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BUFFER REPLACEMENT AND SIMULTANEOUS SACCHARIFICATION AND FERMENTATION ON BIOBUTANOL PRODUCTION FROM LIGNOCELLULOSIC BIOMASS

PENGGANTIAN BUFFER DAN SISTEM SAKARIFIKASI DAN FERMENTASI SERENTAK PADA PRODUKSI BIOBUTANOL DARI BIOMASSA LIGNOSELULOSA

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ABSTRAK

Biomassa lignoselulosa merupakan bahan baku yang sangat baik untuk menghasilkan biobutanol. Ampas tebu, jerami padi, dan tandan kosong kelapa sawit (TKKS) merupakan potensi yang belum dimanfaatkan untuk menghasilkan biobutanol sebagai campuran/substiusi bensin. Namun demikian, proses produksi biobutanol melalui fermentasi dari biomassa lignoselulosa merupakan proses yang memakan waktu dan energi, yang berujung pada tingginya biaya produksi. Penelitian ini dimaksudkan melakukan optimasi proses produksi biobutanol yang dapat menurunkan biaya produksi yang merupakan faktor penting dalam skala industri. Optimasi dilakukan dengan mengganti larutan buffer pada proses hidrolisis enzim dengan akuades serta percobaan sakarifikasi dan fermentasi serentak atau Simultaneous Saccharification and Fermentation (SSF). Hasil penelitian menunjukkan bahwa penggunaaan akuades dalam hidrolisis enzim dapat menghemat biaya pemakaian buffer sitrat sekitar Rp. 41.726/liter hidrolisat. Biobutanol dari semua biomassa jerami padi, ampas tebu, dan TKKS lebih tinggi perolehannya jika menggunakan sakarifikasi dan fermentasi serentak dibandingkan dengan hidrolisis dan fermentasi terpisah. Penggunaan sakarifikasi dan fermentasi serentak juga dapat memotong waktu produksi selama 3 hari dan mengemat listrik sekitar 32,4 kWh.

Kata Kunci: *bahan bakar nabati, biobutanol, biomassa lignoselulosa, optimasi, sakarifikasi dan fermentasi serentak*

ABSTRACT

Lignocellulosic biomass is excellent feedstock for biofuel such as biobutanol. Bagasse, rice straw, and empty fruit bunch (EFB) oil palm are untapped potential for biobutanol production as gasoline blending/ substitution. However, biobutanol production by fermentation from lignocellulosic biomass is a process that consumes time and energy which leads to high production costs. This research is intended to optimize biobutanol production that reduces production costs, an important factor on an industrial scale. Optimization is conducted by replacing the buffer solution in enzymatic hydrolysis with distilled water and by using Simultaneous Saccharification and Fermentation (SSF). The results showed that the buffer replacement with distilled water can reduce cost by approximately 41,726 IDR/liter hydrolysate. Biobutanol contents from all biomass of bagasse, rice straw, and EFB oil palm are higher using SSF compared to Separate Hydrolysis and Fermentation (SHF). The SSF system can cut production time by 3 days and save electricity of 32.4 kWh.

Keywords: biofuel, biobutanol, lignocellulosic biomass, optimization, Simultaneous Saccharification and Fermentation

I. INTRODUCTION

Lignocellulosic biomass is composed of cellulose, hemicellulose and lignin, as well as other minor components. Both cellulose and hemicellulose fractions are polymers of sugars, and thereby a potential source of fermentable sugars (Harmsen et al. 2010). Lignocellulosic biomass is excellent feedstock for biofuel such as biobutanol due to solventogenic clostridia which are able to convert a wide range of carbohydrates to biobutanol (Ezeji et al. 2007).

Indonesia has great biodiversity and an abundance of biomass to produce biofuel. Bagasse, rice straw, and empty fruit bunch (EFB) oil palm are untapped potential for biobutanol as gasoline blending/ substitution. Sugarcane production in Indonesia reached 3.1 million tons in 2014, an increase of 5.4% per year. Based on the material balance of sugarcane processing, the production of biomass from this process is comprised of 954,000 tons bagasse, 136,000 tons of molasses, and 109,000 tons of filter cake. In 2014 Indonesian Crude Palm Oil (CPO) stock of 30 million tons is available. Based on the material balance of the palm oil processing, biomass production from this process is comprised of 25.7 million tons of Empty Fruit Bunches (EFB) oil palm, 17 million tons of fibers, and 7 million tons of shells. Rice husk and rice straw were mostly only burned after harvesting regardless of the fact that the rice plants produced 50% straw and 20% husk. Data in 2013 showed that the rice plants produced 35 million tons of rice straw and 14.3 million tons of rice husk (Hambali *et al*. 2015). Carbohydrate composition from bagasse, rice straw, and EFB oil palm showed that all biomass have cellulose and hemicellulose which can convert to simple sugars by hydrolysis (Rani & Sari 2012). The simple sugars derived from hydrolysis will be consumed by solventogenic microorganism and produce solvent, such as biobutanol.

The biobutanol production of cellulosic materials by fermentation is a process that consumes time and energy which leads to high production costs. The high cost arises from the hydrolysis and separation products processes. The major challenge in making biobutanol using lignocellulosic biomass as raw material is the conversion into monosaccharides/ simple sugars. Microorganisms do not have the enzymes to digest lignocellulose (Aleksic 2009), therefore the pretreatment, such as acid hydrolysis and/or enzymatic hydrolysis, is required before the fermentation process (Harmsen et al. 2010). The pretreatment is an important step and has been recognized as one of the most expensive processing steps in the fermentation of biomass. The process of pretreatment is required to liberate the cellulose from lignin bonding and cellulose crystalline structure and make it easier to interact with the enzyme that converts carbohydrates into sugar polymers (Mosier et al. 2005). It is important that the energy required in butanol recovery should be lower than the energy content of the product (butanol) (García et al. 2011).

This research is intended to optimize biobutanol production that reduces production costs by replacing the citrate buffer in enzymatic hydrolysis with distilled water and by using Simultaneous Saccharification and Fermentation (SSF). Optimum conditions for enzymes are very important to hydrolyse hemicelluloses into monomers. A strong buffer will be required because solventogenic bacteria produce acidic products such as butyric acid and acetic acid. Butyric acid will be effectively converted into butanol product at optimum pH (Lee et al. 2008). During SSF, less number of reactors, time, and cost will be required compared to SSF (Thirmal & Dahman 2011). It is expected that biobutanol production optimization can reduce time and energy consumed, thus it can reduce production costs and improve the technoeconomics of biobutanol production to be applied on an industrial scale.

II. METHODOLOGY

A. Strain, Media, and Cultivation Methods

Clostridium beijerinckii NBRC 103909 from Japan was used as a solventogenic bacteria strain. Reinforced Clostridial Medium was used as growth medium whereas Tryptone Glucose Yeast was used as a fermentation medium/starter. P2 medium comprising buffer, mineral, vitamin, and yeast extract as used in Qureshi et al. (2008) were added as nutrition. The entire processes of cultivation and medium transfer were carried out anaerobically in an Anaerobic Chamber (COY Technologies). Details of the cultivation method were described in previous research (Kussuryani & Rani 2015).

B. Biomass Substrate

Three biomasses of bagasse, rice straw, and EFB oil palm were collected based on an agricultural statistics database. The bagasse originated from a sugar refinery in Lampung, the rice straw originated from a paddy field in Central Java, and the EFB oil palm originated from a palm oil refinery in Riau. The

biomass feedstocks were dried, chopped and milled.

C. Acid Hydrolysis by Dilute Acid Pretreatment

Dilute Acid Pretreatment (DAP) were conducted using 1% H_2SO_4, at 130°C, for 30 minutes. The biomass weight for hydrolysis process is 10% (w/v) dry weight and pH was adjusted to 4.8-5.0 using 10M NaOH. Screening of DAP condition from several cellulosic biomass has been done in previous research (Rani & Sari 2012).

D. Enzymatic hydrolysis using citrate buffer and distilled water

After acid hydrolysis, biomass residue was treated by enzymatic hydrolysis using powder enzymes of Cellulase C1184 and Xylanase X2753 (from Sigma) at pH 4.8, 50°C, 120 rpm, for 72 hours. Citrate buffer and distilled water were tested as solution in the enzymatic hydrolysis process. Citrate buffer application for enzymatic hydrolysis was described in several references such as López et al. (2011). A solution of 0.1 M citrate buffer pH 4.8 was comprised of 400 mL of 0.1 M tri-Sodium Citrate dihydrate and 600 mL of 0.1 M Citric Acid monohydrate (Promega 2012). The hydrolysis time of 72 hours should give enough time to produce sugar. According to Banerjee et al. (2011), glucose was reaching the highest yield in enzymatic hydrolysis after 48 hours with 10% biomass loading.

E. Separate Hydrolysis and Fermentation (SHF)

Separate Hydrolysis and Fermentation (SHF) was performed after acid hydrolysis by using a mixture of liquid enzymes of Celluclast® 1.5 L, Novozyme 188, and Viscozyme® L (from Sigma). The enzymatic hydrolysis process was conducted at pH 4.8, 50°C, 120 rpm for 72 hours. The hydrolysate from DAP and enzymatic hydrolysis were then combined and fermented anaerobically at 35°C for 96 hours. P2 medium stock solutions and culture starter were added prior to fermentation. At the end of the fermentation, the solvent products (butanol, ethanol, and acetone) were recovered from the fermentation broth by distillation.

F. Simultaneous Saccharification and Fermentation (SSF)

After acid hydrolysis, the mixture of hydrolysate and cellulose residues was adjusted to pH 5.0. Three liquid enzymes of Celluclast® 1.5 L, Novozyme 188, and Viscozyme® L (from Sigma), microbial culture, and P2 medium were added simultaneously. The SSF process takes place anaerobically at 35°C for 96 hours. At the end of fermentation, the solvent products (butanol, ethanol, and acetone) were recovered from the fermentation broth by distillation.

G. Analytical methods

Fermentation products (butanol, ethanol, and acetone) were analyzed by Gas Chromatography-Mass Spectrophotometer (GC-MS) with Flame Ionization Detector (FID). Carbohydrate analyses (reducing sugar, cellulose, and hemicellulose) were conducted at the Center for Food & Nutrition Studies, Gadjah Mada University, Yogyakarta. Reducing sugar contents were analyzed using the Nelson-Somogyi method whereas cellulose/hemicellulose contents were analyzed using the *Chesson* method.

III. RESULTS AND DISCUSSION

A. Buffer Replacement in Enzymatic Hydrolysis

The sugar content after enzymatic hydrolysis by using distilled water from all biomasses was higher than by using the buffer solution (Fig. 1). The highest sugar content was found in EFB oil palm using distilled water (4.99%) compared to 3.86% using the buffer solution, while rice straw and bagasse showed a slight difference between distilled water and the buffer solution, 3.81% and 3.77% (rice straw), 3.45% and 3.33% (bagasse), respectively. The sugar content in EFB oil palm using distilled water is the closest to the standard content for butanol fermentation which use 5-7% w/v fermentable sugar.

Lower concentration and insignificant difference in sugar content in enzymatic hydrolysis by using citrate buffer and distilled water is caused by several factors, such as water amount for enzyme powder, the change of enzymes optimal pH, and

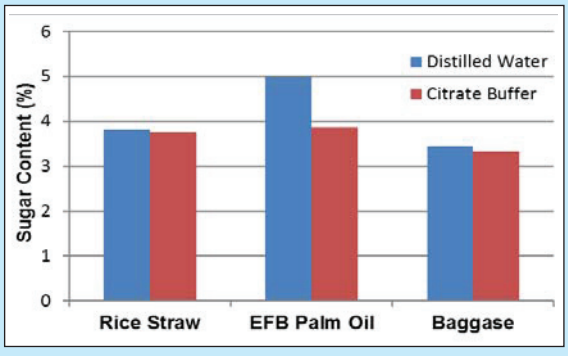

Figure 1
Sugar yield after enzymatic hydrolysis using buffer and distilled water.

Citrate Buffer Cost

Load of 0.1 M tri-Sodium Citrate dihydrate:

$$= \frac{29.41\ \text{g}}{1000\ \text{mL}} \times 400\ \text{mL} = 11.764\ \text{g/L}$$

Chemical Price of Sodium Citrate*

$$= \frac{928{,}000\ \text{IDR}}{500\ \text{g}} = 1{,}856\ \text{IDR/g}$$

Cost of Sodium Citrate

$= 11.764\ \text{g} \times 1{,}856\ \text{IDR}$

$= 21{,}834\ \text{IDR}$

Load of 0.1 M Citric acid monohydrate:

$$= \frac{21.01\ \text{g}}{1000\ \text{mL}} \times 600\ \text{mL} = 12.606\ \text{g/L}$$

Chemical Price of Citric Acid*

$$= \frac{789{,}000\ \text{IDR}}{500\ \text{g}} = 1{,}578\ \text{IDR/g}$$

Cost of Citric Acid

$= 12.606\ \text{g} \times 1{,}578\ \text{IDR}$

$= 19{,}892\ \text{IDR}$

Based on Merck Millipore pricelist per 1st January 2015 (p.a.)

Total cost of citrate buffer solution is 41,726 IDR /liter hydrolysate

Figure 2
Calculation of citrate buffer cost.

Table 1
Solvent yield from fermentation using SHF and SSF treatment

Solvent (%)	Bagasse		Rice Straw		EFB Oil Palm	
	SHF	SSF	SHF	SSF	SHF	SSF
Ethanol	0	0	0	0	0	3.06
Acetone	0.04	0.05	1.61	0.93	0.03	2.13
Butanol	1.73	1.84	1.28	1.78	1.85	53.16

the unpredictable enzyme reaction. For optimum activity in such media, the amount of water required to be added depends upon multiple factors and the need for optimization of water level arises, thus the untreated enzyme powders performed poorly (Gupta et al. 2013). Some enzymes require a unique buffer environment and are more problematic about having their optimal buffer than other enzymes (Dubey 2014). The enzyme reaction itself can cause pH shifts and consequently a continuous decrease of the activity (Bisswanger 2014).

The higher sugar production by using water in enzymatic hydrolysis indicated a potency for buffer replacement in biobutanol production from lignocellulosic biomass on an industrial scale. The use of water in enzymatic hydrolysis process for buffer replacement can reduce production costs by about 41,726 IDR per liter of hydrolysate, or about 834,520 IDR per batch hydrolysate (20 liter). Buffer cost calculations are detailed in Figure 2.

B. SHF and SSF

The higher biobutanol yield in all biomasses was obtained using SSF rather than SHF (Table 1). Bioethanol was not detected in bagasse and rice straw after SHF and SSF processes. This may be caused by an insignificant amount of bioethanol yield which evaporated during the distillation process. The highest biobutanol from EFB oil palm by SSF process (53.16 %) indicated that the SSF process

in this research is appropriate for EFB oil palm as fermentation feedstock. It also indicates that the SSF process using EFB oil palm will generate optimum sugar with low inhibitor compound. Thus, C. beijerinckii can produce high butanol yield. Further studies in the SSF process especially for bagasse and rice straw are required to enhance biobutanol yield from various lignocellulosic biomasses.

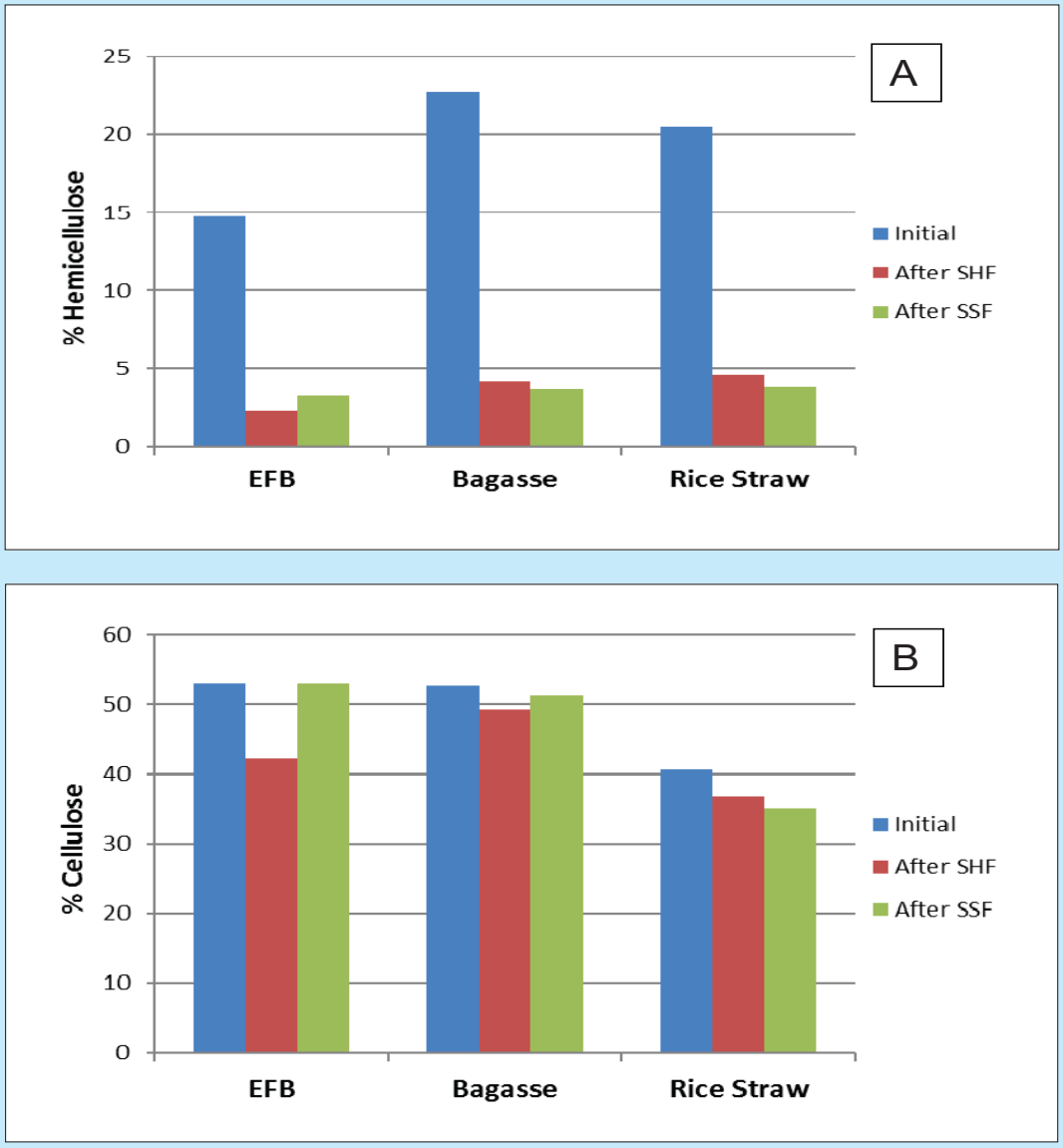

Fig. 3
Hemicellulose (A) and cellulose (B) content before and after fermentation.

Table 2
SHF and SSF Operation Condition

No.	Methods	Operation Condition
1.	SHF	Hydrolysis process at pH 4.8, 50°C, 120 rpm for 72 hours/3days and then fermented anaerobically at 35°C for 96 hours.
2.	SSF	Fermented anaerobically at 35°C for 96 hours.

The solvent yields from SSF experiments are lower than in a previous study which obtained 2.7 g/L butanol (10.55% butanol yield) by water pretreatment SSF and 2.61 g/L butanol (10.22% butanol yield) by no chemical pretreatment SSF (Thirmal & Dahman, 2011). Using distillation process for biobutanol separation lowered the solvent yield due to azeotroph butanol-water. Based on the equilibrium reaction, the fermentation broth must be in large amount to distillate in order to recover butanol rather than water. There are various methods of butanol separation from the fermentation yield. The proven separation processes for biobutanol recovery are gas stripping and liquid-liquid extraction (Ezeji et al. 2007).

Hemicellulose content in all biomasses has a significant reduction after SHF and SSF processes (Fig. 3A). On the other hand, cellulose content from both processes is not significantly different before and after fermentation and even tends to be higher than the initial amount (Fig. 3B). It indicated that hemicelluloses were completely broken down and converted to fermentable sugars followed by sugar consumption by C. beijerinckii whereas the higher cellulose content after fermentation indicated that the breakdown of lignin bond and crystalline structure increased cellulose content but was not completely converted into fermentable sugar. This phenomenon can found in similar studies such as Xu (2009) where cellulose content increased after corn stover hydrolysis. Increases in fermentable sugar can be achieved by several processes such as using various amorphogenesis-inducing agents for enhancing the enzymatic saccharification/enzymatic hydrolysis of cellulose (Arantes & Saddler 2010).

The carbohydrate composition based on hemicellulose and celulose content in this research has a different composition from previous work by Rani & Sari (2012). The difference is caused by variation distribution of carbohydrates in biomass based on the plant species, age, growth conditions, and with certain parts of the plant (Carvalho 2009).

The operation condition of SHF and SSF is shown in Table 2.

The application of the SSF system can cut production time by 3 days from enzymatic hydrolysis process time and save on electricity costs by about 32.4 kWh from heater operation (approx. 450W) at 50°C for 72 hours.

IV. CONCLUSION

Production of biobutanol from bagasse, rice straw, and EFB oil palm are higher using SSF compared to SHF. The SSF system can cut production time by 3 days and save electricity of 32.4 kWh. To increase biobutanol yield, research on fermentation biotechnology such as metabolic engineering and separation process must be enhanced to produce fuel grade butanol with a low production cost.

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