



Study on Optimal Conditions for Reducing Sugars Production from Recycled Paper Sludge by Diluted Acid and Enzymes

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Abstract

Recycled paper sludge (RPS) is basically made up of secondary poor-quality non-recyclable paper fibres (fibres too short to be retained on fibre screens and paper machines). The high lignocellulosic content of this sludge material offers therefore an opportunity as feedstock of bio-products. The effects of various operating variables including pretreatment temperature, pretreatment time, the concentration of diluted acid and the solid-to-liquid ratio and the enzymatic digestibility were optimized using response surface methodology. Recycled paper sludge was dried and milled into small pieces by blender, ultracentrifugal mill and disc mill. Paper sludge was pretreated using aqueous-diluted acid solution at high temperatures to enable production of the maximum amount of fermentable sugars for enzymatic hydrolysis. The results showed that 1.5% (w/v) recycled paper sludge was pretreated by diluted sulfuric acid at 135°C under pressure 15 lb/in² for 30 min producing highest yield of reducing sugars while 0.1 M hydrochloric acid and 0.25 M acetic acid pretreatment produced lower yields of reducing sugars under the same conditions. The optimal reaction conditions, which resulted in an pretreated disc milled-paper sludge with 0.1 M sulfuric acid and enzymatic digestibility of 66.67 %, were found to be at pH 5.5, 55 °C, 24 h. The effects of different commercial cellulases and the additional effect of a non-cellulolytic enzyme, xylanase, were also evaluated.

Keywords: paper sludge, reducing sugars, pretreatment

1. Introduction

Solid wastes generated from industrial sources are heterogeneous in composition, ranging from inert inorganic (such as produced in mining and collieries) to organic (in industries producing basic consumer products) and may include even hazardous constituents

(as in pesticide industry). Sludge is the largest by-product of the pulp and paper industry and disposal of sludge is a major solid waste problem for the industry (1, 2). It was predicted that a global shift in paper and paperboard production would result in the Asia-Pacific region emerging as a major producer of papermill sludge. Global production of papermill sludge was predicted to

rise over the next 50 years by between 48 and 86% over current levels (3). The nature of sludge generated from paper industries is mainly depends on the raw materials used in different unit processes. Sludge generated from the industrial sources contains a large number of ingredients, some of which are toxic. Solid waste is generated from the both large and small categories of paper mills. Solid waste from paper industries is generated usually in various stages of paper production viz., (the raw material handling and preparation sections as sludge from the effluent treatment plants, causticizing section in the chemical recovery unit in the form of lime mud).

The bioconversion of crops and residues to fuels and chemicals is receiving increased interest due to the perceived need for the reduction of consumption and importation of petroleum fuels. These wastes may be hydrolyzed by acids or enzymes to lower molecular weight carbohydrates and finally to monomeric sugars. Most of the effort has been aimed at producing ethanol by hydrolysis of lignocellulosic materials and subsequent fermentation of sugars in the hydrolyzate. Recycled paper sludge (RPS) is the main by-product from processing paper of recycled paper.

In Thailand, approximately 70 ton per day of paper sludge is produced (4). RPS is the solid waste stream of the papermaking industry containing the short cellulose fibers, which leave the process. Usually this stream is deposited off, which has a significant cost-increasing factor on the paper production. Another option for utilizing the organic content of this waste stream is heat and electricity generation by direct combustion. However, the high water and inorganic matter content, which can be as high as 30 wt.% dry matter, would result in substantial energy loss. Producing a value added product from the cellulose present in the paper sludge, could provide an economically more attractive

option. Due to the high and rather accessible cellulose content (50–60%) of paper sludge, it could be a potential feedstock for a value added product (5).

The aim of the study was to obtain soluble reducing sugars by diluted acid and enzymatic hydrolysis of RPS and to determine the optimal conditions of each treatment. The composition of sugars released allowed a comparison of efficiency of different diluted acid and hydrolytic enzymes.

2. Materials and Methods

2.1 Substrate

RPS from the factory of paper production was washed by tap water and dried at 90 °C in hot-air oven for 3 days. The dry paper sludge was milled into small pieces by blender, ultracentrifugal mill and disc mill.

2.2 Dilute acid pretreatment of RPS

The following conditions were used in the pretreatment of RPS by dilute acid. The concentration of RPS was 1.5% (w/v) in 0.01-0.25 M of sulfuric acid, hydrochloric acid and acetic acid. The temperature was varied from 135°C and reaction time was 30 min

2.3 Enzymatic hydrolysis of RPS

RPS at 1.5% in 0.02 M citrate-phosphate buffer was added with 10 mU of cellulase. The enzymatic reaction was studied to determine the optimal conditions by varying temperature between 40-70°C, pH between 3-7 and incubation time between 3-48 hr. 1.5% RPS in 0.02M citrate-phosphate buffer was added with 10 mU of xylanase. The enzymatic reaction was studied to determine the optimal condition by varying temperature between 40-70°C, pH between 3-7 and incubation time between 3-15 hr. RPS at 1.5% in 0.02 M citrate-phosphate buffer was added with 10 mU of pectinase. The enzymatic reaction was studied to determine the optimal condition by varying temperature between

40-70°C, pH between 3-7 and incubation time between 1-5 hr. The enzymes in this study were purchased from Sigma-Aldrich, Inc, USA and Acellulase (cellulolytic enzyme) was purchased from Genecor, Inc, Germany. The hydrolysates were centrifuged to remove any suspended or unhydrolysed materials. Each experiment was done triplicate. The reducing sugars present in the hydrolysates were measured by Nelson-Somogyi method (6).

2.4 Enzyme unit (U) definition

For cellulase, one unit will liberate 1 micromole of glucose from cellulose in one hr at pH 5.0 at 37°C (2 hr incubation time). For xylanase, one unit will liberate 1 micromole of reducing sugar measured as xylose equivalents from xylan per min at pH 4.5 at 30°C. For pectinase, one unit will liberate 1 micromole of galacturonic acid from polygalacturonic acid per

min at pH 4.0 at 25°C. Reducing sugars yield (%) was represented the amount of g of reducing sugars per 100 g of RPS. All determinations were replicated three times to estimate mean values and standard deviations.

3. Results and Discussion

RPS at 1.5% in diluted acids were pretreated under varying conditions, then % reducing sugars yield was determined (Figure 1). The reducing sugar yields were at 32.6, 39.5 and 7.2% from pretreatment at 135°C for 30 min with 0.1 M sulfuric acid, 0.05 M hydrochloric acid and 0.25 M acetic acid, respectively. The dilute sulfuric acid produced the highest yield of reducing sugars. Therefore, the diluted sulfuric acid seemed to be suitable for RPS pretreatment.

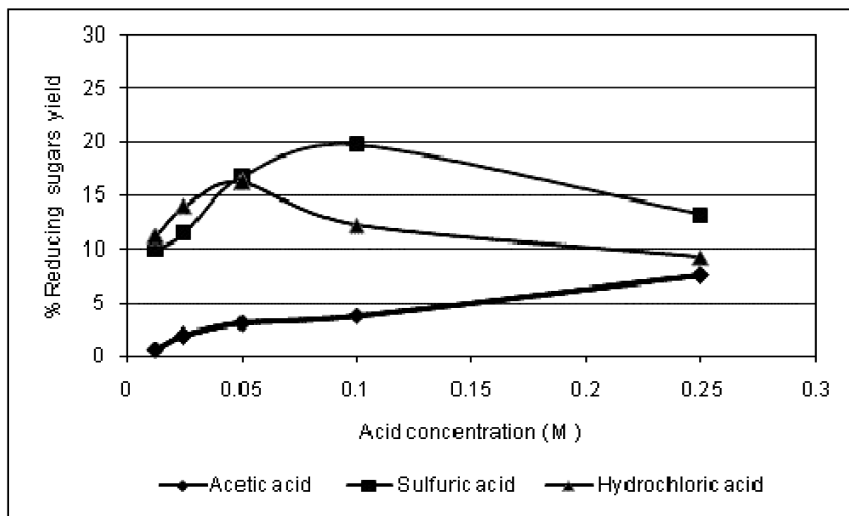


Figure 1. Percentage of reducing sugars yield from 1.5% disc mill RPS pretreated by various concentration of diluted acid at 135 °C for 30 min.

After 0.1 M sulfuric acid pretreatment, the optimum of the pretreated RPS was investigated using different hydrolytic enzymes as shown in Figures 2 to 5. The optimum conditions for cellulase (Sigma) reaction was found to be 24 hr incubation at pH 5.0, 40-70 °C,

which produced maximum yield reducing sugars of 52.4% at 50 °C (Figure 2) while the optimum conditions for Acellulase reaction was found to be 24 hr incubation at pH 5.0, 40-70°C, which produced maximum yield reducing sugars of 67.7% at 50 °C (Figure 3). The

optimum condition for xylanase reaction was found to be 9 hr incubation at pH 4.0, 50°C, which produced 4.6% yield of reducing sugars (Figure 4). The optimum conditions for pectinase reaction was found to be 3 hr incubation at pH 4.0, 40°C which produced 7.8% yield of reducing sugars (Figure 5).

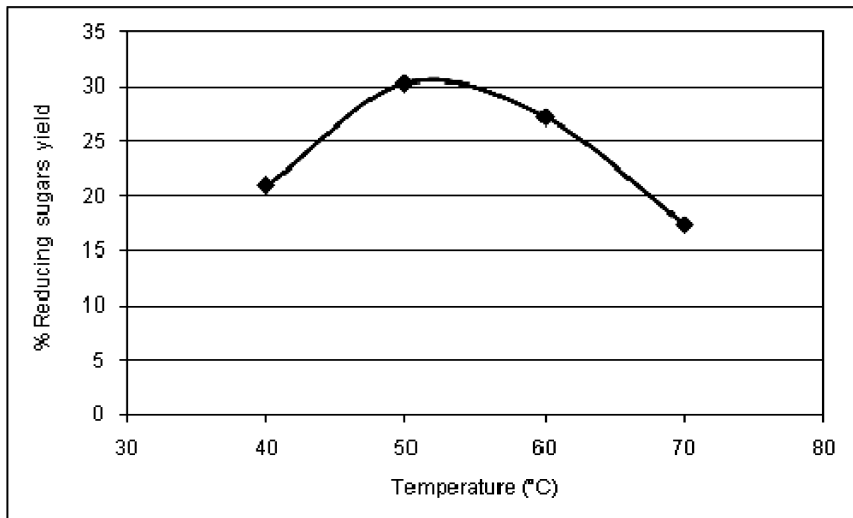


Figure 2. Percentage of reducing sugars yield from 1.5% pretreated disc mill and diluted sulfuric acid RPS incubated with cellulase (Sigma) reaction at pH 5.0, 40–70°C for 24 hr.

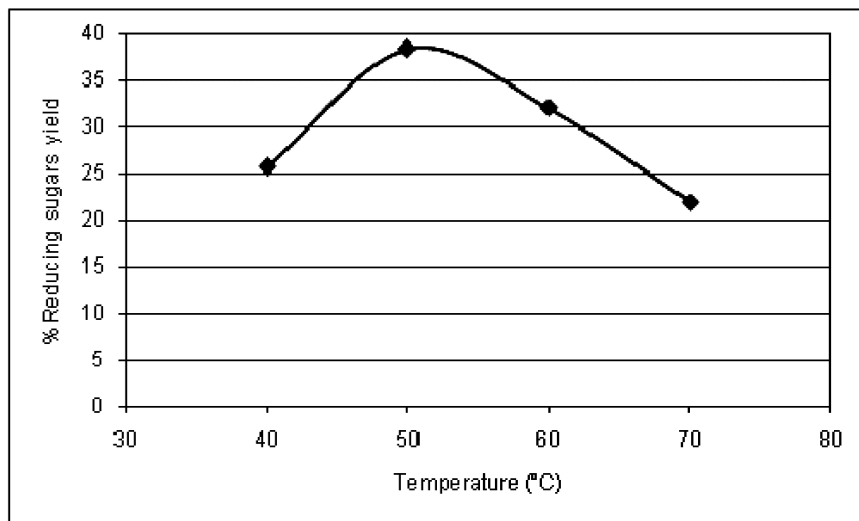


Figure 3. Percentage of reducing sugars yield from 1.5% pretreated disc mill and diluted sulfuric acid RPS incubated with AcCellulase (Genecor) reaction at pH 5.0, 40–70°C for 24 hr.

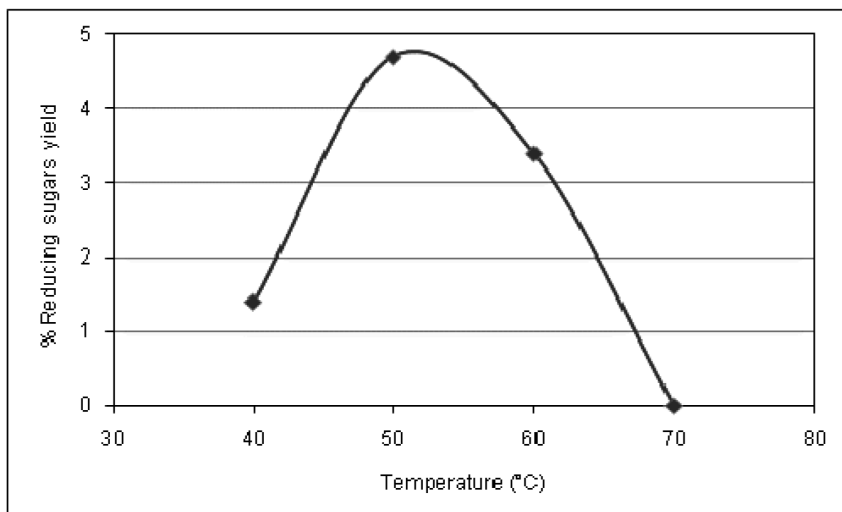


Figure 4. Percentage of reducing sugars yield from 1.5% pretreated disc mill and diluted sulfuric acid RPS incubated with xylanase reaction was at pH 4.0, 40–70 °C for 9 hr.

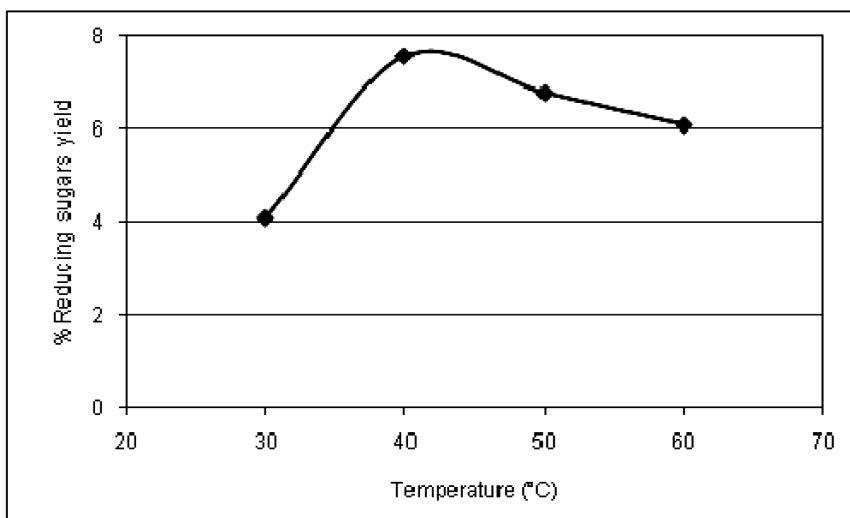


Figure 5. Percentage of reducing sugars yield from 1.5% pretreated disc mill and diluted sulfuric acid RPS incubated with pectinase at pH 4.0, 30 – 60 °C for 3 hr.

Table 1 shows the production of reducing sugars and glucose from un-milled waste paper sludge pretreated either with distilled water pH 5.5 or 0.1 M sulfuric acid at 135°C under pressure of 15 lb/in² for 30 min and the yield of reducing sugars and glucose from the pretreated RPS after enzymatic hydrolysis for 48 h at 50°C. The highest yield of reducing sugars was 5.76 g/L obtaining from diluted acid pretreatment after enzymatic hydrolysis. So, the dilute-acid pretreatment is more efficient in the improving enzymatic hydrolysis than just using steam. The main objective of the biomass pretreatment is to make the cellulose more accessible to enzyme.

Table 1. Yield of sugar from enzymatic hydrolysis of blender RPS.

Pretreatment	Reducing sugars (g/L)
distilled water	0.77
0.1 M sulfuric acid ^a	1.83
0.1 M sulfuric acid	5.76

The dried RPS of this substrate composed of 60.09 % cellulose, 11.18 % hemicelluloses, 9.74 % lignin. Generally, biomass cannot be saccharified by enzymes without a pretreatment because the lignin in plant cell walls forms a barrier against enzyme attack (7). An ideal pretreatment would reduce the lignin content and the crystallization of cellulose while increasing the surface area for enzymatic hydrolysis. The milling pretreatment either with blender rotating at 1,000 rpm

or Ultra-centrifugal mill rotating at 6,000 rpm or Disc mill rotating at 12,000 rpm was investigated for enable production of the fermentable sugars from enzymatic hydrolysis. From the results, the highest amounts of reducing sugars was obtained from disc mill PRS with the concentration of 10.08 g/L (67.7% reducing sugars yield). So, this pretreatment method suggested the effectiveness on hemicelluloses and lignin components and resulted in efficient hydrolysis.

Table 2. Yield of sugar from enzymatic hydrolysis of pretreated RPS.

Pretreatment	Reducing sugars (g/L)
Blender	5.76
Ultra-centrifugal mill	6.04
Disc mill	10.08

When the recycled paper is used for the process of production of paper, the residue is RPS. Hydrolysis of the cellulosic fraction results in hydrolysate rich in hexoses and pentoses (8). From this study, the best enzymatic reaction was found to be Acellulase (cellulolytic enzyme from Genecor company), producing the highest production of reducing sugars. This might be due to large amount of cellulose in the RPS. Cellulose is known to be highly resistant to enzymatic hydrolysis but these difficulties can be overcome by employing a suitable chemical or mechanical pretreatment prior to hydrolysis (9). In comparison between enzyme hydrolysis, it was shown that the RPS could be used to produce sugar by Acellulase enzyme better than cellulase

(Sigma). This might be due to higher specificity activity of Acellulase than the cellulase (Sigma). Increase of the sugars produced in the enzymatic hydrolysis might be maximized by sequential adding of the other hydrolytic enzymes, xylanase and pectinase, in the reactor during period of time of hydrolysis at the optimum condition of the enzymatic reaction.

However, one of the drawbacks of this process is that the enzymatic hydrolysis come from high cost of enzyme employed. From the experiment, it was found that under the optimal conditions, Acellulase treatment were capable of producing the amount of reducing sugars from RPS more than the other hydrolytic enzymes and diluted acid pretreatment also increase the sugar

yield. However, in this experiment, the RPS pretreated by milling to flour was taken part in hydrolysis by these hydrolytic enzymes to produce reducing sugars. Therefore the hydrolysis by combining of these hydrolytic enzymes should be produce higher yield of sugars and from these results, hydrolysis by different enzymes could produce sugars in difference amount suggesting different contents of RPS compositions and the reducing sugar products might be used for further applications.

4. Conclusions

The three diluted acids; sulfuric acid, hydrochloric acid and acetic acid; were evaluated for the acid hydrolysis for the production of reducing sugars from cassava peels. sulfuric acid at 0.1 M was found to yield significantly more reducing sugars than hydrochloric acid and acetic acid. Under optimal conditions, Acellulase (Genecor) was capable to produce reducing sugars RPS than the other hydrolytic enzymes; cellulase (Sigma), xylanase and pectinase. In this study, comparison of the reducing sugars produced in the reaction mixture of acid pretreatment and enzymatic hydrolysis found that the enzymatic treatment of Acellulase reaction produced more reducing sugars than the cellulose (Sigma) hydrolysis and RPS pretreated by disc mill combined with using aqueous-diluted acid solution at high temperatures produced the highest reducing sugars yield after enzymatic hydrolysis.

5. Acknowledgements

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6. References

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