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Kinetic study of substrate dependency for higher butanol production in acetone–butanol–ethanol fermentation

Hideaki Shinto [a], Yukihiro Tashiro [a], Genta Kobayashi [b], Tatsuya Sekiguchi [c], Taizo Hanai [d], Yuki Kuriya [d], Masahiro Okamoto [d,e], Kenji Sonomoto [a,f,*]

[a] Laboratory of Microbial Technology, Division of Microbial Science and Technology, Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan
[b] Division of Microbial Technology, Ariake Sea Research Project, Saga University, 1 Honjo-cho, Saga 840-8502, Japan
[c] Department of Information Engineering, Faculty of Engineering, Maebashi Institute of Technology, 460-1 Kamisadorimachi, Maebashi 370-0816, Japan
[d] Laboratory for Bioinformatics, Graduate School of Systems Life Sciences, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan
[e] Laboratory of Bio-process Design, Department of Bio-Systems Design, Bio-Architecture Center, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan
[f] Laboratory of Functional Food Design, Department of Functional Metabolic Design, Bio-Architecture Center, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

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ABSTRACT

A kinetic simulation model of acetone–butanol–ethanol (ABE) fermentation of xylose ($Model_{XYL}$) was proposed by substituting Embden–Meyerhof–Parnas (EMP) pathway equations in the glucose model ($Model_{GLC}$) by pentose phosphate (PP) pathway equations of xylose utilization. We estimated the equation parameters of the PP pathway and set other equation parameters to the same as those in $Model_{GLC}$, by which $Model_{XYL}$ exhibited an r^2 value of 0.901 between the experimental time course of metabolites with initial xylose concentrations ranging from 40.7 to 292 mM and the calculated values. The results with the developed model suggested that *Clostridium saccharoperbutylacetonicum* N1-4 has a robust metabolic network in acid- and solvent-producing pathways. Furthermore, sensitivity analysis revealed that slow substrate utilization would be effective for higher butanol production; this coincided with the experimental results. Therefore, we consider the proposed model to be one of the best kinetic simulation candidates describing the dynamic metabolite behavior in ABE production.

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1. Introduction

Because of the seriousness of energy and environmental problems, there is an increasing focus on the use of biomass as a renewable energy source. Recently, fermentative energy production from several sources of biomass has been carried out. Acetone–butanol–ethanol (ABE)-producing clostridia could produce acetone, butanol, and ethanol from several biomass types such as palm oil waste [1], domestic waste [2], and abundant agricultural crops [3,4]. Since butanol has remarkable features such as hydrophobicity, high energy content, and ease of storage and transportation, it has been proposed as a substitute and supplement of gasoline as a transportation fuel. Currently, considerable research has been conducted on the ABE fermentation system for high butanol production [5–7].

The metabolic pathways of ABE-producing clostridia consist of two distinct characteristic phases, namely, acidogenesis and solventogenesis. A diagrammatic representation of the metabolic pathways of *Clostridium acetobutylicum* ATCC 824^T by Jones and Woods [8] is summarized in Figs. 1 and 2. Hexose sugars are metabolized via the Embden–Meyerhof–Parnas (EMP) pathway with the conversion of 1 mol of hexose to 2 mol of pyruvate, with net production of 2 mol each of ATP and NADH (Fig. 1). On the other hand, pentose sugars are metabolized via the pentose phosphate (PP) pathway and are converted to pentose-5-phosphate and dissimilated by means of the transketolase (TK)–transaldolase (TA) sequence, resulting in the production of fructose-6-phosphate and glyceraldehyde-3-phosphate (G3P), which then enter the glycolytic pathway (Fig. 2). We used *Clostridium saccharoperbutylacetonicum* N1-4 in this study. This strain does not possess the phosphoketolase pathway; therefore, the fermentation of 3 mol of

* Corresponding author at: Laboratory of Microbial Technology, Division of Microbial Science and Technology, Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan. Tel.: +81 92 642 3019; fax: +81 92 642 3019.
E-mail address: sonomoto@agr.kyushu-u.ac.jp (K. Sonomoto).

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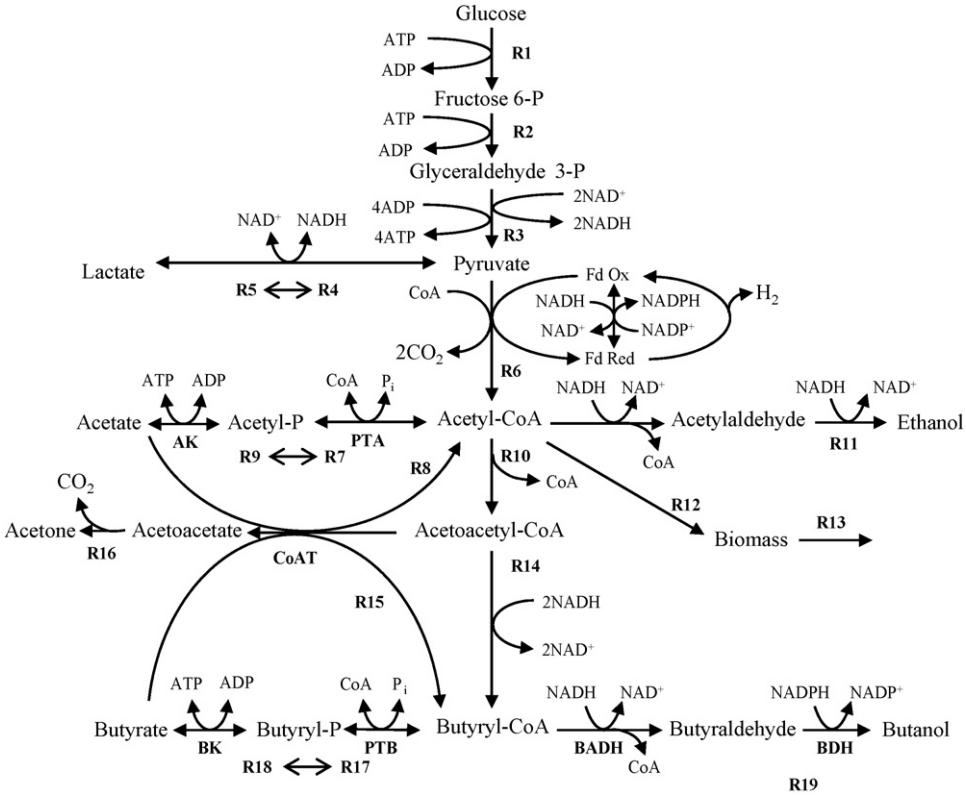

Fig. 1. Metabolic pathways in *Clostridium acetobutylicum* ATCC 824^T with glucose. Enzymes are indicated in bold and abbreviated as follows: PTA, phosphotransacetylase; AK, acetate kinase; CoAT, CoA transferase; PTB, phosphotransbutyrylase; BK, butyrate kinase; BADH, butyraldehyde dehydrogenase; BDH, butanol dehydrogenase.

pentose yields 5 mol of pyruvate, 5 mol of ATP, and 5 mol of NADH. In general, during acidogenesis, ABE-producing clostridia grow exponentially, and acetic and butyric acids are produced with ATP formation. Further, in the subsequent solventogenesis, cell growth attains a stationary phase; organic acids are reassimilated; and acetone, butanol, and ethanol are produced. ABE fermentation includes substrate inhibition by glucose and xylose and product inhibition by butanol [8,9]; these lead to low productivity and yield of solvents. On the other hand, Tashiro et al. [10] have experimentally demonstrated the acceleration of butanol production by addition of butyrate. As described above, since ABE-producing clostridia possess complicated metabolic function, the detailed metabolic network is still unknown.

Metabolic pathway modeling is one of the most successful scientific approaches for improving the metabolic capabilities of industrially relevant microorganisms during their cultivation [11,12]. However, since the metabolic pathway involved in ABE fermentation is very complicated, very few models describing this pathway have been published. Papoutsakis [13] developed a stoichiometric model for this pathway, which could be used to calculate or estimate the rates of reactions occurring within the pathway in several ABE-producing clostridia. Desai et al. [14] analyzed the contribution of acid-formation pathways in the metabolism of *C. acetobutylicum* ATCC 824^T by using metabolic flux analysis. Previously, we developed a kinetic model of metabolic pathways that describes the dynamic behaviors of the metabolites in ABE fermentation of glucose by *C. saccharoperbutylacetonicum* N1-4 by using a novel simulator, Biochemical Engineering System analyzing Tool-KIT (WinBEST-KIT) [15]. We performed sensitivity analysis with the developed model and observed that substrate utilization directly contributed to butanol production and slow utilization of the substrate was more effective for high butanol production [15].

In this study, to assess the effect of the substrate utilization rate on butanol production, we compared the dynamic behavior of two types of fermentation of glucose and xylose by each kinetic model. By substituting some rate equations of substrate utilization in the model of ABE fermentation of glucose, we could develop the kinetic simulation models of ABE fermentation of xylose. By sensitivity analysis, we found that compared to the other pathways, the substrate utilization pathway has the greatest contribution to higher butanol production. Furthermore, results of the simulation suggested that *C. saccharoperbutylacetonicum* N1-4 has a robust metabolic network in acid- and solvent-producing pathways.

2. Materials and methods

2.1. Bacterial strain

C. saccharoperbutylacetonicum N1-4 ATCC 13564 was used in this study [5]. The culture was maintained in the form of spores in fresh potato glucose (PG) medium at 4 °C. To prepare the seed culture, 1 ml of spore suspension was aseptically transferred into 9 ml of PG medium. Next, this mixture was subjected to heat shock by placing it in boiling water for 1 min and was subsequently cultivated at 30 °C for 24 h [1].

2.2. Media

We used tryptone–yeast extract–acetate (TYA) medium for pre-culture and main culture. The composition of this medium per liter of distilled water [5] is as follows: 5–50 g glucose or xylose, 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 $FeSO_4{\cdot}7H_2O$. In all experiments, the initial pH of the medium was adjusted to 6.5 with 1 M NaOH and sterilized at 115 °C for 15 min.

2.3. Culture conditions

Batch culture was carried out with a 300-ml working volume including an inoculum size of 10% in static conditions at 30 °C in a 500-ml Erlenmeyer flask. After inoculation, the broth was sparged with filtered oxygen-free nitrogen gas to maintain strict anaerobic conditions. Samples were periodically withdrawn. In the

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