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Enhancing acetone biosynthesis and acetone–butanol–ethanol fermentation performance by co-culturing *Clostridium acetobutylicum*/*Saccharomyces cerevisiae* integrated with exogenous acetate addition

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HIGHLIGHTS

- ABE fermentation strategy focused on enhancing bio-acetone production proposed.
- Simultaneously enhancing bio-synthesis of butanol and acetone in ABE fermentation.
- Co-culturing *C. acetobutylicum*/*S. cerevisiae* with exogenous acetate addition.
- Amino acids accumulation and NADH regeneration rate in *C. acetobutylicum* optimized.
- Glucose consumption by *C. acetobutylicum* induced by exogenous acetate addition.

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ABSTRACT

Acetone is the major by-product in ABE fermentations, most researches focused on increasing butanol/acetone ratio by decreasing acetone biosynthesis. However, economics of ABE fermentation industry strongly relies on evaluating acetone as a valuable platform chemical. Therefore, a novel ABE fermentation strategy focusing on bio-acetone production by co-culturing *Clostridium acetobutylicum*/*Saccharomyces cerevisiae* with exogenous acetate addition was proposed. Experimental and theoretical analysis revealed the strategy could, enhance *C. acetobutylicum* survival oriented amino acids assimilation in the cells; control NADH regeneration rate at moderately lower level to enhance acetone synthesis but without sacrificing butanol production; enhance the utilization ability of *C. acetobutylicum* on glucose and direct most of extra consumed glucose into acetone/butanol synthesis routes. By implementing the strategy using synthetic or acetate fermentative supernatant, acetone concentrations increased to 8.27–8.55 g/L from 5.86 g/L of the control, while butanol concentrations also elevated to the higher levels of 13.91–14.23 g/L from 11.63 g/L simultaneously.

1. Introduction

Acetone, an important platform chemical, is widely used as valuable solvent, chemical intermediate and fuel additive (Köhler et al., 2015; Wu et al., 2015). Acetone requirements have grown rapidly in recent years and about 6.7 million tons of acetone was produced world-wide in 2010 (Zhou et al., 2012). Nowadays, approximately 83% of acetone is produced by non-renewable fossil resources (Sifniades et al., 2011). Considering the future fossil resources dismiss and global greenhouse effect by continuously utilizing fossil resources, acetone production by renewable alternatives is strongly attractive (Zhou et al., 2012). Up to now, acetone–butanol–ethanol (ABE) fermentation by *Clostridium* spp. seems to be the only candidate for acetone bio-production using renewable biomass resources. A report demonstrated that ABE fermentation based acetone production could have the potential to replace about 70% of fossil resources (Wu et al., 2007). In ABE fermentation, butanol, acetone and ethanol are roughly produced at a mole ratio of 6:3:1, selectively increasing synthesis of butanol and repressing that of acetone (enhancing butanol/acetone ratio) has been one of the major targets pursued by most of the researchers (Jiang et al., 2009; Xu et al., 2015). However, increasing butanol/acetone ratio could not relieve the products purification loads, as acetone and ethanol are still counted in statistics of overall solvents productivity/amount and substrate conversion yield.

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As a result, it is necessary to consider a way to improve overall economics or performance of ABE fermentation process based on other evaluation criteria or in different points of view.

As a criteria, life cycle assessment (LCA) is generally used to evaluate a bioprocess by comprehensively considering the products diversity and their commercial values, greenhouse gas (GHG) emissions, as well as all physical and economic aspects on a set of energy and material inputs (Köhler et al., 2015). The LCA system of corn-based butanol as a potential transportation fuel has been established by a research group in Argonne National Laboratory, which indicated that when considering acetone as a valuable chemical, the GHG emissions would be largely reduced (Wu et al., 2007). From the LCA focused perspective, enhanced bio-acetone production by ABE fermentation shows promising energy and environmental benefits (Köhler et al., 2015; Wu et al., 2007). It was reported that a higher acetone content in ABE solvents mixture could improve the combustion property of the diesel blended with 20% (v/v) of the mixture and significantly reduce the soot (NO_X, SO_X, etc.) emission (Wu et al., 2015). A review report clearly indicated that, LCA of corn-based butanol strongly relies on the evaluation of acetone as valuable platform chemical and energy carrier. In addition, the co-product credit of bio-acetone would substitute a significant portion of chemically derived acetone, allowing ABE fermentation process to be more products diversifying (Köhler et al., 2015). Therefore, the LCA focused ABE fermentation could be actually recognized as maximizing butanol production while imposing larger evaluation weight coefficient on acetone synthesis simultaneously (the targeted or aimed ABE fermentations), or in other word, is to largely enhance bio-acetone production while maintaining butanol synthesis at equivalent or even higher levels. ABE fermentation by *Clostridium acetobutylicum* is characterized with the features: (1) severe butanol inhibition; (2) NADH dependent butanol production but non-NADH dependent acetone synthesis; (3) favorable solvents productions and cells survival under enriched intracellular accumulations of amino acids families of aspartic and aromatic acids (Heluane et al., 2011; Masion et al., 1987). Based on those features, development of the aimed ABE fermentation strategy featured with moderately lower NADH regeneration rate and enriched intracellular accumulations of those favorable amino acids is possible.

In *C. acetobutylicum* metabolism, the reductive power (NADH) formed in the glycolysis pathway and the associated electron transport shuttle system. Converting all of carbon substrate or most of it into acetone is impossible, as NADH accumulation without consumption would completely collapse the entire metabolism system. Butanol must be produced to consume NADH and to keep the mass balance of NADH. However, controlling NADH regeneration rate at a moderately lower level would have the following advantages for the above mentioned targeted ABE fermentations: (1) acetone/butanol ratio could be raised; (2) the NADH dependent butanol synthesis could continue as long as cells could survive and NADH is available, though butanol synthesis rate may reduce; (3) the cells would strive to operate with highest energy efficiency and thus reduce cellular energy load which is beneficial for cells survival (Lee and Papoutsakis, 1999); (4) acetone is much less toxic (to cells) than butanol, high acetone concentration would not deteriorate ABE fermentation performance. Acetone production is catalyzed by CoA transferase (CoAT) without any NADH involvement, while formation of one mole butanol needs four moles NADH (Peralta-Yahya et al., 2012). In ABE fermentation when the cells grown on modified-P2 medium with acetate addition, the specific enzyme activity of CoAT could be maintained at higher levels and the cells could produce acetone at high rate (Chen and Blaschek, 1999). Our previous study also revealed that exogenous acetate addition could alter carbon flux distributions and strengthen the metabolic strength of the butyrate closed-loop leading to a higher acetone/butanol ratio in turn (Li et al., 2014). In other report, acetone production was enhanced by 1.95-fold and acetone/butanol ratio increased from 0.38 to 0.51 in ABE fermentation by *Clostridium saccharoperbutylacetonicum* N1-4 when adding 4.0 g/L acetate (Gao et al., 2015). Their GC–MS analysis results indicated that both butanol and acetone could be formed from the added acetate. It has been very clear that exogenous acetate addition could promote acetone/butanol ratio in ABE fermentation. However, its real role in regulating NADH regeneration rate and enriching the intracellular accumulations of favorable amino acids remained unknown.

As one of effective fermentation methods, co-culturing technique using two microorganisms has been widely used in productions of platform chemicals and bio-fuel (Kiyoshi et al., 2015; Li et al., 2013a). Among the potential co-culturing systems for ABE fermentation, adaptively adding viable *Saccharomyces cerevisiae* cells into *C. acetobutylicum* culture broth could be considered as a novel and special co-culturing system, and a promising protocol for enhancing ABE fermentation performance. A report indicated that, in *S. cerevisiae*, butanol specifically inhibited protein synthesis at the translation initiation step by perturbing the eukaryotic initiation factor 2B. In this case, the intracellular amino acids could only preserve or exist in the "amino acids pools" (an intracellular storage unit in preservation of the amino acid, Ashe et al., 2001). As a result, we speculated that co-culturing *C. acetobutylicum* with *S. cerevisiae* could improve ABE fermentation performance: (1) the amino acids favorable for *C. acetobutylicum* survival and butanol synthesis might be secreted by *S. cerevisiae* under its stress environment and then assimilated by *C. acetobutylicum*; (2) *C. acetobutylicum* would compete with *S. cerevisiae* on substrate utilization for its survival, so that NADH regeneration pattern could be altered to create a favorable reductive power environment for our targeted ABE fermentation. A simplified metabolic network map covering both *C. acetobutylicum* and *S. cerevisiae* metabolisms was summarized in Fig. 1.

In this study, by carefully investigating the favorable amino acids secretions, NADH regeneration patterns, the enhanced utilization ability of *C. acetobutylicum* on glucose and whether most of the extra consumed glucose could be directed into acetone/butanol synthesis routes, under the environments of co-culturing *C. acetobutylicum*/*S. cerevisiae* integrated with exogenous acetate addition, a novel ABE fermentation strategy of co-culturing *C. acetobutylicum*/*S. cerevisiae* with exogenous acetate addition was proposed, attempting to enhance concentrations of acetone and butanol simultaneously. The effectiveness of the proposed strategy was testified by adding either synthetic acetate or the acetate fermentative supernatant.

2. Methods

2.1. Microorganisms and media

Clostridium acetobutylicum ATCC 824 was used for ABE fermentation. Corn meal (5%, w/v) medium was used for the storage and germination of the strain spores. The ABE fermentation medium was 15% (w/v) of corn flour, which was hydrolyzed using the same method described in our previous study (Li et al., 2012). *S. cerevisiae* was obtained from Angel Yeast Co., China. It was maintained at 4 °C on yeast extract-malt extract-glucose (YMG) agar. The medium for yeast culture contained (g/L): glucose 20, yeast extract 8.5, $(NH_4)_2SO_4$ 1.3, $MgSO_4{\cdot}7H_2O$ 0.1, and $CaCl_2{\cdot}2H_2O$ 0.06. *Acetobacter pasteurianus* CGMCC 1.1810 (ATCC 33445) was purchased from China General Microbiological Culture Collection Center (CGMCC) and maintained at 4 °C on yeast extract-glucose (YG) agar. The seed medium of *A. pasteurianus* cultivation consisted