of understanding such processing trade-offs surrounding enzymatic hydrolysis.

Enzymatic hydrolysis efficiency is the primary biomass processing production indicator and is therefore a major factor affecting commercialization of cellulosic biofuel production. Although enzyme prices have decreased, enzyme loadings still need to be minimized in order to reduce production costs. Though enzymes play a critical role in the hydrolysis process, other factors such as pH and temperature also affect the overall rates and yields. A model developed in this study using SAA-pretreated non-pelleted corn stover shows the interaction between cellulase and hemicellulase loadings on hydrolysis rates. Therefore, many combinations of enzyme loadings can be used to achieve similar hydrolysis rates.

Processes designed to optimize yield may not generate the maximum economic return. This research shows optimal enzyme loadings to maximize economic return are lower than loadings to maximize hydrolysis rates. Economic trade-offs are also seen for cellulase and hemicellulase loadings under different hydrolysis conditions (temperature and pH). Use of higher temperatures as is typical for separate hydrolysis and fermentation (SHF, at 50 °C) leads to higher hydrolysis rates. At the same enzyme loadings, SHF yields are higher than a lower temperature process of simultaneous saccharification and fermentation (SSF, at 37 ºC). To maximize profit, SHF can use higher enzyme loadings than SSF. However, economically optimal enzyme loadings will change with fluctuations in individual enzyme costs as well as the ethanol selling price. Fluctuations in ethanol price shows a greater impact on economically optimal cellulase loadings than on hemicellulase loadings. Cellulase enzyme loading is more sensitive to enzyme price than hemicellulase loading at lower processing temperatures. Understanding all these trade-offs allows processors adjusting enzyme loadings to increase return based on enzyme costs, ethanol price, and process temperature.

The development of economical but effective pretreatment methods is still a significant barrier for cellulosic biorefineries. Biomass is required to go through high-severity pretreatment process for higher sugar yields. Pelleted corn stover shows the potential to lower pretreatment severity and costs. Pelleted corn stover allows pretreatment solid loadings twice as high as non-pelleted biomasss without reducing glucose yields, which assists in lowering the process cost. Required severity of SAA-pretreatment in terms of temperature, time and ammonia concentration is also reduced for pelleted corn stover compared to non-pelleted feedstock while still producing 90% or higher glucose yields. The pelleting process increases SAA-pretreatment efficacy by modifying the biomass structure. Pretreatment temperature is a more important parameter for achieving high glucose yields than either pretreatment time or ammonia concentration within the design space of this study. However, trade-offs still occur in different components of pretreatment severity: temperature, time and ammonia concentration. Different combinations of pretreatment components can be used to reach high yields, which gives flexibility to processors to reduce the operating costs. High glucose yields from pelleted corn stover using low-severity pretreatment suggests economic and environmental benefits of biomass densification beyond those associated with transportation and supply logistics.

This research took a life cycle assessment (LCA) approach for examining the fossil energy use and GHG emissions from processing pelleted and non-pelleted corn stover in an industrial scale biorefinery using SAA pretreatment for sugar production. Significant differences were found between pelleted and non-pelleted biomass in terms of fossil energy requirements, GHG emissions, water and chemical use for the pretreatment. Although the temperature is a key parameter in SAA pretreatment for achieving high glucose yields, pretreatment residence time is the most important parameter in terms of energy requirements. Soaking in aqueous ammonia pretreatment of non-pelleted biomass using the range of parameters in this this study is unrealistic based on high energy requirements for maintaining reactor temperatures for an extended pretreatment residence time (24 h). However, use of pelleted biomass makes the unrealistic pretreatment a potential option because of lower required pretreatment residence times (<4 h), which significantly decreases the amount of energy required during pretreatment. Higher pretreatment solid loadings with pelleted biomass drastically reduces the number of pretreatment reactors compared to non-pelleted feedstock. For a one million Mg yr^{-1} facility, 9 pretreatment reactors (250,000 gal) would be required for pelleted biomass, while 59 of the same size reactor would be needed for baled biomass. The extraordinarily high number of reactors required makes SAA pretreatment of baled biomass impractical. Assessment of fossil energy requirements and GHG emissions using an LCA approach for densified and non-densified biomass also provides insight in system efficiency and points to critical area for improvements.

There is no single pretreatment method which is clearly both economical and environmentally friendly. Extensive research is ongoing for the development of such a pretreatment technique. Soaking in aqueous ammonia pretreatment is effective, to an extent, for efficient conversion of cellulosic biomass into fermentable sugars using less severe pretreatment temperatures (below 60 °C) compared to other high temperature (typically, above 180 °C) pretreatments such as dilute acid. Lower temperature pretreatment has significant benefits of lowering capital and operating costs as extreme materials of construction is not required. However, significant pretreatment temperature reductions prolong required pretreatment residence time, making this pretreatment unrealistic for non-pelleted biomass despite of high efficiency of sugar production. An enormous number of reactor requirements for non-pelleted biomass is also not feasible. This study shows the potential of SAA pretreatment using pelleted biomass to maintain yields while reducing energy requirements and the number of reactors needed for pretreatment. More research on SAA with slightly higher temperatures (70 to 90 °C) and lower retention times than used in this study may further improve the feasibility of this pretreatment for pelleted biomass.

To make biorefineries cost effective, it is necessary to look at the overall system efficiency. Though individual process optimizations are important, analysis of how each process impacts subsequent processes and overall system efficiency is critical for increasing the technical feasibility and economic viability of cellulosic biorefineries.

RECOMMENDATIONS FOR FUTURE WORK

There are many trade-offs in a commercial scale biorefinery, such as capital costs, and energy, chemical and water use; these trade-offs influence the overall conversion process and the economical viability of the biorefinery. However, a biorefinery that is economically viable in the short term but not environmentally sustainable may not be economically viable in the long term. Therefore assessing reduction of fossil fuel use, and GHG emissions are critical components of assessing a biorefinery's viability. Life cycle assessment (LCA) is used for evaluating the environmental impacts of a process, but it does not address production costs. Future research incorporating LCA results with a detailed techno-economic analysis (TEA) would be valuable for assessing feasibility of cellulosic biorefinery.

This study included a comparative life cycle of pelleted and non-pelleted corn stover and demonstrated that feedstock form plays a significant role in the pretreatment process and environmental impact of the processing chain. This work could be expanded upon to conduct a full LCA analysis for the well-to-wheel supply chain. Biorefinery processing steps are inter-related and trade-offs of different process parameters impact the cost, efficiency, and environmental impact of the overall system. Other processing units, such as hydrolysis, fermentation, distillation, wastewater treatment, and combined heat and power from lignin combustion need to be incorporated to account for the impact of those processes in the overall system.

Techno-economic analysis is a tool to determine biofuel production costs for economic feasibility assessments. Various conversion technologies are characterized by TEA through process flow diagrams, rigorous material and energy balances, and estimates of capital and operating costs via these technologies. The environmental and economical outputs will vary significantly with the type and form of biomass, and the conversion technology. This would enable a more complete understanding of economic and environmental trade-offs associated with fermentable sugar production from pelleted and non-pelleted biomass.

Capital and operating cost estimates of soaking in aqueous ammonia (SAA) pretreatment will help determine the practicality of using this pretreatment in a commercial setting. Soaking in aqueous ammonia pretreatment allows less costly pretreatment reactors than dilute acid pretreatment, as comparatively lower temperatures and less corrosive chemicals are used. This study showed the potential for use of pelleted biomass in an industrial scale biorefinery. Pelleted biomass substantially reduced the number of reactors, the volume of chemicals, and the energy inputs within the design space of this study. Cost reductions associated with chemicals, reactor use, energy consumption, and chemical recovery are still needed to estimate capital and operating costs associated with SAA pretreatment of pelleted biomass.

Low severity SAA pretreatment of pelleted biomass shows promise of reducing fossil energy use and GHG reduction without reducing yields. More research on SAA with slightly higher temperatures and lower retention times may further improve the feasibility of SAA pretreatment for pelleted biomass. Investigating the impact of other low severity pretreatments such as hot-water pretreatment, steam explosion, ultra-dilute acid pretreatment, or ultrasound pretreatment on pelleted biomass would be helpful. Given the benefits demonsrated with reductions in SAA pretreatment parameters, we may also be able to lower the severity parameters of conventional pretreatments or increase the effectiveness of less conventional ones.

Logistics systems are essential for efficient biomass harvesting, storage, handling, and transportation. Biomass collection is typically performed in a limited period of the year. Therefore, storage of baled or pelleted biomass for year-round supply to biorefinery also needs to be determined. This study incorporated only transportation in the LCA, but other parts of supply logistics can be integrated into the system. Trucks were considered for transportation model of this study for pelleted and non-pelleted biomass; further study with different modes of transportation could be useful for reducing biomass transportation cost and energy.

Substantial effort is still needed for establishment of a technically feasible and environmentally viable biorefinery. Analyzing the overall biorefinery system in terms of yields, energy inputs and outputs, and economic and environmental sustainability is critical. A major challenge of performing these analyses is the lack of reliable process data. The data required for these analyses are based primarily on models or lab scale experiments with a wide variety of assumptions. First commercial cellulosic ethanol plant in U.S., “Project Liberty”, run by POET-DSM has a capacity of producing 20–25 million gallons of cellulosic ethanol from corn stover. Once more data is acquired from commercial scale biorefineries, future work will need to model at an industrial scale for developing economically feasible and environmentally viable biorefineries.