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Effectiveness of Low-Cost Bioreactors Integrated with a Gas Stripping System for Butanol Fermentation from Sugarcane Molasses by *Clostridium beijerinckii*

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Abstract: The effectiveness of column bioreactors for butanol fermentation from sugarcane molasses by *Clostridium beijerinckii* TISTR 1461 was investigated. This fermentation was operated at an initial pH of 6.5 and temperature of 37 °C under anaerobic conditions. A 1-L bubble column bioreactor was used with various gas circulation rates ranging from 0.2 to 1.0 L/min. The highest butanol concentration (P_B, 8.72 g/L), productivity (Q_B, 0.24 g/L·h) and yield ($Y_{B/S}$, 0.21 g/g) were obtained with a gas circulation of 0.2 L/min. To improve butanol production efficiency, gas-lift column bioreactors with internal and external loops at 0.2 L/min of circulating gas were used. Higher P_B (10.50–10.58 g/L), Q_B (0.29 g/L·h) and $Y_{B/S}$ (0.22–0.23 g/g) values were obtained in gas-lift column bioreactors. These values were similar to those using a more complex 2-L stirred-tank bioreactor (P_B, 10.10 g/L; Q_B, 0.28 g/L h and $Y_{B/S}$, 0.22 g/g). Hence, gas-lift column bioreactors have potential for use as low-cost fermenters instead of stirred-tank bioreactors for butanol fermentation. When the gas-lift column bioreactor with an internal loop was coupled with a gas stripping system, it yielded an enhanced P_B and sugar consumption of approximately 9% and 7%, respectively, compared to a system with no gas stripping.

Keywords: renewable energy; butanol production; sugarcane molasses; *Clostridium* sp.; bubble column bioreactor; gas-lift column bioreactor; gas stripping system

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1. Introduction

Due to increasing concerns over the environmental issues associated with the impact of petroleum fuel emissions and decreasing of fossil fuel reserves, renewable energy resources such as butanol are of interest. Butanol has superior properties compared to other fuels such as ethanol. These properties include a higher boiling point, higher energy capacity and a less caustic nature, as well as a lower vapor pressure and evaporation rate [1]. Thus, butanol is considered one of the most suitable candidate biofuels. Additionally, butanol is a multipurpose chemical feedstock that can be widely utilized in the manufacture of plastics, polymers, lubricants, brake fluids, and synthetic rubber, as well as in cosmetics and food [2].

Butanol can be produced via an acetone-butanol-ethanol (ABE) fermentation from various raw materials. The fermentation consists of two phases, acidogenesis and solventogenesis. Solventogenic *Clostridia*, including *C. saccharoperacetobutylicum*, *C. saccharobutylicum*, *C. beijerinckii* and *C. acetobutylicum*, are commonly used as butanol-producing strains in ABE fermentations. However, *C. acetobutylicum* and *C. beijerinckii* are the primary butanol producing strains used in industrial ABE fermentations [3,4].

One of the main costs for butanol fermentations is for raw materials [5,6]. Hence, abundant and low-cost agricultural raw materials with high carbohydrate contents and micronutrients, including vitamins and minerals, are required. In Thailand, sugarcane molasses, a by-product from sugar production, is produced at a level of approximately 3,500,000 metric tons/year [7]. It consists of many sugars that can be used as carbon sources as well as trace elements for microbial growth. Additionally, sugarcane molasses requires no pretreatment prior to use. Thus, it is a very appropriate substrate for butanol production [8,9].

One of the main problems of butanol production is low concentration and productivity due to butanol toxicity during microbial growth [10]. High butanol concentrations can damage cell membranes and make them permeable to ADP (adenosine diphosphate) and some ions, leading to cell lysis [11]. To address this problem, gas stripping can be used to remove butanol from the fermentation broth and alleviate product inhibition. It is a simple and effective technique with low energy consumption that is easy to integrate into a fermentation system [12,13].

Mechanical agitation is one of the major methods of mixing in bioreactors for ABE fermentations [14]. Normally, ABE fermentations are performed using stirred-tank bioreactors since they provide good mixing and are easy-to-control systems. Nevertheless, these bioreactors are expensive, require high energy inputs and are highly complex in their operation. Hence, bubble column and gas-lift column bioreactors are alternatives that can be used for butanol production due to their lower energy consumption, shear stress and costs than mechanically stirred-tank bioreactors [15]. Bubble column and gas-lift column bioreactors are simple in design and operation, have no mechanical agitation and are easy to operate. Circulation of gas in a column bioreactor provides agitation in pneumatically mixed bioreactors [16]. Nonetheless, the use of bubble column and gas-lift column bioreactors for butanol production has rarely been reported.

Thus, the aim of this research was to study the capabilities of column bioreactors for butanol production from sugarcane molasses by *C. beijerinckii* TISTR 1461. Additionally, butanol fermentation using a column bioreactor coupled with gas stripping was investigated to enhance butanol production.

2. Materials and Methods

2.1. Microorganism and Inoculum Preparation

C. beijerinckii TISTR 1461 was purchased from the Thailand Institute of Scientific and Technological Research (TISTR), Pathum Thani, Thailand. It was preserved as spore suspension and kept in sterile distilled water at 4 °C. The spore suspension (~1×10^6 spores/mL) was heat shocked in a hot water bath at 80 °C for 1 min and thereafter immediately swirled in an iced-water bath for 1 min to prevent cell damage [13]. Afterward, a 5% (v/v) spore suspension was transferred into 10 mL of sterile cooked meat medium (CMM) (Oxoid, Basingstoke, Hants, UK) and incubated at 37 °C for 16–19 h under static conditions to produce highly motile vegetative cells. CMM consists of 1 g of CMM and 0.08 g of glucose in 10 mL of distilled water. The CMM was sterile and sparged with oxygen free nitrogen (OFN) gas to attain anaerobic conditions before use [17]. Vegetative cells in the exponential phase of growth at a level of 5% (v/v) exhibited an optical density at 600 nm of 0.5. They were inoculated in a tryptone-glucose-yeast extract (TGY) medium and incubated at 37 °C for 4–6 h before use as an inoculum for butanol production [13,17]. TGY medium is comprised of 5 g of tryptone (Oxoid, Basingstoke, Hants, UK), 1 g of glucose (BDH, Leuvn, Belgium), 5 g of yeast extract (Oxoid, Basingstoke, Hants, UK) and 1 g of K_2HPO_4 (BDH, Leuvn, Belgium) in 1 L distilled water. The TGY medium was autoclaved and purged with OFN gas in the same manner as the CMM medium before use.

2.2. Raw Materials

Sugarcane molasses containing 80 °Bx of total soluble solids was used as a substrate for butanol production. It was obtained from Mitr Phu Viang Sugar Factory, Nong Rua, Khon Kaen, Thailand. Its chemical composition was determined by the Central Laboratory (Thailand) Co., Ltd., Khon Kaen, Thailand and is shown in Table 1. The molasses was stored at −20 °C to protect it from bacterial growth before use.

Table 1. Composition of sugarcane molasses.

Composition	Concentration		Analytical Method
Protein [a]	6.77	g/100 mL	[18] [b]
Phosphorus (P) [a]	483.19	mg/L	ICP-MS
Potassium (K) [a]	28133.60	mg/L	
Sodium (Na) [a]	1405.29	mg/L	
Calcium (Ca) [a]	8110.65	mg/L	
Magnesium (Mg) [a]	4498.04	mg/L	
Iron (Fe) [a]	150.74	mg/L	
Manganese (Mn) [a]	79.90	mg/L	
Copper (Cu) [a]	0.25	mg/L	
Zinc (Zn) [a]	29.93	mg/L	
Molybdenum (Mo) [a]	0.10	mg/L	
Nickel (Ni) [a]	10.81	mg/L	
Boron (B) [a]	44.78	mg/L	
Cobalt (Co) [a]	2.57	mg/L	
Sulfur (S)[a]	5214.58	mg/L	Turbidimetry method
Sucrose	445.60	g/L	HPLC (modified from [19])
Glucose	128.20	g/L	
Fructose	99.46	g/L	

[a] Central Laboratory (Thailand) Co., Ltd., Khon Kaen, Thailand, [b] Association of Official Analytical Chemists [18].

Dried spent yeast (DSY) obtained from Beer Thip Brewery Factory (1991) Co., Ltd., Bang Baan, Phra Nakhon Sri Ayutthaya, Thailand was stored at room temperature. It was used as a nitrogen supplement for butanol fermentation.

2.3. Butanol Production Medium

Sugarcane molasses was diluted with distilled water to achieve a 50 g/L total sugar concentration. Then, 6 g/L DSY was added before sterilization at 110 °C 28 min (modified from [20]). The pH of the sterile medium was adjusted to 6.5 by dropwise addition of 8 N NaOH (modified from [21]). It was then used as a butanol production (BP) medium. Furthermore, sugarcane molasses with no DSY supplementation was used as a control treatment.

2.4. Batch Fermentation Conditions

2.4.1. Butanol Fermentation in Screw-Capped Bottles and a Stirred-Tank Bioreactor

The sterile BP media with and without DSY supplementation were purged with OFN gas to create anaerobic conditions. A 5% (v/v) inoculum in the exponential phase of growth in TGY medium had an optical density at 600 nm of 0.5. It was rapidly transferred into BP medium in a 1-L screw-capped bottle (0.7 L of working volume) or a 2-L stirred-tank bioreactor (Biostat®B, B. Braun Biotech, Melsungen, Germany) (1.4 L of working volume). Temperature and agitation rate of the system were controlled at 37 °C and 150 rpm, respectively [13].

2.4.2. Butanol Fermentation in a Bubble Column Bioreactor under Various Gas Circulating Rates

An 800 mL aliquot of sterile BP medium in the 1.2-L bubble column bioreactor (Figure 1A) was purged with OFN gas. Then, a 5% (v/v) inoculum was added into the BP medium. The bubble column bioreactor used in this study was a 1.2-L jacketed glass column with a length of 55 cm and an internal diameter of 5.45 cm. Column tempera-

ture during fermentation was controlled at 37 °C. Mixing and agitation were achieved by varying gas circulating rates using a peristaltic pump at flow rates of 0.2, 0.5 and 1.0 L/min.

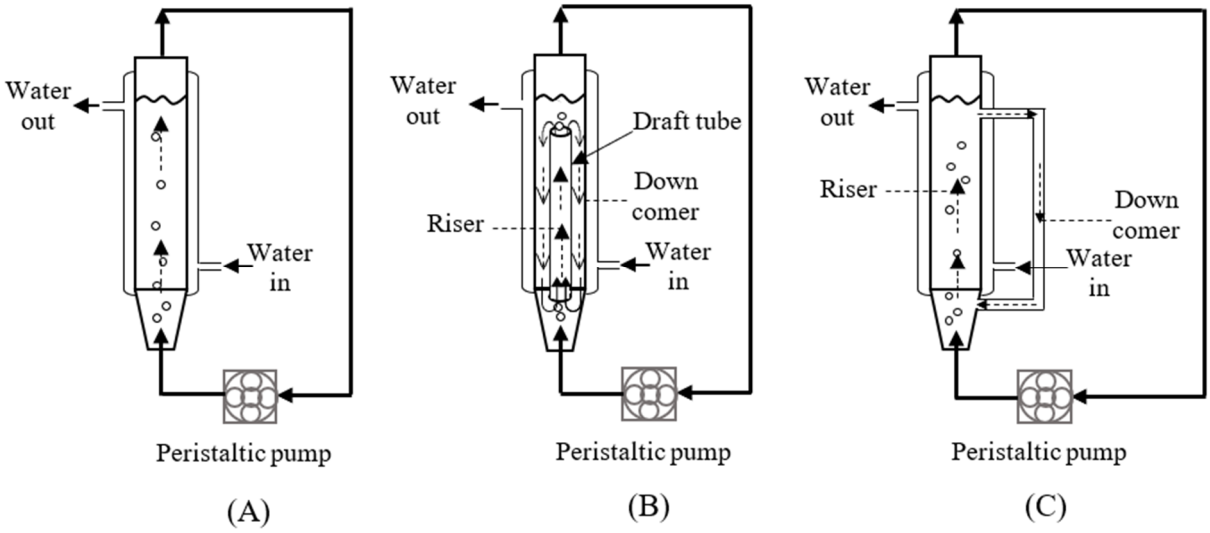

Figure 1. Configuration of bubble column and gas-lift column bioreactors for butanol fermentation. (**A**) bubble column; (**B**) gas-lift column bioreactor with internal loop and draft tube and (**C**) gas-lift column bioreactor with external or outer loop.

2.4.3. Butanol Fermentation in Gas-Lift Column Bioreactors

Butanol production was carried out using gas-lift column bioreactors with internal and external loops under the optimal gas circulation condition obtained in Section 2.4.2. The gas-lift column bioreactor with internal loop consisted of a 1.2-L jacketed glass column (5.45 (i.d.) × 55 cm) with a draft tube (2.92 (i.d.) × 23 cm) inside (Figure 1B), whereas the gas-lift column bioreactor with external loop consisted of a 1.2-L jacketed glass column connected with a small tube (1 (i.d.) × 50 cm) (Figure 1C).

2.4.4. Butanol Fermentation in an Internal Loop Gas-Lift Column Bioreactor Integrated with a Gas Stripping System

The gas-lift column bioreactor internal loop for butanol fermentation was connected to a gas stripping system, as shown in Figure 2. The fermentation was carried out as previously described in Section 2.4.3. After 24 h of fermentation, the gas stripping system was started and controlled at a gas flow rate of 1.0 L/min. The temperature of the condenser (Pyrex, Stoke-on-Trent, Staffs, UK; condenser 40 (i.d.) × 450 mm and cooling coil 0.60 (i.d.) × 1500 mm) was controlled at −8 °C using a cooling bath [13]. Samples in the bioreactor and receiving flask were collected for analyses during the fermentation.

2.5. Analytical Methods

During all fermentations, samples were taken from bioreactors for analyses at regular time intervals. All experiments were carried out in triplicate. The samples were centrifuged at 10,000 rpm for 10 min to remove bacterial cells and other particles. The levels of total organic solvents (acetone, butanol and ethanol) and organic acids (acetic and butyric acids) in the supernatant were measured using a gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) with a stainless-steel column [2.0 mm (i.d.) × 3 m] containing Porapak Q, 80/100 mesh (Resteck, Bellefonte, PA, USA). A flame ionization detector (FID) was used to detect solvents and acids. H_2 gas was used as a fuel gas. The injector and detector temperatures were 220 and 230 °C, respectively. N_2 was used as a carrier gas at a pressure of 150 kPa for 10 min at 160 °C, followed by an increase of 15 °C/min to 180 °C. It was held at 180 °C for 20 min. Iso-butanol was used as an internal standard (modified from [21]). Total sugar levels were determined using a phenol-sulfuric acid method [22]. pH was measured using a pH meter (FiveEasy plus, Mettler Toledo, Columbus, Ohio,

USA). Cell morphology was observed under light microscopy. Butanol yield ($Y_{B/S}$) and butanol productivity (Q_B) were calculated as follows: $Y_{B/S} = P_B/TS$ and $Q_B = P_B/t$, where P_B is the butanol titer produced (g/L), TS is the total sugars consumed (g/L), and t is the fermentation time (h) giving the highest butanol titer.

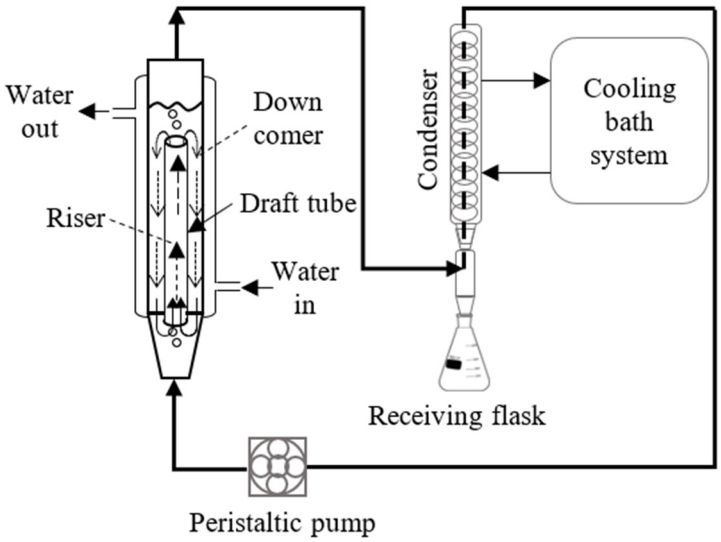

Figure 2. Configuration of an internal loop gas-lift bioreactor integrated with a gas stripping system for butanol production.

3. Results and Discussion

3.1. Butanol Fermentation from Sugarcane Molasses in Screw-Capped Bottles and a Stirred-Tank Bioreactor

Butanol fermentations in screw-capped bottles and a stirred-tank bioreactor were carried out as control experiments. Batch butanol fermentation profiles using sugarcane molasses with and without DSY addition in a 1-L screw-capped bottle are shown in Figure 3. Under a condition with no DSY addition, pH decreased during the fermentation due to acetic and butyric acid production, suggesting that acetate kinase and butyrate kinase were active, respectively [23]. The results implied that ATP was generated, and cells were active until 12 h. During this period, an acidogenesis phase was observed. Then, acids were converted into solvents and concurrently increased the pH during the solventogenesis phase (Figure 3A). With no DSY addition, 7.30 ± 0.33 g/L of butanol (P_B) was produced after 48 h of fermentation, whereas acetone and ethanol were detected at 3.85 ± 0.16 and 1.23 ± 0.02 g/L, respectively (Figure 3B). At the end of the fermentation, 13.01 ± 0.56 g/L of total sugars remained (Figure 3C), corresponding to ~74% sugar consumption. This might have been due to insufficient nitrogen in sugarcane molasses. Under this condition, P_B and ABE concentrations (P_{ABE}) were 7.30 ± 0.33 g/L and 12.09 ± 0.11 g/L, respectively. This corresponds to a $Y_{B/S}$ of 0.20 ± 0.01 g/g and Q_B of 0.15 ± 0.01 g/L·h When sugarcane molasses was supplemented with DSY (Figure 3A), the acidogenesis phase occurred within 12 h, as observed in a fermentation using molasses without DSY supplementation. However, the fermentation time to produce butanol and ABE was within 36 h, suggesting that addition of DSY promoted the rate of butanol production or Q_B. These results were similar to those of Mechmech et al. [24], who found that use of yeast extract as a nitrogen source reduced fermentation time and promoted cell growth of *C. acetobutylicum* ATCC 824, producing butanol from a xylose-P2 medium. Under DSY addition, P_B and P_{ABE} increased to 9.13 ± 0.54 g/L and 13.29 ± 0.46 g/L, respectively (Figure 3B,C), while $Y_{B/S}$ (0.22 ± 0.01 g/g) and Q_B (0.22 ± 0.01 g/L·h) were improved. Additionally, the sugar consumption increased to 80%.

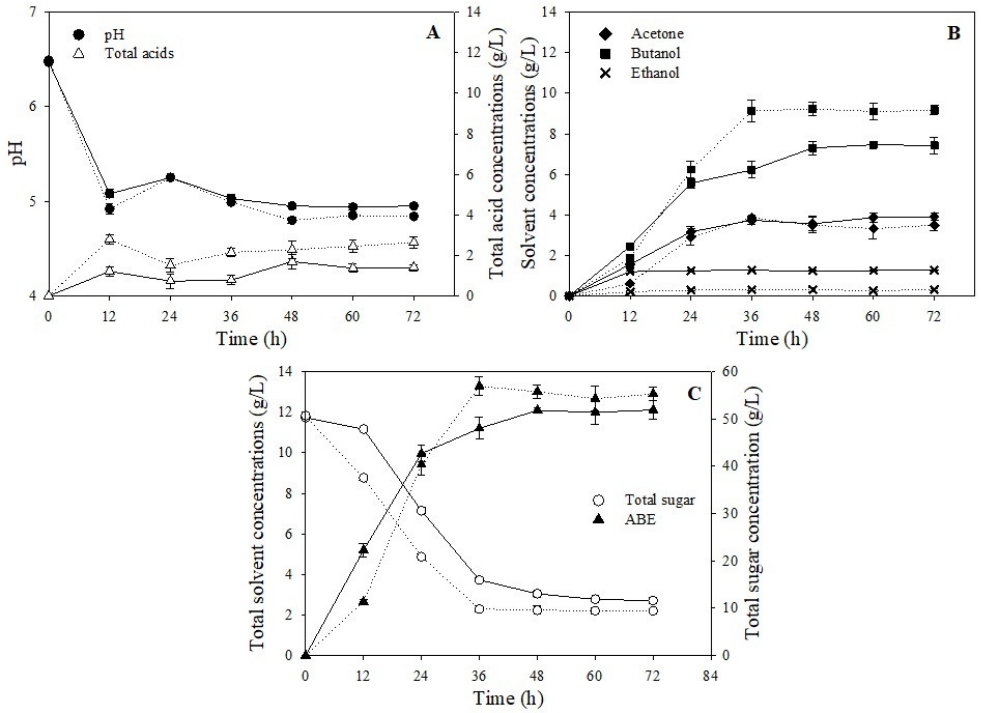

Figure 3. Profiles of ABE fermentation from sugarcane molasses by *C. beijerinckii* TISTR 1461 in 1-L screw capped bottles without (solid line) and with (dotted line) DSY addition. (**A**) pH and total acid, (**B**) acetone, butanol and ethanol and (**C**) total solvent and total sugar.

When a butanol fermentation from sugarcane molasses supplemented with DSY was carried out in a 2-L stirred-tank bioreactor, butanol fermentation profiles were similar to that in a screw capped bottle, but the P_B and P_{ABE} increased to 10.10 ± 0.30 g/L and 15.58 ± 0.32 g/L, respectively (Figure 4). This was approximately 10–17% higher than in screw-capped bottles, perhaps due to the positive effect of mixing. In the stirred-tank bioreactor, a 6-blade turbine with 4 baffles was used to improve mixing, as well as heat and mass transfer, whereas only a magnetic bar was used in the screw-capped bottles. In the stirred-tank bioreactor, $Y_{B/S}$ and Q_B were 0.23 ± 0.01 g/g and 0.28 ± 0.01 g/L·h, respectively. Nevertheless, $Y_{B/S}$ (0.22 ± 0.01 g/g) values in the screw-capped bottles and stirred-tank bioreactor were not significantly different, suggesting that the bioreactors did not affect the metabolic pathway of butanol fermentation from sugarcane molasses by *C. beijerinckii* TISTR 1461.

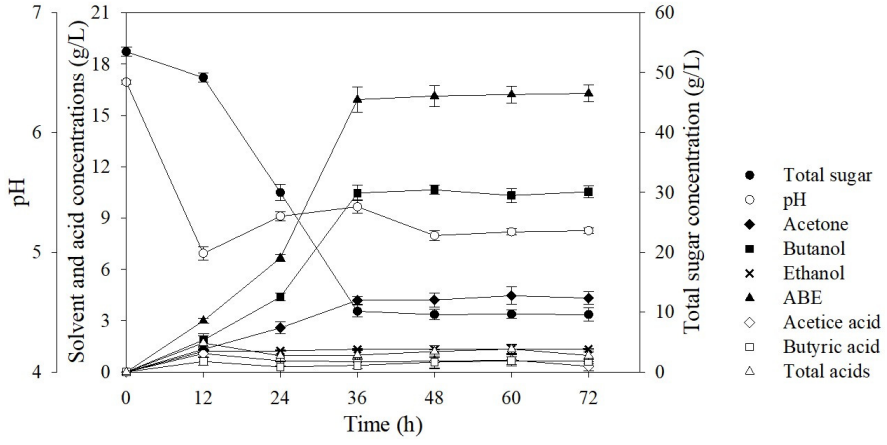

Figure 4. Profiles of ABE fermentation from the sugarcane molasses supplemented with DSY by *C. beijerinckii* TISTR 1461 in a 2-L stirred tank bioreactor.

3.2. Butanol Fermentation from Sugarcane Molasses in Column Bioreactors

3.2.1. Effects of Gas Circulation on Butanol Fermentation in a Bubble Column Bioreactor

A bubble column bioreactor was used in this study to evaluate butanol production and reduce the cost of the bioreactor. Mixing in the bubble column was obtained via buoyant forces caused by gas circulation. Gas circulation rates affect flow patterns and mixing. Hence, H_2 and CO_2 from the headspace of the broth in the bubble column were circulated and returned to the bottom of the column at rates of 0.2, 0.5 and 1.0 L/min. The results showed that pH values during fermentation at gas circulation rates of 0.5 and 1.0 L/min were lower than at 0.2 L/min after 12 h of fermentation, which corresponds to the higher acid concentrations produced in the broth at 0.5 and 1.0 L/min (Figure 5A,B and Table 2). This might have been due to lower acid conversion to solvents during the 12–36 h period of fermentation under various conditions (Figure 5C,D). Under all conditions tested, sugar consumption ranged from 25 to 85% (Figure 5E). Additionally, the patterns of butanol and ABE production were similar under various gas circulation rates (Figure 5C,D). The butanol yields under all conditions tested were not significantly different (Table 2), implying that the metabolic pathway of butanol production had not changed. In the current study, a maximal P_B of 8.72 ± 0.18 g/L was obtained at 0.2 L/min of circulating gas (Table 2). This implies that the best mixing occurred at a lower gas circulation rate. This is supported by Doran [25], who reported that churn flow occurred, and poor blending was observed at higher gas circulation rates, resulting in lower butanol production. Additionally, it was reported that homogeneous flow occurs only at low gas flow rates and when the bubbles leaving the sparger are evenly distributed across the column cross-section [24]. The highest Q_B, 0.24 ± 0.00 g/L·h, was achieved at a gas circulation rate of 0.2 L/min. However, lower gas circulation rates were not examined in this study because cell and DSY sedimentation occurred during the fermentation. Consequently, a gas circulation rate of 0.2 L/min was used in subsequent experiments to boost butanol production in gas-lift column bioreactors.

Table 2. Butanol production in a bubble column bioreactor under various gas circulation rates after 36 h of fermentation.

Fermentation Results	Gas Circulating Rate (L/min)		
	0.2	**0.5**	**1.0**
Acetone (g/L)	4.42 ± 0.11 ^{c}	2.23 ± 0.20 ^{b}	1.91 ± 0.21 ^{a}
Butanol (g/L)	8.72 ± 0.18 ^{c}	4.70 ± 0.21 ^{b}	2.23 ± 0.25 ^{a}
Ethanol (g/L)	1.25 ± 0.01 ^{a}	1.24 ± 0.01 ^{a}	1.27 ± 0.02 ^{a}
ABE (g/L)	14.48 ± 0.43 ^{c}	8.18 ± 0.42 ^{b}	5.44 ± 0.40 ^{a}
Total acids * (g/L)	2.22 ± 0.02 ^{a}	3.94 ± 0.20 ^{b}	3.82 ± 0.23 ^{b}
$Y_{B/S}$ (g/g) **	0.21 ± 0.01 ^{a}	0.21 ± 0.01 ^{a}	0.20 ± 0.01 ^{a}
Q_B (g/L·h) **	0.24 ± 0.00 ^{c}	0.13 ± 0.01 ^{b}	0.06 ± 0.01 ^{a}

* Acetic and butyric acids. The experiments were performed in triplicate and the results were expressed as mean values ± SD, ^{a,b,c} Mean followed by the same letter within the same row are not significantly different using Duncan's multiple range test at a level of 0.05. ** $Y_{B/S}$ = butanol yield, Q_B = butanol productivity.

To elucidate these results, the cell morphology of *C. beijerinckii* TISTR 1461 under gas circulation rates of 0.2, 0.5 and 1.0 L/min was observed under light microscopy. The results showed that the cells appeared clostridial in form or cigar-shaped more than as forespores or prespores under a gas circulation of 0.2 L/min. Forespores and spores were observed at gas circulation rates of 0.5 and 1.0 L/min (data not shown). This indicates that high butanol concentrations were produced when the clostridial form was observed. It was reported that forespores appeared and solvents were not produced when *C. beijerinckii* TISTR 1461 was grown on sweet sorghum juice, while cells of the clostridial form emerged and high levels of solvents were also produced [26].

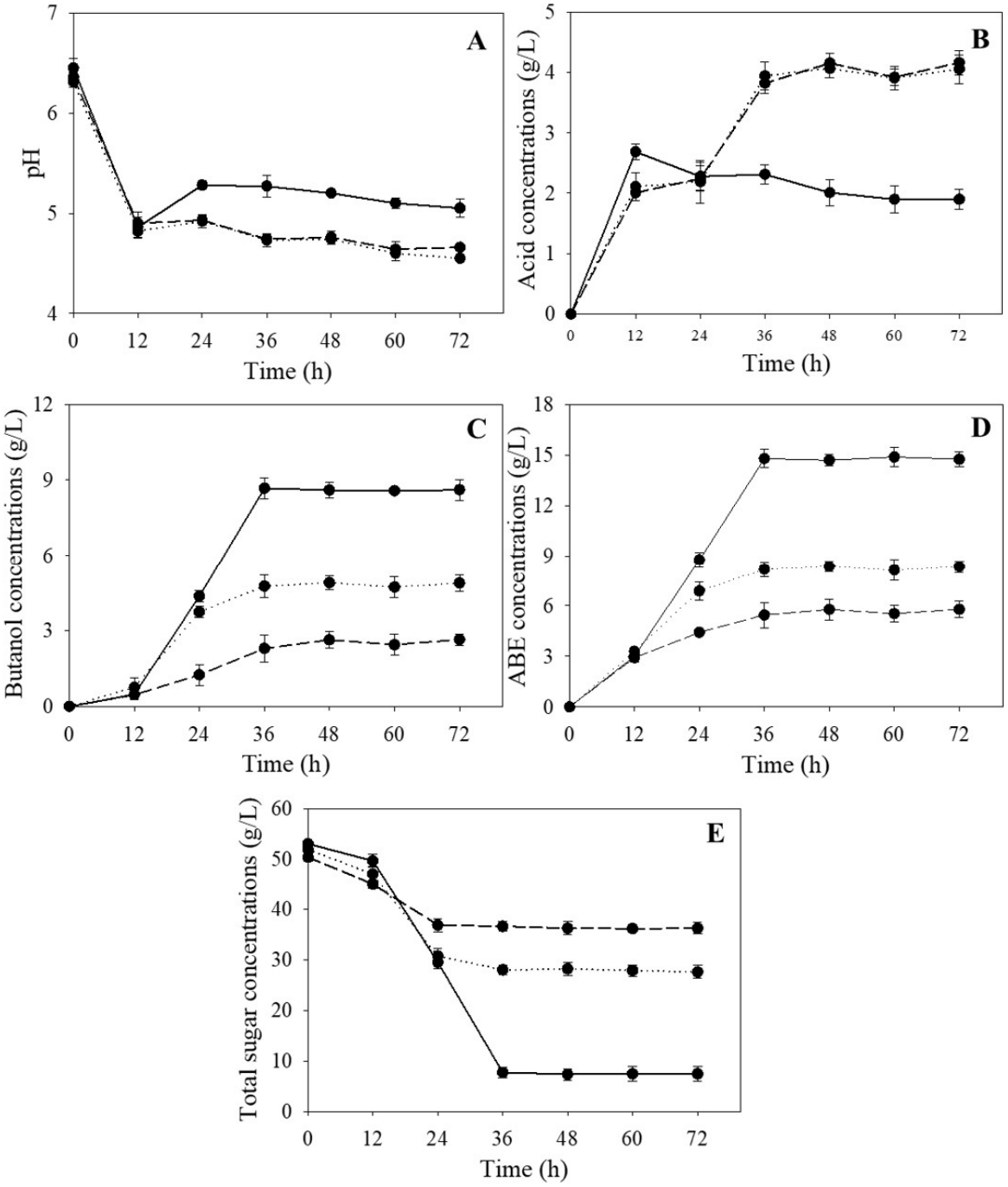

Figure 5. Profiles of ABE fermentation from sugarcane molasses by *C. beijerinckii* TISTR 1461 under various gas circulation rates: 0.2 (solid lines), 0.5 (dashed lines) and 1.0 (dotted lines) L/min; (**A**) pH; (**B**) acid; (**C**) butanol; (**D**) ABE and (**E**) total sugars.

3.2.2. Batch Butanol Fermentation in Gas-Lift Column Bioreactors

From Section 3.2.1, it was found that a bubble column could be used as a low-cost bioreactor for butanol fermentation. However, the butanol production efficiency (P_B and Q_B) using the bubble column bioreactor was approximately 16% lower than that using a standard bioreactor or stirred-tank bioreactor (Table 3 and Figure 6). Lower butanol production in the bubble column bioreactor might have been due to insufficient mixing. Hence, internal and external loop gas-lift column bioreactors were further investigated to promote mixing in the column bioreactors for butanol fermentation. The results showed that P_B values of

10.58 ± 0.28 and 10.50 ± 0.19 g/L and P_{ABE} values of 17.15 ± 0.42 and 16.26 ± 0.43 g/L were obtained using the internal and external loop bioreactors, respectively, and Q_B values (0.29 ± 0.01 g/L·h) were not different in both gas-lift column bioreactors (Figure 6). The P_B and Q_B of the gas-lift column bioreactors increased by approximately 20–21%, respectively, compared with those of the bubble column bioreactor. Butanol fermentations in various bioreactors are compared in Figure 6. The efficiency of butanol production in terms of P_B and Q_B in both gas-lift column bioreactors was not significantly different from the stirred-tank bioreactor, but they were markedly higher than those in the bubble column. These might be due to different patterns of liquid flow in the column bioreactors. Gas disengages at the top of the gas-lift column bioreactors, leaving a denser bubble-free liquid to recirculate through a downcomer or downward direction. Liquid circulates in gas-lift column bioreactors as a result of the density difference between the riser and downcomer [25]. These factors cause mixing in column bioreactors. The results suggest that gas-lift bioreactors with internal and external loops can be used instead of stirred-tank bioreactors for butanol fermentation. The $Y_{B/S}$ values under all conditions tested were not significantly different, suggesting that the type of bioreactor did not affect the butanol production pathway of *C. beijerinckii* TISTR 1461 using sugarcane molasses as a substrate. However, sugar utilization of the bacterium for butanol fermentation was not complete with approximately 12% of sugar remaining at the end of fermentation. This result might have been due to butanol toxicity to bacterial cells [10]. There are many methods that can be used to improve butanol production by protecting the bacterial cells from butanol toxicity or product inhibition such as genetic engineering [27], gas stripping systems [13,28], adsorption [29] and pervaporation techniques [30]. Gas stripping was chosen in subsequent experiments due to its simplicity and compatibility with the ABE fermentation [13].

Table 3. Sugar consumption and product formation in ABE fermentations using various bioreactors after 36 h of fermentation.

Fermentation Results	Bioreactors			
	Stirred-Tank	**Bubble Column**	**Internal Loop Gas-Lift**	**External Loop Gas-Lift**
Acetone (g/L)	4.11 ± 0.04 ^{a}	4.42 ± 0.31 ^{a}	5.44 ± 0.19 ^{b}	4.40 ± 0.26 ^{a}
Butanol (g/L)	10.10 ± 0.30 ^{b}	8.72 ± 0.18 ^{a}	10.58 ± 0.28 ^{b}	10.50 ± 0.19 ^{b}
Ethanol (g/L)	1.26 ± 0.01 ^{a}	1.25 ± 0.01 ^{a}	1.13 ± 0.12 ^{a}	1.39 ± 0.07 ^{a}
ABE (g/L)	15.58 ± 0.32 ^{b}	14.48 ± 0.23 ^{a}	17.15 ± 0.42 ^{d}	16.26 ± 0.43 ^{c}
Total acids * (g/L)	0.97 ± 0.02 ^{a}	2.22 ± 0.02 ^{c}	1.47 ± 0.20 ^{b}	2.52 ± 0.11 ^{d}
Sugar consumption (%)	82.12 ± 1.52 ^{a}	84.56 ± 1.04 ^{a,b}	88.20 ± 2.05 ^{c}	85.84 ± 0.23 ^{b,c}

* Acetic and butyric acids. The experiments were carried out in triplicate, The results are shown as mean values ± SD, ^{a,b,c,d} Mean followed by the same letter within the same row are not significantly different using Duncan's multiple range test at a level of 0.05.

Comparison of batch butanol production efficiency from sugarcane molasses in this study with other research using the same raw material [31], a glucose medium [10,32], and other raw materials [33–35] are shown in Table 4. Typically, butanol fermentations are operated in stirred-tank bioreactors due to their good mixing and control systems. The results showed that butanol production of *C. beijerinckii* TISTR 1461 using a stirred-tank bioreactor was relatively high. However, to reduce the operational complexity and costs of stirred-tank bioreactors, gas-lift column bioreactors are alternatives for butanol fermentation. P_B and $Y_{B/S}$ values in stirred-tank bioreactors are in the range of 6.90–15.68 g/L and 0.16–0.26 g/g, respectively, depending on species of microorganism, raw materials and environmental conditions for fermentation. In this study, the butanol fermentation efficiency using sugarcane molasses by *C. beijerinckii* TISTR 1461 in internal and external loop gas-lift column bioreactors was similar to those using stirred-tank bioreactors. Their P_B and $Y_{B/S}$ values were 10.50–10.58 g/L and 0.22 g/g, respectively. This clearly demonstrates that a gas-lift column bioreactor has potential for use as an alternative low-cost fermenter for butanol production.

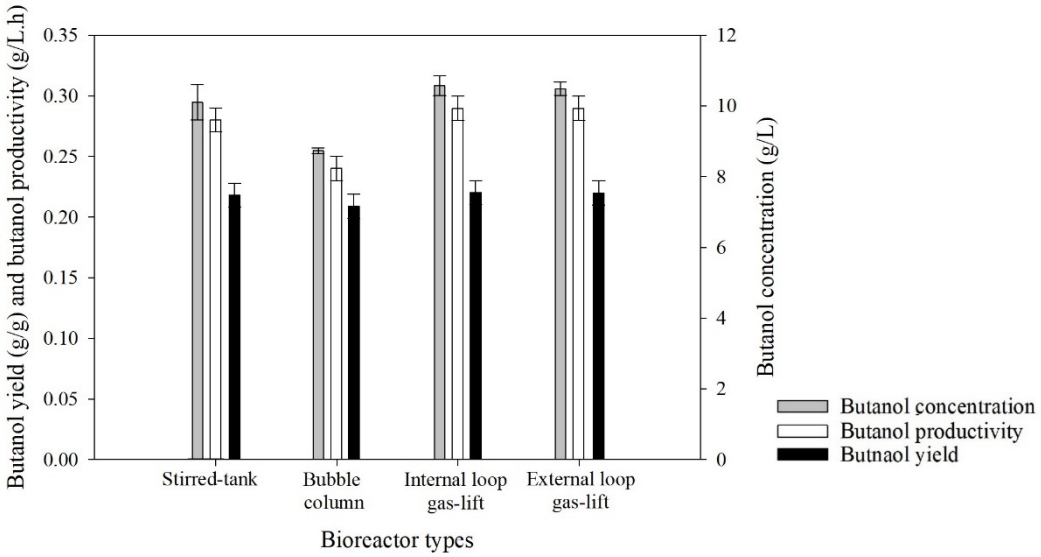

Figure 6. Comparison of butanol fermentation efficiency from sugarcane molasses by *C. beijerinckii* TISTR 1461 in various bioreactors.

Table 4. Comparison of batch butanol fermentation efficiency from various studies.

Feedstock	Microorganism	Bioreactor	P_B (g/L) *	$Y_{B/S}$ (g/g) *	References
Sugarcane molasses (ITS ** = 60 g/L)	*C. saccharobutylicum* DSM13864	Stirred-tank	11.86	0.22	[31]
Glucose (ITS = 60 g/L)	*C. beijerinckii* IB4	Stirred-tank	15.68	0.26	[10]
Waste starch (ITS = 60 g/L)	*C. acetobutylicum* NRRL B-519	Stirred-tank	9.90	0.16	[33]
Corn stalk juice (ITS = 60 g/L)	*C. acetobutylicum* ABE 1201	Stirred-tank	13.71	0.23	[34]
Sugarcane-sweet sorghum juices (ITS = 75 g/L)	*C. acetobutylicum* DSM 792	Stirred-tank	10.50	0.18	[35]
Glucose (ITS = 35 g/L)	*C. acetobutylicum* DSM 6228	Stirred-tank	6.90	0.19	[32]
Sugarcane molasses (ITS = 50 g/L)	*C. beijerinckii* TISTR 1461	Stirred-tank	10.10	0.22	This study
Sugarcane molasses (ITS = 50 g/L)	*C. beijerinckii* TISTR 1461	Internal loop gas-lift	10.58	0.22	This study
Sugarcane molasses (ITS = 50 g/L)	*C. beijerinckii* TISTR 1461	External loop gas-lift	10.50	0.22	This study

* P_B = butanol concentration, $Y_{B/S}$ = the butanol yield, ** ITS = initial total sugar concentration.

Regarding fermentation costs, a gas-lift column bioreactor is a lower-cost bioreactor compared to a stirred-tank bioreactor (STR) in terms of both capital costs and operating costs (energy costs). The price of a standard (basic) 2-L STR (laboratory scale) in Thailand is ∼USD 30,000 (personal contact), whereas the price of the 1.2-L column bioreactor used in this study is ∼USD 7000, which is ∼4-fold lower than the STR. In terms of energy used in this study for 72 h of the fermentation, the energy cost of the STR (for control unit and cooling system) was USD 25.8, whereas it cost USD 18.6 for the column bioreactor (for a peristaltic pump and cooling system). So, the energy cost of the operation using the column bioreactor was about 28% lower than of the STR. The difference of the cost will be greater in larger scale bioreactors. However, butanol yields obtained from both systems are not

different (Tables 3 and 4). This shows that a gas-lift column bioreactor can be used as a low-cost bioreactor for butanol production.

3.2.3. Butanol Fermentation in an Internal Loop Gas-Lift Column Bioreactor Integrated with a Gas Stripping System

Due to the complexity of an external loop gas-lift bioreactor coupled with a gas stripping system, an internal loop gas-lift bioreactor was chosen for this purpose in a butanol fermentation (Figure 2). The gas stripping system was started after 24 h of fermentation, before the onset of butanol toxicity (~8 g/L) [13] (Figure 7). The results showed that the fermentation time that achieved the highest P_B with gas stripping system was ~12 h longer (Figure 8). Nonetheless, butanol fermentation profiles of both conditions were similar in the first 36 h. Using the gas stripping system, the P_B and P_{ABE} values were 11.50 ± 0.13 g/L and 17.78 ± 0.11 g/L, respectively (Figure 7), corresponding to $Y_{B/S}$ and Q_B values of 0.23 ± 0.01 g/L and 0.24 ± 0.00 g/L·h, respectively. The P_B and total sugar consumption values using the gas stripping system were approximately 9 and 7% higher than those with no gas stripping, respectively (Figure 8). These results indicate that the gas stripping system under this condition can enhance butanol fermentation in an internal loop gas-lift column bioreactor.

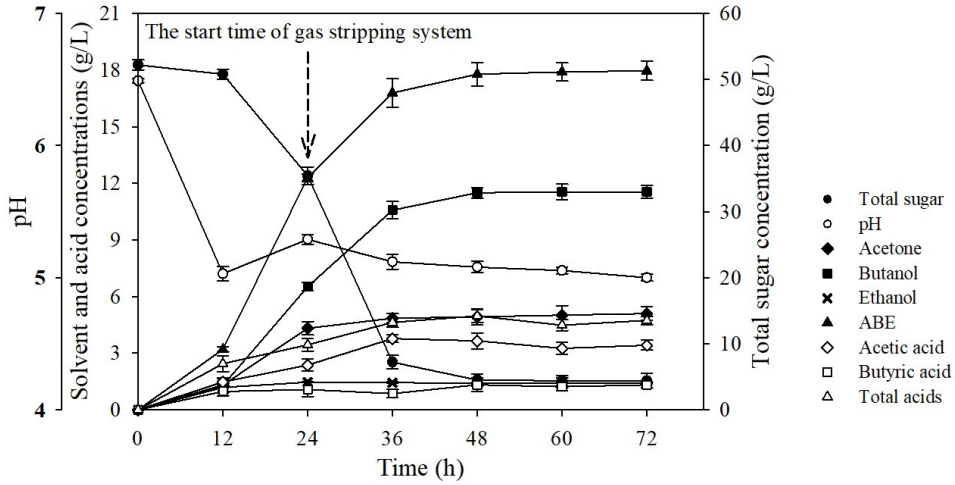

Figure 7. Profiles of ABE fermentation from sugarcane molasses by *C. beijerinckii* TISTR 1461 using an internal loop gas-lift bioreactor integrated with a gas stripping system.

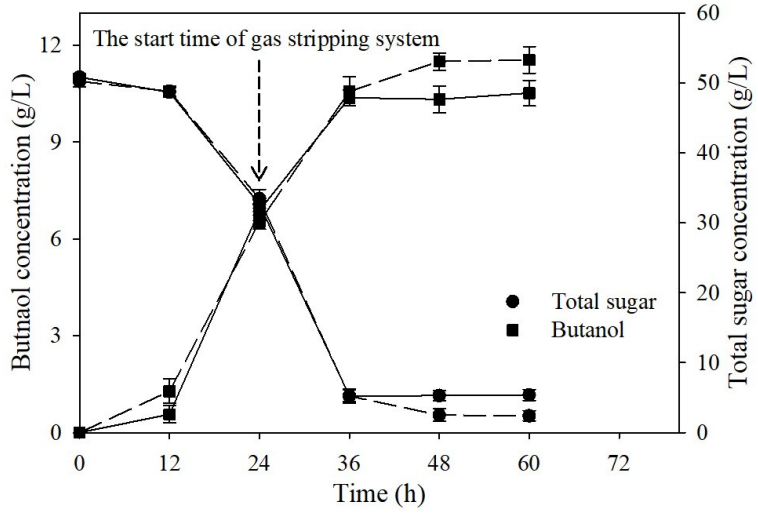

Figure 8. Comparison of butanol production and sugar consumption from sugarcane molasses using an internal loop gas-lift bioreactor coupling with a gas stripping system (dashed lines) and without gas stripping (solid lines).

Complete sugar consumption was not observed in this study. This might have been due to the presence of non-fermentable sugars in the fermented broth (2 g/L of sugar remaining). Higher P_B values may be obtained if the initial sugar concentration is increased. Butanol production from sugarcane molasses at higher initial sugar concentrations to increase the P_B value should be further examined.

4. Conclusions

Gas-lift column bioreactors with internal and external loops were successfully used as low-cost bioreactors to produce butanol from sugarcane molasses by *C. beijerinckii* TISTR 1461. Gas circulation in the column bioreactors affected butanol production. The P_B (10.50–10.58 g/L), $Y_{B/S}$ (0.22–0.23 g/g) and Q_B (0.29 g/L·h) values using the gas-lift column bioreactors were not different from those using a complex bioreactor or stirred-tank bioreactor. The fermentation using an internal loop gas-lift column bioreactor coupled with a gas stripping system improved butanol production under the conditions tested. These findings will be useful for the enhancement of butanol production using gas-lift column bioreactors with lower energy consumption, shear stress and costs compared to stirred-tank bioreactors.

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References

1. Yu, L.; Wu, H.; Zhao, W.; Qian, Y.; Zhu, L.; Lu, X. Experimental study on the application of n-butanol and n-butanol/kerosene blends as fuel for spark ignition aviation piston engine. *Fuel* **2021**, *304*, 121362. [CrossRef]
2. de Jong, E.; Stichnothe, H.; Bell, G.; Jørgensen, H. *Bio-Based Chemical*; IEA Bioenergy: Paris, France, 2020; Volume 42, ISBN 978-1-910154-69-4.
3. Patakova, P.; Linhova, M.; Rychtera, M.; Paulova, L.; Melzoch, K. Novel and neglected issues of acetone-butanol-ethanol (ABE) fermentation by clostridia: *Clostridium* metabolic diversity, tools for process mapping and continuous fermentation systems. *Biotechnol. Adv.* **2013**, *31*, 58–67. [CrossRef] [PubMed]
4. Dahman, Y.; Syed, K.; Begum, S.; Roy, P.; Mohtasebi, B. 14-Biofuels: Their characteristics and analysis. In *Biomass, Biopolymer-Based Materials, and Bioenergy*; Verma, D., Fortunati, E., Jain, S., Zhang, X., Eds.; Woodhead Publishing: Sawston, UK, 2019; pp. 277–325, ISBN 978-0-08-1024263.
5. Ezeji, T.C.; Qureshi, N.; Blaschek, H.P. Acetone butanol ethanol (ABE) production from concentrated substrate: Reduction in substrate inhibition by fed-batch technique and product inhibition by gas stripping. *Appl. Microbiol. Biotechnol.* **2004**, *63*, 653–658. [CrossRef] [PubMed]
6. Kumar, M.; Gayen, K. Developments in biobutanol production: New insights. *Appl. Energy* **2011**, *88*, 1999–2012. [CrossRef]
7. Office of the Cane and Sugar Board. Status of Sugarcane and Sugar in Thailand. Available online: http://www.ocsb.go.th/th/cms/detail.php?ID=5351&SystemModuleKey=country (accessed on 11 April 2018).
8. Thanapornsin, T.; Sanchanda, P.; Laopaiboon, L.; Laopaiboon, P. Batch butanol fermentation from sugarcane molasses integrated with a gas stripping system: Effects of sparger types and gas flow rates. *Asia Pac. J. Sci. Technol.* **2018**, *23*, 1–12. [CrossRef]
9. Narueworanon, P.; Laopaiboon, L.; Laopaiboon, P. Capability of immobilized *Clostridium beijerinckii* TISTR 1461 on lotus stalk pieces to produce butanol from sugarcane molasses. *Processes* **2021**, *9*, 573. [CrossRef]
10. Jiang, W.; Zhao, J.; Wang, Z.; Yang, S.T. Stable high-titer n-butanol production from sucrose and sugarcane juice by *Clostridium acetobutylicum* JB200 in repeated batch fermentation. *Bioresour. Technol.* **2014**, *163*, 172–179. [CrossRef]

11. Abdehagh, N.; Tezel, F.H.; Thibault, J. Separation techniques in butanol production: Challenges and developments. *Biomass Bioenergy* **2014**, *60*, 222–246. [CrossRef]
12. Ezeji, T.C.; Liu, S.; Qureshi, N. Mixed sugar fermentation by Clostridia and metabolic engineering for butanol production. In *Biorefineries: Integrated Biochemical Processes for Liquid Biofuels*, 1st ed.; Qureshi, N., Hodge, D., Vertes, A., Eds.; Elsevier: Amsterdam, The Netherlands, 2014; Volume 1, pp. 191–204, ISBN 978-0-444-59498-3.
13. Wechgama, K.; Laopaiboon, L.; Laopaiboon, P. Enhancement of batch butanol production from sugarcane molasses using nitrogen supplementation integrated with gas stripping for product recovery. *Ind. Crops Prod.* **2017**, *95*, 216–226. [CrossRef]
14. Ranjan, A.; Mayank, R.; Moholkar, V.S. Process optimization for butanol production from developed rice straw hydrolysate using *Clostridium acetobutylicum* MTCC 481 strain. *Biomass Convers. BioRefin.* **2013**, *3*, 143–155. [CrossRef]
15. Chisti, M.Y. *Airlift Bioreactors*, 1st ed.; Elsevier Applied Science: New York, NY, USA, 1989; p. 345, ISBN 1-85166-320-7.
16. Özcan, E.; Sargın, S.; Göksungur, Y. Comparison of pullulan production performances of air-lift and bubble column bioreactors and optimization of process parameters in air-lift bioreactor. *Biochem. Eng. J.* **2014**, *92*, 9–15. [CrossRef]
17. Daengbussadee, C.; Laopaiboon, L.; Kaewmaneewat, A.; Sirisantimethakom, L.; Laopaiboon, L. Novel methods using an *Arthrobactor* sp. to create anaerobic conditions for biobutanol production from sweet sorghum juice by *Clostridium beijerinckii*. *Processes* **2021**, *9*, 178. [CrossRef]
18. Latimer, G.W. *Official Methods of Analysis of AOAC International*, 19th ed.; AOAC International: Rockville, MD, USA, 2012; ISBN 0935584838.
19. Sirisantimethakom, L.; Laopaiboon, L.; Danvirutai, L.; Laopaiboon, P. Volatile compounds of a traditional Thai rice wine. *Biotechnology* **2008**, *7*, 505–513. [CrossRef]
20. Narueworanon, P.; Laopaiboon, L.; Phukoetphim, N.; Laopaiboon, P. Impacts of Initial Sugar, Nitrogen and Calcium Carbonate on Butanol Fermentation from Sugarcane Molasses by *Clostridium beijerinckii*. *Energies* **2020**, *13*, 694. [CrossRef]
21. Areesirisuk, A.; Laopaiboon, L.; Khongsay, N.; Laopaiboon, P. Improvement of gas chromatographic analysis for organic acids and solvents in acetone-butanol-ethanol fermentation from sweet sorghum juice. *Afr. J. Biotechnol.* **2010**, *9*, 6422–6429. [CrossRef]
22. Scherz, H.; Bonn, G. *Analytical Chemistry of Carbohydrate*; Georg Thieme Verlag: New York, NY, USA; Wiley: New York, NY, USA, 1998; ISBN 3131023511.
23. Lütke-Eversloh, T.; Bahl, H. Metabolic engineering of *Clostridium acetobutylicum*: Recent advances to improve butanol production. *Curr. Opin. Biotechnol.* **2011**, *22*, 634–647. [CrossRef]
24. Mechmech, F.; Marinova, M.; Chadjaa, H.; Rahni, M.; Akacham, N.B.; Gargouri, M. Alfalfa juice as a nitrogen source or supplement for acetone-butanol-ethanol production by *Clostridium acetobutylicum*. *Ind. Crops Prod.* **2015**, *78*, 73–81. [CrossRef]
25. Doran, P.M. *Bioprocess Engineering Principles*, 2nd ed.; Academic Press: London, UK, 1995; p. 202, ISBN 9780122208515.
26. Sirisantimethakom, L.; Laopaiboon, L.; Sanchanda, P.; Chatleudmongkol, J.; Laopaiboon, P. Improvement of butanol production from sweet sorghum juice by *Clostridium beijerinckii* using an orthogonal array design. *Ind. Crops Prod.* **2016**, *79*, 287–294. [CrossRef]
27. Zheng, J.; Tashiro, Y.; Wang, Q.; Sonomoto, K. Recent advances to improve fermentative butanol production: Genetic engineering and fermentation technology. *J. Biosci. Bioeng.* **2015**, *119*, 1–9. [CrossRef]
28. Plaza, P.E.; Coca, M.; Yagüe, S.L.; Gutiérrez, G.; Rochón, E.; García-Cubero, M.T. Bioprocess intensification for acetone-butanol-ethanol fermentation from brewer's spent grain: Fed-batch strategies coupled with in situ gas stripping. *Biomass Bioenergy* **2022**, *156*, 106237. [CrossRef]
29. Xue, C.; Liu, F.; Xu, M.; Tang, I.C.; Zhao, J.; Bai, F.; Yang, S.T. Butanol production in acetone-butanol-ethanol fermentation with in situ product recovery by adsorption. *Bioresour. Technol.* **2016**, *219*, 158–168. [CrossRef] [PubMed]
30. Kießlich, S.; Sarchami, T.; Munch, G.; Gao, K.; Rehmann, L.; Kujawski, W.; Johnson, E. Pervaporative butanol removal from PBE fermentation broths for the bioconversion of glycerol by *Clostridium pasteurianum*. *J. Membr. Sci.* **2017**, *535*, 79–88. [CrossRef]
31. Ni, Y.; Wang, Y.; Sun, Z. Butanol Production from Cane Molasses by *Clostridium saccharobutylicum* DSM 13864: Batch and Semicontinuous Fermentation. *Appl. Biochem. Biotechnol.* **2012**, *166*, 1896–1907. [CrossRef] [PubMed]
32. da Conceição Gomes, A.; Rodrigues, M.I.; de França Passos, D.; Machado de Castro, A.; Maria Mello Santa Anna, L.; Pereira, N. Acetone-butanol-ethanol fermentation from sugarcane bagasse hydrolysates: Utilization of C5 and C6 sugars. *Electron. J. Biotechnol.* **2019**, *42*, 16–22. [CrossRef]
33. Kheyrandish, M.; Asadollahi, M.A.; Jeihanipour, A.; Doostmohammadi, M.; Rismani-Yazdi, H.; Karimi, K. Direct production of acetone–butanol–ethanol from waste starch by free and immobilized *Clostridium acetobutylicum*. *Fuel* **2015**, *142*, 129–133. [CrossRef]
34. Chang, Z.; Cai, D.; Wang, Y.; Chen, C.; Fu, C.; Wang, G.; Qin, P.; Wang, Z.; Tan, T. Effective multiple stages continuous acetone-butanol-ethanol fermentation by immobilized bioreactors: Making full use of fresh corn stalk. *Bioresour. Technol.* **2016**, *205*, 82–89. [CrossRef]
35. Rochón, E.; Ferrari, M.D.; Lareo, C. Integrated ABE fermentation-gas stripping process for enhanced butanol production from sugarcane-sweet sorghum juices. *Biomass Bioenergy* **2017**, *98*, 153–160. [CrossRef]