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Enzymatic hydrolysis of paper mill primary sludge as a potential source of simple sugars for lignocellulosic biorefinery

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ABSTRACT

This study examined the enzymatic hydrolysis of lignocellulosic biomass materials extracted from the primary sludge of a pulp and paper mill. The main objective was to investigate residual primary sludge as low-cost feedstock for the production of ethanol and chemicals. Primary sludge was collected from a newspaper mill and treated with commercial cellulase. Two Statistical Designs of Experiment (DOE) were used to determine optimal reaction conditions. The sludge consistency, its pH, the use of acid or buffer to set the pH, the ionic strength, the need for biocide addition, and the enzyme dosage were the conditions analyzed to optimize the enzymatic hydrolysis. The first DOE determined the optimal pH control strategy. Sodium acetate buffer concentrations of 250 mM at pH 5 gave higher results compare to trials with sulphuric acid and acetic acid. Addition of biocide was proven to be ineffective under these conditions. The second DOE determined that the best conditions for hydrolysis yield are an enzyme concentration of 300 µL per gram of dried sludge and a 8.5% sludge consistency. Under optimal conditions, hydrolysis yield reached 58±3% with a sugar concentration of 68±4 mM mM.
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KEYWORDS

Lignocellulosic;
Biosolids;
Enzymatic hydrolysis;
Sugar recovery;
Biomass;
Bio-ethanol;
Biorefinery;
Primary sludge.

INTRODUCTION

Rapid industrialization has provoked a surging demand for primary energy, resulting in the depletion of fossil fuel and causing important environmental impacts. The production of ethanol and other chemicals from residual lignocellulosic materials could be a profitable solution. Using residual primary sludge from pulp and paper as raw material would be inexpensive and cost-effective, perhaps even costless if savings in sludge disposal fees are factored in. The main objective of this study was to investigate residual primary sludge as a low-cost feedstock for the production of ethanol and chemicals.

The pulp and paper industry generates approximately 4 to 5 millions of tons of sludge in the United States every year[1]. In 2007, pulp and paper mills in Quebec alone generated approximately 3 million metric tons of residual matter, 14.6% of which were primary sludge (PS)[2]. Sludge is disposed of in landfills or incinerated,

which represents significant costs and comes with important environmental impacts. Paper sludge contains solid waste residues comprising wood pulp fibres and fines (small wood pulp particles) that were not recovered in the paper, inorganic compounds, extractables, and additives used in the papermaking process. The characteristics of fine particles allow them to be converted into value-added products. The composition of the organic portion is similar to the pulp used in the fabrication process. Most of the mills in Quebec produce mechanical pulp used for the manufacture of newsprint and mechanical pulp printing specialties. The fines fraction therefore contains cellulose, hemicelluloses, and a good quantity of lignin. Furthermore, fines have a high specific surface area. Because of these features, fines can be more easily digested by cellulase type enzymes in comparison to the fibre fraction[3]. On the other hand, enzymatic hydrolysis of materials containing more lignin is thought to be less efficient. The presence of lignin interferes by forming a physical barrier that restricts enzyme access to the cellulose component. Lignin also adsorbs to and deactivates cellulose[4].

Cellulase is the enzyme responsible for the hydrolysis of cellulose and hemicellulose. Enzyme complexes generally consist of three components. Endo-β-glucanase (EG) attaches to regions of low crystallinity by generating free chain ends. Exo-β-glucanase degrades the free chain into cellobiose. Finally, β-glucosidase hydrolyzes cellobiose to produce glucose[5]. Cellulase activity is influenced by reaction conditions such as the size of the particles, substrate concentration, enzyme concentration, temperature, pH, and pH adjustment methods[6]. Bioconversion of pulp mill sludge has been studied; pH was adjusted with hydrochloric acid[7] or sulphuric acid[8]. Using a strong acid or a strong base is not as efficient in stabilizing pH variations when compared to trials with a buffer.

Kraft pulp mill sludge has been considered as a candidate for use in ethanol production processes[9]. Enzymatic hydrolysis presents a total reducing sugar (TRS) yield of 8-32% compared to the theoretical maximum yield. This yield is significantly inferior to kraft pulp from the same mill. The presence of calcium carbonate in the PS influences pH and cause variations that affect the enzyme and make it less efficient. In kraft pulping using alkaline conditions, lignin is almost completely hydrolyzed by cleavage reactions such as aryl ether linkages[10]. Paper recycling mill sludge can have TRS yields of 90%[11]. Finally, enzymatic hydrolysis of municipal sludge with 29.3% cellulose concentration had a TRS yield of 31.1% without pre-treatment and a 54.2% yield when pre-treated with acid or alkaline[6]. Yields were low despite small quantities of lignin. Other chemical substances or reaction conditions are assumed to interfere with cellulase.

In this analysis an attempt was made to determine the enzymatic hydrolysis of mechanical pulp sludge containing high concentrations of lignin. The use of a buffer compared to strong and weak acids was studied. Statistical Designs of Experiment (DOE) were used to optimise reaction conditions.

EXPERIMENTAL

Materials

Primary sludge samples were provided by a newsprint and mechanical pulp printing specialties mill located in the city of Trois-Rivieres, province of Quebec, Canada. The sludge came from the pulp mill primary effluent clarifier. Thermomechanical pulp (TMP) was sampled at the same mill. The kraft pulp used was market hardwood pulp produced at a mill located in the east of Canada.

A commercial enzyme complex for cellulosic biomass hydrolysis was used. The pH of this brown liquor ranges between 4.8 and 5.2. The complex contains multiple enzyme activities; mainly exoglucanase, endoglucanase, hemi-cellulase, and beta-glucosidase. The activity of the enzyme complex was expressed in carboxymethycellulose (CMC U) activity units. One CMC U unit of activity liberates 1 μmol of reducing sugars (expressed as glucose equivalents) in one minute under the specific assay conditions of 50°C and pH 4.8. Beta-glucosidase is reported in pNPG units. One pNPG unit denotes 1 μmol of Nitrophenol liberated from para-nitrophenyl-B-D-glucopyranoside in 10 minutes at 50°C and pH 4.8. As mentioned by the supplier: Endoglucanase activity is 2500 CMC U/g, Beta-glucosidase activity is 400 pNPG U/g. Based on information given by the supplier, the enzyme complex has the best operational stability in the following ranges: temperature: 50 - 65°C ; pH: 4.0 - 5.0.

Methods

Sludge properties were analysed using the following Technical Association of the Pulp and Paper Industry (TAPPI) standards:

Lignin: Acid-Insoluble Lignin in Wood and Pulp, Test Method T 222 om-06

Ash: Ash in Wood, Pulp, Paper and Paperboard: Combustion at 525 Degrees C, Test Method T 211 om-07

Extractives: Solvent Extractives of Wood and Pulp, Test Method T 204 cm-07

Total suspended solids: Measuring, Sampling, and Analyzing White Waters, Test Method T 656 cm-07

Total reducing sugars analysis was conducted using a colormetric method with 4-hydroybenzoylhydrazine (PAHBAH) according to the method described by Lever et al.[12]

Enzymatic hydrolysis was performed in a temperature controlled shaking water bath at 50ºC. Samples were prepared in 50 ml glass tubes according to the procedure described in the DOE and placed in the bath. Samples were taken at 0, 1, 2, 5, 8, 12, 24, 32, 48, 56, and 72 hour time intervals and used to calculate reaction kinetics.

RESULTS AND DISCUSSION

TABLE 1 presents the composition of the primary sludge (PS), thermomechanical pulp (TMP), and kraft pulp.

TABLE 1 : Composition of materials

	PS	TMP	Kraft
Lignin (%)	24.5	33.7	3.5
Ash (%)	22.9	Negligeable	0.1
Lignin (% organic materials)*	33.0	33.7	3.5
Extractibles (%)	2.9	1.3	0.29
TRS (%)**	49.7	65.0	95.5
Particles	Mostly TMP fines and Kaolin clay, few refined Kraft fines	TMP fibres and fines	Kraft long fibres

*** Lignin/(100-Ash)*100; ** By difference**

It should be noted that the PS used was mainly composed of fines comparable to those present in the TMP and the kraft pulp. Kaolin clay quantities were enriched with this process. Higher levels of extractives were found compared to the other samples. These additional extractives were mostly due to the effects of concentration and surface deposition during the pulping and papermaking process. The concentrations of lignin in the organic fraction in the PS and the TMP were equal, which demonstrates that these fines came from this process. This factor helped in evaluating the effect of particle size by comparing the results of PS and TMP. Also, the kraft pulp used made it possible to study material without lignin.

A three-level factorial DOE (DOE-01) first identified optimal conditions for PS hydrolysis. The pH, pH control strategy, and the need for addition of biocide (sodium triazide) were tested. Enzyme dosage, buffer concentration, TSS, and temperature were held stable. TABLE 2 presents variables and constants:

TABLE 2 : DOE-01 variables and constants

	-1	0	1
pH	4.0	4.5	5.0
Neutralization	Sulfuric acid	Acetate buffer	Acetic acid
Biocide (% to PS)	NA	0	0.02

Enzyme dosage = 115 µl/g dry PS; Acetate buffer concentration = 150 mM; Temperature = 50 °C; TSS = 6,5%

Figure 1 provides evidence that the results could be interpreted reliably, since the predictions were close to the observations.

Figure 2 shows that the best results were reached without addition of biocide, at pH 5.0 with an acetate buffer. Optima are represented by the red dotted lines. The desirability function was fitted to determine the optimum hydrolysis yield (HY). HY stands for maximal hydrolysis yield of TRS obtained, compared to the other TRS values as represented in TABLE 1. HY was not increased by addition of biocide. It would seem that no micro organisms consume the TRS product. Decreased HY would have indicated chemical interaction between the enzymes and biocide. HY was higher when an acetate buffer was used compared to the addition of weak or strong acid. The buffer kept the pH stable throughout the hydrolysis compared to using a weak or strong acid. These results indicated that pH stability was important. For this reason, a higher buffer concentration can be used to improve the results. The best HY yields were reached at pH 5.0. This parameter has to be optimized

at superior levels. The HY optimal value of 13% was insufficient. Other parameters were modified in order to increase HY. The TSS and enzyme dosage were optimized to obtain better HY

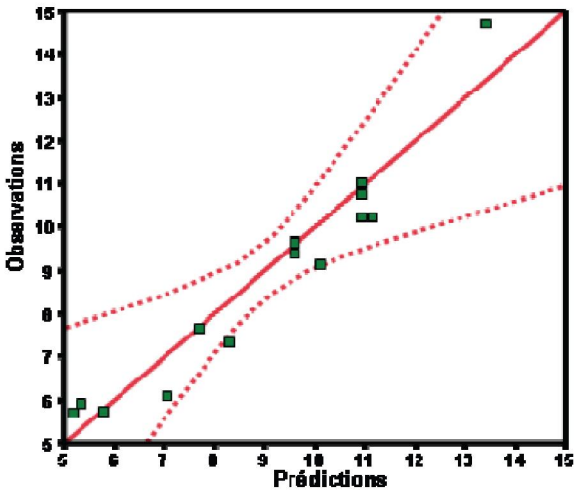

Figure 1 : Observations vs predictions plot of HY for DOE-01 after 72 hours

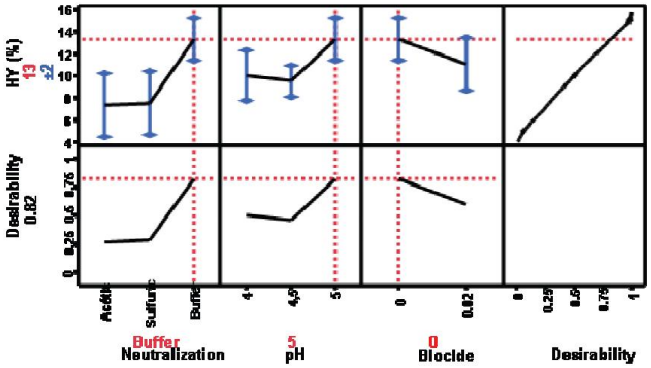

Figure 2 : DOE-01 HY optimum condition and results after 72 hours

Buffer concentration, pH, TSS, and enzyme dosage influence were analyzed in an attempt to obtain superior HY. A second central composite design (CCD) was used. TABLE 3 presents DOE-02 variables and constants.

TABLE 3 : DOE-02 variables and constants

	-2	-1	0	1	2
Buffer concentration (mM)	100	150	200	250	300
pH	4.50	4.75	5.00	5.25	5.50
TSS (%)	5	6.5	8	9.5	11
Enzyme dosage (µl/g. PS)	85.5	114.0	142.5	171.0	199.5

Temperature = 50 °C

Results in Figure 3 show that the effects of the different variables can be measured with sufficient precision.

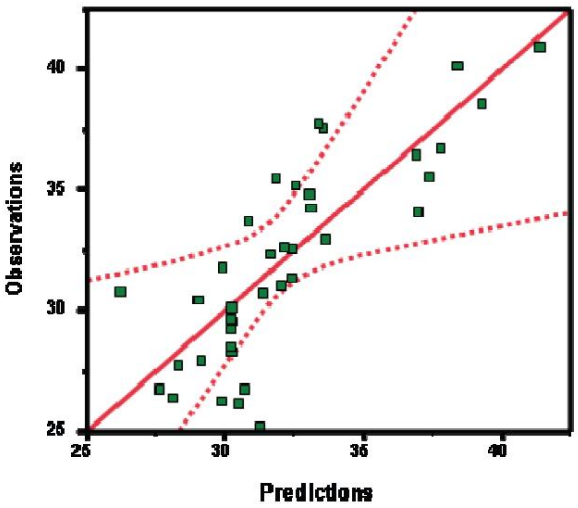

Figure 3 : Observations vs predictions plot of HY for DOE-02 after 72 hours

Confidence intervals (blue dash), in Figure 4 show that buffer concentration and pH were not significant. Buffer concentrations of 150 mM gave the best results in terms of TRS concentration and HY. Maximum yield was reached at pH 5.0, and it was not necessary to optimize any further. Augmenting TSS gave superior concentration of TRS in the solution because less water was added. On the other hand, HY decreased when TSS was augmented. More concentrated solutions affected enzyme active site mobility. Enzyme dosage was the only dominant variable. Figure 4 shows that HY increased as enzyme dosage increased.

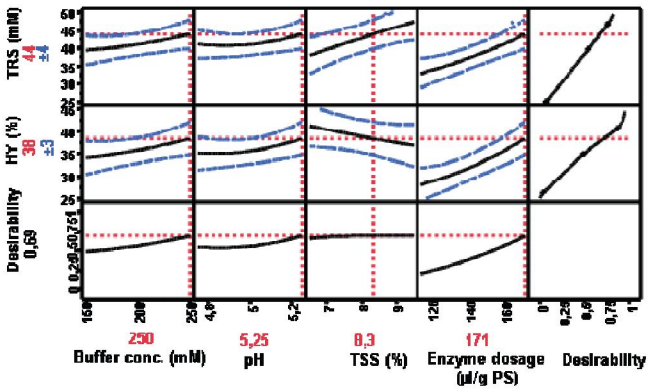

Figure 4 : DOE-02 optimum conditions and results for HY and TRS after 72 hours

Enzyme dosage was increased to 400 µL/g. Buffer concentration was kept at 200mM, pH at 5.0, and TSS at 8.5%. Maximal TRS concentrations of 68±4 mM were reached with an enzyme concentration of 300 µL per gram of dried sludge

and a 8.5% sludge consistency. Figure 5 shows enzyme dosage did not need to be increased above 300 µl/g because the HY of 59±3 reached was more than the one reached at higher dosage. The HY of 59±3 reached was more than the HY of 38±3% reached with DOE-02.

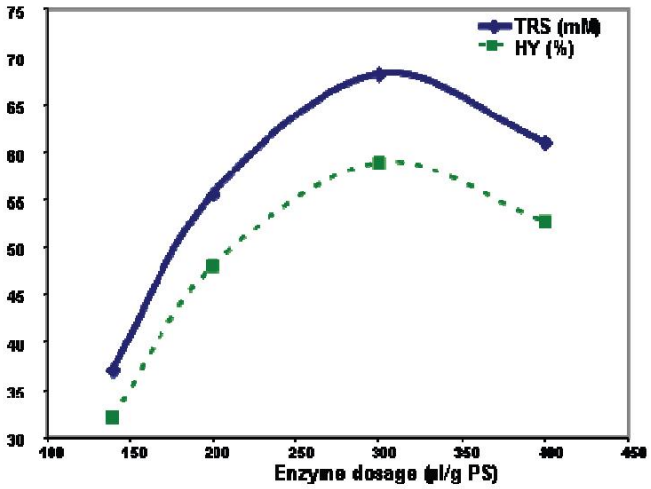

Figure 5 : Optimum conditions and results for HY and TRS at enzyme dosage interval after 72 hours

This result is superior to the 8-32% obtained with sludge for ethanol production from a kraft pulp mill[9]. In the above case enzyme efficiency was decreased by pH variations caused by calcium carbonate in the PS. Enzymatic hydrolysis of municipal sludge with a cellulose content of 29.3% had an HY of 31.1% without pre-treatment and 54.2% with an acid or alkaline pre-treatment[6]. Yield was low despite small quantities of lignin. Other chemical substances or reaction conditions are assumed to have interfered with cellulase. The primary sludge used in the present study had a higher level of TRS and was less heterogeneous.

Results presented in TABLE 4 demonstrate that at similar conditions the HY of mechanical pulp was lower. Hydrolysis was more difficult with long fibres than with fines. Meanwhile, kraft pulp reached a yield efficiency close to 100%, despite being mainly composed of long fibres. It follows that lack of lignin in kraft pulp is the determining factor.

TABLE 4 : PS, TMP, and kraft comparison

	Primary Sludge	TMP	Kraft
Hydrolysis Yield (%)	58	36	98

Enzyme dosage (µl/g. PS) = 300; TSS (%) = 8.5; pH=5.0; Buffer concentration (mM) = 200; Temperature (°C) = 50

Enzyme accessibility is therefore principally linked to the presence of lignin. Particle size is primordial but plays a less important role.

The next study will allow us to analyse enzymatic hydrolysis kinetic in order to better understand the different reaction rates and thus optimize time and temperature conditions. This study is foundational for understanding process and industrial applications.

CONCLUSIONS

1. Primary sludge (PS) is a potential source of reducing sugars for use in biofuel or chemical production processes. Using green technology such as enzymatic hydrolysis and industrial biomass residue means we can envision joining the green side.
2. Under optimal conditions and without pre-treatment the hydrolysis yield (HY) reached 58±3% with a total reducing sugar (TRS) concentration of 68±4 mM.
3. Superior results were obtained with PS containing a majority of TMP fine fibres, in comparison to TMP containing both fines and long fibres.
4. HY decreased in the presence of lignin in PS compared to results obtained with kraft pulp. Thus enzyme accessibility is directly related to the presence of lignin.
5. Higher results were reached with an acetate buffer and pH of 5.0 compared to trials with strong or weak acids at the same pH. A buffer concentration of 150 mM was enough to reach an optimal pH control strategy and gave more final TRS concentration and HY.
6. The addition of biocide had no significant impact under these conditions. It would seem that no microorganisms were consuming TRS products under the conditions of testing.

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