Biobutanol Production by *Clostridium acetobutylicum* ATCC 824 Using Oil Palm Frond (OPF) Juice

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Abstract—The aim of the present paper is to study the production of biobutanol from oil palm frond (OPF) juice by *Clostridium acetobutylicum* ATCC 824. The fermentation was carried out anaerobically in batch mode. The effect of total sugars concentration in OPF juice (10 – 60 g/L), temperature (32 – 42 ^{o}C), and initial pH (5 – 7) were investigated. The highest biobutanol yield (0.24 g/g) was obtained at 50 g/L of total sugars concentration, 37^{o}C, and pH 6 with a biobutanol production value as high as 9.24 g/L. However, the production decreased to 3.09 g/L with a yield of 0.13 g/g when a higher value of initial total sugars concentration (60 g/L) was used. On the other hand, the production yield also low when the fermentation conditions were set at 42^{o}C (0.04 g/g) and pH 5 (0.13 g/g). The results presented herewith showed that OPF juice is promising as a renewable carbon source for biobutanol production. The suitable fermentation environment can enhance the yield of biobutanol production.

Keywords— Biobutanol; Clostridium acetobutylicum; oil palm frond juice

1. Introduction

Energy and environmental concerns such as global warming, attributed to the use of fossil fuels, has brought significant attention for the production of biofuels from biomass. This was due to the fact that biomass is renewable, abundant, and limitless, and its use is often regarded as carbon neutral, the combustion of biofuels derived from biomass releases fewer greenhouse gases, such as carbon dioxide. In addition to that, the production of biobutanol from biomass has brought significant attention due to the increasing crude oil prices and growing awareness of environmental problems such as global warming. Since fossil fuel is not renewable, the source will be depleted in the near future. For this reason, most of the countries in the world are pursuing alternative fuel sources to lessen the dependency on fossil fuel.

The oil palm (*Elaeis guineensis*) was grown in Malaysia since 1870 and nowadays becoming as world's second largest producer (39% of world production) and exporters (44% of world exports) with production 10.8 million tonnes per year [1,2]. Oil palm industry generates about 70 million ton of biomass residue including oil palm frond (OPF), oil palm trunk (OPT), empty fruit bunches (EFB), palm kernel shells, mesocarp fibre and palm oil mill effluent (POME) [2]. OPF is one of lignocellulosic biomass which is prune during harvesting to allow cutting of ripe fruit branches, thus, there is no harvesting cost required. Maximising energy recovery from OPF wastes is desirable because of OPF is currently underutilised which create a major disposal problem for both environmental and economic reasons. OPF juice contained renewable sugars such as glucose, sucrose, and fructose which can be obtained by using simple sugarcane pressing machine [3]. It was reported that 50 wt. % of OPF juice (12.5 kg) could be obtained from 25 kg of fresh OPF and the sugars content was as high as 53.95 g/l of glucose, 20.46 g/l of sucrose and 1.68 g/l of fructose [3]. OPF juice was reported as favorable substrate for fermentation for the production of bioplastics and biofuel [3, 4]. Therefore, this lignocellulosic biomass has potential to be utilized into other valued products efficiently and effectively like biobutanol.

The most common biobutanol producing bacteria are C*lostridium acetobutylicum, Clostridium saccaroperbutylacetonicum, Clostridium saccharoacetobutylicum, Clostridium aurantibutyricum, Clostridium pasteurianum, Clostridium sporogenes, Clostridium cadaveris, and Clostridium tetanomorphum* [5]. *C. acetobutylicum* was the most reported in acetone-butanol-ethanol (ABE) fermentation for synthesis of biobutanol with higher yields [5, 6]. It is an anaerobic, gram-positive, and spore-forming microorganism. It has an ability to produce acetone, butanol, ethanol, as a final product under anaerobic condition, using different carbohydrate sources including monosaccharides, and polysaccharide [6].

Renewable biobutanol is produced from ABE fermentation that is a proven industrial process that uses solventogenic clostridia to convert sugars or starches into solvents. The fermentation occurs in two stages; the first is a growth stage in which acetic and butyric acids are produced and the second stage is characterized by acid re-assimilation into ABE solvents. This study was carried out to explore the potential of OPF juice for production of biobutanol and to study the effect of some environmental parameters including total initial sugars concentration, initial pH medium and temperature by *C. acetobutylicum* ATCC 824.

2. METHODOLOGY

A. Strain and Culture Media

Clostridium acetobutylicum ATCC 824 was obtained from American Type Culture Collection (ATCC) in freeze-dried form and maintained on Reinforced Clostridium Medium (RCM) broth. Spore suspension was prepared by transferring 1 mL of glycerol stock into 90 mL RCM for 3 days at 37^{o}C under anaerobic conditions. This culture was then transferred on RCM medium with subsequent heat shock for 90 s in 90^{o}C water bath and incubated at 37^{o}C for 18-24 h to be used as inoculum. The inoculum was deemed to be ready for fermentation when the optical density was between 1.5 and 2.0.

B. Substrate and Medium Preparation

OPF juice was prepared by pressing the fresh OPF (without leaves), and centrifuge to remove the precipitate by following the previous method described earlier by Zahari et al. [3]. 50 kg of fresh OPF (without leaves) were collected from oil palm plantation at Felda Lepar Hilir, Kuantan, Pahang. Fresh OPF was cut into small size and pressed using sugarcane pressing machine. Then, OPF juice was centrifuged at 10,000 rpm for 10 minutes and filtered to remove the solid particles. The precipitate (pellet) was decanted and the supernatant (OPF juice) was used in the fermentation. OPF juice was then distributed up to required working volume into 125 ml serum bottles, sparged with oxygen-free nitrogen gas, sealed and sterilized at 121^{o}C for 15 minutes. It was then mixed with synthetic P2 medium in known proportion to prepare the final fermentation medium. Synthetic P2 medium had the following composition (in g/L): yeast extract, 5; KH_2PO_4, 0.5; K_2HPO_4, 0.5; para aminobenzoic acid, 0.001; thiamin, 0.001; biotin, 1 x 10^{-5}; $MgSO_4.7H_2O$, 0.2; $MnSO_4.7H_2O$, 0.01; $FeSO_4.7H_2O$, 0.01; NaCl, 0.01; and ammonium acetate, 2.2.

C. Preparation of sugar concentration

Table 1 shows the sugar concentration of renewable sugars in OPF juice determined by HPLC. It was observed that glucose was the major sugar component in OPF juice at 48.19 g/L. Meanwhile, the other sugars were sucrose (8.48 g/L) and fructose (1.68 g/L). The high sugar content indicates its suitability as a renewable carbon source for the production of biobutanol through ABE fermentation. The total sugar concentration was then diluted into the required concentration based on each experiment.

Table 1: Amount of sugar contained in OPF juice

OPF juice	Sugar (g/L)			Total sugar (g/L)
	Glucose	Sucrose	Fructose	
Sample 1	47.78	8.37	11.78	67.93
Sample 2	48.60	8.58	12.04	69.22
Average	48.19	8.48	11.91	68.58

D. Biobutanol production

The fermentation was conducted in 125 ml serum bottles containing 90 ml of medium flushed with N_2 gas and sterilized at 121^{o}C for 15 min. Upon cooling, 10% (v/v) inoculum of *C .acetobutylicum* were added into the medium aseptically prior to fermentation.. The fermentation conditions (initial total sugars concentration, initial pH medium, and temperature) were varied based on the set of experiments. 2 ml of samples were withdrawn at intervals for analysis of acetone, butanol, ethanol (ABE), organic acids and sugar. The samples were centrifuged at 8,500 rpm, 4^{o}C for 20 min before analysis.

E. Analytical methods

The products including solvents and acids were analyzed by gas chromatography (Agilent Technologies, 6890N network GC system). A microliter of the sample was injected into a 30 m x 0.32 mm x 0.5 µm HP-INNOWAX capillary column at 250^oC. The column carrier was helium, 40 cm/sec, 11.7 psi (60^oC) with 2.5 ml/min constant flow. A flame ionization detector (FID) at 275^oC was used for signal detection. Glucose, sucrose, and fructose were determined by HPLC(Agilent; 1200) with Rezex ROA – organic acid H+ (8%) column (Phenomenex) (300 x 7.80 mm) with a flow rate of 0.5 ml/min and RI detector at 30^oC. The mobile phase consists of 0.005 M H_2SO_4, recommended for the column used.

3. RESULTS AND DISCUSSION

A. Biobutanol production from OPF juice

The results for biobutanol production and other solvents from OPF juice containing 50 g/L of total initial sugars concentration by *C.acetobutylicum* ATCC 824 is shown in Table 2.The fermentation using a synthetic medium containing the same total initial sugars concentration as in OPF juice was also performed as the control. The results showed that the biobutanol and ABE production from OPF juice were almost comparable with that produced from the synthetic sugars at similar total initial sugars concentration. As shown in Table 1, the biobutanol production from OPF juice was 9.24 g/L, with a total ABE concentration of 15.69 g/L, whereby the biobutanol and ABE production from synthetic sugars were 10.91 g/L and 17.69 g/L, respectively.

Table 2: Comparison of biobutanol production using OPF juice and synthetic sugars.

Parameters	Medium	
	Control	OPF Juice
Acetone (g/L)	5.43	4.87
Butanol (g/L)	10.91	9.24
Ethanol (g/L)	1.35	1.59
Total ABE (g/L)	17.69	15.69
Acetic acid (g/L)	2.06	3.86
Butyric acid (g/L)	1.04	0.60
Total acid (g/L	3.10	4.46
Sugar consumption (g/L)	40.95	38.29
Butanol productivity (mg/L/h)	75.76	64.14
ABE productivity (mg/L/h)	122.85	108.96
Butanol yield (g butanol/g sugar)	0.27	0.24
ABE yield (g ABE/g sugar)	0.43	0.41

B. Effect of sugar concentration

To investigate the effect of total initial sugars concentration in the OPF juice, the OPF juice was prepared to obtain various initial levels of total sugars ranging from 10 to 60 g/L. Table 3 reveals that the biobutanol production was increased when the total initial sugars in OPF juice increased from 10 g/L until 50 g/L and decreased when the total initial sugars in OPF juice were set at 60 g/L.. The biobutanol production increased from 0.17 to 9.24 g/L with an improved yield from 0.03 to 0.24 g/g. The highest productivity of biobutanol and ABE obtained were 64.14 and 108.96 mg/L/h, respectively. At total initial sugars concentration of 60 g/L, the production of biobutanol and ABE drastically decreased to 3.09 and 4.90 g/L only. Similar to this study, Khamaiseh et al. [7] found that the production of biobutanol increased drastically with increasing date fruit concentration from 10 g/L to 50 g/L with highest amount of butanol 11 g/L. The low amount of solvents production at low initial sugar concentration is because of low amount of carbon source to be consumed in the fermentation [7]. The lower production at higher level date fruit concentration from 60 g/L to 100 g/L is reported that substrate inhibition gets predominant at higher sugar concentration and higher concentration of acids produced during acidogenesis stage like in this study which lower production at 60 g/L of sugar concentration in OPF juice [7].

Komonkiat and Cheirsilp [8] also reported that the butanol production increased from 5.93 to 12.76 g/L at initial sugar concentration 30 g/L and 50 g/L in the oil palm sap. The production of butanol drastically decreased when increasing the sugar concentration up to 70 g/L and 90 g/L with 3.02 and 2.97 g/L of butanol only. This was due to the inhibitory effect of a high concentration of sugar [8].

Table 3: Effect of sugar concentration in OPF juice on biobutanol production.

Parameters	Different total sugar concentration (g/L)					
	10	20	30	40	50	60
Acetone (g/L)	0.00	0.43	1.12	2.11	4.87	1.52
Butanol (g/L)	0.17	1.23	2.73	5.42	9.24	3.09
Ethanol (g/L)	0.07	0.22	0.40	0.60	1.59	0.29
Total ABE (g/L)	0.24	1.88	4.25	8.14	15.69	4.90
Acetic acid (g/L)	2.43	4.73	4.25	5.43	3.86	7.97
Butyric acid (g/L)	1.65	3.52	3.75	2.30	0.60	4.46
Total acid (g/L	4.07	8.25	8.00	7.74	4.46	12.42
Sugar consumption (g/L)	6.34	18.63	23.56	32.96	38.29	24.06
Butanol productivity (mg/L/h)	1.18	8.64	18.98	37.66	64.14	21.46
ABE productivity (mg/L/h)	1.67	13.06	29.51	56.50	108.96	34.05
Butanol yield (g butanol/g sugar)	0.03	0.07	0.12	0.16	0.24	0.13
ABE yield (g ABE/g sugar)	0.04	0.10	0.18	0.25	0.41	0.21

C. Effect of pH

Initial pH of the medium has been shown to perform a significant effect on fermentation process and the production of biobutanol from OPF juice by *C. acetobutylicum* ATCC 824.. Initial pH values of 5, 6 and 7 were tested in this experiment to determine the most suitable and optimum initial pH for solvents production. As shown in Table 4, the highest amounts of acetone, butanol, and ethanol were produced at pH 6, with 4.87 g/L, 9.24 g/L and 1.59 g/L respectively. Compare to the other initial pH 5 and 7, the butanol production was found to be lower compared to the production at pH 6 with only 4.62 g/L and 4.34 g/L. This result was similar as reported by Ibrahim et al. [9] which got higher amounts of ABE at pH 6 compared to pH 5, 5.5, and 6.5. Initial pH 5.5 – 6 was speculated as the best metabolic state for solvent production because it can robust growth without creating an early inhibitory acidic environment which can cause acid crash in solventogenesis stage [10].

Table 4: Effect of pH on biobutanol production

Parameters	Different pH		
	5	6	7
Acetone (g/L)	1.60	4.87	1.68
Butanol (g/L)	4.62	9.24	4.34
Ethanol (g/L)	0.44	1.59	0.41
Total ABE (g/L)	6.66	15.69	6.43
Acetic acid (g/L)	5.69	3.86	5.96
Butyric acid (g/L)	3.25	0.60	3.05
Total acid (g/L	8.94	4.46	9.00
Sugar consumption (g/L)	37.36	38.29	31.98
Butanol productivity (mg/L/h)	32.06	64.14	30.16
ABE productivity (mg/L/h)	46.27	108.96	44.68
Butanol yield (g butanol/g sugar)	0.13	0.24	0.14
ABE yield (g ABE/g sugar)	0.18	0.41	0.20

D. Effect of temperature

Table 5 shows the effect of temperature on the performance of OPF juice as fermentation substrate for ABE production by *C.acetobutylicum.* It was observed that the butanol increased with an increased in temperature at 32^{o}C and 37^{o}C. The production was 6.15 g/L and 9.24 g/L. At higher temperature 42^{o}C, the butanol production was found at the lowest value, as low as 0.87 g/L. *C.acetobutylicum* have lost the ability to continue the acidogenesis and solventogenesis stage at high temperature [11]. Therefore, the most favorable temperature for *C.acetobutylicum* to produce solvents was at 37^{o}C.

Table 5: Effect of temperature on biobutanol production.

Parameters	Different temperature (°C)		
	32	37	42
Acetone (g/L)	2.36	4.87	0.32
Butanol (g/L)	6.15	9.24	0.87
Ethanol (g/L)	0.63	1.59	0.18
Total ABE (g/L)	9.14	15.69	1.38
Acetic acid (g/L)	5.81	3.86	9.05
Butyric acid (g/L)	2.18	0.60	3.78
Total acid (g/L	7.99	4.46	12.82
Sugar consumption (g/L)	45.21	38.29	20.79
Butanol productivity (mg/L/h)	42.73	64.14	6.04
ABE productivity (mg/L/h)	63.49	108.96	9.56
Butanol yield (g butanol/g sugar)	0.14	0.24	0.04
ABE yield (g ABE/g sugar)	0.20	0.41	0.07

4. CONCLUSIONS

This study has shown that OPF juice can be used as renewable substrate for butanol production. The maximum productivity of butanol was obtained 64.14 mg/L/h using 50 g/L of sugar concentration in OPF juice with total ABE produced was 15.69 g/L. Based on the one factor at time (OFAT) method, it was showed that the most favourable environment conditions for the fermentation of biobutanol from OPF juice were at 50 g/L of total sugars concentration, initial pH 6 and temperature 37^{o}C. In future, an experiment using factorial analysis will be conducted in order to further clarify the interaction effects of several parameters on biobutanol production using OPF juice.

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