Effect of alkali pretreatment on bioconversion of waste money bill to glucose for bio-ethanol production

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Running title: Bio-ethanol production from waste money bill pretreated with NaOH

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Abstract

Renewable energy resources and technologies have the potential to provide long-lasting solutions of the global energy-requirements faced by the economic and environmental sectors of a nation. Therefore, waste money bills were used as renewable energy source for the production of bio-ethanol. In this study, different concentrated NaOH 0.5%. 1.0%, 2.0%, 3.0% and 0.0% (as a control) were used for 10, 20 and 30 mins at 121 °C/15 psi in an autoclave. Saccharification and fermentation (aerobic and anaerobic) were carried out through commercial enzyme Celluclast 1.5 L, Novozymes 188 and *Saccharomyces cerevisiae* KCCM 11304 respectively. The results of pretreatment showed that the NaOH pre-treated substrate

enhanced enzyme action and released more amount of glucose. The amount of glucose was found with the increasing concentration of NaOH and time 44996.95±6.30, 46763.10±3.56, 53421.32±4.72, 63431.25±6.95 and 56850.98±6.75 ng/μl for 30 min respectively. As for bioethanol, the conversion rate of NaOH resulted 1010.08±4.71, 1050.25±4.37, 1109.49±4.39, 1139.25±3.26 and 1020.77±3.89 ppm for aerobic; 16730.54±6.67, 17076.45±6.25, 17516.17±4.49, 19782.68±6.19 and 17973.39±7.50 ppm for anaerobic and 18935.02±4.59, 19895.45±5.39, 21912.95±4.83, 24895.21±6.72 and 18961.21±4.90 ppm for anaerobic condition with benzoic acid for respective condition. Thus, the results of the present work clearly revealed that with the increasing of alkali concentration might be more effective for bio-ethanol production from waste money bill, which is economic and environmental friendly.

Key words: Waste money bill, pretreatment, enzymatic hydrolysis, fermentation and bioethanol

1. Introduction

Energy and environmental issues are currently among the major concerns facing the global community. Fuel ethanol productivity has increased remarkably due to the need for several countries to reduce oil imports, boost rural economies and improve air quality (Meng et al. 2010). Burning fossil fuels such as coal, natural gas and oil releases CO_2, which is a major cause of global warming (Yat et al. 2008). The accelerated depletion of fossil resources, global warming, and the lack of alternatives to replace fuels and chemicals derived from fossil resources has led to increased interest in the conversion of lignocellulosic materials into bio-fuels and bioproducts (Goldemberg 2006). The emerging second generation bioethanol as an oxygenated fuel additive focuses on the use of non-edible sources such as lignocellulosic biomass (grasses, sawdust, wood chips, stems and leaves, sewage sludge, paper mill

sludge, solid waste and crop residues) for its renewable nature, abundance and low cost (Saha et al. 2005).

Plant biomass consists of 40–55% cellulose, 25–50% hemicellulose and 10–40% lignin, depending on whether the source is hardwood, softwood, or grasses (Sun and Cheng 2002). Waste paper sludge is a solid residue from the wood pulping and papermaking industries, which contains large quantities of short cellulose fibers (Yuya et al. 2010). These residues are usually disposed of by incineration or landfill, which cause substantial financial burdens and are a source of various environmental problems. Incineration has a significant cost-increasing factor because of its low energy efficiency, caused by high moisture content (Fan and Lynd 2006., Scott et al. 1995., Sjode et al. 2007). Therefore, this byproduct could be a potentially attractive raw material for bio-ethanol production due to its high and accessible cellulose content (Fan et al. 2003).

Since paper sludge constitutes large amounts of carbohydrates, a potential source – waste money bill (WMB) - is believed to be one of the most important raw materials for sugar as cellulosic feedstock. In Korea, on average, approximately 1,509 tons of waste money bill (disposed weight) are incinerated per year, and for the last three years, the incineration cost was 203,483 $, while the disposal cost of waste bank notes and the checking cost (only sell revenue-generating print in waste) were 588 $/ton and 37-47 $/ton respectively. Therefore, in this study, the utilization of high solid-content money bills as an alternative substrate for bioethanol production using SHF process was investigated.

2. Materials and methods

Waste money bills

WMBs were collected from Korea Minting, Security Printing & ID Card Operating Corp, Technology Research Institute in Korea. Sample was washed with distilled water to stop potential microbial growth and air-dried at 105 °C overnight and homogenized using a blender (figure 1).

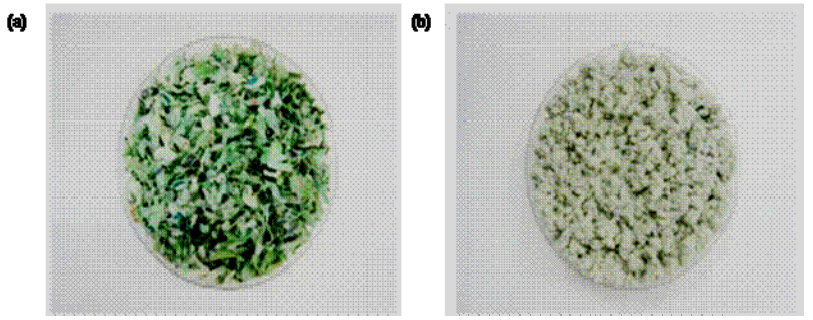

Fig.1. Waste money bill (WMB) chips (a) and (b) is powder state of WMB

Enzymes and inoculum preparation

Celluclast 1.5 L (Cellulase from *Trichoderma reesei* ATCC 26921), novozyme 188 (Cellobiase from *Aspergillus niger*) from Sigma-Aldrich Co and yeast strain *Saccharomyces cerevisiae* KCCM 11304 were purchased from KCCM. The yeast strain was maintained on YM medium (3.0 g yeast extract, 3.0 g malt extract, 5.0 g peptone, 10.0 g dextrose, 16.0 g agar and 1.0 L distilled water). The culture was incubated at 30 ºC for three days.

Pre-hydrolysis of WMB with sodium hydroxide

This experimental was conducted by investigating different parameters; NaOH concentration (0.5% 1.0%, 2%, 3%, and 0.0% v/v), pre-treatment time (10, 20 and 30 min), and temperature (121 ºC). For pretreatment, 2 g WMB with different diluted NaOH was autoclaved at 121 ºC for respective time. Whatman filter paper was used for the filtration.

Hydrolysis of pretreated products of WMB with enzymes

Enzymatic hydrolysis was carried out in 100 ml conical flasks maintained at 50 °C and 150 rpm for 72 hrs by an automated shaking incubator. According to NREL (NREL, 2008), 1.0 g of pretreated WMB was separately used through 0.1 M sodium citrate buffer (pH 4.8), 400 µl/g Celluclast 1.5 L, 200 µl/g Novozyme 188 and 600 µl sodium azide. Then the liquid fraction (hydrolysates) was centrifuged at 3500 rpm for 15 mins. This filtrated supernatant was used for sugar analysis.

Separation and fermentation of hydrolysate

For fermentation, 8 ml of the hydrolysate was mixed with one loopful of yeast cells in a glass vial at 30 ºC, 120 rpm for 96 hrs. The cultivation was conducted under aerobic and anoxic conditions at pH 6.5.

3. Analytical methods

High-Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) analysis

Glucose content was determined by HPLC (Model Agilent 1100 series, USA) using RID1 A, Refractive Index Detector and carbohydrate column (Agilent Technologies, USA). Ethanol was measured by GC (Model Agilent 6890N, USA) using FID1 A, Flame Ionization Detector and HP-5 column (Agilent Technologies, USA).

Statistical analysis

For statistical computations, all experiments were carried out in triplicate and

each sample was analyzed in duplicate. The results are expressed as Mean value and SE (standard error).

4. Results and discussions

Effect of NaOH pre-treatment method on glucose and ethanol content (benzoic acid)

Different doses of NaOH (0.5 1.0, 2.0, 3.0, v/v and 0.0 %) after enzymatic saccharification generated more glucose (44996.95±6.30, 46763.10±3.56, 53421.32±4.72, 63431.25±6.95 and 56850.98±6.75 ng/µl) for 30 mins residence time compare to 10 and 20 mins glucose production (42676.08±7.27, 42730.19±5.49, 51232.25±4.44, 59285.24±6.88 and 53831.686.88±4.41 ng/µl; and 42962.92±3.74, 43698.08±6.30, 51422.27±3.89, 60291.05±4.49, and 55942.01±7.29 ng/µl) respectively (figure 2). Azam Je and Taherzadeh MJ. (2009) mentioned that NaOH pretreatment of cotton fibers were carried out with 0–20% NaOH at 0, 23 and 100 ºC, followed by enzymatic hydrolysis up to 4 days. In general, higher concentration of NaOH resulted in a better yield of the hydrolysis. In this study, indicated that increasing diluted NaOH concentrations (3%) found more glucose after saccharification followed by 0.0, 0.5, 1.0 and 2.0%. As for ethanol, in the presence of low concentrated benzoic acid 0.4 mM with such respective condition produced 16867.06±4.24, 17676.42±7.56, 18230.05±5.28, 20284.90±7.16 and 17931.35±8.16 ppm for 10 mins; 16931.69±5.29, 17876.12±5.47, 18831.98±6.06, 21284.01± 5.71 and 18271.29± 4.60 ppm for 20 min and 18935.02±4.59, 19895.45± 5.39, 21912.95± 4.83, 24895.21± 6.72 and 18961.21± 4.90 ppm for 30 mins (figure 2). Warth (1989) stated that in the presence of benzoic acid, several yeasts, such as *S. cerevisiae,* exhibited an adaptive higher tolerance to benzoic acid at

low concentrations (up to 0.4 mM) stimulated ethanol production, because cells are very permeable to the undissociated form of benzoic acid and other weak-acid type preservatives. In this study, observed that increasing diluted NaOH concentrations (3%) found highest yield of ethanol after fermentation followed by 0.0, 0.5, 1.0 and 2.0%. The overall production yield of glucose and bioethanol from different treatments (table 1).

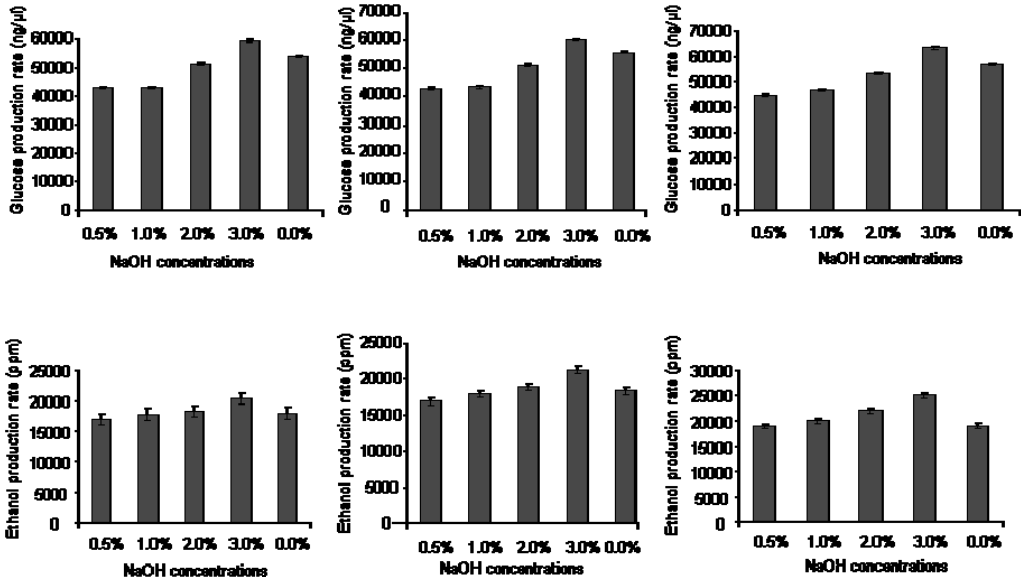

Fig.2. Effect of different NaOH concentrations and water treatment of WMB for glucose and ethanol production during respective time. Error bars indicate standard error (SE).

Table 1. Effect of different concentrated NaOH and water treatment for glucose and ethanol production between aerobic and anaerobic conditions.

Pretreatment Temp (°C)	Time (min)	NaOH conc. (%v/v)	Glucose (ng/μl)	Bioethanol (ppm)		
				Aerobic	Anaerobic	Anaerobic (+benzoic acid)
		0.5	42676.08 ±7.27	994.81±4.27	15530.20 ±6.15	16867.06 ±4.24
		1.0	42730.19 ±5.49	1066.58± 5.62	15875.85 ±7.07	17676.42 ±7.56
	10	2.0	46763.10 ±3.56	1101.62± 3.22	16115.15 ±5.87	18230.05 ±5.28
		3.0	**59285.24 ±6.88**	**1121.65± 4.22**	**18281.35 ±5.23**	**20284.90 ±7.16**
121		0.0	53831.88 ±4.41	1013.67± 3.46	17821.05 ±4.86	17931.35 ±8.16
		0.5	42962.92 ±3.74	999.44±2.80	15733.10 ±6.77	16931.69 ±5.29
		1.0	43698.08 ±6.30	1061.91± 6.10	16076.52 ±6.24	17876.12 ±5.47
	20	2.0	51422.27 ±3.89	1108.96± 4.52	16284.15 ±5.43	18831.98 ±6.06
		3.0	**60291.05 ±4.49**	**1131.65± 3.15**	**18480.26 ±5.48**	**21284.01 ± 5.71**
		0.0	55942.01 ±7.29	1009.41± 4.34	17873.72 ±5.85	18271.29 ± 4.60
		0.5	44996.95 ±6.30	1010.08± 4.71	16730.54 ±6.67	18935.02 ±4.59
		1.0	46763.10 ±3.56	1050.25± 4.37	17076.45 ±6.25	19895.45 ± 5.39
	30					

2.0	53421.32 ±4.72	1109.49± 4.39	17516.17 ±4.49	21912.95 ± 4.83
3.0	**63431.25 ±6.95**	**1139.25± 3.26**	**18480.26 ±5.48**	**24895.21 ± 6.72**
0.0	56850.98 ±6.75	1020.77± 3.89	17873.72 ±5.85	18961.21 ± 4.90

5. Conclusion

With the growing global population, increased waste production is a reality faced by all communities. Using dilute NaOH hydrolysis is the most efficient pre-hydrolysis treatment on the selected waste used in this study. Therefore, increasing NaOH concentration for pretreatment of WMB is supposed to be suitable for enzymatic saccharification and bio-ethanol production. It can be used as an alternative sustainable waste management option in order to minimize the waste produced, and maximize energy/material recovery and to meet the needs of environmentally, economically and socially sustainable.

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