Conversion of Paper Mill Residuals to Fermentable Sugars

Malek Alkasrawi,[a,b,*] Zayed Al-Hamamre,[c] Mohammad Al-Shannag,[c] Md Joynal Abedin,[d] and Eric Singsaas [b]

The state of Wisconsin has existing pulp mill infrastructure capable of converting wood into biofuel and value-added products such as lumber, pulp, and paper. Each day, pulp and paper mills produce a waste material that is commonly referred to as sludge. Sludge is typically sent to a landfill or concentrated for burning to produce steam. The primary material present in pulp and paper mill sludge is fiber, which is mostly cellulose. This study showed how to convert pulp mill waste to fermentable sugars using commercially available enzymes. Preliminary economic analysis has shown that sludge can be converted into a fermentable sugar with chemicals costing less than $0.10 per pound of sugar produced.

Keywords: Enzymatic hydrolysis; Paper sludge; Hydrogen peroxide; Saccharification; Biofuel

Contact information: a: Department of Paper Science and Engineering, University of Wisconsin, Stevens Point, WI 5448 USA; b: Wisconsin Institute of Sustainable Technology, University of Wisconsin, Stevens Point, WI 5448 USA; c: Department of Chemical Engineering, The University of Jordan, 11942 Amman, Jordan; d: Advanced Fuels Center, Montana State University-Northern, Havre, MT 59501, USA;
** Corresponding author: Malek.Alkasrawi@uwsp.edu*

INTRODUCTION

Over 500 tons of paper mill sludge (PMS) are produced every day in the state of Wisconsin (Gurram *et al.* 2015). Sludge is a waste product that every paper mill generates and disposes of in one way or another. Sludge consists of a variety of components, and one of the largest is fiber from several points along the papermaking process (Gurram *et al.* 2015). Some facilities dry the sludge and pelletize it to burn for energy. Other facilities dry the sludge just enough to load into a truck and dispose of in a landfill or apply to fields as a fertilizer (Hubbe *et al.* 2010). Both of these strategies have their downfalls. Landfilling sludge that contains a significant amount of fibrous material is a poor choice from an economic perspective because much of the end product in papermaking is thrown away. Landfilling sludge is also another expense to the papermaking process. Burning sludge is undesirable because there is a low energy value in sludge and the energy gained is largely offset by the energy needed to dry and pelletize the material prior to being burned.

Paper mill sludge is a good source of cellulose for fermentable sugar production because of its pretreatment and location. Any source of woody biomass needs pretreatment to bring lignin content from 20 to 30 wt% to around 5 wt%. Sludge has a very low lignin content because the pretreatment stage has already been taken care of through kraft pulping (Soucy *et al.* 2014). The cost of the kraft pulping pretreatment is offset by the production of pulp, rendering the sludge essentially a free material that is produced to throw away. Another advantage is that sludge does not need to be harvested and shipped. It is already on-site for further processing.

Paper sludge is a residual solid material composed of short fibers, clays, fillers, and other contaminants produced by the pulp and paper industry. The major component of these fines rejects is cellulose. This represents an attractive feedstock for developing technologies based on the processing of cellulosic biomass featuring enzymatic hydrolysis. It is possible to fractionate the PMS for pure fiber production, which can enhance the enzyme digestibility (Kang *et al.* 2010; Chen *et al.* 2014a). Chen *et al.* (2014b) presented PMS as an economical competitive process for ethanol production depending on the fractionation process and the sludge type. PMS to ethanol may represent an interesting approach for the waste management, meanwhile generating additional high added value product and reducing the landfilling costs (Hubbe *et al.* 2010; Chen *et al.* 2014b). The objective of this work is to investigate the conversion of paper sludge from pulp mill waste to fermentable sugars using commercial enzymes. Acid hydrolysis was used to determine the total sugars available in the sludge. Four different commercial enzymes were used to hydrolyze treated and untreated sludge samples. In other words, this research was used to turn sludge from a waste material into a value-added product by coming up with an economical method to convert cellulose to soluble sugars (Fig. 1). To execute this process, a pretreatment stage using hydrogen peroxide and Hydrite's Hydritreat, a catalyst, was studied to improve enzyme efficiency. Commercial cellulase enzymes were used to convert sludge to soluble sugars by saccharification. The primary sugars that result from these experiments were glucose and xylose. Conditions were optimized to lower the cost for a commercial process.

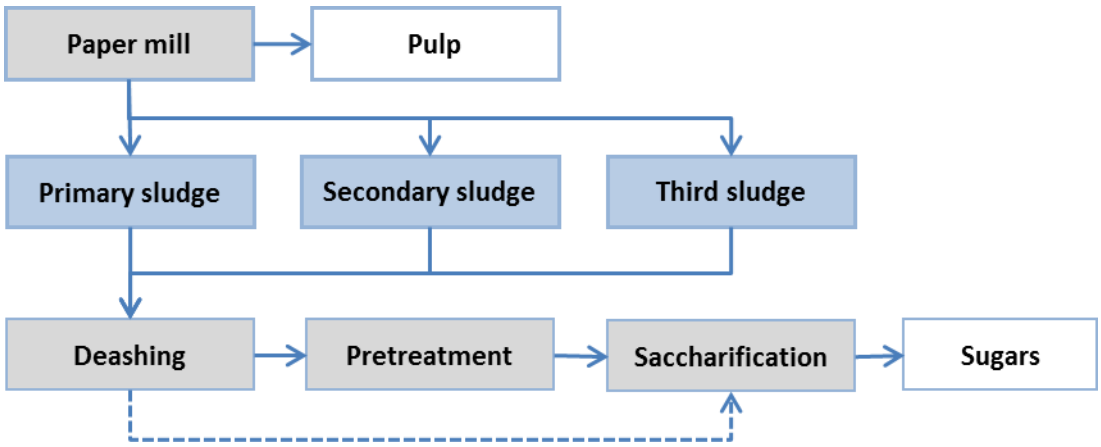

Fig. 1. Schematic diagram for paper mill sludge conversion to sugars. The alternative scenario, shown as a dashed line, represents saccharification without pretreatment.

EXPERIMENTAL

Sludge Description

The effluent treatment plant (ETP) of Thilmany's Kaukauna facility in Wisconsin, USA produces 10 to 40 tons of sludge every day. The paper mill generates three types of sludge during the digestion of red pine (softwood) for pulp production. The three types of sludge were collected and mixed with each other for further bioconversion to sugar. The first sludge, primary sludge, was collected at 50 °C and 4 wt% consistency from the sludge pit that contained the contents of the mill's main sewer. The second sludge, secondary sludge, was collected at 1 wt% solids in the secondary clarifier. The third sludge stream

was the combined sludge, which contained both primary and secondary sludge streams at 40 wt% solids (Fig. 1). The sludge combination was stored at 4 °C for further use.

Sludge Deashing (Fiber Regeneration)

The aim of this step was to obtain an ash-free fiber to facilitate the enzymatic hydrolysis to monomeric sugars with the highest yield. Mechanical pretreatments were employed to remove ash from the sludge using a Pulmac Masterscreen (Pul-mac International, Montpelier, VT, USA) according to the procedure described by Gurram *et al.* (2015). Ten grams of sludge sample was dried at 105 °C overnight and then dispersed in 500 mL of distilled water using a standard disintegrator (Labtech Instruments Inc, Laval, Canada) for 5 min at 11,500 rpm. The sludge suspension was added to a Masterscreen and run for about 1 h. Sludge from the mill was screened with a 0.008" screen to remove non-fibrous materials since this type of sludge does not contains fibers small than 0.008''. The fibrous material was stored at 4 °C for further use. The de-ashing method was efficient in removing more the 99% of the total ash.

Ash and Moisture Content

The sludge sample was dried overnight in an oven at 105 °C to calculate the moisture content, which is calculated from the differences in weights of the original and the oven dried sample. After moisture determination, the dried sludge was ashed in a muffle furnace at 900 °C for 3 h, and the remaining ash was weighed after cooling the sample in a desiccator.

Total Sugar and Lignin Determination

The sugar and lignin composition of the fiber was determined by implementing laboratory analytical procedures (LAP) developed by the National Renewable Energy Laboratory (NREL) (Sluiter *et al.* 2010). Initially, the fiber samples (de-ashed sludge) were dried at 105 °C overnight prior to acid addition. Each sample was then ground to a size that can pass through a 40-mesh screen, using a Wiley Mill (Thomas Scientific, Swedesboro, NJ, USA). A 3-mL aliquot of chilled 72% (w/v) sulfuric acid was added to 0.7 ± 0.1 g of substrate in a 250-mL beaker. The acid was mixed well with a glass stir rod and each beaker was placed in a 30 °C water bath for 1 h. After one hour, ultrapure water was used to dilute the samples to a final volume of 87 mL. The material was then autoclaved at 121 °C for 1 h in an autoclave (Tuttnauer/Brinkmann VWR International, Arlington Heights, IL) to hydrolyze all sugars from cellulose and hemicellulose. Klason lignin content was determined by filtering the sterilized solution through a crucible. Five sugar monomers were quantified with ion chromatography.

Pretreatment of Deashed Sludge

The deashing procedure is essential for removing the ash that interfere with enzymatic hydrolysis. Chen *et al.* (2014a,b) demonstrated that removal of 82 to 98% of the ash from fiber was able to increase the sugar yields from 39 to 69%. In the present work the fiber material (deashed sludge) was chemically treated in a Quantum Mark V Mixer (Quantum Technologies Inc., Lake Forest, CA, USA) at 20 wt% solids loading, at 35 °C for 1 h at a neutral pH. Four hundred grams (oven dry) fiber was loaded into the mixer, followed by an addition of 2.5% (w/w fiber) hydrogen peroxide (Sigma Aldrich, USA) and 2.5% (w/w fiber) Hydritreat HS DP (Hydrite Chemical Co., Brookfield, WI, USA). After

1 h, the pretreated fiber was washed with water in a Buchner funnel to remove chemical residues.

Saccharification

Four different cellulase enzymes were studied to determine how much glucose and other sugars could be liberated from the fiber. The main goal was to compare the efficiency of enzymatic hydrolysis on pretreated and non-pretreated fibers. An additional goal was find the optimal dosage of the enzyme that could be truly helpful in minimizing the additional cost of the enzymes. Pretreated and untreated fibers were hydrolyzed with commercially available cellulase enzymes: Accelerase 1500 (77 FPU/mL) and GC220 (116 FPU/mL) both obtained from DuPont Industrial Biosciences, San Jose, CA, USA. The Spezyme CP (Genecore, USA) with activity of 59 FPU/mL and CTEC2 (150 FPU/mL) were obtained from Novozymes, Denmark. The enzymatic activities were measured according to Adney and Baker (1996). All enzymes were dosed separately in the range of 5 to 90 µL/g to determine the optimal enzyme load that converted fiber to sugar most efficiently, during the first stage. Subsequently, fibers were subjected to an optimal load of enzymatic hydrolysis on untreated (control) and pretreated fiber (peroxide and Hydritreat). In a 50-mL shake flask, 1.0 g of substrate (5% (weight fiber/volume of liquid) solids loading) was suspended in 20 mL of 0.5 M citrate buffer. The pH was adjusted to 5.5 with sodium hydroxide. The reaction flasks were covered with parafilm and placed in an incubated orbital shaker at 50 °C for 48 h**.**

Sugar Analysis

All sugars obtained from compositional analysis and saccharification tests, including glucose, xylose, arabinose, galactose, and mannose, were quantified against a fucose internal standard. The polymeric sugar concentration was calculated from the concentration of the corresponding monomeric sugar, using an anhydro correction of 0.88 (or 132/150) for C-5 sugars (xylose and arabinose) and a correction of 0.90 (or 162/180) for C-6 sugars (glucose, galactose, and mannose). An ion chromatography system (Dionex ICS 3000, Thermo Scientific, Waltham, MA, USA) with an electrochemical detector with a gold electrode, two guard columns (an Amino Trap Bio1C and a Carbo Pac PA1 (Dionex)) and a 4 x 250 mm Carbopac PA1 column (Dionex) maintained at 25 °C was used. The eluents were 3 mM sodium hydroxide, 300 mM sodium hydroxide, and ultrapure water. The eluents were partly degassed using vacuum and were run through the column at a flow rate of 1 mL/min.

Economic Analysis

The purpose of economic analysis was to compare the chemicals treatment cost used versus not carrying out the chemical treatment; the overall process cost was not considered. The cost calculation of chemical addition was based on the chemical cost in U.S. $ per dry paper mill sludge. The cost was compared to a relative cost of the commercial enzyme per dry paper mill sludge. This approach provides a baseline comparison relative to the amount of sludge used. However, this cost is a relative and it assumes that the capital and operation expenditures are fixed. A detailed cost analysis of industrial PMS to ethanol based on engineering process simulation software has been presented by Chen *et al.* (2014b). The main Commercial sugar is sold for approximately $0.25/lb; raw sugars $0.14/lb; corn sugar $0.10/lb; and cellulosic sugar $0.09/lb (Ruth and Wooley 2000). These costs were used as a baseline to guide the process costs, taking into

consideration only the chemical costs of the process. The single largest cost in the process is the enzyme, so that was the primary cost in the calculations (Nguyen *et al.* 2015). Chemical costs were obtained from local vendors, but this price is subject to further reduction if purchased for larger quantities. According to various studies, the enzyme cost is inconsistent, and it varies from $0.10 to 1.5/gal ethanol (Klein-Marcuschamer *et al.* 2012). In the present study, the enzyme cost was related to the cost as presented by Nguyen *et al.* (2015) and Klein-Marcuschamer *et al.* (2012).

RESULTS AND DISCUSSION

Table 1 shows the sugar content of the three different sludge samples that were analyzed. Generally, paper mill sludges are high in glucan. For the primary sludge, the glucan content was detected at 49.2 wt%, while in the secondary sludge it was 7.5 wt%. This is because the secondary sludge stream contained the top layer of the sludge pit, in which the primary sludge had settled down. On the other hand, the lignin content in the secondary sludge was higher in comparison to that of the primary sludge due to variation in the conditions of digestion and the presence of the sludge pit in the secondary sludge. However, the cellulose and lignin variations between the two types of sludge could be attributed to the nature of process configuration at paper mill and the way in disposing the sludge. The combined sludge showed less sugar in comparison to the primary sludge and, at the same time, a lower lignin fraction compared to the secondary sludge.

Table 1. Sugar and Lignin Mass Fractions for Each Sludge Sample from Acid Hydrolysis

Sludge sample	Mass composition (% w/w)					
	Glucan	Araban	Galactan	Xylan	Mannan	Lignin
Primary	49.2	0.2	0.1	6.1	1.2	43.0
Secondary	7.5	0.0	0.2	0.4	0.0	74.0
Combined	34.5	0.2	0.2	4.7	1.2	41.6

The optimal dosage of all enzymes was found to be 90 µL/g of fiber (data not shown here). A very low sugar yield was obtained with all enzyme types from non-treated fibers. The chemical pretreatment of fiber before saccharification appeared to have a positive effect on all of the enzyme activities. Figure 2 shows marked improvement of the sugar yield with chemical pretreated fiber in contrast to that of the non-pretreated fiber. This is most likely because chemicals such as H_2O_2 and Hydritreat have greatly reduced the degree of polymerization of fiber. Hydritreat is a catalyst composed of a polydentate ligand complexed with an iron metal ion. The catalyst can be iron methylglycine diacetate or iron-2,3,4,5,6 pentahydroxyhexanoate (Baures *et al.* 2008). Iron chelates with low redox potentials such as iron-EDTA (Schwarzenbach and Heller 1951; Burkitt and Gilbert 1990) and iron- DTPA (Bottari and Anderegg 1967) are generally associated with rapid decomposition of H_2O_2 and high oxidative product yield (·OH), whereas iron acetate (Friedheim and Michaelis 1931) iron hexanoate, and benzoate (Schwarzenbach and Heller 1951) have high redox potentials, which are characterized by slow, incomplete H_2O_2 decomposition and a low yield of oxidative product from substrate (Winston *et al.* 1984). Even though the iron chelates used in the present experiment as iron catalysts, such as iron acetate and iron hexanoate, have high redox potentials, a significant amount of hydroxyl

free radicals can be formed by the interaction of H_2O_2 with a catalyst comprising a ligand complexed with a metal ion such as iron methyl glycine diacetate or iron 2,3,4,5,6 pentahydroxyhexanoate (Baures *et al.* 2008).

Hydrogen peroxide and Hydritreat pretreatment depolymerized fiber into polysaccharides with lower molecular weights and weakened the fibers by reacting substrates with hydroxyl radicals, which opened up more sites for enzymatic reactions. The hydroxyl radicals are well known for being highly reactive oxidants capable of depolymerizing cellulose (Hammel *et al.* 2002). Our present finding agrees with several research studies (Shafizadeh and Bradbury 1979; Perez *et al.* 2002), but very little has been applied to fibers recovered from paper mill sludge. Hydroxyl radicals are extremely reactive species that attack both lignin and carbohydrates, which are broken down for more efficient saccharification (Perez *et al.* 2002). This reaction would greatly lower the costs of the process because of a substantial increase in the final yield of sugars (over 50 wt% for the CTEC2), which would include both hexoses (C-6) and pentoses (C-5). Even though the percentage of hexose is higher than pentose (Table 1), both types of sugars could potentially be used for ethanol production utilizing genetically modified yeast. Alternatively, the joint sugar steam could be utilized for butane production since the *Clostridium* strain can utilize both C-6 and C-5 efficiently. The efficiency of glucose conversion was increased by 90.88% due to chemical treatment. Hyditreat, so far, has never been tested before for pretreating cellulosic fiber as an enhancer for enzymatic hydrolysis. Hyditreat is a relatively cheap chemical; it has been used for treating wastewater from paper mills.

Among the enzymes, the highest sugar yield with pretreated fiber was obtained with CTEC2, as shown in Fig. 2. Interestingly, GC220 gave better sugar yield compared to CTEC2 on non-pretreated fiber. This may be attributed to some product feedback inhibition (Eriksson *et al.* 2002). Another possible explanation is that the enzymes with various activities perform differently with different feedstocks (Jørgensen *et al.* 2007). Chandra *et al.* (2007) and Zheng *et al.* (2014) reported that various types of cellulase with high filter paper unit (FPU) activity exhibited variations in sugar yield with different types of fibers.

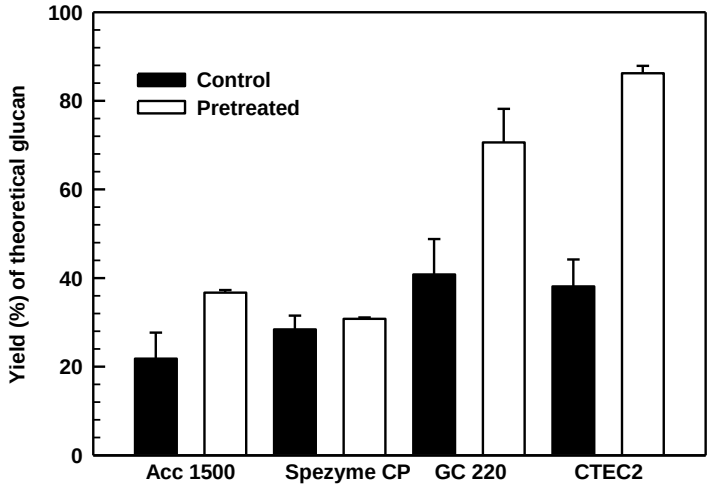

Fig. 2. Total glucan yield of control (untreated) and chemically pretreated fibers with various enzymes

Table 2 shows the details of the cost analysis. All of the cost figures refer to the cost to treat one ton of dry sludge. Replicates with sludge samples, taken at different days, show a chemical cost to produce sugars (C-6 and C-5) ranging from $167 to $231 per ton. The data in Table 2 show that it costs $167 for chemicals and enzymes to produce one ton of sugar using the pretreatment method. This is an approximate cost for producing sugar on a pilot or commercial scale with respect to ethanol cost obtained by Gnansounou and Dauriat (2010). Energy costs of the process were not explored thoroughly, but it can be said that using sludge for a value added by-product of the pulp and paper mill is remarkably more energy efficient than hauling the material away for land-filling or spending energy to make steam (Kang *et al.* 2010). However, there will be another challenging arise while scale up this process is the generation of large wastewater streams due to de-ashing step process.

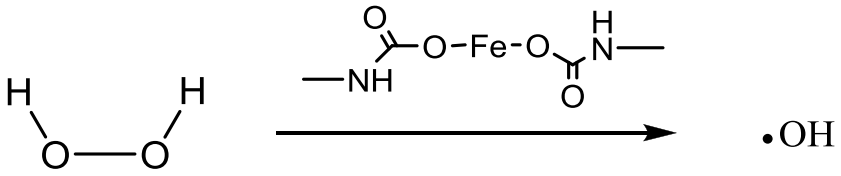

Fig. 3. Reaction of Hydritreat catalyst with hydrogen peroxide to form hydroxyl radicals

Table 2. Economic Analysis of Pretreatment with CTEC2 Enzyme

Property	Control	Pretreated
408 theoretical total sugars/ ton dry sludge	179.5	367.2
Cost of hydrogen peroxide ($/ton dry sludge)	0.00	10
Cost of Hydritreat HS DP catalyst ($/ton dry sludge)	0.00	10
Cost of Novozymes (CTEC2 enzyme) ($/ton dry sludge)	41.55	41.55
Total chemical cost ($/ton dry sludge)	41.55	61.55
Total chemical ($/ton sugar liberated)	231	167
Assuming 1 dry ton PMS contains 0.408 ton total sugars. **Control**: enzymatic hydrolysis on non chemical pretreated PMS. **Pretreated**: enzymatic hydrolysis on chemical pretreated PMS		

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CONCLUSIONS

1. Cellulase enzymes were used effectively to convert cellulose present in sludge from a softwood kraft pulp mill to glucose and other sugars with the help of chemical pretreatment. CTEC2 enzyme proved to be the most economically viable for this stage.
2. Chemical pretreatment depolymerized fiber by reacting substrates with hydroxyl radicals, which opened up more sites for enzymatic reactions.
3. Converting sludge to sugar is a process that has a lot of economic potential. Cellulase enzymes can be used to produce sugar at a cost competitive with commercial sugar.
4. Pulp and paper mills could greatly benefit by adding this process to their facility. Not only would the mill reduce costs by cutting their sludge transportation costs in half, but they would also be adding another revenue stream to their business.

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