Biobutanol production from oil palm frond juice in 2 L stirred tank bioreactor with in situ gas stripping recovery

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Biobutanol production from oil palm frond juice in 2 L stirred tank bioreactor with in situ gas stripping recovery

F M Asri, N Masngut and M A K M Zahari*

Faculty of Chemical and Process Engineering Technology, College of Engineering Technology, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Malaysia.

*E-mail: ahmadkhushairi@ump.edu.my

Abstract. Biobutanol has generated renewed interest of being a sustainable alternate fuel. As butanol can fully miscible with diesel fuel even at low temperatures, better fuel extender than ethanol, low vapour pressure, low miscibility with water and has higher price, making it most potential solvent compared to acetone and ethanol. Previous study shows that biobutanol can be biosynthesized from oil palm frond (OPF) juice by *Clostridium acetobutylicum* ATCC 824 in the lab scale experiment. Thus, this paper focused on the synthesis and characterization of the OPF juice as fermentation substrate for biobutanol production in 2 L batch fermentation using 50 g/L OPF juice. 14.0 g/L biobutanol was produced in batch fermentation. The research was further extended with fed-batch experiment and in situ gas stripping. 71.5% acetone, 36.5% biobutanol and 18.4% ethanol were stripped out from the fermentation broth during first gas stripping interval (42-48 h). After 120 h, total of 395 g/L biobutanol was stripped out. Thus, it was proven that OPF juice can be used as an alternative carbon source for biobutanol production while fed-batch fermentation with in situ gas stripping method improved the biobutanol production from OPF juice.

1. Introduction

The United States Department of Energy (DOE) and United States Department of Agriculture (USDA) define sustainable biofuels as production of biofuels which are economically competitive, preserve the natural reserves, reduce greenhouse emissions and secure social well-being. According to International Energy Agency (IEA) report, biofuels be it liquid or gaseous fuels, helped in reducing CO_2 emissions in the transportation sector. Currently, biofuels contribute only 2% of energy share in transportation, however, due to rapid growth in the biofuels industry, it is predicted that biofuels would supply 27% of the total transport fuel need by 2050 [1].

Butanol is expected to play a major role in the next generation of biofuels [2]. Biobutanol which is butanol derived from fermentation, has been claimed to be “superior biofuel”, as it can be blended into standard gasoline similarly to ethanol but with several advantages. These include higher energy content and lower vapour pressure, which make storage and transportation easier. It is also immiscible with water, has a better blending ability with gasoline and diesel fuel, and can be used in conventional internal combustion engines without modification [2]. With scientific advances in microbiology and dramatic improvements in process technology nowadays, the advanced ABE process is able to produce higher purity n-butanol with a carbon footprint that is over 45% better than petroleum-based butanol.

Despite these promising results, there are some drawbacks that affect its widespread use such as the high cost of substrates, low productivity, low concentrations (less than 20 g/L) due to solvent toxicity,

and the high cost of product recovery [3]. High cost of the substrate, low productivity and product yield due to slow fermentation, and uneconomical product recovery are the three areas of greatest factors that affect the economic decline of butanol fermentation [2, 4, 5]. In order to reintroduce an environmentally safe biological process, a cost-effective non-food feedstock with a successful genetically engineered *Clostridia* strain and an effective recovery method are needed.

Previous study shows that biobutanol can be biosynthesized from oil palm frond (OPF) juice by *Clostridium acetobutylicum* ATCC 824 in the lab scale experiment [6]. Under optimized conditions, the highest biobutanol yield was 0.3054 g/g after 144 hours of incubation period obtained in 100 mL serum bottle. However, the potential of OPF juice for biobutanol production at 2 L bioreactor scale has yet to be studied. One of the major bottlenecks faced many researchers to produce biobutanol in large scale is the problem of scaling up the process for industrial scale production. For instance, most of the fermentation process performance could not sustain at larger scale compared to the lab scale. In addition to that, there was no research conducted on the biobutanol recovery from the previous work using oil palm frond juice.

One of the efforts to overcome the issue of butanol toxicity is by coupling the fermentation with in situ butanol recovery. Among the available separation technologies, gas stripping appears to be more economical and superior for separating butanol from ABE fermentation broth. However, it is not applicable to the highly concentrated-substrate batch fermentation over 162 g/L [7] due to the substrate inhibition. In this case, fed-batch fermentation appears to solve the substrate toxicity problem. It is an industrial technique where low substrate concentration is used at initial and concentrated substrate was added after the substrate is consumed to keep the substrate inhibitory under levels. The limitation to fed-batch fermentation is, it can cause butanol toxicity. Therefore, fed-batch fermentation with in situ product recovery methods should be carried out simultaneously to avoid butanol toxicity.

2. Methodology

2.1. Chemical and biological materials

All chemicals are of analytical grade. *Clostridium acetobutylicum* ATCC 824 was procured from American Type Culture Collection (ATCC) in freeze-dried form.

2.2. OPF juice preparation

Fresh OPF (without leaves) was shredded and pressed using conventional sugarcane press machine repeatedly to extract the juice. The obtained juices were subsequently filtered to remove fibrous debris. It was then centrifuged at 15,000 × *g* for 15 min at 4 °C (Thermo Fisher Scientific, NC, USA) and the supernatant was filtered using a mixed cellulose ester membrane filter with the pore size between 3 and 5 µm (Cole Parmer, Illinois, USA). The supernatant was analyzed using High Performance Liquid Chromatography (HPLC) to determine the sugar content and its elemental constituents using CNHS Analyzer (vario MACRO cube, Elementar, Germany) following ASTM D-5291 method [8]. Nutrients and heavy metals in the OPF and OPF juice were determined using in-house method based on APHA 3010 and the content was then determined using Inductively Coupled Plasma (ICP) (Perkin Elmer, 7300 DV, USA).

2.3. Reinforced Clostridial Medium (RCM) Preparation

RCM was used to revive the bacteria and for inoculum development. 38 g of RCM powder was liquefied in 1 L of deionized water. Solutions were distributed each into 100 mL serum bottles and sparged with oxygen-free nitrogen gas. Serum bottles were fitted with butyl rubber stopper and sealed with aluminium cap before sterilized at 121 °C for 15 min.

2.4. P2 medium

P2 medium containing buffer (KH_2PO_4, K_2HPO_4, and $C_2H_7NO_2$), minerals ($MgSO_4$-$7H_2O$, $MnSO_4$-H_2O, $FeSO_4$-$7H_2O$, and NaCl) and vitamin (*para*-amino-benzoic acid, thiamine, biotin) was prepared according to table 1 [9].

Table 1. Composition of P2 medium [9].

Nutrient contents	Composition (g/L)
Yeast extract	1
Potassium dihydrogen phosphate, KH_2PO_4	0.5
Dipotassium hydrogen phosphate, K_2HPO_4	0.5
Ammonium acetate, $C_2H_7NO_2$	2.2
Magnesium sulfate heptahydrate, $MgSO_4$-$7H_2O$	0.2
Manganese sulfate heptahydrate, $MnSO_4$-H_2O	0.01
Iron (II) sulfate heptahydrate, $FeSO_4$-$7H_2O$	0.01
Sodium chloride, NaCl	0.01
Para-amino-benzoic acid	1 mg
Thiamine	1 mg
Biotin	0.01

2.5. Inoculum Preparation

The inoculum was prepared from a *Clostridium acetobutylicum* spore suspension. The spore suspension was heat-shocked by immersing in 90 °C water bath for 90 s before used. After that, inoculum was initiated by inoculating 10% (v/v, volume of spore suspension per volume of media) into sterile deoxygenated RCM aseptically. The mixture was allowed to grow in static conditions for 16-18 h at 37 °C. The inoculum was deemed to be readily used in the fermentation when the optical density at 600 nm gave a reading between 1.5 and 2.0 and its pH was in the range of 5.2-5.5 [10].

2.6. Research design

A five-level-three-factor central composite design (CCD) was employed in this study. The variable and the selected levels for the fermentation process were: inoculum size (10-30%); temperature (27-47 °C); agitation speed (0-200 rpm). The research design was performed using software Design Expert Version 7.1.6. (Stat-Ease Inc., Statistic made easy, Minneapolis, MN, USA).

2.7. Batch fermentation in 1 L Schott Bottle

The anaerobic batch fermentation was conducted in 1 L Schott (Duran) bottle using 50 g/L of total sugars in OPF juice with modified upper cap consisted of both inlet and outlet ports; one for sample withdrawal and nitrogen sparging, the other one for by-product gas out. The initial pH of OPF juice was adjusted to pH 6.0 with an addition of 2M NaOH before autoclaved. Upon cooling the medium, the bottle was sparged with nitrogen to remove the oxygen. 1 g/L of autoclaved yeast extract and filter-sterilized P2 solutions were aseptically added into the sterilized medium. Subsequently, the batch culture was initiated by inoculation of 16-h grown *Clostridium acetobutylicum* ATCC 824 into the medium. The fermentation bottles were kept in an incubator shaker (Scigenics Biotech, Orbitek) under specific temperature control and different agitation rate as per CCD design table generated by the software. Total 20 set of experiments were done under uncontrolled pH and each experiment was conducted in duplicate and measurements were average values.

2.8. Fed-batch fermentation in 2 L stirred tank bioreactor

Fed-batch fermentation with in situ gas stripping product recovery was set up as in figure 1. Fed-batch fermentation was carried out at 1.5 L working volume. The initial pH of the fermentation broth was adjusted to pH 6.0. The batch culture was initiated by inoculation of medium with 20% (v/v, volume of fresh inoculum per volume of media). Nitrogen gas was flushed through the broth to initiate anaerobic condition. When sugar concentration reached below 20 g/L in the fermentation broth, a fresh OPF juice, additional yeast extract and P2 medium was fed for extended fed-batch fermentation. Throughout the process, the fermentation was maintained at 37 °C and pH was uncontrolled.

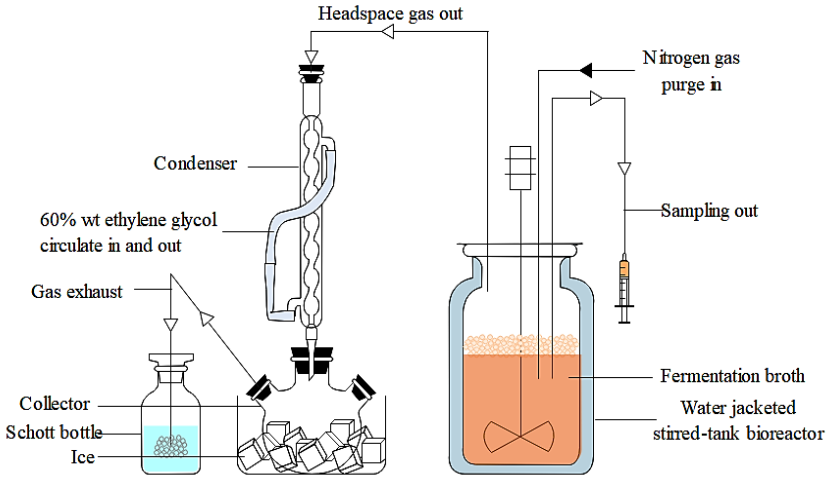

Figure 1. Fed-batch fermentation with in situ gas stripping biobutanol set up.

Gas stripping was first initiated at the intervals of 42-58 h of fermentation followed with second, third and fourth intervals at 66-72 h, 90-96 h and 114-120 h. The nitrogen gas was purged to circulate the headspace fermentation gas through the 36 cm height x 4 cm width condenser. 60%.wt ethylene glycol was circulated at -10 °C in the condenser. The bottom of the condenser was attached to an ace three-neck round-bottom flask that act as a collector of the condensate. The flask was kept at 0 °C in an iced box. One side arm of the flask was closed while the other side arm was fitted with silicon tubing to act as a gas exhaust. The tubing was left in the Schott bottle containing distilled water to check any product loss. The condensate was collected at the end of each gas stripping cycle. The product yield, titer and productivity from the fermentation was estimated based on total sugar consumption and products present in the fermentation broth and condensate collected from gas stripping.

2.9. Analysis procedures

All analysis was made on the supernatants of the samples. The optical density of sample was measured at 600 nm using UV/visible spectrophotometer (Hitachi).

2.9.1. High Performance Liquid Chromatography (HPLC). The sugars present in the OPF juice and fermentation broth were analyzed using an organic acid analysis column (Bio-Rad Monitoring Fermentation) and Agilent Technologies 1200 series high performance liquid chromatography (HPLC) equipped with a refractive index detector (RID) at 60 °C. The mobile phase used was 0.0005N H_2SO_4 at 0.8 mL/min.

2.9.2. Gas Chromatography with Flame Ionization Detector (GC-FID). The separation and concentration of acids and solvents were determined by GC-FID Agilent Technologies 6890N series an Agilent 7693 series autosampler. An internal standard method was used in which 10 g/L methanol was mixed at 1 to 1 ratio with the sample's supernatant liquid [9]. Sample was then auto injected into a capillary column HP INNOWax (30 m × 0.25 mm id × 0.25 µm). Helium was fed constantly at 2.5 mL/min as a carrier gas.

3. Results and Discussion

3.1. Extraction and Characterization of OPF Juice

From 30 kg fresh OPF pressed using sugarcane press machine, 50 wt. % of OPF juice was obtained. pH of untreated OPF juice was around 4.60 to 4.70. Sugars composition in OPF juice was presented in Table 2. Glucose concentration was around 48.67 ±2.25 g/L while sucrose and fructose were 18.12 ± 0.85 and 10.91 ± 0.15 g/L, respectively. Thus, average total amount of sugars found in OPF juice was 76.21 ±

2.25 g/L. The highest butanol yield from butyrate occurs in a medium rich in glucose, thus implied that OPF juice has good impression to generate high solvent yield [11].

Table 2. Comparison of the sugar content analysis in OPF juice.

Glucose (g/L)	Sucrose (g/L)	Fructose (g/L)	Total sugar (g/L)	References
48.67	18.12	9.42	76.21	This study
53.95	20.46	1.68	76.09	[12]
40.50	NA	NA	40.50	[13]

Table 3 showed the elemental constituents (carbon, nitrogen, hydrogen, sulphur), nutrient and heavy metals content in OPF and OPF juice. Based on the results obtained by CNHS analyzer and ICP, OPF and OPF juice contained high carbon which is 46.2% and 41.52%, respectively and suitable C/N ratio >15. Several compounds found in OPF were similar to P2 medium components, for examples, potassium (K), phosphate (P), calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), zinc (Zn), aluminium (Al) and sodium (Na). This indicated that OPF juice is suitable to be used as a renewable carbon source for production of various bioproducts.

Table 3. Nutrients and metallic elements compositions.

Composition	OPF juice	OPF juice[a]	OPF juice[b]
C (%)	41.52	39	0.023
N (%)	1.47	0.8	0.0097
C/N	28.24	50	–
S (%)	0.66	0.4	ND
P (%)	NM	0.02	0.119
K (%)	0.35	2.3	19.20
Ca (%)	0.043	2.9	12.17
Mg (%)	0.025	0.5	2.90
B (ppm)	15.91	2	3.80
Mn (ppm)	7.27	2	22.60
Cu (ppm)	0.45	2	0.30
Fe (ppm)	12.27	66	73.25
Zn (ppm)	ND	9	0.50
Al (ppm)	10.28	NM	NM
Mg (ppm)	246.6	NM	NM
Na (ppm)	ND	NM	NM

*Data was the mean of duplicate samples
*NM = Not measured
*ND = Not detected
[a] [12]
[b] [14]

3.2. Optimization of biobutanol production in 1 L batch fermentation

Inoculum size (A), temperature (B) and agitation speed (C) were studied in this optimization process with biobutanol concentration (Y) as the response. Five levels of numeric factor variation were used. The five levels consisted of plus and minus alpha (axial point), plus and minus 1 (factorial design) and the centre point. By using Design Expert 7.1.6 software, 20 sets of experiments were obtained and quadratic model was proposed. Full central composite design for optimization process with three of its variables, and both responses; actual response obtained from experimental works are presented in table 4.

Table 4. CCD results for batch ABE fermentation in 1 L Schott bottle.

Standard Order	Factors						Response
	Inoculum size, A		Temperature, B		Agitation speed, C		Butanol yield, Y (g/g)
	Coded value	Actual value (%)	Coded value	Actual value (°C)	Coded value	Actual value (rpm)	Actual value
1	-1	15	-1	32	-1	50	0.189
2	+1	25	-1	32	-1	50	0.180
3	-1	15	+1	42	-1	50	0.169
4	+1	25	+1	42	-1	50	0.177
5	-1	15	-1	32	+1	150	0.141
6	+1	25	-1	32	+1	150	0.159
7	-1	15	+1	42	+1	150	0.075
8	+1	25	+1	42	+1	150	0.084
9	-2	10	0	37	0	100	0.150
10	+2	30	0	37	0	100	0.158
11	0	20	-2	27	0	100	0.093
12	0	20	+2	47	0	100	0.065
13	0	20	0	37	-2	0	0.169
14	0	20	0	37	+2	200	0.050
15	0	20	0	37	0	100	0.171
16	0	20	0	37	0	100	0.169
17	0	20	0	37	0	100	0.158
18	0	20	0	37	0	100	0.160
19	0	20	0	37	0	100	0.150
20	0	20	0	37	0	100	0.190

It was apparent that an increased in inoculum size from 10% (v/v) to 30% (v/v) resulted in an increased of biobutanol production. Similar trend was found which showed that an increase in inoculum size from 5% to 15% (v/v) elevated biobutanol production [15]. This previous study reported an optimum inoculum size of 16.2% (v/v) using oil palm decanter cake hydrolysate as the substrate. Increasing the inoculum size shorten the lag phase of the clostridial cell growth. This improves clostridial growth by triggering the solventogenic phase faster and increased biobutanol production [16]. However, further increased in inoculum size had no considerable effects on lag phase of microbial growth and cell activity which consequently resulted in the production of biobutanol in concentration close to best inoculum size [17]. The optimum inoculum size was varied depending upon the type of microorganism and substrate used. In this study, the best inoculum size for the production of biobutanol from OPF juice using *Clostridium acetobutylicum* ATCC 824 was 20% (v/v).

As the agitation speed increased from 0 to 200 rpm at an interval of 50 rpm, the biobutanol yield decreased. The maximum biobutanol yield (0.19 g/g-sugar) was observed at agitation speed of 50 rpm. The present finding was equal to the previous study where an increase in agitation speed subsequently reduced the ability of the bacteria to breakdown the glucose or carbohydrate uptake and thus affecting the production of biobutanol [18]. In this study, the best condition for biobutanol production is at 50 rpm agitation speed with highest biobutanol reached up to 0.19 g/L.

The optimum temperature for fermentation of *Clostridium acetobutylicum* strains strongly depends not only on the type of strain, but strongly influenced by various factors such as, medium, substrate, pH and others. The reported optimal growth temperature for most of Clostridia was between 30 and 37 °C. *Clostridium acetobutylicum* have lost the ability to continue the acidogenesis and solventogenesis stage at high temperature [18]. Clostridia grow optimally between 20 and 45 °C. Beyond this range resulted in low efficiency on producing acids as well as solvents. It was observed that the biobutanol yield was

low outside the temperature range (27 and 47 °C) with yield of 0.06 g/g-sugar and 0.09 g/g-sugar respectively than the optimum temperature of 37 °C which is 0.19 g/g-sugar yield.

3.3. Analysis of variance (ANOVA)

From the ANOVA data in table 5, the Model F-value of 10.37 and Prob>F value less than 0.0500 indicated that the model terms were significant. There was only a 0.05% chance the "Model F-Value" this large occurred due to the noise. In this case, B, C, BC, B^2 and C^2 were significant model terms. While, the model term of A, AB, AC and A^2 were not significant, as their p-value more than 0.05. Values greater than 0.1000 indicated the model terms were not significant. The "Lack of Fit p>F " of 0.1643 implied the Lack of Fit is not significant relative to the pure error. There is a 16.43% chance that a "Lack of Fit p>F" this large could occurred due to the noise.

R^2 obtained was 0.9032 (90.32%). Thus, there was only 0.10% of variability response in this study. The presented R^2 value, therefore, implied a very good fit between experimental and predicted values. Adj R-Squared was 0.8161. For a second-order model to be a good predictive model, the adjusted R-square ≥ 0.8 (Myers et al., 2009). The Pred R-Squared of 0.4066 is not as close to the Adj R-Squared as one might normally expect. Adequate precision ratio of 9.343 for this model indicated an adequate signal, which supported the fitness of the model.

Table 5. Analysis of variance (ANOVA).

Source	Sum of squares	Degree of freedom	Mean square	F-value	Prob>F	
Model	0.033	9	3.652E-003	10.37	0.0005	significant
A	1.056E-004	1	1.056E-004	0.30	0.5960	
B	3.011E-003	1	3.011E-003	8.55	0.0152	
C	0.015	1	0.015	43.65	<0.0001	
AB	7.411E-006	1	7.411E-006	0.021	0.8875	
AC	1.044E-004	1	1.044E-004	0.30	0.5980	
BC	1.773E-003	1	1.773E-003	5.04	0.0487	
A^2	4.168E-005	1	4.168E-005	0.12	0.7379	
B^2	0.010	1	0.010	28.85	0.0003	
C^2	3.960E-003	1	3.960E-003	11.25	0.0073	
Residual	3.521E-003	10	3.521E-004			
Lack of Fit	2.528E-003	5	5.055E-004	2.54	0.1643	Not significant
Pure Error	9.935E-004	5	1.987E-004			
Cor Total	0.036	19				
Std. Dev.	0.019		R-Squared		0.9032	
Mean	0.14		Adj R-Squared		0.8161	
C.V.%	13.15		Pred R-Squared		0.4066	
PRESS	0.022		Adeq Precision		9.343	

*A = inoculum size
*B = Temperature
*C = Agitation speed

3.4. Fed-batch fermentation kinetics with in situ gas stripping

Fed-batch fermentation was applied to processes where a high substrate concentration is toxic to culture. Since butanol is toxic to *Clostridium* cells, the fed-batch fermentation technique cannot be applied unless one of the feasible product recovery technique was applied for simultaneous separation of product. Thus, in order to overcome both substrate and solvent inhibitions, fed-batch fermentation was carried out with in situ gas stripping. A batch fermentation in 1 L working volume was first conducted for 42 h before gas stripping started, followed with first fed time. The additional fresh OPF juice, yeast extract and P2

medium was fed again after the second gas stripping finished by 72 h. Next after that was just gas stripping parts with no more feeding.

Over this period, a total of 38.1 g/L substrate was consumed in fed-batch fermentation. Normally, only 60% of sugars was used for ABE production [19] and the rest was used for cell growth. The fermentation produced 6.69 g/L of butanol, 3.48 g/L of acetone, and 0.87 g/L of ethanol with total ABE of 11.04 g/L. Gas stripping not only eliminate butanol toxicity but altered the fermentation kinetic as well. Typical ABE fermentations produce butanol and acetone at a 2:1 ratio. However, the butanol/acetone ratio was significantly higher than 2 in this study at the end of 240 h fermentation. Good acid reassimilation has been one of the reasons to this increment in evident by the stable concentration levels of both acetic and butyric acids in the fermentation broth throughout the fed-batch fermentation. In this study, the overall biobutanol and ABE productivities in fed-batch fermentation with in situ gas stripping were significantly higher compared to batch fermentation without gas stripping. Biobutanol and ABE productivities by fed-batch fermentation increased 11% and 17%, respectively. Clearly, gas stripping not only prevented the biobutanol toxicity but increased the fermentation rate and the biobutanol yield as well. Table 6 shows the kinetic data for batch fermentation without gas stripping and fed-batch fermentation with gas stripping in 2 L stirred tank bioreactor.

Table 6. Kinetic data for batch fermentation without gas stripping and fed-batch fermentation with gas stripping in 2 L stirred tank bioreactor.

	Batch without gas stripping	Fed-batch with gas stripping
Substrate (g/L)	50	50
Fermentation time (h)	144	42
Substrate consumed (g/L)	49.0	38.1
Substrate consumption rate (g/L/h)	0.34	0.91
Acetic acid produced (g/L)	2.14	3.37
Butyric acid produced (g/L)	0.72	1.42
Total acid produced (g/L)	2.86	4.40
Total acid yield (g/g)	0.06	0.13
Acetone production (g/L)	3.86	3.48
Butanol production (g/L)	7.83	6.69
Ethanol production (g/L)	1.18	0.87
Total ABE production (g/L)	12.9	11.0
Acetone yield (g/g)	0.08	0.09
Butanol yield (g/g)	0.16	0.18
Ethanol yield (g/g)	0.02	0.02
Total ABE yield (g/g)	0.26	0.29
Biobutanol productivity (g/L/h)	0.05	0.16
ABE productivity (g/L/h)	0.09	0.26

3.5. Gas stripping effects on ABE fermentation

All previous studies on integrated ABE fermentation with gas stripping were conducted at relatively low butanol concentrations of 5 g/L or less to minimize butanol toxicity [20]. Based on butanol production profile from batch fermentation, the butanol production of 5 g/L started at 42 h. Thus, gas stripping was started at 42 h as well to intermittently remove ABE from the fermentation when the butanol concentration was 5 g/L and higher. The stripping process in the whole system took out at four time intervals; 42-48, 66-72, 90-96, and 114-120 h. Acids and solvents production profile for fed-batch fermentation in 2 L stirred tank reactor with in situ gas stripping is presented in figure 2. The shaded area in the graph was the time when gas stripping being applied (First gas stripping: GS 1, second gas stripping: GS 2, third gas stripping: GS 3, fourth gas stripping: GS 4).

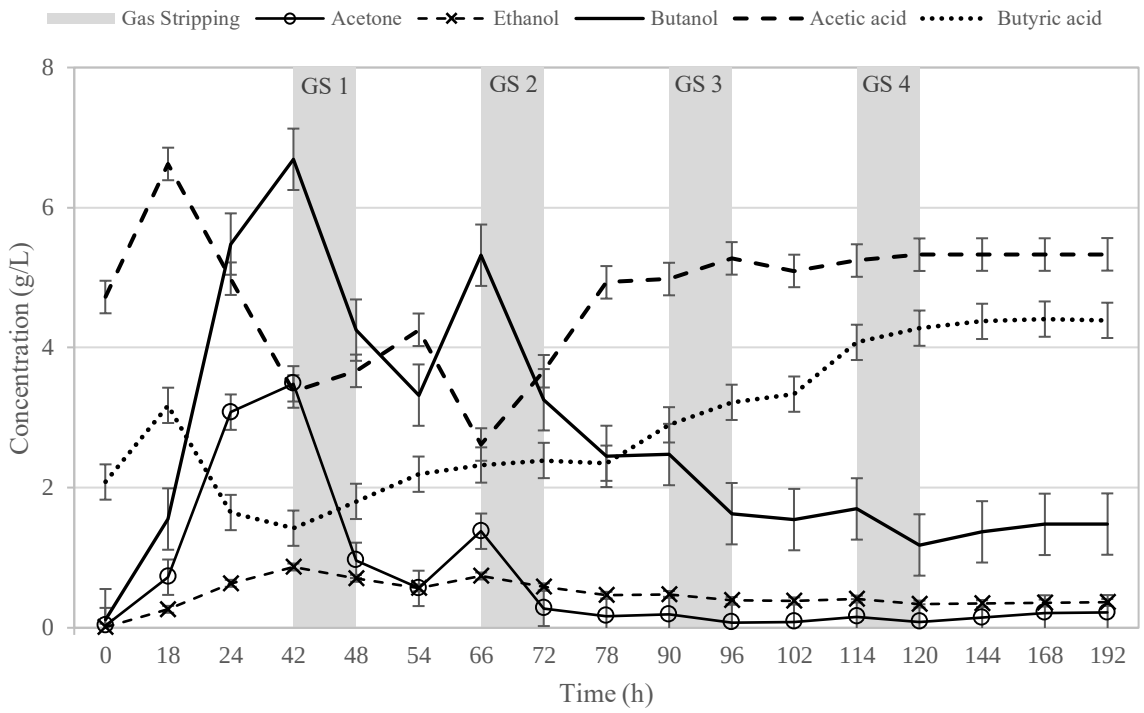

Figure 2. Acids and solvents produced from fed-batch ABE fermentation in 2 L stirred tank bioreactor with in situ gas stripping method.

Based on the graph in figure 2, after which the gas stripping has been initiated, ABE concentrations in the reactor decreased dramatically. This indicates that ABE were successfully stripped out of the reactor. The butanol concentration in the fermentation broth fluctuating between 3 and 5 g/L in the first 72 h, indicating a dynamic equilibrium between solvent production and removal. During first gas stripping, about 10.53 g/L acetone, 135.15 g/L butanol and 10.03 g/L ethanol have been stripped. The stripped butanol and ethanol concentrations was higher in second gas stripping with 136.39 g/L and 14.11 g/L of butanol and ethanol were stripped out, respectively.

There is no further ABE production after 72 h. When the third gas stripping was done, only butanol and ethanol left to be stripped. Total ABE concentration that were successfully stripped and percent recovery is presented in table 5 and Table 6 respectively. The product stripped is displayed in figure 3.

Table 7. ABE concentration that have been stripped from gas stripping.

Gas stripping intervals (h)	Acetone (g/L)	Butanol (g/L)	Ethanol (g/L)
42-48	10.53	135.15	10.03
66-72	8.64	136.39	14.11
90-96	1.25	80.26	8.03
114-120	1.02	42.32	5.75

Table 8. Percent recovery of ABE.

Gas stripping intervals (h)	Acetone (%)	Butanol (%)	Ethanol (%)
42-48	71.5	36.5	18.4
66-72	79.7	38.7	21.6
90-96	57.9	34.0	18.8
114-120	50.0	30.6	17.1

(a) (b) (c)

Figure 3. (a) First strip (b) second strip (c) third strip.

From table 7, the results indicate that gas stripping removed only volatile compounds, which are mainly ABE and did not remove acetic and butyric acid at 37 °C. It was found that the gas stripping process is more selective in separating butanol from water since butanol is the highest concentration in the condensate collected, although butanol has a higher boiling temperature and lower vapor pressure than acetone, ethanol, and water at 37 °C.

The second gas stripping produced higher ABE production and substrate consumption rates, likely due to increased viable cell density in the fed-batch fermentation. However, solvent production decreased after the third gas stripping, indicating decreased in cell viability. It was probably because the dead cells and non-active cells were present with active solvent producing cells in the fermenter [9]. In this experiment, butanol production ceased after extended fermentation hours starting from 72 h and onwards. This finding was matched with previous study where they claimed that there is an inhibitory effect on cell growth and acid production due to the removal of solvents [10]. At 72 h, butanol concentration reached 5.32 g/L. At relatively low butanol concentrations of 5 g/L or less, sporulation occurred and caused culture degeneration [20]. Therefore, to ensure better yield of butanol removal by gas stripping, the butanol production should be maintained above 8 g/L because the gas stripping is more effective in separating butanol from the aqueous solution at higher concentrations. Butanol goes through phase break when the concentration is higher than 8%. Butanol in the condensate boost to 150 g/L when the butanol concentration in the solution was nearly 8 g/L [21].

The product separation results in this study were compared with other methods from previous researches in order to evaluate the performance of gas stripping in recovering the product from the fermentation. Table 9 shows the comparison of several fermentation strategies from other research studies with different modes of fermentation and recovery method used in product recovery. Gas stripping was found to be the most practical process for biobutanol recovery.

Table 9. ABE concentration obtained from gas stripping.

Substrate concentration (g/L)	Fermentation strategy	Concentration (g/L)			References
		Acetone	Butanol	Ethanol	
150 Glucose	Continuous, integrated with pervaporation	5.1	152.0	1.1	[22]
100 Glycerol	Batch, in situ membrane distillation	N.A	29.8	N.A	[23]
86.2 Glucose	Fed-batch, ex situ adsorption	6.7	18.2	0.9	[24]
600 Glucose	Fed-batch, in situ gas Stripping	58	703.4	14	[25]
50 OPF juice	Fed-batch, in situ gas stripping	10.53	135.15	10.03	(This study)

4. Conclusions

OPF juice can be used as an alternative carbon source for biobutanol production at bioreactor scale. The findings have suggested the optimum operating parameters which leading to high yield and productivity of biobutanol, at 20% inoculum size, 37 °C fermentation temperature and 50 rpm agitation speed. It is evident that the overall biobutanol and ABE productivities in fed-batch fermentation with in situ gas stripping were significantly higher compared to batch fermentation without gas stripping. Biobutanol and ABE productivities by fed-batch fermentation increased 11% and 17%, respectively. Clearly, gas stripping not only prevented the biobutanol toxicity but increased the fermentation rate and the biobutanol yield as well.

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