Optimization of Conditions for High Production of 3-HPA through Mathematical Modelling of Series Reactions by Resting *Lactobacillus reuteri*

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Glycerol can be converted to 3-HPA and 1,3-propanediol by resting *Lactobacillus reuteri*. The main catalytic pathway of glycerol is dehydration and reduction. In this study, a novel mathematical model was set up to describe the series reactions of glycerol transformation by *L. reuteri*. In the model, the cell deactivation rate was proposed by a first order equation with different constants for step one and step two of series reactions. The biotransformation process was satisfactorily simulated by the kinetic model proposed. By comparison of cell deactivation and transformation rate constants under different conditions, the optimal conditions for 3-HPA production by resting cell were obtained. The results showed that the 3-HPA/glycerol yield was highest when the biotransformation was done at glycerol concentration of 200 mmol L^{-1}, pH 6.2 and at 37 °C by resting cell with a concentration of 25.3 g L^{-1}. Under the optimal transformation condition for resting cell of *L. reuteri*, the inactivation rate constants of cell (k_1, k_1') and conversion rate constants (k_2, k_3) in step one and step two are 0.72 ± 0.04 h^{-1}, 12.54 ± 0.63 h^{-1}, 0.073 ± 0.002 L g^{-1} h^{-1}, 0.11 ± 0.01 L g^{-1} h^{-1}, respectively.

Key words:
Reuterin, *Lactobacillus reuteri*, kinetics, mathematical model

Introduction

Reuterin is a low-molecular mass, neutral, water-soluble compound, which consists of an equilibrium mixture of monomeric, hydrated monomeric and clyclic dimeric forms of 3-hydroxypropionealdehyde (3-HPA) in aqueous solution.[1,2] Reuterin can be used for food preservation because of its broad spectrum antimicrobial activity towards a range of food-borne pathogens and spoilage organisms, including both gram-positive and gram-negative bacteria, yeasts, moulds and protozoa.[3] The possible use of reuterin has been investigated in milk, cheese[4] and meat.[5,6] It is also a precursor in the production of industrial chemicals such as 1,3-propanediol (1,3-PD) and acrylic acid.[7,8] Though 3-HPA can be produced by chemical and biological methods, researchers are more interested in the latter due to it being environmentally friendly. 3-HPA is normally an intracellular metabolic intermediate from glycerol in genera of bacteria (*Bacillus*, *Klebsiella*, *Citrobacter*, *Enterobacter*, *Clostridium*).[9] Generally, glycerol can be metabolized *via* two parallel pathways. The first is the oxidation of glycerol to dihydroxyacetone (DHA) by glycerol dehydrogenase (GDH, EC 1.1.1.6), and the second is the conversion of glycerol by glycerol dehydratase (GDHt, EC 4.2.1.30) to 3-HPA which is further reduced to 1,3-PD by a NADH-linked oxidoreductase (PDOR, EC 1.1.1.202). Accumulation of 3-HPA in small amounts in the fermentation process will cause growth cessation.[10] The inhibitory effects of 3-HPA on the activity of the enzymes GDHt and PDOR have been well investigated.[11,12]

Lactobacillus reuteri is a heterofermentative lactic acid bacterium, which can not grow on glycerol as sole carbon source but can utilize glycerol as an alternate hydrogen acceptor.[13] The second pathway is the sole way of glycerol utilization in *L. reuteri*. The yield of reuterin was not high when glycerol/glucose cofermented by *L. reuteri* even at a glycerol concentration higher than 400 mmol L^{-1}.[14] A two-step process was proposed for high 3-HPA accumulation. *L. reuteri* cells were the first propagated in optimal conditions for cell growth and 3-HPA was subsequently produced by washed cells in a pure aqueous glycerol solution. Talarico *et al.*[15] reported a two-step fermentation process for reuterin production by non-growing *L. reuteri* cells of 10 mg DCM mL^{-1}. Using this method, Lüthi-Peng *et al.*[16,17] obtained that 170 mmol L^{-1} 3-HPA was produced with a dry cell mass of 30 g L^{-1}, representing 85 % conversion of the glycerol supplied (200 mmol L^{-1} in H_2O), within 2 h of incubation.

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Doleyres *et al.*[18] studied the effect of biomass concentration, temperature, glycerol concentration, anaerobic/micro-aerophilic conditions and incubation time on 3-HPA accumulation, but the 1,3-PD concentration was not shown. Rasch *et al.*[19] developed a model which was able to predict the oscillatory behaviour of *L. reuteri* during reuterin production in a chemostat and to describe satisfactorily the evolution of glucose and glycerol concentrations but not those of biomass and reuterin in a batch system. Tobajas *et al.*[20] proposed a model describing the kinetics of glycerol, reuterin and 1,3-propanediol in the reuterin production process by resting cells of *Lactobacillus reuteri* PRO 137. A spontaneous inactive rate of cells was evaluated for several biomass (2 and 5 CDM g L^{-1}) and glycerol (150–300 mmol L^{-1}) concentrations.

The kinetic model based on resting cell biotransformation has been developed by Tobajas *et al.*,[20] but the cell deactivation rate of synchronization is used in his model. Nevertheless, 3-HPA is an intermediate of reactions from glycerol to 1,3-PD catalyzed by enzymes of GDHt and PDOR. The inactivation rate of GDHt and PDOR should be different. The relationship among the substrate, key enzymes, intermediate and target product needs considering in kinetic analysis. This work deals with the mechanism of biotransforming conditions leading to high rate production of 3-HPA from glycerol by resting cells. In this work, a novel kinetics model describing catalysis of the whole resting cell including GDHt and PDOR will be proposed. The difference cell inactivation rates between the first step of glycerol to 3-HPA and the second step of 3-HPA to 1,3-PD are considered. The model parameters will be calculated under the conditions of glycerol concentration, cell concentration, pH, temperature and harvesting time. The reaction rate constant and cell inactive rate constant in two steps will be compared to thoroughly expound the series reaction from glycerol to 1,3-PD.

Materials and methods

Strain and culture conditions

Lactobacillus reuteri strain CG001 was kept frozen at –80 °C in a solution containing 6 % skim milk powder and 10 % glycerol. For inoculum preparation, 1 % of the frozen *L. reuteri* was propagated in MRS medium at 37 °C and 200 rpm for 15 h under anaerobic conditions in a thermostatized orbital shaker.

The culture medium employed was a modified MRS broth containing 20 g L^{-1} yeast extract, 20 g L^{-1} glucose, 1 g L^{-1} di-ammonium hydrogen citrate, 7 g L^{-1} sodium acetate, 3 g L^{-1} K_2HPO_4, 0.18 g L^{-1} $MnSO_4 \cdot 4H_2O$, 0.2 g L^{-1} $MgSO_4 \cdot 7H_2O$, 1 g L^{-1} Tween 80. The temperature was set 38 °C and the initial pH was set 6.2 as we have optimized in other work. Batch cultures of free cells were inoculated with 2 % (v/v) cells from the stationary growth phase. Anaerobic conditions were established by continuously flushing with filter-sterilized nitrogen.

Glycerol biotransformation

Cells were harvested after 16 h by centrifugation at 7500 rpm for 10 min at 10 °C, washed twice with deionized water and re-suspended in a glycerol solution in the buffer. The effect of bioconversion conditions including time (10, 30, 60, 120, 240 min), initial cell concentration (11.1, 16.6, 20.8, 25.3 g L^{-1}), glycerol concentration (150, 250, 350, 450 mmol L^{-1}), temperature (20, 37, 45 °C), pH (6.2, 7.2, 7.5) and cell harvest time (8, 16, 24 h) on the production of 3-HPA was investigated independently.

Analytical methods

Biomass concentration was determined by optical density measurements at 600 nm which were converted to cell dry mass using a previously obtained calibration curve. Glycerol and 1,3-propanediol were determined by a Agilent 1100 HPLC system with an Aminex HPX-87H column (300 mm × 7.8 mm, Bio-Rad, Palo AHO, CA, USA). Separation proceeded at 65 °C and 0.025 mol L^{-1} H_2SO_4 was used as eluent at a flow-rate of 0.6 mL min^{-1}. The components were identified and quantified using a refractive index detector. 3-HPA was determined using the colorimetric method described by Circle *et al*.[21] Briefly, 1 mL sample was mixed with 0.75 mL of DL-tryptophan solution (0.01 mol L^{-1} solution in 0.05 mol L^{-1} HCl) and 3 mL 37 % HCl. Mixtures containing samples and standards were incubated for 20 min in a water bath at 37 °C and the optical density was measured at 560 nm. A precise quantification of 3-HPA was determined by a calibration curve using acrolein as standard.

Kinetic models

In glycerol metabolism by resting cell of *L. reuteri*, glycerol is firstly transformed to 3-HPA by B_{12}-dependent glycerol dehydratase, and then 3-HPA is catalyzed to 1,3-PD by NADH-dependent oxidoreductase. These series reactions can be indicated by the biochemical reaction eq. (1).

$$G \xrightarrow[X_1]{k_2} R \xrightarrow[X_2]{k_3} P \qquad (1)$$

In the above equation, G is glycerol, R is 3-HPA and P is 1,3-PD. In the reaction, whole-cell

is treated as the catalyst. Tobajas *et al.*[20] assumed same cell decay rate in two reactions and developed a model to describe biomass decay in the process. Actually, the whole-cell carries out two reactions when glycerol is presented. The whole cell should have different deactivation rate in the series reactions. For the first reaction, the activity of coenzyme B_{12} and glycerol dehydratase in cells is important, so an overall deactivation constant of k_1 (h^{-1}) is assigned to describe the cell deactivation in the first step. For the second reaction, the activity of coenzyme NADH and 1,3-PD oxidoreductase in cells is crucial, so an overall deactivation constant of k_1' (h^{-1}) is given to describe the cell deactivation in the second step. If active biomass decay is described by using a first order equation which is typical of biomass death phase, the active cell decay rate can be expressed as eq. 2 for step one and eq. 3 for step two.

$$-\frac{dX_1}{dt} = k_1 X_1 \tag{2}$$

$$-\frac{dX_2}{dt} = k_1' X_2 \tag{3}$$

X_1 and X_2 are the instantaneous active biomass concentrations of the first and second step respectively. Eq. 2 and eq. 3 are integrated for an initial biomass concentration, X_0, yielding eq. 4 and eq. 5. The time evolution of active cell can be depicted by eq. 4 and eq. 5.

$$X_1 = X_0 e^{(-k_1 t)} \tag{4}$$

$$X_2 = X_0 e^{(-k_1' t)} \tag{5}$$

In series reactions, glycerol is transformed to 3-HPA and 1,3-PD. The glycerol consumption rate depends on active biomass concentration (X_1) of step one and glycerol concentration, which can be described as eq. 6.

$$-\frac{dG}{dt} = k_2 G X_1 = k_2 G X_0 e^{(-k_1 t)} \tag{6}$$

Where G is glycerol concentration (mmol L^{-1}) and k_2 is kinetic constant (L g^{-1} h^{-1}) for glycerol consumption rate. A similar production rate equation is expressed for 1,3-PD based on the substrate of 3-HPA and the resting cell as eq. 7. Then the variation rate of intermediate 3-HPA can be expressed differently between 3-HPA generation rate in step one and 3-HPA consumption rate in step two as eq. 8.

$$\frac{dP}{dt} = k_3 R X_2 = k_3 R X_0 e^{(-k_1' t)} \tag{7}$$

$$\begin{aligned} \frac{dR}{dt} &= k_2 G X_1 - k_3 R X_2 = \\ &= k_2 G X_0 e^{(-k_1 t)} - k_3 R X_0 e^{(-k_1' t)} \end{aligned} \tag{8}$$

Where R is 3-HPA concentration (mmol L^{-1}) and k_3 is kinetic constant (L g^{-1} h^{-1}) for 1,3-PD production rate. Eq. 8 can be transformed to eq. 9 by the merger.

$$\begin{aligned} \frac{dR}{dt} &= X_0\left(1 - \frac{k_3 R e^{(-k_1' t)}}{k_2 G e^{(-k_1 t)}}\right) k_2 G e^{(-k_1 t)} = \\ &= X_0\left(1 - \frac{k_3}{k_2}\frac{R}{G} \cdot e^{(k_1 - k_1')t}\right) k_2 G E^{(-k_1 t)} \end{aligned} \tag{9}$$

When $\frac{dR}{dt} = 0$, the increasing of 3-HPA stopped and the eq. 9 is transformed to eq. 10 and eq. 11.

$$\frac{k_3}{k_2}\frac{R}{G} \cdot e^{(k_1 - k_1')t} = 1 \tag{10}$$

$$\frac{R}{G} = \frac{k_2}{k_3} \cdot e^{(k_1' - k_1)t} \tag{11}$$

With the reaction carrying on, 3-HPA concentration increases and glycerol concentration decreases. R/G does not increase with the time until the reactions stop. At the time the reaction stops, the value of $\frac{k_2}{k_3} \cdot e^{(k_1' - k_1)t}$ determines the final 3-HPA yield. In eq. 11, the value of $\frac{k_2}{k_3}$ is the ratio of reaction rates between step 1 and step 2. The value of $e^{(k_1' - k_1)t}$ is the ratio of cell catalytic activity between step 1 and step 2. By comparing the value of $\frac{k_2}{k_3}$ and $(k_1' - k_1)$ under different reaction conditions, the optimum conditions can be deduced.

The model parameters were estimated by non-linear regression analysis and optimized by using simplex and least-squares method implemented in SCIENTIST software.[22] They were evaluated by fitting the mathematical model to the experimental data, where the expression for the calculated sum of squares is presented by the equation of $\sum_{i=1}^{n}(y_i - \bar{y})^2$ where n is the number of data point, and $\bar{y}$ is the algebraic mean of the y data column. Y data column is concentration of glycerol, 3-HPA and 1,3-PD. A program was written in SCIENTIST that performed the optimum search of kinetic parameters to obtain the unknown parameters. The Simplex method provides a useful means for locating a region of the minimum. Runge-Kutta algorithm implemented in the SCIENTIST software was used for simulation.

Results and discussion

The effect of cell harvest time on glycerol transformation

The activity of enzyme and coenzyme is significantly different when cells are harvested at different cell growth phases. To determine the effect of cell harvest time on glycerol trasnformation, the biotransformation is done by resting cell harvested at 8 h, 16 h and 24 h respectively. The model constants were calculated by fitting the concentration of glycerol, 3-HPA and 1,3-PD, presented in Table 1. The cell inactivation rate of the first step is faster than that of the second step for cells harvested in logarithmic phase, in contrast to cells harvested in quiescent phase. The catalytic properties of cells harvested at 24 h are similar to those harvested at 16 h. From the results, cells harvested from logarithmic phase deactivate more quickly in step 1 and step 2, thus most of the 3-HPA converted to 1,3-PD. The quiescent cells accumulate more 3-HPA. Both experimental data and model fitting for the kinetic profiles are given in Fig.1(a)–(c). The values of all kinetic parameters used in the model of the batch biotransformation kinetics are listed in Table 1.

The effect of initial glycerol concentration on glycerol transformation

Substrate concentration is an important factor that affects the catalytic activity of resting cells. Here the glycerol concentrations of 150, 250, 350, 450 mmol L^{-1} are investigated. Model constants are calculated by fitting experimental data under different glycerol concentration. The cell deactivation constants in the first step increase gradually with increasing concentration of glycerol, which means that the inactivation of resting cell accelerated. This coincides with the conclusion in the literature that glycerol is the suicide substrate of glycerol dehydratase. Glycerol concentrations higher than 250 mmol L^{-1} rapidly reduce cell activity, although the whole-cell catalysis rate constant increases with the increase of glycerol concentration. Considering the cell inactivation and catalytic rate constant in all, 3-HPA accumulation is seriously reduced at initial glycerol concentration above 250 mmol L^{-1}.

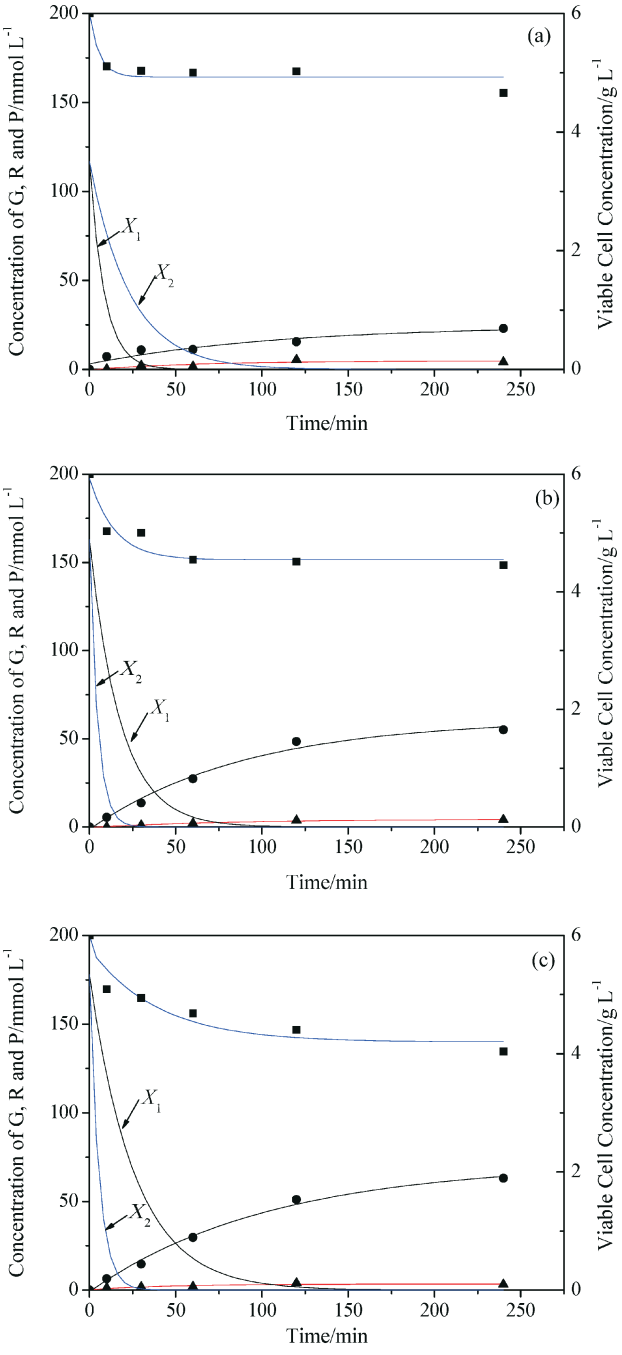

Fig. 1 – *Time-evolution of glycerol (■), 3-HPA (●) and 1,3-PD (▲) during bioconversion of 200 mmol L^{-1} glycerol with pH 6.2 at 30 °C in different growth periods of L. reuteri CG001: (a) 8 h; (b) 16 h; (c) 24 h. X_1 and X_2 represent viable biomass concentration of the two reactions predicted by the model, the other three solid lines are fitted results.*

Table 1 – *Fitting values of several kinetic constants during bioconversion with L. reuteri CG001 in different growth phases*

X_0 (g L^{-1})	k_1 (h^{-1})	k_1' (h^{-1})	k_2 (L g^{-1} h^{-1})	k_3 (L g^{-1} h^{-1})	k_2/k_3	$k_1' - k_1$
3.51 (8 h)	7.02±0.24	2.58±0.06	0.48±0.02	0.42±0.02	1.14	–4.44
4.89 (16 h)	3.36±0.06	12.78±0.72	0.18±0.01	1.14±0.06	0.16	9.42
5.34 (24 h)	2.28±0.12	10.98±0.48	0.12±0.01	0.78±0.06	0.15	8.7

*G_0 = 200 mmol L^{-1}, pH = 6.2, ϑ = 30 °C. The meaning of errors are standard deviations.

According to the value of k_2/k_3 and $k_1' - k_1$, the optimal glycerol concentration for high 3-HPA/glycerol yield should lie between 250 mmol and 350 mmol. Both experimental data and model fitting for the kinetic profiles are given in Fig. 2(a)–(d). The values of all kinetic parameters used in the modeling of the batch biotransformation kinetics are listed in Table 2.

The effect of pH on glycerol transformation

The value of pH is an important factor that affects the enzyme reaction rate. It also has an important influence on series reaction catalyzed by whole-cell. The time profile of glycerol, 1,3-PD, 3-HPA concentration are compared under the pH of 6.2, 7.2, 7.5. Model parameters are calculated by fitting experimental data. The results show that catalytic reaction rate constant of two reactions reaches maximum at pH 7.2, which coincides with the optimal pH of key enzymes "glycerol dehydratase[23]" and "1,3-PD oxidoreductase[24]". However, this pH is not optimal for the production of 3-HPA because the inactivation rate of bacteria is greater at pH 7.2. Although the catalytic rate constant decreases by half at pH 6.2, the inactivation rate constant of bacteria in the first step also reduces by half. Overall, it is more conducive to 3-HPA accumulation at pH 6.2. Both experimental data and model fitting for the kinetic profiles are given in

Table 2 – *Fitting values of several kinetic constants during bioconversion with different glycerol concentrations*

G_0 (mmol L^{-1})	k_1 (h^{-1})	k_1' (h^{-1})	k_2 (L g^{-1} h^{-1})	k_3 (L g^{-1} h^{-1})	k_2/k_3	$k_1' - k_1$
150	0.72±0.03	8.04±0.48	0.048±0.002	0.84±0.06	0.057	7.32
250	1.02±0.05	42.24±1.32	0.054±0.003	4.32±0.21	0.013	41.22
350	5.16±0.12	13.38±0.36	0.192±0.006	4.32±0.12	0.044	8.22
450	5.82±0.18	10.92±0.48	0.180±0.006	2.52±0.12	0.072	5.10

*X_0 = 5.14; pH = 6.2; ϑ = 30 °C. The meaning of errors are standard deviations.

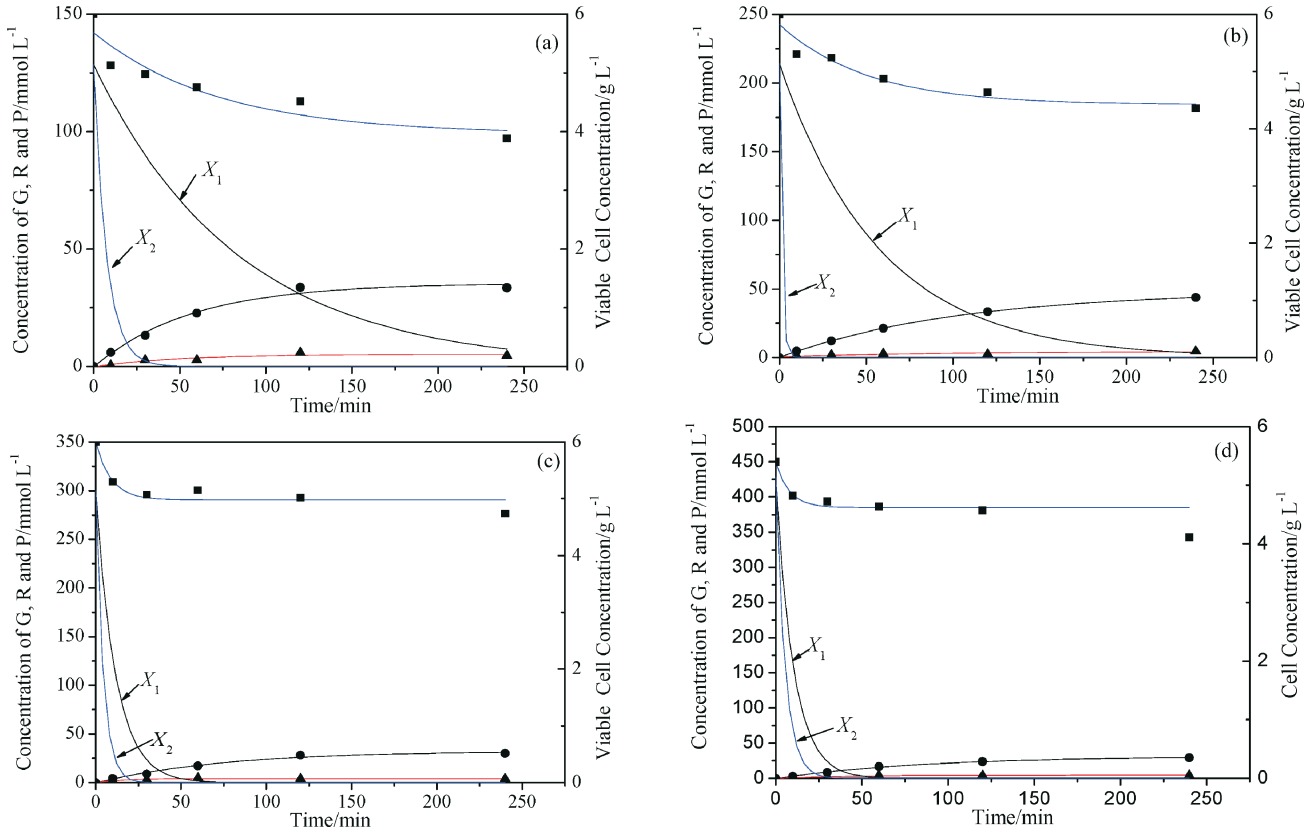

Fig. 2 – *Time-evolution of glycerol (■), 3-HPA (●) and 1,3-PD (▲) during bioconversion of different initial glycerol concentrations with pH 6.2 at 30 °C: (a) 150 mmol L^{-1}; (b) 250 mmol L^{-1}; (c) 350 mmol L^{-1}; (d) 450 mmol L^{-1}. X_1 and X_2 represent viable biomass concentration of the two reactions predicted by the model, the other three solid lines are fitted results.*

Fig. 3(a)–(c). The values of all kinetic parameters used in the modeling of the batch biotransformation kinetics are listed in Table 3. Fig. 3 shows that 3-HPA concentration reaches its maximum 30.5 mmol L^{-1} when 200 mmol L^{-1} glycerol is converted by 5.63 g L^{-1} resting cell at pH 6.2.

The effect of temperature on glycerol transformation

The model parameters are calculated by fitting time profile of glycerol, 1,3-PD and 3-HPA concentration under different biotransformation temperature. The effect of temperature in the catalytic activity of resting cells is complex, due to the number of factors that condition the rate of the process. As the temperature increases, cell deactivates rapidly. The cell deactivation rate and the biotransformation rate constant of step one reach their summit at 37 °C, and those of step two reach summit at 45 °C. The optimum temperature for the dehydration of glycerol to 3-HPA by resting cell is 37 °C which coincides with the glycerol dehydratase from *Citrobacter freundii*[25] and *Klebsiella pneumoniae*.[26] The optimum temperature for glycerol dehydratase from *L. reuteri* is not reported. The optimum temperature for the reduction of 3-HPA to 1,3-PD by resting cell is observed to shift from 37 °C for 1,3-PD oxidoreductase[24] in buffer to 45 °C for resting cell, probably due to a higher enzyme stability in the resting cell. Both experimental data and model fitting for the kinetic profiles are given in Fig. 4(a)–(d). The values of all kinetic parameters used in the modeling of the batch biotransformation kinetics are listed in Table 4. The figures show that 3-HPA concentration reaches its maximum of 52.9 mmol L^{-1} at the temperature of 37 °C.

The effect of cell concentration on glycerol transformation

The bacteria concentration reflects the concentration of catalyst for the resting cell transformation. The time evolution of glycerol, 3-HPA and 1,3-PD concentration at different cell concentration of 11.1, 16.6, 20.8, 25.3 g L^{-1}, are depicted

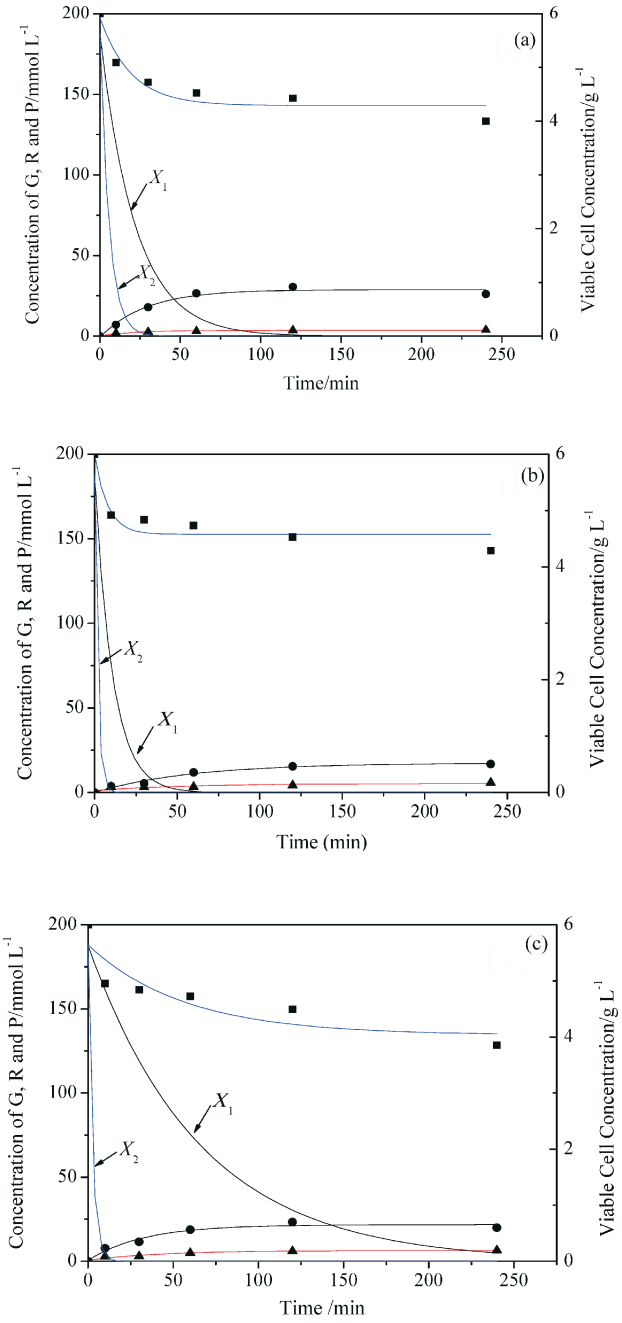

Fig. 3 – *Time-evolution of glycerol (■), 3-HPA (●) and 1,3-PD (▲) during bioconversion of 200 mmol L^{-1} glycerol with different pH at 30 °C: (a) pH 6.2; (b) pH 7.2; (c) pH 7.5. X_1 and X_2 represent viable biomass concentration of the two reactions predicted by the model, the other three solid lines are fitted results.*

Table 3 – *Fitting values of several kinetic constants during bioconversion at different pH*

pH	k_1 (h^{-1})	k_1' (h^{-1})	k_2 (L g^{-1} h^{-1})	k_3 (L g^{-1} h^{-1})	k_2/k_3	$k_1' - k_1$
6.2	2.76±0.13	10.62±0.50	0.162±0.008	0.90±0.04	0.18	7.86
7.2	5.52±0.26	31.14±1.51	0.270±0.013	5.52±0.28	0.05	25.62
7.5	0.90±0.04	23.22±1.16	0.054±0.002	2.46±0.12	0.02	22.32

*X_0 = 5.63 g L^{-1}, G_0 = 200 mmol L^{-1}, ϑ = 30 °C. The meaning of errors are standard deviations.

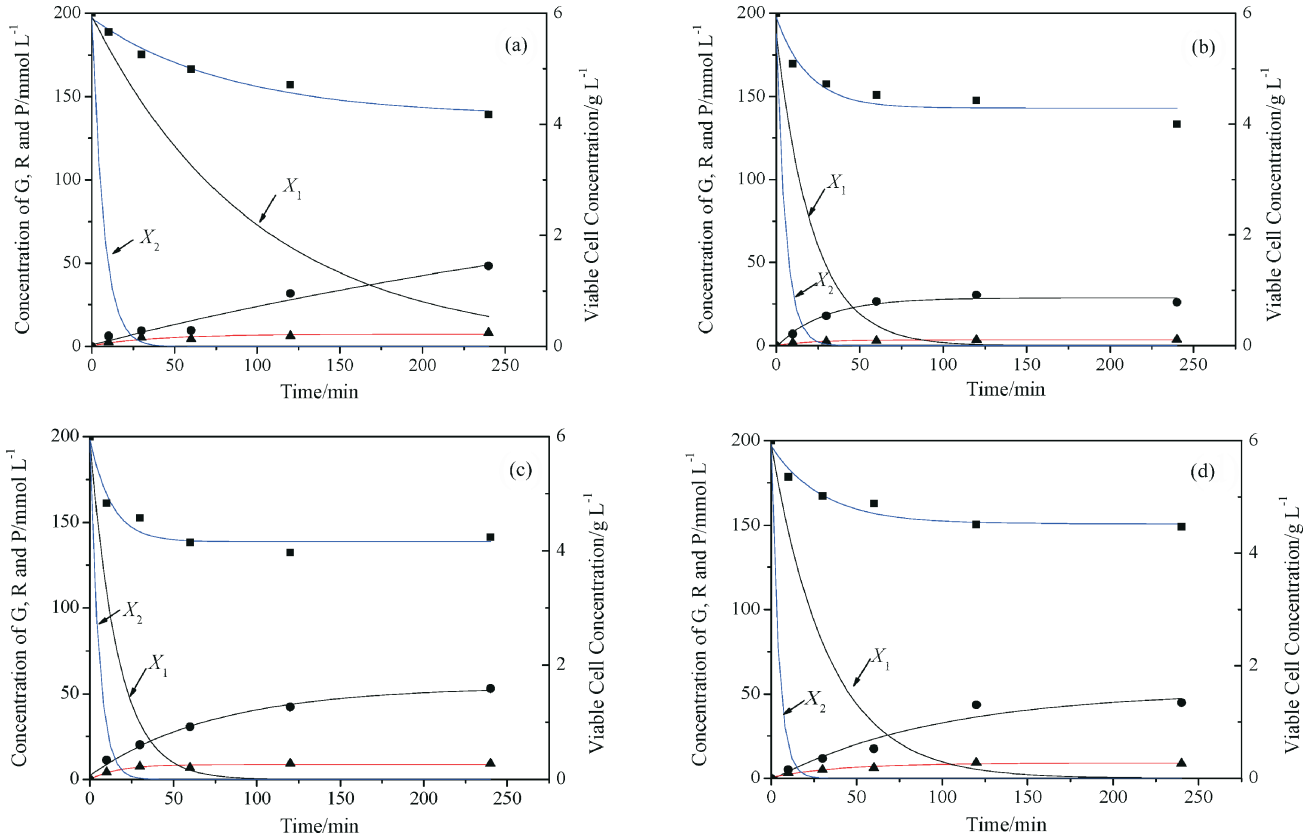

Fig. 4 – *Time-evolution of glycerol (■), 3-HPA (●) and 1,3-PD (▲) during bioconversion of 200 mmol L^{-1} glycerol with pH 6.2 at different temperatures: (a) 20 °C; (b) 30 °C; (c) 37 °C; (d) 45 °C. X_1 and X_2 represent viable biomass concentration of the two reactions predicted by the model, the other three solid lines are fitted results.*

Table 4 – *Fitting values of several kinetic constants during bioconversion at different temperatures*

Temp. (°C)	k_1 (h^{-1})	k_1' (h^{-1})	k_2 (L g^{-1} h^{-1})	k_3 (L g^{-1} h^{-1})	k_2/k_3	$k_1' - k_1$
20	0.60±0.03	8.58±0.42	0.036±0.002	1.62±0.08	0.022	7.98
30	2.76±0.13	10.62±0.53	0.162±0.008	0.90±0.04	0.18	7.86
37	3.60±0.18	11.34±0.57	0.222±0.011	1.92±0.09	0.116	7.74
45	1.80±0.09	13.86±0.68	0.084±0.004	4.44±0.22	0.019	12.06

*X_0 = 5.94 g L^{-1}, G_0 = 200 mmol L^{-1}, pH = 6.2. The meaning of errors are standard deviations.

at Fig. 5. Model constants are calculated by fitting experiment data. The results show that cell concentration has little effect on the cell inactivation rate constant of the first step, but the conversion rate constant of the second step is affected largely at cell concentration above 20.8 g L^{-1}. When the bacteria concentration reaches 25.3 g L^{-1} , the conversion rate constant of the first step reaches a maximum, and the conversion rate constant of the second step decreases to a minimum, so the highest 3-HPA concentration of 195.7 mmol L^{-1} and 3-HPA/glycerol molar yield of 97.8 % are obtained. Both experimental data and model fitting for the kinetic profiles are given in Fig. 5(a)–(d). The values of all kinetic parameters used in the modeling of the batch biotransformation kinetics are listed in Table 5. Increasing the amount of resting cell can greatly accelerate the overall reaction rate, which leads to a higher 3-HPA/glycerol yield.

Conclusion

In the development of an overall model for biotransformation profiles, a relatively good fit is achieved by the model for the complete data set from series reactions. According to the predictive value, the model provides an acceptable description of the resting cell biotransformation over a range of conditions. The cell deactivation constants (k_1, k_1')

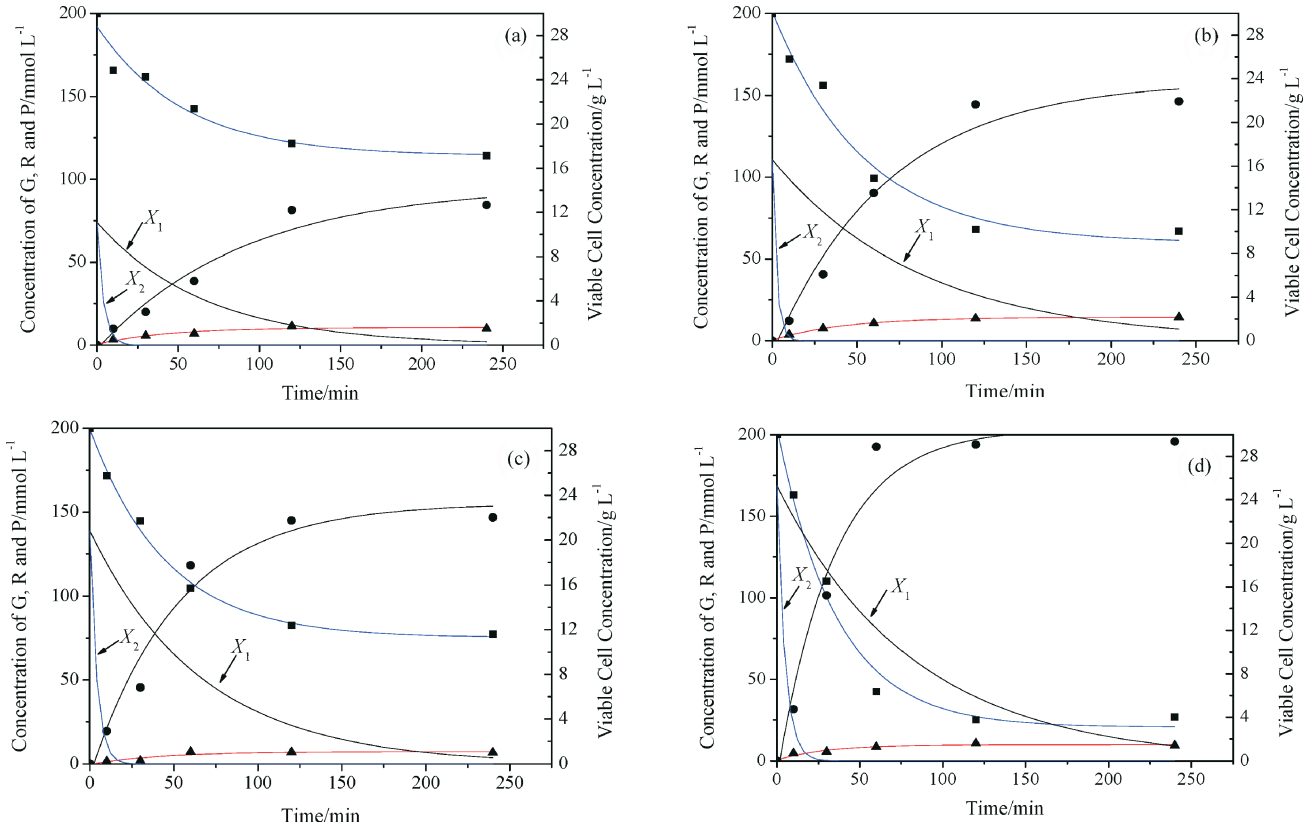

Fig. 5 – *Time-evolution of glycerol (■), 3-HPA (●) and 1,3-PD (▲) during bioconversion of 200 mmol L^{-1} glycerol with pH 6.2 at different conversion CDMs: (a) 11.1 g L^{-1}; (b) 16.6 g L^{-1} ; (c) 20.8 g L^{-1}; (d) 25.3 g L^{-1}. X_1 and X_2 represent viable biomass concentration of the two reactions predicted by the model, the other three solid lines are fitted results.*

Table 5 – *Fitting values of several kinetic constants during bioconversion with different CDMs*

X_0 (g L^{-1})	k_1 (h^{-1})	k_1' (h^{-1})	k_2 (L g^{-1} h^{-1})	k_3 (L g^{-1} h^{-1})	k_2/k_3	$k_1' - k_1$
11.1	0.90±0.05	15.54±0.78	0.044±0.002	1.76±0.14	0.025	14.64
16.6	0.68±0.03	23.64±1.18	0.053±0.003	2.54±0.18	0.021	22.96
20.8	0.90±0.05	15.12±0.76	0.044±0.002	0.25±0.01	0.176	14.22
25.3	0.72±0.04	12.54±0.63	0.073±0.002	0.11±0.01	0.664	11.82

*G_0 = 200 mmol L^{-1}, pH = 6.2, ϑ = 30 °C. The meaning of errors are standard deviations.

and biotransformation rate constants (k_2, k_3) used in the model are significant in judging the effect of conditions on series reactions. From the comparison of the simulation curves generated by the model and the experimental data obtained from various biotransformations in the ranges of initial cell concentration of 11.1–25.3 g L^{-1}, initial glycerol concentration of 150–450 mmol L^{-1}, temperature of 20–45 °C, pH of 6.2–7.5 and cell harvest time of 8–24 h, the maximum 3-HPA/glycerol yield was achieved at glycerol concentration of 200 mmol L^{-1}, pH 6.2 and 37 °C by resting cell with a concentration of 25.3 g L^{-1}. There is evidence of some minor deviation from experimental data. However, these variations are not considered systematic in nature and appear to occur due to experimental errors within particular studies. It is possible that some inhibitory effects (not currently included in the model structure) may have an influence on the biotransformation kinetics.

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List of symbols

X_0 – initial biomass concentration, g L^{-1}

X_1 – instantaneous active biomass concentrations of the first step, g L^{-1}

X_2 – instantaneous active biomass concentrations of the second step, g L^{-1}

G – glycerol concentration, mmol L^{-1}

R – 3-HPA concentration, mmol L^{-1}

P – 1,3-PD concentration, mmol L^{-1}

k_1 – overall deactivation constant in the first step, h^{-1}

k_1' – overall deactivation constant in the second step, h^{-1}

k_2 – kinetic constant for glycerol consumption rate, L g^{-1} h^{-1}

k_3 – kinetic constant or 1,3-PD production rate, L g^{-1} h^{-1}

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