Mechanical Pretreatment of Corncobs for Bioethanol Production by a Twin-Screw Extruder

Jun Zheng

Supervisor
Dr. Lars Rehmann
The University of Western Ontario

Graduate Program in Chemical and Biochemical Engineering

A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy

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MECHANICAL PRETREATMENT OF CORNCOBS FOR BIOETHANOL PRODUCTION BY A TWIN-SCREW EXTRUDER

(Thesis Format: Integrated Article)

by

Jun Zheng

Graduate Program in Chemical and Biochemical Engineering

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The School of Graduate and Postdoctoral Studies
The University of Western Ontario
London, Ontario, Canada

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Abstract

Extrusion pretreatment of lignocellulosic biomasses is a physical continuous pretreatment method for subsequent fermentative ethanol production. Twin-screw elements (conveying, kneading and reverse) have different effects on enzymatic digestibility according to their geometries and functions. The first study evaluated the effects of individual functional screw elements on enzymatic digestibility after extrusion. It was found that extrusion enhanced enzymatic digestibility under all tested condition. Reverse and kneading screw elements however resulted in lignin re-distribution over the cellulose fibres, blocked pores (shown via SEM) and resulted in less digestible biomass, compared to conveying screw elements. Lignin removal via NaOH reversed this effect and the highest digestibility was found for biomass extruded in the presence of reverse screw elements (the most severe conditions tested in this study), indicating that lignin redistribution can counteract otherwise positive effects of extrusion pretreatment.

Based on these finding a process was developed for separating xylose from lignocellulosic biomass (steam-exploded corncobs) in a co-rotating twin-screw extruder, through water addition and solid-liquid separation. Eight screw configuration profiles were evaluated to define the best performance on xylose recovery. Subsequently, operating conditions (barrel temperature, screw speed and water flow rate) were examined with respect to xylose recovery and specific mechanical energy consumption. It was found that liquid/solid separation highly depended on the position and spacing of reverse screw elements. In addition, more xylose was recovered as the screw speed decreased and water flow rate and barrel temperature increased. Furthermore, operating conditions influenced the specific mechanical energy consumption of the motor drive.

The extruder was then used to produces differently treaded biomass samples, and a
response surface method (RSM)-based model using IV-optimal design was used to study the combined effects of various enzymatic hydrolysis process variables (enzyme loading, surfactant addition, and hydrolysis time) with two differently extruded corncobs (7% xylose removal, 80% xylose removal) on glucose conversion. The results showed that the extrusion process led to an increase in cellulose crystallinity, while structural changes could also be observed via SEM. A quadratic polynomial model was developed for predicting the glucose conversion and the fitted model provided an adequate approximation of the true response as verified by the analysis of variance (ANOVA).

**Keyword:** Extrusion Pretreatment, Enzymatic Digestibility, Lignin, Screw Element, NaOH, Screw Configuration Profile, Xylose Recovery, Specific Mechanical Energy, Corn cob, Mechanical Pretreatment.
Co-Authorship Statement

Chapter 2: Extrusion Pretreatment of Lignocellulosic Biomass: A Review

Authors: J. Zheng, L. Rehmann

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Chapter 3: The Effects of Twin-screw Elements on Enzymatic Digestibility of Corncobs after Pretreatment in a Twin-screw Extruder

Authors: J. Zheng, K. Choo, L. Rehmann

This chapter was submitted for publication in the journal *Biomass and Bioenergy*

All experiments were planned and conducted by J. Zheng, under supervision of supervisors Dr. Rehmann, and co-supervisor Dr. Choo. The manuscript was written by J. Zheng and subsequent revised and edited by Dr. Rehmann.

Chapter 4: The Effects of Screw Configuration on Xylose Removal from Lignocellulosic Biomass via a Twin-screw Extruder: Feasibility Study and Influence of Operating Conditions

Authors: J. Zheng, K. Choo, L. Rehmann

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All experiments were planned and conducted by J. Zheng, under supervision of supervisors Dr. Rehmann, and co-supervisor Dr. Choo. The manuscript was written by J. Zheng and subsequent revised and edited by Dr. Rehmann.
Chapter 5: Enzymatic Hydrolysis of Steam Exploded Corncob Residues after Pretreatment in a Twin-screw Extruder

Authors: J. Zheng, K. Choo, C. Bradt, R. Lehoux, L. Rehmann


All experiments were planned and conducted by J. Zheng, under supervision of supervisors Dr. Rehmann, and co-supervisor Dr. Choo. A subset of the experiments was conducted in collaboration with the industrial partner GreenField Specialty Alcohols and the work at GFSA was supervised by Mr Brandt and Lehoux. The manuscript was written by J. Zheng and subsequent revised and edited by Dr. Rehmann.
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Nomenclature

HPLC: high-performance liquid chromatography
C_{HPLC}: the concentration of glucose as detected by HPLC (mg/ml)

DF: dilution factor

NREL: national renewable energy laboratory

FPU: filter paper unit

DM: dry matter

V_{filtrate}: the volume of the buffer solution (ml)

M_{total}: the total amount of glucose in the substrate (g)

R_{ave.sugar}: the average sugar standard recovery (%)

I_{max}: the maximum intensity peak for the crystalline region of the biomass (i.e., cellulose),
I_{min}: the minimum intensity peak for the amorphous region (i.e., cellulose, hemicelluloses, and lignin).

n: an integer

λ: the wavelength of incident wave (1.54056 for CuKa; 1.78896 for CoKa)

d: the spacing between the planes in the atomic lattice

θ: the diffraction angle

Y: predicted response

β_0: interception coefficient

β_i: coefficient of each independent variable

x_i: independent variables

ε: random error
Chapter 1. Introduction

1.1 Introduction

Environmental, long-term, economic and national security concerns associated with the use of fossil fuels have strengthened the interest in alternative, nonpetroleum-based sources of energy, such as sunlight, rain, wind energy, etc. in the past two decades (Yat et al., 2008). Biomass appears to be the only suitable and renewable primary energy source that can provide alternative liquid transportation fuels. Currently, ethanol is being produced from sugar-and starch-based materials such as sugarcane and corn, but its sustainability has been considerably debated (Sun and Cheng, 2002). Another interesting alternative raw material for the second generation production of ethanol is lignocellulosic biomass, which offers large-scale availability with low cost, limited conflict with food crops and lower fossil fuel inputs (Lynd et al., 2002). The main composition of lignocellulosic biomass is cellulose, hemicelluloses and lignin. Cellulose is the most abundant of biopolymers on earth (Zhang and Lynd, 2006; O’Sullivan, 1997). A worldwide annual production of $1 \times 10^{10}$ MT was estimated (Sanchez and Cardona, 2008). Lignocellulosic feedstocks could be grouped into five main categories: (1) agricultural residues, (2) dedicated energy crops, (3) municipal solid wastes, (4) forestry residues, and (5) food processing and other industrial wastes. Ethanol from various types of lignocellulosic biomass is now considered the most promising mid-term fuel-additive to be blended with gasoline in dedicated engines (Hamelinck et al., 2005).

Lignocellulosic biomass can be converted into fermentable sugars for fermentative ethanol production. However, this bioconversion is further complicated due to recalcitrance caused by the association of cellulose, hemicelluloses and lignin in the
biomass (Jorgensen et al., 2007). Cellulose chains are embedded in a cross-linked matrix of hemicellulose wrapped by lignin on the outside, making the sugars inaccessible to hydrolyzing enzymes (De Vries and Visser, 2001). Therefore, pretreatment is required to reduce the recalcitrance of lignocellulosic biomass by opening or partially breaking up the recalcitrant structure, while minimizing the chemical degradation of fermentable sugars and enhancing the enzymes’ accessibility to the cellulose during the enzymatic hydrolysis step. In addition, the cost and efficiency of enzymatic hydrolysis and fermentation will be affected by pretreatment since it is the first major unit operation in the process of bioconversion. An effective pretreatment should have the following criteria: reducing cost and minimizing energy requirements, preserving hemicellulose fractions (mainly pentose), avoiding the degradation of sugars and minimizing the formation of inhibitors for further fermentation steps, and recovery of lignin for valuable co-products productions (National Research Council, 1999).

A large number of pretreatment methods have been proposed generally on a wide variety of lignocellulosic biomasses for bioethanol production since different feedstocks have different physical-chemical characteristics. These pretreatment methods are usually divided into physical, chemical, physical-chemical and biological, such as steam explosion (Cara et al., 2008), dilute acid pretreatment (Chen et al., 2007), organosol pretreatment (Sun and Cheng, 2002) and alkali pretreatment (Chang and Holtzapple, 2000). Some articles have generally reviewed biomass pretreatment (Alvira et al., 2010; Hendriks and Zeeman, 2009; Carvalheiro et al., 2008; Taherzadeh and Karimi, 2008; Yang and Wyman, 2008; Moiser et al., 2005; Sun and Cheng, 2002).
In the present, the extrusion process, commonly used in the polymer industry, has been used as a continuous physical pretreatment method towards bioethanol production by means of heat, compression and shear forces, which can disrupt and modify biomass structures during its passage through the extruder. The most significant factor influencing the extent of mixing, product transportation, residence time distribution, extrudate properties and mechanical energy input during extrusion processing is the screw configuration, which is based on the arrangement of different characteristics of screw elements (pitch, stagger angle and length), such as conveying, kneading and reverse screw elements (Karunanithy and Muthukumararappan, 2011, 2010; Abe et al., 2007; Rizvi and Mulvaney, 1992).

Several studies recently have expanded the usage of twin-screw extruders for the continuous extraction of vegetable oil from sunflower and soy (Amalia Kartika et al., 2010, 2006, 2005; Amalia Kartika, 2008; Wang and Johnson, 2001; N'Diaye et al., 1996), while, a modified twin-screw extruder incorporated with a filtration device was considered as a thermal-mechano-chemical reactor and a liquid/solid separator for hemicellulose extraction from lignocellulosic biomass (N’Diaye and Rigal, 2000; N’Diaye et al., 1996). The advantages of using a twin-screw extruder for lignocellulosic biomass fractionation include (1), low cost and good monitoring and control of all variables (Abe et al., 2007); (2), no sugar degradation products (De Vries and Visser, 2001); (3), good adaptability to different process modifications (Rizvi and Mulvaney, 1992) and (4), high continuous throughput (Rizvi and Mulvaney, 1992).

The enzymatic hydrolysis process is usually catalyzed by cellulase enzymes and the process is affected by many factors including cellulose fibre protection by hemicelluloses
and lignin, cellulose crystallinity, degree of polymerization, degree of acetylation of hemicelluloses and the accessible surface area of the biomass (McMillan, 1994). The presence of hemicelluloses and lignin makes the cellulase enzymes’ access to cellulose difficult, which will reduce hydrolysis efficiency. Therefore, the structure of cellulosic biomass must be pretreated prior to enzymatic hydrolysis to make cellulose more accessible to enzymatic conversion (Gao and Rehmann, 2014; Mosier et al., 2005).

The objectives of this thesis were therefore to:

1. To investigate the effects that individual functional screw elements have on biomass structure and its enzymatic digestibility.

2. To develop screw configurations for an extrusion process of steam exploded corncobs in order to maximize xylose removal at a reasonable amount of energy input.

3. To characterize and optimize the enzymatic digestion of biomass after extrusion pretreatment.
Chapter 2. Literature Review of Extrusion Pretreatment

Jun Zheng and Lars Rehmann

With minor editorial changes to fulfill formatting requirements, this chapter is substantially as it appears in: *Int. J. Mol. Sci.* 15: 18967-18984 (2014).

2.1 Conversion technology for cellulosic ethanol production

The cellulosic ethanol production process for lignocellulosic biomass consists of the following main steps: pretreatment, hydrolysis, fermentation and product recovery (Figure 2-1). Pretreatment is required to alter the lignocellulosic structure, so that hemicelluloses and lignin will be removed and the crystalline structure of cellulose will also be disrupted. Thus, a higher hydrolysis rate will be achieved more rapidly with greater yields (Hsu et al., 1980). Hydrolysis refers to the processes that convert the remaining cellulose into glucose by acid or enzymatic reaction; the fermentation process will ferment glucose and pentose sugars produced from cellulose and hemicelluloses respectively into ethanol by yeasts or bacteria. Finally, the ethanol will be separated and recovered by a distillation process to remove any remaining water from the ethanol. The lignin and other bio-products from the recovery process can be used to produce energy for the ethanol production process (DOE, 2011).
2.2 Pretreatment of lignocellulosic biomass

2.2.1 Physical pretreatment

Physical pretreatment technologies include mechanical comminution. The objective of the mechanical pretreatment involves particle size reduction and the breaking of the crystallinity of cellulose by grinding, milling, or chipping in order to increase the surface area and reduce the degree of polymerization (Sun and Cheng, 2002). These processes can be used to improve the enzymatic hydrolysis of lignocellulosic feedstocks, but the required power consumption is relatively higher than the theoretical energy content available in the biomass. Therefore, this type of pretreatment becomes economically unfeasible for full-scale production (Sun and Cheng, 2002).

2.2.2 Chemical pretreatment

The chemical process comprises acidic, alkaline, ozone, organosolv and ionic liquids. Acid pretreatment technologies include concentrated and diluted acid pretreatment. The
concentrated acid pretreatment is less attractive as it can produce inhibitory compounds and cause corrosion problems on the equipment. Dilute acid pretreatment is used to solubilize a great amount of hemicelluloses and lignin to improve the enzyme’s accessibility to the cellulose. However, this process also has the ability to form some sugar degradation compounds like furfural, HMF and phenolic acids, which can inhibit both enzymatic hydrolysis and fermentation (Klinke et al., 2004).

Alkaline pretreatment uses alkaline catalysts, such as sodium, potassium, calcium and ammonium hydroxides. The major effect of this pretreatment is the removal of lignin from the lignocellulosic feedstocks to increase cellulose digestibility, exhibiting minor cellulose and hemicellulose solubilization in comparison to the acid process (Carvalheiro et al., 2008). It has also been reported that alkaline pretreatment forms less sugar degradation than acid pretreatment. Pretreatment of biomass with aqueous ammonia at elevated temperatures can efficiently remove the lignin content and minimize the interaction with hemicelluloses to increase the surface area and pore size. Ammonia pretreatment comprises the ammonia fiber explosion-method (AFEX), ammonia recycle percolation (ARP) and soaking in aqueous ammonia (SAA). However, the disadvantage of alkaline pretreatment is that the alkali will be converted into irrecoverable salts or incorporated the biomass during the pretreatment reactions, resulting in a large amount of alkali, which is a challenge for alkaline pretreatment (Kumar and Wyman, 2009).

The organosolv pretreatment process is normally performed with organic solvents or mixtures of organic solvents to extract lignin from lignocellulosic biomass, such as ethanol, methanol, acetone and ethylene glycol. The advantage of this process is to produce a relatively high-quality lignin as a by-product, which could be used for higher-
value applications of lignin. However, the solvent has to be removed and recovered in order to reduce cost and environmental impact, which could inhibit enzymatic and fermentation processes (Zhao et al., 2009a).

Ionic liquids (IL) pretreatment has shown a great potential in dissolving crystalline cellulose at considerably mild conditions, generating more digestible and less recalcitrant materials for the enzymes (Zhao et al., 2009b). Although ILs have demonstrated new ways for the efficient utilization of lignocellulosic biomass, there are many barriers before putting these into reality practice. For example, the high cost of ILs, lack of toxicological data and inhibitor generation results suggest that more research is required to solve these challenges (Swatloski, et al., 2002).

2.2.3 Physical-chemical pretreatments

The physical-chemical pretreatment process comprises steam explosion, liquid hot water, ammonia fiber explosion, CO₂ explosion, and oxidation delignification among others. Steam explosion is considered the most applied pretreatment process for lignocellulosic biomass. In this process, the biomass is subjected to high pressure saturated steam for a determined period of time, followed by a sudden pressure reduction. The partial hemicelluloses will be hydrolyzed and the lignin matrix will be disrupted due to the explosive decompression. Drawbacks of steam explosion include the formation of degradation products that may inhibit enzymatic hydrolysis and fermentation (Garcia-Aparicio et al., 2006).

Liquid hot water pretreatment requires that biomass is pretreated with water at high temperature (160-230°C) and pressure (5MPa) without employing any catalysts or
chemicals. Pressure is used to maintain water in a liquid state (Mosier, et al., 2005). This hot water pretreatment solubilizes mainly the hemicellulose followed by lignin becoming partially depolymerized to make the cellulose more accessible and to minimize the formation of inhibitors. The major advantage of this pretreatment is the lower concentration of solubilized hemicelluloses and lignin products due to high water input. Therefore, the concentration of degradation products is reduced (Mok and Antal, 1992).

In the ammonia fiber explosion (AFEX) pretreatment process, biomass is treated with liquid ammonia as the catalyst at low temperature and high pressure (Teymouri, 2005). The pressure will be suddenly reduced to disrupt biomass fiber and partially decrystallize cellulose. The advantage of this process is to decrease cellulose crystallinity (Laureano-Perez et al., 2005), reduce lignin content and remove some hemicellulose to increase the digestibility of biomass after AFEX (Galbe and Zacchi, 2007). However, the cost and recovery of ammonia will drive the cost of the pretreatment (Holtzapple et al., 1994, 1991).

CO$_2$ explosion is also used for lignocellulosic biomass pretreatment. This method is similar to the steam and AFEX explosion processes. It uses high pressure CO$_2$ as a supercritical fluid to effectively remove lignin, disrupt the structure of cellulose and hemicelluloses increasing the accessible surface area of the biomass, which in turn increases the digestibility of the biomass. After enzymatic hydrolysis, glucose yields of CO$_2$ explosion are lower than those obtained from steam and ammonia explosion (Sun and Cheng, 2002).

Oxidation delignification pretreatment employs oxidizing agents, such as hydrogen peroxide, ozone, oxygen or air to remove lignin and hemicelluloses from lignocellulosic
biomass. These oxidizing agents will react selectively with lignin’s aromatic ring to make lignin convert into carboxylic acids, which will inhibit the fermentation. A part of the hemicelluloses will be degraded by oxidative treatment, which cannot be used for sugar production (Garcia-Cubero et al., 2009; Sun and Cheng, 2002).

2.2.4 Biological pretreatment

Biological pretreatment normally employs micro-organisms (e.g. white rot basidiomycetes) to degrade lignin. During pretreatment, the microorganisms secrete extracellular lignin-degrading enzymes, such as peroxidases and lactases. These enzymes are capable of removing a considerable amount of lignin and very little cellulose from the biomass. The biological pretreatment is inexpensive under mild conditions, but the hydrolysis rate is low compared to other technologies, ranging from several hours to a few days (Balan et al., 2008). Current effects in biological pretreatment are combined with other pretreatments to develop novel microorganisms for rapid hydrolysis (Kumar and Wyman, 2009).

2.3 Extrusion pretreatment of lignocellulosic biomass

2.3.1 Overview of extrusion pretreatment

Extrusion is defined as an operation of creating objects of a fixed, cross-sectional profile by forcing them through a die of the desired cross-section. The material will experience an expansion when it exits the die. The extrusion process has been expanded as one of the physical continuous pretreatment methods towards bioethanol production due to its significant improvements of sugar recovery from different biomass feedstocks.
Extrusion pretreatment has some advantages over other pretreatments: (1) low cost and provides better process monitoring and control of all variables (Abe et al., 2007), (2) no sugar degradation products (De Vrije et al., 2002), (3) good adaptability to different process modifications (Rizvi and Mulvaney, 1992), and (4) high continuous throughput (Rizvi and Mulvaney, 1992). It seems therefore that extrusion pretreatment is more feasible for the pretreatment of lignocellulosic biomass towards bioethanol production.

A screw extruder is based around screw elements, including (1), forward screw elements, which principally transport bulk material with different pitches and lengths with the least degree of mixing and shearing effect; (2), kneading screw elements, which primarily exert a significant mixing and shearing effect with different stagger angles combined with a weak forward conveying characteristics; and (3), reverse screw elements, designed with a reverse flight to push the material backward, which carries out extensive mixing and shearing effects (Rigal, 1996). The arrangement of different pitches, lengths, stagger angles, positions and spacings define a screw configuration, which is a main factor influencing the extent of mixing, product transportation, extrudate properties, mechanical energy input and residence time distribution during extrusion processing (Gautam and Choudhury, 1999a, 1999b; Amalia Kartika et al., 2006, 2005). With different screw configurations, the twin screw extruder can conduct diverse functions and processes in a single step, such as material transporting, heating, mixing, shearing, grinding, chemical reaction, drying and liquid-solid separation (Amalia Kartika, 2010).

Different types of extruders, such as single-screw extruders and twin-screw extruders, have been widely examined for different lignocellulosic biomass, resulting in subsequently high enzymatic hydrolysis rates. The extrusion pretreatment process can be
used as a physical pretreatment for the bioconversion of biomass to ethanol production; it also can be conducted in a large number of systems with or without the addition of chemical solutions. Karunanithy and Muthukumarappan (2011b, 2010b) report that extruder parameters, such as compression ratio, screw speed, and barrel temperature, had significant effects on sugar recovery. Extruders will provide high shear, rapid heat transfer, and effective mixing in a short residence time. The physical and chemical structure of the material will be disturbed and changed during the passage through the extruder barrel, resulting in a large specific area to increase the accessibility of cellulose for enzymatic action (Zhan et al., 2006). An extrusion pretreatment can also be combined with other treatments to improve sugar recovery. Zheng et al. (2014) evaluated an extrusion process with a dedicated filtration device after steam explosion treatment of corncobs for hemicelluloses separation.

Lignocellulosic biomass can be treated with chemical solutions such as acid and alkali during the extrusion process (Miller and Hester, 2007a, 2007b; Green et al., 1988); however, the acid would cause corrosion problems due to the construction material of the extruder, thus, acid resistant stainless steel alloys such as AL6XN, would be required for extruder screws and barrel fabrication (Miller and Hester, 2007a, 2007b). Alkali pretreatment is a preferred method due to its role as a delignification agent with less degradation of carbohydrates. Among different alkali employed on lignocellulosic biomass, such as sodium, potassium, calcium and ammonium hydroxides, Morrison (1991, 1988) reports that sodium hydroxide is the most known alkali in lignocellulosic biomass pretreatment and can cleave ester linkages and solubilise some hemicelluloses and lignin. Alkali pretreatment can be conducted by soaking the biomass in a sodium
hydroxide solution at room temperature or by adding it to the extruder using a volumetric pump. Table 2-1 summarizes different kinds of extrusion pretreatments of lignocellulosic biomass on sugar yields.

**Table 2-1,** Effects of different kinds of extrusion pretreatment of lignocellulosic biomass on sugar yields. Sugar yields presented in the table were produced from different feedstocks and achieved under different operating conditions, the basis of the percentage values can also vary between each study. The results show the typical range and are for reference purposes, detailed comparisons should only be drawn between highly similar conditions and materials.

<table>
<thead>
<tr>
<th>Extruion Pretreatment</th>
<th>Lignocellulose</th>
<th>Extruder</th>
<th>Extrusion Conditions</th>
<th>Sugar Yield</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn cobs</td>
<td>Single-screw extruder</td>
<td>Screw speed: 75rpm, temp: 125°C</td>
<td>Glucose: 75%, xylose: 49%, combined sugar: 61%</td>
<td>Karunanithy and Muthukumarappan (2010a)</td>
<td></td>
</tr>
<tr>
<td>Switchgrass, big bluestem, prairie cord grass</td>
<td>Single-screw extruder</td>
<td>Screw speed: 200, 200, and 150 rpm, temperature of 75, 150, and 100°C, respectively</td>
<td>Combined sugar: 28.2% for switchgrass, 66.2% for big bluestem, 49.2% for prairie cord grass</td>
<td>Karunanithy et al., (2013)</td>
<td></td>
</tr>
<tr>
<td>Switchgrass, prairie cordgrass</td>
<td>Single-screw extruder</td>
<td>Screw speed: 50 rpm, temp: 150°C, moisture content: 15% for switchgrass; screw speed: 50 rpm, temp: 50°C, moisture content: 25% for prairie cordgrass</td>
<td>Combined sugar: 45.2% for switchgrass Glucose: 61.4%, xylose: 84.3%, combined sugar: 65.8% for prairie cordgrass</td>
<td>Karunanithy and Muthukumarappan (2010b)</td>
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</tr>
<tr>
<td>Wheat bran, soybean hull</td>
<td>Twin-screw extruder</td>
<td>Screw speed: 7Hz or 3.7Hz, temp: 150°C or 110°C</td>
<td>Glucose: 41-60%</td>
<td>Lamsal et al., (2010)</td>
<td></td>
</tr>
<tr>
<td>Big bluestem</td>
<td>Single-screw extruder</td>
<td>Screw speed: 150 rpm, temp: 180°C, moisture content: 20% wb, particle size: 8 mm</td>
<td>Glucose: 71.3%, xylose: 78.5%, combined sugar: 56.9%</td>
<td>Karunanithy and Muthukumarappan (2011a)</td>
<td></td>
</tr>
<tr>
<td>Switchgrass</td>
<td>Single-screw extruder</td>
<td>Screw speed: 155 rpm, temp: 176°C, moisture content: 20% wb, particle size: 8 mm</td>
<td>Glucose: 41.4%, xylose: 62.2%, combined sugar: 47.4%</td>
<td>Karunanithy and Muthukumarappan (2010b)</td>
<td></td>
</tr>
<tr>
<td>Corn stover</td>
<td>Twin-screw extruder</td>
<td>Screw speed: 80 rpm, moisture content: 27.5%, enzyme</td>
<td>Glucose: 48.79%, xylose: 24.98%, combined</td>
<td>Zhang et al., (2011)</td>
<td></td>
</tr>
<tr>
<td>Feedstock</td>
<td>Pretreatment</td>
<td>Equipment Type</td>
<td>Dosage</td>
<td>Sugar</td>
<td>Reference</td>
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<tr>
<td>Soybean hulls</td>
<td>Acid Pretreatment</td>
<td>Twin-screw extruder</td>
<td>0.028 g</td>
<td>40.07%</td>
<td>Yoo (2001)</td>
</tr>
<tr>
<td>Douglas fir</td>
<td>Acid Pretreatment</td>
<td>Batch-type kneader</td>
<td>0.028 g</td>
<td>54.2%</td>
<td>Lee et al., (2010a)</td>
</tr>
<tr>
<td>Soy hull</td>
<td>Acid Pretreatment</td>
<td>Single-screw extruder</td>
<td>0.028 g</td>
<td>62.5%</td>
<td>Karunanithy and Muthukumarappan (2009)</td>
</tr>
<tr>
<td>Municipal solid wastes</td>
<td>Acid Pretreatment</td>
<td>Extruder type reactor</td>
<td>0.028 g</td>
<td>60%</td>
<td>Green et al., (1988)</td>
</tr>
<tr>
<td>Pine sawdust</td>
<td>Acid Pretreatment</td>
<td>Twin-screw extruder</td>
<td>0.028 g</td>
<td>44.4%</td>
<td>Miller and Hester (2007a)</td>
</tr>
<tr>
<td>Pine sawdust</td>
<td>Alkali Pretreatment</td>
<td>Twin-screw extruder</td>
<td>0.028 g</td>
<td>50%</td>
<td>Miller and Hester (2007b)</td>
</tr>
<tr>
<td>Rice straw</td>
<td>Alkali Pretreatment</td>
<td>Twin-screw extruder</td>
<td>0.028 g</td>
<td>83.7%</td>
<td>Chen et al., (2011)</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>Alkali Pretreatment</td>
<td>Extrusion type mixer</td>
<td>0.028 g</td>
<td>90-92%</td>
<td>Carr and Doane (1984)</td>
</tr>
<tr>
<td>Corn stover</td>
<td>Alkali Pretreatment</td>
<td>Twin-screw extruder</td>
<td>0.028 g</td>
<td>86.8%</td>
<td>Zhang et al., (2012)</td>
</tr>
<tr>
<td>Switchgrass</td>
<td>Alkali Pretreatment</td>
<td>Single-screw extruder</td>
<td>0.028 g</td>
<td>90.5%</td>
<td>Karunanithy and Muthukumarappan (2009b)</td>
</tr>
<tr>
<td>Big bluestem</td>
<td>Alkali Pretreatment</td>
<td>Single-screw extruder</td>
<td>0.028 g</td>
<td>90.1%</td>
<td>Karunanithy and Muthukumarappan</td>
</tr>
<tr>
<td>Biomass</td>
<td>Extruder Type</td>
<td>Particle Size</td>
<td>NaOH Concentration</td>
<td>Combined Sugar</td>
<td>References</td>
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<tr>
<td>Corn stover</td>
<td>Twin-screw extruder</td>
<td>4 mm</td>
<td>0.2 g/g biomass</td>
<td>89.9%</td>
<td>Dale et al., (2011c)</td>
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<tr>
<td></td>
<td>Twin-screw extruder</td>
<td></td>
<td></td>
<td>Digestibility: increased up to 32%, lignin reduction: 12.5%</td>
<td>Liu et al., (2013)</td>
</tr>
<tr>
<td>Populus tremuloides</td>
<td>Twin-screw extruder</td>
<td></td>
<td>0.02 g/g biomass</td>
<td>Hemicellulose extraction: 90%</td>
<td>Kadam et al., (2009)</td>
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<td></td>
<td></td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Corn stover</td>
<td>Twin-screw extruder</td>
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<tr>
<td>Douglas fir, Eucalyptus</td>
<td>Twin-screw extruder</td>
<td></td>
<td></td>
<td>Glucose: 5 times higher than HCW treatment alone</td>
<td>Lee et al., (2010)</td>
</tr>
<tr>
<td>Prairie cordgrass</td>
<td>Twin-screw extruder</td>
<td></td>
<td></td>
<td></td>
<td>Ana da Silva et al., (2013)</td>
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<tr>
<td>Bagasse</td>
<td>Twin-screw extruder</td>
<td></td>
<td></td>
<td>Glucose: 90%</td>
<td></td>
</tr>
</tbody>
</table>

2.3.2 General background of single and twin screw extrusion

The screw extruder is a well known technology in the production, compounding, and processing of plastics; it also can be used in food processing industries, such as pet food, cereals and bread. The single screw extrusion process consists of an Archimedean screw in a fixed barrel. It can be classified as a smooth barrel, grooved and/or pin barrel screw...
extruder. Both are employed when melting and pressure build up are required. However, the mixing ability of single screw extruders is limited to distributive mixing and dispersive mixing (spatial rearrangement of solids or fluids changes in physical properties, such as particle size reduction) (Kohlgrüber, 2008). These could be achieved by some types of twin screw extruders using relatively high shear stress screw elements, i.e., kneading disks (Kalyon and Malik, 2007).

Twin-screw extruders consist of two parallel screws with the same length placed in a stationary barrel section. Twin-Screw extruders can be classified according to their direction of screw rotation, i.e., co-rotating or counter-rotating for which the screws rotate in either similar or opposite directions, respectively. Twin-screw extruders can be further subdivided into fully, partial or non-intermeshing based on the relative position of the screws (Riaz, 2000). In contrast to a single screw extruder, the flights scrape the inside of the barrel, and at the same time, maintain a certain clearance between the barrel and screw. The fully intermeshing co-rotating extruders possess the channel, tip, lobal pools, apex and intermesh mixing regions that give rise to very high normal shear. However, the single screw extruder lacks intermesh and apex regions (MDDI, 2000). Therefore, the co-rotating, fully-intermeshing twin screw extruder is a dominant application for biomass processing (Kalyon and Malik, 2007).

2.4 Studies of single and twin screw extrusion for biomass pretreatment

2.4.1 Physical pretreatment

Screw design strongly influences work done on the material and amount of shear force generated during extrusion processes such as compression ratio, screw speed and
barrel temperature. Karunanithy and Muthukumarappan (2010a) conducted pretreatments through a single screw extruder by varying different extruder temperatures (25, 50, 75, 100, and 125°C) and screw speeds (25, 50, 75, 100, and 125 rpm) on the pretreatment of corn cobs while varying enzymes and their ratios. As a result, 75, 49, and 61% of glucose, xylose, and combined sugar recovery were obtained, respectively, at 75 rpm and 125°C using a 1:4 cellulase and β-glucosidase combination. These results were 2.0, 1.7, and 2.0 times higher than the control sample. Similarly, Karunanithy et al. (2013) investigated the effects of screw speeds (100, 150, and 200 rpm), barrel temperatures (50, 75, 100, 150, and 200°C) and cellulase with β-glucosidase (1:1 to 1:4) on sugar yield from selected warm season grasses, such as switchgrass, big bluestem, and prairie cordgrass. The highest values of 28.2, 66.2, and 49.2% of combined sugar yield were obtained for switchgrass, big bluestem, prairie cordgrass at screw speeds of 200, 200, and 150 rpm and at barrel temperatures of 75, 150, and 100°C, respectively, when the ratio of cellulase and β-glucosidase was 1:4. The properties of biomass material also had significant effects on sugar recoveries. Karunanithy and Muthukumarappan (2010b) evaluated the effect of compression ratio (2:1 and 3:1), screw speed (50, 100, and 150 rpm), and barrel temperature (50, 100, and 150°C) on the sugar recovery from switchgrass and prairie cordgrass while varying moisture contents (15%, 25, 35 and 45% wb). The highest sugar recovery from switchgrass after enzymatic hydrolysis was 45.2% at a screw speed of 50 rpm, a barrel temperature of 150°C with a moisture content of 15%. The maximum glucose, xylose and combined sugar recovery of 61.4%, 84.3%, and 65.8% were achieved for prairie cordgrass at a screw speed of 50 rpm, a barrel temperature of 50°C with a moisture content of 25%. In addition, both treatment conditions resulted in low
concentration of glycerol and acetic acid (0.02–0.18 g/L) for both biomasses. Lamsal et al. (2010) investigated two physical pretreatment methods, grinding and thermo-mechanical extrusion, on wheat bran and soybean hull. A higher reduction in sugar yield from extrusion was obtained compared to grinding for wheat bran, but not for soybean hulls. The best combinations of screw speed and barrel temperature were 7Hz/150°C and 3.7Hz/110°C leading to higher sugar yields. Later the effects of extrusion temperature (100, 110, 120 and 130°C), screw speed (50, 60, 70 and 80 rpm) with three moisture contents (10, 12.5 and 15% wb) for soy hulls were studied on a single screw extruder, resulting in 62.5%, 68.6% and 62.4% recoveries for glucose, xylose and combined sugars respectively at a barrel temperature of 130°C, a screw speed of 60 rpm with moisture content of 12.5% wb were 1.7, 1.4 and 1.8 times higher than untreated samples (Karunanithy and Muthukumarappan, 2009c). Karunanithy and Muthukumarappan (2011a) optimized the extrusion pretreatment parameters of barrel temperature (45-225°C), screw speed (20-200 rpm) and big bluestem parameters with moisture content (10%–50% wb) while varying particle size (2-10 mm) for enzymatic hydrolysis for maximum sugar recovery using a single screw extruder. They recovered 71.3%, 78.5%, and 56.9% of glucose, xylose, and combined sugars, respectively at a barrel temperature of 180°C, a screw speed of 150 rpm, moisture content of 20% wb with particle size of 8 mm. As a result, 68.5% of surface area of the optimum pretreated big bluestem was increased compared to that of the control sample. Karunanithy and Muthukumarappan (2011b) also evaluated the effects of parameters of switchgrass such as particle size (2, 4, 6, 8, and 10 mm) and moisture content (10, 20, 30, 40, and 50% wb) over a range of screw speeds (20–200 rpm) and barrel temperatures (45–225°C) on a single screw
extruder. The results show that moisture content, screw speed and barrel temperature had significant effects on sugar recoveries. They recovered 41.4, 62.2 and 47.4%, of glucose, xylose, and combined sugar recoveries, respectively, at a barrel temperature of 176°C, a screw speed of 155 rpm, moisture content of 20% wb with particle size of 8 mm. Zhang et al. (2011) investigated the effect of the extrusion pretreatment method on corn stover and the intrinsic factors contributing to the improvement of sugar recovery, such as moisture content (22.5%, 25%, 27.5% wb) and screw speed (40-140 rpm) using a twin-screw extruder on sugar recovery. The maximum glucose, xylose, and combined sugar recovery were 48.79%, 24.98%, and 40.07%, respectively at 27.5% of moisture content with a screw speed of 80 rpm and an enzyme dosage of 0.028 g enzyme/g dry corn stover. These results were 2.2, 6.6, and 2.6 times higher than that of untreated corn stover, respectively. The cellulose network was exposed because of the lignin destruction and the specific surface area of pretreated materials was significantly amplified over the control samples. Yoo et al. (2011) conducted a thermo-mechanical pretreatment process on soybean hulls and compared with two traditional pretreatment methods, dilute acid and alkali hydrolysis. By comparison, 95% cellulose was converted to glucose when the optimum processing conditions were set up at a screw speed of 350 rpm, a barrel temperature of 80°C with 40% moisture content in the soybean hulls. The conversion from cellulose to glucose was increased by 69.5%, 128.4 and 132.2% for dilute acid, alkali and extrusion pretreatments, respectively. Lower fermentation inhibitors in the extrusion pretreated substrate such as furfural, 5-(hydroxymethyl)-2-furaldehyde (HMF), were found than those reported from the acid hydrolyzed substrate.
Fibrillation of Douglas-fir was performed using water with mechanical kneading forces instead of chemicals for biomass pretreatment in a batch type kneader with twin screw elements. Douglas-fir was milled in a ball milling for 20 minutes and then kneaded at 40 °C at 90 rpm in a batch-type kneader by adding water for 30 minutes. The results showed the surface area of cellulose was increased and the glucose yield from the fibrillated products by enzymatic hydrolysis was 54.2%, much lower than the extrusion process with chemicals (Lee et al., 2010a).

2.4.2 Acid pretreatment

Extruders can be used as an acid hydrolysis reactor. Acid pretreatment is effective for converting cellulose and hemicelluloses into monomeric sugars. For example, an extruder type reactor was used for dilute acid hydrolysis for municipal solid wastes and the optimal glucose yields reached 60% at temperatures of 230°C, pressures of 30-32 atm, pH values of 0.50 and reaction times of 8-15s (Green et al., 1988). A twin screw extruder reactor was also used for concentrated-acid hydrolysis of pine sawdust to break down and convert cellulose into low molecular weight carbohydrates. Experiments were controlled at 110 rpm screw speed, 60°C barrel temperature, 780 psi head pressure and sawdust to acid feed rate ratio of 1 to 0.8 (70 wt%. H_{2}SO_{4}). Consequently, 38.2% of dry sawdust solids were converted to soluble liquids and 44.4% of cellulose was converted to soluble monomer sugars and oligosaccharides (Miller and Hester, 2007a). Later the same operating conditions were applied with different acid concentrations (5 to 30 wt%.) and temperatures (110°C, 120°C and 130°C). An acid-resistant stainless alloy, AL6XN, was used for extruder screws and barrel fabrication. As a result, 50% of theoretical glucose
was achieved at a temperature of 130°C and 5 wt%. in 25 minutes and 41% of the theoretical glucose was converted in three minutes at a temperature of 130°C with more concentrated acid conditions of 30 wt%. (Miller and Hester, 2007b). The twin screw extrusion process also can be combined with the hot water extraction process at a bench scale to prepare monomeric xylose hydrolysate. The effects of screw speed (30-150 rpm), barrel temperature (80-160°C) and dilute sulfuric acid (1-3 wt%) of the twin screw extruder was evaluated on the structural properties of extruded rich straw, sugar concentration and conversion rate, after which 83.7% of the xylan was converted to monomeric xylose when the optimal conditions of the extruder process was at 120°C and 40 rpm with 3% sulphuric acid and the optimal condition of the extraction was 130°C for 20 min. Finally, an 80% yield of the total saccharification was achieved after enzymatic hydrolysis (Chen et al., 2011).

2.4.3 Alkali pretreatment

Alkali pretreatment can be performed at a lower temperature and pressure compared to other chemical pretreatment methods. The process in the extruder also does not cause as much sugar degradation (Moiser et al., 2005). Carr and Doane (1984) employed an extrusion type mixer as a pretreatment method for wheat straw in order to disrupt the lignin-hemicellulose and cellulose complex. The milled wheat straw was treated with various chemical solutions, such as anthraquinone, anthrahydroquinone, hexamethylenediamine, hexamethylenetetramine, sodium hydroxide, ferrous ammonium sulfate and sodium sulfide. These chemicals were metered into the barrel by a high-pressure diaphragm. They acted as delignification agents without degrading the
carbohydrates, so that 64-72% of lignin and 36-43% of the pentosans were achieved when these chemicals used alone or in selected combinations. The conversion rate from cellulose to glucose reached 90-92% at catalyst loadings of 0.157 g/g (NaOH), 0.003 g/g (antrahydroquinone (AHQ)), 0.127 g/g (NaOH), or 0.05 g/g (Na₂S). Similarly, Zhang et al. (2012) also evaluated the effect of twin screw extrusion to promote sugar yields from corn stover with different concentrations of alkali loadings (0.004, 0.012, 0.013 and 0.04 g/g biomass). Corn stover was mixed with alkaline solutions in a mechanical mixer and then fed into the extruder. The optimum glucose and xylose sugar yields were 86.8% and 50.5%, respectively, at an alkali loading ratio of 0.04 g/g biomass and screw speed of 80 rpm. These results were 3.9 and 13.3 times higher than the raw material. In addition, the alkali combined extrusion process produced more pores. Karunanithy and Muthukumarappan (2009b) also carried out pretreatments through a single screw extruder by varying different extruder parameters for maximum sugar recovery from switchgrass. The maximum glucose, xylose, and combined sugar recovery were 90.5, 81.5, and 88%, respectively, at the optimized conditions of 180°C barrel temperature, 118 rpm screw speed, 0.02 g/g (alkali/biomass), and particle size of 6 mm. Similarly, Karunanithy and Muthukumarappan (2011c) evaluated the combined effect of alkali soaking and extrusion on big bluestem using a single screw extruder. Big bluestem was soaked in different alkali concentrations at room temperature for 30 min and then extruded while varying barrel temperatures (45-225°C) and screw speeds (20-200 rpm) and particle size of big bluestem (2-10mm). Consequently, 90.1%, 91.5%, and 89.9% of glucose, xylose and combined sugar were recovered, respectively, at a barrel temperature of 90°C, a screw speed of 155 rpm, 0.2 g/g (alkali/biomass) with particle size of 4 mm. Dale et al. (1999)
conducted the extrusion pretreatment of corn stover with an ammonia solution injection on a twin screw extruder. The technique takes advantage of explosive depressurization resulting in a rapid expansion of liquid ammonia gas that disrupted biomass fibres. The digestibility was increased up to 32% over that of completely untreated material and 23% over extruded material without an ammonia solution. An average of 12.5% in lignin reduction was achieved from extrusion treated material. Liu et al. (2013) performed the alkaline twin screw extrusion pretreatment of corn stover for fermentable sugar production with a biomass/liquid ratio of 1/2 (wt.) at a temperature of 99°C. They used 0.06 g/g biomass of NaOH and converted 83% of glucan and 89% of xylan, respectively; in addition, 71% lignin removal was achieved. N’Diaye et al. (1996) employed a modified Clextral twin screw extruder to extract hemicelluloses through a filter from the hardwood Populus tremuloides. They added 0.02 g/g of a sodium hydroxide solution using a volumetric pump and the screw speed was fixed at 124 rpm for all experiments, subsequently, 90% of the initial hemicelluloses were extracted. Later the same application was performed using a continuous pilot-scale biomass fractionation extrusion process on corn stover. The countercurrent and cocurrent process fractionates corn stover into three streams. Countercurrent prehydrolysis of hemicelluloses at 210°C mainly extracted hemicellulosic sugars and cocurrent flow catalyzed by NaOH (0.06 g/g biomass) at 220°C was used to remove lignin. The overall process was employed to produce a pure cellulose stream and low-molecular weight lignin (Kadam et al., 2009).
2.4.4. Combined pretreatment

Biomass extrusion can be utilized as a stand-alone pretreatment method, or in combination or sequence with other pretreatment techniques. Lee et al. (2010) evaluated extrusion processes after hot-compressed water (HCW) treatment of Douglas fir and Eucalyptus. HCW effectively removed hemicelluloses and lignin while the extrusion process improved the micro/nano fibrillation. Douglas fir and Eucalyptus were pretreated under temperature ranging from 140°C to 180°C at a pressure of 1MPa. The reaction time was 30 minutes and the water to starting material weight was 5:1. The water insoluble residue was then subjected to a twin-screw extruder at room temperature with a screw speed of 45-120 rpm. The produced glucose levels were five times higher than those obtained by HCW treatment alone. Zheng et al. (2014) evaluated a modified twin-screw extruder incorporated with a dedicated filtration device after steam explosion. The ground corncobs were pretreated by steam explosion, and hemicelluloses were hydrolyzed largely to xylose. Subsequent solid liquid separation in a twin-screw extruder resulted in the removal of 80% xylose along with other inhibitors such as soluble lignin (100 rpm screw speed, 100°C barrel temperature, mass flow rate of 4 kg/h). Enzymatic saccharification of the remaining solids resulted in 88% glucose conversion was. Similarly, Brudecki et al. (2013) investigated the effects of sequential extrusion and clean fractionation processing. Ground Prairie Cordgrass was extruded in a single screw extruder under the optimized conditions (90°C barrel temperature, 65 rpm screw speed, 20% moisture content, 8 mm particle size). A clean fractionation process was implemented after the extrusion process to fractionate the extruded material into cellulose, hemicelluloses and lignin fractions. Different proportions of organic solvent
mixtures including methyl isobutyl ketone (MIBK), ethanol and water with sulfuric acid were used. Consequently, 92% glucose was yielded, and 87% lignin and 95% xylan were removed under the optimal conditions of 39 min, 129°C, 0.69% catalyst and 28% MIBK. Ionic liquids are considered as promising solvents for lignocellulosic biomass pretreatment due to their unique solubilization properties. A combination of extrusion in the presence of ionic liquids was performed (Ana da Silva et al., 2013). The extrusion process was carried out using a twin-screw extruder at 140°C with a screw speed of 15 rpm in the presence of 1-ethyl-3-methylimidazolium acetate ([Emim][Ac]). Different bagasse loadings were examined and the results showed that more than 90% glucose was yielded after 24 h of enzymatic hydrolysis from the pretreated baggases at a loading of 25 wt% for 8 min at 140°C (Ana da Silva et al., 2013).

2.5 Economic analysis of extrusion pretreatment methods

Many pretreatment technologies of lignocellulosic biomass have been studied to improve ethanol yield (Saha and Cotta, 2007). Thermo-mechanical extrusion has been used for lignocellulosic biomass pretreatment, resulting in a higher efficiency of enzymatic hydrolysis. An economic, feasible assessment of the extrusion pretreatment methods should be evaluated in order to design an industrial bio-ethanol production plant. However, a comparison of the economic feasibility of each pretreatment is very difficult due to different underlying assumptions (Foust et al., 2009). Kazi et al. (2010) carried out a quantitative data analysis of different pretreatment technologies for the production of ethanol from lignocellulosic materials, including dilute acid, 2-stage dilute-acid, hot water, and ammonia fibre explosion (AFEX). This analysis was based on a short-term
commercial viability of biochemical ethanol production and each pretreatment process was embedded in a full facility model. Publicly available experimental data was used for the total capital investment and product value estimation. Biomass corn stover was used for all pretreatment technologies in the analysis. An ASPEN Plus simulation model of a full bio-ethanol production facility was used for each pretreatment model. In the dilute-acid pretreatment, concentrated sulfuric acid was diluted to 1.1% and the pretreatment reactor operated at 12.1 atm pressure and 190°C; in the 2-stage dilute-acid pretreatment, the first stage solubilizes most of the hemicelluloses and dilute acid hydrolyzes a fraction of cellulose and remaining hemicelluloses in the second stage; in the hot water pretreatment, the corn stover was chopped and washed from the pretreatment section and mixed with hot water in a plug flow pretreatment reactor when the pressure and temperature were maintained at 12.5 bars and 190°C, respectively, for a residence time of 5 min; in the AFEX pretreatment, the corn stover was treated with liquid ammonia, where the pressure was maintained at 17.2 bars and the temperature was held at 60°C for 5 min. The pressure was released rapidly to make the fibres explode. The analysis in terms of pretreatment cost on total capital, installed equipment investment and the ethanol annual capacity, yield and product value are given in Table 2-2. The dilute-acid pretreatment had the highest ethanol yield (289 l/Mg) while the ethanol yield for other pretreatments varied between 177 and 250 l/Mg. In addition, the dilute-acid pretreatment has the lowest product value of $1.36/LGE among all pretreatments.
Table 2-2, Techno-economic analysis for each pretreatment technology. Adapted from Kazi et al. with permission from Elsevier, copyright 2010.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Total capital investment ($ million)</th>
<th>Total installed equipment cost ($ million)</th>
<th>Ethanol production million/year</th>
<th>Ethanol yield (l/Mg)</th>
<th>Product value ($/LEG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilute acid</td>
<td>376</td>
<td>164</td>
<td>202</td>
<td>289</td>
<td>1.36</td>
</tr>
<tr>
<td>2-Stage dilute-acid</td>
<td>391</td>
<td>173</td>
<td>124</td>
<td>177</td>
<td>1.75</td>
</tr>
<tr>
<td>AFEX</td>
<td>386</td>
<td>167</td>
<td>175</td>
<td>250</td>
<td>1.47</td>
</tr>
<tr>
<td>Hot water</td>
<td>327</td>
<td>156</td>
<td>148</td>
<td>211</td>
<td>1.77</td>
</tr>
</tbody>
</table>

LEG: Liter of gasoline equivalent. PV: Ethanol production cost, including a 10% return on investment.

Yoo (2011) analyzed the technical and economic competitiveness of thermo-mechanical extrusion pretreatment with dilute acid hydrolysis for cellulosic ethanol production. The Monte Carlo simulator model was employed to estimate the sugar yield and production cost over a year of production. Biomass soybean hull was used as raw material in the analysis. In the dilute acid hydrolysis, the biomass was run through in three reactor trains consisting of a pre-steamer, blow tank, and reactor, and the solids coming from the reactor were separated from the slurry using a pressure filter for enzymatic saccharification (Aden et al., 2002). In the extrusion process, the biomass was hydrolyzed and softened by steam and water in a preconditioner, and wet extruded pellets were mixed with additional water in a tank for further enzymatic saccharification. The production cost of each pretreatment was performed at the plant scale under some well-defined assumptions. The results of total fixed capital, pretreatment direct fixed capital and the ethanol production of each pretreatment are given in Table 2-3. As a result, 53.7 million gal/year of ethanol was produced from the extrusion process, which was around 23.4% more ethanol than that produced from the acid hydrolysis process at 43.5 million
gal/year; this is due to the higher glucose conversion efficiency from the extrusion pretreatment process. Yoo et al. (2011) report that 94.8% and 69.2% cellulose to glucose conversion was achieved utilizing extrusion and dilute acid hydrolysis pretreatments, respectively. However, if the feedstock was replaced by corn stover, the amount of hemicelluloses would be two times higher than the amount of soybean hulls, and thus more pentose could be fermented to cellulosic ethanol. The total fixed capital investment for each pretreatment was 174 million and 191 million, respectively, and the total pretreatment equipment capital costs for extrusion and dilute acid hydrolysis were estimated at about 25 million and 27 million, respectively, due to the fact that the extrusion process is simpler and required less equipment. From the results, it's proven that extrusion pretreatment is a promising pretreatment technology for cellulosic ethanol production compared to dilute acid hydrolysis due to lower capital, pretreatment cost and higher sugar conversion efficiency (Yoo, 2011).

**Table 2-3, Capital cost for each pretreatment technology (Yoo, 2011).**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Pretreatment direct fixed capital, $million</th>
<th>Total fixed capital, $million</th>
<th>Ethanol production million gal/year</th>
<th>Total fixed capital $/gal annual capacity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilute acid</td>
<td>27.0</td>
<td>191</td>
<td>43.5</td>
<td>4.39</td>
</tr>
<tr>
<td>Extrusion</td>
<td>25.0</td>
<td>174</td>
<td>53.7</td>
<td>3.24</td>
</tr>
</tbody>
</table>

The underlying assumptions of each economical model have to be carefully evaluated when comparing different pretreatment techniques, as can be illustrated by the different assumptions for the capital costs of a diluted acid process of 376 million by Kazi et al. (2010) and 191 million by Yoo (2011) made in different years for otherwise similar projects. Ultimately, no commercial cellulosic ethanol plant has been built to date using
extrusion pretreatment. The Beta Renewables Company started the world’s first commercial cellulosic ethanol plant in Italy with a production capacity of 75 million liters in 2013. The plant is based on the steam explosion and hot water pretreatments from local wheat straw, rice straw and Arundo donax. Few companies have started to test cellulosic ethanol plants on a demonstration scale using some pretreatment methods, such as steam explosion, liquid hot water and dilute acid hydrolysis (Table 2-4) (Dina et al., 2013). The PureVision Technology employs a modified continuous countercurrent extruder reactor (CCER) to rapidly fractionate biomass, such as corn stover and wheat straw, into xylose, lignin, and a digestible solid cellulose fraction that can be converted to glucose in minutes. It yields 80% of total mixed sugars. The company is now proceeding from its experimental concepts to its half-a-tonne-a-day continuous extruder reactor. The first commercial scales 250 tons per day CCER will be built in 2014 (Pure Vision Technology, 2011).
**Table 2-4,** Worldwide production of bioethanol. Adapted with permission from Dina et al., (2013).

<table>
<thead>
<tr>
<th>Company</th>
<th>Location</th>
<th>Products</th>
<th>Status</th>
<th>Raw material</th>
<th>Pretreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abengoa</td>
<td>Spain</td>
<td>4000t/year EtOH</td>
<td>Demo facility, operational since 2008</td>
<td>Wheat straw, barley, corn stover</td>
<td>Acid catalyzed steam explosion</td>
</tr>
<tr>
<td>Clariant</td>
<td>Germany</td>
<td>1000t/year EtOH</td>
<td>Demo facility, operational since 2012</td>
<td>Agriculture residues, wheat straw</td>
<td>Thermo-mechanical</td>
</tr>
<tr>
<td>Inbicon</td>
<td>Denmark</td>
<td>4300t/year EtOH</td>
<td>Demo facility, operational since 2009</td>
<td>wheat straw</td>
<td>Hydrothermal (Liquid hot water)</td>
</tr>
<tr>
<td>Blue Sugar Corp</td>
<td>USA</td>
<td>4500 t/year EtOH</td>
<td>Demo facility, operational since 2008</td>
<td>Sugarcane bagasse and other biomass</td>
<td>Thermo-mechanical</td>
</tr>
<tr>
<td>BP Biofuels</td>
<td>USA</td>
<td>4200t/year EtOH</td>
<td>Demo facility, operational since 2009</td>
<td>dedicated energy crops,</td>
<td>Biochemical (steam explosion and mildly acidic conditions)</td>
</tr>
<tr>
<td>Iogen</td>
<td>Canada</td>
<td>1600t/year EtOH</td>
<td>Demo facility, operational since 2004</td>
<td>Straw (wheat, barley, oat), corn stover,</td>
<td>Modified steam explosion</td>
</tr>
</tbody>
</table>

2.6 Conclusion

The main purpose of pretreatment is to remove hemicelluloses and lignin, to increase the accessible surface area for enzymes and to descrystallize cellulose. The advantages of extrusion pretreatment technologies have been listed and discussed above. An efficient and economical likely has to be developed feedstock specific due to the varying types of biomass. Few quantitative economical analyses of data of different pretreatment technologies were found and discussed. In this review, the feasibility and economical analysis of the extrusion pretreatment offers only an initial glimpse into future
investigations in pretreatment technology. None of the cellulosic ethanol from the extrusion pretreatment technology has been commercialized yet. Uncertainties and limitations are unavoidable in the economic analysis and comparison of conversion technologies. Therefore, identifying the economic impact of different pretreatments related to productivity, capital cost, and operating cost, as well as defined assumptions, is important when conducting the economic analysis of bioethanol. This will help obtain reliable and creditable cost predictions. Further improvements on pretreatment, enzymatic hydrolysis, and fermentation should be researched in order to reduce the production costs.

2.7 Reference


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67. PureVision Technology, Inc.  


Chapter 3. The Effects of Twin-Screw Elements on Enzymatic Digestibility of Corncobs after Pretreatment in a Twin-screw Extruder

Jun Zheng, Kim Choo and Lars Rehmann

With minor editorial changes to fulfill formatting requirements, this chapter is substantially as it has been submitted to: Biomass and Bioenergy

3.1 Preface to Chapter 3

The literature review presented in Chapter 2 clearly shows that extrusion-based biomass pretreatment can be an economically competitive option, at least when compared to other pretreatment methods. The results achieved in the literature vary substantially based on the type of biomass used and the objective of the respective study. The addition of chemical reagent to the extruder appears to have advantageous effects on biomass digestibility, while the impact of individual screw elements on enzymatic digestibility has not been specifically studied. An extrusion process that does not add any chemical agents other than water would have operation advantages as it would lower operating cost, simply the process, not require product purification/detoxification and agent recovery. The requirements on the extruder material would also be lower, reducing the capital investment costs. It was therefore an objective in this study to avoid reactive extrusion and the addition of chemical agents to the process. The current Chapter evaluates the effect of individual functional screw-elements on the biomass structure and enzymatic digestibility of otherwise similarly pretreated biomass. Such information is required in order to establish a rational screw configuration for a proposed extrusion-based pretreatment process.
3.2 Introduction

Long-term economic, environmental and geopolitical concerns associated with the use of and access to fossil fuels, have resulted in increased research on alternative, nonpetroleum-based sources of liquid fuels in the past decades (Yat et al., 2008) and lignocellulosic-based biofuels are considered a logical evolution of current starch-based ethanol processes (Dalgaard et al., 2006; Lynd, et al., 2002; Wyman 1999).

Conversion of lignocellulosic biomass into fermentable sugars for further biofuel production is hindered by the close association of cellulose, hemicelluloses and lignin (Jørgensen et al., 2007). Typically pretreatment steps are required to separate the three main fractions and to increase enzymatic accessibility to the cellulose during the subsequent hydrolysis step (Zhao et al., 2008; Zhu et al., 2006). A wide range of pretreatment methods have been studied generally on different lignocellulosic biomass sources for improving their hydrolysis, such as steam explosion, organosolvent and lime treatment. However, most of these technologies have high processing cost and can produce sugar degradation products due to the use of severe reaction conditions (Alvira et al., 2010; Kumar et al., 2009). Recently, the extrusion process, commonly used in the polymer industry, has been expanded as a continuous physical pretreatment method towards bioethanol production by means of heat, compression and shear forces, which can disrupt and modify biomass structures during its passage through an extruder. Several studies have shown that significant improvements on sugar recovery at low cost, and good adaptability to different process modifications were obtained from the extrusion pretreatment (Karunanithy and Muthukumarappan, 2011, 2010; Abe et al., 2007; Rizvi and Mulvaney, 1992).
The most significant factor influencing the product transportation, extent of mixing, residence time distribution, product properties and mechanical energy consumption during extrusion processing is the screw configuration. Screw-configurations are highly flexible and have to be specifically developed for each process. Screw elements can vary in pitch, stagger angle and length, and are typically categorized based on their functionality as conveying, kneading and reverse elements. Conveying screw elements largely function to transport and compress the bulk material. The number of flights per unit length, as well as pitches or helix angles can be varied, influencing the residence time and the degree of fill. Kneading elements have a significant mixing and shearing effect combined with weak forward feeding characteristics; while reverse screw elements push the material backward with a reverse flight, producing high local pressures. Reverse screw elements exert extensive mixing and shearing effects on the biomass and also exert a strong axial compression along with upstream conveying screw elements (Wang, 2000; Gautam and Choudhury, 1999a, 1999b; Choudhury et al., 1998; Gogoi et al., 1996; Rigal, 1996). With different screw configurations, a variety of functions and processes, such as material conveying, crushing, mixing, melting, shearing, chemical reactions, drying and liquid-solid separation can be conducted in a single unit (Amalia et al., 2010). Several studies employed twin-screw extruders with complex screw configurations to maximize pretreatment efficiency. Subsequently, the sugar yields were shown to be much higher than untreated materials. However, none of these studies investigated the effects of individual functional screw elements on enzymatic digestibility, rendering rational screw design and generally applicable conclusions challenging. Biomass is exposed to heat, compression and shear forces, leading to
physical disruption and chemical changes during the extrusion process. Lignin in particularly can melt and re-condense on the surface of the biomass, thus reducing enzymatic digestibility. It can be removed via sodium hydroxide (NaOH), often used as a pretreatment process from lignocellulosic feedstocks (Xu et al., 2011; Wang et al., 2010). The same technique can be used to evaluate the contribution of possibly re-located lignin on biomass digestibility. The objective of this research was therefore to investigate the effect of individual functional screw elements on the subsequent digestibility of extruded biomass. An extruder was therefore configured with conveying screw elements only, and subsequent a single element was replaced with functional screws in order to isolate the effect of a single screw.

3.3 Materials and methods

3.3.1 Materials

Corn cobs were harvested from a farm near Chatham, Ontario, Canada (42.453367, -82.241060) during the fall harvest in 2013. Kernels were removed at the R&D facilities of GreenField Specialty Alcohols (Chatham, Ontario) followed by cleaning and grinding to particle sizes of 0.5 to 1 cm$^3$ and drying to a moisture content of 8%. The biomass was stored at 4 °C and processed via extrusion within less than 4 weeks. The composition of structural carbohydrates and lignin of untreated and treated corn cobs were determined according to NREL methods outlined by Sluiter et al. (2011). All composition values in this study were based on dry matter. Before extrusion pretreatment, ground corn cobs were adjusted by adding water to retain a moisture content of 40%.
All other chemicals (e.g., citric acid, sodium hydroxide anhydrous, sodium citrate, xylose and glucose) were of analytical grade and purchased from Sigma-Aldrich (USA). Cellic CTec 2 cellulose enzyme was obtained from Novozyme (Denmark).

3.3.2 Twin screw extruder

Experiments were carried out with a pilot-scale Leistritz co-rotating twin-screw extruder (American Leistritz Extruder Corp, USA). The extruder has twelve modular barrels, each 200 mm in length. Different twin-screws have segmental screw elements each between 15 mm and 90 mm in length. Thermal induction and cooling water circulation are used for barrel heating and cooling, respectively. Feed flow rate, screw speed and barrel temperature were monitored. The ground corncobs were fed into the extruder by a gravimetric feeder (Brabender Technology Company, Canada) and fixed at a mass flow rate of 4 kg h\(^{-1}\) for all experiments. The operating conditions of the extrusion process were maintained at a screw speed of 1.67 Hz and a barrel temperature of 75 °C. Fig.3-1 shows the schematic modular barrel of the twin-screw extruder. Three screw configuration profiles were tested in this study as shown in Fig.3-2. All conveying screw elements were configured to have a constantly decreasing pitch to enhance the degree of fill in Profile 1. A conveying screw element with a length of 30 was replaced by two forward kneading screw elements and a combination of a kneading and reverse screw element in zone 10 for Profiles 2 and 3, respectively. A circular die of 1.98 cm diameter was employed for all extrusion experiments.
**Figure 3-1.** The schematic modular barrel of the twin-screw extruder.

**Figure 3-2.** Screw configurations development with different screw elements.


GFA-2-XX<sup>a</sup>-XX<sup>b</sup>: G= co-rotating, F=conveying, A=free-meshing, 2=number of threads, XX<sup>a</sup>=pitch, XX<sup>b</sup>=length of screw element, x2: two same elements.

KB2<sup>a</sup>-2<sup>b</sup>-30-XX-Li: KB=kneading block, 2<sup>a</sup>=number of kneading segments, 2<sup>b</sup>=number of threads, 30=length of kneading block, XX=twisting angle of the individual kneading segments, Li=reverse element.
3.3.3 Enzymatic hydrolysis

Batch enzymatic hydrolysis was carried out in 20 mL screw capped glass vials with Cellic CTec 2 enzyme (Novozyme, Denmark). The enzyme activity was measured to be 168.2 FPU ml^-1 (Filter Paper Unit) according to NREL standard procedures (Adney and Baker, 2008). Applied enzyme loadings were varied from 2.85 to 3.59 FPU g^-1 of the extruded corncobs determined based on the total amount of glucan in each sample. The hydrolysis mixture consisted of 100 g L^-1 substrate in a 0.1 mol L^-1 sodium citrate buffer at a pH of 5.0 and the total batch volume was 10 mL. All vials were incubated at 50 °C in an incubation shaker (Infors HT, Switzerland) at 4.34 Hz from 24 to 96 h. All experiments were conducted in triplicate and data is presented as the average plus minus standard deviation. The reaction was monitored by taking 50 µL samples every 24 h and placed at -20°C for 10 min to denature enzyme activity. Each sample was diluted 15 fold with 5x10^-3 mol L^-1 H_2SO_4 and the slurry was filtered through 0.2 μm cellulose acetate membrane (VWR International, USA). 1 ml of liquid was transferred to a HPLC vial for sugars analysis.

3.3.4 Carbohydrate and lignin analysis

The structural carbohydrate composition of ground, extruded and alkali treated corncobs were determined by a quantitative procedure (Sluiter et al., 2011). Monomeric sugars were quantified by an Agilent 1260 Infinity high-performance liquid chromatography (HPLC) using a MetaCarb H Plus Column 300x7.8 mm (Agilent Technologies, USA), equipped with a refractive index detector at 55 °C. Column temperature was maintained at 60 °C and 5x10^-3 mol L^-1 H_2SO_4 was used as a mobile
phase at a flow rate of 0.7 mL min\(^{-1}\). The glucan conversion after enzymatic hydrolysis was calculated by comparing the total amount of glucose produced in the hydrolyzate to the total amount of glucan in the substrate, as measure after full acid hydrolysis. Mannose and galactose were co-eluted with xylose and not separately quantified. Lignin was measured according to standard NREL methodology (Sluiter et al., 2011).

3.3.5 Sodium hydroxide pretreatment after extrusion

Extruded corncobs were treated with 10 g L\(^{-1}\) sodium hydroxide solutions at a solid loading of 100 g L\(^{-1}\) in a 150 mL flask sealed with aluminum foil. Samples were treated in an autoclave (AMSCO Eagle Series 2041 Gravity; American Sterilizer Co., Erie, USA) for 60 min at 121 °C. The treated biomass was recovered through a filtration system with 0.45 µm filter paper (Millipore, Billerica, USA). Wet solids were washed with 800 mL of distilled (DI) water (100 mL DI water g\(^{-1}\) biomass) through vacuum filtration in order to neutralize the pH of the substrate, and then the solids were dried under ambient conditions for 48 h, and stored at 4 °C in sealed plastic bags for subsequent enzymatic hydrolysis. A small portion of the dried substrate was used to determine the lignin content.

3.3.6 Particle size measurement

Particle size measurements of ground and extruded corncobs were carried out via sieving (VWR Scientific, West Chester, USA) in triplicates, the sieve size varied from 2000 µm to 38 µm. Extruded corncobs were dried in the air until the moisture content was less than 5% prior to sieving. 300 g of each sample were sieved under mechanical
shaking for 20 min. The finest particles were collected by a well-fitting pan at the base. Any particles caught in the sieve were removed by a sieve brush. The mass of samples retained on each sieve was weight and recorded, including the base.

3.3.7 Scanning electron microscopy (SEM)

The surface properties and microstructure of ground, extruded and alkalized corncobs samples (dried powder of particle size <150 µm) were conducted using a FIB/SEM Crossbeam (Zeiss/Leo 1540XB, Oberkochen, Germany) at an accelerated voltage of 1.0 kV. After air-drying, 5 nm of amorphous osmium metal was deposited on the surface of the sample before imaging using an osmium plasma coater (OPC-80T, SPI supplies, West Chester, USA) to enhance the secondary electron yield. The representative images were acquired at magnifications ranging from 500 to 5,000x.

3.3.8 Crystallinity measurement

The diffraction patterns of biomass samples were obtained using an X-ray powder diffractometer. Samples of particle size less than 150 µm-mesh screen were scanned and the cellulose crystallinity was determined by Rigaku (USA) in conjunction with a CoKα radiation source. Samples were scanned at an angular incidence of 5-45° (2θ) range of diffraction angle at a speed of 5° (2θ) min⁻¹. Biomass crystallinity is expressed via its crystallinity index (CrI), determined through the X-ray diffraction patterns based on the following relationship (Cao and Tan, 2005).

\[
\text{CrI} = \frac{I_{\text{max}} - I_{\text{min}}}{I_{\text{max}}} \times 100\% \quad (1)
\]
Where $I_{\text{max}}$ represents the maximum intensity peak for the crystalline region of the biomass (i.e., crystalline cellulose), $I_{\text{min}}$ represents the minimum intensity peak for the amorphous region (i.e., amorphous cellulose, hemicelluloses, and lignin).

For the estimation of the CrI from CuKα to CoKα radiation source, the intensity of the crystalline and amorphous portions was converted based on Bragg’s Law:

$$\theta = \text{Arc Sin} \left( \frac{n\lambda}{2d} \right)$$

(2)

Where $n$ is an integer, $\lambda$ is the wavelength of the incident wave (1.54056 for CuKα; 1.78896 for CoKα), $d$ is the spacing between the planes in the atomic lattice, and $\theta$ is the diffraction angle.

In this study, the intensity of the crystalline and amorphous portions were at $2\theta = 26^\circ$ and $19^\circ$ respectively.

3.4 Results and discussion

3.4.1 Carbohydrate analysis

The compositions of the ground, extruded and alkalized corncobs are shown in Table 3-1. Slight variation can be seen in the analytical results when different screw elements were used. The experiments were conducted on different days, as equipment cleanup and changes in the screw configuration of the pilot-scale extruder required substantial time. The differences might be due to the heterogeneity of the starting material, or simply daily fluctuation in the analytical results. Table 3-1 also shows an expected decrease in lignin content after sodium hydroxide pretreatment. This reduction is enhanced for the extruded samples. The lignin fraction is highly affected under the heat, compression and shear in the extruder. Physicochemical changes, such as lignin melting and re-condensation or
pseudo-lignin formation by the combination of carbohydrates and lignin degradation products on the surface of corncobs have been reported in the literature (Kumar et al., 2009; Zhu et al., 2009), Zhang et al. (2012) reported that the shear rate developed during the extrusion process at 1.34 Hz is enough for lignin softening. Some researchers reported that the apparent lignin content after extrusion is higher compared to the raw material due to the formation of pseudo-lignin (Sannigrahi et al., 2011), which was not observed here. The measured xylan and glucan values are in good agreement with values reported by Kumar et al. (2009). Delignification typically results in improved enzymatic hydrolysis of lignocelluloses, as lignin removal can significantly enhance the exposure of cellulose to the enzymes (Gao and Rehman, 2014; Jeoh et al., 2007). The Lignin content of extruded corncobs was reduced to approximately 14% after the sodium hydroxide pretreatment (21% without extrusion), demonstrating that lignin is much easier to remove from the extruded corncobs; either its composition or location within the biomass is likely effected by the extruder. The severity of the extrusion appears to enhance the lignin removal during NaOH treatment, as can be seen in case of the reverse screw elements. The data shows that biomass extrusion can enhance subsequent delignification, and that the choice of screw configuration might further control the achievable degree of delignification.
Table 3-1. Composition analysis of untreated and treated corn cobs with different pretreatment methods.

<table>
<thead>
<tr>
<th>Biomass Sample</th>
<th>Extrusion Pretreatment (%)</th>
<th>Alkaline Pretreatment (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
<td>Xylose</td>
</tr>
<tr>
<td>Ground Corncobs</td>
<td>39.39±0.72</td>
<td>29.18±0.64</td>
</tr>
<tr>
<td>Conveying</td>
<td>41.94±0.35</td>
<td>30.40±0.43</td>
</tr>
<tr>
<td>Kneading</td>
<td>42.59±0.58</td>
<td>31.24±0.58</td>
</tr>
<tr>
<td>Reverse</td>
<td>41.25±0.16</td>
<td>31.35±0.20</td>
</tr>
</tbody>
</table>

3.4.2 Particle size measurements

Extrusion of biomass can result in particle size reduction. Smaller particles tend to result in higher enzymatic hydrolysis rate due to increased surface area. However, substrate surface area is divided into external surface area affected by shape and particle size, and internal surface area, which is dominated by capillary structures, such as intraparticulate pores and interparticulate voids (Marshall and Sixsmith, 1974). Particle size reduction will change fibre length and width, leading to change in the substrate external surface area. Karunanithy and Muthukumarappan (2011) confirmed that a combination of high screw speed, low moisture content, particle size, screw elements and high barrel temperature might have increased the shear forces and friction between the biomass and barrels, leading to fibre length reduction during the extrusion process. The cell walls will be opened up through the particle size reduction process, which increases the effective surface area to volume ratio, thus allowing enzymes to access active substrate sites (Mansfield et al., 1999); meanwhile, the affinity between cellulose and
enzymes will be enhanced by particle size reduction, thus resulting in a higher enzymatic hydrolysis rate. Numerous studies have investigated the impact of substrate particle size on enzymatic hydrolysis for cellulose to glucose conversion, enzymatic hydrolysis generally increased as the substrate particle size was reduced (Zhu et al., 2009; Dasari and Eric Berson, 2007; Mooney et al., 1999; Elshafei et al., 1991). Particle size reduction can be achieved by physical and mechanical pretreatment methods, such as milling, grinding and extrusion (Kumakura and Kaetsu, 1978). The particle size distribution of the ground and extruded corncobs were determined and cumulative mass fractions for all samples are shown in Fig.3-3. As expected, the distribution was effected by the extrusion process. The results show a clear reduction in particle size after extrusion, while there was no measurable differences observed between the respective screw configurations.

![Figure 3-3](image)

**Figure 3-3.** Particle size measurement of untreated and extruded corncobs with different screw elements (cumulative mass fraction).
3.4.3 SEM images of the ground, extruded and alkalized corncobs

SEM images of the biomass are shown in Fig. 3-4. Extrusion pretreatment can disrupt the pore size distribution of corncobs, so that some irregular pores in the outer secondary cell wall region were observed on the surface of extruded corncobs (Fig. 3-4 C vs. A). Some of the pores might be caused by moisture evaporation. Extrusion pretreatment provides mixing, shear force and heat to corncobs; therefore, some of the moisture present deeply inside the particles can evaporate by the sudden pressure reduction at the end of the extrusion process, resulting in expansion and a porous structure of the extruded material (Zhan, et al., 2006). Disruption of physical and chemical structures within the cell walls will change the layered and porous structures, resulting in internal surface area changes (Chandra et al., 2007). These large pores will increase cell wall porosity, reduce threshold pressure, increase permeability and percolation probability, all of which allow large and small cellulase components to easily diffuse into these pores and have a synergistic effect on the cellulase enzyme system, thus are expected to improving the efficiency of the enzymatic hydrolysis rate (Zhao and Chen, 2013). As shown in Fig. 3-4 C, the extruded corncobs with all conveying screw elements produced many pores. However, the extruded corncobs with kneading screw elements produced less and smaller pores (Fig. 3-4D). Pores on extruded corncobs with reverse screw elements were barely visible while some pores were blocked (Fig. 3-4 F). The extrusion pretreatment might cause lignin condensation or pseudo-lignin complex formation when the pretreatment temperature is above the range for lignin phase transition. Although the operating temperature is only 75 °C, the reverse screw generates high shear forces during the extrusion process, possibly resulting in high local temperatures. High temperatures can
melt lignin and evenly deposit it on the surface of the extruded corncobs to co-exist with cellulose. The accessibility of the enzyme to the pore structure of the substrate is an important factor impacting the enzymatic digestibility. Lignin re-localization appears to have blocked the pore structure when the material was extruded with reverse screw elements, potentially hindering subsequent enzymatic digestibility (Zhu et al., 2009). Alternatively, the lack of observable pores might have been caused by pore collapse under high pressure, as generated by the functional screw elements. Sodium hydroxide-based lignin removal was undertaken in order to investigate if pores were still present and covered with lignin or if pore collapse had occurred. Fig. 3-4 B, D, F, H reveal the structural changes of ground and extruded corncobs treated with NaOH. The extruded corncobs clearly exhibit more pores after the NaOH pretreatment, especially evident for the reverse screw elements, indicated that blocked pores were freed from lignin. These findings suggest that extruders can substantially influence the lignin fraction of biomass. Extrusion treatment with functional screw elements highly increases the susceptibility of lignin for subsequent removal (Table 3-1), beneficial if such as step is part of the overall pre-treatment process. However, the reason for the easier removal is likely the re-distribution of lignin on the fibre surface, which might be a hindrance for direct enzymatic conversion.
Figure 3-4. SEM images of untreated, extruded and NaOH pretreated corncobs before enzymatic hydrolysis. [A]: untreated corncobs; [B]: extruded corncobs by all conveying screw elements; [C]: extruded corncobs by kneading screw elements; [D]: extruded corncobs by reverse screw elements; [E]: Ground corncobs pretreated by NaOH; [F]: extruded corncobs with conveying screw elements pretreated by NaOH; [G]: extruded corncobs with kneading screw elements pretreated by NaOH; [H]: extruded corncobs with kneading screw elements pretreated by NaOH.
3.4.4 Crystallinity measurement

Cellulose crystallinity is an important factor affecting the enzymatic digestibility of cellulose (McMillan, 1994) and it is generally assumed that amorphous cellulose is more susceptible to enzymatic hydrolysis than the crystalline form (Mosier et al., 2005). The crystallinity index (CrI) was calculated from XRD data as the mean of three replicates and the results are shown in Table 3-2. The crystallinity index increased slightly after extrusion and was not significantly different among the extruded corncob samples. The CrI’s increase after NaOH treatment is due to the removal of amorphous lignin and the increase is within the range typically reported in the literature (Maeda et al., 2011; Sannigrahi, et al., 2010; Kim and Holtzapple, 2006; Sinitsyn et al., 1991).

Table 3-2, Crystallinity index of biomass samples prior and after lignin removal (average of triplicates ± standard deviation).

<table>
<thead>
<tr>
<th>Biomass Sample</th>
<th>CrI (-)</th>
<th>Prior NaOH</th>
<th>Post NaOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground Corn Cobs</td>
<td>0.303 ± 0.02</td>
<td>0.382 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Conveying</td>
<td>0.331 ± 0.03</td>
<td>0.409 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Kneading</td>
<td>0.332 ± 0.01</td>
<td>0.422 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Reverse</td>
<td>0.332 ± 0.02</td>
<td>0.452 ± 0.03</td>
<td></td>
</tr>
</tbody>
</table>

3.4.5 Enzymatic digestibility testing of corncobs after different pretreatment methods

The degree of enzymatic conversion of glucan to monomers among untreated, extruded and alkalized corncobs was evaluated. Glucan conversion over a 96 h time period is shown in Fig.3-5. Untreated corncobs only showed 9 % glucan conversion after 24 h and 12% after 96 h under the given conditions. The enzymatic digestion of extruded corncobs yielded significantly higher total glucan conversion than that of untreated corncobs (approximately 50% higher at 24h), irrespectively of the screw configuration. This increase might have been caused by the smaller particle size of the extruded...
corn cobs. Extruded corn cobs with kneading and reverse screw elements exhibited similar glucan conversion of approximately 19% at 96 h compared to 12% for untreated corn cobs. Extruded corn cobs with all conveying screw elements had a significantly higher glucan conversion of 22% at 96 h. These results are in line with the observed blockage of pores through lignin relocalization on the fibre surface in the case of the reverse screw elements, as seen via SEM (Fig.3-4). Lignin coverage of the fibres might have generally reduced accessibility and in addition prevent the cellulase accessibility to the inner layers, effects also observed during other thermochemical pretreatment methods (Li et al., 2014; Kumar et al., 2013). This hypothesis was further tested through NaOH-based lignin removal.

The efficiency of lignin removal via sodium hydroxide was strongly affected by the extrusion process (Table 3-1). Approximately 10% lignin was removed from untreated corn cobs, while 31%, 30% and 37% lignin were removed from extruded corn cobs with conveying, kneading and reverse screw elements, respectively. Lignin removal resulted in increased enzymatic hydrolysis of all corn cob samples. As shown in Fig.3-5, the enzymatic digestibility of untreated corn cobs, and extruded corn cobs with all conveying, kneading and reverse screw elements after the alkali pretreatment was increased substantially achieving 41%, 56%, 64% and 68% glucan conversion, respectively (24 h). The digestibility increased with the severity of the extrusion process, as would be expected from a mechanical pretreatment process. The fact that the same effect did not occur prior to de-lignification clearly shows that redistribution of lignin through an extruder can mask otherwise positive effects of the extrusion process. The possibility of lignin redistribution therefore has to be considered when designing a screw configuration.
for a biomass extrusion process, unless subsequent delignification will occur. This study isolated the effect of single screw elements, while a typical screw configuration for extruder-based biomass pretreatment would involve a large number of functional screw elements. The effect observed here would likely be enhanced further under such conditions.

Figure 3-5. Enzymatic glucan conversion of corncobs before (a) and after (b) NaOH treatment. Ground corncobs ∇, extruded with all convening elements □, extruded with kneading elements ◇ and extruded with reverse screw elements △.
3.5 Conclusion

This study demonstrates that individual screw elements in a twin-screw extruder can affect lignocellulosic biomass in multiple ways, some these effects can enhance enzymatic digestibility while others decrease it. The negative effect of lignin redistribution increases with the severity of the extrusion process, however subsequent lignin removal shows that the same conditions will enhance digestibility through other physicochemical changes in the biomass.

3.6 Reference


Chapter 4. The Effects of Screw Configuration on Xylose Removal from Lignocellulosic biomass via a Twin-screw Extruder: Feasibility Study and Influence of Operating Conditions

Jun Zheng, Kim Choo and Lars Rehmann

With minor editorial changes to fulfill formatting requirements, this chapter is substantially as it has been submitted to: Biomass and Bioenergy

4.1 Preface to Chapter 4

The previous chapter has shown that extrusion pretreatment under very mild conditions can have a positive effect on enzymatic digestibility. However it also showed that more aggressive screw configuration might result in the lignin relocation, possibility interfering with enzymatic digestibility. However, the positive effects appear to outweigh the negative and therefore and attempt was made of developing an extrusion process with an integrated solid/liquid separator in order to separate xylose from the cellulose fibers. The hemicellulose fraction hydrolyses easily during steam-explosion, and under elevated temperature in the extruder. Removing the hemicellulose fraction is one of the set pretreatment goals, as xylose typically interferes with the cellulase enzymes used during saccharification prior to fermentation. The cost of the enzymatic saccharification can be substantially reduced if xylose is removed prior to this step. The yeast strains used in industrial ethanol fermentations typically cannot ferment C5 sugars either, hence prior removal of this fraction makes economical sense, especially as dedicated C5 fermenting organisms are currently being developed. The following chapter therefore aims to
hydrolyze the hemicellulose fraction of the biomass in the extruder and to separate the dissolved xylose from solid cellulose through a dedicated filter block.

4.2 Introduction

Twin-screw extruders are widely used in the mixing, compounding, and processing of plastics. It is also a popular unit operation for agro processing industries, such as pet food, cereals and snacks, which allows the extrusion and cooking to occur simultaneously in a single step (White, 1991; Harper, 1989). A twin-screw extruder consists of two parallel screws with the same length placed in a figure-of-eight shaped barrel section. Twin-screw extruders can be classified according to their direction of screw rotation (co-rotating and counter-rotating). It can also be further subdivided into full, partial or non-intermeshing based on the relative position of the screws. The co-rotating, intermeshing, self-wiping twin-screw extruder occupies a dominant position among extruders (Riaz, 2000).

Extruders are currently gaining importance in the bioresource industry, such as biodiesel and lignocellulosic ethanol production. In the present day, several studies have expanded the usage of twin-screw extruders for the continuous extraction of vegetable oil from sunflower and soy (Amalia Kartika et al., 2010, 2006, 2005; Amalia Kartika, 2008; Wang and Johnson, 2001; N'Diaye et al., 1996), while, recently, a modified twin-screw extruder incorporating a filtration device was considered as a thermal-mechano-chemical reactor and a liquid/solid separator for hemicellulose extraction from lignocellulosic biomass (N’Diaye and Rigal, 2000; N’Diaye et al., 1996). Much experience has been obtained successfully extracting hemicellulose via different solvents. Wingerson and
Chin (2012) have recently reported a fractionation technology with organic or inorganic solvents, such as alcohols for separating hemicellulose from lignocellulosic biomass into three streams for a xylose-rich liquor, lignin-rich liquor and a solid cellulose fraction using a modified twin-screw extruder.

The advantages of using a twin-screw extruder for lignocellulosic biomass fractionation include (1), low cost and good monitoring and control of all variables (Abe et al., 2007); (2), no sugar degradation products (De Vries and Visser, 2001); (3), good adaptability to different process modifications (Rizvi and Mulvaney, 1992) and (4), high continuous throughput (Rizvi and Mulvaney, 1992). A suitable screw configuration is required to achieve a process with these advantages. Screw configuration varies from process to process and are based on the arrangement of different screw elements, including (1), conveying screw elements, which transport bulk material with different pitches and lengths and have no mixing effect; (2), kneading elements, which exert significant mixing and shearing in combination with weak forward conveying; and (3), reverse screw elements, designed with a reverse flight to push the material backward, which exerts extensive mixing, shearing, and increases local pressure. The pitch, length, stagger angle and position of screw elements define a screw configuration which is a dominant factor influencing the extent of mixing, extrudate properties and mechanical energy input during extrusion processing (Gautam and Choudhury, 1999a, 1999b; Rigal, 1996). With different screw configurations, the twin-screw extruder can conduct diverse functions and processes in a single step, such as material transporting, heating, mixing, shearing, grinding, chemical reaction, drying and liquid-solid separation (Amalia Kartika et al., 2010).
Hemicellulose extraction from lignocellulosic biomass is an important pre-treatment step when considering lignocellulosic ethanol production, as subsequent enzymatic saccharification of the cellulose fraction is typically inhibited by the presence of hemicellulose (Zheng et al., 2014; Zhang et al., 2012). Although hemicellulose extraction via organic solvents has been demonstrated in the literature (N’Diaye and Rigal, 2000; N’Diaye et al., 1996), little data is available on purely aqueous systems, especially with respect to operating conditions and energy input. It was therefore the objective of this study to evaluate the effect of screw configurations and operating conditions on hemicellulose separation from steam-exploded corncobs (a waste product from the sweet corn industry and potential feedstock for lignocellulosic ethanol production), with additional consideration of the required energy input.

4.3 Materials and methods

4.3.1 Materials

Corncobs were collected from local farms in Chatham, Ontario, Canada and pretreated via steam explosion prior to the extrusion process. In brief, the corncobs were ground (particle size between 0.5 and 1cm³) and fed into a batch reactor, and exposed to 235 psig for 8 min prior to explosive decomposition. High temperature steam will convert acetyl groups in hemicelluloses into acetic acid which will hydrolyze xylan polymer into xylose oligomer or monomers (Garrote et al., 2002, 2001). The steam-exploded corncobs were dried by air drying to a final moisture content of 40% and were used as raw material for the extrusion process.
Other chemicals (e.g., sulfuric acid, acetic acid, ammonium hydroxide, myo-inositol, sodium borohydride, acetic anhydride, 1-Methylimidazole, dichloromethane, sodium sulfate anhydrous and xylose) were of analytical grade and purchased from Sigma-Aldrich (Canada).

### 4.3.2 Sugar analysis

Total monomeric xylose sugar was quantified by an Agilent 7890A gas chromatograph (Agilent, Palo Alto, CA, USA) with a DB 225 capillary column (30m×0.25mm I.D. and film thickness of 0.15 µm,) equipped with a flame ionization detector (FID) (Agilent, Palo Alto, CA, USA). A 10 ml filtrate sample from each extrusion process was hydrolyzed by 3 ml 72% (w/w) H₂SO₄ covered with parafilm at 30°C in a water bath for 1 hr. Upon completion, the acid was diluted to 4% (w/w) by adding 74ml water and autoclaved for 1 hr at 121°C and 15psi. Samples were removed from the autoclave and placed in an ice bath to cool to room temperature. 10ml of myo-inositol solution (10 g/L) was added to each sample. Each sample was then neutralized with a solution of 43 ml water and 11 ml of NH₄OH and filtered using Whatman #4 filter paper (Whatman Limited, Japan). 2 ml of neutralized solution was added to 35 mg of NaBH₄ in a 125ml Erlenmeyer flask covered with parafilm and placed in a 40°C water bath for 2 hr. 1ml of glacial acetic acid was then pipette into the flask. 2ml of 1-methylimidazolide and 20 ml of acetic anhydride were added and mixed on a stir plate for 20 min. After 20 min, a 100 ml ice water mixture (70mL water and top up to 100mL with ice chips) was added and stirred for at least another 20min. 10ml, 5ml, 5ml of DCM (Dichloromethane) were added separately to extract a total of 20 ml DCM and finally, the
DCM solvent was evaporated by Roto-evaporator (Sigma-Aldrich, Canada) until 2 ml remained followed by the addition of 2ml of DCM. Samples were filtered through 0.45μm nylon filter vials (Alltech Associates Inc., Deerfield, IL, USA) in 5ml syringe (filled with sodium sulphate), 1ml filtrate was transferred to GC vial (Alltech Associates Inc) and kept refrigerated at 4°C until analyzed. Helium was used as the carrier gas at a constant flow rate of 1.2 ml min⁻¹ and a pressure of 13.2 psi. The nitrogen gas flow rate was maintained at 25 ml min⁻¹. The injector temperature was maintained at 230°C. The oven temperature was adjusted to 220°C at the start of injection and hold for 20 min. Data were acquired and processed using an Agilent GC ChemStation software ver. 3.03.02 (Agilent, Palo Alto, CA, USA) (Englyst et al., 1982; Krull and Inglett, 1980; Borchardt and Piper, 1970).

4.3.3 Twin screw extruder

Experiments were conducted with a pilot-scale Leistritz Co-Rotating Twin-Screw Extruder (American Leistritz Extruder Corp, USA). The extruder is composed of twelve modular barrels, each 200 mm in length and individually temperature controlled. The extruder was further equipped with designed filtration device for liquid/solid separation (barrel 9), a water injection port (barrel 7) and two pressure sensors (barrels 8 and 10). A schematic is shown in Figure 4-1. The steam-exploded corncobs were fed into the extruder inlet port (barrel 0) by a gravimetric feeder (Brabender Technology Company, Canada) at a mass flow rate of 4 kg/h for all experiments. The liquid and solid flow rates at the exit of the extruder were measured and filtrate samples were collected and stored at 4°C for xylose analysis.
4.3.4 Mechanical energy measurement

The total energy input to the extrusion system consists of mechanical energy provided by the motor drive and thermal energy, coming from the heating system. Both inputs can be measured separately and the sum is the total energy consumption of the extrusion process (Karunanithy and Muthukumarappan, 2012; Rauwendaal, 2008). An industrially relevant measure is the mechanical energy consumed by extruder motor drive. Therefore the torque transmitted through the shaft of the extruder was quantified with a torque transducer connected to the extruder drive (Power Monitor 1000, Allen Bradley, Canada). The actual torque caused by the biomass processing was determined from operational torque measurement subtracted by no-load torque measurement. The power consumed by the motor drive was obtained by multiplying the actual torque (N m) with the screw speed (s\(^{-1}\)) and 2 \(\pi\) (Karunanithy and Muthukumarappan, 2012, 2011; Rosentrater et al., 2009; Harper, 1989). The specific mechanical energy (SME) was defined as the amount

Figure 4-1. Schematic of modified twin-screw extruder.
of mechanical energy consumed by the motor drive per kg of steam exploded corncobs during extrusion:

\[
\text{SME (kWh/kg)} = \frac{\text{Mechanical Energy (kWh)}}{\text{Mass Flow Rate (kg/h)}}
\]  \hfill (1)

Furthermore xylose specific energy (XSE) was defined as the amount of specific mechanical energy needed to remove 1% xylose under given extrusion conditions:

\[
\text{XSE (kWh/kg,%) = } \frac{\text{SEM (kWh/kg)}}{\% \text{ Xylose Recovery}}
\]  \hfill (2)

4.3.5 Screw configuration study

A suitable screw configuration for high xylose recovery was developed based on data gathered at eight distinct screw configurations as summarized in Figure 4-2. Various combinations of conveying, kneading and reverse screw elements at different positions and spacing were used in order to manipulate the pressure profile in the extruder and therefore the liquid recovery at the filter block. Screw configuration profile 1 consisted of conveying screw elements with a large pitch (GFA 2-40-90) directly below the feed hopper (barrel 0-2) and the pitch was reduced by GFA 2-30-90 in order to compress the material and achieve a high degree of filling in barrels 3 and 4, followed by kneading screw elements oriented at 90° and 60° in zones 5 and 8, respectively, to break down large solids of biomass and mix biomass and water to achieve a homogeneous distribution (water addition in barrel 7, hence conveying elements in barrels 6 and 7). Liquid solid separation occurred in barrel 9 and large pitch conveying elements (GFA 2-40-90) were used in barrels 9-10 followed by a reduced length element (GFA 2-40-60) in zone 11. Screw configuration profile 2 was developed to increase the pressure in the filter
zone for liquid/solid separation using reverse screw elements after the filter. Two reverse screw elements were placed in zones 10 following two kneading screw elements oriented 30° to mix and distribute the material in order to avoid plugging. In screw configuration profile 3 two additional reverse screw elements before two 30° kneading screw elements were placed in zone 11 to further enhance the pressure around the filter block. These four reverse screw elements were separated by a short regular conveying screw element (GFA 2-30-30). In addition, kneading screw elements were added from zone 0 to zone 8 to enhance mixing. Screw configurations profiles 4 and 5 were based on profile 3 to evaluate the best location of the two reverse screw elements in zone 10.

Screw configuration profiles 6, 7 and 8 were designed to evaluate the effect of developing high pressure downstream of the filter-block. For screw configuration profile 6, one 60° kneading screw element was replaced by a reverse screw element in zone 8; another two 60° kneading screw elements were replaced by two 90° kneading screw elements in zone 8 on screw configuration profile 7. One 90° kneading screw element was replaced by a reverse screw element on screw configuration profile 8 to further increase the pressure.

For all experiments, barrel temperature, screw speed, water flow rate, and feed flow rate were kept at 100°C, 100rpm, 2.9kg/h, and 4kg/h, respectively. To ensure a steady state operation, the extruder was operated for 30 min before any solid and liquid samples were collected. Upon achieving steady state, the filtrate (mixture of water, xylose, suspended solids and other carbohydrate components) and extruded corncobs (mainly composed of cellulose) were immediately collected over a period of 30 min. The filtrate
was further treated for xylose analysis via GC. The xylose recovery was calculated based on the following equation:

\[
\text{Xylose Recovery (\%)} = \frac{\text{Total Xylose in Filtrate (kg/h)} \times 100\%}{\text{Total Xylose in Steam Exploded Corncobs (kg/h)}}
\] (3)

**Figure 4-2.** Screw configurations development on xylose recovery.


K1: KB2-15-30 \(^\circ\), K2: KB2-15-60 \(^\circ\), K3: KB2-15-90 \(^\circ\), K4: KB2-15-30 \(^\circ\)-Li.

GFA-2-XX\(^a\)-XX\(^b\): G= co-rotating, F=conveying, A=free-meshing, 2=number of threads, XX\(^a\)=pitch, XX\(^b\)=length of screw element, x2: two same elements.

KB2\(^a\)-2\(^b\)-30-XX-Li: KB=kneading block, 2\(^a\)=number of kneading segments, 2\(^b\)=number of threads, 30=length of kneading block, XX=twisting angle of the individual kneading segments, Li=reverse element.
4.3.6 Operating conditions study

All experiments were performed with screw configuration 8 at various barrel temperatures (65-100 °C), screw speeds (75-100 rpm), water flow rates (0-2.9 kg/h) at a fixed biomass feed rate of 4 kg/h. The choices of these operating conditions were based on preliminary studies (data not shown). A response surface method (RSM) using IV-optimal design was generated based on the operating conditions above. Response surface plots were generated for xylose recovery, specific mechanical energy consumption and xylose specific energy consumption. The experimental data was used to fit a second order polynomial model as described in detail elsewhere (Jimenez-Contreras et al., 2009) which was used to generate surface plots.

4.4 Results and discussion

4.4.1 Composition of the raw material

The main chemical composition of the steam-exploded corncobs is presented in Table 4-1. The glucan content was 55 % and the xylan content 23%. Monomeric xylose is required to be removed during the extrusion process. Arabinose, galactose, and mannose were found in minor fractions (<5.0%).

Table 4-1, Carbohydrate composition analysis of steam exploded corncobs.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Steam Exploded Corncobs (DM %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>55.4±1.61</td>
</tr>
<tr>
<td>Xylose</td>
<td>22.8±1.29</td>
</tr>
<tr>
<td>Arabinose</td>
<td>2.4±0.07</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.6±0.05</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.3±0.03</td>
</tr>
</tbody>
</table>
4.4.2 Results of screw configuration studies on xylose recovery

The position, spacing, and number of reverse screw elements greatly affected xylose recovery. Table 4-2 shows the effects of each screw configuration on the pressure profile and the resulting liquid/solid separation and xylose recovery. No liquid/solid separation was achieved when only conveying and kneading screw elements were applied (profile 1). Filtrate was obtained when the pressure in zone 10 was increased by placing two reverse screw elements in this zone (profile 2). Reverse screw elements have very strong flow restricting effects, enhancing the degree of filling and generating pressure. Placing reverse screw elements in zones 10 and 11 enhanced the pressure and the mechanical energy input further (profile 3). Different locations of two reverse screw elements in zone 10 had significant different effects on xylose recovery. The testing results from the screw configuration profiles 4 and 5 showed that the best location of these two reverse screw elements was the middle of zone 10 according to the amount of filtrate and xylose obtained. The pressure in zone 10 increased to 350-450 psig on screw configuration profile 5, the filtrate was continuously flowing and extruded corncobs were very dry.

An increased pressure downstream of the filter block was developed in order to further increase liquid separation. The results with screw configuration profile 6 show that the pressure in zone 8 increased to 85 psig when a reverse screw element was added in that zone. The filtrate was continuously flowing at 2.2 kg/h and the xylose recovery was increased to 60%. The pressure did not further increase when two more 90° kneading screw elements were placed in zone 8 (profile 7) with similar filtrate flow rates. Adding two additional reverse screw elements in zone 8 further increased the pressure to 150 psig (profile 8), and 4.5 kg/h of filtrate were collected with 80 % xylose recovery. The
extrusion process could run continuously with consistent xylose recovery over the operation time of 1.5h.

Screw configurations profile 8 was the best screw configurations tested in this study, producing the highest amount of filtrate. The xylose recovery was highly dependent on the amount of filtrate obtained. As higher filtrate flow rate was collected, more xylose recovery was achieved.

**Table 4-2, Effects of screw configuration development on xylose recovery.**

<table>
<thead>
<tr>
<th>Screw Configuration Profile</th>
<th>Pressure 1 (psig)</th>
<th>Pressure 2 (psig)</th>
<th>Filtrate (kg/h)</th>
<th>Xylose Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>41</td>
<td>125-175</td>
<td>0.3</td>
<td>2.3</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
<td>200-250</td>
<td>0.8</td>
<td>12.3</td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>250-350</td>
<td>1.3</td>
<td>16.1</td>
</tr>
<tr>
<td>5</td>
<td>47</td>
<td>350-450</td>
<td>1.6</td>
<td>32.5</td>
</tr>
<tr>
<td>6</td>
<td>85</td>
<td>350-450</td>
<td>2.2</td>
<td>59.7</td>
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<tr>
<td>7</td>
<td>85</td>
<td>350-450</td>
<td>2.2</td>
<td>62.1</td>
</tr>
<tr>
<td>8</td>
<td>150</td>
<td>350-450</td>
<td>4.5</td>
<td>79.8</td>
</tr>
</tbody>
</table>

4.4.3 The effects of operating conditions on xylose recovery

Xylose recovery in a twin-screw extruder, as well as the resulting energy consumption of the motor, is governed by the operating conditions. The effects of barrel temperature, screw speed, and water flow rate (zone 7) were investigated for screw configuration 8. The operating conditions were chosen based on a response surface method (RSM) using IV-optimal design and response surface plots of a 2nd order polynomial regression models were used to visualize the interaction effect of these three factors.

Fig.4-3a-c shows the response surfaces of the combined effects of screw speed and water flow rate at a constant barrel temperature of 82.5°C on xylose recovery, specific
mechanical energy and xylose specific energy. Enhancing the screw speed did not have a positive effect on xylose recovery, on the contrary, xylose recovery increased slightly with decreasing screw speeds. A change in the screw speed at a constant feed rate affects the residence time in the twin-screw extruder by changing the degree of fill within the extruder elements (Karunanithy and Muthukumarappan, 2010). Decreasing the screw speed increases the residence time and induces a higher degree of fill. This effect pushes the biomass across restricting elements slower, thus higher pressures can be developed (Amalia Kartika et al., 2005; Davidson et al., 1983) and more xylose was recovered. The specific mechanical energy did not seem to be affected by the screw speed in this case (decreased torque at higher rpm) as shown in Fig.4-3b. As anticipated, xylose recovery increased significantly with increasing water flow rates; however increasing the flowrate above 1.45 kg/h had a negative effect on xylose recovery at a low screw speed of 75 rpm. Moderate water flow rates helped remove free xylose from the surface of steam exploded corncobs, while higher water flow rate reduced the friction between biomass and extruder, which decreased the pressure developed by the screw elements, thus, less xylose (liquid) was recovered. Similar effects of moisture have been reported elsewhere (Chen et al, 2010). The specific mechanical energy was increased with an increase of the water flow rate from 0 to 1.45 kg/h due to the increased pressing efficacy of the reversed screw elements, thus, the residence time in the whole extruder was increased which required more energy for the motor to push biomass (Amalia Kartika et al., 2008). The specific mechanical energy decreased when the water flow rate was further increased from 1.45 to 2.90 kg/h due to the friction reduction between the biomass and the extruder. More water was contained in the extruder and biomass easily pushed through the extruder
which required lower energy for the motor drive. Fig.4-3c shows that the xylose specific energy is slightly affected by the screw speed, but significantly affected by the water flow rate. As the water flow rate increased, the xylose specific energy decreased indicating less energy was required to recover extra xylose; as the water flow rate further increased, less xylose was recovered, and the xylose specific energy increased indicating the energy consumption is faster than xylose recovery.

The surface plots of the combined effect of barrel temperature and water flow rate on xylose recovery, specific mechanical energy and xylose specific energy at a constant screw speed of 87.5 rpm are shown in Fig 4-3d-f. The xylose recovery is a function of both the barrel temperature and water flow rate. The barrel temperature had stronger effects than the water flow rate. The xylose recovery increased exponentially when the barrel temperature was increased from 65 to 100°C. This is due to the fact that an increase in barrel temperature through the barrel heaters would introduce more energy into the biomass and facilitated the friction development, which induced increased biomass fluidity and breaking of the walls of steam exploded corncobs (Karunanithy and Muthukumarappan, 2010; Yu et al., 2009). Acetyl groups in hemicelluloses will be converted into acetic acids, which will hydrolyze xylan and xylose-oligomers in steam exploded corncobs into monomeric xylose under high barrel temperature (Garrote et al., 2001, 2002), which is then removed through the solid/liquid separator. The xylose recovery was not just influenced by the barrel temperature; it was also affected by the relative quantity of water in the flow rates investigated. Xylose recovery was not significantly affected by changes in operating temperature without adding water; however, xylose recovery increased dramatically when the water flow rate increased from
0 to around 1.45 kg/h at a barrel temperature of 100°C. Further increases in the water flow rate resulted in decreasing xylose recovery, as discussed above. The barrel temperature strongly correlates with biomass viscosity and hence the achievable degree of fill. Higher barrel temperature causes biomass to be less viscous; hence a higher degree of fill can be achieved, resulting in higher torque on the motor (Gautam and Choudhury, 1999b). The lower viscosity also results in less restricted flow across the restricting elements, possible reducing the required energy input, however in this case the first effect was dominant and the overall specific mechanical energy of the motor drive increased. The water flow rate had a small effect on the specific mechanical energy at a barrel temperature of 65°C. As the barrel temperature increased, the water flow rate was clearly correlated to the barrel temperature. Below 80°C, the specific mechanical energy varied little, however, the specific mechanical energy increased dramatically when the water flow rate was increased from 0 to 1.45 kg/h and the barrel temperature increased from 80 to 100°C. The specific mechanical energy decreased sharply when the water flow rate was increased further, with water working similar to a lubricant in the extruder (Chen et al., 2010; Yeh and Jaw, 1998; Hayashi et al., 1992). Fig. 4-3f shows the effects of barrel temperature and water flow rate on xylose specific energy. The xylose specific energy increases due to higher energy requirements as the barrel temperature decreased from 100°C to 65°C. The xylose specific energy stays constant as the water flow rate increased at a barrel temperature of 100°C indicating that the xylose recovery is highly proportional to the energy consumption of the motor drive.

The effect of screw speed and barrel temperature on xylose recovery at a constant water flow rate of 1.45 kg/h is visualized Fig 4-3g-i. The plots confirm that higher barrel
temperatures and lower screw speeds result in enhanced xylose recovery, while the specific mechanical energy increase as barrel temperature increased. In addition, the specific mechanical energy did not change as the screw speed changed in the experimental range at a lower barrel temperature, but it changed significantly with increased screw speeds at a higher barrel temperature. At a barrel temperature of 100°C, the specific mechanical energy increased when the screw speed increased. The highest specific mechanical energy was observed at 100 rpm and 100°C of barrel temperature. Fig.4-3i highlights this by showing the xylose specific energy as a function of barrel temperature. The xylose specific energy increased as the barrel temperature decreased and did not change as the screw speed changed.
4.5 Conclusion

Monomeric xylose can feasibly be recovered without organic solvents from the steam-exploaded corncobs with co-rotating twin screw extruders. The screw configuration and operating conditions play an important role in xylose recovery. Xylose removal can
be enhanced by decreasing the screw speeds and increasing water flow rate and barrel temperature. The specific energy consumption of the extruder is well correlated with the amount of xylose recovered. However, there is no general proportionality, hence optimization of the xylose specific energy consumption and other can be undertaken.

4.6 Reference


Chapter 5. Enzymatic Hydrolysis of Steam Exploded Corncob Residues after Pretreatment in a Twin-Screw Extruder

Jun Zheng, Kim Choo, Chris Bradt, Rick Lehoux and Lars Rehmann

With minor editorial changes to fulfill formatting requirements, this chapter is substantially as it appears in: *Biotech. Rep.* 3: 99-107 (2014)

5.1 Preface to Chapter 5

The previous Chapter established an extrusion process with a designed filter block for biomass pretreatment that can achieve very high xylose recovery without organic solvents from the steam exploded corncobs. However it also showed that operating conditions such as screw speed, water flow rate, and barrel temperature played an important role in xylose recovery. It is therefore expected that the overall enzymatic hydrolysis rate of the extruded biomass can be increased, as it is well known that the removal of hemicellulose will increase the enzymatic hydrolysis of cellulose. The enzymatic hydrolysis rate is dependent on multiple process parameters; therefore, the condition for enzymatic digestion has to be optimized. The following chapter therefore aims to study the combined effects of various enzymatic hydrolysis process variables, including enzyme loading, surfactant concentration, hydrolysis time and two extruded corncobs (7% xylose removal, 80% xylose removal) using a response surface method (RSM)-based model with the IV-optimal design. The characteristics of these two extruded materials were also examined by SEM and XRD.
5.2 Introduction

Bioconversion of lignocellulosic biomass to ethanol is considered to be one of the most important alternatives to petroleum based liquid fuels (Himmel et al., 2007; Hahn-Hägerdal et al., 2006; Hamelinck, et al., 2005; Mosier et al., 2005; Sun and Cheng, 2002). Lignocellulosic biomass are highly abundant, have high energy potential and are low cost materials for ethanol production. Typical sources are forest products, agricultural residues, municipal solid waste, and dedicated energy crops (Hsu et al., 2010; Palonen et al., 2004).

Corncobs, a byproduct of corn grain production, were once used for heat, animal feed and manure for agricultural production in some parts of Europe, while in the United States, corncobs are currently being used as a potential feedstock for cellulosic ethanol production due to its low lignin and high carbohydrate contents. Moreover, corncobs have a high heating value (HHV) producing approximately 8000 Btu/lb. The average corncob yield is about 14% of grain yield, which represents about 16% of the total corn stover in a field (Roth and Gustafson, 2014; Latif and Rajoka, 2001; Barl et al., 1991).

Among the different technologies (Luque et al., 2014; Schwab et al., 2013) available for the conversion of lignocellulosic biomass to suitable fermentation substrates, the enzymatic conversion of cellulose seems to be the most promising approach to get a high yield of fermentable sugars (El-Zawawy et al., 2011) because it is highly specific and does not produce substantial amounts of unwanted byproducts (Wen et al., 2004). The enzymatic hydrolysis process is usually catalyzed by cellulase enzymes and the process is affected by many factors including cellulose fibre protection by hemicelluloses and lignin, cellulose crystallinity, degree of polymerization, degree of
acetylation of hemicelluloses and the accessible surface area of the biomass (McMillan, 1994). The presence of hemicelluloses and lignin makes the cellulase enzymes’ access to cellulose difficult, which will reduce hydrolysis efficiency. Therefore, the structure of cellulosic biomass must be pretreated prior to enzymatic hydrolysis to make cellulose more accessible to enzymatic conversion (Gao and Rehmann, 2014; Mosier et al., 2005). Various physical, chemical, physico-chemical and biological pretreatment methods have been well-investigated for ethanol production from lignocellulosic biomass (Tian et al. 2011; Hendriks and Zeeman, 2009; Sun and Cheng, 2002). The purpose of the pretreatment is mainly to increase the accessibility of the enzymes to cellulose the by solubilisation of hemicelluloses or/and lignin, and by decreasing the degree of polymerization and cellulose fibre crystallinity (Gil et al., 2010). Moreover, adding surfactants has also improved the effectiveness of the cellulose hydrolysis (Alkasrawi et al., 2003; Eriksson et al., 2002).

To improve the rate of enzymatic hydrolysis, researchers have focused on the study of multiple enzymatic hydrolysis process parameters, including substrate concentration, and reaction conditions, such as hydrolysis time, pH, temperature and addition of surfactants (Sun and Cheng, 2002). Optimal parameters are highly dependant on the physicochemical structure of the digested biomass, and different pre-treatment methods will produce substantially different biomass. Pretreatment in a twin-screw extruder can be used (among other things) to hydrolyze and remove the hemicellulose fraction (Lehoux and Bradt, 2013, 2012; Dottori et al., 2013). However, the effect of xylose removal via extrusion pretreatment, along with other process parameters on the enzymatic hydrolysis of corncobs, has not yet been systematically characterized. In the present study, two
differently extruded corncobs with 7% xylose removal and 80% xylose removal, respectively, were used as a source of enzymatic hydrolysis. The characteristics of these two materials were examined by SEM and XRD. A response surface method (RSM)-based model using IV-optimal design was used to study the combined effects of various enzymatic hydrolysis process variables (enzyme loading, surfactant addition, and hydrolysis time) with these two extruded corncobs (7% xylose removal, 80% xylose removal).

5.3 Materials and methods

5.3.1 Materials

Corncobs were obtained from local farmers in Chatham, Ontario, Canada. Corncobs were cleaned and ground to the particle size of 0.5 to 1 cm³ and moisture was adjusted to 50% dry matter. Corncobs were then fed into a continuous steam explosion pretreatment reactor (GreenField Ethanol, Chatham). The reactor was set at a temperature of 205°C with pH 4.8 in a system pressurized with saturated steam. The overall retention time of the corncobs during pretreatment was 5 min. Hemicellulose was hydrolyzed to xylose or xylo-oligosaccharides under these conditions. The pressure of the reactor was rapidly released to atmospheric pressure, thus the pressurized corncobs were flashed into a cyclone separator, which increased the accessible surface area of the fibres for the enzymes. Pretreated corncobs with 80% moisture content were collected and adjusted to 60% by air drying for further xylose removal during the extrusion process.

All other chemicals (e.g., acetic acid, sodium sulfate anhydrous, tetracycline, cycloheximide, glucose and xylose) were of analytical grade and purchased from Sigma
Aldrich (USA). The Cellic CTec 2 cellulose enzyme was obtained from Novozyme (Canada).

5.3.2 Xylose removal during extrusion process

Experiments were conducted with a Leistritz Co-Rotating Twin Screw Extruder (American Leistritz Extruder Corp, USA). The extruder was composed of twelve modular barrels that were each 200 mm long. The barrels were electrically heated using thermal induction and cooled by water circulation. Barrel temperature, water flow rate, feed flow rate and pressure were monitored from a control panel. The material was fed into the extruder inlet port (Barrel 0, Fig. 5-1) at 4 kg/hr by a gravimetric feeder (Brabender Technology, Canada). Water was injected into Barrel 8 by a positive displacement pump (Milton Roy, USA). A solid/liquid separator was positioned in Barrel 9 to collect the filtrate mainly containing dissolved xylose. Two pressure sensors were positioned in Barrels 8 and 10, respectively, to detect the pressure on both sides of the filter. Two screw configuration profiles (Fig. 5-1a & 5-1b) were used to produce the extruded corncobs with 7% and 80% xylose removals, respectively. These two screw configuration profiles were built by placing conveying, kneading and reverse screw elements at different positions and intervals. The conveying screw elements were used for material transportation and their smaller pitch could compress the products and achieve a high degree of filling within each barrel. Kneading screw elements oriented at different angles were used to break down large solids and to mix biomass and water to achieve a homogeneous distribution. In addition, reverse screw elements carrying the materials in the opposite direction were placed immediately before and after the filter to
increase forward and backward pressure. The only differences between these two screw configuration profiles concerned their backward pressure development zones, situated in Zone 11. The backward pressure development zone was composed of two reverse screw elements for Profile A, but only one for Profile B, which caused lower backward pressure, resulting in less xylose removal. All experiments were conducted at a barrel temperature of 100°C, screw speed of 100rpm, and a Liquid/Solid ratio of 1.2.
Figure 5-1. Screw configuration profiles used to achieve 7% (a) and 80% (b) xylose removal.


GFA-2-XXa-XXb: G= co-rotating, F=conveying, A=free-meshing, 2=number of threads, XXa=pitch, XXb=length of screw element, x2: two same elements.

KB2a-2b-30-XX-Li: KB=kneading block, 2a=number of kneading segments, 2b=number of threads, 30=length of kneading block, XX=twisting angle of the individual kneading segments, Li=reverse element.
5.3.3 Carbohydrate analysis

The concentration of glucose was quantified by an Agilent 1260 Infinity high-performance liquid chromatography (HPLC) using a MetaCarb H Plus Column 300x7.8mm (Agilent Technologies, USA), equipped with a refractive index detector. Before analysis, hydrolyzed liquid samples were subjected to 50x dilutions and filtered through a 0.2 μm cellulose acetate membrane (VWR International, USA). The column temperature was maintained at 60°C and the flow rate was 0.7 ml/min (5 mM H$_2$SO$_4$). The glucose conversion was calculated by comparing the amount of glucose produced in the hydrolyzate to the total amount of glucose monomers present in the pretreated biomass.

5.3.4 Enzymatic hydrolysis

Enzymatic hydrolysis of extruded corncobs was conducted in 100-ml screw capped glass vials with the Cellic CTec 2 enzyme obtained from Novozyme (Canada). The enzyme activity was measured to be 168.2 FPU/ml. Applied enzyme loadings varied from 1.8 to 7.2 FPU/g DM of the extruded corncobs with 80% xylose removal and from 1.1 to 4.4 FPU/g DM of the extruded corncobs with 7% xylose removal. The enzyme loading was determined based on the total cellulose amount in each extruded corncob. The hydrolysis mixture consisted of 12 % (w/v) dry matter-buffer and 0.1 M sodium citrate buffer (pH 5.0), which was supplemented with 40 µl tetracycline and 30 µl cycloheximide to prevent microbial contamination during digestion. Tween 80 (Sigma-Aldrich, USA) was used in these hydrolysis experiments to enhance the enzymatic hydrolysis of extruded corncobs. All vials were incubated at 50°C in a rotary shaker.
(Infors HT, Switzerland) at 140 rpm from 48h to 96h. Each experiment was conducted in triplicate. 50 µl of an aliquot sample was withdrawn from each reaction mixture at different hydrolysis times according to the experimental design and kept at -20°C for 10 min to denature enzyme activity. Each sample was diluted, filtered and 1 ml was transferred to a HPLC vial for glucose analysis.

5.3.5 Scanning electron microscopy (SEM)

The surface properties and microstructure of untreated and pretreated corncob samples were observed using Scanning Electron Microscopy (SEM) (Hitachi S-4800) at an accelerated voltage from 1.0 to 5.0 kV. After air-drying, the surface of the sample was covered with a thin layer of gold before observation using a sputter coater (Emitech K550X, UK) for 3 min to make it more conductive for charge. Digital images were obtained at magnifications ranging from 600x to 20,000x.

5.3.6 Crystallinity measurement

The crystallinity index is a helpful measure of the relative degree of crystallinity (Lynd et al. 2002; Zhang and Lynd, 2004). X-ray diffraction (XRD) was used for phase identification of the untreated and pretreated corncobs. Samples were ground to pass through a 150 µm-mesh screen and the crystallinity was determined by Rigaku (USA) using the CoKα radiation source. Samples were scanned at a speed of 5° (2θ)/ min for the continuous run in the 5 to 45° (2θ) range.

The crystalline index (Crl) of cellulose samples was determined through the X-ray diffraction patterns based on the following relationship (Cao and Tan, 2005):
\[
\text{CrI} = \frac{I_{\text{max}} - I_{\text{min}}}{I_{\text{max}}} \times 100\% 
\] 

(1)

Where \(I_{\text{max}}\) represents the maximum intensity peak for cellulose I at \(2\theta\) around 26\(^0\), \(I_{\text{min}}\) represents the minimum intensity peak for the amorphous region (cellulose II) at \(2\theta\) around 19\(^0\) based on Bragg’s law conversion from the CuK\(\alpha\) radiation source.

5.3.7 Experimental design

A response surface method (RSM)-based model using IV-optimal design with four factors was chosen to evaluate the effect of the selected variables on the response pattern and to determine the optimum combination of enzyme loading (2\%-8\%), Tween 80 concentration (0\%-6\%) and hydrolysis time (24h – 72h) with extruded corncobs with different xylose removals (7\% and 80\%) were used to maximize glucose conversion from pretreated corncobs. Each factor level was selected based on preliminary studies. Preliminary results from a full factorial design had shown significant curvature in Table B3, hence a IV-optimal design was chosen. The ratio of the total amount of glucose produced in the hydrolyzate to the total theoretical amount of glucose in the steam-exploded corncobs (analyzed after acid hydrolysis) was chosen as the response for analysis. The experimental design was developed using the software Design Expert, version 8.0.7.1 (Stat Ease, Inc., USA). The resulting 22 experimental conditions, as well as three center point replicates for each type of biomass, were tested in triplicate and data is presented as the average of triplicates ± standard deviation. All experiments were performed fully randomized, and the data was fitted via linear regression to a second order model:
\[ y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i \leq j \leq k} \beta_{ij} x_i x_j + \varepsilon \]  

(2)

Where \( y \) is the predicted response, \( x_i \) represents the independent variables, \( k \) is the number of variables, \( \beta_0 \) is the interception coefficient, \( \beta_i \) represents the linear coefficient of each independent variable, \( \beta_{ii} \) represents the coefficients of the quadratic terms, \( \beta_{ij} \) represents the coefficients of the interaction effects and \( \varepsilon \) is the random error.

Analysis of the variance (ANOVA) was performed and the significance of each variable, the interaction, and quadratic effects were determined based on a significance of \( \alpha = 0.05 \) using the \( F \)-test. The fitted model was evaluated by \( R^2 \), adjusted \( R^2 \), adequate precision and the lack of fit coefficient for determining the adequacy. In addition, the fitted model was validated by performing experiments using the identified conditions of the significant variables (Adel et al., 2010).

5.4 Results and discussion

The carbohydrate composition of the investigated corncobs before and after steam explosion and after different extruder treatments was measured after acid hydrolysis (Englyst et al., 1982; Krull and Inglett, 1980; Borchardt and Piper, 1970). The data are shown in Table 5-1 (based on total dry matter). The relative glucose content, which was the largest fraction of monosaccharides, increased from 41% to 66% and 58%, respectively, depending on different extrusion process conditions. The hemicelluloses fraction was largely hydrolyzed to xylose under high temperature and pressure during the steam explosion pretreatment. 7% xylose removal from the steam exploded corncobs was achieved through the extrusion process at a barrel temperature of 65°C and a screw speed of 100 rpm without adding water, while 80% xylose removal was achieved when the
barrel temperature increased to 100°C and water was injected at Barrel 8 at 2.9 kg/h. Arabinose, galactose, and mannose were found in minor fractions (<5.0%).

**Table 5-1,** Carbohydrate composition of corncob samples after different treatment conditions (average of triplicates ± standard error).

<table>
<thead>
<tr>
<th></th>
<th>Ground Corncobs (%)</th>
<th>Steam Exploded Corncobs (%)</th>
<th>Extruded Corncobs with 80% Xylose Removal</th>
<th>Extruded Corncobs with 7% Xylose Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>41.3±0.75</td>
<td>55.4±1.61</td>
<td>65.5±1.50</td>
<td>58.1±1.52</td>
</tr>
<tr>
<td>Xylose</td>
<td>29.0±1.25</td>
<td>22.8±1.29</td>
<td>7.4±0.10</td>
<td>19.4±1.00</td>
</tr>
<tr>
<td>Arabinose</td>
<td>4.2±0.18</td>
<td>2.4±0.07</td>
<td>1.5±0.07</td>
<td>2.3±0.06</td>
</tr>
<tr>
<td>Galactose</td>
<td>1.5±0.10</td>
<td>0.6±0.05</td>
<td>0.2±0.03</td>
<td>0.6±0.03</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.4±0.03</td>
<td>0.3±0.03</td>
<td>0.3±0.03</td>
<td>0.2±0.02</td>
</tr>
</tbody>
</table>

5.4.1 Biomass characterization

SEM images of untreated and extruded corncobs with different xylose removals at different magnifications are shown in Fig.5-2. Based on the differences from SEM micrographs, it was evident that the untreated corncobs exhibited a highly fibrillar, ordered and rigid surface structure and the surface was relatively smooth (Fig. 5-2A and B); however, after the extrusion pretreatment, the corncobs were separated into differently irregular fibres with different dimensions and some internal areas were fully exposed, thus increasing the internal surface area. At the same time, the surface of extruded corncobs was more chapped, cracked and coarser structures compared to the images in the untreated corncobs. In addition, some pores were observed on the surface of extruded corncobs which could be caused by moisture evaporation under the high temperature (Fig. 5-2C, D, E and F). Extrusion pretreatment provides mixing, shear force and heat to corncobs; therefore, moisture can evaporate and deeply penetrate corncobs particles during extrusion (Zhan et al., 2006).
Figure 5-2. SEM images at various magnifications for untreated and extruded corncobs. [A, B] untreated corncobs with no xylose removal, [C, D] extruded corncobs with 7% xylose removal, [E, F] extruded corncobs with 80% xylose removal.

The structures of untreated and extruded corncobs were examined using a powder X-ray diffractometer (XRD). The crystal structure of cellulose can be changed by various pretreatments by disrupting inter-and intra-chain hydrogen bonding of cellulose fibrils...
(Mosier et al., 2005). The diffractogram results show in Fig. 5-3 that the untreated and extruded corn cobs have the typical cellulose I and cellulose II allomorph characteristics at \( \theta = 26^\circ \) and \( \theta = 19^\circ \), respectively. For untreated corn cobs, the crystalline peak predominates over the amorphous peak, likely due to the presence of higher crystalline cellulose content in untreated corn cobs, a form of cellulose which is difficult for enzymatic hydrolysis. The crystallinity index (CrI) for different treatments was calculated from the XRD data by means of three replicates and were 0.304 ± 0.02, 0.462 ± 0.03 and 0.510 ± 0.007 for untreated, ‘7% xylose removed’ and ‘80% xylose removed’, respectively. After the extrusion pretreatment, the peak height of the extruded corn cobs increased and became sharper, showing that the amount of cellulose increased, which could be confirmed from the composition analysis in Table 1 and indicates a higher crystallinity degree in the extruded corn cobs. The crystallinity increase after pretreatment might be caused by the removal of amorphous components of lignin and hemicelluloses, consistent with values typically reported in the literature. This also confirms that the extrusion pretreatment is an effective method to expose cellulose to enzymatic conversion.

An increase in the crystallinity of the extruded corn cobs is corresponding to an increase in the rigidity of the cellulose structure, which causes higher tensile strength of fibres (Maeda et al., 2011, Alemdar and Sain, 2008, Kim and Holtzapple, 2006). The extruded corn cobs with 80% xylose removal have higher crystallinity at \( \theta = 26^\circ \) compared to the extruded corn cobs with 7% xylose removal, as more amorphous xylose was removed during the extrusion process, thus the content of crystalline cellulose in extruded corn cobs with 80% xylose removal is higher than extruded corn cobs with 7% xylose removal confirmed in Table 5-1. This conclusion was also confirmed by the statistical
test of Analysis of Variance (ANOVA) (F value >Fcrit) using Microsoft Office Excel 2007. Sun et al. (2014) reported that switchgrass treated with certain ionic liquids increased crystallinity index by reducing amorphous cellulose, hemicelluloses and lignin, resulting in a higher hydrolysis rate by using the Cellic CTec 2 and HTec2. Hall et al. (2010) tested the enzymatic hydrolysis rate of the pure cellulosic Avicel and found that the hydrolysis rate increased with a decreasing crystallinity index by endo- and exocellulases. However, the relationship between the crystallinity index of extruded biomass and its corresponding enzymatic hydrolysis rate is not well understood. A biomass with high crystallinity index may not necessarily negatively affect the enzymatic hydrolysis rate (Kim and Holtzapple, 2006).
Figure 5.3. X-ray diffraction diagram of untreated and treated corncobs. [A] untreated corncobs with no xylose removal, [B] extruded corncobs with 7% xylose removal, [C] extruded corncobs with 80% xylose removal.

5.4.2 Enzymatic hydrolysis

The test conditions for enzymatic hydrolysis were chosen based on a statistical experimental design using a response surface method (RSM)-based model with IV-
optimal design. The tested conditions and the resulting glucose conversion are shown in Table 5-2. The results of the quadratic response surface model are shown in Table 5-3.

The F value of the model is 405.10 which is very high compared to the critical value (2.80), indicating that the model is highly significant. The value of “Prob>F” was less than 0.0001, supporting that the model is significant. The significance of each parameter coefficient was determined by P-values (Prob>F) if their-values were<0.05. The smaller the P values, the more significant the corresponding coefficient. Among the independent variables, enzyme loading, hydrolysis time, Tween 80 concentration and ‘extruded corncobs with different xylose removals’ had significant effects on glucose conversion. The quadratic effects of enzyme loading and hydrolysis time also had significant effects on glucose conversion. An adjusted R² of 0.99 confirms the model’s adequacy and no significant lack of fit was detected based on the P value. The signal to noise ratio for all experiments was greater than 4, indicating an adequate signal, which could be used to navigate the design space.

Based on the selected significant variables, the regression analysis yielded the following quadratic model, which was an empirical relationship between glucose conversion and the test variables in terms of coded units (-1 to +1):

\[
Y = +7.27 + 1.33X_1 + 0.14X_2 + 0.52X_3 + 0.37X_4 + 0.13X_1X_3 + 0.071X_2X_4 + 0.076X_3X_4 - 0.38X_1^2 - 0.16X_3^2
\]  

(3)

Where, Y is the square root of glucose conversion (%); X₁, X₂, X₃ and X₄ are enzyme loading, tween 80 Concentration, hydrolysis time and, ‘extruded corncobs with different xylose removals (7%, 80%)’ respectively.
Table 5-2, Glucose release from extruded biomass under different conditions, based on a response surface method (RSM) using IV-optimal design.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Enzyme loading (% w/w)</th>
<th>Tween 80 concentration (% w/w)</th>
<th>Different xylose removals (%)</th>
<th>Hydrolysis time (hr)</th>
<th>Glucose conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>0</td>
<td>80</td>
<td>24</td>
<td>56.67±1.34</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>3</td>
<td>7</td>
<td>48</td>
<td>49.87±1.75</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>3</td>
<td>80</td>
<td>48</td>
<td>61.06±0.73</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>6</td>
<td>80</td>
<td>24</td>
<td>32.31±2.11</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>0</td>
<td>7</td>
<td>24</td>
<td>52.18±1.68</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>0</td>
<td>80</td>
<td>72</td>
<td>38.23±1.31</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>3</td>
<td>80</td>
<td>48</td>
<td>62.4±0.66</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>6</td>
<td>7</td>
<td>24</td>
<td>24.3±1.74</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>0</td>
<td>80</td>
<td>48</td>
<td>26.95±1.02</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>0</td>
<td>7</td>
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Table 5-3, Analysis of variance of 2\textsuperscript{nd} order model.

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<th>Source</th>
<th>Sum of squares</th>
<th>Degrees of freedom</th>
<th>Mean square</th>
<th>F value</th>
<th>P value</th>
<th>Remark</th>
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<td>0.28</td>
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<td>0.09</td>
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<td>Lack of Fit</td>
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<td>1.37</td>
<td>0.4111</td>
<td>Not significant</td>
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<td>Pure Error</td>
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<td>9.03x10\textsuperscript{-4}</td>
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<td>Adj-Squared</td>
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<td>69.64</td>
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</tbody>
</table>

5.4.3 Combined effects of variables on glucose conversion

Surface plots were generated to further illustrate the interaction of corresponding parameters. The effect of Tween 80 concentration and enzyme loading on the enzymatic hydrolysis of extruded corncobs is shown in Fig.5-4. For the extruded corncobs with 7\% xylose removal, as can be seen in Fig.5-4a & b, the glucose conversion was not affected significantly in the presence of the Tween 80 when the enzyme loading and hydrolysis time were varied ($P = 0.05$). This indicates that xylose might be the major factor limiting enzymatic hydrolysis. For the extruded corncobs with 80\% xylose removal, the effect of
Tween 80 was very small at 24h (Fig.5-4c). However, when the hydrolysis time was prolonged to 72h (Fig.5-4d), increasing Tween 80 concentration resulted in a significant increase in glucose conversion at a high level of enzyme loading ($P < 0.05$). However as the hydrolysis time increases it would be expected to see a decrease of the hydrolysis rate due to cellulosic substrate decrease, increase of potentially inhibitory end- and by-products and general enzyme deactivation (Gregg and Saddler, 1996); potentially more evident at low enzyme loadings. The plot shows that a higher hydrolysis yield was obtained in the presence of a high level of Tween 80 concentration. For example, the difference in the glucose conversion was changed from 36 to 42% when the enzyme loading was 2%, and a higher difference was obtained from 80 to 88% when the Tween 80 concentration increased to 6% at an enzyme loading of 8%. In addition, the surfactant also could prevent the unproductive binding of cellulase to lignin by absorbing into the surface of lignin. This enabled the more active enzyme to only react with cellulose to improve the glucose conversion (Eriksson et al., 2002).
Figure 5-4. Response surface plot showing interaction effects of Tween 80 concentration and enzyme loading (a,b: constant hydrolysis time of 24h and 72h, respectively with extruded corncobs with 7% xylose; c,d: constant hydrolysis time of 24h and 72h, respectively with extruded corncobs with 80% xylose removal).

The combined effect of enzyme loading and hydrolysis time at fixed Tween 80 concentration (3%) is shown in Fig.5-5. As can be seen from Fig.5-5A, the conversion of glucose increased from 22% to 29% at an enzyme loading of 2% with extruded corncobs with 7% xylose removal, but increased from 51% to 68% at 8% enzyme loading when increasing hydrolysis time from 24 to 72h. The effects of hydrolysis time on the glucose conversion of extruded corncobs with 80% xylose removal were also observed (Fig. 5-
5b). When enzyme loading was at 2%, glucose conversion was only 28% at the hydrolysis time of 24h. Increasing the amount of cellulase significantly improved the glucose conversion to 59% when enzyme loading increased from 2% to 8%. Enzyme crowding on the cellulose surface, an effect that can result in lower hydrolysis rates at increasing enzyme concentrations (Warden et al., 2011), was not observed under the experimental conditions. An increase in hydrolysis time from 24 to 72h at 2% enzyme loading only resulted in a slight increase in the glucose conversion. This might be due to not enough cellulase reaching adsorption saturation for a certain amount of cellulose hydrolysis in the reaction mixture. Further increases in the enzyme loading would slow down the glucose conversion due to more unused cellulase in the mixture solution. Thus, as expected, glucose conversion could be increased with longer hydrolysis times at a higher enzyme loading.
Figure 5-5. Response surface plot of the combined effects of hydrolysis time and enzyme loading on the glucose conversion. (a: constant Tween 80 concentration (3%) with extruded corncobs with 7% xylose removal; b: constant Tween 80 concentration (3%) with extruded corncobs with 80% xylose removal).

The effect for xylose removal (designated as a categorical parameter) could be visualized in two-dimensional plots as shown in Fig.5-6. Fig. 5-4 and 5-5 already show that high xylose removal clearly resulted in enhanced enzymatic digestibility. Fig.5-6
highlights this by showing the model results for glucose conversion as a function of hydrolysis time and Tween 80 surfactant concentration, respectively, for both types of biomass, while the remaining variables were at their center points. These findings are consistent with several studies showing that cellulose conversion by enzymatic hydrolysis can be facilitated if a high percentage of hemicelluloses are removed (Kabel et al., 2007; Yang and Wyman, 2004; Palonen et al., 2004a).

**Figure 5-6.** 2D plot showing interaction effects of extruded corncobs with different xylose removals with hydrolysis time, Tween 80 concentration on the glucose conversion, respectively. (a: constant Tween 80 concentration (3%) with enzyme loading of 5%; b: constant hydrolysis time of 48h with enzyme loading of 5%; dash line: extruded corncobs with 7% xylose removal; solid line: extruded corncobs with 80% xylose removal. Symbols represent measured data.
5.4.4 Model validation

In order to confirm the validity and applicability of the second-order polynomial regression model obtained from the experimental data, six confirmation runs were carried out as listed in Table 5-4 to compare the difference between the predicted and measured values. The results in Table 5-4 show that the difference is below 3%. A plot of predicted versus measured values as shown in Fig.5-7 also verifies the overall good fit of the suggested models, indicating that the proposed model could be a useful and accurate model to express the actual relationship between the response and significant variables to predict the glucose conversion.

Table 5-4, Operating conditions and predicted and measured response of confirmation experiments.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Enzyme loading (%)</th>
<th>Tween 80 concentration (%)</th>
<th>Different xylose removals (%)</th>
<th>Hydrolysis time (h)</th>
<th>Predicted glucose conversion (%)</th>
<th>Measured glucose conversion (%)</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>6.00</td>
<td>80</td>
<td>72</td>
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<tr>
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<td>4</td>
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<td>3.00</td>
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<td>59.65±1.04</td>
<td>+2.15</td>
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<td>3.00</td>
<td>7</td>
<td>72</td>
<td>28.59</td>
<td>29.45±2.09</td>
<td>+2.92</td>
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</tbody>
</table>
Figure 5-7. Predicted versus measured values for glucose conversion.

5.5 Conclusion

Twin-screw extruders can be used as a pre-treatment method for lignocellulosic biomass to produce material with varying xylose contents. The xylose content can be controlled based on the employed screw configuration, as demonstrated for steam-exploded corncobs. The extrusion process further led to an increase in cellulose crystallinity, while structural changes were also observed via SEM. The effects of residual xylose (7% and 80% removal through extrusion process), enzyme loading, surfactant addition, and hydrolysis time on enzymatic hydrolysis could be described with a 2\textsuperscript{nd} order polynomial model, based on data generated through a response surface method (RSM)-based model using IV-optimal design. All independent variables and the interaction effects of enzyme loading and hydrolysis time, hydrolysis time and xylose content, Tween 80 concentration and xylose content, the quadratic terms of enzyme
loading as well as the quadratic term of hydrolysis time had a significant effect on enzymatic hydrolysis.

5.6 Reference


cholinium and imidazolium ionic liquids by chemistry and computation. Green Chem. DOI: 10.1039/c3gc42401d.


Chapter 6. Conclusions

The experimental results described in this work indicate that twin-screw extruders can be used as a pre-treatment method for lignocellulosic biomass to produce material with varying xylose contents, thus, enhancing the enzymatic hydrolysis.

Individual screw elements (conveying, kneading and reverse) in a twin-screw extruder can affect lignocellulosic biomass in multiple ways, some these effects can enhance enzymatic digestibility while others decreases it. The negative effect of lignin re-distribution increases with the severity of the extrusion process, however subsequent lignin removal shows that the same conditions will enhance digestibility through other phytochemical changes in the biomass.

Monomeric xylose can feasibly be recovered without organic solvents from the steam-exploded corncobs with co-rotating twin screw extruders. The screw configuration and operating conditions played an important role on xylose recovery. Xylose removal can be enhanced by decreasing the screw speeds and increasing water flow rate and barrel temperature. The specific energy consumption of the extruder is well correlated with the amount of xylose recovered. However, there is no general proportionality; hence optimization of the xylose specific energy consumption and other can be undertaken.

The xylose content can be controlled based on the employed screw configuration, as demonstrated for steam-exploded corncobs. The extrusion process further led to an increase in cellulose crystallinity, while structural changes were also observed via SEM. The effects of residual xylose (7% and 80% removal through extrusion process), enzyme loading, surfactant addition, and hydrolysis time on enzymatic hydrolysis could be described with a 2nd order polynomial model, based on data generated through a response
surface method (RSM)-based model using IV-optimal design. All independent variables and the interaction effects of enzyme loading and hydrolysis time, hydrolysis time and xylose content, Tween 80 concentration and xylose content, the quadratic terms of enzyme loading as well as the quadratic term of hydrolysis time had a significant effect on enzymatic hydrolysis.
Chapter 7. Recommendations

In the author’s view, the extrusion pretreatment process equipped with a filtration device using a twin-screw extruder is a promising method for xylose removal, which will increase the digestibility. Future studies may be carried out as follows:

a). The screw configuration used for 80% xylose removal should be revised. The current screw configuration consists of too many kneading and reverse screw elements. The number of these severe screw elements should be minimized or replaced by conveying screw element to form a new configuration, which can achieve the same xylose removal rate.

b). The filter should be studied in the future such as the porosity. The current filter was blocked slowly by large solids after the extrusion process run a few hours, thus no additional xylose was recovered. The pore size of the filter should be adjusted according to the particle size of the solids and make sure the extrusion process can continuously run with the same xylose removal rate.

c). Optimization of the xylose specific energy consumption should be performed to minimize the energy consumption while achieve the highest xylose recovery.

d). The reason explained in this paper for lower digestibility caused by the reverse screw element was the pseudo-lignin formation during the extrusion process. More testing should be done to proof this. Furthermore, the mechanism of pseudo-lignin formation during the extrusion process should also be investigated.

e). High concentration of recovered xylose during the extrusion process should be researched on fermentation for bioethanol production.
f). The economic analysis of extrusion pretreatment of corncobs for bioethanol production related to productivity, capital cost, and operating cost, as well as defined assumptions should be conducted, in order to obtain reliable and creditable cost predictions.

g). It is recommended that the extrusion pretreatment of corncobs for bioethanol production should be tested at a larger scale.
Appendix A

A1. Response surface methodology

Modeling of extrusion pretreatment processing involves consideration of process parameters and product properties. Thus, extrusion pretreatment process modeling is a multiple input and multiple output process. However, mathematical modeling of the biomass extrusion process has become a difficult task as large number of process variables are involved and nonlinear relationships might also be involved. The response surface methodology (RSM) has been widely used as an effective modeling tool of extrusion processing. It is a collection of mathematical and statistical techniques for developing, improving and optimizing the response influenced by multiple variables. If there is more than one response, RSM will help find the compromise optimum conditions for all responses (Ferreira et al., 2009).

A2. Statistical analysis

In general, the relationship between the true response and independent variables is unknown. Experimenters usually start with a low order polynomial to approximate experimental results over a small region. If the response is not curved in that small region, a first order model can be used to reasonably approximate of the response. A first order model with independent variables can be expressed as follows:

\[ Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \epsilon \]  \hspace{1cm} (1)

\( Y \): predicted response

\( \beta_0 \): interception coefficient
\( \beta_i \): coefficient of each independent variable

\( x_i \): independent variables

\( \epsilon \): random error.

If there is a curvature in the response surface, then a more highly structured model, such as a second-order model should be studied. The proposed model for response with independent variables can be expressed as follows:

\[
y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{1 \leq i < j \leq k} \beta_{ij} x_i x_j + \epsilon
\]  

(2)

Where \( y \) is the predicted response, \( x_i \) represents the independent variables, \( k \) is the number of variables, \( \beta_0 \) is the interception coefficient, \( \beta_i \) represents the linear coefficient of each independent variable, \( \beta_{ii} \) represents the coefficients of the quadratic terms, \( \beta_{ij} \) represents the coefficients of the interaction effects and \( \epsilon \) is the random error.

The experimental data was analyzed using Design Expert 8.0.7.1 for determining the regression coefficient of each independent variable. A proposed polynomial equation will mathematically describe the relationship between the response and the variables. The significance was checked by the analysis of the variance (ANOVA). The significance of each parameter, and the interaction and quadratic effects were determined based on significance (\( \alpha = 0.05 \)) using the \( F \)-test. The fitted model was evaluated by normal plot, \( R^2 \) and adjusted \( R^2 \) and lack of fit coefficient for determining the adequacy.

A3. Response surface model validation

As observed from the experimental results in Table A1, the twin screw extruder with a filtration device was successfully employed to recover xylose within the ranges of the
input variables. The fitted model has to be checked to determine whether the model provides an adequate approximation of the true response surface. The analysis of variance for the data is presented in Table A2. As observed, F value of the xylose recovery is 79.71, which is very high compared to the critical value (2.96), and this indicates that the model for xylose recovery was highly significant. The value of $R^2$ can be used to estimate how well the model fits the data by measuring the percentage of the variation of $y$ around $\bar{y}$ that is explained by the regression equation. The $R^2$ lies in the interval $[0, 1]$. The closer $R^2$ is to 1, the better the estimation of regression equation that fits the data. However, only 71% of variation in xylose recovery was explained by the proposed models, indicating that estimated regression equations did not fit the data well. A large value of $R^2$ did not always imply that the regression model was a good one because adding a variable to the model always increased $R^2$ no matter that the variable was statistically significant. Hence, adjusted $R^2$ was used to calculate and check the model adequacy, because adjusted $R^2$ will not increase when variables are added, it will often decrease when unnecessary variables are added. Table A2 shows that adjusted $R^2$ for xylose recovery was around 67%; therefore, it is confirmed that the model of xylose recovery did not fit the data well. Curvature measures the true response surface whether it is linear or nonlinear. The results from Table A2 show that the curvature of xylose recovery is significant on the p value. This confirms that a second-order model should be used for xylose recovery in approximating the true response surface with curvature. The lack of fit measures the failure of the model to represent data in the experimental domain at points which are not included in the regression. As evident from Table A2, the lack of fit for xylose recovery was significant, and this was confirmed from the $p$ value. Adeq
Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The response of xylose recovery here was greater than 4, indicating an adequate signal, which can be used to navigate the design space. Therefore, based on the above discussion, the estimated regression model of xylose recovery should be augmented to a higher model that provides extra information for the quadratic effects estimation. Table A3 shows the six runs required for the next step.

**Table A1**, Experimental design with actual values of factors and observed responses.

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<thead>
<tr>
<th>Run #</th>
<th>Temperature (°C)</th>
<th>Screw Speed (rpm)</th>
<th>L/S Ratio</th>
<th>Screw Configuration</th>
<th>Xylose Recovery (%)</th>
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<td>87</td>
<td>0.6</td>
<td>Low shear</td>
<td>71.31</td>
</tr>
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<td>75</td>
<td>1.2</td>
<td>Low shear</td>
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<td>0.6</td>
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<tr>
<td>11</td>
<td>65</td>
<td>100</td>
<td>1.2</td>
<td>High shear</td>
<td>2.27</td>
</tr>
<tr>
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<td>0</td>
<td>High shear</td>
<td>7.18</td>
</tr>
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<td>87.5</td>
<td>0.6</td>
<td>High shear</td>
<td>67.84</td>
</tr>
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<td>82.5</td>
<td>87.5</td>
<td>0.6</td>
<td>Low shear</td>
<td>66.84</td>
</tr>
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<td>15</td>
<td>100</td>
<td>75</td>
<td>0</td>
<td>High shear</td>
<td>62.10</td>
</tr>
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<td>65</td>
<td>75</td>
<td>0</td>
<td>High shear</td>
<td>16.05</td>
</tr>
<tr>
<td>17</td>
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<td>100</td>
<td>0</td>
<td>Low shear</td>
<td>32.50</td>
</tr>
<tr>
<td>18</td>
<td>100</td>
<td>100</td>
<td>1.2</td>
<td>High shear</td>
<td>79.76</td>
</tr>
<tr>
<td>19</td>
<td>65</td>
<td>75</td>
<td>1.2</td>
<td>High shear</td>
<td>11.35</td>
</tr>
<tr>
<td>20</td>
<td>100</td>
<td>75</td>
<td>0</td>
<td>Low shear</td>
<td>62.01</td>
</tr>
<tr>
<td>21</td>
<td>100</td>
<td>100</td>
<td>1.2</td>
<td>Low shear</td>
<td>69.67</td>
</tr>
<tr>
<td>22</td>
<td>65</td>
<td>100</td>
<td>1.2</td>
<td>Low shear</td>
<td>8.00</td>
</tr>
</tbody>
</table>
Table A2. Analysis of variance of fitted model for different responses.

<table>
<thead>
<tr>
<th>Response</th>
<th>Source</th>
<th>F value</th>
<th>P value</th>
<th>R²/Adj R²</th>
<th>Adeq Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylose Recovery</td>
<td>Model</td>
<td>79.71</td>
<td>&lt;0.0001</td>
<td>0.7298/0.6662</td>
<td>9.467</td>
</tr>
<tr>
<td></td>
<td>Lack of fit</td>
<td>13.58</td>
<td>0.0111</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Curvature</td>
<td>102.06</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table A3. Additional runs for estimation of quadratic effects.

<table>
<thead>
<tr>
<th>Run #</th>
<th>Temperature (°C)</th>
<th>Screw Speed (rpm)</th>
<th>L/S Ratio</th>
<th>Screw Configuration</th>
<th>Xylose Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>87.5</td>
<td>0.6</td>
<td>High shear</td>
<td>2.73</td>
</tr>
<tr>
<td>2</td>
<td>82.5</td>
<td>75</td>
<td>0.6</td>
<td>High shear</td>
<td>24.73</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>87.5</td>
<td>0.6</td>
<td>High shear</td>
<td>58.97</td>
</tr>
<tr>
<td>4</td>
<td>82.5</td>
<td>100</td>
<td>0.6</td>
<td>Low shear</td>
<td>23.72</td>
</tr>
<tr>
<td>5</td>
<td>82.5</td>
<td>87.5</td>
<td>1.2</td>
<td>High shear</td>
<td>7.43</td>
</tr>
<tr>
<td>6</td>
<td>82.5</td>
<td>87.5</td>
<td>0</td>
<td>High shear</td>
<td>37.39</td>
</tr>
</tbody>
</table>

The six experiments were performed using another batch of pretreated corn cobs with the same pretreatment conditions. The results of the quadratic response surface model are shown in Table A4. The F value of the model is 42.43, which is very high compared to the critical value, indicating that the model is highly significant. The value of “Prob>F” is less than 0.005 supporting that the model is significant. The significance of each parameter coefficient was determined by p-values. The smaller the p-values, the more significant the coefficient (Fannin et al., 1981). In this case, temperature has the greatest effect while screw configuration has no effect. The quadratic effects of screw speed and L/S ratio and interaction effect of temperature and L/S Ratio has also significant effects on xylose recovery. The goodness of fit of the model was checked by the coefficient of determination $R^2$, adjusted determination coefficient and adeq Precision. The value of the coefficient of determination ($R^2=0.9049$) implies that 90.49% of the total variations are explained by the model. The value of the adjusted coefficient of determination (Adj.
\( R^2 = 0.9049 \) also supports the significance of the model. A ratio of 15.972 of the adeq precision indicates an adequate signal for navigating the design space. The "Lack of Fit F-value" of 10.64 implies its significance. There is only a 1.70% chance that a "Lack of Fit F-value" this large could occur due to noise.

Based on the selected significant variables, the quadratic model for the xylose recovery in terms of actual factors is shown as follows:

\[
\text{Xylose Recovery} = -727.77113 + 1.21125X_1 + 15.3872X_2 + 10.28104X_3 + 0.54744X_1X_3 - 0.089432X_2^2 - 43.85762X_3^2
\]

Where \( X_1 \) is barrel temperature, \( X_2 \) is screw speed, \( X_3 \) is L/S Ratio.
### Table A4, Analysis of variance of fitted model for different responses.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>Degrees of freedom</th>
<th>Mean square</th>
<th>F value</th>
<th>Prof&gt;F</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>18007.62</td>
<td>6</td>
<td>3001.27</td>
<td>42.23</td>
<td>&lt;0.0001</td>
<td>Significant</td>
</tr>
<tr>
<td>X₁</td>
<td>13068.59</td>
<td>1</td>
<td>13068.59</td>
<td>183.90</td>
<td>&lt;0.0001</td>
<td>Significant</td>
</tr>
<tr>
<td>X₂</td>
<td>195.10</td>
<td>1</td>
<td>195.10</td>
<td>2.75</td>
<td>0.1131</td>
<td>Significant</td>
</tr>
<tr>
<td>X₃</td>
<td>51.38</td>
<td>1</td>
<td>51.38</td>
<td>0.72</td>
<td>0.4052</td>
<td></td>
</tr>
<tr>
<td>X₁X₃</td>
<td>528.66</td>
<td>1</td>
<td>528.66</td>
<td>7.44</td>
<td>0.0130</td>
<td>Significant</td>
</tr>
<tr>
<td>X₂²</td>
<td>643.96</td>
<td>1</td>
<td>643.96</td>
<td>9.06</td>
<td>0.0069</td>
<td>Significant</td>
</tr>
<tr>
<td>X₃³</td>
<td>822.11</td>
<td>1</td>
<td>822.11</td>
<td>11.57</td>
<td>0.0028</td>
<td>Significant</td>
</tr>
<tr>
<td>Residual</td>
<td>1421.24</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>1388.62</td>
<td>16</td>
<td>86.79</td>
<td>10.64</td>
<td>0.0170</td>
<td>Significant</td>
</tr>
<tr>
<td>Pure Error</td>
<td>32.62</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- R-Squared 0.9268
- Adj-Squared 0.9049
- Pre R-Square 0.8055
- Adeq Precision 15.972

### Reference

Appendix B

B1. Response surface methodology

Modeling of enzymatic hydrolysis processing involves consideration of process parameters and product properties. Thus, enzymatic hydrolysis modeling is a multiple input and multiple output process. However, mathematical modeling of enzymatic hydrolysis becomes a difficult task as large numbers of process variables are involved and nonlinear relationships might also be involved. The response surface methodology (RSM) has been widely used as an effective modeling tool of enzymatic hydrolysis. It is a collection of mathematical and statistical techniques for developing, improving and optimizing the response, and influenced by multiple variables. If there is more than one response, RSM will help find the compromise optimum conditions for all responses (Ferreira et al., 2009).

B2. Experimental design

A general full factorial design with four factors was selected to screen and evaluate the significant variables that affect the response pattern and to determine the optimum combination of enzyme loading, surfactant concentration, hydrolysis time with extruded corn cobs with different xylose removals for maximizing glucose conversion from pretreated corn cobs. The ranges and levels of the independent variables are shown in Table B1. Each factor level was selected based on preliminary studies. The ratio of the total amount of glucose produced in the hydrolyzate to the total amount of glucose in the steam exploded corn cobs was chosen as a response for analyzing. The experimental design was developed using the Design Expert software, version 8.0.7.1 (Stat Ease, Inc.,
USA), which resulted in a total of 22 runs with center points. These runs were performed fully randomly to minimize the effect of any unexplained variability in the observed responses. The response was tested for linear and quadratic models to obtain the best fitting model. The effects of the main factors and their interactions were evaluated by the experimental plan. The experimental design with actual independent variables and observed responses are shown in Table B2.

**Table B1**, Range of each independent variable in the full factorial design.

<table>
<thead>
<tr>
<th>Level of variables</th>
<th>Enzyme loading (% w/w)</th>
<th>Hydrolysis time (hr)</th>
<th>Tween 80 concentration (% w/w)</th>
<th>Different xylose removals (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low level</td>
<td>2</td>
<td>48</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>High level</td>
<td>8</td>
<td>72</td>
<td>6</td>
<td>80</td>
</tr>
</tbody>
</table>
Table B2, Factors and the response of the full factorial design used for response surface methodology.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Enzyme loading (%. w/w)</th>
<th>Tween 80 concentration (%. w/w)</th>
<th>Different xylose removals (%)</th>
<th>Hydrolysis time (hr)</th>
<th>Glucose conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>0</td>
<td>80</td>
<td>24</td>
<td>56.67±1.34</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>3</td>
<td>7</td>
<td>48</td>
<td>49.87±1.75</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>3</td>
<td>80</td>
<td>48</td>
<td>61.06±0.73</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>6</td>
<td>80</td>
<td>24</td>
<td>32.31±2.11</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>0</td>
<td>7</td>
<td>24</td>
<td>52.18±1.68</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>0</td>
<td>80</td>
<td>72</td>
<td>38.23±1.31</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>3</td>
<td>80</td>
<td>48</td>
<td>62.4±0.66</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>6</td>
<td>7</td>
<td>24</td>
<td>24.3±1.74</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>0</td>
<td>80</td>
<td>24</td>
<td>26.95±1.02</td>
</tr>
<tr>
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<td>2</td>
<td>0</td>
<td>7</td>
<td>72</td>
<td>29.79±1.67</td>
</tr>
<tr>
<td>11</td>
<td>8</td>
<td>6</td>
<td>80</td>
<td>24</td>
<td>63.94±0.98</td>
</tr>
<tr>
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<td>2</td>
<td>6</td>
<td>7</td>
<td>72</td>
<td>31.23±1.25</td>
</tr>
<tr>
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<td>5</td>
<td>3</td>
<td>7</td>
<td>48</td>
<td>47.99±1.63</td>
</tr>
<tr>
<td>14</td>
<td>8</td>
<td>0</td>
<td>80</td>
<td>72</td>
<td>82.03±0.83</td>
</tr>
<tr>
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<td>5</td>
<td>3</td>
<td>7</td>
<td>48</td>
<td>46.37±1.80</td>
</tr>
<tr>
<td>16</td>
<td>8</td>
<td>0</td>
<td>7</td>
<td>72</td>
<td>69.98±1.21</td>
</tr>
<tr>
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<td>0</td>
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<td>24</td>
<td>24.15±1.76</td>
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<td>3</td>
<td>80</td>
<td>48</td>
<td>62.2±0.85</td>
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<td>6</td>
<td>7</td>
<td>24</td>
<td>55.45±1.46</td>
</tr>
<tr>
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<td>2</td>
<td>6</td>
<td>80</td>
<td>72</td>
<td>42.83±0.77</td>
</tr>
<tr>
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<td>8</td>
<td>6</td>
<td>7</td>
<td>72</td>
<td>72.92±0.91</td>
</tr>
<tr>
<td>22</td>
<td>8</td>
<td>6</td>
<td>80</td>
<td>72</td>
<td>88.41±0.64</td>
</tr>
</tbody>
</table>
### Table B3, Analysis of variance of fitted model for the response.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>Degrees of freedom</th>
<th>Mean square</th>
<th>F value</th>
<th>Prob&gt;F</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>35.59</td>
<td>7</td>
<td>5.08</td>
<td>982.11</td>
<td>&lt;0.0001</td>
<td>Significant</td>
</tr>
<tr>
<td>Enzyme Loading</td>
<td>27.73</td>
<td>1</td>
<td>27.73</td>
<td>5356.71</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Hydrolysis Time</td>
<td>4.23</td>
<td>1</td>
<td>4.23</td>
<td>816.59</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Extruded Corn Cobs</td>
<td>1.64</td>
<td>1</td>
<td>1.64</td>
<td>316.69</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Surfactant Concentration</td>
<td>0.31</td>
<td>1</td>
<td>0.31</td>
<td>58.99</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Enzyme Loading x Hydrolysis Time</td>
<td>0.28</td>
<td>1</td>
<td>0.28</td>
<td>54.03</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Hydrolysis Time with Extruded Corn Cobs</td>
<td>0.11</td>
<td>1</td>
<td>0.11</td>
<td>22.01</td>
<td>0.0005</td>
<td></td>
</tr>
<tr>
<td>Extruded Corn Cobs x Surfactant Concentration</td>
<td>0.079</td>
<td>1</td>
<td>0.079</td>
<td>15.30</td>
<td>0.0021</td>
<td></td>
</tr>
<tr>
<td>Curvature</td>
<td>1.28</td>
<td>2</td>
<td>0.64</td>
<td>123.72</td>
<td>&lt;0.0001</td>
<td>Significant</td>
</tr>
<tr>
<td>Residual</td>
<td>0.062</td>
<td>12</td>
<td>5.178E-003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack of Fit</td>
<td>0.026</td>
<td>8</td>
<td>3.252E-003</td>
<td>0.36</td>
<td>0.8977</td>
<td>Not significant</td>
</tr>
<tr>
<td>Pure Error</td>
<td>0.036</td>
<td>4</td>
<td>9.028E-003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-Squared</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.9636</td>
<td></td>
</tr>
<tr>
<td>Adj-Squared</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.9454</td>
<td></td>
</tr>
<tr>
<td>Pre R-Square</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.9327</td>
<td></td>
</tr>
<tr>
<td>Adeq Precision</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24.936</td>
<td></td>
</tr>
<tr>
<td>C.V.%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.41</td>
<td></td>
</tr>
</tbody>
</table>
The fitted model has to be checked if the model provides an adequate approximation of the true response surface. The analysis of variance (ANOVA) for the data is presented in Table B3. As observed, F value of the glucose conversion is 982.11, which is very high compared to the critical value (2.96). Moreover, the value of probability (Prob) is greater than the F value (<0.0001) indicating that the model for glucose conversion was highly significant. The coefficient of determination R² can be used to estimate how well the model fits the data by measuring the percentage of the variation of y around \( \bar{y} \) that is explained by the regression equation. R² lies in the interval [0, 1]. The closer R² is to 1, the better the estimation of the regression equation fits the data. As 96.36% of variation in glucose conversion rate was explained by the proposed models, this indicates that the estimated regression equations fit the data well. A large value of R² did not always imply that the regression model was a good one because adding a variable to the model always increased R² no matter which variable is statistically significant. Hence, adjusted R² was used to calculate and check the model adequacy because adjusted R² will not increase when variables are added; it will often decrease when unnecessary variables are added. The adjusted R² value was used to correct the R² value for the sample size and the number of terms. A high value of adjusted R² indicates the high significance of the model. Table 5 shows that adjusted R² for the glucose conversion rate was around 94.54%; therefore, it is confirmed that the model glucose conversion rate did fit the data well. Curvature measures the true response surface whether it is linear or nonlinear. The results from Table 5 show that the curvature of glucose conversion is significant to the P value. The smaller the p-values, the more significant the corresponding coefficient. This confirms that a second-order model should be used for glucose conversion in
approximating the true response surface with curvature. The lack of fit measures the failure of the model to represent data in the experimental domain at points which are not included in the regression. As evident from Table B3, the lack of fit glucose conversion was not significant as confirmed by the $P$ value. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The response of glucose conversion here was greater than 4, indicating an adequate signal, which can be used to navigate the design space. A relatively lower CV (4.41) indicated a better precision and reliability of the experiments. Therefore, based on the above discussion, the estimated regression model of glucose conversion should be augmented to a higher model that provides extra information for the quadratic effects estimation. Table B4 shows the additional six runs required to perform for next step.

**Table B4.** Additional 6 runs for quadratic effects estimation

<table>
<thead>
<tr>
<th>Trial</th>
<th>Enzyme loading (% w/w)</th>
<th>Tween 80 concentration (% w/w)</th>
<th>Different xylose removals (%)</th>
<th>Hydrolysis time (h)</th>
<th>Glucose conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>3</td>
<td>7</td>
<td>24</td>
<td>36.57±0.59</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>3</td>
<td>80</td>
<td>24</td>
<td>45.89±2.05</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>3</td>
<td>80</td>
<td>48</td>
<td>71.93±1.11</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>3</td>
<td>80</td>
<td>48</td>
<td>32.38±1.23</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>0</td>
<td>80</td>
<td>48</td>
<td>54.15±1.15</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>6</td>
<td>80</td>
<td>48</td>
<td>60.57±1.60</td>
</tr>
</tbody>
</table>

The six experiments were performed by the same batch of the extruded corn cobs with the same pretreatment conditions. The results of the quadratic response surface model are shown in Table 5-3.
Reference

Appendix C

C1. Steam explosion process

Figure C1. Steam explosion process.
The ground corncobs 10 (0.5 to 1 cm$^3$ particle size) with a moisture content of 50% DM were pre-conditioned by preheating to a temperature of about 95°C for about 10-60 minutes with steam 20 at atmospheric pressure in a conditioning container 30. Steam and air were vented through an air vent 35. Corncobs were compressed through a Modular Screw device 40. Air and inhibitory extracts were removed through an air vent 50 and 55, respectively. The corncobs were then fed into a pressurized upflow tube 70. Pressurized saturated steam was directly injected 60 or indirectly injected 61 into the upflow tube at a temperature of 205°C until the desired pressure was achieved. The preheated corncobs were moved through the upflow tube and finally discharged into a pretreatment reactor 80 for a residence time of 5 min at 205°C. Condensate and liquids were collected at the bottom of the pretreatment reactor through a purge discharge control valve 95. Pretreated corncobs were continuously discharged into a Co-rotating Twin Screw extruder 100 by adding hot water 90. Hemicellulose was extracted 110 and the washed corncobs were flashed into a reactor for future enzymatic hydrolysis (Dottori et al., 2013).

**Reference**

Appendix D

D1. Photographs of the twin-screw extruder.

Figure D1. The modular barrel of the twin-screw extruder.
D2. A designed filtration device

Figure D2. A designed filtration device for xylose removal.
Curriculum Vitae

Name: Jun Zheng

Post-secondary Education and Degrees:

- University of Toronto
  Toronto, Ontario, Canada
  2005-2009 B.A.

- The University of Western Ontario
  London, Ontario, Canada
  2009-2010 M.Eng.

- The University of Western Ontario
  London, Ontario, Canada
  2010-2014 Ph.D.

Honours and Awards:

- Western Graduate Research Assistant Scholarship (WGRA)
  2010-2014

Related Work Experience:

Teaching Assistant
The University of Western Ontario
2010-2014

Research Assistant
The University of Toronto
2008-2009
Assisted in the research project in the production ethyl esters from white mustard oil via an aqueous process

Extracted ester and applied trans-esterification reaction to test the conformity between the extracts and the desired standards

Research Assistant
The University of Western Ontario
2010-2014
Researched the effect of ionic liquid (1-butyl-3-methylimidazolium) on the pretreatment of ground switchgrass for the for enzymatic hydrolysis

Recovered a solid fraction enriched in cellulose and 88% of glucose and
25% hemicelluloses contained in the raw switchgrass were released by enzymatic hydrolysis

**Research Assistant**  
Lambton College  
2011-2012  
Participated in the mechanical pretreatment of steam exploded Corncobs for xylose removal for ethanol production by a Twin-screw extruder

Successfully developed a screw configuration which could 80% xylose from steam exploded corncobs results

Studied the effects of screw elements on enzymatic hydrolysis rate

Greenfiled Company made a major design to their continuous cellulose pre-treatment system replacing 5 pieces of existing best in class equipment by two Twin-screw extruders

**Research Assistant**  
The University of Western Ontario  
2010-2014  
Carried out the enzymatic hydrolysis and studied the combined effects of various enzymatic hydrolysis process variables including enzyme loading, Tween 80 concentration and hydrolysis time with two differently extruded corncobs

Examined the properties of extruded corncobs with differently extruded corncobs by FTIR, XRD and SEM

**Publications:**


