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The Effect of Initial Butyric Acid Addition on ABE Fermentation by *C. acetobutylicum* NCIMB 619

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Abstract: The addition of organic acids to the growth medium has been shown to stimulate solvent production and protect against the degeneration of ABE-producing clostridia. The objective of this study is to demonstrate the effect of introduction of butyric acid in the fermentation media on the solvent production by *C. acetobutylicum* NCIMB 619. In this study, batch cultures were carried out in Reinforced Clostridial Media (RCM) containing 0.0015 and 2.0 g L^{-1} of butyric acid, simultaneously with a control media (without addition of butyric acid) at 37°C for 72 h. It was found that the presence of butyric acid during the early stage of the fermentation process favor the solvent yield to glucose utilization up to 71%. By adding butyric acid, the concentration of acetone and butanol generated was improved significantly. In the case of 2.0 g L^{-1} butyric acid addition, acetone and butanol production was increased approximately 5 and 1.6 folds, respectively compared to control culture. The result obtained in this study showed that the addition of butyric acid not only promoted solvent production, but also induced butanol production at the initial stage of fermentation by *C. acetobutylicum* NCIMB 619.

Key words: ABE fermentation, *C. acetobutylicum*, butyric acid

INTRODUCTION

The production of butanol by Acetone-Butanol-Ethanol (ABE) fermentation used to be one of the largest bioprocesses until the 1950s, but later it was replaced by the less expensive petroleum-based chemical synthesis. In recent years, interest in bio-based butanol has been revived primarily due to concerns with fossil fuel depletion and microbial production of butanol is considered to be a potential source of liquid fuels.

The metabolic pathway of solvent-producing Clostridium consists of two distinct phases, acidogenesis and solventogenesis (Jones and Woods, 1986). In general, during acidogenesis, cell growth is exponential and organic acids are produced together with hydrogen gas, which results in pH reduction. Solventogenesis followed subsequently where cell growth enters the stationary phase and the organic acids are reutilized to produce acetone, butanol and ethanol. This reutilization of organic acids increases the pH value of the broth. It was reported that the addition of organic acids in the broth triggered the metabolic transition from the acidogenic phase to solventogenic phase (Bahl *et al*., 1982; Huang *et al*., 2004; Tashiro *et al*., 2004). Therefore, in the present study, butyric acid was introduced early in the fermentation media to demonstrate its contribution in solvent production.

MATERIALS AND METHODS

ABE fermentation: *C. acetobutylicum* NCIMB 619 obtained from British Culture Collection NCIMB Ltd., Scotland, UK was used in this study. Laboratory stocks of *C. acetobutylicum* were routinely maintained and kept as a single colony as spore suspensions in liquid Reinforced Clostridial Media (RCM) at 4°C. The bacterial reactivation were performed by transferring stock culture into fresh deoxygenized RCM liquid medium and incubated at 37°C for 30 h. Followed by inoculum production by inoculating 10% (v/v) of reactivated culture in another volume of fresh deoxygenized RCM liquid medium and grew in the similar temperature for 10 h. All procedures were carried out anaerobically and inoculum with optical density of 1.2 to 1.4 at 680 nm was used in the fermentation. Different volumes of pure butyric acid were added into the media prior to fermentation process which

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took place subsequently in three 250 mL flasks (including control culture) at a temperature of 37°C for 72 h without stirring. Periodically, samples were withdrawn for analysis purposes.

Analytical method: The pH reading was determined by pH meter (Mettler Toledo, USA) while the cell concentration was estimated by optical density with a UV-spectrophotometer at 680 nm. The concentration of acetone, butanol and ethanol was evaluated by Agilent Technologies gas chromatograph model 6890N, equipped with Flame Ionization Detector (FID) and capillary column Equity™-1 (Supelco) with dimension of 30×0.32 m×1.0 μm. The oven temperature was programmed at 40°C (8 min) and later increased to 130°C (4°C min^{-1}) for 5 min. The detector and injector were set at 250°C with the carrier gas (helium) flow rate of 1.5 mL min^{-1}. The reducing sugar concentration in the sample was measured using DNS method.

RESULTS

To investigate the effect of addition of butyric acid, batch cultures were carried out in RCM medium containing 0, 0.0015 and 2.0 g L^{-1} butyric acid. Figure 1a and b show the effect of initial addition of butyric acid on pH of the broth, cell optical density and reducing sugar concentration. The culture without acid addition demonstrated a common pattern of bacterial growth with 3 h of lag phase, followed by the exponential cell growth and peaked at 12 h of fermentation time before it reduced gradually to the end of fermentation. The addition of butyric acid, as low as 0.0015 g L^{-1} enhanced the bacterial growth as the OD reading rose directly upon incubation, with higher value compared to that in the control media (Fig. 1b). This was probably contributed by the changes of initial pH value towards 5.0 which favors the bacterial growth. Moreover, the exponential growth was extended up to 24 h with the addition of 2.0 g L^{-1}. This supported the findings of earlier studies that the early presence of organic acid offered a protection against the degeneration of ABE-producing clostridia (Bahl *et al*., 1982; Chen and Blaschek, 1999a, b; Hartmanis *et al*., 1984; Husemann and Papoutsakis, 1990).

The amount of solvents (ABE) produced in the medium containing 2.0 g L^{-1} was greater than those produced in the medium containing other butyric acid concentrations (Table 1). Furthermore, with additional butyric acids, the glucose utilization rate was lower and results in higher solvent yield.

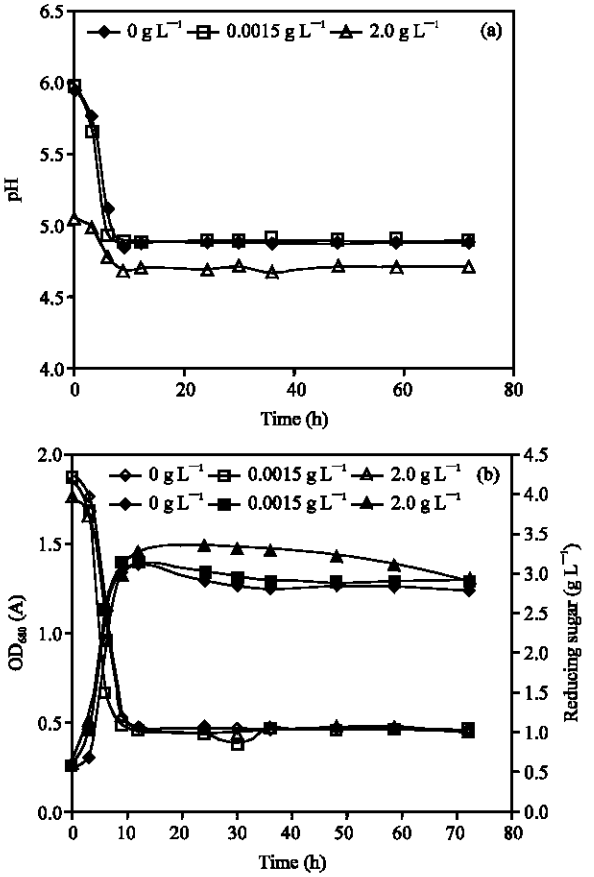

Fig. 1: Effect of initial addition of butyric acid on (a) pH of the broth and (b) cell optical density and reducing sugar concentration, Open symbols: Reducing sugar concentration, Closed symbols: Cell optimal density at 680 nm

Table 1: Effect of butyric acid in ABE fermentation

Butyric acid (g L^{-1})	Acetone (g L^{-1})	Production[a] (g L^{-1}) Butanol	Production[a] (g L^{-1}) Ethanol	Solvent yield[b] (g g^{-1})	Productivity[c] (g/L/h)
0	0.008	0.149	0.067	0.066	0.018
0.0015	0.011	0.156	0.094	0.071	0.003
2.0	0.040	0.237	0.056	0.113	0.006

[a]Maximum solvent production, [b]Total solvent production per glucose utilization and [c]Total solvent production over fermentation time

DISCUSSION

Butyric acid in broth, particularly undissociated butyric acid, has been shown to trigger solvent production by *C. acetobutylicum* (Huang *et al*., 2004; Tashiro *et al*., 2004; Girbal *et al*., 1995). Furthermore, the increase in yield and production of solvents were also reported upon the addition of butyric acid and acetic acid to cultures of *C. acetobutylicum*, *C. beijerinckii* and *C. saccharoperbutylacetonicum* N1-4 (Bahl *et al*., 1982;

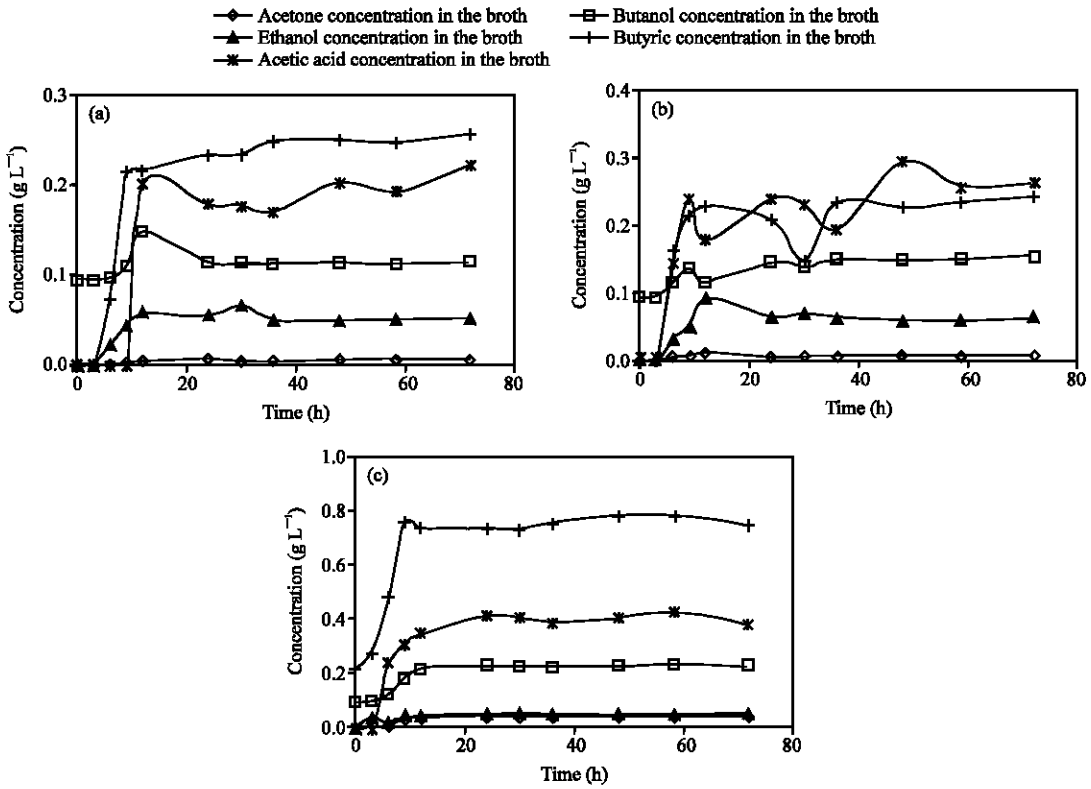

Fig. 2: Solvent production profiles over 72 h fermentation with butyric acid initial addition at several concentrations: (a) 0 g L^{-1}; (b) 0.0015 g L^{-1} and (c) 2.0 g L^{-1}

Tashiro *et al*., 2004; Chen and Blaschek, 1999a, b; Hartmanis *et al*., 1984; Husemann and Papoutsakis, 1990).

Besides that, previous studies have shown that pH of the fermentation media has a significant influence on ABE fermentation where it determine the organic acid or solvent production (Bahl *et al*., 1982; Huang *et al*., 2004; Tashiro *et al*., 2004; Holt *et al*., 1984; Somrutai *et al*., 1996). It is reported that organic acid production is enhanced at higher pH while solvents are mainly produced at lower pH. The addition of butyric acid helped to maintain the media at low pH value which induced a metabolic shift from acidogenesis to solventogenesis and thus, enhanced the total solvent production.

Figure 2a-c show the solvent production profiles over 72 h fermentation with butyric acid initial addition at several concentrations. By adding butyric acid, the concentration of acetone and butanol generated was improved significantly. With 0.0015 g L^{-1} of butyric acid addition, the ethanol production followed the similar trend. As the concentration of butyric acid increased to 2.0 g L^{-1}, the ethanol production reduced to 0.056 g L^{-1}. This was comparable with the previous study using *C. saccharoperbutylacetonicum* N1-4 where the ethanol production in the presence of butyric acid was lower than in its absence (Tashiro *et al*., 2004). In the case of 2.0 g L^{-1} butyric acid addition, acetone and butanol production was increased approximately 5 and 1.6 folds, respectively compared to conventional batch culture (without butyric acid addition). Obviously, the addition of butyric acid affected acetone production the most compared to butanol and ethanol.

As for the addition of 0.0015 g L^{-1} of butyric acid, the growth profiles (pH value, Optical Density (OD) reading and reducing sugar concentration) and solvent production profiles was just slightly improved to those in the absence of butyric acid. This is because the acid concentration was too low to provide a significant effect. From the plots of Fig. 2, it was apparent that solvent production was ceased at fermentation time of 12 h for the medium containing 0, 0.0015 and 2.0 g L^{-1} of butyric acid. This outcome could be explained by relating the importance of sufficient glucose concentration for solvent production. After 12 h of fermentation, the remaining glucose concentration in the broth was approximately 1.0 g L^{-1}. At this point, the solvent production was ceased due to glucose insufficiency.

CONCLUSION

The result obtained in this study showed that the initial addition of butyric acid promoted solvents production by *C. acetobutylicum* NCIMB 619. Upon butyric acid addition, more organic acids were presented in the broth and subsequently converted into solvents. Apart from that, with the introduction of butyric acid, the initial pH was adjusted to a lower value which favors solvent production. Total solvent concentration of 0.3 g L^{-1} was obtained with acetone and butanol production was increased approximately 5 and 1.6 folds, respectively.

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REFERENCES

Bahl, H., W. Andersch, K. Braun and G. Gottschalk, 1982. Effect of pH and butyrate concentration on the production of acetone and butanol by *Clostridium acetobutylicum* grown in continuous culture. J. Applied Microbiol. Biotechnol., 14: 17-20.

Chen, C.K. and H.P. Blaschek, 1999a. Acetate enhances solvent production and prevents degeneration in *Clostridium beijerinckii* BA101. Applied Microbiol. Biotechnol., 52: 170-173.

Chen, C.K. and H.P. Blaschek, 1999b. Effect of acetate on molecular and physiological aspects of *Clostridium biejerinckii* NCIMB 8052 solvent production and strain degeneration. Applied Environ. Microbiol., 65: 499-505.

Girbal, L., C. Croux, I. Vasconcelos and P. Soucaille, 1995. Regulation of metabolic shifts in *Clostridium acetobutylicum* ATCC 824. FEMS Microbiol. Rev., 17: 287-297.

Hartmanis, M.G.N., T. Klason and S. Gatenbeck, 1984. Uptake and activation of acetate and butyrate in *Clostridium acetobutylicum*. Applied Microbiol. Biotechnol., 20: 66-71.

Holt, R.A., G.M. Stephens and J.G. Morris, 1984. Production of Solvents by *Clostridium acetobutylicum* cultures maintained at neutral pH. Applied Environ. Microbiol., 48: 1166-1170.

Huang, W.C., D.E. Ramey and S.T. Yang, 2004. Continuous production of butanol by *Clostridium acetobutylicum* immobilized in a fibrous bed bioreactor. Applied Biochem. Biotechnol., 113: 887-898.

Husemann, M.H.W. and E.T. Papoutsakis, 1990. Effects of propionate and acetate additions on solvent production in batch cultures of *C. acetobutylicum*. Appied Environ. Microbial., 56: 1497-1500.

Jones, D.T. and D.R. Woods, 1986. Acetone-Butanol Fermentation Revisited. Microbiol. Mol. Biol. Rev., 50: 484-524.

Somrutai, W., M. Takagi and T. Yoshida, 1996. Acetone-butanol fermentation by *C. aurantibutyricum* ATCC 17777 from a model medium for palm oil mill effluent. J. Fermen. Bioeng., 81: 543-547.

Tashiro, Y., K. Takeda, G. Kobayashi, K. Sonomoto, A. Ishizaki and S. Yoshino, 2004. High butanol production by *C. saccharoperbutylacetonicum* N1-4 in fed-batch culture with pH-stat continuous butyric acid and glucose feeding method. J. Biosci. Bioeng., 98: 263-268.