Jurnal Teknologi Industri Pertanian
23 (3):184-189 (2013)

ETHANOL PRODUCTION USING SSF METHOD FROM PAPER-BASED MATERIAL EXPOSED TO VARIOUS PHYSICAL TREATMENTS

PRODUKSI ETANOL DENGAN METODE SSF DARI BAHAN BAKU BERBASIS KERTAS YANG MENDAPAT PERLAKUAN FISIK BERBEDA

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ABSTRAK

Penelitian ini bertujuan untuk mengidentifikasi produktivitas bioetanol dari unbeaten pulp, beaten pulp, non-printed paper dan laser-printed paper dengan menggunakan metode sakarifikasi dan fermentasi simultan (simultaneous saccharification and fermentation – SSF). Khamir yang digunakan adalah Saccharomyces cerevisiae, diaplikasikan dalam konsentrasi 10%, 15% dan 20%, dengan waktu inkubasi selama 3, 4, dan 5 hari. Konsentrasi etanol dianalisis menggunakan kromatografi gas, kemudian digunakan untuk menghitung rendemen etanol dan konversi selulosa. Kadar karbohidrat sampel yang cenderung tinggi (lebih dari 70%) mengindikasikan bahwa pulp yang digunakan berpotensi baik untuk produksi bioetanol. Konversi selulosa (8,24%) dan produksi etanol (5,4%) tertinggi ditemukan pada beaten pulp yang difermentasikan dengan konsentrasi khamir 15% dan waktu inkubasi 3 hari. Perlakuan beating diasumsikan dapat meningkatkan rendemen etanol, namun demikian, bahan kimia dan mineral aditif pada kertas menghambat proses hidrolisis enzimatik dan tinta menghambat fermentasi gula sederhana.

Kata kunci:bioetanol, pulp putih, penggilingan, kertas bercetak laser,SSF

ABSTRACT

Bioethanol production from unbeaten pulp, beaten pulp, non-printed paper and laser printed paper by the use of simultaneous saccharification and fermentation (SSF) method was carried out in the this research. The yeast used was *Saccharomyces cerevisiae* which was applied in various concentrations (10 5, 15%, and 20%) and incubation times (3, 4, and 5 days). Gas chromatography was used to quantity ethanol concentration and this was used to calculate ethanol yield and cellulose conversion. Carbohydrate contents of samples were relatively high (over 70%) indicating that the pulps have good potential for bioethanol production. The highest cellulose conversion (8.24%) and ethanol production (5.4%) were obtained from beaten pulp fermented at 15 % yeast concentration and incubated for 3 days. Beating treatment was assumed beneficial to increase ethanol yield, however chemical and mineral additives in paper appeared to inhibit enzymatic hydrolysis processes and ink inhibited fermentation of simple sugar.

Keywords: bioethanol, bleached pulp, beaten pulp,laser printed paper, *SSF*

INTRODUCTION

Cellulose-based bioethanol or second generation bioethanol (Millati *et al.*, 2008) has been expected to solve the problem arising from competition between food and energy production based on the same feedstock. Furthermore, bioethanol production from cellulose has been considered beneficial from the standpoint of food security, reducing greenhouse gasses and renewable energy sources (Nzelibe and Okafoagu, 2007).

The source of cellulosic biomass is diverse and classified into four main groups, i.e. wood/forests, municipal solid waste, agricultural waste and paper waste (Demirbas, 2003). Paper waste from bleached pulp is probably the most appropriate to be converted into bioethanol due to its lowest lignin content as compared to the other sources of previously mentioned biomass (Kadam and McMillan, 2003). The presence of lignin in biomass hinders its conversion process into bioethanol. Lignin and polysaccharides is associated through physical and chemical attachment, and this association giving a physical barrier to cellulose degradation by external factors (Dawson and Boopathy, 2008). This physical barrier is considered as the main problem in the hydrolysis of cellulose to produce simple sugars fermentable by microorganism into bioethanol.

Although waste paper can be recycled, the properties of its new products have been known to be inferior (Vynios *et al*., 2009). Conversion of waste paper into bioethanol could be more beneficial from economic and environmental stand points.

Biomass conversion into ethanol can be carried out through simultaneous saccharification and fermentation (SSF) process, in which hydrolysis of biomass into individual sugar and fermentatation

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of the sugar into ethanol occurred simultaneously in the same reactor. Simulatneous fermentation of hydrolysis products significantly reduced the inhibiton of proceeding hydrolysis process of lignocellulosic materials (Sun and Cheng, 2002). It has been reported that simultaneous fermentation increased cellulose conversion eficiency by 25% and thus reduced production cost when compared to that of separate saccharification and fermentation process (Koesnandar, 2001).

Fermentation influenced by the types of microorganism and the substrates. *Saccharomyces cerevisiae* is probably the most common fermenter used in the production of bioethanol. The yeast has been proven to be relatively tolerant to high ethanol concentration, capable of surviving in high temperature and low pH, stable during fermentation processes, and capable of producing high ethanol yield (Hector *et al.*, 2011). Boonsawang *et al.* (2011) have successfully produced bioethanol from palm pressed fiber with SSF operated at 30-40^{o}C. In an aerobic growth condition, *Saccharomyces* yeast preferred hexose sugar (glucose, mannose and galactose) based substrate than that of xylose based (Millati *et al*., 2008).

The present research was intended to determine bioethanol productivity of unbeaten pulp, beaten pulp, non-printed paper and laser printed paper by the use of simultaneous saccharification and fermentation (SSF) method. Hydrolysis process in SSF has been known to be less problematic as compared to its fermentation process. It was expected that the present research will complete the information regarding optimum condition to convert wood-based lignocellulosic into bioethanol.

MATERIALS AND METHODS

Paper and pulp handsheets were slashed, soaked in distilled water and homogenized in a mixer. The resulting pulp was then screened and air dried for about two weeks. Upon drying, the content of holocellulose, cellulose and hemicelluloses were determined following the methods of Browning (1967) and the solid content of sample (oven dry weight) was determined based on TAPPI T 264 cm-97. Experiments were carried out based on cellulose content of 6% (w/v) with 10 mL volume of medium in a 20 mL caped reaction tube (vial) in accordance with the SSF Experimental Protocols of NREL (Dowe and McMillan, 2008). Saccharification and fermentation were carried out simultaneously. Saccharification was done by the use of cellulase enzyme with enzyme activity of 4.5-7 IU/mL and fermentation was done by the use of a 10^3 CFU/mL of *Saccharomyces cerevisiae*'s filtrate. Medium was buffered with 0.5 mL sodium citrate 1 M to stabilize its pH. NPK 0.04% (w/v) and ZA 0.15% (w/v) were used as the nutrition of yeast growth. Upon autoclaving of the medium, cellulase and *S. cerevisiae* filtrate were simultaneously added. The amount of cellulase added was twice of cellulose content (v/w) or 2 mL/g of oven dried pulp sample. *S. cerevisiae* addition (10%, 15%, and 20%) was carried out in a sterilized chamber (laminar flow). SSF was run at 40^{o}C in a water bath-shaker and was then incubated for 3, 4, and 5 days. Upon incubation, the medium was heated for 5 minutes in a boiling water to deactivate the enzyme and yeast.

Ethanol concentration was determined by the use of a GC 2014 Shimadzu using flame ionization detector and a column of RTX WAX 906952 (0.25 mm i.d. and 30 m length). The column oven was operated at 150^{o}C. The injector and detector temperatures were maintained at 180^{o}C and 200^{o}C, respectively. The column flow of 0.65 mL/min and total flow of 55.5 mL/min was used during the processes. Ethanol was detected at 2.8-3.0 minutes retention time. Ethanol concentration was calculated based on a standard curve previously prepared at the concentration of (%-v/v) 0.00, 0.05, 0.10, 1.00 and 5.00. The standard curve was depicted as the ratio between chromatogram of ethanol and injected standard concentration shown in Figure 1.

Ethanol yield and conversion degree of cellulose can be determined after the determination of ethanol concentration. Yield and degree of conversion can be calculated by the following formulas (Dowe and McMillan, 2008):

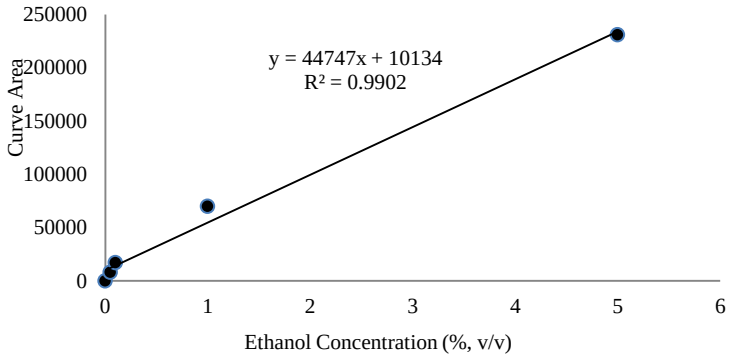

Figure 1. Standard curve of Ethanol

$$Yield\ (\%-v/w) = \frac{ethanol\ \ concentration\ (\%-v/v) x volume\ of\ \ ethanol\ \ distillate}{oven\ dried\ \ sample(g)}$$

$$Cellulose\ \ Conversion(\%) = \frac{EtOH\ \ concentration\ (\%-v/v)\, x\, vol.distillate\ \ xSG\ \ of\ \ EtOH}{oven\ dried\ \ cellulose\ \ in\ \ medium(g)\ x\ 1.111\ x\ 0.51}$$

In which1.111 is cellulose conversion into glucose equivalent and 0.51 is correction factor of glucose into ethanol based on biochemical stoichiometric of yeast.

RESULTS AND DISCUSSION

Raw Materials Characteristics.

Raw materials were characterized based on their carbohydrate components before conversion into ethanol. Table 1 indicates the characteristic of carbohydrate from unbeaten pulp, beaten pulp, non-printed paper and laser printed paper of the present experiments.

Cellulose content of beaten pulp was higher than that of other samples. Beaten pulp used in the present experiments was a dissolving pulp for rayon production that requires high content of cellulose. Hemicelluloses is not expected to be present in dissolving pulp (Smook, 1992) and intentionally removed. The content of hemicelluloses in beaten pulp was lower than that found in unbeaten pulp used for papermaking. Paper additives might be the cause of lower content of cellulose and hemicelluloses in pulp from non-printed and laser printed paper. Laser printed paper contains ink that further lower the content of its holocellulose.

Although cellulose content of laser-printed paper in the present experiment was the lowest, but it is still relatively high (74.86%) and a potential raw material for ethanol production. Its cellulose content is much higher than these of several lignocellulosic biomasses such as wheat straw (28.52%) (Zhuang *et al.*, 2009) and bagasse (33.60%) (Singh*et al.*, 2007). Theoretically, with the conversion factor of cellulose into ethanol as high as 0.51 (Demirbas, 2003), a maximum of 42.42% of laser-printed paper can be converted into ethanol.

Ethanol Content and Cellulose Conversion

Ethanol concentration can be a measure of fermentation effectivity and the degree of cellulose conversion indicates the level of SSF performance. Cellulose conversion is a ratio between the amount of produced ethanol and the amount of ethanol possibly produced from all cellulose in medium. Cellulose conversions in the present experiments are depicted as Figure 2.

Analysis of variance on ethanol concentration indicated that the types of pulp and incubation time did not significantly influence ethanol concentration (p>0.05). However, ethanol concentration was significantly (p<0.05) influenced by yeast concentration. Further statistical evaluation (advance Duncan test) indicated that ethanol concentration at 10% yeast concentration was different to that at 15% and 20% yeast concentration. Since there was no significant difference in ethanol concentration resulted from 15% and 20% yeast treatment, it can be assumed that optimum ethanol concentration can be resulted from fermentation with 15% yeast concentration for 3 days incubation.

Figure 2 indicates that the degree of cellulose conversion (or ethanol concentration) tends to decrease with increasing incubation time. Maximum ethanol concentration of the present experiment was achieved with 3 days incubation, which is in agreement with that found by Dodic *et al.* (2009) who converted sugar beet into bioethanol. Incubation for 3 days might be the yeast exponential phase (Gozan *et al.*, 2007) where maximum conversion of cellulose occurred. Beyond 3 days incubation the yeast may decline in phase (death phase) that could decrease cellulose conversion. Advance reaction product of ethanol, such as acetic acid produced in stationary phase (Gozan *et al.*, 2007) can inhibit yeast proliferation and promote yeast death, as well.

The highest conversion degree of 8.24% was achieved by fermenting saccharification product of beaten pulp with 15% yeast concentration for 3 days incubation. Recycled enzyme with activity ranged from 4.5-7.0 IU/mL used in the hydrolysis process of the present experiment could be the origin of the resulting low conversion degree. Hydrolysis is the limiting performance of SSF (Sun and Cheng, 2002). Thus, high enzyme activity is required in saccharification stage to gain elevated degree of conversion. Enzyme activity of 4.5-7.0 IU/mL indicates that every unit of cellulose used is capable of producing only 4.5-7.0 µmol glucose per minute. Conversion degree of current cellulosic material could be increased by the use of cellulase with a better activity, such as that from *Trichodermareesei* (the best cellulase resulting fungi) that has enzyme activity in the ranged of 250-430 IU per gram of cellulase (Chahal, 1985). Furthermore, SSF process was carried out in a closed reactor that possibly contributed to the low conversion degree. In a closed reactor, carbon dioxide is concentrated in the medium and that inhibit the fermentation performance of microbes (Fardiaz, 2002).

Table 1. Carbohydrates composition and total solid content of samples under investigation

Biomass types	Holocellulose (%)	Cellulose (%)	Hemicelluloses*) (%)	Solid content (%)
Unbeaten Pulp	96.2	88.8	7.4	27.3
Beaten Pulp	98.1	91.3	6.8	26.9
Non-printed Paper	81.8	76.6	5.3	32.6
Laser-printed Paper	79.7	74.9	4.9	33.0
Average	**99.0**	**82.9**	**6.1**	**-**

Note:$^{*)}$*hemicelluloses was calculated as the difference between holocellulose and cellulose content*

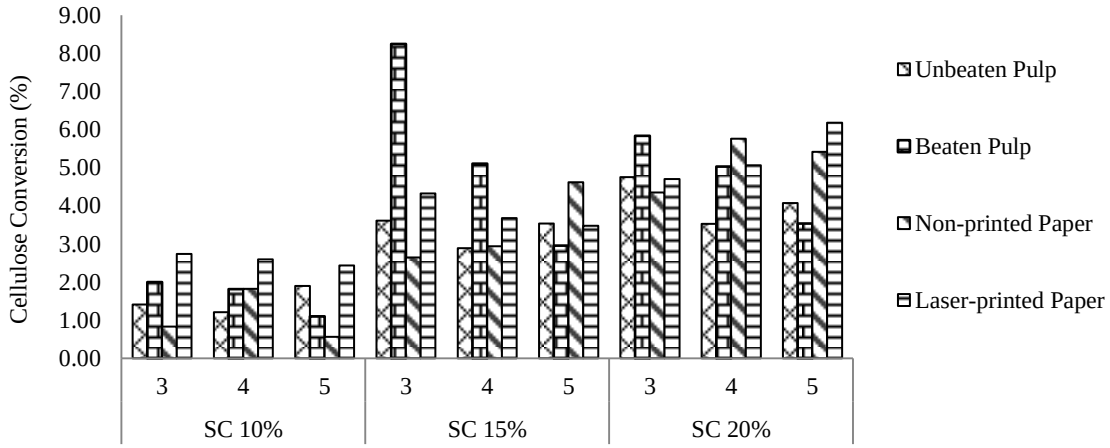

Figure 2. Influence of yeast concentration and incubation time on cellulose conversion

Compromising temperature between hydrolysis and fermentation processes in SSF has been assumed to bring about that hydrolysis and fermentation does not take place at themost favorable temperature (Saddler, 1993). This is another possible cause of low conversion degree.

Ethanol Yield

The yield was indicated in % (v/w) of raw material. Correlation between yield and concentration of ethanol was linear. The implication was that the responses of yield and concentration were similar in all three treatments with its respective level. Table 2 indicates the resulting yield of the present research.

Table 2 indicates that the highest yield of fermentation of simple sugars from unbeaten and beaten pulp was achieved at 20% and 15% yeast concentrations, respectively, with 3 days of incubation. It seemed that beating process increased cellulose porosity (Smook, 1992) that assisted hydrolysis of polysaccharide to produce higher amount of reducing sugar.

The highest yield for fermentation of hydrolysis products of non-printed paper was achieved at 20% yeast concentration for 4 days incubation. The present results have shown that physical treatment given to pulp during papermaking process did not influence the requirement of yeast and incubation time in fermentation of saccharification products. Therefore, non-fibrous additives (chemical and mineral such as rosin, urea-formaldehyde, melamine-formaldehyde etc.) addition during stock preparation in papermaking processes (Smook, 1992) possibly inhibited simple sugar fermentation into ethanol. Chemical additives and minerals can influence the acidity of paper. Changes in acidity during fermentation process reduced the activity of *S. cerevisiae* (Demirbas, 2003). Fermentation of hydrolysis products of laser-printed paper at 20, % yeast concentration required 5 days incubation to produce a maximum yield. Since ink in paper did not influence enzymatic hydrolysis (Hakim, 2007; Marques *et al.*, 2003), this increase of incubation time possibly brought about by inhibition properties of ink in paper to fermentation process by *S. cerevisiae*. Current experiment indicated that 303.77 grams of laser-printed paper is required to produce 1L ethanol. The yield of ethanol from the waste of laser-printed paper was only about 3.3% (v/w).

Table 2. The yield of ethanol resulted from different yeast concentration and incubation time

Yeast concentration (%-v/v)	Incubation time (days)	Yield (%-v/w) Raw material types			
		Unbeaten Pulp	Beaten Pulp	Non-printed Paper	Laser-printed Paper
10	3	0.9	1.3	0.5	1.5
	4	0.8	1.2	1.0	1.4
	5	1.2	0.7	0.3	1.3
15	3	2.3	**5.4***)	1.4	2.3
	4	1.8	3.3	1.6	2.0
	5	2.2	1.9	2.5	1.9
20	3	**3.0***)	3.8	2.4	2.5
	4	2.2	3.3	**3.1***)	2.7
	5	2.6	2.3	2.9	**3.3***)

*Note: *) = maximum yield*

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

Optimum condition of ethanol production by the SSF process for beaten pulp and unbeaten pulp was by the use of 15% and 20% yeast concentration, respectively, for the same incubation time of 3 days. Those of non-printed paper and laser-printed paper required the use of 20% yeast concentration for 4 and 5 days incubation time, respectively. The highest cellulose conversion of the present experiment was 8.24% and the highest ethanol yield of 5.4% (v/w) was obtained from beaten pulp.

Beating process improved the hydrolysis of pulp and resulted in a higher ethanol production. Non-fibrous additives in paper were thought to inhibit fermentation process and reduced ethanol production. Ink did not influence enzymatic saccharification, but it inhibit fermentation of simple sugar into ethanol.

Recommendations

Further works should be focused on the endeavors of increasing hydrolysis level of cellulose extracted from laser printed recycled paper. Appropriate mechanical (beating process) and chemical swelling of the pulp can be tried to further reduce the cellulose crystallinity in order to facilitate enzyme and/or chemical during hydrolysis process.

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