



Yeast extract promotes phase shift of bio-butanol fermentation by *Clostridium acetobutylicum* ATCC824 using cassava as substrate

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HIGHLIGHTS

- ▶ A severe delay of phase shift was found in fermentation using cassava substrate.
- ▶ Yeast extract added during delay period promoted initiation of solventogenesis.
- ▶ Performances of traditional and extractive runs were improved by yeast extract.
- ▶ Real-time PCR was investigated to analyze transcriptional levels of genes.
- ▶ This process can produce butanol from cassava, a cheap non-grain crop.

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ABSTRACT

When fermenting on cassava (15–25%, w/v) with *Clostridium acetobutylicum* ATCC824, a severe delay (18–40 h) was observed in the phase shift from acidogenesis to solventogenesis, compared to the cases of fermenting on corn. By adding yeast extract (2.5 g/L-broth) into cassava meal medium when the delay appeared, the phase shift was triggered and fermentation performances were consequently improved. Total butanol concentrations/butanol productivities, compared to those with cassava substrate alone, increased 15%/80% in traditional fermentation while 86%/79% in extractive fermentation using oleyl alcohol as the extractant, and reached the equivalent levels of those using corn substrate. Analysis of genetic transcriptional levels and measurements of free amino acids in the broth demonstrated that timely and adequate addition of yeast extract could promote phase shift by increasing transcriptional level of *ctfAB* to 16-fold, and indirectly enhance butanol synthesis through accelerating the accumulation of histidine and aspartic acid families.

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1. Introduction

Biofuel is one of the attractive means to prevent a further increase of carbon dioxide emissions. Currently, gasoline is blended with ethanol at various percentages (Durre, 2007). However, compared to bio-ethanol, bio-butanol has several advantages such as being less hygroscopic, less volatile, and miscible with gasoline/diesel (Qureshi and Blaschek, 2001b; Qureshi and Ezeji, 2008). Renewable butanol is produced from the fermentations of carbohydrates in a process often referred as ABE fermentation, for solvent products of acetone, butanol and ethanol are formed at a ratio of 3:6:1 (w/w). ABE fermentation is a proven industrial process that uses solventogenic clostridia to convert starches or sugars

into solvents (Green, 2011). However, the relatively high cost of conventional starch (corn) or sugar (molasses) substrates at arable land competing with foods production and the very low total solvents concentration (2%) have been identified as a major factor affecting the economic viability of ABE fermentation (Jones and Woods, 1986; Lee et al., 2008; Qureshi and Blaschek, 2001a; Qureshi et al., 2001; Wang and Blaschek, 2011). Some researchers have also attempted to use waste agricultural residues as the substrates, but cells were unable to grow on such feedstocks unless they were subject to a complex enzymatic hydrolysis pre-treatment (Qureshi et al., 2008, 2010a,b; Thirml and Dahman, 2012). Among the available starch substances for bio-butanol production, cassava is very attractive because of being inexpensive, highly productive, and particularly non-competitive with foods for arable land (Cock, 1982). Although the utilization of cassava as a source of starch for production of butanol seems promising, recent reports indicated that butanol productivity when fermenting on cassava without any supplement is much lower than that of using corn starch or glucose (Gu et al., 2009; Lu et al., 2012; Thang et al., 2010).

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