BIOCONVERSION OF KNOT REJECTS FROM A SULPHITE PULP MILL TO ETHANOL

Xiao Zhang,[a.b] Maobing Tu,[c] Michael Paice,[a] George Sacciadis,[a] Zhihua Jiang,[a] Naceur Jemaa,[a] and Alain Thibault[a]

Knot rejects obtained from pulp screening after sulphite pulping are difficult to dewater, which makes landfilling expensive and burning inefficient. The rejects were found to contain up to 50% cellulose, which is very susceptible to enzymatic hydrolysis to glucose. Knot rejects were hydrolyzed at 20% consistency in a laboratory peg mixer with cellulase enzyme. The thick slurry was liquefied within the first hour of mixing and resulted in a glucose concentration of over 100 g/L after 24 hours of reaction. This solution was fermented by yeast to give an ethanol concentration of over 5%. The laboratory results were confirmed at pilot scale with a mortar mixer (high consistency) or stirred tank reactor (medium consistency) at the 400 L and 6000 L scales, respectively. It was found that washing the knots with ammonia resulted in increased glucose conversion. Enzyme costs could be lowered by separating the enzyme from the hydrolyzed sugars by membrane ultrafiltration and recycling the enzyme to the subsequent batch of substrate. The combination of high-consistency hydrolysis and enzyme recycling minimizes capital investment, energy requirements, and enzyme costs, which are significant factors in the overall economic viability of cellulose conversion to ethanol.

Keywords: Bioconversion; Enzyme hydrolysis; Ethanol; High consistency; Fermentation; Pulp mills; Knots; Rejects

Contact information: a: FPInnovations Paprican Division, 570 St-Jean Boul. Pointe Claire, Quebec, Canada, b: Center for Bioproducts and Bioenergy, Washington State University, 2710 University Drive, Richland, WA 99354,USA, c: School of Forestry & Wildlife Sciences, Auburn University, AL 36849, USA
**Corresponding author: xiaozhang@tricity.wsu.edu*

INTRODUCTION

Recently, bioconversion of lignocellulosic biomass to ethanol and bioproducts has attracted unprecedented research and investment interest. Despite decades of research effort, several technical and economic bottlenecks still prohibit the commercialization of this bioconversion process. Major cost bottlenecks are associated with the feedstock, pretreatment, and enzyme hydrolysis (Fig. 1). While these process elements are essential to most biomass feedstocks, knot rejects generated from the sulphite pulping process represent a unique substrate that has already been pretreated and has little or negative value. This makes knot rejects a promising lignocellulosic substrate for developing a cost-competitive cellulosic ethanol bioconversion process.

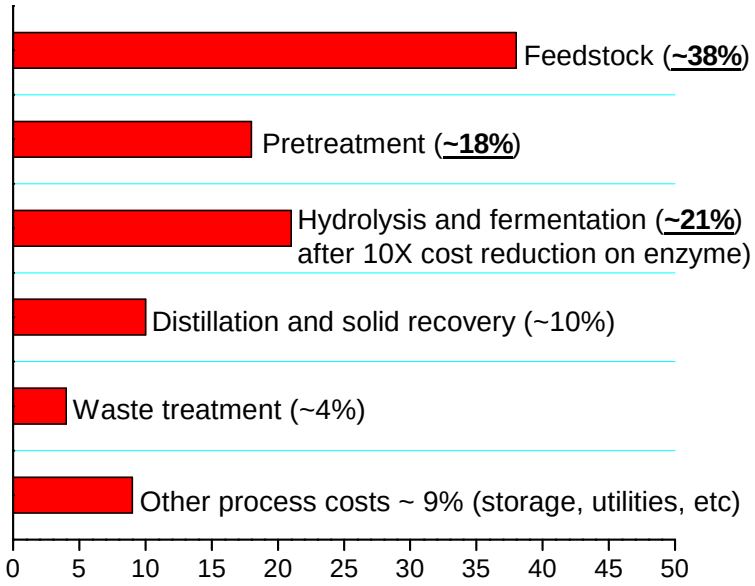


Fig. 1. Cost breakdown for a lignocellulosic biomass-to-ethanol process (Aden and Foust 2009)

Knot rejects obtained from pulp screening after sulphite pulping (Fig. 2) consist of botanical knots, insufficiently cooked chips, and variable amounts of cellulose fibres. Depending on the feedstock, the solids fractions of knot rejects contain up to 50% cellulose and 30% phenolic compounds. The cellulose portion is held tightly by the phenolic core, which makes it difficult to separate the fibres from the knot core. Knot rejects are a major solid waste stream; the amount of heat generated from burning knot rejects is small due to their poor dewatering efficiency, while sending rejects to landfill results in additional costs to the mill. Mills have attempted to "extract" fibres from this material with little success. Finding cost-effective ways of utilizing knot rejects presents a great challenge. Our preliminary studies have shown that these knot rejects contain a significant amount of cellulose and are very susceptible to enzymatic hydrolysis to glucose, which can be readily fermented to ethanol. The knot rejects also contain a significant quantity of lignans, a group of biologically active compounds. Hydrolysis of knot rejects was found to provide an effective means of removing cellulose, thus facilitating lignan extraction.

A previous study has evaluated the technical and economic feasibility of bioconversion of knot rejects to ethanol using cellulase, and a low return on investment (ROI), 25%, was determined (Helle *et al.* 2007). Although the economic assessment was based on small scale experiments (up to 250 mL shake flask), Helle's study did identify two major economic bottlenecks which hinder the implementation of the knot bioconversion process: 1) the high capital cost of the hydrolysis tank and 2) the high cost of cellulase enzyme required for effective hydrolysis.

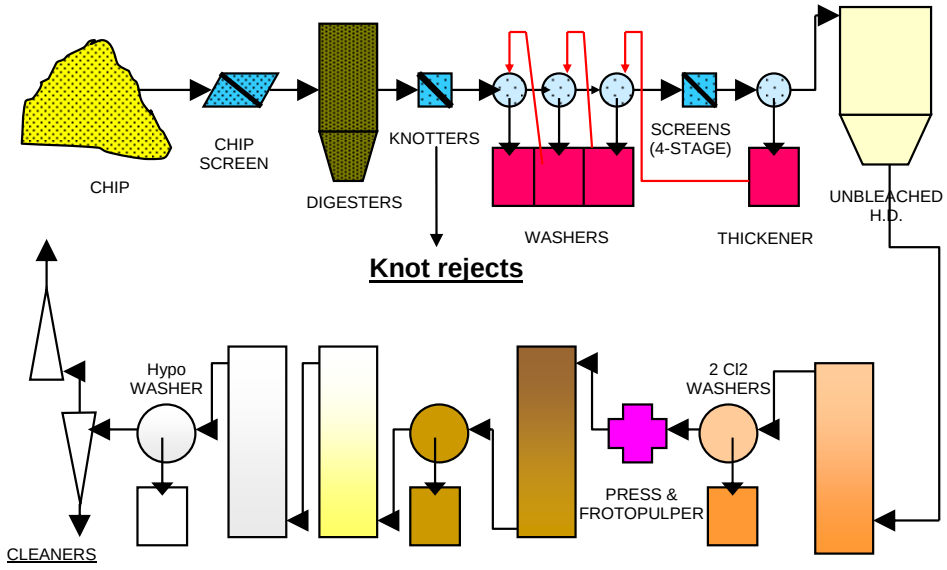


Fig. 2. Sulphite pulping process diagram

Our research approach was designed to address these two issues. An effective way to reduce the capital cost of hydrolysis tanks is to carry out enzymatic hydrolysis at high substrate loadings. Recently, we have developed high consistency hydrolysis (HCH) utilizing a peg mixer as the hydrolysis vessel. Hydrolysis of organosolv-pretreated poplar (OPP) at 20% substrate consistency for 48 h gave a high glucose concentration, 158g/L in the final hydrolysate (Zhang *et al.* 2009). The size of the hydrolysis vessel can be reduced four-fold by increasing solids loading from 5% to 20%. HCH also brings other benefits, including reduced operating costs for hydrolysis and fermentation, and reduced energy consumption during distillation/evaporation and other downstream processes. A previous techno-economic assessment suggested that an increase in substrate loading from 5% to 8% (w/w) can reduce the total ethanol production cost by nearly 20% (Stenberg *et al.* 2000; Wingren *et al.* 2003). In addition, in the case of Mill A, the high glucose concentration stream obtained from HCH of knot rejects could be used as a rich sugar supplementation during hardwood runs to maintain the viability of the yeast and stabilize production rate.

The high amounts and cost of the cellulase enzymes employed in the cellulose hydrolysis is another major economic bottleneck of the bioconversion process. In recent years, a significant amount of research has been focused on improving the production and efficiency of the enzymes for cellulose hydrolysis (Chen *et al.* 1993; Chand *et al.* 2005; Ahameda and Vermette 2008). A recent joint research effort between the U.S. Department of Energy (DOE) and two major enzyme supplier companies, Genencor International and Novozymes, has resulted in a significant reduction in the cost of enzymes (Sandra and Cherry 2007; Mitchinson 2004) through a number of measures, including the elimination of downstream processing steps, the use of less expensive carbon sources, the improvement of specific activity through site-directed mutagenesis, etc. While further reduction in enzyme cost through microbial genetic engineering

remains a long-term prospect (Galbe and Zacchi 2002), a more practical approach is to devise recycling strategies to allow maximum reuse of the enzyme. Previous studies have shown that cellulase enzymes can be reused for more than six consecutive cycles without any significant loss in enzyme activity (Tu *et al.* 2007). Enzymes are macromolecules with a molecular weight typically above 10,000 Da, while the glucose is smaller, with a molecular weight of 180 Da. Therefore, one of the possible enzyme recycling strategies is to apply membrane technology to separate the enzyme from hydrolysis products (e.g. glucose).

The overall objective of the current study was to develop an economically feasible hydrolysis scheme to produce fermentable sugars. The knot rejects used in this study were obtained from a Paprican Member Company mill (Mill A). This pulp mill has been producing ethanol from spent sulphite liquor (SSL) since 1991, with a current capacity of 18 million litres of ethanol per year. However, changing wood species, primarily between softwood and hardwood, has a major impact on the ethanol production rate and yeast viability. Softwood SSL contains a relatively high hexose content, which is readily fermentable by the yeast *Saccharomyces cerevisiae*, while hardwood SSL consists mainly of pentoses, which cannot be assimilated by *Saccharomyces cerevisiae*. The low ethanol output during hardwood runs disrupts ethanol production, and low fermentable sugar concentrations decrease the viability of the yeast. Although the sugar composition of HW and SW SSLs is different, knot rejects derived from both HW and SW contain a similar cellulose content. Hydrolysis of knot rejects to provide an additional glucose stream for fermentation would help maximize the ethanol production capacity and more importantly, would allow Mill A to continue ethanol production during hardwood runs.

EXPERIMENTAL

Substrates

Knot rejects were obtained from a Paprican Member Company mill in Eastern Canada. The total lignin content (acid-soluble lignin and acid-insoluble lignin) of knot rejects was measured following PAPTAC standard procedures G.8 and G.9. The filtrate obtained from lignin analysis was collected and used for sugar analysis. The sugar monomers in the filtrate, including arabinose, galactose, glucose, xylose, and mannose, were separated by an anion exchange column (Dionex CarboPac™ PA1) on a Dionex DX-600 Ion Chromatograph system (Dionex, Sunnyvale, CA) equipped with an AS50 auto-sampler and a GP50 gradient pump (Zhang *et. al.* 2009). The sugar monomers were detected and quantified by an on-line pulsed amperometric detector.

Enzymes

A cellulase enzyme mixture was obtained from Novozymes North America (Franklinton, NC). This enzyme preparation contains 124 filter paper units (FPU) and 400 cellobiose units (CBU) per milliliter of the solution.

Enzymatic Hydrolysis at Laboratory Scale

The hydrolysis experiments at laboratory scale were carried out in 500 mL flasks. The reaction solution contained 200 mM acetate buffer (pH 4.8) with differing concentrations of substrates and enzyme dosages described above. All the flasks were placed in a controlled environment incubator shaker (New Brunswick Scientific Co., Edison, NJ. USA). The enzymatic hydrolyses were carried out at 50°C with a rotating speed of 200 rpm for up to 96 h at various substrate consistencies. The cellulose-to-glucose conversion yield is defined as the glucose amount in the liquid phase product divided by the cellulose content (as glucose) in the substrate.

Enzymatic hydrolysis in a peg mixer was carried out under the same treatment conditions as in shake flasks (temperature, pH and enzyme dosage) except that the mixing speed was set at 20 rpm. Prior to the hydrolysis, the substrate, enzyme, and buffer were mixed thoroughly in a Hobart mixer before being transferred to the peg mixer.

Enzymatic Hydrolysis at Pilot Scale

Enzymatic hydrolysis at the pilot scale was carried out under the same treatment conditions as the shake flasks (temperature, pH, and enzyme dosage).

Enzyme Recycling at Laboratory and Pilot Scale

Enzyme recovery at the laboratory scale was carried out with a Koch laboratory membrane filtration unit (model LabCell CF-1) with an effective membrane surface area of 28 cm^2. The system is equipped with a feed tank holding 500 mL and a feed/recirculation pump. The membrane used to retain the enzymes was manufactured by Koch Membrane Systems (HFK-131) and had a molecular weight cut off (MWCO) of 10,000 Da. The hydrolysate was maintained at 45°C and the pressure around 40 psig during filtration. To provide a substrate with minimized inhibitory effects from the knot phenolic compounds, the knot rejects for this experiment were treated with ammonia and subsequently washed with DI water prior to the enzyme recycling experiment.

Enzyme recovery at the pilot scale was carried out using a Koch pilot membrane system and an ITT Industries (PCI Membrane Systems) membrane module consisting of 18 tubular membranes, with an effective membrane surface area of 0.9 m^2. The membranes used to retain the enzymes were manufactured by ITT Industries and had a MWCO of 6,000 Da. The hydrolysate was maintained at a temperature of 40°C and the pressure was maintained around 80 psig.

Data Analysis and Number of Sample Replicates

All the results reported for chemical compositions and batch hydrolysis are the mean values of at least four replicates based on two batches of repeated experiments under the same conditions. Three samples were taken for each time point during the enzyme hydrolysis.

RESULTS AND DISCUSSION

Laboratory-Scale Study

Enzymatic hydrolysis and fermentation of knot rejects

The chemical compositions of the knot rejects before and after washing are shown in Table 1. Knot rejects consist mainly of cellulose and phenolic compounds (acid soluble and insoluble lignin). A significant amount of acid-soluble lignin fraction can be removed by water washing. This fraction is likely to be phenolic compounds adsorbed on the reject fibre surfaces.

Table 1. The Chemical Composition of Unwashed and Washed Rejects from Knot Screening

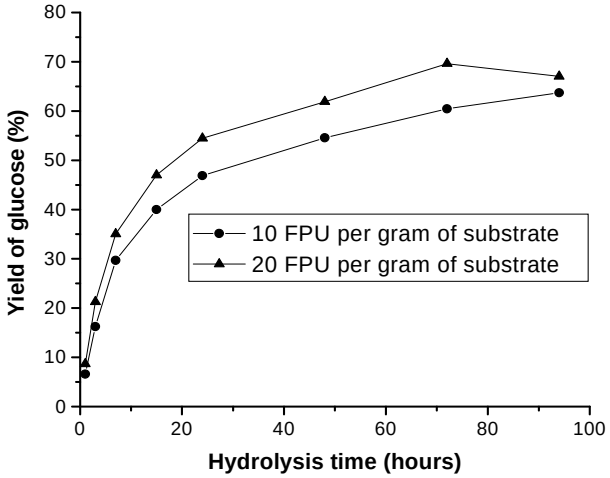
Percentage (%) based on dry weight of knot rejects	Unwashed sample	Washed sample
Extractives (acetone)	1.9	1.8
Lignin (acid insoluble)	18.3	18.0
Lignin (acid soluble)	13.4	8.2
Galactose	0.4	0.2
Glucose	52.9	64.7
Xylose	3.7	4.4
Mannose	4.3	3.9

The high cellulose content makes knot rejects an attractive substrate for hydrolysis and fermentation to ethanol. Knot rejects hydrolysis was first carried out at 2% substrate consistency with two enzyme loadings, 10 FPU and 20 FPU per gram of knot rejects (Fig. 3). It is apparent that the sugar concentration reached a plateau after 48 h of hydrolysis. We found that approximately 60% of the cellulose present in the knot rejects was converted to glucose within this time period. The hydrolysability of the knot rejects was also tested at higher solids loadings, 5 and 10% (Fig. 4). The increase in substrate consistency led to an increase in glucose concentration in the hydrolysate. For example, the glucose concentrations were 8 g/L, 17 g/L, and 31 g/L after 48 h hydrolysis of knot rejects at 2%, 5%, and 10% substrate consistencies, respectively. All these hydrolysis experiments were performed in shake flasks, which are typical settings for laboratory enzymatic hydrolysis. Further increase in substrate consistency to 20% was not successful with shaking flasks due to a rheological problem. A high-consistency hydrolysis process was previously developed using a peg mixer to resolve the rheological problem associated with mixing lignocellulosic substrates at high solids content (Zhang *et. al.* 2009). Applying a peg mixer for HCH of knot rejects resulted in a considerably higher glucose concentration, 110 g/L at 20 FPU/g enzyme loading (Fig. 5). Fermenting this hydrolysate led to the production of over 5% ethanol (w/v) in concentration (data not shown). Lowering the enzyme dosage to 10 FPU/G caused only a small drop in the glucose concentration; however, a much lower glucose concentration was obtained at 3

FPU/g of enzyme loading (Fig. 5). Reducing enzyme dosage from 10 to 3 FPU/G also resulted in a significant increase in the liquefaction time from 30 min to 2 h.

Besides providing a concentrated glucose stream, applying HCH enables knots hydrolysis to be carried out in smaller tanks, which would significantly reduce capital investment. The concentrated sugar stream obtained from HCH can be supplemented to the hardwood SSL, thereby maintaining the viability of yeast and ethanol production rate.

A: Cellulose-to-glucose conversion yield



B: Glucose concentration in the hydrolysate

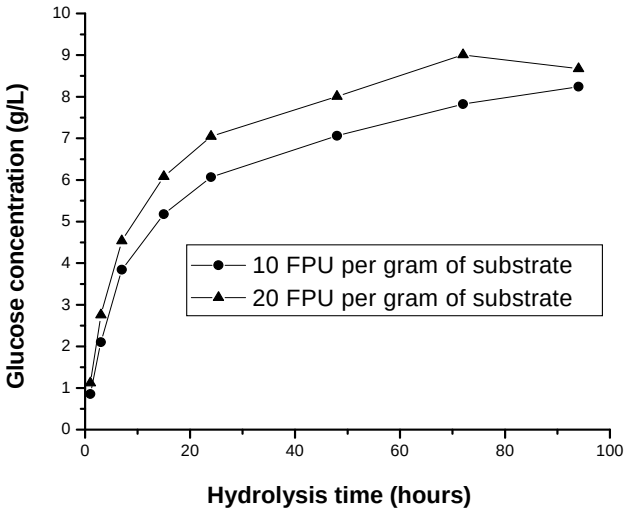
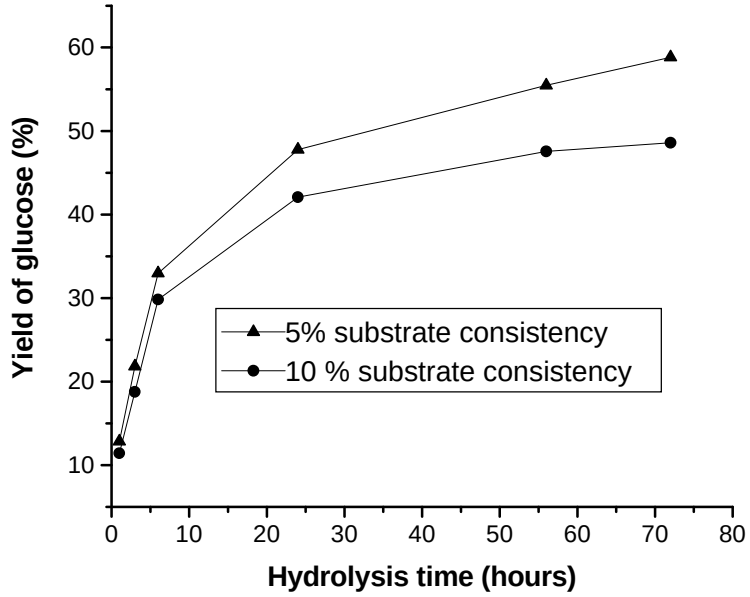


Fig. 3. The production of glucose during cellulase hydrolysis of knot rejects at 2% substrate consistency

A: Cellulose-to-glucose conversion yield



B: Glucose concentration in the hydrolysate

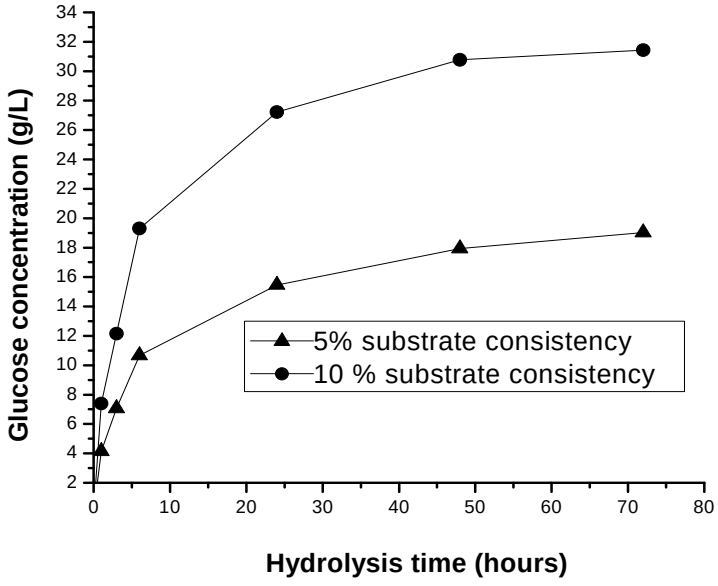


Fig. 4. The production of glucose during cellulase hydrolysis of knot rejects at 5% and 10% substrate consistencies with an enzyme dosage of 20 FPU/g

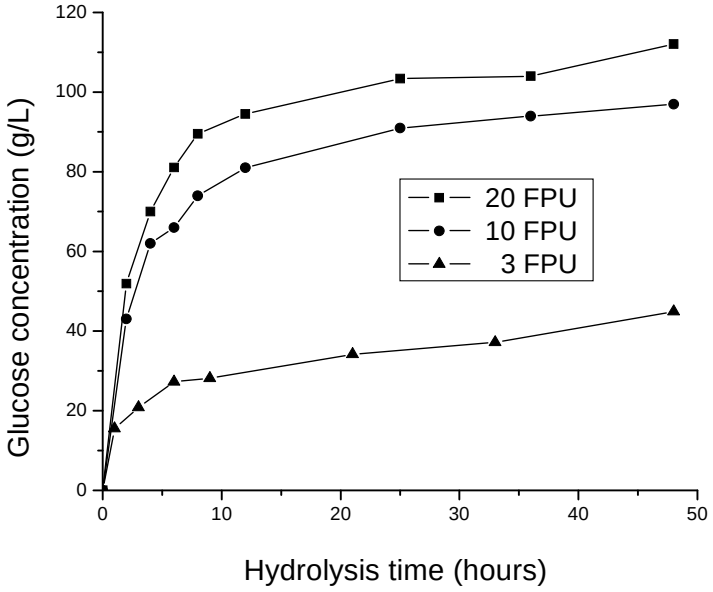


Fig. 5. Hydrolysis of knot rejects at 20% consistency with a peg mixer at different enzyme dosages

S*trategies to reduce enzyme dosages for knot hydrolysis*

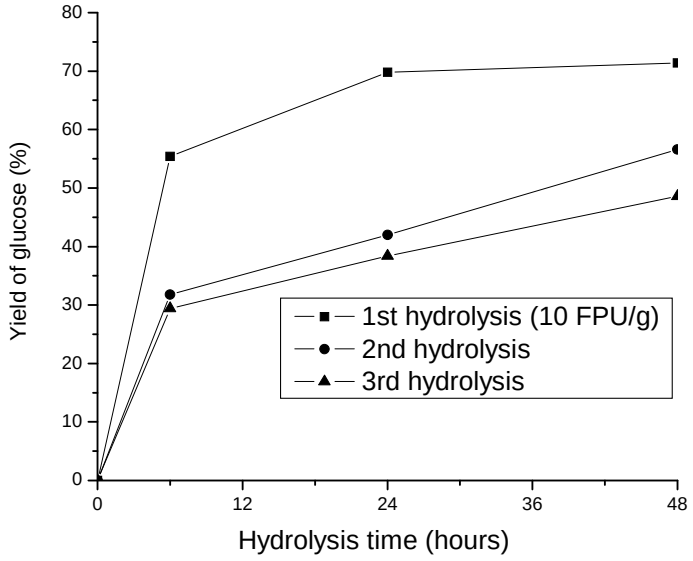
Enzyme cost has been recognized as a major factor affecting the overall economics of the lignocellulose-to-bioethanol process (Fig. 1). Despite significant progress toward reducing the cost of cellulase for biomass conversion, major enzyme producers are still unwilling to provide an attractive price for "commercial cellulase for bioconversion". It is apparent that a further reduction in enzyme dosage is required to improve the overall process economy.

Effective hydrolysis of cellulose to glucose requires an array of hydrolytic enzymes, including at least three groups of glycoside hydrolases: endoglucanase, cellobiohydrolase (exoglucanase), and β–glucosidase (Lynd *et. al.* 2002; Sun and Cheng 2002). Several enzyme recycling strategies have been evaluated in the past to maximize the reuse of different enzyme fractions (Ramos *et. al.* 1993; Tu *et. al.* 2006; Gregg *et. al*. 1998). Most endoglucanases and cellobiohydrolases contain cellulose binding domains (CBDs), whose function is to attach the enzymes onto the cellulosic substrates. A simple and practical recycling strategy for these enzymes is to recover and reuse solid residues after batch hydrolysis for the subsequent batch of hydrolysis.

Microfiltration (MF) and ultrafiltration (UF) type membranes are typically used to remove large particles or suspended solids from a liquid solution (Ho and Sirkar 1992). In this study, the potential of using membrane filtration to recover and reuse cellulase was determined. As shown in Fig. 6, the first batch (1st) hydrolysis was carried out at 10% substrate consistency using 10 FPU/g enzyme loading. After 48 h of hydrolysis, the hydrolysate was collected and filtered through a 10 kDa membrane in an ultrafiltration unit (500 mL capacity). The enzymes were retained in the concentrate while glucose was passed through the membrane into the permeate. The hydrolysate was thus concentrated five times and reused in the second batch of hydrolysis without adding fresh enzyme. The

recycling was repeated in the same manner to carry out the third batch of hydrolysis. As shown in Fig. 6A, the yield of glucose production from cellulose dropped from 71% to 57% and 49% after the second and third batch of hydrolysis, respectively. In another set of enzyme recycling experiments, 2 FPU/g of fresh enzymes was supplemented to the second and third batch of hydrolysis. As shown in Fig. 6B, the addition of 2 FPU/g of fresh enzyme was able to help maintain the glucose conversion rate.

A: Without further enzyme addition



B: With 2 FPU/g enzyme addition

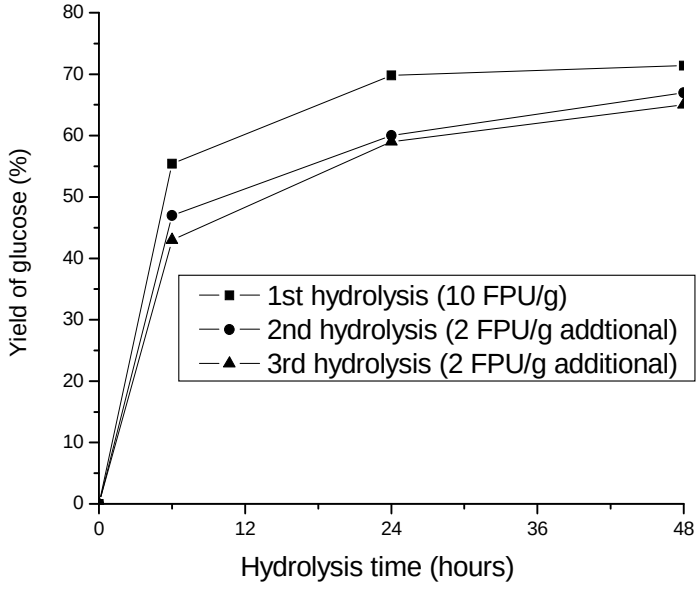


Fig. 6. Recovery and reuse cellulase in subsequent batch of knots hydrolysis

These laboratory results demonstrate that knot rejects are susceptible to cellulase hydrolysis, thereby providing an opportunity to increase ethanol production and bring in additional revenue to Mill A. The knots hydrolysate obtained from high-consistency hydrolysis can be directly used for fermentation or can be added to hardwood spent sulphite liquor to improve process stability. An enzyme loading of 10 FPU/g of substrate was found to be the optimum dosage. Reducing enzyme dosage to 2 FPU/g of knots can be achieved by recycling enzymes in the hydrolysate through membrane filtration.

Pilot Trial

In order to verify the results from the laboratory study, and to obtain more precise measurement of the potential sugar and ethanol production at an industrial scale, a pilot trial was undertaken to:

1) determine the size of the mixer and retention time required for liquefaction, and
2) determine the volume and concentration of glucose produced after enzymatic hydrolysis.

The pilot trials were carried out (Fig. 7) at two scales, 400 L and 6000 L. Besides addressing the above two main questions, the pilot trial also included experiments to determine: 1) a suitable stream for washing the knot rejects prior to enzyme hydrolysis; 2) the feasibility of enzyme recycle after batch hydrolysis.

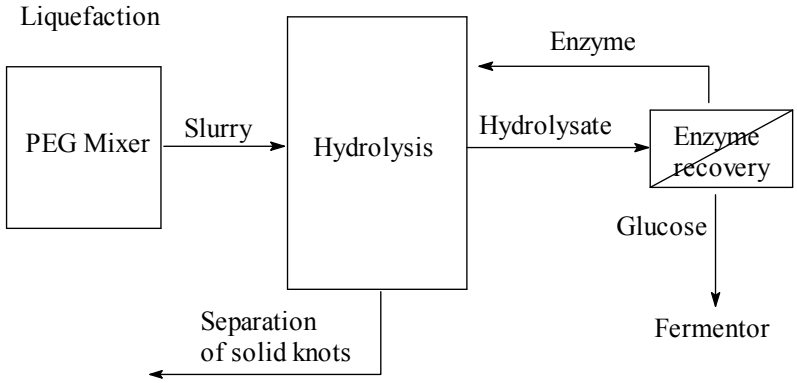


Fig. 7. Pilot trial scheme

The effect of different washing liquids on knot rejects hydrolysability

As mentioned earlier, washing knot rejects prior to hydrolysis removed a considerable amount of phenolic compounds that are known to inhibit cellulase activity. Introducing additional fresh water to wash knots is unlikely to be a practical approach in pulp mill operations. Therefore, an existing mill stream had to be identified for knot washing. Three mill streams were collected: AC condensate, stillage, and weak liquor. AC condensate is a stream collected from the spent sulfite liquor evaporators. Stillage is a residue stream collected after ethanol evaporation, and the weak liquor is the spent sulphite liquor prior to the fermentation after pH adjustment. Washing of knot rejects using these streams was carried out at 80°C with or without the addition of liquid ammonia. The hydrolysability was compared to knots without washing, knots washed by

each of the liquors, and knots washed with liquor in the presence of NH_3. As shown in Fig. 8, washing knots with AC condensate improved the glucose yield by 20% after 48 h. The addition of liquid ammonia led to a further 10% increase in hydrolysability. As mentioned earlier, the knot rejects contain a significant quantity of phenolic compounds, which have inhibitory effects on cellulase enzymes. Although water washing can remove a fraction of these compounds, a significant amount still remains. Liquid ammonia can react with phenolic compounds and alleviate their negative effect on cellulase activity (Tengborg *et. al.* 2001). Washing knots with stillage gave a similar improvement to the AC condensate (Fig. 9). However, the glucose conversion rate increased to almost 80% by adding ammonia to the stillage during washing. Ammonia supplementation was also applied on weak liquor and compared to water. In both cases, a 75% conversion rate was reached after 48 h of hydrolysis (Fig. 10). It should be mentioned that a temperature above 80°C is required to maximize the reaction rate between ammonia and phenolic compounds. At the mill, the temperature of weak liquor and stillage is around 30°C, while the temperature of AC condensate is above 90°C. Although there is a higher sugar yield obtained from weak liquor and stillage, washing knot rejects with AC condensate appears to be more practical.

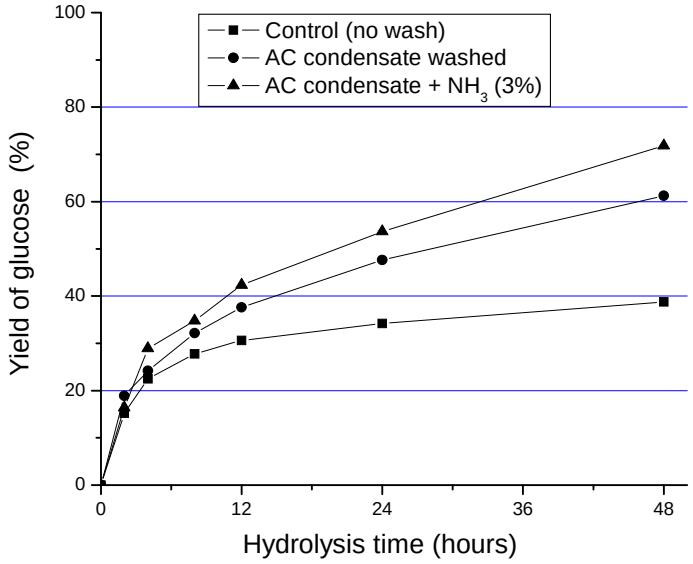


Fig. 8. The hydrolysability of knot rejects after washing with AC condensate (AC) and AC with liquid ammonia

Liquefaction of knot rejects at pilot scale

Liquefaction is a critical step occurring at the beginning of high-consistency hydrolysis to break down the fibre network in the presence of cellulase enzyme. Although the effectiveness of using a peg mixer for knot rejects liquefaction was demonstrated, it was difficult to find pilot-size equipment to provide enough material for the large-scale hydrolysis trial. A mortar mixer, which is typically used for mixing cement offers a similar mixing effect on knot rejects liquefaction as a peg mixer. A 170 L mortar mixer was used to process up to 30 kg (o.d) knot rejects per batch. Based on the enzyme dosage

tested at the laboratory scale, three enzyme dosages, 10, 7, and 3 FPU per gram of knots were selected. The liquefaction of knot rejects was carried out in the mortar mixer. At 10 FPU/g enzyme loading, it only took 20 minutes for the knots to liquefy (Fig. 11), while the same effect requires 30 minutes at 7 FPU/g enzyme loading. At 3 FPU/g loading, the liquefaction took over 1.5 h to occur and little sugar was released during the subsequent hydrolysis.

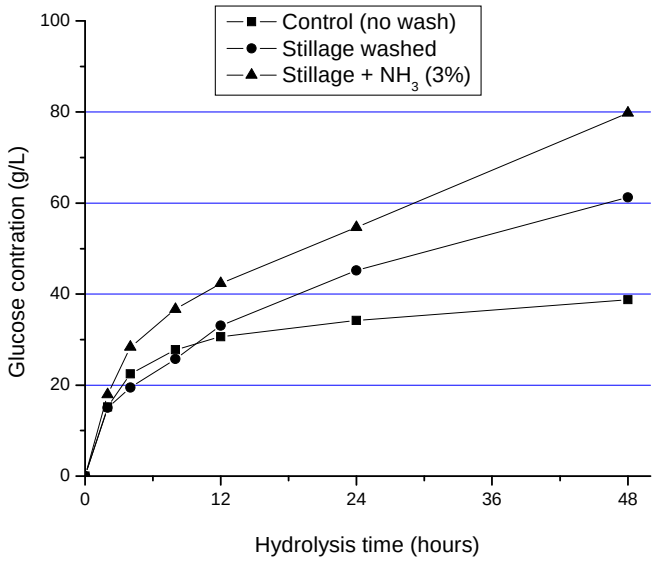


Fig. 9. Hydrolysability of knot rejects after washing with stillage (SL) and SL with liquid ammonia

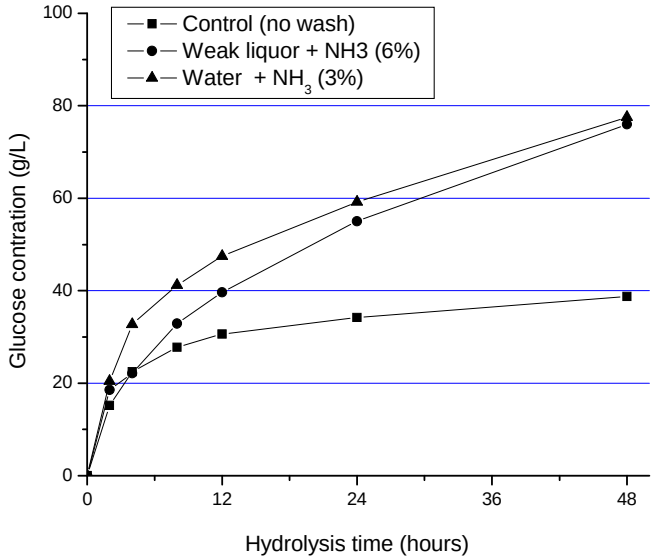


Fig. 10. Hydrolysability of knot rejects after washing with weak liquor (WL) and water with liquid ammonia

Hydrolysis of knot rejects at 20% consistency in a 400 L tank

As already shown, hydrolysing knot rejects at 20% consistency in a laboratory peg mixer resulted in a hydrolysate containing 110g/L of glucose after 48 h. It was also observed that there was a significant amount of solid residue remaining after 48 h. These solids mainly consist of unhydrolyzed cellulose and phenolic compounds. At 20% consistency, these residues occupy a significant part of the total volume, which makes it difficult to accurately measure the true volume of the hydrolysate. The pilot trial enabled us to accurately measure the liquid-to-solid ratio and thus to determine the true glucose production rate (concentration × liquid volume).

A: 0 minute 10 FPU

B: 20 minutes 10 FPU

C: 30 minutes 10 FPU

Fig. 11. Knots liquefaction in a mortar mixer

The hydrolysis of knot rejects at 20% consistency was carried out in a 400 L tank using a newly developed cellulase from a major enzyme supplier. The enzyme loading was 10 FPU per gram of knot rejects. The knot rejects (45 kg in a total volume of 225 L) were first liquefied in the mortar mixer and then transferred to a tank to continue the hydrolysis for 48 h. The production of glucose during the hydrolysis process was determined (Fig. 12). The glucose concentration reached 12.2% and 14.25% after 24 and

48 h of hydrolysis, respectively. The higher concentration of glucose compared to that obtained from laboratory experiments was probably due to better mixing at the larger scale. The liquid-to-solid ratio was also determined after 24 and 48 h of hydrolysis by centrifuging 100 g of the reaction mixture at 2000 g for 30 minutes. This centrifuge condition was selected to represent liquid and solid separation that can be obtained by overnight settling. It was found that the liquid-to-solid ratio was 0.48 vs. 0.56 (48 kg of liquid versus 56 g of wet solid) after 24 h of hydrolysis, and the ratio increased to 0.58 vs. 0.42 after 48 h of hydrolysis. Based on this, we calculated the glucose production from enzyme hydrolysis of knot rejects at 20% substrate consistency by the following equation:

Glucose concentration (g/L) × total volume (L) × percentage of liquid (%)
= glucose production (g)

Thus, the glucose production after 48 h of hydrolysis of 45 kg of knot rejects equates to 142.5 × 225 × 0.58 = 18.6 kg. Therefore, it can be calculated that one tonne of knot rejects will yield 413 kg of glucose at a concentration of 14%. The cellulose to glucose conversion yield was about 63%.

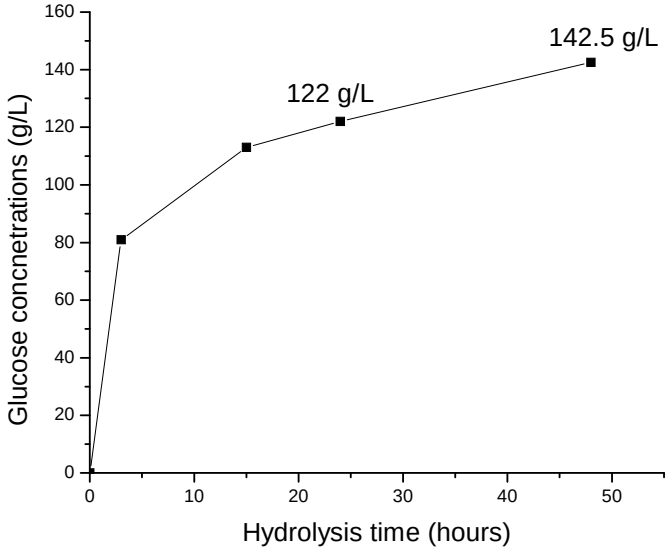


Fig. 12. Glucose concentration during cellulase hydrolysis of knot rejects at 20% consistency in 400 L tank (10 FPU/g enzyme loading)

Hydrolysis of knot rejects at medium consistencies

High-consistency hydrolysis of knot rejects can produce a highly concentrated glucose stream after 48 h; however, it does not maximize the cellulose-to-glucose conversion yield. The low yield is caused by the end-product inhibition resulting from the high glucose concentration in the hydrolysate (Xiao *et al.* 2004; Galbe and Zacchi 2002). The unhydrolyzed residues can be sent back for further hydrolysis to improve the overall conversion yield.

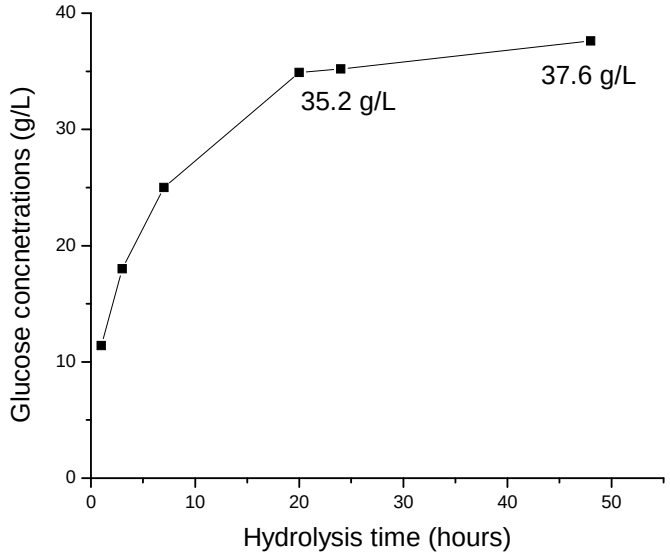


Fig. 13. Glucose concentration during enzyme hydrolysis of knot rejects at 7% consistency in 400 L tank (10 FPU/g enzyme loading)

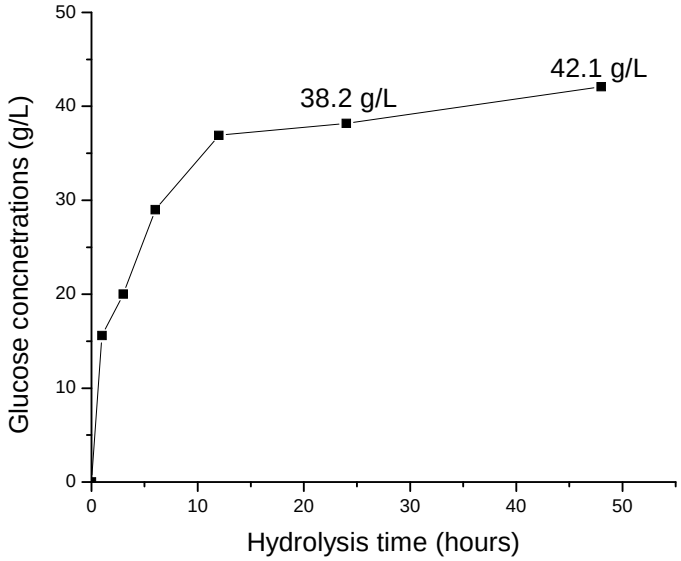


Fig. 14. Glucose concentration during enzyme hydrolysis of knot rejects at 8% consistency in 6000 L tank (10 FPU/g enzyme loading)

To determine if a higher cellulose-to-glucose conversion yield can be achieved at an industrial scale, we also carried out knot rejects hydrolysis at medium consistencies in 400 L and 6000 L tanks. The final consistency in the 400 L tank was 7%, which included 14.6 kg (o.d.) of knot rejects in a total volume of 208 L. At this consistency, liquefaction

is not required. As in high-consistency hydrolysis, an enzyme loading of 10 FPU/g of knot rejects was used. The glucose concentration during 48 h of hydrolysis is shown in Fig. 13. The hydrolysis reached a plateau after 24 h of hydrolysis with a glucose concentration of 35.7 g/L. Extending hydrolysis to 48 h only yielded an additional 2.4 g/L of glucose. Although low in sugar concentration, the cellulose-to-glucose yield reached about 83% after 48 h of hydrolysis. After the hydrolysis, 205 L of hydrolysate was collected and about 5.4 kg of solid residue was obtained.

Hydrolysis of knot rejects at medium consistency was repeated in a 6000 L tank, essentially scaling up the volume by approximately 10 times. The stock consisted of about 144 kg (o.d.) of knot rejects in a total volume of 1800 L. The consistency was approximately 8%. A similar glucose concentration profile was obtained as with the 400 L tank. The glucose concentrations were about 38.2 g/L and 42.1 g/L after 24 and 48 h of hydrolysis, respectively (Fig. 14). The similar hydrolysis results obtained from 400 L and 6000 L suggest that there is a linear relation between the two sizes. Based on these results, it can be calculated that hydrolysis of one tonne of knot rejects at medium consistency can yield 526 kg of glucose at 4% concentration after 48 h. There was only a small increase in sugar concentration from 24 to 48 h. Hydrolysis of knot rejects at 15% consistency was also attempted. It was apparent that the mixer in the 6000 L tank can effectively mix the knot rejects at this consistency; however, due to safety concerns, we were not able to continue the enzyme hydrolysis for 48 h. Enzyme hydrolysis of knot rejects removed the cellulose fibres attached to the knot core leaving a smooth residue (Fig. 15).

Fig. 15. The physical appearance of knot rejects before and after enzymatic hydrolysis

Enzyme recycling at the pilot scale

As mentioned earlier, a 10 FPU/g loading is the optimum enzyme dosage for batch hydrolysis of knot rejects. Recycling cellulase through membrane separation can reduce the enzyme loading to 2 FPU/g in subsequent batches of hydrolysis. One major concern related to membrane separation is fouling. During the hydrolysis, the substrates

are broken down into particles of various size ranges. These particles can deposit on the membrane surface and thus reduce the separation efficiency. Membranes are semi-permeable barriers used to filter particles, ions, molecules, or other components from a solvent. Commercial membranes are made from polymers, ceramics, and stainless steel (Ho and Sirkar 1992). Both are pressure-driven and depend on tangential flow across the filtering surface to keep the surface clear of deposition. The objective of the pilot trial was to address this concern by testing enzyme recovery using a pilot-scale tubular membrane system. The unit was equipped with 6 kDa MWCO membrane tubes with a total surface area of 0.9 m^2. One hundred litres of hydrolysate was collected from the 6000 L tank after 48 h of hydrolysis at 8% consistency. The filtrate was circulated in the membrane system for 3 h to separate the sugars from the enzymes.

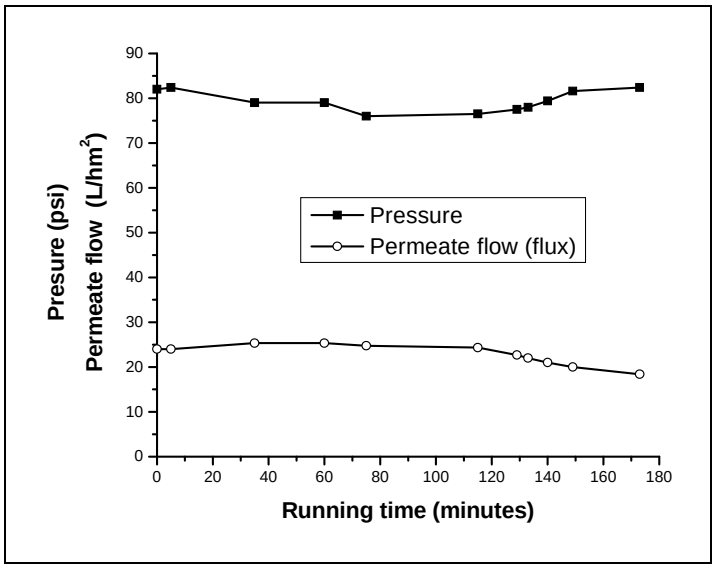


Fig.16. Pressure and flux rate during membrane filtration of knots hydrolysis for enzyme recycling.

Table 2. Sugar and Protein in the Hydrolysate, Permeate and Concentrate

	Hydrolysate	Permeate	Concentrate
Volume (L)	100	65	35
Glucose (g/L)	31.4	31.4	32.9
Protein content (g/L)	0.31	0.02	0.71

The permeate flow rate and system pressure were monitored during the filtration (Fig. 16). The filtration rate, or flux, is a measure of the volume passing through the membrane per unit area. The permeate flow was maintained at about 20 L per hour per m^2 during the filtration. There was little pressure fluctuation during the three hours of filtration. The initial pressure was 82 psi and varied between 76 psi to 82 psi during the three hours, suggesting that there was an increasing resistance to flow through the

membrane as the solution was concentrated. About 65 L of permeate was collected after three hours from the initial 100 L of filtrate. The glucose concentration and protein content in the permeate and the remaining concentrates were determined after 3 h (Table 2). It is apparent that membrane filtration is effective in retaining almost all the enzym, as indicated by the increase in protein concentration. As shown from the laboratory experiments, reusing the concentrate stream back to hydrolysis would significantly reduce the enzyme cost.

CONCLUSIONS

1. The results obtained from this study have clearly demonstrated that hydrolysis of knot rejects at high consistency can produce a highly concentrated sugar stream (over 14%). Hydrolysis of one tonne of knot rejects at 20% solids content can produce approximately 413 kg of glucose after 48 hours.
2. Lowering the substrate consistency for enzyme hydrolysis to 7 or 8% results in a higher glucose production, approximately 526 kg per tonne of knot rejects, but much lower sugar concentration, about 4%, after 48 hours of hydrolysis.
3. The high-consistency hydrolysis and enzyme recycling technologies developed here significantly reduce the capital and operational cost of hydrolysis. The optimum enzyme dosage for effective knot hydrolysis is 10 FPU per gram of knot rejects, and this dosage can be lowered to 2 FPU/g by incorporating membrane filtration for enzyme recycle.

ACKNOWLEDGMENTS

The financial support of Natural Resources Canada and FPInnovations – Paprican Member Companies are greatly appreciated. We would also like to thank Novozymes North America Inc. for providing the enzymes.

REFERENCES CITED

Ahameda, A., and Vermette, P. (2008). "Culture-based strategies to enhance cellulase enzyme production from *Trichoderma reesei* RUT-C30 in bioreactor culture conditions," *Biochem. Eng. J.* 40(3), 399-407.

Aden, A and Foust, T. (2009). "Technoeconomic analysis of the dilute sulfuric acid and enzymatic hydrolysis process for the conversion of corn stover to ethanol," Cellulose. 16(4), 535–545

Chand, P., Aruna, A., Maqsood, A. M., and Rao, L. V. (2005). "Novel mutation method for increased cellulase production," *J. Appl. Microbiol*. 98(2), 318-323.

Chen, S., and Wayman, M. (1993). "Use of sorbose to enhance cellobiase activity in a *Trichoderma reesei* cellulase system produced on wheat hydrolysate," *Biotechnol. Tech*. **7**(5), 345-350.

Galbe, M., and Zacch,i G. (2002). "A review of the production of ethanol from softwood," *Appl. Microbiol. Biotechnol*. 59(6), 618-628.

Gregg, D. J., Boussaid, A., and Saddler, J. N. (1998). "Techno-economic evaluations of a generic wood-to-ethanol process: Effect of increased cellulose yields and enzyme recycle," *Bioresource Technol*. 63(1), 7-12.

Helle, S. W., Petretta, R. A., and Duff, S. J. B. (2007). "Fortifying spent sulfite pulping liquor with hydrolyzed reject knots," *Enzyme Microb. Technol*. 41, 44-55.

Ho, W. S. W., and Sirkar, K. K. (eds.), (1992). *Membrane Handbook*, Van Nostrand Reinhold, New York.

Lynd, L. R., Weimer, P. J., van Zyl, W. H., and Pretorius, I. S. (2002). "Microbial cellulose utilization: Fundamentals and biotechnology," *Microbiol. Mol. Biol. R.*, 66(3), 506-577.

Mitchinson, C. (2004). "Improved cellulases for the biorefinery: A review of Genencor's progress in the DOE subcontract for cellulase cost reduction for bioethanol," Stanford GCEP Biomass Energy Workshop, Genencor International, Inc. (2004). http://gcep.stanford.edu/pdfs/energy_workshops_04_04/biomass_mitchinson.pdf

Ramos, L. P., Breuil, C., and Saddler, J. N. (1993). "The use of enzyme recycling and the influence of sugar accumulation on cellulose hydrolysis by *Trichoderma* cellulases," *Enzyme Microb. Tech*. 15(1), 19-25.

Sandra, T., and Cherry, J. (2007). "Process and challenges in enzyme development for biomass utilization," *Adv. Biochem. Eng./Biotechnol.* 108, 95-120.

Stenberg, K., Bollok, M., Reczey, K., Galbe, M., and Zacchi, G. (2000). "Effect of substrate and cellulase concentration on simultaneous saccharification and fermentation of steam-pretreated softwood for ethanol production," *Biotechnol. Bioeng*. 68(2), 204-210.

Sun, Y., and Cheng, J. Y. (2002). "Hydrolysis of lignocellulosic materials for ethanol production: A review," *Bioresource Technol*. 83(1), 1-11 (2002).

Tengborg, C., Galbe, M., and Zacchi, G. (2001). "Reduced inhibition of enzymatic hydrolysis of steam-pretreated softwood," *Enzyme Microb. Tech*. 28(9-10), 835-844.

Tu, M. B., Chandra, R. P., and Saddler, J. N. (2007). "Recycling cellulases during the hydrolysis of steam exploded and ethanol pretreated lodgepole pine," *Biotechnol. Progr*. 23, 1130-1137.

Wingren, A., Galbe, M., and Zacchi, G. (2003). "Techno-economic evaluation of producing ethanol from softwood: Comparison of SSF and SHF and identification of bottlenecks," *Biotechnol. Prog*. 19(4), 1109-1117.

Xiao, Z. Z., Zhang, X., Gregg, D. J., and Saddler, J. N., "Effects of sugar inhibition on cellulases and beta-glucosidase during enzymatic hydrolysis of softwood substrates," *Appl. Biochem. Biotechnol*. 113-116, 1115–1126 (2004).

Zhang, X., Qin, W. J., Paice, M. G., and Saddler, J. N. (2009). "High consistency enzymatic hydrolysis of hardwood substrates," *Bioresource Technol*. 100(23), 5890-5897.

Article submitted: Sept. 18, 2009; Peer review completed: Oct. 8, 2009; Revised version received, accepted, and published: Nov. 6, 2009.