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RESEARCH PAPER

Enhanced Production of Butanol and Isopropanol from Sugarcane Molasses Using *Clostridium beijerinckii optinoii*

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Abstract Sugarcane molasses was studied as a substrate for butanol and isopropanol fermentation by *Clostridium beijerinckii optinoii* in 10 L batch fermentations. Using 3% glucose as a carbon source, the solvent concentrations (butanol and isopropanol) and solvent yields were 10.03 g/L and 0.43 g/g, respectively, with a sugar utilization of 81.7%. However, on 2.5% glucose medium supplemented with an additional 0.5% sugar supplied as sugarcane molasses, sugar consumption was 100% and the solvent concentrations (13.37 g/L) and solvent yields (0.45 g/g) were both higher. Sugarcane molasses (3%) with or without invertase and without P2 vitamin/mineral solution produced a solvent concentration of 12.15 ~ 12.81 g/L and solvent yield of. 0.39 ~ 0.41 g/g with sugar consumption of 94.88 ~ 100%. This work demonstrated the value of sugarcane molasses as a supplementary nutrient for glucose fermentation for butanol and isopropanol production using *C. beijerinckii optinoii* as well as its value as a low cost carbon and media source.

Keywords: *Clostridium beijerinckii optinoii*, butanol, sugarcane molasses

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1. Introduction

Butanol can be produced from the sugars in biomass [1,2]. As a potential biofuel, butanol has several advantages over ethanol including higher energy content, transportable through existing pipelines, and ease of blending with gasoline. Unlike ethanol, butanol does not absorb water allowing it be stored and distributed using the existing petrochemical infrastructure and to by-pass the blend wall seen with ethanol [3]. Butanol has been tested as a jet fuel (or army helicopter fuel) as a 50/50 blend with current jet fuel.

A number of *Clostridium* strains including *Clostridium acetobutylicum, Clostridium butylicum, Clostridium beijerinckii* produce butanol [4]. In typical acetone, butanol, ethanol (ABE) fermentations, *Clostridium* strains produce acetone, butanol, and ethanol at a 3:6:1 (weight ratio) producing a total solvent concentration of up to 2.5% (weight per volume). This production level is limited by product inhibition. There have been numerous attempts to manipulate the genetics of *Clostridium* strains to produce higher yields of butanol and to reduce the acetone production in order to simplify downstream processing [5,6]. However, attempts to block acetone production to increase selectivity for butanol production usually result in loss of butanol, as acetone and butanol share a common intermediate (acetoacetyl-CoA) [7,8].

Although genetic studies of *Clostridium* strain ABE fermentation are plentiful, there are few reports on substrate optimization to reduce the cost of butanol fermentation. In fact, a crucial factor in butanol fermentation is substrate cost, which runs about 60 ~ 70% of direct manufacturing expenses [9]. Industrial fermentation of any material is ultimately at the mercy of the feedstock costs. Normally low cost agricultural byproducts, such as sugarcane molasses are favored. Sugarcane molasses, a byproduct of raw sugar

production, is used in a range of fermentation industries, because of cost, carbohydrate content, and range of minor compounds that support microbial growth. Sugarcane molasses is the feedstock of choice for ABE fermentation for commercially produced butanol [10].

Clostridium beijerinckii optinoii is a butanol producing bacteria obtained from an isolate previously identified as *Clostridium* sp. Prazmowski 1880 AL (Code No., NCCBNr 84049) obtained from Centraalbureau voor Schimmelcultures, Utrecht, Netherlands. The *optinoii* isolate does not match the carbohydrate utilization or product profiles of *C. saccharoperbutylacetonium* N1-504. Rather, it was closer to *Clostridium beijerinckii* with regard to carbohydrate consumption. Interestingly *C. beijerinckii optinoii* produces mainly butanol and isopropanol with very little ethanol/acetone, unlike other ABE organisms, where the ratio is 6:3:1 (butanol:acetone:ethanol, weight ratio) [11].

In this study, sugarcane molasses as byproduct of Louisiana sugar mills (USA), was applied as a substrate and/or supplementary nutrient for the production of butanol and isopropanol by *C. beijerinckii optinoii*. Sugarcane molasses was compared with a glucose medium in batch fermentations to see how sugarcane molasses affects sugar utilization, solvent yield, and productivity of butanol and isopropanol.

2. Materials and Methods

2.1. Bacterial strain

Clostridium beijerinckii sp. *optinoii* was isolated from a strain identified as *Clostridium* sp. Prazmowski 1880 AL (Code No., NCCBNr 84049) obtained from Centraalbureau voor Schimmelcultures, Utrecht, Netherlands. *Clostridium* sp. Prazmowski 1880 AL is cross-listed as *Clostridium saccharoperbutylacetonicum* N1-504 (ATCC 27022). *The C. beijerinckii* sp. *optinoii* isolate was not a match to *C. saccharoperbutylacetonium* N1-504 based on reported carbohydrate utilization and product profiles, but was closer to *Clostridium beijerinckii*. Therefore, it was named as *Clostridium beijerinckii optinoii* and is deposited with ATCC, Accession No. PTA-11285 [11].

2.2. Inoculum and fermentation medium

Spores of the organism were stored as suspensions at -60°C in 20% glycerol (v/v). *C. beijerinckii optinoii* spores (200 μL) were heated for 10 min at 80°C followed by cooling to room temperature on ice. The heat shocked spores (100 μL) were inoculated into 9 mL inoculum medium in 10 mL serum bottles capped with butyl rubber stoppers. The inoculum medium was composed of: 5 g/L glucose, 5 g/L peptone, 5 g/L yeast extract, 5 g/L sodium thioglycolate, 5 g/L monobasic potassium phosphate, and 0.002 g/L methylene blue (adjusted to pH 6.5 with dilute sodium hydroxide). The spore suspensions were allowed to grow in an anaerobic chamber for 30 h at 36ºC. A GasPak anaerobic sachet was used to remove oxygen within the anaerobic chamber.

The inoculum suspension (1.0 mL) was then transferred to 99 mL of fermentation medium as a pre-culture in a 120 mL serum bottle capped with butyl rubber stoppers, and was incubated in a shaking incubator at 36ºC at 100 rpm for 24 h. The pre-culture (100 mL) was then used to inoculate a bioreactor (10 L working volume) at a 1% inoculum volume. The fermentation medium consisted of 30 ~ 70 g carbon sources (fermentable sugars such as sucrose, glucose, and fructose), 1 g tryptone, 1 g yeast extract, and 10 mL of filter-sterilized P2 stock solution, per liter. The P2 stock solution used in the fermentation medium was composed of buffer: 50 g/L KH_2PO_4, 50 g/L K_2HPO_4, and 220 g/L ammonium acetate; vitamins: 0.1g/L para-amino-benzoic acid, 0.1 g/L thiamin, and 0.001 g/L biotin; and minerals: 20 g/L $MgSO_4 \cdot 7H_2O$, 1 g/L $MnSO_4 \cdot H_2O$, 1 g/L $FeSO_4 \cdot 7H_2O$, and 1 g/L NaCl. The sugarcane molasses, as a carbon source, was prepared by adding 705 g of sugarcane molasses syrup (Brix 82) to a 10 L fermentation volume to bring the fermentable sugar concentration to 30 g/L. Sugarcane molasses was collected and combined from eleven raw sugar mills in Louisiana. All other media components were purchased from Sigma-Aldrich (St. Louis, MO, USA). According to HPLC analysis, sugarcane molasses syrup contains sucrose (34.8%, based on solids), fructose (6.8%), glucose (3.9%), and the other factors (54.5%, such as ash salts and unknown nutrients).

2.3. Bioreactor batch fermentations

Batch fermentations (10 L working volumes) were performed in duplicate, using a 14 L New Brunswick CelliGen 310 Benchtop Bioreactor (New Brunswick Scientific, Enfield, CT, USA). After autoclaving and cooling the fermentation medium (9.8 L, pH 6.5) in the bioreactor at 120°C for 30 min, invertase (300 Unit ≥ mg, Sigma-Aldrich) was used to invert molasses. Invertase solution (150 mg dissolved and filtered in 10 mL of 20 mM sodium acetate buffer, pH 5.5) was added into a bioreactor and reacted for 3 h with 200 rpm at 36°C. Then, the bioreactor was sparged with nitrogen gas at 10 PSIG for one hour to establish anaerobic conditions prior to adding P2 (100 mL) and inoculum solution (100 mL) to start the fermentation. The complete conversion of sucrose into glucose and fructose was confirmed by HPLC (described in analytical methods). Nitrogen gas was not used after inoculation. The bioreactor agitation speed was 200 rpm and the temperature was maintained at 36°C throughout fermentation. The pH was

not controlled or monitored automatically by the reactor. Cell density was determined at 660 nm using a DU series 800 UV/visible spectrophotometer (Beckman and Coulter, Fullerton, CA, USA).

2.4. Analytical methods

An Agilent 7890A Gas Chromatography System (Agilent Technologies, Santa Clara, CA) was used to determine the solvent (butanol, isopropanol, acetone, ethanol) and acid (acetic and butyric) concentrations. The injection size was 1.0 μL into a Zebron ZB Waxplus (Phenomenex, Torrance, CA) capillary GC column, 60 m × 0.25 mm ID with 0.25 μm film thickness. The injection was split 40:1 with a flow of 1.4 mL/min through the column. The initial temperature of 35°C was held for one minute, then raised by 10°C/min up to 150°C, held for ten minutes, raised by 10°C/min up to 180°C, held for five minutes and lowered by 40°C/min to 35°C for a total run time of 34.125 min. The analytes were determined with a flame ionization detector (Agilent Technologies, Santa Clara, CA) held at 280°C.

High performance liquid chromatography (Agilent 1200 HPLC with a differential refractive index detector at 45°C, BioRad Aminex HPX-87K) at 85°C eluted with water at 0.6 mL/min) was used for quantitative analysis of carbohydrates. A three-point curve made of sucrose, glucose, and fructose was used to standardize the instrument.

3. Results

3.1. Batch fermentations with 3% glucose

The 10 L batch fermentations of *C. beijerinckii optinoii* with a 3% glucose carbon source (initial sugar, 28.34 g/L) are shown in Figs. 1A and 1B. *C. beijerinckii optinoii* showed a normal growth curve starting from lag phase (8 h) to log phase (8 ~ 40 h), followed by the stationary phase. Mainly butanol (6.45 g/L) and isopropanol (3.45 g/L) were produced with very little ethanol/acetone (less than 0.2 g/L, data not shown). Glucose was not completely consumed, leaving behind 18.3%, even after 90 h fermentation.

3.2. Batch fermentation with 2.5% glucose and 0.5% sugarcane molasses

Sugarcane molasses was treated with invertase to convert sucrose to monomeric sugars and then used to supplement the glucose in the media making the final sugar concentration was 3.0%. The batch fermentations (Figs. 1C and 1D) showed faster growth curves, reaching an A_{660} of 2.1, whereas,

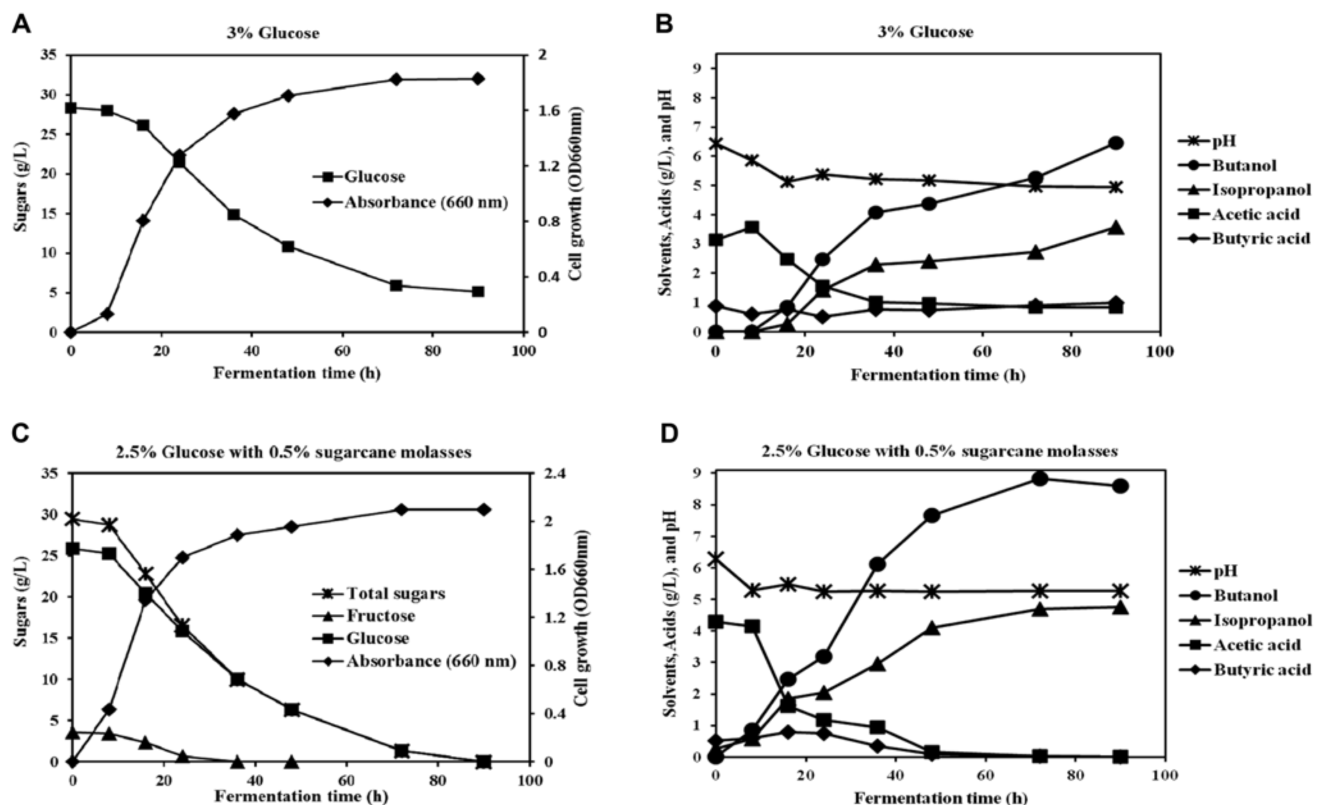

Fig. 1. Batch fermentation of *C. beijerinckii optinoii*. (A) Glucose and cell growth (OD660nm) and (B) solvents, acids, and pH using 3% glucose. (C) Residual sugar and cell growth (OD660nm) and (D) solvents, acids, and pH using a 2.5% glucose with 0.5% sugarcane molasses.

only an A_{660} of 1.8 was achieved on 3% glucose medium. Fermentable sugars (initial fermentable sugars, 29.47 g/L), including fructose from sugarcane molasses, were completely consumed, whereas in the standard glucose media there was always a residual 0.51% glucose. *C. beijerinckii optinoii* also produced higher butanol (8.60 g/L) and isopropanol (4.77 g/L), although solvent yield (0.43 g/g) is quite similar with 3% glucose fermentation (0.45 g/g). When comparing acid production patterns, neither acetic acid nor butyric acid were detected at the end of the fermentation (72 ~ 90 h fermentation time) (Fig. 1D). This indicates that acids may have been metabolized into butanol and isopropanol in this fermentation. Both acids were maintained at 1 g/L after 48 h on 3% glucose fermentation (Fig. 1B). These results indicate that sugarcane molasses contains supplementary nutrients that help this strain grow and produce butanol and isopropanol.

3.3. Batch fermentation on 3% sugarcane molasses

C. beijerinckii optinoii grown on 3% sugarcane molasses solutions, with or without invertase treatment, are compared in Fig. 2. Initial sugars (32.05 g/L) of 3% sugarcane molasses medium, without invertase treatment, contained sucrose (24.50 g/L, 76.4% of the total sugars), fructose (4.78 g/L), and glucose (2.77 g/L). In Fig. 2A, monomeric sugars disappeared within 36 h followed by a slow decrease in the sucrose content. Sucrose (6.68% of the original sucrose) was still present after 90 h. Interestingly, all the sugars were utilized at the same time, perhaps indicative that *C. beijerinckii optinoii* metabolizes sucrose, glucose, and fructose without catabolic repression. Fermentation of 3% invertase treated sugarcane molasses showed that fructose was metabolized at the same time as glucose (Fig. 2C), which is different from the typical catabolic repression pattern shown in other organisms [12].

Although growth was quicker on invertase treated sugarcane molasses, the final solvent yield (0.41 g/g) after 90 h of fermentation was same as with sugarcane molasses without invertase (0.41 g/g), indicating that the invertase accelerated the initial growth converting the sucrose to the more digestible monomeric sugars.

3.4. Batch fermentation on 3% sugarcane molasses without P2 solution

Based on the consideration that sugarcane molasses might be considered, not just a sugar source, but as a crucial supplementary nutrient of *C. beijerinckii optinoii*, we tested the fermentation of invertase treated sugarcane molasses

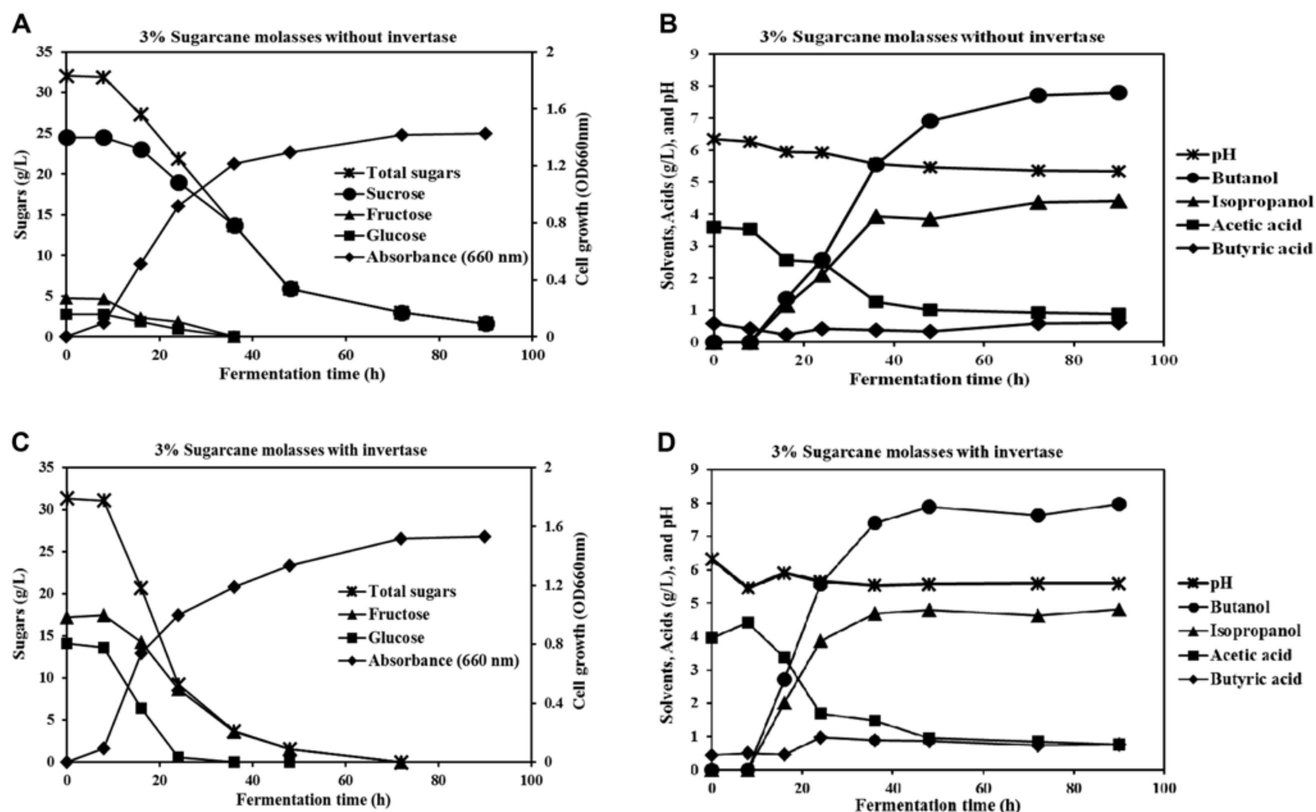

Fig. 2. Batch fermentation of *C. beijerinckii optinoii*. (A) Residual sugars and cell growth (OD660nm) and (B) solvents, acids, and pH using 3% sugarcane molasses without invertase. (C) Residual sugar and cell growth (OD660nm) and (D) solvents, acids, and pH using 3% sugarcane molasses with invertase.

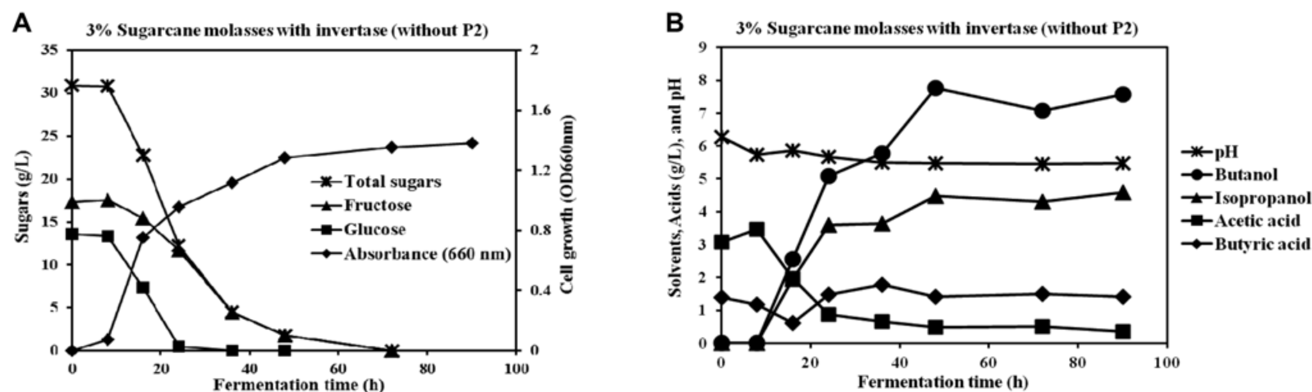

Fig. 3. Batch fermentation of *C. beijerinckii optinoii*. (A) Residual sugars and cell growth (OD660nm) and (B) solvents, acids, and pH using 3% sugarcane molasses with invertase and without P2 solution (supplementary vitamin and minerals).

without adding the mineral P2 solution. Generally, P2 solution is used to supplement the nutrients for *Clostridium* strains. It contains potassium phosphate, vitamins, and minerals (See methods 2.2).

C. beijerinckii optinoii showed a similar growth curve pattern (Fig. 3A) on this media and produced a slightly lower A_{660} of 1.38 compared to 3% sugarcane molasses with invertase with P2 solution (A_{660} = 1.53) (Fig. 2C). Although all fermentable sugars (39.93 g/L) were consumed within 72 h, the solvent yields of butanol (0.39 g/g) and isopropanol (0.41 g/g) were slightly lower than with sugarcane molasses fermentation with P2 solution.

4. Discussion

Most *Clostridium* strains such as *Clostridium acetobutylicum*, *Clostridium butylicum*, and *Clostridium beijerinckii* produce ABE in a 3:6:1 (weight ratio), up to 2.4% (weight per volume) total solvents [10,13,14]. Acetone is considered an undesirable product when the goal is a biofuel. Acetone cannot be used as an alternative fuel due to its corrosive nature which may damage engine parts, especially rubber and plastic. Further, it is hard to separate in downstream processing. *C. beijerinckii optinoii* has some benefits compared other ABE organisms because it produces primarily butanol (6.45 g/L) and isopropanol (3.45 g/L), with small amounts of acetone/ethanol.

Similarly to *C. beijerinckii optinoii*, *C. beijerinckii* NRRL B593 was reported as a butanol and isopropanol producing species that produces 8.4 g/L of butanol and 4.5 g/L of isopropanol with low traces of acetone/ethanol (0.3 g/L) [5]. On the solvent yield, *C. beijerinckii optinoii* was greater (0.43 g/g) than that reported for (0.36 g/L) *C. beijerinckii* NRRL B593. Sugar utilization (81.72%) was also greater, but glucose utilization was not complete (Fig. 1A) and total glucose consumption was lower than that of *C. beijerinckii* NRRL B593. Shaheen *et al.* observed that ABE solvent yields decreased as the concentration of glucose increased from four *Clostridium* strains including *C. beijerinckii* [15]. *C. beijerinckii optinoii* also showed low glucose utilizations (24.06%) as glucose concentration increased (data not shown).

This study aims to elucidate the role of sugarcane molasses in butanol fermentation by *C. beijerinckii optinoii.* Sugarcane molasses has been using for ABE production by *Clostridium* strains because of its low cost as a by-product of sugar production. Shaheen *et al.* showed that *C. beijerinckii* NCP P260 produced ABE solvent concentration up to 24.4 g/L using Australian blackstrap molasses at sugar concentrations up to 7.5%, while solvent yield remained constant at around 0.32 (g/g), and the solvent production was inhibited above 7.5% fermentable sugars [15]. Ni *et al.* also used sugarcane molasses from Jiangmen Sugarcane Chemical Plant Co., Ltd (Guangdong, China) and they reported that *C. saccharobutylicum* DSM 13864 produced 17.88 g/L of ABE solvents from 6% sugars with 0.33 g/g of solvent yield [16].

Compared to these ABE producing species, *C. beijerinckii optinoii* produced less g/L of solvents (12.19) due to a lower sugar concentration (3% initial sugars), but the solvent yield was higher at 0.41 g/g, indicating that sugar concentrations were not optimum to reach the highest solvent yield. Through *C. beijerinckii optinoii* fermentation of sugarcane molasses without or with invertase (Fig. 2), we found that invertase sped up the sugar consumption, but it did not affect the solvent yields (both 0.41 g/g). Rather, the P2 solution affected the solvent yield (0.39 g/g, without P2 solution in Table 1).

The growth of *C. beijerinckii optinoii* was inhibited on 7% sugars from sugarcane molasses (data not shown). Sugarcane molasses is a complex solution and may contain inhibitors such as salts, colorants, and heavy metals [9,17]. Sugarcane molasses also contains some buffering salts,

Table 1. Batch fermentation comparison of *C. beijerinkii optinoii* on various medium conditions.

	3% Glucose	2.5% Glucose +0.5% Molasses[a]	3% Sugarcane molasses		
			without invertase	with invertase	with invertase (without P2)
Initial sugars (g/L)	28.34	29.47	32.05	31.32	30.93
Sugar consumed (g/L)	23.16	29.47	30.04	31.32	30.93
Sugar utilization (%)	81.72	100	94.88	100	100
Acetic acid (g/L)	0.83	-	0.89	0.75	0.36
Butyric acid (g/L)	0.99	-	0.61	0.75	1.40
Butanol (g/L)	6.45	8.60	7.79	7.98	7.57
Isopropanol (g/L)	3.45	4.77	4.40	4.83	4.58
Final solvents (g/L)	10.03	13.37	12.19	12.81	12.15
Solvent yield (g/g)	0.43	0.45	0.41	0.41	0.39
Productivity (g/L/h)[b]	0.11	0.15	0.14	0.14	0.14

[a]Sugarcane molasses syrup was added by calculating three fermentable sugars (sucrose, fructose, and glucose).
[b]The productivity was calculated based on 90 h batch fermentation time.

vitamins, minerals, and other unknown nutrients which might be beneficial for microbial growth. Abou-Zeid *et al.* reported that the good yields of acetone and butanol were obtained using Egyptian black strap molasses supplemented with $(NH_4)_2SO_4$ [18]. *C. beijerinckii optinoii* also showed the best growth and solvent production when 0.5% sugarcane molasses was used to supplement a 2.5% glucose fermentation medium, indicating that sugarcane molasses contains some crucial supplementary nutrients to help this strain grow or metabolize sugar for the efficient production of butanol and isopropanol.

During the batch fermentations by *C. beijerinckii optinoii*, butanol and isopropanol concentrations increased as acetate concentrations decreased during 8 ~ 42 h fermentation times. This phenomenon was commonly observed in all fermentation processes in this study, which may be a function of the complex metabolic pathway where organic acids are converted to solvents during the solventogenesis phase. In ABE producing *Clostridium* organisms, acetate is converted to acetone through several intermediates, such as acetyl CoA, acetoacetyl CoA, and acetoacetate [7]. Unlike most *Clostridium* strains, *C. beijerinckii optinoii* produces isopropanol instead of acetone, as it seems to have a secondary alcohol dehydrogenase capable of converting acetone to isopropanol [19]. The production of isopropanol instead of acetone has several benefits to the *Clostridium* organism. The most obvious advantage is that acetone is more toxic to the organism than isopropanol and also the reduction of acetone to isopropanol regenerates $NAD(P)^+$ which helps maintain an electrochemical balance within the cell. Additionally the conversion of NAD(P)H to $NAD(P)^+$ also decreases the acidity of the fermentation broth preventing a so called "acid crash" that if the pH drops too quickly, the bacteria cannot recover and switch to the solventogenesis phase in order to raise the pH of the fermentation [20]. Owing to these benefits of isopropanol as well as an attractive fuel, researchers have been developing engineered *Clostridium* strains capable of converting acetone to isopropanol by introducing the secondary alcohol dehydrogenase within the cell through DNA manipulation [21].

Due to the production limits in batch fermentation systems, an immobilized cell system will be developed to produce solvents continuously using sugarcane molasses and to improve the solvent productivity in further studies.

5. Conclusion

Unlike other ABE producing *Clostridium* strains, *C. beijerinckii optinoii* produced butanol and isopropanol up to 13.37 g/L of final solvents and 0.45 g/g of solvent yield using a 2.5% glucose supplemented with 0.5% sugarcane molasses in 10 L batch fermentation. Compared to 3% glucose fermentation, sugarcane molasses can be used as a crucial supplementary nutrient or as a cheap carbon source without supplementary nutrients for efficient butanol and isopropanol fermentation.

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References

1. Lu, C., J. Dong, and S. T. Yang (2013) Butanol production from

wood pulping hydrolysate in an integrated fermentation-gas stripping process. *Bioresour. Technol.* 143: 467-475.
2. Qureshi, N. and T. C. Ezeji (2008) Butanol, 'a superior biofuel' production from agricultural residues (renewable biomass): Recent progress in technology. *Biofuels Bioprod. Bioref.* 2: 319-330.
3. Lee, S. Y., J. H. Park, S. H. Jang, L. K. Nielsen, J. Kim, and K. S. Jung (2008) Fermentative butanol production by clostridia. *Biotechnol. Bioeng.* 101: 209-228.
4. Keis, S., R. Shaheen, and D. T. Jones (2001) Emended descriptions of *Clostridium acetobutylicum* and *Clostridium beijerinckii*, and descriptions of *Clostridium saccharoperbutylacetonicum* sp. nov. and *Clostridium saccharobutylicum* sp. nov.. *Int. J. Syst. Evol. Microbiol.* 51: 2095-2103.
5. Collas, F., W. Kuit, B. Clement, R. Marchal, A. M. Lopez-Contreras, and F. Monot (2012) Simutaneous production of isopropanol, butanol, ethanol and 2,3-butanediol by *Clostridium acetobutylicum* ATCC 824 engineered strains. *AMB Express* 2: 45-54.
6. Lee, J., Y. S. Jang, S. J. Choi, J. A. Im, H. Song, J. H. Cho, D. Y. Seung, E. T. Papoutsakis, G. N. Bennett, and S. Y. Lee (2012) Metabolic engineering of *Clostridium acetobutylicum* ATCC 824 for isopropanol-butanol-ethanol fermentation. *Appl. Environ. Microbiol.* 78: 1416-1423.
7. Gheshlaghi, R., J. M. Scharer, M. Moo-Young, and C. P. Chou (2009) Metabolic pathways of clostridia for producing butanol. *Biotechnol. Adv.* 27: 764-781.
8. Han, B., V. Gopalan, and T. C. Ezeji (2011) Acetone production in solventogenic *Clostridium* species: New insights from non-enzymatic decarboxylation of acetoacetate. *Appl. Microbiol. Biotechnol.* 91: 565-576.
9. Qureshi, N. and H. P.Blaschek (2001) ABE production from corn: A recent economic evaluation. *J. Ind. Microbiol. Biotechnol.* 27: 292-297.
10. Merwe, A. B. V. D., H. Cheng, J. F. Gorgens, and J. H. Knoetze (2013) Comparison of energy efficiency and economics of process designs for biobutanol production from sugarcane molasses. *Fuel* 105: 451-458.
11. Day, D. F., A. K. Hoogewind, S. S. Randhava, J. Oswald, L. Madsen, and M. Kim (2013) Method for producing butanol and isopropanol. *US Patent* 2013/0149757 A1.
12. Yao, R., H. Kurata, and K. Shimizu (2013) Effect of *cra* gene mutation on the metabolism of *Escherichia coli* for a mixture of multiple carbon sources. *Adv. Biosci. Biotechnol.* 4: 477-486.
13. Ezeji, T., N. Qureshi, and H. P. Blaschek (2004) Butanol fermentation research: upstream and downstream manipulations. *Chem. Rec.* 4: 305-314.
14. Roffler, S., H. Blanch, and C. Wilke (1987) Extractive fermentation of acetone and butanol: Process design and economic evaluation. *Biotechnol. Prog.* 3: 131-140.
15. Shaheen, R., M. Shirley, and D. T. Jones (2000) Comparative fermentation studies of industrial strains belonging to four species of solvent-producing Clostridia. *J. Mol. Microbiol. Biotechnol.* 2: 115-124.
16. Ni, Y., Y. Wang, and Z. Sun (2012) Butanol production from cane molasses by *Clostridium saccharobutylicum* DSM 13864: Batch and semicontinuous fermentation. *Appl. Biochem. Biotechnol.* 166: 1896-1907.
17. Bae, S. O. and M. Shoda (2005) Production of bacterial cellulose by *Acetobacter xylinum* BPR2001 using molasses medium in a jar fermentor. *Appl. Microbiol. Biotechnol.* 67: 45-51.
18. Abou-Zeid, A. A., M. Fouad, and M. Yassein (1978) Microbiological production of acetone-butanol by *Clostridium acetobutylicum*. *Zentralbl Bakteriol Naturwiss* 133: 125-134.
19. Dai, Z., H. Dong, Y. Zhu, Y. Zhang, Y. Li, and Y. Ma (2012) Introducing a single secondary alcohol dehydrogenase into butanol-tolerant *Clostridium acetobutylicum* Rh8 switches ABE fermentation to high level IBE fermentation. *Biotechnol. Biofuels* 5: 44-54.
20. Maddox, I. S., E. Steiner, S. Hirsch, S. Wessner, N. A. Gutierrez, J. R. Gapes, and K. C. Schuster (2000) The cause of "acid crash" and "acidogenic fermentations" during the batch acetone-butanol-ethanol (ABE-) fermentation process. *J. Mol. Microbiol. Biotechnol.* 2: 95-100.
21. Jang, Y. S., A. Malaviya, J. Lee, J. A. Im, S. Y. Lee, J. Lee, M. H. Eom, J. H. Cho, and D. Y. Seung (2013) Metabolic engineering of *Clostridium acetobutylicum* for the enhanced production of isopropanol-butanol-ethanol fuel mixture. *Biotechnol. Prog.* 29: 1083-1088.