

Utilization of Glucose Recovered by Phase Separation System from Acid-hydrolysed Oil Palm Empty Fruit Bunch for Bioethanol Production

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ABSTRACT

Oil palm empty fruit bunch (OPEFB) is one the most abundant lignocellulosic wastes produced throughout the year in the palm oil industry. A new process of separating lignocellulose components after acid hydrolysis (known as phase separation system) has been previously developed, by which lignin and carbohydrate can be completely and rapidly separated in 60 minutes between 25 and 30°C. In this process, cellulose is completely hydrolyzed to oligosaccharides and remains in the acid phase. The maximum glucose yield of 53.8% was obtained by hydrolysis, with 4% acid after autoclaving at 121°C for 5 minutes. This work focused on the separation of monosaccharide (glucose) from cellulose fraction, which was subsequently used as a substrate for ethanol production. For this purpose, different types of nitrogen sources were evaluated, with yeast extract as the best nitrogen source (93% of theoretical yield) as compared to palm oil mill effluent (POME) and sludge powder for the growth of acid tolerant *Saccharomyces cerevisiae* ATCC 26602. Batch and repeated batch fermentation of *S. cerevisiae* ATCC 26602 using OPEFB hydrolysate gave 0.46 g glucose g ethanol⁻¹, representing 87% of theoretical yield with a productivity of about 0.82 g⁻¹ l⁻¹ h⁻¹ and 0.48 g glucose g ethanol⁻¹, representing 89% of theoretical yield with productivity of about 2.79 g⁻¹ l⁻¹ h⁻¹, respectively.

Keywords: Bioethanol, oil palm empty fruit bunch, phase separation system, acid hydrolysis, glucose

INTRODUCTION

Lignocellulose waste material, such as oil palm empty fruit bunch (OPEFB), is well known for its potential as a renewable resource for the production of food, feed and fine chemicals. Approximately 15 million tonnes of OPEFB is

generated annually by palm oil mills in Malaysia (Rahman *et al.*, 2006). In practice, OPEFB is burnt in incinerator to obtain bunch ash or is dumped for mulching in the oil palm plantation. With technologies such as diluted acid hydrolysis and enzymatic hydrolysis, cellulose from waste

Received: 28 January 2010

Accepted: 6 May 2010

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materials is converted into glucose, leaving lignin as a by-product without any further use as value added-product. Most of lignins are burnt for power generation. Only a portion is used for various purposes, such as dispersant, pelletizing materials, molding stabilizers and concrete grinding additives (Funaoka *et al.*, 1995). A new process of separating lignocellulose into lignin and carbohydrate moieties, with the conversion to highly reactive forms at room temperature, has been developed in previous study (Funaoka & Abe, 1989). This process includes phase-separation reaction system comprising of phenol derivatives and concentrated acid. In this process, lignocellulose material is rapidly separated into two main phases, called organic phase and aqueous phase. The acid solution is a solvent for carbohydrate and as a catalyst for the fragmentation and phenolation of lignin, while phenol derivatives act as phenolation agents, a barrier to minimize the attack of acid on the lignin and a solvent for the lignin fraction. Through this process, the lignin is converted into lignophenol. Lignophenol derivatives have currently been used for immobilizing proteins, specifically enzymes and they are also used in fibre composite (Funaoka, 1998). In order to develop the total usage of lignocellulosic materials, the carbohydrate (i.e. the by-product of the lignophenol) production can be utilized rather than left them unused. Currently, ethanol has gained a great interest as an alternative energy due to the concern of global warming and depletion of fossil fuel. Therefore, it is a good approach to convert these carbohydrates from biomass to ethanol. It has been estimated that OPEFB composed of 45-50% cellulose, a sugar polymer made of hexose sugar glucose (Rahman *et al.*, 2006; Ariffin *et al.*, 2008). The objective of this study was to utilize glucose recovered during phase separation process of acid-hydrolysed OPEFB for ethanol production.

MATERIALS AND METHODS

Sample Preparation

Shredded OPEFB was obtained from Seri Ulu Langat Palm Oil Mill, Dengkil, Selangor,

Malaysia. The sample was degreased by soaking it with detergent overnight and washing was repeated until no trace of oil was observed. The sample was then placed in oven at 40°C. The sample was ground and sieved to 40 mesh. The extraction with ethanol:benzene (2:1) was done to remove extractives, such as terpene and tannin in the OPEFB fibre.

Phase Separation System

Ten ml *p*-cresol (a phenol derivative) was added to the treated OPEFB fibre and stirred for 10 minutes. Twenty ml of 72% sulfuric acid was then added to the mixture, and this was followed by vigorous stirring at room temperature (29-30°C) for 60 minutes. The mixture was then centrifuged at 3500 rpm, 25°C for 15 minute, as reported in the previous study by Funaoka *et al.* (1995).

Hydrolysis of Aqueous Phase for Substrate Preparation and Bioethanol Production

The aqueous phase from the phase separation process was separated and subjected to further treatment by adding water and hydrolyzed by boiling for 4 hours or autoclaving at 121°C for 5 minutes. The sample was then neutralized with Ca(OH)₂ and filtered to remove the precipitated CaSO₄. In addition, the sample was also concentrated using an evaporator (EYELA, Japan). The aqueous phase hydrolyzate was used as a substrate for ethanol production, as summarized in *Fig. 1*.

Microorganism and Medium

The yeast *Saccharomyces cerevisiae* ATCC 22062 was used for ethanol fermentation. It was kept at -30°C in Yeast Medium Broth (YMB) with 30% glycerol. The cells were transferred and maintained in the medium containing (in 1 L medium): 10.0 g glucose, 3 g yeast extract, 3 g malt extract, 5 g peptone and 20.0 g agar at 4°C and sub-cultured every 2 weeks. The growth medium consisted of (in 1 l medium) 30.0 g glucose, 5 g yeast extract and 3g peptone. Meanwhile, the fermentation medium consists of

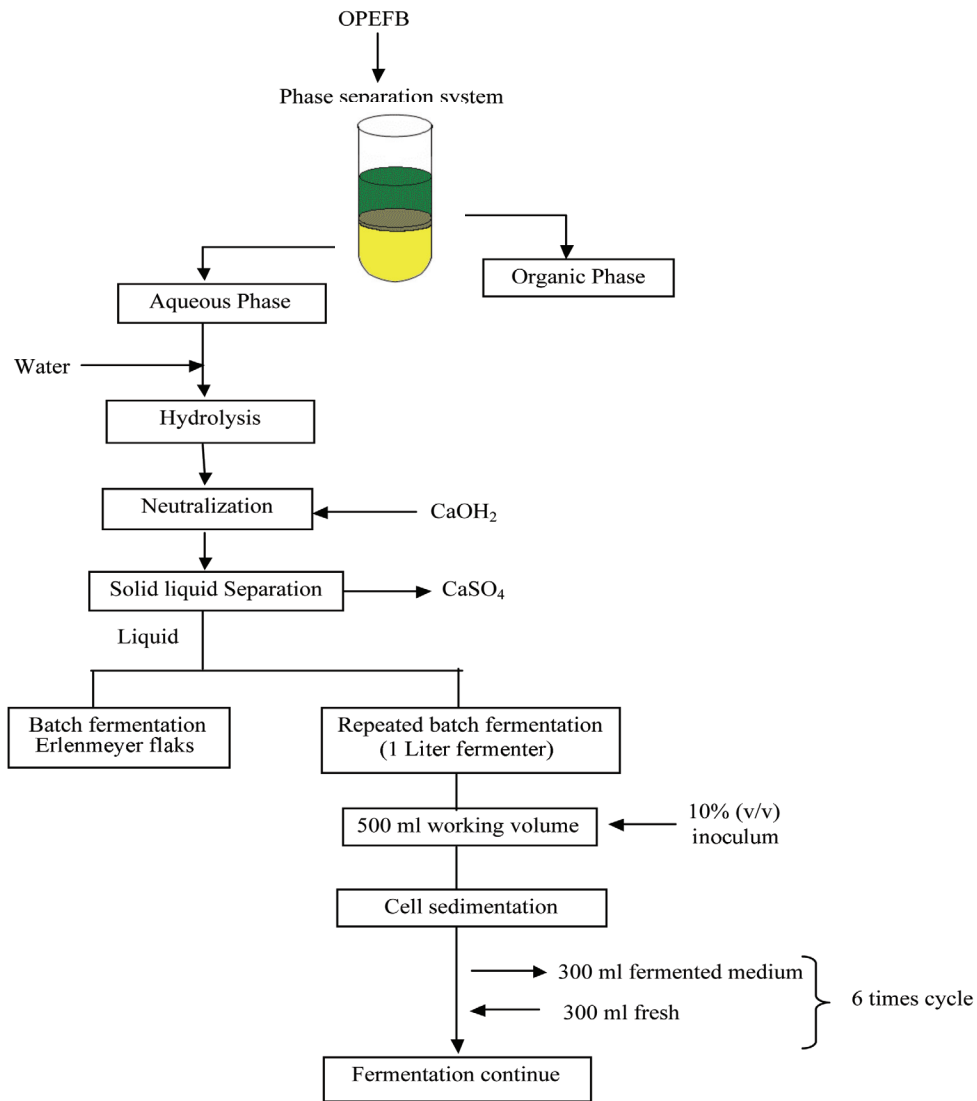


Fig. 1: Flow chart of the overall process for ethanol production from the OPEFB through phase separation system

hydrolysate containing (in 1 L medium) 5 g yeast extract, 5 g KH_2PO_4 , 1.5 g NH_4Cl , 0.7 g Mg_2SO_4 , and 1.7 g KCl. The concentrations of glucose ranging from 32 g l^{-1} to 62 g l^{-1} were used. The fermentation medium was filter sterilized using $0.25\mu\text{m}$ membrane filter.

Samples Analysis

Determination of major components of extracted and non-extracted OPEFB was done by acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (NDF) (Wong *et al.*, 2008).

The concentration of ethanol was analyzed using gas chromatography (Model GC-17A, Shimadzu) with BPX capillary column and flame ionization detection. Meanwhile, the temperature of the injector and detector was set at 150°C and 200°C, respectively. The oven temperature was maintained at 40°C for 1 minute and then raised to 130°C at a gradient of 20°C min⁻¹. Helium gas was used as the carrier gas at a flow rate of 0.5 mL min⁻¹. The ethanol standards were prepared using a mixture of commercial grade ethanol (Wako) and propanol as an internal standard. The amount of carbohydrates was determined according to the phenol sulfuric method (Liu *et al.*, 1972). Viable cell, cell dry weight and glucose composition were measured in the fermentation process as described previously (Ma *et al.*, 2007).

RESULTS AND DISCUSSION

Determination of the OPEFB composition

The results of the OPEFB composition in this study were compared with the previous study (Table 1). Nonetheless, the treatment of OPEFB by extracting with ethanol-benzene did not show significant changes on the major component of the OPEFB (Table 2). The composition of cellulose, hemicellulose and lignin of the extracted and non-extracted OPEFB were about 45.06%, 28.51% and 12.39% corresponding to the composition of the OPEFB done in a previous study by Rahman *et al.* (2006). The composition of the OPEFB was also compared

with the other types of lignocellulosic materials (Table 3). It was found that the OPEFB cellulose composition was corresponded to the cellulose measured in hardwood (pine). However, the OPEFB lignin was found to correspond to the lignin in grass (switch grass). Therefore, it is suggested that the OPEFB could be categorized between hardwoods and grasses in term of their lignocelluloses content. Typically in hardwoods, the composition of cellulose, hemicellulose and lignin were about 40-50%, 15-30% and 10-25%, respectively (Malherbe & Cloete, 2000).

Phase Separation System

For a complete hydrolysis of lignocellulosic materials, the cellulose must be swollen by treatment with concentrated acid such as more than 65% of sulfuric acid or more than 85% of phosphoric acid (Mikame & Funaoka, 2006). For the OPEFB used in this study, the total sugar obtained from acid phase was about 0.73 g, which is 73% of raw material and 98% of total carbohydrate, after 60 minutes of treatment in the phase separation system. A previous study on separating lignocellulose of Yezo spruce (*Picea jezoensis*), through phase separation system by Mikame and Funaoka (2006) whereby the total sugars composition obtained after 60 minutes of treatment, was about 75% of raw materials and 104% of total carbohydrate (Table 4). Based on the simple total material balance (Fig. 2), a small part of carbohydrate was not hydrolyzed in which hydrolysis of cellulose was strongly dependent on the degree of crystallinity

TABLE 1
Major components of the oil palm empty fruit bunch (on a dry basis)

Main Fraction	Composition (%)		
	This study	Ariffin <i>et al.</i> (2008)	Rahman <i>et al.</i> (2006)
Cellulose	45.06	51.28	42.85
Hemicellulose	28.51	28.18	24.01
Lignin	12.39	15.17	11.70
Others (extractives, ash, etc)	14.04	5.37	21.44

The composition of the OPEFB obtained was compared with the values obtained from other references. Note: All data were average of duplicated samples.

TABLE 2
Major components of the oil palm empty fruit bunch, with and without extraction with ethanol-benzene

Main Fraction	With extraction	Without extraction
Cellulose	45.06	45.73
Hemicellulose	28.51	28.47
Lignin	12.39	12.07
Others (ash. etc)	11.03	10.72
Extractives	-	3.01

Note: All data were average of duplicated samples

TABLE 3
Major components of the types of lignocellulose materials (on a dry basis)

Main Fraction	Composition (%)			
	OPEFB	Softwood (Hybrid Poplar)	Hardwood (Pine)	Grasses
Cellulose	45.06	44.55	44.70	31.98
Hemicellulose	28.51	21.90	18.55	25.19
Lignin	12.39	27.67	26.44	18.13
Others (extractives, ash. etc)	14.04	5.88	10.31	24.7

References: Malherbe and Cloete (2002); Hamelink *et al.* (2005)

TABLE 4
Total sugar recovered from the phase separation process

	Total sugar recovered	
	% total carbohydrate	% raw materials
This study	73	98
Mikame and Funaoka (2006)	75	104

and swelling state of cellulose (Xiang *et al.*, 2003). As cellulose contains high crystalline structure, hydrolysis of cellulose structure was not done completely. Therefore, some parts of cellulose, having a high crystalline structure, were not hydrolyzed and remained at interface. The release of glucose was suggested from hemicellulose and cellulose chain. Lignin, which had been transformed into lignophenol, was further purified. As lignophenol was one of the main products of this process, the yield

was also calculated. The amount of lignophenol obtained was 0.13 g lignophenol g lignin⁻¹.

Aqueous Phase Hydrolysate as Substrate

In phase separation system, when the stirring of reaction mixture stopped, the system was quickly separated into the organic phase containing lignophenol derivatives and the aqueous phase containing partially hydrolyzed carbohydrates. Heating time and temperature were examined to

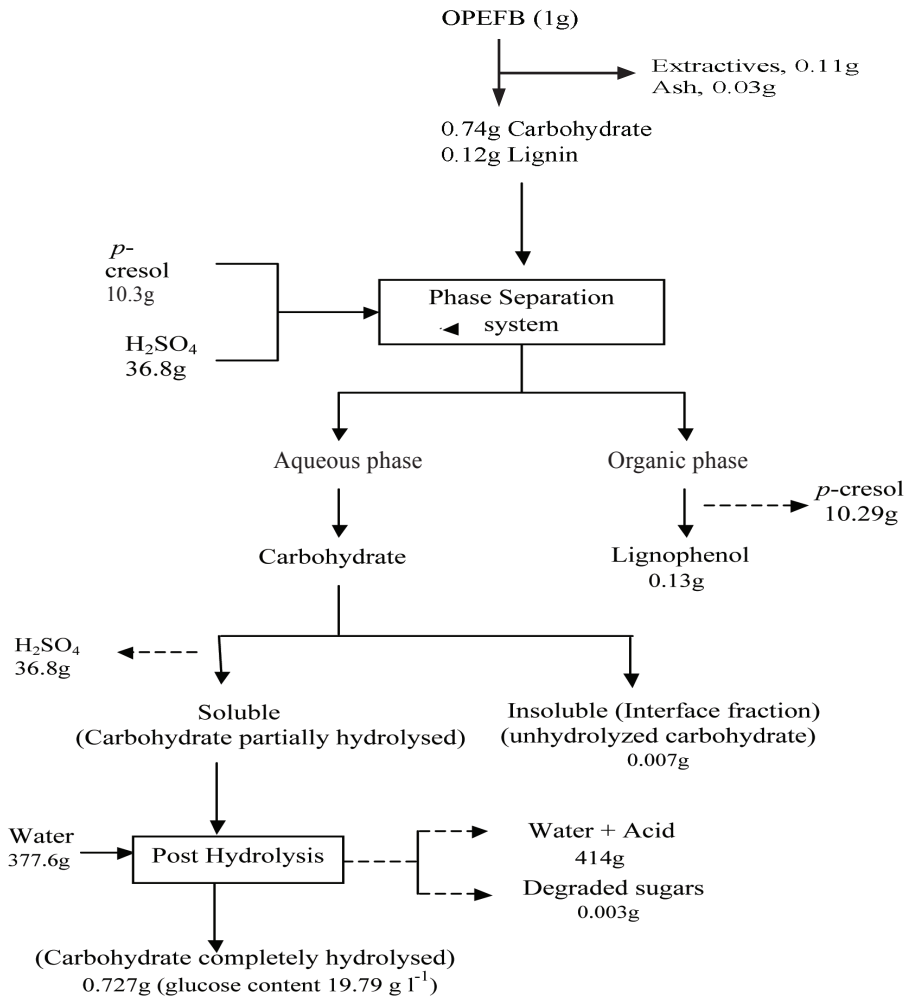


Fig. 2: The simplified materials balance of lignophenol and sugar in the phase separation system process

get maximum amount of glucose. The maximum amount of glucose obtained was 17.91 g l⁻¹ yielding 79.1% based on cellulose and 48.1% based on raw material respectively, when hydrolysis occurred at 100°C. It was reported that, 80% of the lignin was separated into the organic phase and 87% carbohydrates were in aqueous phase (Funaoka *et al.*, 1995; Funaoka,

1998). With very small quantities of dissolved lignin, the hydrolysis process of cellulose to sugar becomes a simple process and can occur in a less acid concentration mixture (Iranmahboob *et al.*, 2002). It was reported that the sugar present in aqueous phase consisted of a mixture of oligosaccharides with various molecular sizes (Mikame & Funaoka, 2006). Therefore,

the aqueous phase was subjected to hydrolysis after acid dilution in order to complete the hydrolysis. In the hydrolysis, heating is needed to accelerate the hydrolysis process in order to completely convert oligosaccharides into monosaccharides. Meanwhile, the production of glucose gradually increased by heightening the temperature. At high acid concentration and heating time, however, cellulose may be degraded and oxidized during the heating process. Thus, optimizing concentration of acid and heating time should be applied during the hydrolysis process.

Hydrolysis of Oligosaccharides in Aqueous Phase for Glucose Production

In order to obtain the maximum amount of glucose, different types of treatment on acid concentrations were examined (see Table 5). Based on the preliminary result, it was found that the best temperature of hydrolyzing carbohydrate in the aqueous phase was 100°C. The effective hydrolysis of carbohydrate is mostly depending on acid concentration and heating time. Meanwhile, the maximum amount of glucose obtained in the hydrolysis of aqueous phase was 19.8 g l⁻¹ when autoclaved at 121°C with 4% acid and 18.5 g l⁻¹ when boiling at 100°C with 3% acid. Nonetheless, only 13.7 g l⁻¹ of the glucose was obtained for the hydrolysis using 4%

acid by boiling at 100°C. The hydrolysis at 5% acid concentration for autoclaving at 121°C and boiling at 100°C gave the glucose concentration of 15.8 g l⁻¹ and 14.9 g l⁻¹, respectively. The results showed that the hydrolysis efficiency by autoclaving gave a higher yield than boiling.

ETHANOL FERMENTATION

The Effect of Nitrogen Source on Ethanol Production

Yeast extract is typically used as a stimulating factor for yeast growth. Since yeast extract is rather expensive, therefore the effects of POME and sludge powder which are wastes from the oil palm industry were therefore evaluated together with yeast extract as nitrogen source in the batch culture in this study. Fifty g l⁻¹ of technical grade glucose was used in this fermentation. The results showed that yeast extract was the best nitrogen source with ethanol production up to 93% of the theoretical yield. Meanwhile, POME gave 63% ethanol production based on the theoretical yield (Table 6). In the case of sludge powder, the ethanol yield was very low. Therefore, POME might have potential to be used as a nitrogen source as it is abundantly available in the oil palm industry.

TABLE 5
Different types of pre-treatment to maximize the production of glucose

Treatment	Yield (%) (g glucose g cellulose ⁻¹)	Yields (%) (g glucose g carbohydrate ⁻¹)	Glucose Concentration (g l ⁻¹)
a) Boiling			
3%	82.1	50.2	18.48
4%	60.7	37.1	13.66
5%	70.3	42.7	15.82
b) Autoclaved			
3%	57.0	34.8	12.82
4%	87.9	53.8	19.79
5%	66.4	40.6	14.94

The sample was treated through hydrolysis by boiling at 100°C for 4 hours or autoclaving at 121°C for 5 minutes.

TABLE 6
Ethanol production by *Saccharomyces cerevisiae* ATCC 22062 with 50 g l⁻¹ glucose using different nitrogen sources in the Erlenmeyer flask fermentation

Nitrogen source (5g l ⁻¹)	Ethanol concentration (g l ⁻¹)	Productivity of ethanol (g l ⁻¹ h ⁻¹)	Ethanol yield (g ethanol g glucose ⁻¹)	Ethanol yield (% of theoretical)	Viable cell (cell ml ⁻¹)
Yeast extract	23.6	0.98	0.48	93	2.16 x 10 ⁸
POME	14.9	0.47	0.32	63	7.8 x 10 ⁷
Sludge powder	7.51	0.24	0.16	23	5.8 x 10 ⁷

TABLE 7
Batch fermentation for ethanol production by *Saccharomyces cerevisiae* ATCC 22062 in the Erlenmeyer flask

Substrate	Productivity of ethanol (g l ⁻¹ h ⁻¹)	Ethanol yield (g ethanol g glucose ⁻¹)	Ethanol yield (% of theoretical)	cell mas (g l ⁻¹)
Technical grade glucose	0.87	0.50	93	7.23
OPEFB glucose	0.82	0.46	87	3.63

Ethanol Production of Glucose from OPEFB in Batch and Repeated-batch Fermentation

The ethanol production by *Saccharomyces cerevisiae*, using glucose from OPEFB by batch fermentation, gave yield up to 82% (Table 7). In several studies, the presence of inhibitory factors in the acid hydrolysis of lignocellulose has been the main concern due to its toxicity in ethanol fermentation (Palmqvist *et al.*, 1999; Martinez, 2000). In this study, however, high yield of ethanol was produced and this indicated that small amounts of inhibitors were generated from the hydrolysis through the phase separation system. The extractive compounds, such as terpene and tannin generated during hydrolysis, and which are toxic to fermentation process, were removed through extraction by ethanol-benzene. In this study, the lignin and its degradation products such as polyaromatic compounds were separated from the hydrolysate in the phase separation process. Therefore, the effects of inhibitory compounds on ethanol fermentation have been reduced and the high yield of ethanol could be obtained. Previously, it was shown that the detoxification of diluted

acid hydrolysate, using CaOH₂ to remove the inhibitory compound and the fermentability of the hydrolysate, was increased, with ethanol yield more than 90% (Milatti *et al.*, 2002). Through the repeated batch fermentation using 62 g l⁻¹ OPEFB glucose, it was found that the maximum ethanol yield increased up to 88% of theoretical value. However, the productivity of ethanol production was found to be higher than in the batch fermentation, which was 2.79 g⁻¹ l⁻¹ h⁻¹. Six time of batch cycle had been done and showed consistent yields throughout the fermentation process (Fig. 3). It was suggested that the increased of productivity with repeated batch fermentation due to the adaptation of yeast on the hydrolysate condition.

CONCLUSION

Complete separations of lignin and carbohydrate were obtained in the phase separation system, comprising of phenol derivatives and concentrated acid. Based on the material balance, it was found that most of the lignin dissolved in organic phase and 0.73 g sugars

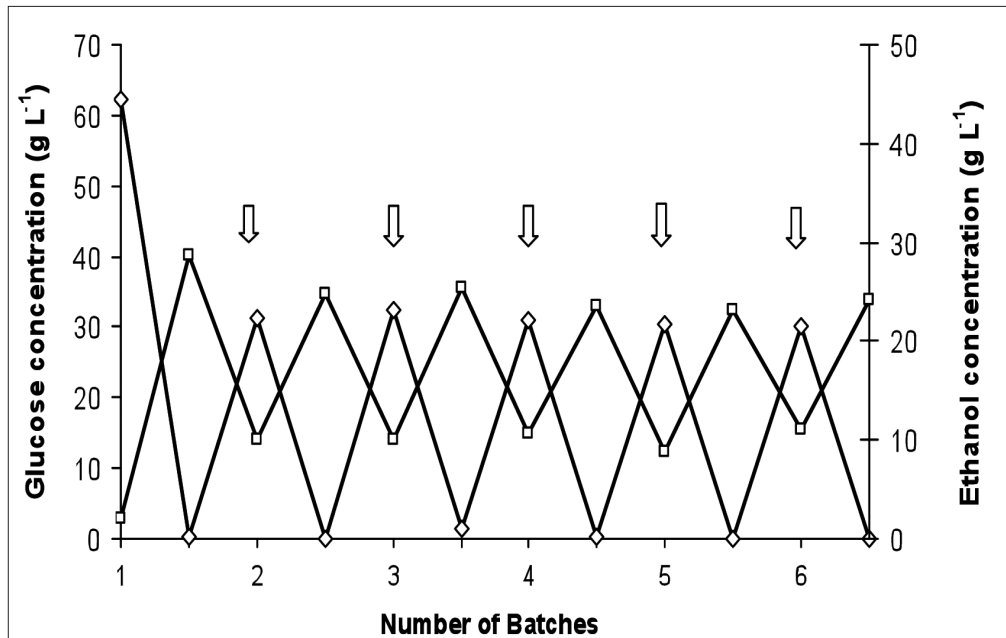


Fig. 3: Time course for ethanol fermentation using 1L bioreactor in repeated-batch fermentation. (EFB's glucose; concentration of ethanol produced;) Arrows indicates the change of one batch to the next.

g carbohydrate⁻¹ was dissolved in the aqueous phase. Meanwhile, oligosaccharides, which were mainly from cellulose in the aqueous phase, were successfully hydrolyzed in diluted acid to form glucose. The maximum glucose produced in the aqueous phase was 87.9% of cellulose content, which was 53.8% of raw material by autoclaving at 121°C for 5 minutes in 4% acid concentration. The sugar was fermented by *Saccharomyces cerevisiae* in the batch and repeated-batch fermentations to produce about 0.46 g glucose g ethanol⁻¹ and 0.47 g glucose g ethanol⁻¹, with the productivity of about 0.82 g l⁻¹ h⁻¹ and 2.79 g l⁻¹ h⁻¹, respectively. It was suggested that sugar, particularly glucose recovered from the OPEFB through phase separation process, had successfully been fermented to produce high yield of ethanol.

ACKNOWLEDGEMENTS

This project was supported by FELDA-UPM-KIT biomass project and the Japan Society for Promotion of Science (JSPS) through Asia core program. The authors wish to thank Universiti Putra Malaysia and the Japanese government for their support.

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