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Kinetic modeling of lactic acid production from molasses using *Enterococcus faecalis* RKY1

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Abstract

A kinetic model has been developed for batch fermentation of cane-sugar molasses for lactic acid production by *Enterococcus faecalis* RKY1. Parameters of the kinetic model have been determined based on experimental data by using genetic algorithm. The values of key kinetic constants are maximum specific growth rate (μ_{max}), 1.6 h^{-1}, growth-associated constant for lactic acid production (α), 0.26 g g^{-1}; maximum specific lactic acid production rate ($q_{p,max}$), 3 g g^{-1} h^{-1}; maximum specific sugar utilization rate ($q_{s,max}$), 3.33 g g^{-1} h^{-1}. It has been observed that the growth of biomass and lactic acid production are affected by lactic acid inhibition and it has been taken into account with an exponential term. However, effects of substrate limitation and substrate inhibition have been found to be relatively small. When compared with batch experimental data, the model provides good predictions for growth of biomass, sugar consumption and lactic acid production profiles on media with initial molasses concentration ranging from 130 to 333 g/l with few exceptions. Effect of pH on kinetic parameters has also been studied. It has been found that the growth of biomass and lactic acid production are strongly influenced by pH. Optimum pH is found to be 7.

Keywords: *Enterococcus faecalis*; Fermentation; Growth kinetics; Lactic acid; Modeling; Molasses

1. Introduction

Lactic acid and its derivatives are largely used in food, pharmaceutical, leather and textile industry or for the production of base chemicals. The continuous increase in its demand has been due to its increasing applications in the preparation of biodegradable polymers like polylactic acid (PLA), medical sutures, and green solvents [1,2]. PLA could be a good substitute for synthetic plastic derived from petroleum feedstock. About 90% of the lactic acid produced world wide every year is made by lactic acid bacterial fermentation and the rest is produced synthetically. The chemical synthesis of lactic acid always results in racemic mixture of lactic acid, which is a major disadvantage. Fermentative production of lactic acid offers the advantages in both utilization of renewable carbohydrates and production of optically pure L- or D-lactic acid depending on the strain selected. Physical properties of PLA are strongly influenced by the isomeric composition of lactic acid [3,4]. Recently, the conversion of renewable raw materials into various chemicals has become a major subject of research and development around the world. The reasons are limited fossil resources of the earth, need to control green house gas emissions and great advances in fundamental research into biochemical and chemical processing, into biotechnological techniques and into the genetic construction of high-performance strains with novel properties, making the biotechnological production of special chemicals more efficient than chemical synthesis. Various renewable resources like cellulose, starch, wheat bran, cheese whey and molasses can be used as substrates in the fermentative production of L-(+)-lactic acid. Renewable resources do not give any net contribution of CO_2 to atmosphere as do the fossil fuel based resources.

Manufacturing cost of lactic acid is greatly influenced by the cost of raw materials. Use of pure substrates like glucose, lactose is not economical due to their higher prices. Natural polysaccharides like starch and cellulose require liquefaction and saccharification processes in order to release fermentative carbohydrates. Waste products from agriculture or forestry have a good potential to be raw materials for lactic acid production. However, the manufacturing cost of lactic acid must be significantly reduced if it could be possible to use a waste product such as molasses as a raw material for the production of lactic

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Nomenclature

K_d	death coefficient (h^{-1})
K_{ip}	substrate inhibition constant for lactic acid production (g/l)
K_{is}	substrate inhibition constant for sugar consumption (g/l)
K_{ix}	substrate inhibition constant for growth of biomass (g/l)
K_{pp}	product inhibition constant for lactic acid production (g/l)
K_{ps}	product inhibition constant for sugar consumption (g/l)
K_{px}	product inhibition constant for growth of biomass (g/l)
K_{sp}	substrate limitation constant for lactic acid production (g/l)
K_{ss}	substrate limitation constant for sugar consumption (g/l)
K_{sx}	substrate limitation constant for growth of biomass (g/l)
P	lactic acid concentration (g/l)
P_{is}	threshold lactic acid concentration for substrate consumption (or lactic acid production) (g/l)
P_{ix}	threshold lactic acid concentration for growth of biomass (g/l)
P_{ms}	maximum lactic acid concentration for substrate consumption (or lactic acid production) (g/l)
P_{mx}	maximum lactic acid concentration for growth of biomass (g/l)
Q	objective function
$q_{p,max}$	maximum specific lactic acid production rate (g/(g h))
$q_{s,max}$	maximum specific sugar utilization rate (g/(g h))
R^2	correlation coefficient
S	sugar concentration (g/l)
S_0	initial sugar concentration (g/l)
t	fermentation time (h)
W_i	weighing factor (l^2/g^2)
X	biomass concentration (g/l)
$Y_{p/x}$	lactic acid yield on growth of biomass
$Y_{p/s}$	lactic acid yield on sugar consumption
$Y_{x/s}$	biomass yield on sugar consumption

Greek symbols

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

acid [5]. A comparative study of lactic acid production processes using molasses as a cheap substrate is summarized in Table 1 with essential parameters and operating conditions. A review of factors affecting lactic acid production from renewable resources has been also published [12]. It has also been observed

Table 1
Comparison of lactic acid production processes using molasses as a substrate

Substrate	Organism	Nutrient composition (g/l)	Fermentation type	pH	Temperature (°C)	Substrate (g/l)	LA (g/l)	$Y_{LA/tot}$ (g/g)	Q_v (g/l h)	Authors; [Ref.]
Cane-sugar molasses	*Enterococcus faecalis* RKY1	Yeast 15	Smf, Batch	7	38	102	95.7	0.949	4.0	Wee et al.; [5]
Cane-sugar (hydrolyzed)	*Lactobacillus delbrueckii* NCIM 2365 mutant Uc-3	Yeast 10	Smf, Batch	7	42	200 (sugar)	120	0.956	3.3	Patil et al.; [6]
Beet-sugar molasses (pasteurized)	*Rhizopus oryzae*	Yeast 10 $(NH_4)_2SO_4$ 2, KH_2PO_4 0.65, $MgSO_4{\cdot}7H_2O$ 0.25	Smf, Batch	7	30	400	49	–	0.29	Bulut et al.; [7]
Beet molasses	*Lactococcus lactis*	Ammonium citrate 0.2, Sodium acetate 0.5, KH_2PO_4 1, $MgSO_4$ 0.1, $MnSO_4{\cdot}4H_2O$ 0.01, $Na_2HPO_4{\cdot}12H_2O$ 4	Smf, Cont.	7.5	37	78 (sugar)	40	0.59	10.6	Ohashi et al.; [8]
Beet molasses	*Lactobacillus delbrueckii* NCIMB 8130	Yeast 50	Smf, Batch	7	45	100 (sugar)	90	0.967	3.67	Kotzamanidis et al.; [9]
Molasses (hydrolyzed)	*Lactobacillus delbrueckii*	–	Smf, Batch	6.2	42	20 (sugar)	16	0.83	–	Aksu et al.; [10]
Beet molasses (pretreated)	*Lactobacillus delbrueckii* IFO 3202	Yeast 10	Smf, Batch	6	45	782 (sugar)	–	0.771	4.83	Göksungur et al.; [11]

Abbreviations: LA, lactic acid; $Y_{LA/tot}$, yield of g LA per g substrate consumed; Q_v, maximum volumetric LA productivity in g LA per l per h; Smf, submerged fermentation; cont., continuous.

from this comparative study that the amount of experimental work done for lactic acid production from cheap substrates is quite high, while modeling and optimization studies for lactic acid production are very few. Mathematical models have been used to predict influence of fermentation operating parameters on cell growth rate, cell concentration, substrate utilization rate and lactic acid production rate. These models are very useful for design, optimization and control of biological processes. However, satisfactory models are rarely available in practice.

In this study, it is proposed to develop a kinetic model for the production of lactic acid from cane-sugar molasses by bacterium *Enterococcus faecalis* RKY1. Effect of pH on kinetic model parameters has also been studied.

2. Kinetic model

Various structured and unstructured kinetic models have been reported in the scientific literature for fermentative production of lactic acid by bacteria or fungi. Unstructured models are much easier to use, and have proven to accurately describe lactic acid fermentation in a wide range of experimental conditions and media. A good model must, however, take into account the effects of substrate limitation, substrate inhibition and product inhibition as well as maintenance energy and cell death on the cell growth and metabolism [13]. However, most of the models developed are mainly for pure substrates. Several kinetic studies are also reported for substrates like, beet molasses, starch, wheat flour, cheese whey for fermentative lactic acid production [13–17]. Models considering the various operating parameters like temperature and pH dependence of lactic acid production have been also reported [14,15,18–20].

Experimental data available in the literature [5] for lactic acid production using *Enterococcus faecalis* RKY1 bacterium has been used for kinetic model development. Various kinetic models available in the literature have been explored in search of the model which can give the best fit to the experimental data. Available models for substrate and product inhibition have also been tried to find out the most suitable terms. The model which can give minimum value of correlation coefficient, R^2 (often referred to as the goodness of fit) and minimum deviation between experimental data and predicted results has been selected.

In the present studies, it has been observed that the kinetic model used for the production of lactic acid by *Lactococcus lactis* NZ133 [21] may be used after suitable modifications.

In this model, Boonmee et al. [21] have taken following assumptions:

(i) Monod kinetic model for specific growth rate is applicable.
(ii) Substrate limitation, substrate inhibition and product inhibition, have been considered. It is assumed that the substrate limitation follows Monod model, while substrate inhibition follows a linear non-competitive kinetic model.
(iii) Different substrate inhibition constants and substrate limitation constants have been used for growth and lactic acid production.
(iv) Luedeking–Piret model [22] for growth-associated and non-growth-associated lactic acid production is used.
(v) Product inhibition is occurring in a linear manner with an initial value (P_i) being a threshold lactic acid concentration before any inhibition occurs and a value P_m being the maximum inhibitory value.
(vi) Different values of product inhibition constants for growth and lactic acid production have been taken.

The kinetic model used by them is given below

$$\frac{dX}{dt} = \frac{\mu_{max} S K_{ix}}{(K_{sx} + S)(K_{ix} + S)} \left(1 - \frac{P - P_{ix}}{P_{mx} - P_{ix}}\right) X \quad (1)$$

$$\frac{dS}{dt} = q_{s,max} \frac{S K_{is}}{(K_{ss} + S)(K_{is} + S)} \left(1 - \frac{P - P_{is}}{P_{ms} - P_{is}}\right) X \quad (2)$$

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + q_{p,max} \frac{S K_{ip}}{(K_{sp} + S)(K_{ip} + S)} \times \left(1 - \frac{P - P_{is}}{P_{ms} - P_{is}}\right) X \quad (3)$$

In the present study, above model has been modified in respect of following aspects:

(i) The cell death coefficient (K_d) is included to take into account viability loss.
(ii) Product inhibition occurs in an exponential manner with a product inhibition constant (K_p) as it has been found to give better results as compared to linear product inhibition term used by Boonmee et al. [21]. Similar exponential product inhibition terms have been also used previously for lactic acid production [17,23].

The modified model for lactic acid production by *Enterococcus faecalis* RKY1 is as follows:

Specific growth rate

$$\mu = \frac{\mu_{max} S K_{ix}}{(K_{sx} + S)(K_{ix} + S)} e^{-P/K_{px}} \quad (4)$$

The rate of biomass production

$$\frac{dX}{dt} = (\mu - K_d) X \quad (5)$$

The rate of sugar consumption

$$\frac{dS}{dt} = q_{s,max} \frac{S K_{is}}{(K_{ss} + S)(K_{is} + S)} e^{-P/K_{ps}} X \quad (6)$$

The rate of lactic acid production

$$\frac{dP}{dt} = \alpha \frac{dX}{dt} + q_{p,max} \frac{S K_{ip}}{(K_{sp} + S)(K_{ip} + S)} e^{-P/K_{pp}} X \quad (7)$$

Several usual assumptions have also been made for the simplification of this model.

(i) When sugar uptake is affected by low sugar concentration, lactic acid production rate is affected in the same way, i.e. $K_{ss} = K_{sp}$.
(ii) When sugar uptake is inhibited by sugar at high concentrations, lactic acid production is inhibited in the same manner, i.e. $K_{is} = K_{ip}$.
(iii) Lactic acid inhibit sugar consumption and lactic acid production in the same fashion, i.e. $K_{ps} = K_{pp}$.

3. Experimental data

The required experimental data are taken from the work of Wee et al. [5], with permission from authors. They carried out batch fermentation experiments using different concentrations 130–333 g/l of cane-sugar molasses (equivalent to 68–170 g/l of sugar) as a substrate at pH 7 and temperature 38 °C. Molasses was used without any pretreatment to make the production process commercially more viable. Fifteen grams per litres yeast was added to the fermentation medium as a source of nitrogen. They used the strain *Enterococcus faecalis* RKY1, an efficient lactic acid producing bacterium, which could metabolize glucose into L-(+)-lactic acid thorough homolactic fermentation pathway with high yield and high productivity. Lactic acid yield obtained based on sugar consumption ($Y_{p/s}$) varies from 0.90 to 0.99 for different initial sugar concentrations. Similarly $Y_{x/s}$ varies from 0 to 0.37. Maximum productivity obtained is 4.3 g/(l h). It was also observed that lactic acid production is growth-associated as well as non-growth-associated and there is an apparent exponential growth phase for biomass.

Data for variation of concentrations of biomass (X), sugar (S) and lactic acid (P) with time for different initial concentrations of molasses (130–333 g/l) and for variation of X, S and P with pH (5–9) of fermentation medium for molasses concentration of 200 g/l have been used in the development of kinetic model and in studies on the variation of pH.

4. Estimation of kinetic parameters

In order to estimate kinetic parameters, it is required to search the values of these parameters for which predicted values of X, S and P are closed to the experimental values, X_{exp}, S_{exp} and P_{exp} within acceptable tolerance at all times during fermentation process. Accordingly the following objective function is formulated.

$$Q = W_X^2 \sum_{\text{All times}} (X - X_{exp})^2 + W_S^2 \sum_{\text{All times}} (S - S_{exp})^2 + W_P^2 \sum_{\text{All times}} (P - P_{exp})^2 \quad (8)$$

W_X, W_S and W_P are weighing factors, which were assumed as the reciprocal of the maximum concentration for respective components, *viz*. X, S and P.

The problem of estimating kinetic parameters can be stated as follows:

Determine kinetic parameters which minimize Q.

Thus, one requires the method to solve model equations for a given set of kinetic parameters, and an appropriate optimization technique. Model differential equations are solved using ODE15s solver of MATLAB to obtain values of X, S and P. This solver uses Gear's method which is quite robust in solving stiff and non-stiff differential equations. The program used to solve model differential equations is written in MATLAB software (student version 7, MathWorks). Genetic algorithm has been used to minimize objective function (Q) given by Eq. (8). Further, the genetic algorithm toolbox of MATLAB was used. Genetic algorithm involves random search over the control variable domain after the problem has been appropriately coded, usually in terms of strings or chromosomes comprising binary numbers. The best few solutions evolve over generations using techniques that mimic genetic evolution. It is superior to other classical numerical approaches as it can handle a variety of parameter optimization problems, including those involving multi-modal, non-linear or discontinuous functions. The advantage of GA lies in the fact that it works without requiring much information about the system, in contrast to the traditional techniques, which need gradients, initial guesses, etc. A discussion of the technique, as well as its major applications, is available in the literature [24,25].

5. Results and discussion

5.1. Estimation of parameters

The experimental data obtained from batch fermentation studies [5] with initial sugar concentrations of 68, 102, 136 and 170 g/l were used for the determination of kinetic parameters. The values, which resulted in the minimum value of the objective function (Q) were determined and are listed in Table 2. The value of maximum specific growth rate (μ_{max}) obtained from the parameter estimation is 1.6 h^{-1}. The same value of μ_{max} has previously been reported for *Streptococcus faecalis* (similar to *Enterococcus faecalis*) grown on glucose [26]. The substrate limitation constants K_{sx} and K_{ss} (Monod or saturation constants) obtained for biomass production and for substrate consumption (also for lactic acid production) are 0.89 and 0.1 g/l, respectively, which are of the same order of magnitude to those previously reported for *Streptococcus faecalis*, 0.22 g/l [26]. The substrate inhibition constant (K_{ix}) found for growth of biomass is 167 g/l, suggesting that the significant inhibition would occur for the sugar concentration higher than 167 g/l. This implies that the effect of substrate inhibition on growth of biomass is significantly less except for the last experimental data set corresponding to initial sugar concentration of 170 g/l. The value of substrate inhibition constant (K_{ss}) found for substrate consumption (or for lactic acid production) is 303.17 g/l. This high value of K_{ss} suggests that the substrate inhibition does not affect lactic acid production and the sugar consumption significantly. Similar conclusions in respect of substrate inhibition have also been reported [21]. In order to take into account the decrease in the biomass concentration towards the end of the some batch fermentations, a cell death coefficient (K_d) has been introduced in the equation for biomass growth (Eq. (5)). However, the esti-

Table 2
Optimum parameter values for kinetic model of *Enterococcus faecalis* RKY1 and their comparison

Kinetic parameter	This work	*Lactococcus lactis* NZ133 grown on lactose. Boonmee et al. [21]	*Streptococcus faecalis* grown on glucose, Ohara et al. [26]
Biomass production model			
μ_{max} (h^{-1})	1.6	1.1	1.6
K_{ix} (g/l)	167.46	304	–
K_{sx} (g/l)	0.89	1.32	0.22
K_{px} (g/l)	17.07	–	9.5
K_d (h^{-1})	0.00318	–	–
Sugar utilization model			
K_{is} (g/l)	303.17	140	–
K_{ss} (g/l)	0.1	2.05	0.22
K_{ps} (g/l)	29.17	–	24.13
$q_{s,max}$ (g/(g h))	3.33	3.42	4.74
Lactic acid production model			
K_{ip} (g/l)	303.17	140	–
K_{pp} (g/l)	29.17	–	12.54
$q_{p,max}$ (g/(g h))	3.00	3.02	6.06
α (g/g)	0.26	0.39	–

mated value of K_d is 0.00318 h^{-1}; this suggests a relatively small effect of cell death rate. The values of product inhibition constants on biomass growth (K_{px}), and on lactic acid production and sugar consumption (K_{pp} and K_{ps}) are found to be 17.074 and 29.1664 g/l, respectively. Product inhibition is caused by both undissociated and dissociated lactic acid. Lactic acid is almost completely dissociated at pH 6 or higher. As the experimental data have been taken at pH 7, therefore, product inhibition shall be due to completely dissociated lactic acid. The inhibition is also evident from significant K_{px} and K_{pp} values (17.074 and 29.1664 g/l). For sugar consumption, the maximum sugar uptake rate ($q_{s,max}$) is estimated to be 3.33 g/(g h). Maintenance metabolism on sugar is observed to be low and it is also evident from the low value of death coefficient. The values of growth-associated term (α) and non-growth-associated term ($q_{p,max}$) in lactic acid production equation (Eq. (7)) are 0.26 and 3.0 g/(g h), respectively. It has been observed that lactic acid production is growth-associated and non-growth-associated both. Rate of production of lactic acid is high during growth-associated phase but amount of production taking place during stationary phase is more. Growth-associated parameter (α) is identical to the total lactic acid yield on biomass ($Y_{p/x}$), whose value is estimated to be 0.26. However, it was not possible to include other yields ($Y_{x/s}$ and $Y_{p/s}$) in the kinetic model due to nutrient effects of complex organic components in the medium (viz. yeast extract, nutrients present in the molasses). Besides, they do not remain constant during the course of fermentation.

5.2. Comparison of parameter values

Kinetic parameter values obtained in this study are compared with the kinetic parameter values reported for lactic acid production from lactose using *Lactococcus lactis* NZ133 [21] which uses a somewhat similar model with different product inhibition terms and no death coefficient, and for lactic acid production from glucose using *Streptococcus faecalis* [26] which uses a similar bacteria in their study. They are listed in Table 2. The difference in the kinetic model parameters obtained between these studies may be attributed due to the use of different substrates, different strains of bacteria, impurities in the untreated molasses, use of different kinetic models, etc.

5.3. Comparison of predicted and experimental data

The model developed in the present study describes satisfactorily kinetics of *Enterococcus faecalis* RKY1. The comparison of experimental data and predicted results are shown in Figs. 1–3. The value of objective function (Q) and correlation coefficient (R^2) were used to determine goodness of fit of the model to experimental data. The magnitude of the value of Q gives idea

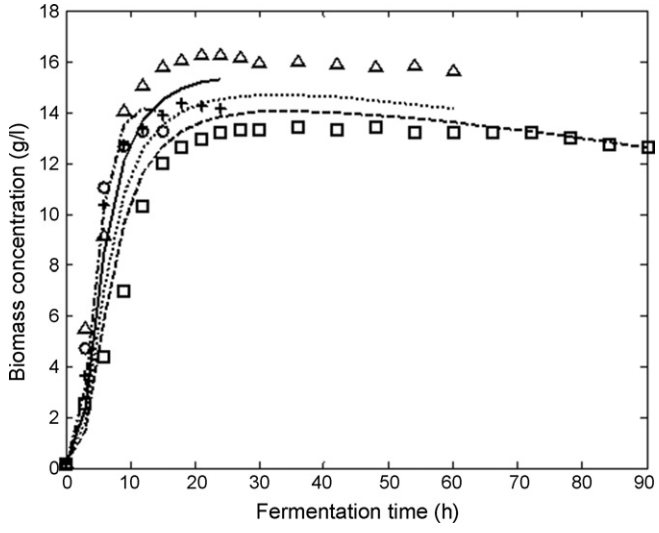

Fig. 1. Experimental data (points) and simulation (lines) of biomass concentration of batch culture of *Enterococcus faecalis* RKY1: 68 g/l sugar (◯), 102 g/l sugar (+), 136 g/l sugar (△), 170 g/l sugar (□).

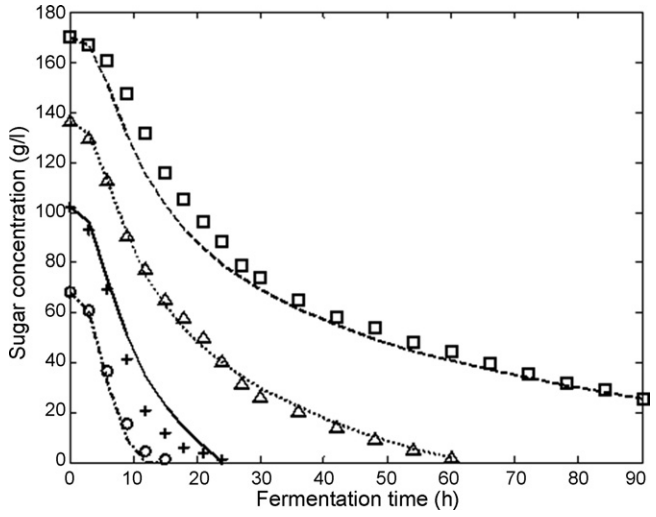

Fig. 2. Experimental data (points) and simulation (lines) of sugar concentration of batch culture of *Enterococcus faecalis* RKY1: 68 g/l sugar (◯), 102 g/l sugar (+), 136 g/l sugar (△), 170 g/l sugar (□).

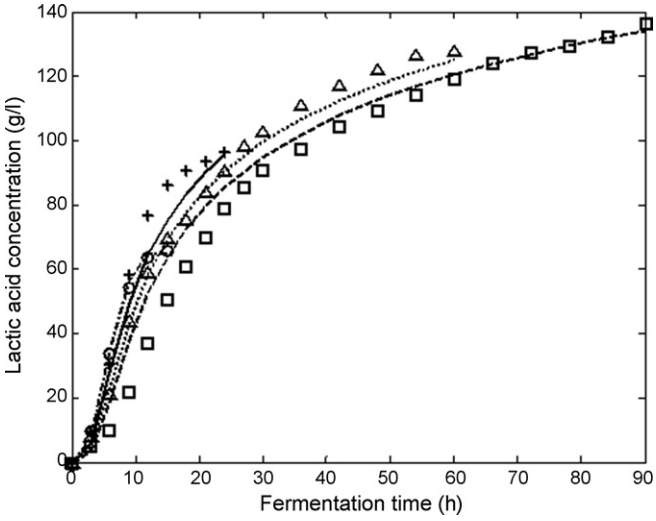

Fig. 3. Experimental data (points) and simulation (lines) of lactic acid concentration of batch culture of *Enterococcus faecalis* RKY1: 68 g/l sugar (◯), 102 g/l sugar (+), 136 g/l sugar (△), 170 g/l sugar (□).

about discrepancy between experimental results and predicted results. Table 3 provides values of Q and R^2 for each experimental data set. The cumulative value of the objective function (total of the values of Q for all data sets) and an average correlation coefficient (R^2) were found to be 0.637 and 0.9624, respectively. This confirms that the model is capable of predicting the experimental results with a good amount of accuracy. However, some discrepancy has been observed between predicted results and the experimental data for biomass concentrations corresponding to initial sugar concentration of 136 g/l.

Table 3
Objective function (Q) and R^2 values of at different initial sugar concentrations

S_0 (g/l)	Q	R^2
68	0.0506	0.9806
102	0.1380	0.9612
136	0.2544	0.9421
170	0.1941	0.9655

Table 4
Kinetic model parameters at different pH

Kinetic parameter	pH				
	5	6	7	8	9
μ_{max}	0.79	1.60	1.60	1.51	0.40
K_{ix}	167.46	167.46	167.46	167.46	167.46
K_{sx}	0.89	0.89	0.89	0.89	0.89
K_{is} (=K_{ip})	303.17	303.17	303.17	303.17	303.17
K_{ss} (=K_{sp})	0.10	0.10	0.10	0.10	0.10
K_{px}	3.79	14.29	17.50	16.67	19.67
K_{ps} (=K_{pp})	6.31	28.00	38.89	36.84	30.56
K_d	0.0035	0.003	0.003	0.003	0.0055
$q_{s,max}$	1.76	2.62	3.00	2.57	1.01
$q_{p,max}$	1.48	2.43	2.67	2.29	0.86
α	0.79	0.15	0.20	0.26	0.71

5.4. Influence of pH on kinetics

The influence of change in pH of the batch fermentation experiments on kinetic model parameters was also studied in this work. Wee et al. [5] have carried out batch fermentation experiments at controlled pH values of 5–9 to observe the effect of change in pH of the fermentation medium keeping other variables and parameters constant. These experimental data were used to check variation of kinetic model parameters with pH. It has been assumed that the substrate limitation constants and substrate inhibition constants do not vary with pH. The values of kinetic parameters obtained for different pH values are listed in Table 4. Variation of μ_{max} with pH shows generally observed dependence of growth rate on kinetics. However, the variation in its value is small between pH 6–8. Effect of the product inhibition (K_{px} and K_{pp}) was observed to be significantly higher at lower pH values. The death coefficient (K_d) increases at extreme values of pH showing that the bacteria favors neutral pH range. Maximum specific sugar utilization rate ($q_{s,max}$) and maximum specific lactic acid production rate ($q_{p,max}$) decreased at extreme values of pH and this can be interpreted as a decrease in the production of lactic acid at extreme pH values. The opposite behavior has been observed for the growth-associated lactic acid production, as the value of α is lower in the neutral pH range. Assumption of total lactic acid term instead of separate protonated form of lactic acid and undissociated form of lactic acid in inhibition equation could be the reason for which few parameters have values which differ erratically according to the pH.

Ohara et al. [20] have also studied the effect of pH in kinetic model parameters for fermentation using *Streptococcus faecalis* and observed similar trends in the variation of the values of μ_{max}, K_d, $q_{s,max}$ and $q_{p,max}$ [26]. They have found optimum pH for fermentation to be 7 which has also been observed in the present work.

The comparison of experimental data and simulated results for pH values ranging from 5 to 9 is shown in Figs. 4–6.

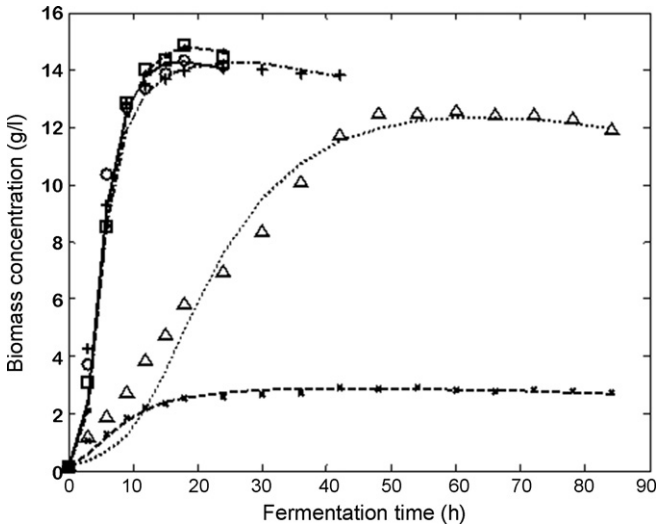

Fig. 4. Experimental data (points) and simulation (lines) of biomass concentration of batch culture of *Enterococcus faecalis* RKY1: pH: 5 (×), pH: 6 (+), pH: 7 (◯), pH: 8 (□), pH: 9 (△).

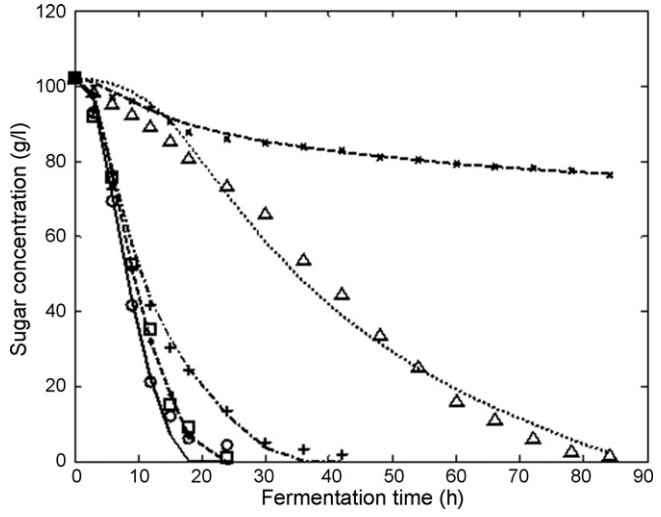

Fig. 5. Experimental data (points) and simulation (lines) of sugar concentration of batch culture of *Enterococcus faecalis* RKY1: pH: 5 (×), pH: 6 (+), pH: 7 (◯), pH: 8 (□), pH: 9 (△).

The values of objective function (Q) and correlation coefficient (R^2) for each experimental data set are reported in Table 5. It can be observed from Figs. 4–6 that the experimental results and the predicted data for various pH values are close to each other.

Table 5
Objective function (Q) and R^2 values at different pH

pH	Q	R^2
5	0.0590	0.9827
6	0.0415	0.9884
7	0.0312	0.9897
8	0.0152	0.9952
9	0.1466	0.9786

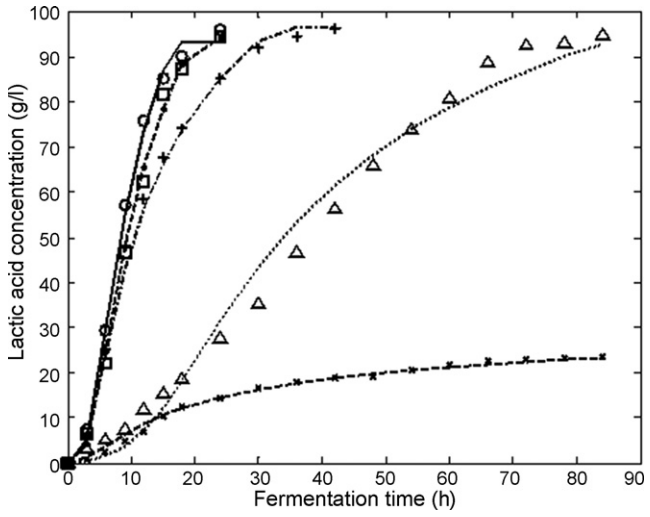

Fig. 6. Experimental data (points) and simulation (lines) of lactic acid concentration of batch culture of *Enterococcus faecalis* RKY1: pH: 5 (×), pH: 6 (+), pH: 7 (◯), pH: 8 (□), pH: 9 (△).

6. Conclusion

A rigorous kinetic model for the production of lactic acid from cane-sugar molasses by using *Enterococcus faecalis* RKY1 has been developed. The model takes into account the substrate limitation and inhibition, product inhibition, growth- and non-growth-associated lactic acid production and cell death rate. It provides specific understanding of biomass growth, sugar consumption and lactic acid production in batch fermentation. Further, the effect of pH on kinetic model parameters has also been determined. It may be concluded that the parameters, namely, μ_{max}, K_d, K_{px} K_{ps}, α, $q_{s,max}$ and $q_{p,max}$ are sensitive to change in pH, and consequently extreme values of pH may adversely effect production of lactic acid. Optimum pH is found to be 7.

This model may be used for the analysis, design and control of batch and continuous fermentation units for lactic acid production by using cheap substrates such as molasses.

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