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Batch butanol fermentation from sugarcane molasses integrated with a gas stripping system: Effects of sparger types and gas flow rates

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Abstract

High butanol levels inhibit microbial metabolism and acetone-butanol-ethanol (ABE) fermentation. Gas stripping is a simple technique to separate solvents from a fermentation broth, which can improve butanol production during fermentation. Sparger types in gas stripping systems may affect the liquid-gas mass transfer. The aim of this study was to investigate the effects of sparger types (porous, ring and nozzle designs) and gas flow rates in a gas stripping system on butanol production from sugarcane molasses by *Clostridium beijerinckii* TISTR1461 in a batch fermentation. The gas stripping was started after 24 h of the fermentation, and the gas hold-up values were used to calculate the liquid-gas mass transfer for the gas stripping system. Results showed that a maximum cumulative butanol concentration (15.33 g/L) and butanol productivity (0.21 g/L.h) were achieved using a ring sparger at a gas flow rate of 1.0 L/min. Under these conditions, the gas hold-up value (0.010) was maximal. The lowest cumulative butanol concentration (13.17 g/L) and butanol productivity (0.18 g/L.h) were obtained using a nozzle sparger, corresponding to a minimal gas hold-up value of 0.003. In conclusion, the results demonstrated that the higher the gas hold-up, the better the gas-liquid mass transfer attained. The fermentation using the gas stripping system increased the butanol concentration by approximately 44% compared to that of a fermentation without gas stripping.

Keywords: butanol fermentation, *Clostridium beijerinckii*, gas hold-up value, gas stripping system, sparger type

1. Introduction

Nowadays, renewable energy forms such as bioethanol, biodiesel, hydrogen and biobutanol are being investigated due to global warming, climate change, air pollution and energy stability issues. Biobutanol is a promising next-generation alcohol fuel. It is considered an attractive biofuel because it clearly has superior properties to ethanol due to its higher energy content, lower vapor pressure, lower volatility, less corrosivity, lower freezing point, higher octane number, higher hydrophobicity and lower water absorption compared to bioethanol. This makes it stable when blended with gasoline for use in internal combustion engines. It provides better fuel economy than ethanol-gasoline blends [1]. Additionally, biobutanol is widely used in many industries. It is an important chemical precursor for paints, polymers and plastics [2].

Biobutanol can be produced via an acetone-butanol-ethanol (ABE) fermentation by *Clostridium* sp., e.g., *C. saccharoperacetobutylicum*, *C. saccharobutylicum*, *C. beijerinckii* or *C. acetobutylicum*. ABE fermentations consist of two phases. The first phase is an acidogenesis phase, in which acid forming pathways are stimulated producing acetate, butyrate and some gasses (H_2 and CO_2) as the major products. This phase occurs during the cell division. The second phase is solventogenesis, in which acetone, butanol and ethanol are produced from acetic and butyric acids in a ratio of 3:6:1 (acetone: butanol: ethanol) [1]. However, the ratio depends on the microbial species used and the fermentation conditions.

The raw materials for butanol production are quite expensive [3-5]. Hence, raw materials with a high carbohydrate content that are low cost and readily available, that can be used in efficient, energetically optimized transformation processes are required for practical butanol production on an industrial scale. Sugarcane molasses, a by-product of sugar production, is one of these materials. In Thailand, it is produced in great quantities, 3,500,000 tonnes per year [6]. It consists of various fermentable sugars (sucrose, glucose and fructose) that can be used as carbon sources and it also has many trace elements necessary for microbial growth. Additionally, among sugarcane juice, sugarcane molasses and sweet sorghum juice, sugarcane molasses is the most suitable substrate for butanol production [7 & 8].

One of the main obstacles to a successful ABE fermentation is product inhibition of microbial metabolism. Additionally, commercial butanol production is not yet economically feasible due to the limitations of low product yield, low productivity and low product titer [9-11]. Butanol has shown an inhibitory effect on microbial growth at concentrations above 6 g/L [3,12-13]. Its toxicity causes cell membrane damage and makes membranes permeable to ADP and some ions, leading to cell lysis [13].

Gas stripping system is a simple technique to separate solvents from a fermentation broth, which can improve butanol production during an ABE fermentation. Additionally, it is effective, easy to integrate with fermentation processes and has a low energy consumption [14 & 15]. Xue et al. [16] reported that gas stripping is essential to attain butanol concentrations higher than 8 g/L in a fermentation broth. This can be done to get a condensate with a butanol concentration higher than its solubility in water (~80 g/L at 20 °C), resulting in more efficient butanol separation.

Normally, a gas stripping system can be modelled using first order kinetics as show in Eq. 1 [17]:

$$R_S = -\frac{dC_S}{dt} = K_S a \cdot C_S \tag{1}$$

where: R_S = gas stripping rate of the solute (g/L·s)
C_S = solute concentration in aqueous solution (g/L)
$K_S a$ = gas stripping rate constant (1/s)
t = time (s)

From this equation, it is seen that R_S is proportional to its concentration in the bulk liquid. This has been confirmed by various studies [17-19].

The role of the stripping rate constant, $K_S a$, is seen in two equations, Eq. 2 and 3 [17 & 20]:

$$K_S a = \frac{\alpha}{V} \cdot \left[\frac{1}{k_1} + \frac{RT}{H' k_g} \right]^{-1} \tag{2}$$

where α = interfacial area (cm^2)
V = total volume of the fluid (cm^3)
k_1 = liquid film mass transfer coefficient of the solute (cm/s)
R = universal gas constant (cm^3 atm/mol·K)
T = absolute temperature (K)
H' = Henry's law constant (cm^3/mol)
k_g = gas film mass transfer coefficient of the solute (cm/s)

$$K_S a = b \frac{Q}{V} (H_c)^m \tag{3}$$

where b and m = power function constants
Q = gas flow rate (L/s)
H_c = dimensionless Henry's constant

From these equations, increasing the interfacial area (α) by decreasing the bubble size at a set flow rate can improve the stripping rate (R_S). This theoretical prediction is supported by Ezeji et al. [19]. They compared two gas bubble sizes in a gas stripping system. It was found that bubble size plays an important role in gas stripping. However, the effects of flow rates on the performance of gas stripping systems remain vague. The stripping rate can be improved by decreasing bubble size, but it is very difficult to accurately measure the bubble size and bubble area experimentally. Hence, the gas hold-up in Eq. 4 can be used to explain the various effects of bubble sizes, depending on the sparger types [21]. Eq. 4 shows the effect of the volume of gas in the fermentation broth when the gas stripping system is started. A high volume of gas in the fermentation broth can increase gas-liquid mass transfer as:

$$\varepsilon = \frac{V_G}{V_L + V_G} \tag{4}$$

where ε = gas hold-up
V_G = total volume of gas bubbles in the bioreactor (m^3)
V_L = total volume of broth in the bioreactor (m^3)

To date, gas stripping systems are widely used for *in situ* solvent removal to reduce product inhibition during an ABE fermentation [8]. However, the effects of various sparger types on the performance of gas stripping systems have not been studied. It is necessary to study this parameter because it has an effect on liquid-gas mass transfer. Thus, the aim of this study was to investigate the effects of sparger types and gas flow rates in a gas stripping system on butanol production from sugarcane molasses by *C. beijerinckii* TISTR1461 in a batch process. Selectivity of gas stripping with different solvents (acetone, butanol and ethanol) were also calculated.

2. Materials and methods

2.1. Microorganism and inoculum preparation

Spores of *C. beijerinckii* TISTR 1461 were preserved as a suspension in sterile distilled water and stored at 4 °C. This suspension, containing 1×10^6 spores/mL, was heat shocked in a hot water bath at 80 °C for 1 min, then immediately immersed in an iced-water for 1 min [8]. Thereafter, the spore suspension of (5%, v/v) was transferred into 10 mL of cooked meat medium (CMM) and incubated at 37 °C for 8-10 h to obtain active vegetative cells. Then, they were transferred into a tryptone-glucose-yeast extract (TGY) medium and incubated at 37°C for 4-6 h [22].

2.2. Culture media and raw materials

Inoculum preparation was done in CMM and TGY media. CMM consists of a cooked meat medium powder, 10 g/L and glucose, 0.8 g/L [23], whereas TGY was composed of tryptone, 5 g/L; glucose, 1 g/L; yeast extract, 5 g/L and K_2HPO_4, 5 g/L (modified from Qureshi and Blaschek [23]). The media were sterilized in an autoclave at 110 °C for 28 min [8]. Afterwards, strictly anaerobic conditions were created using oxygen-free nitrogen (OFN) gas before *C. beijerinckii* TISTR1461 was transferred into the media.

Sugarcane molasses (Mitr Phu Viang Sugar Co., Ltd. Khon Kaen, Thailand) was used as a substrate for a butanol production medium. It contained 80 °Bx of total soluble solids, and its composition was reported in Wechgama et al. [8]. The molasses was stored at −20 °C before use to prevent bacterial growth. Dried spent yeast (DSY), obtained from Beer Thip Brewery Factory (1991) Co., Ltd., Bang Baan, Phra Nakhon Sri Ayutthaya, Thailand, was used as a nitrogen supplement. It was kept at room temperature before use in the experiments. The composition of DSY was analyzed and is presented in Tables 1.

Table 1 The composition of dried spent yeast (DSY). [24]

Composition [a]	Concentration (%, dry weight)
Total carbohydrate	41.92
Protein	41.75
Total fat	2.95
Crude fiber	0.16
Ash	6.08
Moisture	7.30

[a] Central Laboratory (Thailand) Co., Ltd., Khon Kaen, Thailand.

2.3 Butanol production medium

Sugarcane molasses containing a total sugar content of 50 g/L supplemented with 6 g/L of DSY and 6.6 g/L of $CaCO_3$ (modified from Sanchanda et al. [25]) was used as a butanol production medium. A 1.2 L volume of the medium was transferred to a 2-L stirred-tank bioreactor (Biostat® B, B. Braun Biotech Melsungen, Germany). After sterilization at 110°C for 40 min, the pH of the fermentation medium was adjusted to 6.5 with the addition of 8 N NaOH before use [8].

2.4 Batch fermentation

The sterile fermentation medium at pH 6.5 was purged with oxygen-free nitrogen (OFN) gas to remove oxygen. Active *C. beijerinckii* TISTR1461 cells (5%, v/v) were then inoculated into the medium and incubated at 37 °C with an agitation rate of 150 rpm. The experiments were performed in triplicate. Samples were collected every 12 h for analysis.

2.5 Batch fermentation integrated with a gas stripping system using various sparger types and gas flow rates

The 2-L stirred-tank bioreactor was connected to gas stripping system as shown in Figure 1. The gas stripping system was started after 24 h of fermentation. The three sparger types, porous, ring and nozzle (Table 2), were studied to determine the butanol fermentation efficiency at a controlled gas flow rate of 1.0 L/min [8]. After determining the best sparger type for use in the gas stripping system, gas flow rates of 0.5, 1.0 and 1.5 L/min were supplied using a peristaltic pump to investigate its effect on butanol production. The temperature of the condenser (Pyrex, USA; condenser 40×450 mm and cooling coil 0.60×1,500 mm) was controlled at -8 °C using a cooling bath. Samples in the bioreactor and receiving flask were collected for analysis during the fermentation.

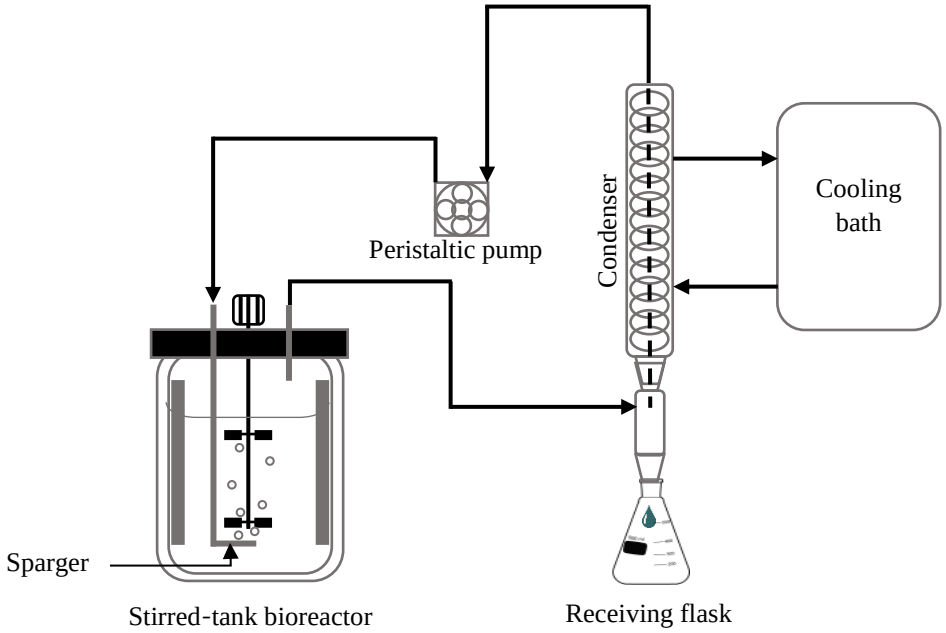


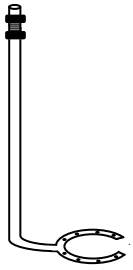
Figure 1 Schematic diagram of a batch ABE fermentation integrated with a gas stripping system. (modified from Wechgama et al. [8]).

2.6 Analytical methods

The supernatant was separated from cells and other particles by centrifugation at 7,400g for 10 min. The supernatant was then analyzed for total sugar, pH, organic acids and organic solvents (acetone, butanol and ethanol). The total sugar content was determined using a phenol-sulfuric acid method [26]. The pH values were measured using a pH meter (Mettler Toledo, USA). Organic acids and organic solvents were analyzed using a gas chromatograph (GC-2014, Shimadzu, Japan) with a stainless steel column packed with Pora-pack Q, 80/100 mesh (Resteck, USA). A flame ionization detector (FID) was used to detect solvents and acids, using H_2 gas as a fuel. The conditions of the injector and detector were described in Wechgama et al. [8]. Iso-butanol was used as an internal standard (modified from Areesirisuk et al. [27]). Cell morphology was observed under light microscopy at various times during the fermentation. The butanol yield ($Y_{B/S}$), butanol productivity (Q_B), total ABE yield ($Y_{ABE/S}$) and ABE productivity (Q_{ABE}) were calculated [8].

Selectivity of gas stripping with different solvents (acetone, butanol and ethanol) were calculated as $\alpha = [y/(1-y)]/[x/(1-x)]$, where x and y are weight fractions of the solvent in fermentation broth and condensate, respectively [28].

Table 2 Configuration of spargers used in the gas-stripping system.

Type of sparger	Shape	Bubble sizes
Porous		Small (1-2 mm) [a]
Ring		Medium (3-4 mm) [a]
Nozzle		Large (5-7 mm) [a]

[a] The bubble sizes in tap water.

3. Results and discussion

3.1 Batch butanol fermentation from sugarcane molasses without gas stripping

Due to the low nitrogen content of sugarcane molasses (6.40 g protein/100 mL, corresponding to 1.02 g nitrogen/100 mL) [8], DSY consisting of 41.92% protein on a dry weight basis as shown in Table 1 (nitrogen 6.68% dry weight) was used as a nitrogen supplement in the sugarcane molasses medium to increase butanol production.

In this study, a batch butanol fermentation from sugarcane molasses medium by *C. beijerinclii* TISTR1461 without gas stripping was performed as a control experiment. The ABE fermentation profiles are shown in Figure 2. In the first 12 h of the fermentation, the pH was reduced due to the production of acetic and butyric acids, implying that an acidogenesis phase had occurred and cells were growing coupling with the generation of ATP in the ABE fermentation pathway. After 12 h of fermentation, the pH slightly increased. During this period, acetone, butanol and ethanol were clearly observed, indicating an occurrence of solventogenesis phase. A butanol concentration (P_B) of 8.60 g/L increased rapidly after 24 h of fermentation, after which it increased more gradually. The maximum P_B (10.66 g/L) was observed at 36 h. At this time, the acetone and ethanol levels were 3.72 and 0.20 g/L, respectively. The total sugar concentration remained at about 22 g/L. Under this condition, the ABE concentration (P_{ABE}), butanol yield ($Y_{B/S}$) and butanol productivity (Q_P) were 14.58 g/L, 0.38 g/g and 0.34 g/L·h, respectively.

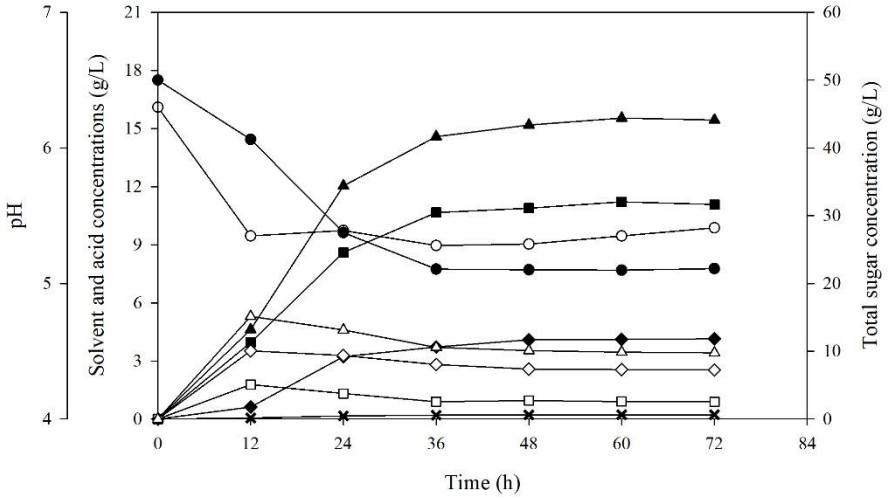


Figure 2 ABE batch fermentation profiles from sugarcane molasses by *C. beijerinckii* TISTR 1461 in a stirred-tank bioreactor: acetone (♦), butanol (■), ethanol (×), ABE (▲), acetic acid (◊), butyric acid (□), total acids (Δ), pH (○) and total sugar (●).

3.2 Effect of various sparger types in a gas stripping system on butanol fermentation

According to the pathway of ABE fermentation [29], the stripped gases consist of H_2, CO_2 and ABE in gas phase. In this study, ethanol was rarely produced during the ABE fermentation (Figure 2), therefore the composition of the stripped gas was H_2, CO_2, acetone and ethanol in gas phase. The performance of porous, ring and nozzle spargers were examined in the gas stripping system (Figure 1). The gas flow rate was controlled at 1.0 L/min in the system to investigate the effect of bubble sizes of various spargers on butanol removal. The bubble sizes of porous, ring and nozzle spargers were measured and reported in Table 2. The bubbles of porous, ring and nozzle spargers were of small, medium and large sizes, respectively. The ABE fermentation profiles using a gas stripping system with various sparger types are shown in Figure 3. In this study, the gas stripping system was started after 24 h of fermentation because a high butanol concentration (8.60 g/L) was detected in the fermentation broth of the control experiment (Figure 2). The results illustrated that fermentation profiles of butanol production before 24 h of fermentation were similar (Figures 3A-3D). This indicated that the operational parameters of all conditions tested were the same during this timeframe. After starting the gas stripping system, the profiles of the remaining sugar concentration and pH in the fermentation broth of all treatments were slightly different (Figures 3A-3B). About 25-29% of the total sugar- remained un-utilized. These results were similar to those of Li et al. [9] and Wu et al. [30], who found that the sugar consumption was not complete in an ABE fermentation from molasses (70 g/L of total sugar) by *C. beijerinckii* L175 and a mutant strain, MUT3, as well as on a glucose medium (70 g/L of total sugar) by *C. acetobutylicum* L7. This might have been due to low butanol removal rate. Hence, sugars could not be simultaneously converted to their products (acids and solvents). Under these conditions, pH decreased from 6.5 to 5.3. Butanol and ABE production after 24 h under each condition was different (Figures 3C-3D). The butanol concentration in the fermentation broth ranged from 9.12-10.20 g/L. The highest of butanol and ABE concentrations (in both fermentation broths and condensates) were 15.33 and 19.46 g/L, respectively, using a ring sparger in the gas stripping system. In the current study, the order of effectiveness of the spargers upon butanol and ABE production was ring > porous > nozzle spargers.

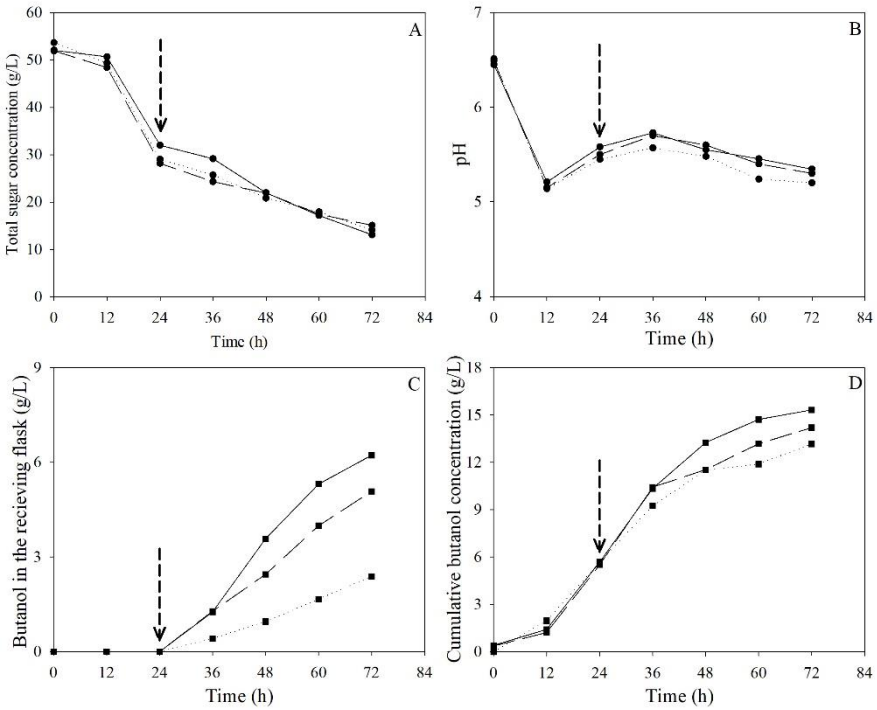


Figure 3 ABE batch fermentation profiles from sugarcane molasses by *C. beijerinckii* TISTR1461 in a stirred-tank bioreactor integrated with a gas stripping system using various spargers: porous (dash lines), ring (solid lines) and nozzle (dot lines) spargers. Total sugar content (A), pH (B), butanol in the receiving flask (C) and cumulative butanol concentration (D). The arrows indicate the starting time of the gas stripping system.

It was found that butanol ($Y_{B/S}$) and ABE ($Y_{ABE/S}$) yields were not significantly different with and without gas stripping systems (Figure 4). These results indicate that metabolic pathways of ABE fermentation were not changed and the gas stripping systems did not disturb the butanol and ABE production pathways. However, the gas stripping system markedly affected butanol productivity. This might have been due to the promotion of butanol production by prolonging the fermentation time using a gas stripping system (Figure 3C).

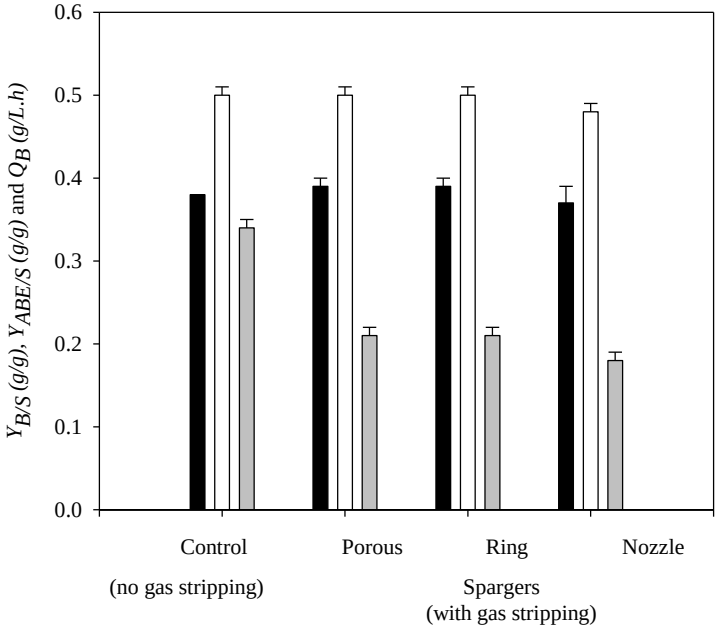


Figure 4 Comparison of ABE fermentation efficiencies from sugarcane molasses using various spargers in a gas stripping system: $Y_{B/S}$ (black bars), $Y_{ABE/S}$ (white bars) and Q_B (gray bars).

Unfortunately, the bubble sizes of gas using various sparger types in this study could not be accurately measured due to the dark color of molasses. Therefore, to explain the effect of sparger type on butanol production, the gas hold-up was calculated. The results showed that the maximum gas hold-up was obtained using a ring sparger (Table 3), corresponding to higher cumulative butanol concentration (Figure 3D). These results implied that high mass-transfer rates were achieved at high gas hold-up values. The order of effectiveness of gas hold-up from high to low was ring > porous > nozzle type spargers. The magnitude of cumulative butanol concentration also exhibited this trend. The porous sparger yielded very small bubble sizes. This can be troublesome in a stirred-tank bioreactor. The gas concentration in these bubbles equilibrates with that in the medium within seconds, so that the gas hold-up no longer reflects the capacity of the system for mass transfer [21]. The results demonstrated that ring sparger in a gas stripping system was the better type for butanol fermentation from sugarcane molasses by *C. beijerinckii*.

Table 3 The parameter values of various sparger types in an ABE fermentation.

Sparger type	V_L (cm^3)	V_G (cm^3)	ε
Porous	1260	7.70	0.006
Ring	1260	13.33	0.010
Nozzle	1260	3.32	0.003

V_L = total volume of broth in the bioreactor, V_G = total volume of gas bubbles in the bioreactor,
ε = gas hold-up
The results are expressed as means of triplicate values.

3.3 Effects of gas flow rate in a gas stripping system on butanol fermentation

In these experiments, the gas flow rates in the gas stripping system using a ring sparger were varied. The gas stripping system was started after 24 h of fermentation. Then, butanol was separated from the fermentation broth and collected in a receiving flask (Figure 5). Total volume of condensate at the gas flow rates at 0.5, 1.0 and 1.5 L/min were 47, 60 and 57 ml, respectively; and the condensate contained acetone and butanol. The results showed that the P_B in the receiving flask using gas flow rates at 0.5 and 1.5 L/min was lower than at 1.0 L/min, corresponding to higher P_B remaining in the bioreactor. These results indicated that mass transfer rates between the liquid and gaseous states of butanol and/or recirculating rate of gas from fermentation broth to the condenser using gas flow rates at 0.5 and 1.5 L/min were lower than that at 1.0 L/min. Lower mass transfer rates resulted in butanol toxicity to the bacterial cells. Therefore, lower cumulative P_B values (13.10 g/L at a gas flow rate of 0.5 L/min, and 13.74 g/L at a gas flow rate of 1.5 L/min) were observed. This might have occurred since the condenser could not completely remove butanol. Therefore, butanol was returned to the fermentation broth. Alternatively, when the gas flow rate was decreased to 0.5 L/min, a lower P_B was observed, implying that a lower mass transfer occurred at a lower gas flow rate. Additionally, this lower P_B corresponded to lower P_{ABE} values of 16.96 and 17.23 g/L using gas flow rates at 0.5 and 1.5 L/min, respectively. Hence, the best gas flow rate to improve the butanol production efficiency was 1.0 L/min. Regarding the selectivity (α) of gas stripping system, the α values for acetone, butanol and ethanol at the gas flow rate of 1.0 L/min were 0.22, 4.69 and 0, respectively; indicating that the condition of gas stripping was the most suitable for butanol removal. The results also illustrated that the $Y_{B/S}$ and $Y_{ABE/S}$ values of this ABE fermentation integrated with a gas stripping system under various gas flow rates were not significantly different (Table 4), indicating that the gas stripping system did not disturb the ABE fermentation. This gas stripping using a flow rate of 1.0 L/min is suitable for batch butanol production from sugarcane molasses by *C. beijarinckii* TISTR1461.

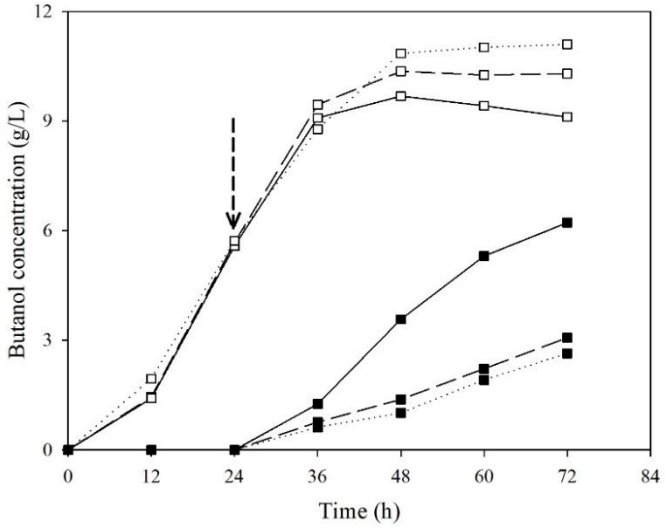


Figure 5 Butanol concentration profiles of an ABE fermentation integrated with gas stripping system under various gas flow rates: butanol concentration in the bioreactor (□) and butanol concentration in the receiving flask (■); 0.5 L/min (dash lines), 1.0 L/min (solid lines) and 1.5 L/min (dot lines). The arrows indicate the starting time of the gas stripping system.

Table 4 The results of an ABE fermentation by *C. beijerinckii* TISTR1461 from sugarcane molasses integrated with a gas stripping system under various gas flow rates at 72 h.

Results	Gas flow rates (L/min)		
	0.5	1.0	1.5
Cumulative acetone (g/L)	3.45 ± 0.23 [a]	4.11 ± 0.34 [b]	3.19 ± 0.52 [a]
Butanol in bioreactor (g/L)	10.30 ± 0.27 [b]	9.14 ± 0.11 [a]	11.10 ± 0.18 [c]
Butanol in condensate (g/L)	3.07 ± 0.17 [b]	6.22 ± 0.12 [c]	2.60 ± 0.20 [a]
Cumulative butanol (g/L)	13.10 ± 0.31 [a]	15.33 ± 0.25 [c]	13.74 ± 0.28 [b]
Cumulative ethanol (g/L)	0.39 ± 0.50 [b]	0.00 ± 0.00 [a]	0.52 ± 0.07 [c]
ABE concentration (g/L)	16.96 ± 0.11 [a]	19.46 ± 0.18 [b]	17.23 ± 0.35 [a]
Sugar consumption (%)	68.13 ± 1.52 [a]	75.00 ± 2.05 [b]	69.70 ± 1.89 [a]
$Y_{B/S}$ (g/g)	0.38 ± 0.01 [a]	0.39 ± 0.01 [a]	0.39 ± 0.01 [a]
$Y_{ABE/S}$ (g/g)	0.50 ± 0.01 [a]	0.50 ± 0.01 [a]	0.49 ± 0.01 [a]

[a, b, and c] Mean followed by the same letter within the same row are not significantly different using Duncan's multiple range test at a level 0.05
$Y_{B/S}$ = butanol yield, $Y_{ABE/S}$ = total ABE yield

Table 5 summarizes the butanol production from various feedstocks in batch fermentations with and without a gas stripping system. Many agricultural residues are potential feedstocks for butanol production (Table 5). Butanol production ranged from 5.58 to 13.70 g/L. In this study, *C. beijerinckii* TISTR1461 successfully used sugarcane molasses as an alternative agricultural residue feedstock for butanol production to produce a relatively high butanol concentration of 10.66 g/L. When the batch fermentation integrated with a gas stripping system, superior butanol production was achieved due to relived end-product inhibition on butanol production. When the gas stripping system was used, the resulting butanol concentration ranged from 9.38 to 16.80 g/L. These results demonstrated that the gas stripping system resulted in improved butanol concentration from approximately 0.8 to 4.67 g/L. In this study, it promoted 4.67 g/L, corresponding to approximately 44% improvement (compared with no gas stripping system).

Table 5 Comparison of butanol fermentation efficiencies with and without a gas stripping system.

Feedstock	Strain	Gas stripping system	P_B (g/L)	Butanol production improvement (g/L)	Reference
Sugarcane molasses	*C. beijerinckii* TISTR1461	-	12.55	1.58	[8]
		+	14.13		
Sweet sorghum juice	*C. acetobutylicum* ABE1201	-	13.20	0.80	[31]
		+	14.00		
Wood pulping hydrolysate	*C. beijerinckii* CC101	-	5.58	3.80	[32]
		+	9.38		
Glucose	*C. beijerinckii* BA101	-	13.70	3.10	[33]
		+	16.80		
Liquefied cornstarch	*C. beijerinckii* BA101	-	13.40	1.70	[33]
		+	15.10		
Sugarcane molasses	*C. beijerinckii* TISTR1461	-	10.66	4.67	This study
		+	15.33		

-, fermentation without gas stripping system
+, fermentation with gas stripping system

3.4 Morphology of C. beijerinckii TISTR 1461

In this study, the morphology of *C. beijerinckii* TISTR1461 cells was investigated at 12 h intervals during batch butanol fermentation. Without a gas stripping system (control experiment), the microorganism showed marked variation in their cell morphology, similar to the results reported by Lütke-Eversloh and Bahl [29] and Schuster et al. [34]. After inoculation and during the acidogenesis phase, rod-shaped cells, sometimes in chains, were observed after 12 h of fermentation. During this period, cells exhibited rapid movement (data not shown). Later, cells converted acids into solvents after 24 h of fermentation. In this phase, clostridial forms of cigar-shaped cells appeared and these cells exhibited sluggish movement. Then, sporulation occurred and forespores were observed at 36 h of fermentation. After 36 h, spore maturation was observed.

The morphology of cells observed in the fermentation using a gas stripping system was similar to the control experiment before the gas stripping system was started after 24 h of fermentation. When gas stripping was done, although high butanol concentration (>10 g/L) at 36 h was detected, forespores were not observed and only cigar-shaped cells were seen (data not shown). The might have been due to butanol removal from the fermentation broth. The cells retained their cigar-shape until 60 h of fermentation. After that, forespores were created at a slower rate than in the control experiment. This result indicated that using a gas stripping system could protect the cells from butanol toxicity, resulting in higher butanol concentrations compared to fermentations without a gas stripping system.

4. Conclusions

Butanol fermentation using a gas stripping system promoted butanol production. The sparger types and the gas flow rates in gas stripping system affected butanol production efficiency. A ring sparger at a gas flow rate of 1.0 L/min gave the highest butanol production from sugarcane molasses by *C. beijerinckii* TISTR1461. Under this condition, the butanol concentration, yield and productivity were 15.33 g/L, 0.39 g/g and 0.21 g/L·h, respectively. The effects of size and temperature of condenser of gas stripping system should be further investigated to enhance butanol production efficiency.

5. Conflict of interest

The authors declare no conflict of interest.

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8. References

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