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Enhancement of batch butanol production from sugarcane molasses using nitrogen supplementation integrated with gas stripping for product recovery

Kitipong Wechgama[a], Lakkana Laopaiboon[b,c], Pattana Laopaiboon[b,c,*]

[a] Graduate School, Khon Kaen University, Khon Kaen, 40002, Thailand
[b] Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen, 40002, Thailand
[c] Fermentation Research Center for Value-Added Agricultural Products, Khon Kaen University, Khon Kaen, 40002, Thailand

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ABSTRACT

Initial pH, sugar and nitrogen are important parameters for bio-butanol production. To improve butanol production efficiency, an L_9 (3^4) orthogonal array design was used to obtain high levels of butanol production from sugarcane molasses by *Clostridium beijerinckii* TISTR 1461. The three parameters optimized were initial pH (5.5–7.5), sugar concentration (40–80 g l^{-1}) and urea level (0.27–1.35 g l^{-1}). The results showed that the optimal conditions were an initial pH of 6.5, a sugar concentration of 40 g l^{-1} and a urea level of 0.81 g l^{-1}. The magnitude of these parameters' influence on butanol and ABE concentrations was initial pH > sugar > urea. The fermentation under the optimal conditions in a 2-L fermenter with agitation rate of 100 rpm revealed that the butanol and ABE concentrations were 12.55 and 17.96 g l^{-1}, respectively. Without nutrient supplementation, they were 7.45 and 11.21 g l^{-1}, respectively, using molasses as a substrate. When a gas stripping system was connected to the fermenter, the butanol and ABE values increased to 14.13 and 18.90 g l^{-1}, respectively, with butanol productivity and sugar utilization of 0.29 g l^{-1} h^{-1} and 90.50%, respectively.

1. Introduction

Nowadays, increasing concerns over global warming have renewed interest in biobutanol as an alternative renewable liquid fuel (Sun and Liu, 2010). Butanol is an excellent biofuel, compared with the currently popular fuel additive, ethanol. Butanol has a lower vapour pressure and higher energy content than ethanol, which makes the former safer for blending with gasoline and offers better fuel economy than ethanol–gasoline blends. Additionally, it has a higher tolerance for water contamination in gasoline blends. Hence, butanol–gasoline blends are less susceptible to separation, which facilitates their use in existing gasoline supplies and distribution channels (Al-Shorgani et al., 2011).

Butanol can be produced by fermentation process called the acetone–butanol–ethanol (ABE) fermentation. The primary microorganisms involved in this fermentation are spore-forming bacteria, i.e., *Clostridium* spp. A typical feature of clostridia solvent production is its biphasic nature. The first phase is an acidogenic phase, in which the acid-forming pathways are activated producing acetate, butyrate, hydrogen and carbon dioxide as major products. This phase usually occurs during the exponential-growth phase of cell division. The second phase is a solventogenic phase in which acetic and butyric acids are assimilated and used in the production of acetone and butanol, respectively (Al-Shorgani et al., 2012).

Sugarcane molasses is the main by-product of sugar production. The quantity of sugarcane molasses available in Thailand is about 3,610,000 tons $year^{-1}$ (Office of the Cane and Sugar Board, 2014). Normally, this sugarcane molasses is used for the production of ethanol, fertilizer, monosodium glutamate and liquor. Molasses without pretreatment contains high levels of fermentable sugars, and its cost is favorable compared with other agricultural raw materials used for alternative energy production (Department of Alternative Energy Development and Efficiency, 2014). Among three agricultural raw materials (sweet sorghum stem juice, sugarcane juice and sugarcane molasses), sugarcane molasses is the most suitable substrate for butanol production (Wechgama et al., 2014).

Initial pH, sugar and nitrogen concentrations are important parameters for growth of *Clostridium* spp. and ABE production

* Corresponding author at: Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen University, Khon Kaen, 40002, Thailand.
E-mail address: patlao@kku.ac.th (P. Laopaiboon).

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(Tashiro et al., 2004; Wanga and Blaschek, 2011; Li et al., 2013). Several researchers reported that the optimum initial pH and initial sugar for butanol production ranged from 5.5 to 7.5 and 40 to 80 g l^{-1}, respectively, depending on *Clostridium* sp. and substrates used (Areesirisuk et al., 2010; Wanga and Blaschek, 2011; Guo et al., 2012; Li et al., 2013; Wal et al., 2013). However, no report of the optimum initial pH and sugar concentration for butanol production from sugarcane molasses by *C. beijerinckii* has been published. Yeast extract (1-5 g l^{-1}) is widely used as a nitrogen source in laboratory scale of butanol production (Qureshi and Blaschek, 1999; Areesirisuk et al., 2010; Abd-Alla and El-Enany, 2012; Li et al., 2012, 2013), but it is relatively expensive when used as the nitrogen source for biofuel production. Therefore, urea was used in this research instead of yeast extract as a low cost nitrogen source for butanol production.

One of the largest problems associated with the ABE production is low product concentration and productivity caused by butanol toxicity and product inhibition (Jiang et al., 2014). Butanol toxicity causes cell membrane damage and makes membranes permeable to ADP (adenosine diphosphate) and some ions, and subsequently causes cell lysis (Abdehagh et al., 2014). Gas stripping is a simple technique to remove butanol from a fermentation broth during the ABE fermentation. It is effective, has low energy requirements and is easy to integrate with the fermentation process to reduce product inhibition (Ezeji et al., 2004; Lu et al., 2012; Xue et al., 2012).

The challenge in this research was to use urea as a nitrogen source instead of the more costly yeast extract and to optimize three parameters using a statistical design (orthogonal array design, OAD) (Taguchi, 1990; Mandali, 1995; Zhu and Ju, 2004). The optimized parameters were initial pH, initial sugar and urea concentrations. This was done to produce high levels of butanol from sugarcane molasses by *C. beijerinckii* TISTR 1461 in batch mode. Additionally, gas stripping was used in the ABE fermentation to further improve butanol production.

2. Materials and methods

2.1. Microorganism and inoculum preparation

C. beijerinckii TISTR 1461 (Wechgama, 2015) was purchased from the Thailand Institute of Scientific and Technological Research (TISTR). It was maintained as a spore suspension and stored at 4 °C in sterile distilled water. A spore suspension containing 1×10^6 spores ml^{-1} was heat shocked in a water bath at 80 °C for 1 min, then immediately cooled in ice-water for 1 min. Then, 0.5 ml of the spore suspension was inoculated into 10 ml of cooked meat medium or CMM (Oxoid, England) and incubated at 37 °C for 16–19 h to obtain highly motile vegetative cells. Vegetative cells (5%, v v^{-1}) at an optical density 600 nm (OD_{600}) of 0.5 were transferred into tryptone-glucose-yeast extract (TGY) medium and incubated at 37 °C for 4-6 h before use as an inoculum for ABE production (Areesirisuk et al., 2010).

2.2. Raw materials

Sugarcane molasses (80 °Bx of total soluble solids) was obtained from Mitr Phu Viang Sugar Co., Ltd. Khon Kaen, Thailand. Its composition was determined by the Central Laboratory (Thailand) Co., Ltd., Khon Kaen, Thailand, as shown in Table 1. The molasses was kept at −20 °C to protect it against microbial contamination before its use as a substrate for butanol production.

2.3. Culture medium

CMM and TGY media were used for inoculum preparation. CMM was composed of CMM powder, 10 and glucose, 0.8 g l^{-1} (modified from Qureshi and Blaschek, 1999). TGY medium was composed of typtone, 5; glucose, 1; yeast extract, 5 and K_2HPO_4, 5 g l^{-1} (Qureshi and Blaschek, 1999). Both CMM and TGY media were autoclaved at 110 °C for 28 min. Before inoculation, the media were purged with sterile oxygen free nitrogen (OFN) gas to create strictly anaerobic conditions.

Table 1
The composition of sugarcane molasses from Mitr Phu Viang Sugar Co., Ltd., Thailand.

Composition	Concentration		Analytical method
Protein[a]	6.40	g 100 ml^{-1}	AOAC, 2005 [b]
Phosphorus (P)[a]	694.17	mg l^{-1}	ICP-MS
Potassium (K)[a]	33540.70	mg l^{-1}	
Sodium (Na)[a]	574.49	mg l^{-1}	
Calcium (Ca)[a]	12085.36	mg l^{-1}	
Magnesium (Mg)[a]	5733.75	mg l^{-1}	
Iron (Fe)[a]	152.44	mg l^{-1}	
Manganese (Mn)[a]	108.99	mg l^{-1}	
Copper (Cu)[a]	1.54	mg l^{-1}	
Zinc (Zn)[a]	1.13	mg l^{-1}	
Molybdenum (Mo)[a]	0.35	mg l^{-1}	
Nickel (Ni)[a]	1.92	mg l^{-1}	
Boron (B)[a]	3.10	mg l^{-1}	
Cobalt (Co)[a]	0.86	mg l^{-1}	
Sulfur (S)[a]	1036.00	mg l^{-1}	Turbidimetry method
Sucrose	364.46	g l^{-1}	HPLC (modified from
Glucose	103.69	g l^{-1}	Sirisantimathakom et al., 2004)
Fructose	123.99	g l^{-1}	

[a] Central Laboratory (Thailand) Co., Ltd., Khon Kaen, Thailand.
[b] Association of Official Analytical Chemists (AOAC, 2005).

Table 2
The L_9 (3^4) orthogonal array design for butanol production.

Run	*Factor A*: Initial pH	*Factor B*: Sugar (g l^{-1})	Blank	*Factor C*: Urea (g l^{-1})
1	0 (6.5)	−1 (40)	0	0 (0.81)
2	1 (7.5)	0 (60)	0	−1 (0.27)
3	0 (6.5)	1 (80)	1	−1 (0.27)
4	−1 (5.5)	1 (80)	0	1 (1.35)
5	0 (6.5)	0 (60)	−1	1 (1.35)
6	1 (7.5)	−1 (40)	1	1 (1.35)
7	−1 (5.5)	0 (60)	1	0 (0.81)
8	−1 (5.5)	−1 (40)	−1	−1 (0.27)
9	1 (7.5)	1 (80)	−1	0 (0.81)

2.4. Butanol production medium

The sugarcane molasses was diluted with distilled water to obtain desired total sugar concentrations (Table 2) before urea addition. The medium (750 ml) was transferred into 1-L air-locked bottles. After sterilization, the pH of the medium was adjusted to desired values (Table 2) by addition of 8 N NaOH.

P2 medium or synthetic butanol production medium was also prepared as the control treatment for the ABE fermentation. P2 medium was composed of glucose, 60 and yeast extract, 1 g l^{-1} and stock solutions A, B and C (Qureshi and Blaschek, 1999). Stock solution A consisted of K_2HPO_4, 50; KH_2PO_4, 50 and ammonium acetate, 220 g l^{-1}. Stock solution B consisted of para-amino-benzoic acid, 0.1; thiamine, 0.1 and biotin, 0.001 g l^{-1}. Stock solution C consisted of $MgSO_4 \cdot 7H_2O$, 20; $MnSO_4 \cdot H_2O$, 1.0; $FeSO_4 \cdot 7H_2O$, 1.0 and NaCl, 1.0 g l^{-1}. Stock solutions A and C were autoclaved at 110 °C for 28 min, whereas stock B was sterilized using a sterile 0.2 μm cellulose acetate membrane. Then, 1% (v v^{-1}) of each stock solution was aseptically added into the sterile P2 medium.

2.5. Preliminary experiments

To study the effect of the nutrients contained in P2 medium (excluding glucose) on butanol production from the sugarcane