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Kinetic modeling of lactic acid and acetic acid effects on butanol fermentation by *Clostridium saccharoperbutylacetonicum*

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ABSTRACT

Kinetic models of acetone-butanol-ethanol fermentation with lactic acid or acetic acid addition were developed and implemented in COPASI for metabolic analysis of acid effects on butanol synthesis. The simulation results were compared with experimental data in batch cultures of *Clostridium saccharoperbutylacetonicum* under various initial lactic acid or acetic acid concentrations. High average correlation coefficients (r^2) of over 0.92 between simulation and experimental results were obtained in both models. According to parameter scan in both models, reducing glucose uptake rate, increasing the conversion rate from glyceraldehyde 3-phosphate (G3P) to pyruvate or from butyryl-CoA (BCoA) to butanol would enhance butanol production. On the other hand, increasing consumption rate of supplemented lactic acid or acetic acid could also contribute to improved butanol synthesis. Overall, the developed kinetic models can accurately predict the dynamic behavior of metabolites in ABE fermentation with lactic acid or acetic acid addition and consequently identify genetic manipulation strategies for higher bio-butanol production in the future.

1. Introduction

Using petroleum products as today's main energy source has brought numerous issues such as uphill depletion of natural resources, environmental pollutions and energy price fluctuations [1,2]. Comparing with petroleum, bio-fuels extracted from biomass such as grains, grass, wood, and agricultural residues can help lessen the issues brought by petroleum [3–5]. Among the biofuels available in the energy market, bio-ethanol has been recognized as the most widely used liquid biofuel for motor vehicles [6]. However, butanol, a versatile four carbon alcohol (C_4H_9OH) has been considered as a superior alternative biofuel to bio-ethanol for its remarkable features, such as higher energy density, hydrophobicity, and compatibility with today's unmodified internal combustion engines [7].

In traditional acetone-butanol-ethanol (ABE) fermentation processes, the metabolism of ABE-producing clostridia can be divided into two distinct phases: acidogenesis and solventogenesis. During acidogenesis, the carbon source is converted into acids including butyric acid, lactic acid, and acetic acid. In the following solventogenesis, the acids are assimilated to produce acetone, butanol, and ethanol [8]. Considering the acid assimilation mechanism in solventogenesis, butyric acid, acetic acid and lactic acid have been recognized as potential substrates to improve butanol production [9]. To date, the mechanism of butyric acid addition on butanol production has been broadly studied [10–13]. However, rare research was conducted regarding the mechanism of lactic acid or acetic acid effects on butanol fermentation.

It has been experimentally proven that lactic acid could contribute to enhanced butanol production. For instance, lactic acid could be utilized along with glycerol to produce butanol by *Clostridium pasteurianum* DSM 525, enhancing butanol production from 6.5 g/L to 8.7 g/L with 0 and 16 g/L lactic acid, respectively [14]. In addition, when 5 g/L lactic acid was added into the glucose medium, butanol production by *C. saccharoperbutylacetonicum* increased to 5.98 g/L comparing with 4.95 g/L in the absence of lactic acid. Moreover, lactic acid addition resulted in a higher yield of 0.531 C-mol butanol/C-mol glucose comparing with 0.467 C-mol/C-mol in the control group [3]. On the other hand, researchers observed mixed results of acetate influence on ABE fermentation using various microorganisms. It was reported that acetic acid led to significant inhibition on cell growth and ethanol production of *Saccharomyces cerevisiae* [15,16]. Whereas, Alsaker et al. [17] demonstrated that the acetate-supplemented medium exhibited significant inhibition on the growth of *C. acetobutylicum* ATCC 824 but similar amounts of butanol and slightly higher levels of acetone were produced as compared to the control [17]; also, supplementation of 4 g/L acetate in glucose containing media increased butanol concentration by 48.3% as well as acetone concentration by 90.5%, suggesting that acetate addition altered the metabolic flux of *C. saccharoperbutylacetonicum* N1-4 [9]. However, although researchers have

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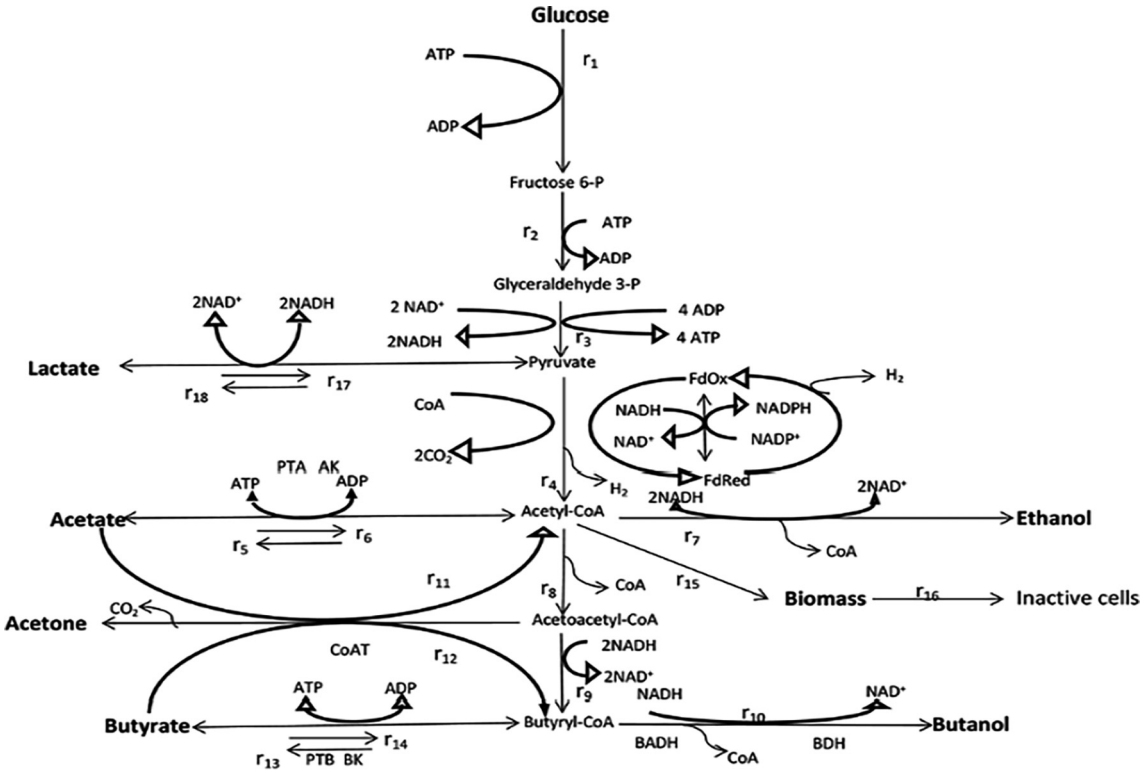

Fig. 1. The metabolic pathways of *C. acetobutylicum* in glucose media. Enzymes are abbreviated as follows: TA, transaldolase; TK, transketolase; PTA, phosphotransacetylase; AK, acetate kinase; CoAT, CoA transferase; PTB, phosphotransbutyrylase; BK, butyrate kinase; BADH, butyraldehyde dehydrogenase; BDH, butanol dehydrogenase; r: reaction rate (Raganati et al. [34]).

experimentally studied the influence of exogenous lactic acid and acetic acid on butanol synthesis [18–20], there is no reported effort on the development of kinetic models to better understand lactic acid/acetic acid effects on ABE fermentation in a systematic way.

Kinetic modeling has long been used to provide crucial information about metabolic capabilities of microorganisms during their cultivation [21–23]. The early work of ABE fermentation modeling mainly focused on the development of stoichiometric equations, which only described the relationships among various products and biomass accumulation in the fermentation process [24–26], but had very limited capacity to predict the fermentation behaviors when the culture conditions changed [24,27]. In contrast, recent kinetic models integrated with biochemical information are more efficient in reflecting system dynamics [28]. To date, two kinetic models reported by Shinto et al. [21,22] described the dynamic behaviors of metabolites in the ABE fermentation by *C. saccharoperbutylacetonicum* N1-4 using glucose and xylose, respectively. The sensitivity analysis results in both models revealed that slow substrate utilization would be beneficial for higher butanol production. Also, another kinetic model was developed by Raganati et al. [34] to investigate the effect of various sugars (mono-, di-, hexose and pentose sugars) on butanol synthesis by *C. acetobutylicum* DSM 792. These kinetic models, however, provided no insights into the effects of lactic acid or acetic acid on butanol synthesis.

The objective of this study was to understand the influence of lactic acid and acetic acid addition on butanol fermentation by *C. saccharoperbutylacetonicum* N1-4 (ATCC 27021). Kinetic models of ABE fermentation taking into account lactic acid/acetic acid effects were developed and implemented in COPASI, an open-source computer software that has been successfully used in microbial kinetic modeling by other researchers [29,30]. The modeling results were compared with experimental data and provided insights into the metabolic pathways of glucose to butanol influenced by lactic acid/acetic acid addition.

2. Materials and methods

2.1. Bacterial strain and culture medium

C. saccharoperbutylacetonicum N1-4 (ATCC 27021) was obtained from American Type Culture Collection (Manassas, Virginia, USA). The culture was maintained in the form of spores at 4 °C in fresh potato glucose medium (PG medium) containing 150 g fresh potato, 10 g glucose, 3 g $CaCO_3$, and 0.5 g $(NH_4)_2SO_4$ per liter of distilled water. The tryptone-yeast extract-acetate (TYA) medium was used as the pre-culture medium, which consisted the following ingredients per liter of distilled water: 20 g glucose, 2 g yeast extract, 6 g tryptone, 3 g CH_3COONH_4, 0.3 g $MgSO_4{\cdot}7H_2O$, 0.5 g KH_2PO_4 and 10 mg $FeSO_4{\cdot}7H_2O$ [31]. The phosphate-free nitrogen medium containing 22.5 g/L glucose, 0.5 g KH_2PO_4, and 10 mg $FeSO_4{\cdot}7H_2O$ in 1 L distilled water [10] was used as the experimental culture medium. Lactic acid or acetic acid was added into the experimental culture medium at concentrations from 0 to 12.5 g/L depending on the experimental design. Pre-culture was inoculated in TYA medium for 24 h, later *C. saccharoperbutylacetonicum* was transferred into the phosphate-free nitrogen medium for main culture. In all experiments, the initial pH was adjusted to 6.5 using 5 M NaOH prior to sterilization [32]. The medium was sterilized at 121 °C for 15 min before use. All chemicals were purchased from Sigma–Aldrich (St. Louis, Missouri, USA) unless specified otherwise.

2.2. Batch culture and analysis

Batch cultures were carried out with three replications in phosphate-free nitrogen medium at 30 °C under anaerobic condition without pH control. Pyrex bottles (250 mL) with silicone septa containing 180 mL culture medium and 20 mL inoculum were used as fermenters. To investigate lactic acid effects on butanol production, the initial lactic acid concentrations were set to 2.5 (27.8 mM), 5 (55.5 mM), 7.5