Modeling and Simulation of Lactic Acid Fermentation with Inhibition Effects of Lactic Acid and Glucose

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Abstract An unstructured mathematical model for lactic acid fermentation was developed. This model was able to predict the inhibition effects of lactic acid and glucose and was confirmed to be valid with various initial concentrations of lactic acid and glucose. Simulation of energy production was made using this mathematical model, and the relationship between the kinetics of energy metabolism and lactic acid production was also analyzed.

Keywords: modeling, lactic acid, glucose, inhibition, energy balance

INTRODUCTION

Lactic acid has wide applications in food and pharmaceutical industries. Apart from these normal uses, the potential use as the source for polylactate polymers in making biodegradable plastics will make market for lactic acid even bigger than before. About half of the world production of lactic acid is made from fermentation, while the remaining portion is created by chemical synthesis [1].

Modeling and simulation is a powerful tool in evaluation of feasibility, design, and optimization of complex fermentation system. The purpose of this work is to develop a mathematical model for the process analysis, prediction, and optimization for lactic acid fermentation.

MATHEMATICAL MODEL

The specific growth rate, μ, and the specific lactic acid production rate, q_P, were described using equations (1) and (2), respectively:

$$\mu = \frac{\mu_{max} S}{k_m + S} \cdot \frac{k_{iS}}{k_{iS} + S} \cdot \left(1 - \frac{P}{P_{cri}}\right)^n \cdot \left(1 - \frac{X}{X_{max}}\right) \quad (1)$$

$$q_p = \alpha \cdot \mu + \beta \quad (2)$$

The byproducts of oligosaccharides and other kinds of organic acids in addition to lactic acid were found in the fermentation broth by the analysis using HPLC (data not shown). The specific byproduct production rate, q'_P, was found to be growth related:

$$q'_P = \gamma \cdot \mu \quad (3)$$

The specific glucose consumption rate for the byproduct synthesis, q'_S, was as follows:

$$q'_S = \frac{q'_P}{Y'_{P/S}} = \frac{\gamma \cdot \mu}{Y'_{P/S}} = \frac{1}{\delta} \cdot \mu \quad (4)$$

where, $\delta = Y'_{P/S}/\gamma$. The total specific glucose consumption rate, q_S, was:

$$q_S = -\frac{\mu}{Y_{X/S}} - \frac{q_P}{Y_{P/S}} - q'_S = -\left(\frac{1}{Y_{X/S}} + \frac{1}{\delta}\right) \cdot \mu - \frac{q_P}{Y_{P/S}} \quad (5)$$

In cultivation using a complex medium, carbon source was used for energy production and product synthesis, but it was not used for cell synthesis, which was confirmed using *Lactobacillus rhamnosus* [2,3]. In this study, a complex medium was utilized, and therefore, the glucose consumption for cell synthesis in Eq. (5) was omitted.

The mass balances for cell, lactic acid and glucose were expressed using Eqs. (6)-(8):

$$\frac{dX}{dt} = \mu \cdot X \quad (6)$$

$$\frac{dP}{dt} = q_P \cdot X \quad (7)$$

$$\frac{dS}{dt} = -q_S \cdot X \quad (8)$$

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MATERIALS AND METHODS

Strain, Medium and Cultivation Conditions

The strain that was used was *Lactobacillus casei* subsp. *rhamnosus* (ATCC 10863). The medium contained yeast extract 15 g/L, sodium acetate 1 g/L, K_2HPO_4 0.3g/L, KH_2PO_4 0.15g/L, $MgSO_4 \cdot 7H_2O$ 0.15g/L, and a certain amount of glucose. The concentration of glucose was described in later part of this paper. $CaCO_3$ of 4% was added for buffering of the pH.

Experiments were done using 100-mL flasks, which contained 50 mL of the medium with 5% of inoculation. It was cultivated at 42°Cand was shaken at 150 rpm in a rotary shaking incubator of Model KMC-8480 SF (Vision Scientific Co., Korea).

Analytical Procedures

Samples of 1.5 mL for each flask were made and centrifuged, the supernatant was stored in the refrigerator until time of analysis for glucose and lactic acid. Glucose and lactic acid concentrations were measured using glucose oxidase-peroxidase and lactic acid oxidase-peroxidase methods, respectively, with an autoanalyzer (Biochemistry Analyzer 2700; YSI, Ohio, USA). Samples of 0.5 mL for each flask were made for the measurement of cell concentration using a spectrophotometer (Spectronic Instruments Co., USA) at the wave length of 600 nm. The samples were diluted from 5 to 50 times to keep the light absorbance value below 0.7. HCl solution of 0.5 M was used in the dilution of the samples in order to dissolve the solid $CaCO_3$, which, in its solid form, can cause interference in the light absorbance measurement. Low concentration of HCl has shown no effect of cell lysis in the sample.

RESULTS AND DISCUSSION

Effect of Lactic Acid Inhibition

Experiments were done using the initial lactic acid concentrations of 0, 30, 50, 70, and 90 g/L, respectively, and the initial glucose concentration of 90 g/L (Fig. 1). Almost no cell growth, lactic acid production, and glucose consumption occurred when the initial lactic acid concentration was higher than 70 g/L, which was defined as P_{cri}. The cell growth, lactic acid production, and glucose consumption were significantly decreased when the initial lactic acid concentration was 30 g/L or higher.

There are several kinds of models describing lactic acid inhibition, but through preliminary testing of the published models, the term of $(1\text{-}P/P_{cri})$ gave the best fit for the experimental data obtained in various initial lactic acid concentrations. The simulation of the specific growth rate as a function of the lactic acid concentration is shown in Fig. 2. It shows that the specific growth rate is sensitive to lactic acid inhibition even in low lactic acid concentrations. The specific growth rate decreases almost

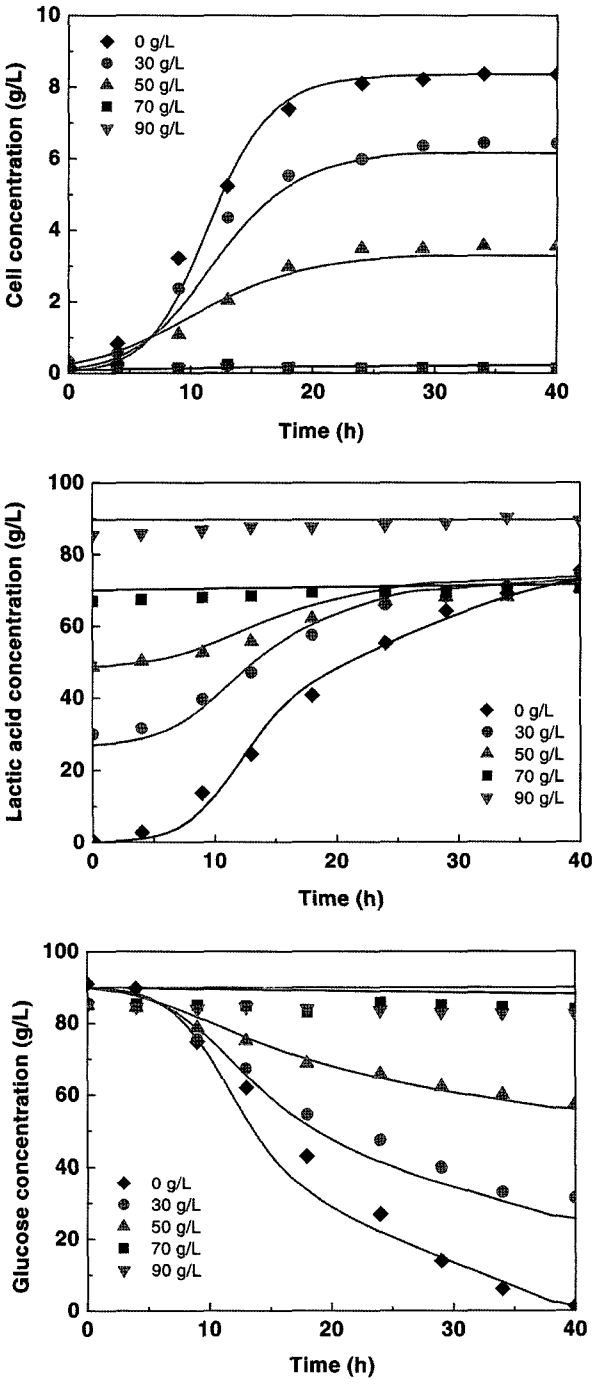

Fig. 1. Lactic acid fermentation under various initial lactic acid concentrations.

linearly with the increase of the lactic acid concentration up to 50 g/L (Fig. 2). When the lactic acid concentration reaches P_{cri} of about 70 g/L, the specific growth rate drops quickly to zero (Fig. 2). The above phenomena are supported by experimental data.

The simulation of lactic acid fermentation under various initial lactic acid concentrations was made using

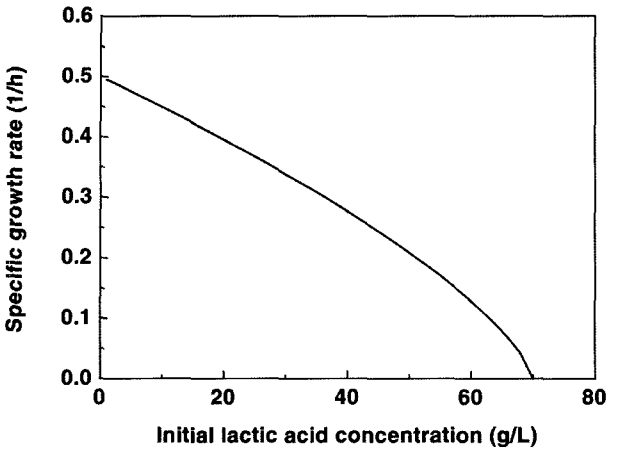

Fig. 2. Simulation of changes of the specific growth rate with lactic acid concentration.

Table 1. Parameter values for lactic acid fermentation

Parameter	Value	Unit
μ_{max}	0.5	1/h
k_m	0.3	g/L
k_{iS}	325	g/L
n	0.7	—
$Y_{P/S}$	1.0	g/g
α	4.5	g/g
β	0.17	g/g/h
δ	0.6	g/g

Eqs. (1), (2) and (5)~(8) (Fig. 1). This model accurately predicted the lactic acid inhibitory effect in a wide range of initial lactic acid concentrations, which confirmed the validity and practicality of this model (Fig. 1). The differential equations of the mathematical model were solved using the fourth order Runge-Kutta method. All calculations were carried out on an IBM compatible computer with Windows Me using self-programmed Visual Basic (Microsoft Co., USA) programs. The model parameters are shown in Table 1.

Lactic acid may decrease the environmental pH, and in turn, the low pH may inhibit cell growth as well as lactic acid production. But, in this experiment, pH, which was buffered by excess calcium carbonate, should not be the main reason for the inhibitory effects. Being the final metabolic product, lactic acid may have feedback inhibitory effects on the metabolism of the cells. Lactic acid exists in the solution in two forms: dissociated (LA^-) and undissociated (LAH). Both forms have an inhibitory effect on the cells, but the undissociated form has a greater inhibitory effect than the dissociated form [4]. This is due to the fact that the undissociated form is more hydrophobic than the dissociated form, and it is easier to be transported across the cell membrane into the cells, resulting in intracellular acidification. The relationship between the concentrations of dissociated and undissociated forms of lactic acid under certain pH can be presented as follows:

$$pK_a = pH - \log\left(\frac{LA^-}{LAH}\right) \tag{9}$$

At a constant pH, the ratio of LAH to LA^- is fixed according to Eq. (9) with pK_a of 3.86 for lactic acid. Therefore, with the increase of the total lactic acid concentration ($LA^- + LAH$) in a pH controlled system, the increase of LAH concentration is much smaller than that in the non pH controlled system (acidic pH). So, it is more reasonable to use the total lactic acid concentration as the model parameter in a pH controlled system than in non-pH controlled system.

Effect of Glucose Inhibition

Initial glucose concentration of 10, 20, 30, and 50 g/L was respectively used in the experiments. The experimental results are shown in Fig. 3. The cell growth in the case of the initial glucose concentration of 50 g/L was a little slower than that of 20 and 30 g/L, showing weak inhibitory effect of glucose. In the case of the initial glucose concentration of 10 g/L, the glucose concentration decreased to very low level before 10 h of cultivation, which was unable to support a high growth rate.

The parameter values of μ_{max}, k_m and k_{iS} were obtained using the linear or nonlinear regression method with the experimental data. The value of $Y_{P/S}$ was obtained by theoretical calculation based on the fact that two lactic acid molecules were produced from one glucose molecule ($Y_{P/S}=2\times M_{Lac}/M_{Gluc}=1$ g/g). The value of δ was calculated from substrate balances. The value of X_{max} was directly measured. All parameter values except the theoretical value of $Y_{P/S}$ were then refined by minimizing the sum of the squared relative errors between the model simulation and experimental data using genetic algorism [5-7]. The data of both experiments of various initial lactic acid concentrations and various initial glucose concentrations were used in the parameter refinement. The parameter refinement was done by computer using the Visual Basic program.

The simulation of the glucose inhibitory effect on the specific growth rate was done using Eq. (10), which was obtained from Eq. (1).

$$\mu = \frac{\mu_{max} S}{k_m + S} \cdot \frac{k_{iS}}{k_{iS} + S} \tag{10}$$

The simulation result showed that glucose inhibitory effect was weak (Fig. 4), which was in agreement with the experimental result. After the specific growth rate reached the highest value of about 0.5 h^{-1} at a glucose concentration of 18 g/L, it decreased slowly with the in-

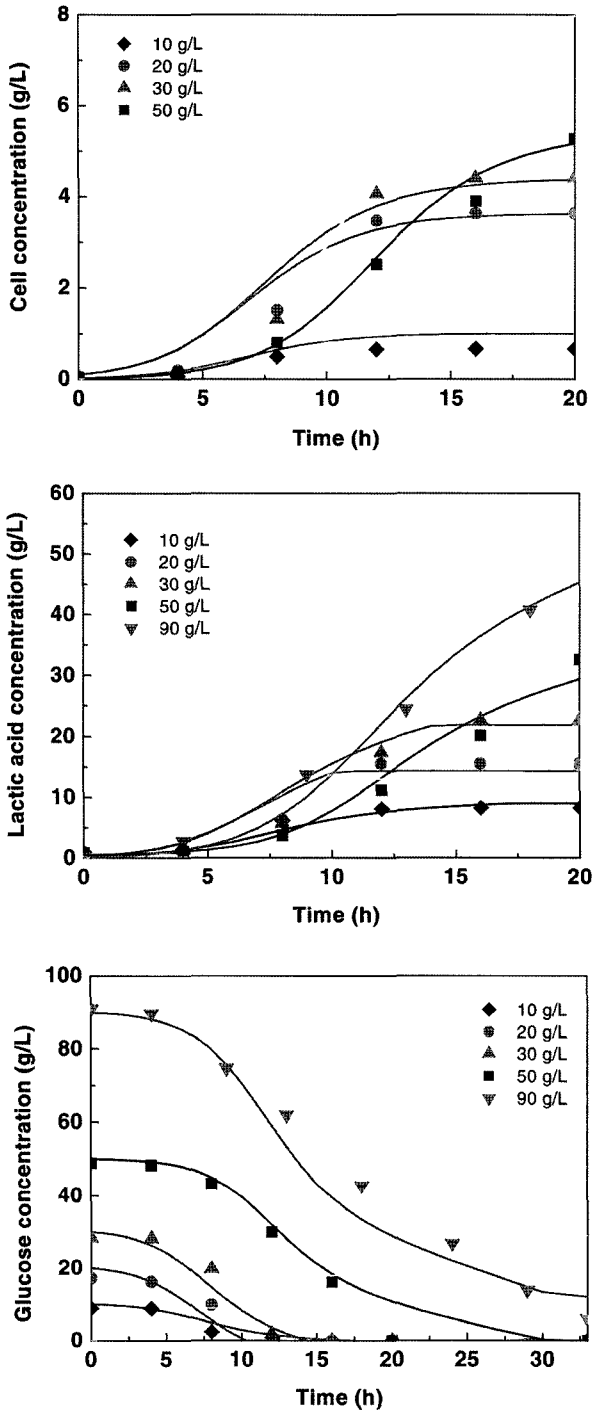

Fig. 3. Lactic acid fermentation under various initial glucose concentrations.

crease of glucose concentration. The simulation of lactic acid fermentation under various initial glucose concentrations was done, and the results are shown in Fig. 3. The model satisfactorily matched the experimental data satisfactorily. The same mathematical model with the same parameter values listed in Table 1 suited the conditions of various initial glucose and initial lactic acid concentrations, which showed the practicality and applicability of the mathematical model.

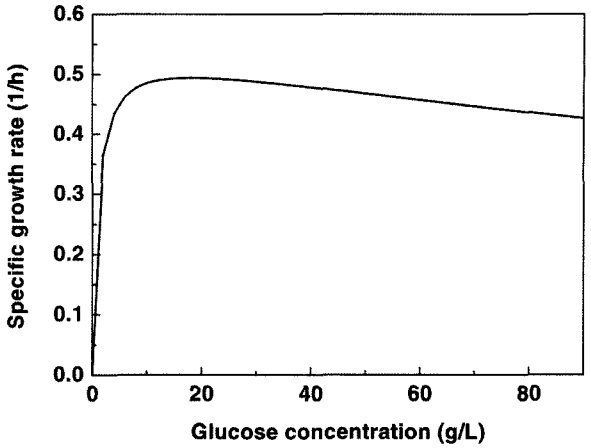

Fig. 4. Simulation of changes of the specific growth rate with glucose concentration.

Most studies on glucose inhibition were done using yeast, and the mechanism was still not clear. Inhibition on the rate of glucose entry into the cell was suggested to be a main reason for this unclear mechanism [8,9]. In this paper, the unstructured model was used, and no mechanism was involved in glucose inhibition, which enabled the model to be simple and practical for applications.

The term of $(1 - X/X_{max})$ from the logistic equation was used in modeling cell growth, which is necessary for modeling the stationary growth phase. Although there were other kinds of equations used in modeling the stationary growth phase [10], this form of the logistic equation was used in this model for the advantage having less model parameters.

Energy Balance in Lactic Acid Fermentation

In anaerobic conditions, *Lactobacillus* metabolizes glucose through the glycolysis pathway, producing two moles of lactic acid and two moles of ATP from one mole of glucose. The ATP produced is used in cell growth and maintenance. The ATP balance could be expressed as follows:

$$q_{ATP} = \frac{2 \cdot q_S}{M_{Gluc}} = \frac{q_P}{M_{ATP}} = \frac{\mu}{Y^*_{ATP}} + m_{ATP} \tag{11}$$

From Eq. (11), Eq. (12) can be obtained:

$$q_P = \frac{M_{Lac}}{Y^*_{ATP}} \cdot \mu + m_{ATP} \cdot M_{Lac} \tag{12}$$

Comparing Eq. (2) with Eq. (12), the following equations can be obtained:

Table 2. The published parameter values in Luedeking-Piret model for lactic acid production

α (g/g)	β (g g^{-1} h^{-1})	Organism
0.392	3.02	*Lactococcus lactis* [13]
2.14	0.526	*Lactobacillus delbrueckii* [14]
3.46	0.223	*Lactobacillus rhamnosus* [15]
4.2 (pH 4)	0.017	*Lactobacillus delbrueckii* [16]
4.9 (pH 5)	0.01	*Lactobacillus delbrueckii* [16]
5.0 (pH 5.5)	0.001	*Lactobacillus delbrueckii* [16]
5.3 (pH 4.2)	0.01	*Lactobacillus bulgaricus* [17]
7.0 (pH 5)	0.01	*Lactobacillus bulgaricus* [17]
6.77	0.285	*Lactobacillus delbrueckii* [18]
8.64	0.321	*Lactobacillus delbrueckii* [19]

$$\alpha = \frac{M_{Lac}}{Y^*_{ATP}} \quad \text{and} \quad \beta = m_{ATP} \cdot M_{Lac} \tag{13}$$

The values of α and β were 4.5 g/g and 0.17 g/g/h, respectively, as shown in Table 1. Therefore, the values of Y^*_{ATP} and m_{ATP} were calculated using Eq. (13), which were 20 g/mol and 2 mmol g^{-1} h^{-1}, respectively. The "apparent" yield of biomass from ATP, $Y_{X/ATP}$, can be obtained by dividing Eq. (12) by μ:

$$\frac{1}{Y_{X/ATP}} = \frac{1}{Y^*_{ATP}} + \frac{m_{ATP}}{\mu} \tag{14}$$

Then, $Y_{X/ATP}$ was calculated to be 18.71 g/mol using equation (14), assuming μ to be μ_{max} of 0.5 h^{-1} as shown in Table 1.

The theoretical calculation of Y^*_{ATP} was about 20 g/mol, but the experimental measurement of Y^*_{ATP} using many kinds of microorganisms and substrates was nearly constant at 10 to 11 g/mol [11], which meant that the calculated result of Y^*_{ATP} in this study was about twice as large than expected. If the value of Y^*_{ATP} were 10 g/mol, the value of α will be 9 g/g according to Eq. (13), which is larger than most of the published values of α (Table 2). The α value of 4.5 g/g in this research was reasonable compared with the published values. The value of Y^*_{ATP} of *Lactobacillus casei* obtained using a complex medium with glucose as the limiting substrate was reported to be 24 g/mol [12], which showed that the result of Y^*_{ATP} of 20 g/mol in this research was still in a reasonable range.

The large value of Y^*_{ATP} was obtained from the production of extra ATP from substrates other than glucose. Fifteen of yeast extract was contained in the cultivation medium, which was possibly used in ATP production. Another explanation could be that the synthesis of the byproduct of acetic acid could produce extra ATP and increase the ATP yield.

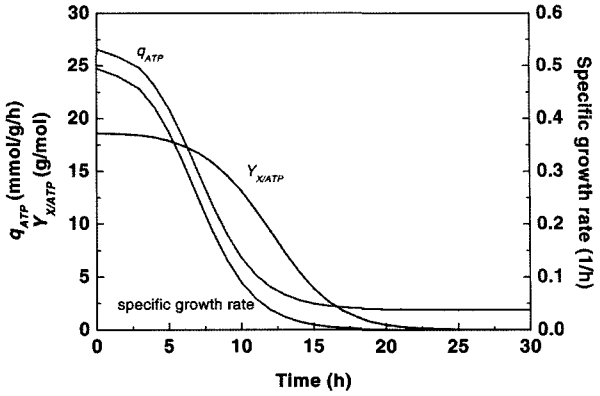

Fig. 5. Simulation of the specific growth rate, specific ATP production rate, and the apparent cell yield from ATP.

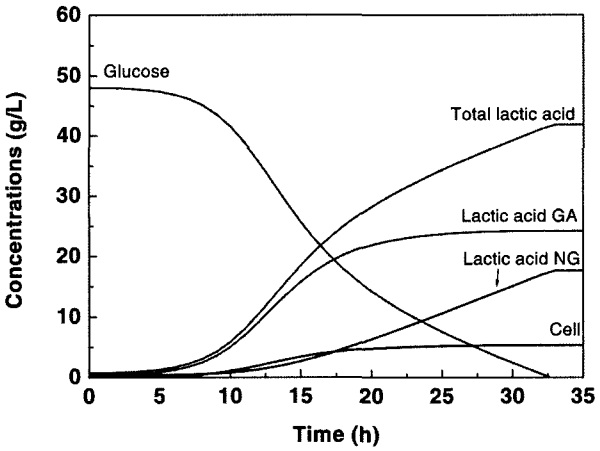

Fig. 6. Simulation for growth associated and non-growth associated lactic acid productions.

Because lactic acid production is related to cell growth and energy production, the simulation of μ, q_{ATP}, and $Y_{X/ATP}$ ($=\mu/q_{ATP}$) under initial glucose concentration of 10 g/L was made (Fig. 5). $Y_{X/ATP}$ decreased after the cells entered into the decreased and stationary growth phases (Figs. 3 and 5). The decrease of $Y_{X/ATP}$ was resulted from the decrease of μ and the increase of the portion of ATP consumed on maintenance in the decelerating and stationary growth phases, according to Eq. (14). The m_{ATP} is related to the non-growth associated (NG) lactic acid production according to Eq. (13). The growth associated (GA) and non-growth associated lactic acid production were simulated using the following equations and was shown in Fig. 6.

$$\frac{dP_{GA}}{dt} = \alpha \cdot \mu \cdot X \quad \text{and} \quad \frac{dP_{NG}}{dt} = \beta \cdot X \tag{15}$$

The simulation showed that the non-growth associated lactic acid production was lower than that of the growth associated due to the small value of m_{ATP}. If q_{ATP}, which

was proportional to q_P, were larger than m_{ATP} in the late production phase, the energy charge would increase and negatively feedback control q_{ATP}, q_S and finally decrease q_P. Cell growth phase is short in batch culture. Although fed-batch culture can prolong the cell growth phase to some extent, it still has limitations. Therefore, uncoupling of the energy metabolism with cell growth, in order to increase the value of m_{ATP} or β, should be a possible way to prolong the lactic acid production phase. A strain of this kinetic type with a low value of growth associated term (α) and a high value of non-growth associated term (β) did exist. The α and β values of *Lactococcus lactis* NZ133 were reported to be 0.392 and 3.02, respectively [13]. This kind of strain also has an advantage in the cultivation systems with a low cell growth rate, such as the immobilized cell or cell retention cultivation systems.

CONCLUSION

The mathematical model developed in this paper is suitable for a wide range of initial lactic acid and glucose concentrations, and it is also simple and practical in the analysis of the fermentation process. The model was able to predict the inhibition effect of lactic acid. The model was confirmed to be valid with various initial concentrations of lactic acid and glucose. Simulation of lactic acid fermentation was made using the mathematical model, and the relationship between the kinetics of energy metabolism and lactic acid production. The mathematical model and simulation is useful in characteristics investigation, analysis as well as optimization of lactic acid fermentation.

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NOMENCLATURE

k_m	glucose saturation constant in Monod equation (g/L)
k_{iS}	glucose inhibition constant (g/L)
LA^-	dissociated lactic acid concentration (M)
LAH	undissociated lactic acid concentration (M)
m_{ATP}	the rate of ATP consumption for maintenance energy (mol g^{-1} h^{-1})
M_{Gluc}	molecular weight of glucose (g/mol)
M_{Lac}	molecular weight of lactic acid (g/mol)
n	constant (–)
P	lactic acid concentration (g/L)
pK_a	dissociation constant for lactic acid (–)

P_{cri} critical concentration of lactic acid for growth inhibition (g/L)
q_{ATP} specific ATP production rate (mol g^{-1} h^{-1})
q_S specific glucose consumption rate (g/g/h)
q_P specific lactic acid production rate (g/g/h)
q'_P specific byproduct production rate
q'_S specific glucose consumption rate for the by product synthesis
S glucose concentration (g/L)
X cell concentration (g/L)
X_{max} experimental value of maximum cell concentration (g/L)
$Y_{X/ATP}$ "apparent" cell yield from ATP (g/mol)
Y^*_{ATP} maximum cell yield from ATP (g/mol)
$Y_{P/S}$ lactic acid yield from glucose (g/g)
$Y'_{P/S}$ byproduct yield from glucose (g/g)
$Y_{X/S}$ cell yield from glucose (g/g)

Greek symbols

α growth associated constant in Luedeking-Piret model (g/g)
β non-growth associated constant in Luedeking-Piret model (g g^{-1} h^{-1})
δ constant (g/g)
γ constant (g/g)
μ specific growth rate (1/h)
μ_{max} maximum specific growth rate (1/h)

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