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Bioconversion of Butyric Acid to Butanol by *Clostridium saccharoperbutylacetonicum* N1-4 (ATCC 13564) in a Limited Nutrient Medium

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Abstract This study was designed to investigate the ability of *Clostridium saccharoperbutylacetonicum* N1-4 to produce butanol in a limited nutrient medium using mixtures of glucose and butyric acid as substrates. Specific combinations of glucose and butyric acid were found to influence the enhancement and retardation of butanol production as well as the reduction and modulation of the number of bacterial cells. Increasing the butyric acid concentration leads to the inhibition of bacterial growth, whereas the presence of (0–5 g/L) butyric acid and (0–10 g/L) glucose enhances the butanol production. The combination of 5 g/L butyric acid with 5 and 10 g/L of glucose was found to be the most suitable, but the use of glucose at concentrations greater than 10 g/L shifted the optimal butyric acid concentrations to 10 and 15 g/L for maximum butanol production signifying the requirement of a specific combination of glucose and butyric acid for enhanced butanol production in the fermentation process. *C. saccharoperbutylacetonicum* N1-4 demonstrated the ability to produce butanol in the absence of glucose, but no acetone or ethanol was produced under these conditions, reflecting the nature of the pathways involved in the production of butanol using only butyric acid. Ten grams per litre of butyric acid was found able to produce 13 g/L of butanol in the presence of 20 g/L of glucose, and 0.7 g/L butanol was produced in the absence of glucose. This study indicates the importance of the glucose to butyric acid ratio to the enhancement of butanol production.

Keywords Butanol · *Clostridium saccharoperbutylacetonicum* N1-4 · Butyric acid bioconversion · Limited nutrient medium · Batch culture

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Introduction

Acetone-butanol-ethanol (ABE) fermentation by clostridia was widely employed on an industrial basis during the first half of the last century, but later, the process could not compete economically with petrochemical synthesis, due to the cost of substrate, the development of the petrochemical industry and the low yield of butanol, due to its heterofermentative nature [8]. The bioconversion of agricultural biomass into biofuel and chemical feedstock into biofuels has attracted interest because the supply of petroleum and fossil fuels is limited, causing oil prices to increase continuously and leading to environmental problems resulting from waste accumulation. The cost of substrate is an important factor in butanol fermentation, and notably, butanol can be produced from various raw materials or renewable agricultural crops, including sago starch [10], corn [14], molasses and whey permeate [4]. Although, current strategies for biomass have focused on the production of ethanol, the production of butanol instead of ethanol offers several advantages for biofuel–gasoline blending. Butanol has many attractive properties as a fuel, compared with other biofuels, such as ethanol and methanol. Butanol has a lower vapour pressure but a higher energy content than ethanol, which makes the former safer for blending with gasoline and offers better fuel economy than ethanol–

gasoline blends. In addition, butanol has a higher tolerance for water contamination in gasoline blends; therefore, butanol–gasoline blends are less susceptible to separation, which facilitates their use in existing gasoline supplies and distribution channels [3, 9]. Specifically, butanol can be shipped through existing pipelines and is far less corrosive than ethanol [3]. Furthermore, branched chain 4-carbon alcohols, including isobutanol, 2-methyl-1-butanol and 3-methyl-1-butanol, have higher octane numbers than *n*-butanol [2]. Also, butanol is sufficiently similar to gasoline to be used directly, or it can be blended with gasoline at higher concentrations than ethanol in any gasoline engine without any modification. Therefore, when compared with ethanol and acetone, butanol is the most promising solvent, and the effort to optimise ABE fermentation to enhance butanol production over ethanol appears to be the more commercially and technologically attractive option [13]. A typical feature of the clostridial solvent production is biphasic fermentation, the first phase of which is the acidogenic phase, during which the acid-forming pathways are activated, and acetate, butyrate, hydrogen and carbon dioxide are produced as major products. This acidogenic phase usually occurs during the exponential-growth phase of cell division. The second phase is the solventogenic phase, during which acids are reassimilated and used in the production of acetone, butanol and ethanol (or isopropanol instead of acetone in some strains of *Clostridium beijerinckii*) [1, 15]. Our study presents a procedure in which an inoculum from the growing medium was transferred to a non-growing medium for butanol production.

Materials and Methods

Microorganism and Inoculum Preparation

Clostridium saccharoperbutylacetonicum N1-4 was obtained from a stock culture maintained at the Biotechnology Lab in the Chemical and Process Engineering Department at UKM. The stock culture was maintained at 4°C as a suspension of spores in potato glucose medium (PG medium). A volume of 1 mL stock culture was transferred into 10 mL of 15% PG medium with subsequent heat shock for 1 min in boiling water, cooled in ice water and incubated for 1 to 2 days at 30°C under anaerobic conditions. The viability and purity of the culture was verified by observation of the colonies' morphology characteristics and by Gram-stain technique to ensure the culture's purity. The culture was then transferred to sterilised Tryptone–yeast extract–acetate medium (TYA), was incubated anaerobically for 15–18 h and was used to prepare the inoculum. The ingredients of TYA and PG media were summarized in Table 1.

Preparation of Medium

The fresh PG medium was prepared from the components mentioned in Table 1. After mixing the substances, the medium was incubated in boiling water for 1 h with interval mixing every 10 min. Next, the medium was filtered through gauze (12×8 mesh size). The medium was sterilised in an autoclave at 121°C for 15 min. TYA medium was used for the pre-culture. The medium was sterilised at 121°C for 15 min. TYA medium used to activate the bacteria from PG medium (contained 20 g/L glucose) and to prepare the inoculum (contained 40 g/L glucose).

Batch Fermentation

Anaerobic batch fermentations were conducted in 15-mL test tubes with a 10-mL working volume of phosphate-free nitrogen medium (Table 1). The initial medium pH was adjusted to 6.2 with 10 M NaOH. The medium was sterilised by autoclaving at 121°C for 15 min., and the medium was sparged with oxygen-free nitrogen before use to ensure anaerobic conditions. *C. saccharoperbutylacetonicum* N1-4 (ATCC13564) was grown in TYA medium in a 250-ml Scott

Table 1 The compositions of TYA, PG and phosphate-free nitrogen media

TYA medium		Potato glucose medium (PG)		Phosphate-free nitrogen medium	
Components	(g/L)	Components	(g/L)	Components	(g/L)
Glucose	20–40	Potato	150	Glucose	0–20
Yeast extract	2	Glucose	10	Butyric acid	0–15
Tryotone	6	$CaCO_3$	3	KH_2PO_4	0.5
CH_3COONH_4	3	$(NH_4)_2SO_4$	0.5	$FeSO_4{\cdot}7H_2O$	0.01
KH_2PO_4	0.3	Distilled water	1 L	Distilled water	1 L
$MgSO_4{\cdot}7H_2O$	0.3				
$FeSO_4{\cdot}7H_2O$	0.01				
Distilled water	1 L				

Duran bottle in anaerobic condition at 30°C for 20 h. This culture was used as an inoculum to various combinations of phosphate-free nitrogen medium. There were five sets of experiments using different glucose concentrations of 0, 5, 10, 15 and 20 g/L and butyric acid concentrations of 0, 1, 5, 10 and 15 g/L with every set of glucose concentration. An inoculum of 10% *v/v* was made to every reaction vessel, and the effects of glucose concentration on butanol production from butyric acid were determined. Five experimental sets were designated by combination numbers represented by the symbol "G". The cultures were incubated at 30°C without shaking in an incubator under anaerobic conditions.

Analytical Procedures

Samples were centrifuged at 10,000 rpm for 15 min. The supernatant was used for determining the concentration of solvent (ABE), glucose and organic acids.

Solvent was measured using a gas chromatograph (7890A GC-System, Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionisation detector and a 30-m capillary column (Equity1; 30 m×0.32 mm×1.0 μm film thickness; Supelco Co, Bellefonate, PA, USA). The oven temperature was programmed to increase from 40°C to 130°C at a rate of 8°C/min. The injector and detector temperatures were set at 250°C and 280°C, respectively. Helium, as the carrier gas, was set at a flow rate of 1.5 mL/min. Butyric acid and acetic acid were analyzed with a high-performance liquid chromatography (HPLC 12000 Series, Agilent technologies, Palo Alto, CA, USA) using a Genessis C18 120A column (25 cm×4.6 mm; Jones Chromatography, Tempe, AZ, USA) at a column temperature of 40°C and 20 mM H_2SO_4 as a mobile phase at a rate of 0.6 ml/min. Detection was accomplished with a UV detector at a wavelength of 220 nm. The glucose concentration in the fermentation broth was determined with a Biochemistry Analyzer (YSI 2700D, YSI Inc. Life Sciences, Yellow Springs, OH, USA). The cell concentration was determined by optical density (OD) at 660 nm.

The yield of butanol to the consumed substrate was calculated using the following equation:

$$Y_{\text{butalnol/substrate}} = B/S_c(\text{C}-\text{mol/C}-\text{mol})$$

Where B/S_c is the yield of butanol to the substrate consumed (C-mol/C-mol), B is the numbers of carbon moles of butanol produced and S_c is the substrate consumed (S_c=carbon moles of butyric acid consumed + carbon moles of glucose consumed).

Results and Discussions

Butanol is considered as an important alternative to the fossil fuel and better than ethanol as it tolerates water contamination and is less corrosive than ethanol [3]. This research is focused on the enhancement of butanol production using a cost-effective medium. A limited nutrient medium without any nitrogen source was used to investigate the ability of *C. saccharoperbutylacetonicum* N1-4 to produce butanol using mixtures of glucose and butyric acid as substrates. The distinctive feature of this study was to inoculate the limited nutrient medium with a (10% *v/v*) of *C. saccharoperbutylacetonicum* N1-4 which was previously grown in TYA medium. Various concentrations of glucose and butyric acid were used to evaluate the effects of glucose on butanol production from butyric acid as substrate by *C. saccharoperbutylacetonicum* N1-4.

Butanol Production from Butyric Acid in the Absence of Glucose

In the first step, glucose concentration was kept at zero, butyric acid concentration was varied from 0 to 15 g/L and the ABE products were analyzed with gas chromatography as described in the "Materials and Methods" section (Table 2). Combinations 1 to 5 G represent the concentrations of butyric acid (0, 1, 5, 10 and 15 g/L) in which glucose concentrations

Table 2 The effect of butyric acid on butanol production by *C. saccharoperbutylacetonicum* N1-4 in phosphate-free nitrogen medium without glucose

G/B.A ratio	Combination symbol	Final pH	Production			Consumed Butyric acid (g/L)	Butanol productivity (g L^{-1} h^{-1})	$Y_{B/S}$* (C-mol/C-mol)
			Acetone (g/L)	Butanol (g/L)	Ethanol (g/L)			
0/0	1 G	4.26	0.00	0.28	0.00	–	0.006	–
0/1	2 G	4.81	0.00	0.80	0.00	1.00	0.017	0.95
0/5	3 G	5.05	0.00	0.75	0.00	1.86	0.016	0.48
0/10	4 G	5.69	0.00	0.70	0.00	3.95	0.015	0.21
0/15	5 G	5.58	0.00	0.77	0.00	4.08	0.016	0.22

G/B.A ratio glucose to butyric acid ratio; $Y_{B/S}$* yield of butanol to substrate

are zero. In the absence of glucose, no acetone or ethanol were observed (1–5 G), but butanol was obtained at concentrations of 0.28, 0.80, 0.75, 0.70 and 0.77 g/L, respectively.

The lowest level of butanol production, generated at a concentration of 0.28 g/L in the absence of glucose and butyric acid (1 G), was attributed to the presence of residual nutrients from the inoculum medium (10% *v*/*v*), which was subjected to the butanol-production pathway. The maximum butanol production was achieved by using the lowest amount of butyric acid (1 g/L), and the butanol yield was significantly decreased, with an increase in the amount of butyric acid in the absence of glucose.

Importantly, in a previous study by Tashiro et al. in 2007, only 0.2 g/L butanol was produced from 10 g/L butyrate in the absence of glucose when a high concentration of washed *C. saccharoperbutylacetonicum* N1-4 cells was used, and no acetone and ethanol were observed [18]. It is noticeable that the strategic use of inoculum medium (10% *v*/*v*) has remarkable contribution to enhance the butanol production by using butyric acid (1 g/L) as compared to the 10 g/L butyrate reported by Tashiro et al.

Although, butyric acid is reported as a triggering substance to solventogenesis in *C. acetobutylicum* [7, 12, 19], whereas this study revealed that the butyric acid can be utilized as a substrate in the absence of glucose. Theoretically, the glucose concentration in the inoculum medium (10% *v*/*v*) was not enough to produce 0.28 g/L of butanol assumed to be produced by the residual butyric acid as a product of butanol pathway. The addition of 1 g/L of butyric acid is not only triggering the solventogenic pathway but also consumed as a substrate to produce butanol. The analysis of the residual butyric acid and estimation of its consumption is evidence that butyric acid has been efficiently utilized as a substrate (Table 2).

Tashiro et al. used a concentrated washed cells of *C. saccharoperbutylacetonicum* N1-4 as an inoculum to produce butanol, while in our study, a fresh inoculum (10% *v*/*v*) of *C. saccharoperbutylacetonicum* N1-4 was used which showed more efficient than their study.

Butanol Production from Butyric Acid in the Presence of 5 g/L Glucose

The effect of glucose on butanol production from butyric acid by *C. saccharoperbutylacetonicum* N1-4 was studied in batch culture using phosphate-free nitrogen medium containing 5 g/L glucose with various concentrations of butyric acid (0, 1, 5, 10 and 15 g/L). In combination 6 G (5 g/L glucose and 0 g/L butyric acid), the cell density (OD of 660 nm) was increased to 1.16, and butanol and acetone were also produced (Table 3). The highest yield of butanol to carbon source was obtained from combinations 6 and 9 G (0.46 C-mol/C-mol) and the highest ABE productivity was 0.146 gL^{-1} h^{-1} from 10 G. In combination 10 G (5 g/L glucose and 15 g/L butyric acid), a significant amount of ethanol was produced, which was the major product (ethanol 3.94 g/L and butanol 2.82 g/L), and the ratio of ABE was 1:10:14.6. Martin et al. reported that the total ABE and the ratio of ABE (acetone/butanol/ethanol) to be produced varied depending on initial acetic and butyric acid concentrations [11].

Fond et al. have reported that the production of acetone and butanol in the fed-batch culture is activated when butyrate and acetate are added to the culture medium; moreover, they become an additional carbon source for solvent production [5]. Our results are in agreement with the results from Fond et al., but the medium they have used is expensive as compared to the limited nutrient medium in this study.

It was also found that addition of butyric acid is not contributing to cell growth but a specific ratio of glucose and butyric acid (5 g/L glucose and 5 g/L butyric acid) enhanced the butanol production in the limited nutrient medium. However, elevated ABE (7.03 g/L) production with a higher yield of ethanol (3.94 g/L) instead of butanol (2.82 g/L) was observed in a different combination (glucose 5 g/L and butyric acid 15 g/L). It is evident that the different combinations of the glucose and butyric acid are responsible to influence the ratio of ABE, and these results

Table 3 The effect of butyric acid on butanol production from by *C .saccharoperbutylacetonicum* N1-4 in the presence of 5 g/L glucose

G/B.A ratio	Combination symbol	Final pH	Production			Total	Consumption (g/L)		ABE productivity (g L^{-1} h^{-1})	$Y_{B/S}$* (C-mol/C-mol)
			Acetone (g/L)	Butanol (g/L)	Ethanol (g/L)	ABE (g/L)	Glucose	Butyric acid		
5/0	6 G	4.32	0.04	1.19	0.00	1.23	4.2	–	0.026	0.46
5/1	7 G	4.25	0.05	1.61	0.00	1.66	4.8	1.00	0.034	0.42
5/5	8 G	5.13	0.12	2.65	0.77	3.54	5.0	4.00	0.074	0.41
5/10	9 G	5.06	0.03	2.06	0.00	2.09	5.0	1.61	0.044	0.46
5/15	10 G	5.44	0.27	2.82	3.94	7.03	5.0	6.82	0.146	0.32

G/*B*.*A ratio* glucose to butyric acid ratio, $Y_{B/S}$* yield of butanol to substrate

Table 4 The effect of butyric acid on butanol production by *C. saccharoperbutylacetonicum* N1-4 in the presence of 10 g/L glucose

G/B.A ratio	Combination symbol	Final pH	Production			Total ABE (g/L)	Consumption (g/L)		ABE productivity (g L^{-1} h^{-1})	$Y_{B/S}$* (C-mol/C-mol)
			Acetone (g/L)	Butanol (g/L)	Ethanol (g/L)		Glucose	Butyric acid		
10/0	11 G	4.52	0.13	2.47	0.75	3.35	9.0	–	0.070	0.44
10/1	12 G	4.30	0.06	2.99	0.76	3.81	9.2	1.00	0.079	0.46
10/5	13 G	4.86	0.18	4.49	0.78	5.45	10.0	1.01	0.114	0.64
10/10	14 G	5.00	0.04	2.39	1.02	3.45	9.5	1.34	0.072	0.34
10/15	15 G	5.27	0.33	4.96	0.79	6.09	9.7	7.78	0.127	0.40

G/B.A ratio glucose to butyric acid ratio, $Y_{B/S}$* yield of butanol to substrate

in agreement with the previous report using growing *C. acetobutylicum* [16].

Butanol Production from Butyric Acid in the Presence of 10 g/L Glucose

In the 3rd set of experiments, the glucose concentration was maintained at 10 g/L and the butyric acid concentration ranged from 0 to 15 g/L. Combinations 11 to 15 G represent the concentrations of butyric acid (0, 1, 5, 10 and 15 g/L), with the glucose concentration maintained at 10 g/L. Combination 11 G was found with increased cell growth and produced the lowest ABE, with 3.35 g/L. The maximum ABE, 6.09 g/L, was produced along with a butanol concentration of 4.96 g/L in combination 15 G. ABE productivity increased from 0.079 to 0.127 gL^{-1} h^{-1} when butyric acid was increased from 1 to 15 g/L (Table 4). Furthermore, combination 13 G (10 g/L glucose and 5 g/L butyric acid) exhibited the highest ABE productivity (0.114 gL^{-1} h^{-1}) and the highest butanol yield $Y_{B/S}$ (0.64 C-mol/C-mol). The lowest amount of butanol in this set was obtained from 14 G (10 g/L glucose and 10 g/L butyric acid), and this combination produced more ethanol than other combinations in the same set (combinations 11, 12, 13 and 15 G; 2.39 g/L butanol and 1.02 g/L ethanol).

It was reported that growth of *C. saccharoperbutylacetonicum* (ATCC 27022) can be inhibited completely by the addition of 8 g/L butyric acid in presence of 10 g/L glucose [17]. This study is also supporting a fact that growing or expensive media is not required when butyric acid is being used as an enhancer of butanol or ABE.

Butanol Production from Butyric Acid in the Presence of 15 g/L Glucose

From previous results in this study, 5 and 10 g/L glucose were compatible with 5 g/L butyric acid, and these combinations exhibited the highest butanol production; hence, in the next experiments, we increased the concentrations of glucose to more than 10 g/L to investigate the effect of increased glucose concentration on butanol production using the same procedures as in previous experiments.

In the 4th set of experiment, glucose concentration was maintained at 15 g/L, and butyric acid concentrations ranged from 0 to 15 g/L (Table 5). In terms of butanol yield, combination 19 G demonstrated the greatest yield (0.92 C-mol butanol/C-mol substrate), followed by combination 17 G (0.75 C-mol/C-mol). The greatest production of butanol was with 15 g/L butyric acid, and the ABE

Table 5 The effect of butyric acid on butanol production by *C.saccharoperbutylacetonicum* N1-4 in the presence of 15 g/L glucose

G/B.A ratio	Combination symbol	Final pH	Production			Total ABE (g/L)	Consumption (g/L)		ABE productivity (g L^{-1} h^{-1})	$Y_{B/S}$* (C-mol/C-mol)
			Acetone (g/L)	Butanol (g/L)	Ethanol (g/L)		Glucose	Butyric acid		
15/0	16 G	4.28	0.11	2.45	0.00	2.57	10.7	–	0.053	0.37
15/1	17 G	4.27	0.55	6.86	0.83	8.23	13.4	1.00	0.172	0.75
15/5	18 G	4.83	0.59	6.18	0.80	7.57	14.0	3.98	0.158	0.52
15/10	19 G	5.03	0.47	7.95	0.84	9.26	10.2	2.84	0.193	0.92
15/15	20 G	5.32	0.53	8.25	0.89	9.67	13.7	8.63	0.201	0.53

G/B.A ratio glucose to butyric acid ratio, $Y_{B/S}$* yield of butanol to substrate

Table 6 The effect of butyric acid on butanol production by *C. saccharoperbutylacetonicum* N1-4 in the presence of 20 g/L glucose

G/B.A ratio	Combination symbol	Final pH	Production			Total ABE (g/L)	Consumption (g/L)		ABE productivity (g L^{-1} h^{-1})	$Y_{B/S}$* (C-mol/C-mol)
			Acetone (g/L)	Butanol (g/L)	Ethanol (g/L)		Glucose	Butyric acid		
20/0	21 G	3.92	0.09	1.90	0.00	1.99	10.5	–	0.041	0.29
20/1	22 G	4.87	1.26	9.05	0.99	11.29	19.0	1.00	0.235	0.72
20/5	23 G	5.27	1.11	10.61	0.95	12.67	19.0	2.14	0.264	0.78
20/10	24 G	5.41	1.47	12.99	0.94	15.41	19.2	1.57	0.321	0.99
20/15	25 G	5.18	1.36	11.09	0.87	13.32	19.7	8.14	0.278	0.58

G/B.A ratio glucose to butyric acid ratio, $Y_{B/S}$* yield of butanol to substrate

productivity of this combination (20 G) was also the highest (0.2 gL^{-1} h^{-1}). Increasing the concentration of glucose to 15 g/L with various concentrations of butyric acid enhanced the production of butanol, although no bacterial growth was observed. The ratio of glucose and butyric acid is not a factor to influence the bacterial efficiency but the ratio as well as appropriate concentration of the G/BA is responsible for enhanced solvent production (Table 4—14 G, 15 G and Table 3—8 G).

Butanol Production from Butyric Acid in the Presence of 20 g/L Glucose

Prior to this experiment, a control batch culture was carried out with *C. saccharoperbutylacetonicum* N1-4 in a TYA medium with 20 g/L of glucose. The total ABE produced from this culture was 3.52 g/L containing 2.11 g/L butanol, 1.20 g/L acetone and 0.21 g/L ethanol.

In the 5th set of experiment, glucose concentration was maintained at 20 g/L, and butyric acid concentrations ranged from 0 to 15 g/L. The 10% inoculum of *C. saccharoperbutylacetonicum* N1-4 produced 12.99 g/L butanol from 20 g/L glucose in the presence of 10 g/L butyric acid, while Tashiro et al. could only produce 7.5 g/L butanol from the same combination using concentrated washed *C. saccharoperbutylacetonicum* N1-4 cells [18].

Furthermore, an increase in cell growth was observed in the absence of butyric acid, and increased glucose utilisation accompanied increased butyric acid concentrations. With 20 g/L glucose, the concentrations of produced butanol was 9.05, 10.61, 12.99 and 11.09 g/L in the presence of 1, 5, 10 and 15 g/L butyric acid, respectively (Table 6). Combination 24 G was found to be an optimal combination, in which 15.41 g/L ABE was produced, with $Y_{B/S}$ of 0.99 C-mol/C-mol.

Although the presence of butyric acid clearly inhibited cell growth, butanol was produced efficiently, which suggested that butanol production is not associated with cell growth. In addition, small quantities of butanol were produced in the absence of glucose compared with that produced in the presence of glucose, indicating that glucose has a pronounced effect and is required in butanol production.

As demonstrated in Fig. 1, the conversion of butyrate to butanol through the CoA transferase pathway requires two molecules of NADH as cofactors of butyraldehyde dehydrogenase and butanol dehydrogenase which resulted from glucose metabolism. This assumption explains the increase in butanol production in the presence of glucose. It has been reported that 0.88 g/L butyric acid is sufficient to stimulate solventogensis [19]. However, the concentrations of butyrate and acetate required to reassimilate and produce solvent by *Clostridium acetobutylicum* were 7.5 and 6.5 g/L, respectively [4]. The stimulation of ABE production was initiated at an earlier stage when an elevated concentration of acids (acetic acid and butyric acid) was initially added to the medium, but increased acid levels do not necessarily improve the final production of butanol [6]. In contrast, the addition of butyric acid to cultures of *C. saccharoperbutylacetonicum*

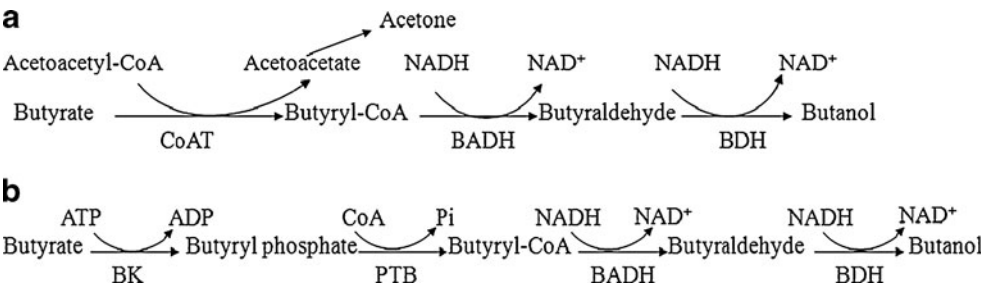

Fig. 1 Two possible mechanisms for the conversions of butyrate to butanol via the CoAT pathways (**a**) and the reverse pathway of butyrate production (**b**) [16]. *CoAT* CoA transferase; *BADH* butylaldehyde dehydrogenase; *BDH* butanol dehydrogenase; *BK* butyratekinase; *PTB* phosphotransbutyrylase

N1-4 increased the concentration of ABE and the production from TYA medium supplemented with 5 g/L butyric acid was found to be optimal [5].

Conclusions

This study introduces a cost-effective, industrial-scale method of butanol production that may also contribute toward the management of fuel shortages, environmental challenges and economic scarcity.

It is observed that increases in cell density are not responsible for the production of ABE, but the precise combination of glucose and butyric acid is the primary factor determining butanol production from butyric acid. It was also observed that when culturing *C. saccharoperbutylacetonicum* N1-4, glucose enhances the production of butanol-acetone-ethanol and the yield of butanol with increasing concentrations of butyric acid, although butyric acid without glucose can produce only butanol. The production of only butanol from butyric acid in phosphate-free nitrogen medium reflects the nature of the pathways required for butanol production by *C. saccharoperbutylacetonicum* N1-4. It can be concluded that an inexpensive nutrient limited medium can be used to produce enhanced butanol or ABE by using 10% inoculum from grown bacterial culture of *C. saccharoperbutylacetonicum* N1-4.

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