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Enhanced butanol production by modulation of electron flow in *Clostridium acetobutylicum* B3 immobilized by surface adsorption

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HIGHLIGHTS

- NAD(P)H that had escaped from the fermentation as H_2 limited the butanol yield.
- NAD(P)H regulation in *C. acetobutylicum* B3 by methyl viologen increased the butanol yield by 37.8%.
- *C. acetobutylicum* B3 cells were immobilized on a cotton towel by surface adsorption.
- The butanol tolerance of the immobilized cells was significantly improved.
- An average of 15.6 g/L butanol was achieved within 12 h in repeated batch fermentation.

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ABSTRACT

The objective of this study was to improve butanol yield and productivity by redox modulation and immobilization of *Clostridium acetobutylicum* B3 cells. Stoichiometric network analysis revealed that NAD(P)H that had escaped from the fermentation as H_2 limited the butanol yield and led to the accumulation of oxidation byproducts, e.g., acetone. Methyl viologen was used as an electron carrier to divert the electron flow away from H_2 production and to reinforce the NAD(P)H supply. Butanol yield was increased by 37.8% with severely diminished acetone production. Immobilization of the cells by adsorption onto a fibrous matrix improved their butanol tolerance and production rate. An average of 15.6 g/L butanol was achieved within 12 h with a solvent productivity of 1.88 g/L/h in repeated batch fermentation. To our knowledge, this is the highest solvent productivity with a relatively high butanol titer produced by a *Clostridium* strain in batch fermentation.

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1. Introduction

The bioproduction of acetone, butanol and ethanol (ABE) by solventogenic clostridia, such as *Clostridium acetobutylicum*, was once the second largest biotechnological industry in the world (Jones and Woods, 1986) and has attracted renewed interest for several economic and environmental reasons in recent years. ABE production by *C. acetobutylicum* strains in a batch culture is characterized by two distinct phases, the acidogenic phase and the solventogenic phase. Acetic and butyric acids are predominantly produced in the acidogenic phase, followed by the solventogenic phase wherein partial acids are reassimilated and solvent formation is observed. Butanol, acetone and ethanol are typically produced in an approximate ratio of 6:3:1 (*w*/*w*). Butanol, an important industrial chemical and excellent alternative to gasoline, is the preferred solvent and attracts the highest price (Green, 2011; Jang et al., 2012). To increase the metabolic flux towards butanol and reduce byproduct formation, genetic modifications of the acids and acetone formation pathways have been explored. Regarding the acetone branch, antisense RNA against *ctf*B was employed to improve the butanol:acetone ratio. However, the mutant strain also exhibited reduced butanol production (Tummala et al., 2003). Similarly, a mutant of *C. acetobutylicum* EA2018 with the *adc* gene disrupted by insertion of the group II intron produced small amounts of acetone but also significantly lower butanol titers as compared to the parent strain (Jiang et al., 2009). In addition, impairment of the acetone pathway in these mutant strains resulted in larger amounts of acetate accumulating in the growth medium (Lütke-Eversloh and Bahl, 2011). Inactivation of the *pta* gene involved in acetate formation did not achieve improved butanol production in *C. acetobutylicum* ATCC824 (Green et al., 1996). In the degenerated strain M5, butanol and ethanol production was restored by

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