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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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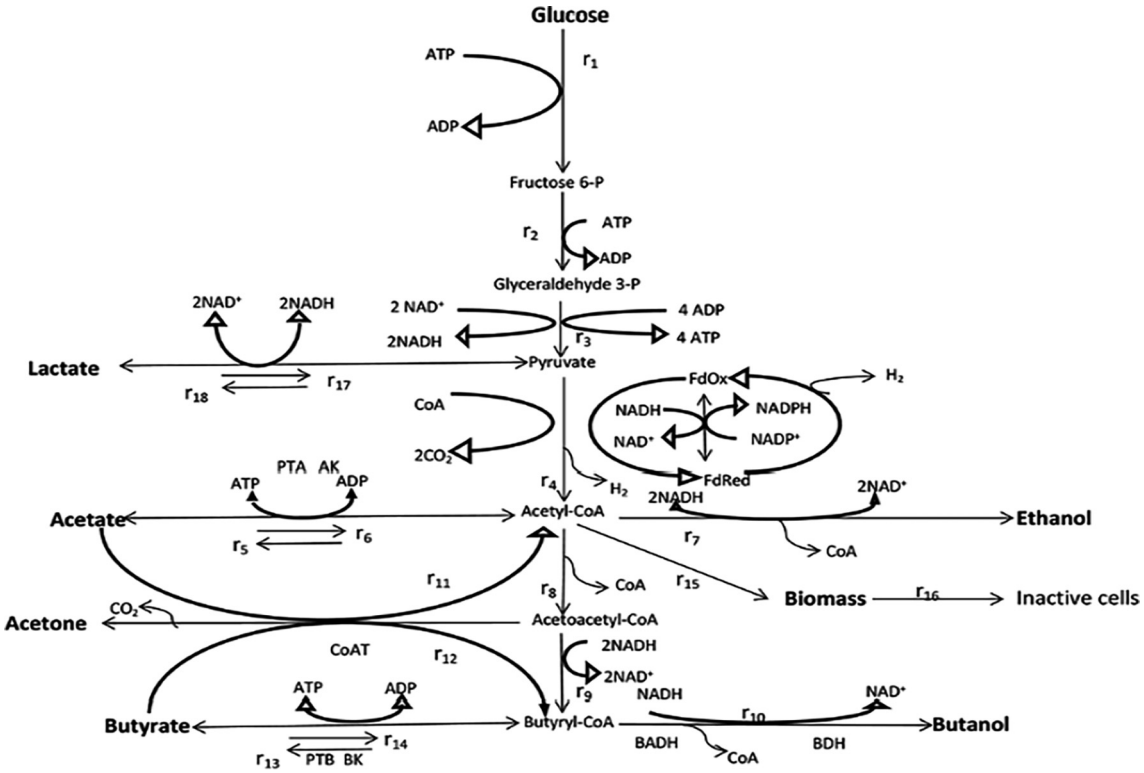

Fig. 1. The metabolic pathways of *C. acetobutylicum* in glucose media. Enzymes are abbreviated as follows: TA, transaldolase; TK, transketolase; PTA, phosphotransacetylase; AK, acetate kinase; CoAT, CoA transferase; PTB, phosphotransbutyrylase; BK, butyrate kinase; BADH, butyraldehyde dehydrogenase; 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

Table 1
The reaction steps from glucose to butanol and associated kinetics considering lactic acid/acetic acid effects.

Name	Reactions	Kinetics	Refs
R1	G → F6P	$r_1 = \frac{V_{max1}[G]}{K_{m1} + [G] + K_{m1}\left(\frac{[G]}{K_{is1}}\right)^2}\left(1-\frac{[B]}{B_{max20}}\right)^{nB1} F$	a
R2	F6P → 2 G3P	$r_2 = \frac{V_{max2}[F6P]}{K_{m2} + [F6P]}F$	b
R3	G3P → Pyr	$r_3 = \frac{V_{max3}[G3P]}{K_{m3} + [G3P]}F$	b
R4	Pyr → AcoA	$r_4 = \frac{V_{max4}[Pyr]}{K_{m4} + [Pyr]}F$	b
R5	AcoA → Acet	$r_5 = \frac{V_{max5}[AcoA]}{K_{m5} + [AcoA]}F$	b
R6	Acet → AcoA	$r_6 = \frac{V_{max6}[Acet]}{K_{m6} + [Acet]}F$	b
R6′	Acet → AcoA	$\boldsymbol{r_6 = \frac{V_{max6}[Acet]}{K_{m6} + [Acet] + K_{m6}\left(\frac{Acet}{Kis6}\right)^2}F}$	b, c
R7	ACoA → E	$r_7 = \frac{V_{max7}[AcoA]}{K_{m7} + [AcoA]}F$	b
R8	ACoA → 1/2AACoA	$r_8 = \frac{V_{max8}[AcoA]}{K_{m8} + [AcoA]}$	b
R9	AACoA → BCoA	$r_9 = \frac{V_{max9}[AAcoA]}{K_{m9} + [AAcoA]}F$	b
R10	BCoA → B	$r_{10} = \frac{V_{max10}[BcoA]}{K_{m10}(1 + K_{a10}/[Butyr]) + [G]}\left(1-\frac{[B]}{B_{max10}}\right)^{nB10}$ F	a
R11	Acet + AACoA → A + ACoA	$r_{11} = V_{max11}\left(\frac{1}{1 + K_{m11A}/[Acet]}\right)\left(\frac{1}{1 + K_{m11B}/[Acet]}\right)$	b
R11′	Acet + AACoA → A + ACoA	$\boldsymbol{r_{11=} = V_{max11}\left(\frac{1}{1 + K_{m11A}/[Acet] + K_{m11A}\left(\frac{Acet}{Kis11}\right)^2}\right)\left(\frac{1}{1 + K_{m11B}/[AACoA]}\right)}$	a, c
R12	Butyr + AACoA → A + BCoA	$r_{12} = V_{max12}\left(\frac{1}{1 + K_{m12A}/Butyr}\right)\left(\frac{1}{1 + K_{m12B}/AAcoA}\right)$	a
R13	BCoA → Butyr	$r_{13} = \frac{V_{max13}[BcoA]}{K_{m13} + [BcoA]}F$	b
R14	Butyr → BCoA	$r_{14} = \frac{V_{max14}[Butyr]}{K_{m14} + [Butyr]}F$	a
R15	ACoA → Biomass	$r_{15} = \frac{V_{max15}[AcoA]}{K_{m15} + [AcoA]}\left(1-\frac{[Acet]}{Acet_{max}}\right)^{nAcetate}\left(1-\frac{[Butyr]}{Butyr_{max}}\right)^{nButyrate}\left(1-\frac{[A]}{A_{max}}\right)^{nA}\left(1-\frac{[E]}{E_{max}}\right)^{nE}\left(1-\frac{[B]}{B_{max15}}\right)^{nB15}$	a
R16	Biomass → Inactive cells	$r_{16} = \frac{V_{max16}[Biomass][B]}{K_{ms16} \times K_{a16} + (K_{ms16} + [Biomass])[B]}$	b
R17	Lactate → Pyr	$\boldsymbol{r_{17} = \frac{V_{max17}[Lactate]}{K_{m17} + [Lactate] + K_{m17}\left(\frac{Lactate}{Kis17}\right)^2}F}$	b, c
R17′	Lactate → Pyr	$\boldsymbol{r_{17} = \frac{V_{max17}[Lactate]}{K_{m17} + [Lactate]}F}$	b
R18/R18′	Pyr → Lactate	$\boldsymbol{r_{18} = \frac{V_{max18}[Pyr]}{K_{m18} + [Pyr]}F}$	b

*R: Reaction; r: reaction rate; The reactions in bold font are the reactions that have been modified in LA modeling. The reactions in bold and marked with ' are the reactions that have been modified in AA modeling.
[a]Raganati et al. [34] [b]Shinto et al. [22] [c]Reed et al. [35].

(83.3 mM), or 10 (111.0 mM) g/L. Acetic acid with initial concentration of 2.5 (41.6 mM), 5 (83.3 mM), 7.5 (124.9 mM), 10 (166.5 mM) or 12.5 (208.2 mM) g/L was added into medium to investigate the effect of acetic acid on butanol production. The concentrations of glucose, acids (lactic acid, acetic acid, and butyric acid), and solvents (acetone, butanol, ethanol) in the fermenter were analyzed every 12 h by a high performance liquid chromatography (Prominence Series HPLC with a refractive index detector, model RID-10A, Shimadzu Corporation, Kyoto, Japan) using a Rezex RHM - Monosaccharide H+ (8%) column (300 × 7.8 mm, Phenomenex, Torrance, CA, USA), at a column temperature of 80 °C. And 0.005 N H_2SO_4 was used as the mobile phase at a flow rate of 0.6 mL/min. Detection was accomplished with the RI detector at the oven temperature of 40 °C. Cell concentration was determined by measuring the optical density (OD) at 562 nm with a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). An OD value of 1.0 was equivalent to 0.301 g of dry cell weight per liter and the average molecular weight of *C. saccharoperbutylacetonicum* was set to 172 g/mol [22].

Data was statistically analyzed with SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA). Multiple one-way analysis of variance (ANOVA) was conducted to evaluate the effect of lactic acid and acetic acid concentration on butanol production by *C. saccharoperbutylacetonicum*. The concentration of lactic acid or acetic acid was used as the independent variable while butanol production was the dependent variable. Tukey's adjustment was applied to the general linear model for determining the level of significance ($P < 0.05$) among various treatments. All experiments were conducted in triplicates and results were expressed as mean ± standard deviation.

2.3. Kinetic model development

Kinetic models were developed using a biochemical network simulator software COPASI, which can convert the biochemical reaction equations into the appropriate mathematical formalism automatically [33]. The developed models were established based on the ABE fermentation pathway from glucose as shown in Fig. 1 [34]. Table 1 shows the rate equations of each metabolic reaction with lactic acid/acetic acid addition. Compared to the previous model developed by Raganati et al. [34], the metabolic network developed in the present study was modified as follows (Fig. 1, Table 1): a reversible pathway (R17 and

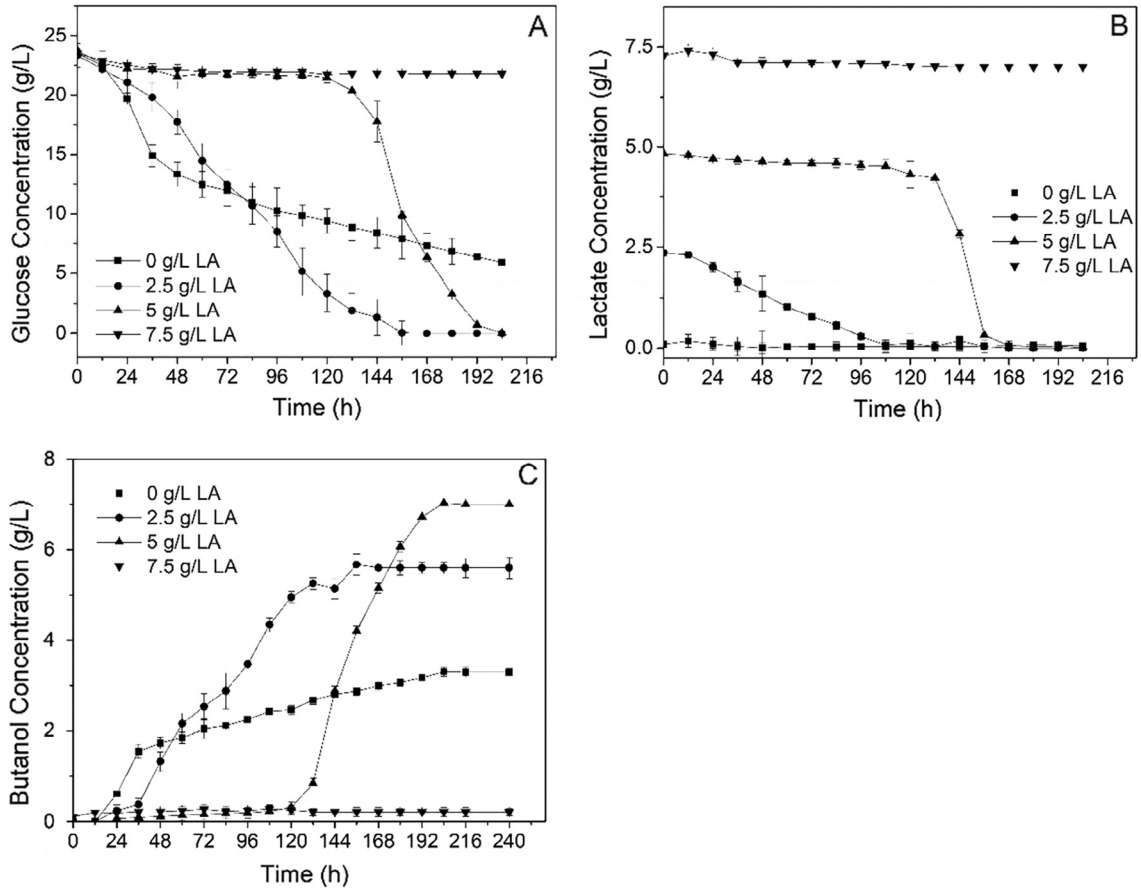

Fig. 2. The effect of lactic acid addition on ABE fermentation. (A) glucose consumption profile; (B) lactic acid consumption profile; (C) butanol production profile. LA = lactic acid.

R18, where R denotes reaction, and the number following R denotes the number of reaction in Table 1, the same hereafter) from pyruvate to lactate was added in both models and substrate (lactic acid) inhibition was considered in R17 when lactic acid was added to the fermenter (denoted as LA model); similarly, acetic acid (substrate) inhibition was also considered in R6′ and R11′ when acetic acid was exogenously added into the medium (denoted as AA model).

Two assumptions were made in the developed kinetic models. Firstly, no reactivation of dead cells was taken into consideration. As shown in Table 1, when butanol concentration approaches the critical value (B_{max}) in R1, R10, and R15, their reaction rates tend to be 0. Therefore, complete inhibition of cell growth and fermentation occurred as butanol reached the critical value (B_{max}). Secondly, reaction 17 in the lactic acid model or reaction 6′ and 11′ in the acetic acid model include a substrate inhibition kinetic. It is believed that an enzyme has two binding sites: the catalytic site and non-catalytic site. The catalytic site of the enzyme is defined as the binding site where the product is produced at a regular rate and the non-catalytic site is expressed as the binding site where the product was produced at a reduced rate. Under high substrate concentrations, it is assumed that one substrate molecule (lactic acid/acetic acid) binds to the catalytic site of the corresponding enzyme, following the other acid molecule binding to the non-catalytic site of the enzyme. Thus, an unproductive ternary complex could be generated under high substrate concentrations and the rate of reaction might decrease consequently [35].

2.4. Determination of model parameters

The values of multiple sets of kinetic parameters were estimated by fitting the experimental data measured during the batch cultures of *C. saccharoperbutylacetonicum* in the media with lactic acid initial concentrations of 0–10 g/L (153.2 mM) or acetic acid concentrations of 0–12.5 g/L (208.2 mM). The maximum reaction rate V_{maxj} and K_{mj} of each reaction step and the values of K_{isj}, K_{aj}, K_{msj}, K_{mjA}, K_{mjB}, B_{maxj}, $Acet_{max}$, $Butyr_{max}$, A_{max}, E_{max}, n_{Bj}, n_{Acet}, n_{Butyr}, n_A, and n_E were assessed. The particle swarm method – an optimization algorithm of COPASI – was used for the parameter estimation [33].

The simulation results were compared to the experimental data according to the assessment of the average squared correlation coefficients (r^2) between them. Moreover, parameter scan was carried out to reveal which reactions had potential impacts on achieving high butanol production. The impact of each parameter on endpoint butanol production was assessed by given a 5% increase in each kinetic parameter in the rate equations with lactic acid concentration of 5 g/L (55.5 mM) in LA model or with acetic acid concentration of 5 g/L (83.3 mM) in AA model as an example.

3. Results and discussion

3.1. Batch fermentation with lactic acid addition

With varying lactic acid concentrations (0–10 g/L) in the glucose medium, the highest butanol production (7.03 g/L with 0.40 C-mol/C-mol yield) was achieved when 5 g/L lactic acid was added (Fig. 2). In general, a higher concentration of lactic acid improved butanol production; however, there was a lactic acid tolerance limit for *C. saccharoperbutylacetonicum* N1-4. Comparing with the control group without lactic acid addition, 2.5 g/L and 5 g/L lactic acid addition increased final butanol concentration by 71.54% and 112.55%, respectively. Besides, the C-mol yield of butanol to substrates increased

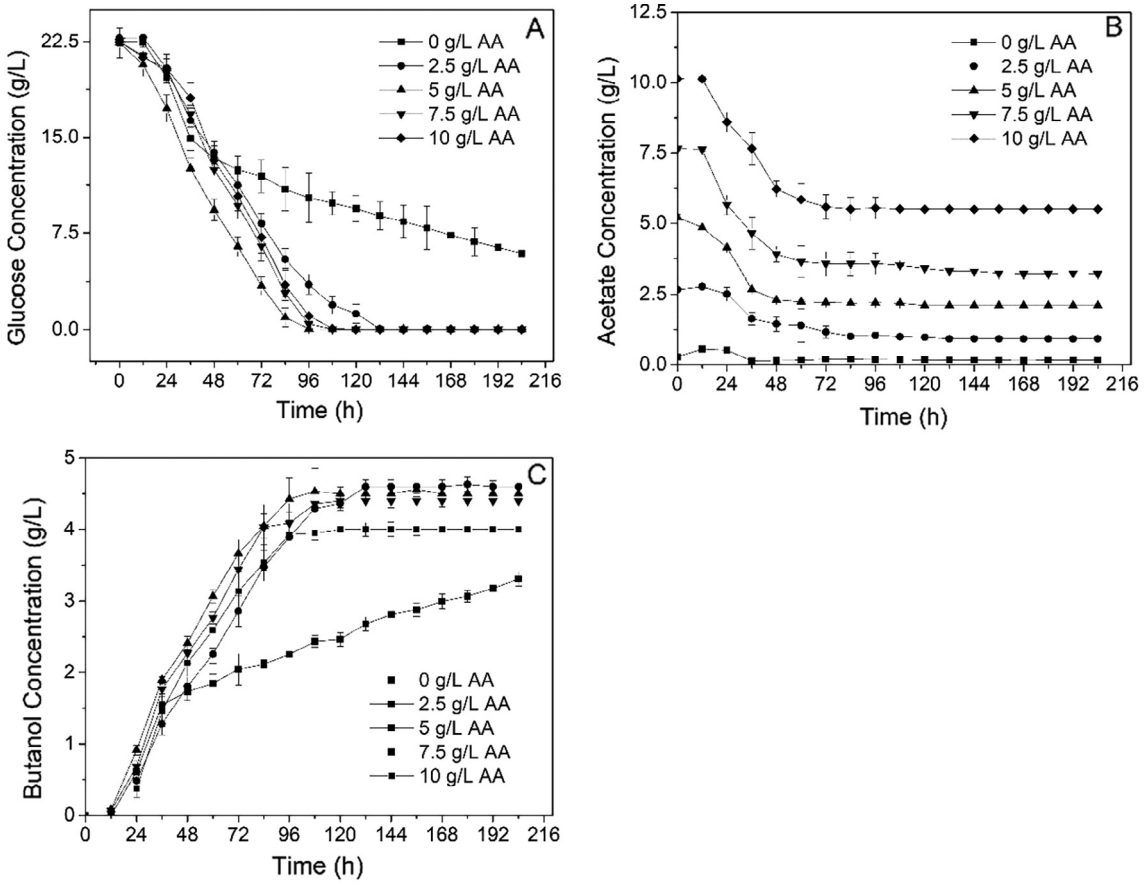

Fig. 3. The effect of acetic acid addition on ABE fermentation. (A) glucose consumption profile; (B) acetic acid consumption profile; (C) butanol production profile. AA = acetic acid.

18.43% (with 2.5 g/L lactic acid) and 31.81% (with 5 g/L lactic acid) compared to the control group. However, when 7.5 g/L or higher concentration of lactic acid (data not shown in Fig. 2) was present in the medium, no butanol production or substrates consumption was observed, indicating that higher than 7.5 g/L lactic acid would be lethal to the cells of *C. saccharoperbutylacetonicum* N1-4. Similar results reported by Oshiro et al. [3] showed that 5 g/L lactic acid addition in the glucose medium increased butanol concentration from 4.95 g/L to 5.98 g/L by *C. saccharoperbutylacetonicum* N1-4; however, addition of over 10 g/L lactic acid sharply reduced not only butanol concentration but also the lactic acid and glucose consumption. Thus, it can be concluded that substrate inhibition occurred when lactic acid reached a threshold level.

On the other hand, a higher concentration of lactic acid resulted in longer lag phase; whereas once the cells adapted to the environment, it took less time in the acid treated groups to reach the maximum butanol production than the control group. Specifically, when 2.5 g/L of lactic acid was present in the medium, butanol production increased its maximum level within 120 h (from 36 h to 156 h). Additionally, with 5 g/L lactic acid addition, butanol concentration reached its highest value within 84 h (from 120 h to 204 h), which was much shorter than 192 h taken in the control group.

3.2. Batch fermentation with acetic acid addition

The addition of acetic acid increased butanol concentration compared with the control group (Fig. 3). The highest butanol concentrations of 4.4–4.6 g/L (comparing with 3.3 g/L in the control group) were achieved under different concentrations of acetic acid, and there was no significant difference in butanol production among 2.5, 5 or 7.5 g/L of acetic acid additions (Fig. 3C). As reported by previous studies, the increase in butanol production with acetate addition could be attributed to the fact that acetate served as not only a buffering agent but also a carbon source [9,36,37]. Gao et al. [9] also reported that the enzyme activities involved in acetate uptake (phosphate acetyltransferase and CoA transferase), acetone formation (acetoacetate decarboxylase) and butanol formation (butanol dehydrogenase) in *C. saccharoperbutylacetonicum* were increased dramatically with acetate addition, resulting in a significant increase in ABE production. Nevertheless, butanol production was totally inhibited when acetic acid concentration exceeded 10 g/L (data not shown), suggesting that substrate inhibition occurred when the concentration of acetic acid was higher than the tolerance capacity of *C. saccharoperbutylacetonicum*.

In all the tested groups, acetic acid was not fully utilized that only 28–54% of acetic acid was consumed (Fig. 3B). In contrast, glucose was used completely after 96–132 h of fermentation (Fig. 3A), moreover, glucose consumption rate (denoted as total glucose consumption over time) increased with acetic acid addition. After 48 h, glucose was consumed at a higher rate in the acetic acid addition groups comparing with the control group. Similarly, Luo et al. [42] reported that total glucose consumption increased by 40% with exogenous acetate addition. Therefore, both the glucose consumption and ABE production could be enhanced by exogenous acetate addition.

C. saccharoperbutylacetonicum N1-4 has a boarder tolerance range to acetic acid than to lactic acid that cells grew well under 10 g/L acetic acid but were totally inhibited under 7.5 g/L of lactic acid. However, comparing with the effects of lactic acid, butanol production and yield were stimulated at a less extent by acetic acid addition. Specifically, when 2.5 g/L lactic acid was added exogenously into the medium, butanol endpoint concentration increased by 72% comparing with 39%

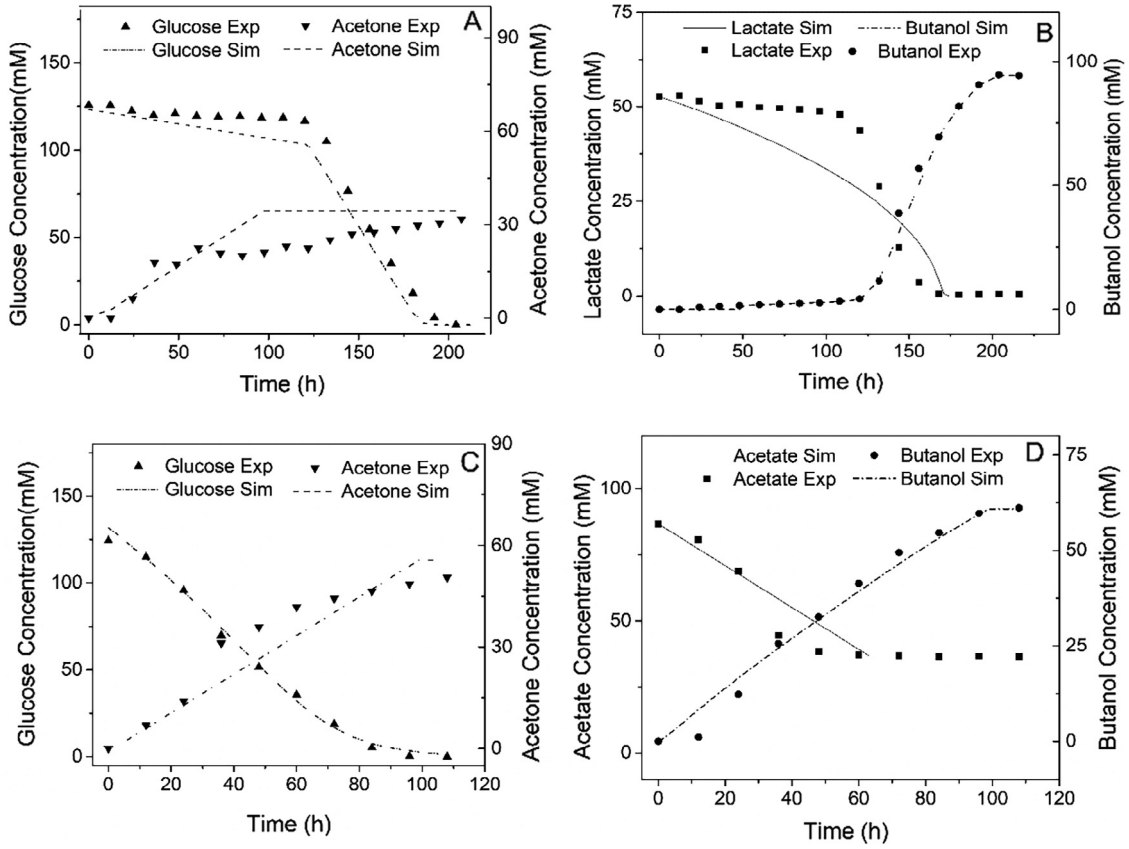

Fig. 4. The comparison between model simulation (Sim) and experimental (Exp) time-course data of target metabolites with 124.9 mM (22.5 g/L) of initial glucose concentration with the addition of 5 g/L (55.5 mM) lactic acid or 5 g/L (83.3 mM) acetic acid: (A) time-resolved concentration of glucose and acetone in the lactic acid treatment group; (B) time-resolved concentration of lactate and butanol in the lactic acid treatment group; (C) time-resolved concentration of glucose and acetone in the acetic acid treatment group; (D) time-resolved concentration of acetate and butanol in the acetic acid treatment group.

Table 2
Average squared correlation coefficients (r^2) between simulation results and experimental data.

Lactate (g/L)	Acetate (g/L)	Glucose	Lactate	Acetate	Acetone	Butanol	Biomass
0	0	0.9697	0.958	0.949	0.8242	0.9681	0.906
2.5	0	0.9925	0.9761	0.9316	0.968	0.951	0.815
5	0	0.9892	0.9068	0.8864	0.8216	0.9151	0.896
7.5	0	0.9064	0.962	0.9913	0.845	0.9747	0.889
10	0	0.9015	0.8647	0.8366	0.954	0.9425	0.875
0	2.5	0.9949	0.9247	0.913	0.979	0.9804	0.9197
0	5	0.9956	0.9574	0.9016	0.8961	0.9824	0.927
0	7.5	0.9963	0.9619	0.9446	0.9028	0.9767	0.9239
0	10	0.9921	0.9551	0.8575	0.9357	0.9716	0.9047

increment in 2.5 g/L acetic acid addition medium. Also, the butanol yield (C-mol/C-mol) was enhanced by 15% in lactic acid addition fermentation, whereas, only 2% increment was observed in acetic acid addition fermentation. Moreover, lactic acid was considered as a more beneficial co-substrate with glucose for butanol production, because only 2 mol NADH are required for conversion from 2 mol lactate to 1 mol butanol comparing with the requirement of 4 mol NADH for the conversion from acetate to butanol [9].

3.3. Comparison between simulation results and experimental time-course data

The estimated kinetic parameters with lactic acid concentration of 5 g/L (55.5 mM) and with acetic acid concentration of 5 g/L (83.3 mM) are presented in Table S1 and Table S2 (In Supplementary Material), respectively. Based on these parameters, the simulation results were obtained from the developed model and were compared with the experimental data (Fig. 4). It can be seen from Fig. 4 that the dynamic behaviors of target metabolites qualitatively matched the corresponding experimental time-course data from the batch cultures in both model.

The correlation coefficient (r^2) between simulation results and experimental data of each metabolite under varying acetic acid or lactic acid concentrations were calculated from the developed model (Table 2). An average correlation coefficient (r^2) of 0.92 and 0.95 of was obtained in the lactic acid model and acetic acid model, respectively, suggesting that the predictions were consistent with the experimental data.

3.4. Parameter scan

The parameter scan results of the lactic acid model are shown in Table 3. The reactions that had recognizable impacts on endpoint butanol concentration were R1, R3, R10, R16, and R17. Using R1 as an example, the increase in V_{max1}, K_{is1}, n_{B1}, B_{max1} caused lower endpoint butanol concentration, but the increase in K_{m1} caused higher endpoint butanol concentration. Seen from Table 1, the increase in V_{max1}, K_{is1}, n_{B1}, B_{max1} and decrease in K_{m1} would result in greater r1, indicating that higher reaction rate of r1 could result in poorer butanol production. Therefore, slower utilization of glucose could be beneficial for high butanol production. Similarly, R16 also had a negative effect on butanol production. In contrast, R3, R10, and R17 showed positive effects on butanol production. Taking R10 as an example, the increase in V_{max10}, n_{B10}, B_{max10} resulted in enhanced butanol production, but vice versa for K_{m10} and K_{a10}. Observed from Table 1, the increase in V_{max10},

Table 3
Percentage change in endpoint butanol production in response to a 5% increase in each parameter in the lactic acid model.

Reaction	Parameter	Percentage	Reaction	Parameter	Percentage
R1*	**V_{max1}**	**−0.11**	R11	V_{max11}	−0.34
	K_{m1}	**0.16**		K_{m11A}	0.17
	K_{is1}	**−0.41**		K_{m11B}	−0.61
	n_{B1}	**−0.05**	R12	V_{max12}	0.16
	B_{max1}	**−0.08**		K_{m12A}	0.34
R2	V_{max2}	0.06		K_{m12B}	0.23
	K_{m2}	0.47	R13	V_{max13}	−0.11
R3#	**V_{max3}**	**0.35**		K_{m13}	−0.16
	K_m	**−0.20**	R14	V_{max14}	0.32
R4	V_{max4}	0.13		K_{m14}	0.14
	K_{m4}	0.30	R15	V_{max15}	−0.34
R5	V_{max5}	0.36		K_{m15}	0.18
	K_{m5}	0.43		$Acet_{max}$	−0.16
R6	V_{max6}	−0.25		A_{max}	−0.11
	K_{m6}	−0.00		B_{max15}	−0.11
R7	V_{max7}	0.21		$Butyr_{max}$	−0.1
	K_{m7}	0.15		E_{max}	−0.25
R8	V_{max8}	0.15		n_A	−0.11
	K_{m8}	−0.01		$n_{Acetate}$	−0.09
R9	V_{max9}	0.18		n_{B15}	−0.11
	K_{m9}	0.5		$n_{Butyrate}$	−0.12
R10#	**V_{max10}**	**0.48**		n_E	0.41
	K_{m10}	**−0.08**	**R16***	**V_{max16}**	**−0.09**
	K_{a10}	**−0.21**		**K_{ms16}**	**0.52**
	n_{B10}	**0.47**		**K_{a16}**	**0.24**
	B_{max10}	**0.65**	**R17#**	**V_{max17}**	**0.59**
R18	V_{max18}	−0.02		**K_{m17}**	**−0.33**
	K_{m18}	−0.23		**K_{is17}**	**0.09**

* : The reactions that had negative effects on butanol production.
# : The reactions that had positive effects on butanol production.

Table 4
Percentage change in endpoint butanol production in response to a 5% increase in each parameter in the acetic acid model.

Reaction	Parameter	Percentage	Reaction	Parameter	Percentage
R1*	**V_{max1}**	**−0.25**	**R11***	**V_{max11}**	**−0.03**
	K_{m1}	**0.06**		**K_{m11A}**	**0.06**
	K_{is1}	**−0.37**		**K_{m11B}**	**0.64**
	n_{B1}	**−0.29**		**K_{is11}**	**0.44**
	B_{max1}	**−0.12**	R12	V_{max12}	0.23
R2	V_{max2}	0.85		K_{m12A}	0.29
	K_{m2}	0.11		K_{m12B}	−0.19
R3#	**V_{max3}**	**0.57**	R13	V_{max13}	0.28
	K_{m3}	**−0.28**		K_{m13}	0.24
R4	V_{max4}	0.64	R14	V_{max14}	0.21
	K_{m4}	0.08		K_{m14}	0.59
R5	V_{max5}	−0.29	R15	V_{max15}	1.80E-03
	K_{m5}	0.11		K_{m15}	0.01
R6#	**V_{max6}**	**0.16**		$Acet_{max}$	0.46
	K_{m6}	**−3.46E-03**		A_{max}	0.7
	K_{is6}	**0.06**		B_{max15}	0.22
R7	V_{max7}	5.10E-03		$Butyr_{max}$	0.61
	K_{m7}	0.05		E_{max}	0.29
R8	V_{max8}	1.41E-02		n_A	0.61
	K_{m8}	0.18		$n_{Acetate}$	−0.06
R9	V_{max9}	0.47		n_{B15}	0.66
	K_{m9}	0.36		$n_{Butyrate}$	0.28
R10#	**V_{max10}**	**0.18**		n_E	−1.1
	K_{m10}	**−1.82**	R16	V_{max16}	0.35
	K_{a10}	**−0.14**		K_{ms16}	0.87
	n_{B10}	**0.69**		K_{a16}	−0.01
	B_{max10}	**0.43**	R17	V_{max17}	0.04
R18	V_{max18}	0.62		K_{m17}	0.46
	K_{m18}	0.14		K_{is17}	0.09

* : The reactions that had negative effects on butanol production.
# : The reactions that had positive effects on butanol production.

n_{B10}, B_{max10} or the decrease in K_{m10} and K_{a10} would result in a higher value of r10, suggesting that increasing the reaction rate of r10 resulted in increased butanol production. Compared with the previous model studied by Shinto et al. [21] with glucose as the sole carbon source, the lactic acid model in this study revealed similar effects of R1 and R10 on endpoint butanol production. Whereas, the effects of conversion from glyceraldehyde 3-P (G3P) to pyruvate (R3) and the conversion from lactate to pyruvate (R17) were not substantial on butanol production in Shinto's model but positive in the present model; and the effect of conversion from biomass to inactive cells (R16) was negative in this model but not substantial in the Shinto's model. Therefore, it can be concluded that with the addition of lactic acid, the significance of some reactions on butanol synthesis were altered. Previous researchers demonstrated that co-factors and energy contents such as NADH, acetyl-CoA, and ATP were needed for the conversion of organic acids to solvents production [3,9,12]. In the present study with lactic acid addition, increasing the rate of R17 and R3 could produce more NADH and ATP (Fig. 1), which can support acids conversions and consequently, butanol synthesis could be enhanced.

Table 4 presents the parameter scan results in the acetic acid model. The reactions that had recognizable impact on endpoint butanol concentration were R1, R3, R6, R10, and R11. Using R11 as an example, the increase in K_{m11A}, K_{m11B}, and K_{is11} caused increasing endpoint butanol concentration, and vice versa for V_{max11}, indicating that reducing r11 resulted in increased butanol production. Therefore, R11 and R1 had negative effects on butanol production. In contrast, R3, R6, and R10 showed positive effects. Compared with the previous model by Shinto et al. [21] with glucose as sole carbon source, the acetic acid model revealed similar effects of R1 and R10 on endpoint butanol synthesis. Whereas, the effect of conversion from G3P to pyruvate (R3) and the conversion from acetate to acetyl-CoA (R6) was not substantial on butanol production in Shinto's model but they were positive in the present model. The conversion from acetate and acetoacetyl-CoA (AACoA) to acetyl-CoA (ACoA) and acetone had negative effects on butanol synthesis in the present model, which was not influential in the Shinto's model.

Parameter scan results in the acetic acid model revealed that: firstly, increasing the reaction rate of G3P to pyruvate can offset the ATP loss caused by lessened metabolic flux to acetate formation. Specifically, since 1 ATP is spent for every acetate consumption, increasing the acetate utilization rate (r6) may increase ATP consumption and result in energy deficiency for ABE production; however, 4 ATP can be generated from the conversion of G3P to pyruvate, therefore, increasing the reaction rate from G3P to pyruvate (r3) could compensate the ATP loss and eventually, butanol synthesis can be improved. Secondly, reducing the glucose uptake rate (r1) may alleviate the 'acid crush' effect. With abundant acids in the medium, no significant switch to the solventogenic phase would be observed (this phenomenon is called "acid crush"), resulting in the termination of ABE fermentation [38]. Whereas, 'acid crush' can be eliminated by reducing the glucose uptake rate and controlling the culture pH [38]. In the present study, when acetic acid was added exogenously into the medium, dampening the glucose uptake rate (R1) can result in higher butanol production, probably by reducing the "acid crash" under high acid concentrations. Thirdly, less acetate conversion to acetone may result in higher butanol production. Gao et al. [9] reported that exogenously added acetate was mainly consumed by the CoA transferase (CoA-T) pathway (R11) rather than by the reverse pathways of acetate formation (R6), leading a higher acetone formation. It was demonstrated in the current research that reducing the reaction rate of R11 and increasing the reaction rate of R6 may increase butanol synthesis, suggesting that less acetate conversion to acetone and more acetate conversion to ACoA could be beneficial for butanol formation.

Comparing with the parameter scan result in the lactic acid model, the acetic acid model revealed similar negative effects of R1 and positive effect of R3 and R10 on endpoint butanol production; indicating

that reducing the glucose uptake rate (r1), enhancing ATP production rate (r3) and butanol formation rate (r10) could result in higher butanol production in despite of acid types. On the other hand, when lactic acid or acetic acid was extrogenously added into the medium, increasing lactic acid or acetic acid consumption rate could enhance butanol synthesis, which suggested that the supplemented acids could shift ABE metabolism to solvents production by acids assimilation. Overall, the model can be used to elucidate the metabolic networks of butanol fermentation by *C. saccharoperbutylacetonicum*.

However, the model was not developed to predict the behavior of *C. saccharoperbutylacetonicum* under a different condition. In other words, the model is different for each different set of data fed to the model. The success of the model is represented by how close the time-course modeling results are to the experimental data, and the usefulness of the model is for understanding the dynamic behavior of metabolites under the specific condition. Additionally, optimal genetic manipulation strategies for higher butanol production by *C. saccharoperbutylacetonicum* can be identified based on the parameter scan results with lactic acid/acetic acid addition. For instance, glucose uptake rate can be reduced by mutating *C. saccharoperbutylacetonicum* into carbohydrate phosphotransferase system (PTS) defective phenotype [39,40] to increase butanol production. In addition, the rate from BCoA to butanol can be enhanced by increasing the expression level of the solventogenic *adhE1* gene encoding butanol dehydrogenase of *C. saccharoperbutylacetonicum*, which may result in elaborated butanol production consequently [41].

4. Conclusions

Kinetic simulation models were developed to accurately predict the dynamic behavior of metabolites in ABE fermentation by *C. saccharoperbutylacetonicum* in lactic acid/acetic acid supplemented media. Increasing lactic acid or acetic acid consumption rate, ATP production rate, butanol formation rate and reducing the glucose uptake rate could enhance butanol synthesis. Overall, the developed models can be used to elucidate the metabolic networks of butanol fermentation with lactic acid/acetic acid addition, and consequently to identify genetic manipulation strategies for higher bio-butanol production in the future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fuel.2018.04.019.

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