ORIGINAL ARTICLE

Simulation of kefiran production of *Lactobacillus kefiranofaciens* JCM6985 in fed-batch reactor

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Abstract

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Kinetics of kefiran production by *Lactobacillus kefiranofaciens* JCM6985 has been investigated. A mathematical model taking into account the mechanism of exopolysaccharides production has been developed. Experiments were carried out in batch mode in order to obtain kinetic model parameters that were further applied to simulate fed-batch processes. A simplification of parameter fitting was also introduced for complicated model. The fed-batch mode allows more flexibility in the control of the substrate concentration as well as product concentration in the culture medium. Based on the batch mathematical model, a fed-batch model was developed and simulations were done. Simulation study in fed-batch reactor resulted that substrate concentration should be controlled at 20 g L^{-1} to soften the product inhibition and also to stimulate utilization of substrate and its hydrolysate. From simulation results of different feeding techniques, it was found that constant feeding at 0.01 L h^{-1} was most practically effective feeding profile for exopolysaccharides production in fed-batch mode.

Key words : batch, fed-batch, kefiran, lactic acid bacteria, model, simulation

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บทคัดย่อ

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การเลียนแบบจำลองเพื่อศึกษาการผลิตคีเฟอรันของเชื้อ *Lactobacillus kefiranofaciens* JCM6985 ในถังหมักแบบกึ่งกะ

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งานวิจัยนี้เป็นการศึกษาจลนพลศาสตร์ของการผลิตคีเฟอรันของเชื้อ *Lactobacillus kefiranofaciens* JCM6985 โดยมีการประยุกต์ใช้กลไกของการผลิตเอ็กซ์โซโพลีแซคคาไรด์ในแบบจำลองทางคณิตศาสตร์ ค่าคงที่ต่าง ๆ ในแบบจำลองคำนวณจากผลการทดลองของการผลิตแบบกะ และนำมาประยุกต์ใช้ในการสร้างและเลียนแบบจำลองของการผลิตแบบกึ่งกะ ในงานวิจัยนี้ยังได้แสดงวิธีการอย่างง่ายในการคำนวณค่าคงที่ของแบบจำลองที่ซับซ้อนด้วย การผลิตแบบกึ่งกะจะช่วยให้สามารถควบคุมความเข้มข้นของซับสเตรตและผลผลิตในอาหารเลี้ยงเชื้อได้ จากสมการทางคณิตศาสตร์ของการผลิตแบบกะทำให้สามารถสร้างแบบจำลองสำหรับการผลิตแบบกึ่งกะได้ และสามารถคำนวณหารูปแบบการเติมซับสเตรตที่เหมาะสมของการผลิตแบบกึ่งกะได้อีกด้วย โดยจากผลการเลียนแบบจำลองของการผลิตแบบกึ่งกะ พบว่าการควบคุมปริมาณซับสเตรตไม่ให้เกิน 20 กรัม/ลิตร จะทำให้ผลของการยับยั้งโดยผลผลิตลดลง และยังเป็นการกระตุ้นให้เกิดการใช้ซับสเตรตและผลิตภัณฑ์จากการย่อยซับสเตรตได้ ซึ่งจากการเลียนแบบจำลองวิธีการเติมซับสเตรตแบบต่าง ๆ พบว่าการเติมซับสเตรตด้วยความเร็วคงที่ที่ 0.01 ลิตร/ชั่วโมง เป็นวิธีการเติมซับสเตรตที่มีประสิทธิภาพที่สุดสำหรับการผลิตเอ็กซ์โซโพลีแซคคาไรด์ในถังหมักแบบกึ่งกะ

ภาควิชาเทคโนโลยีชีวภาพอุตสาหกรรม คณะอุตสาหกรรมเกษตร มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่ จังหวัดสงขลา 90112

Bioprocess engineers have strived for many years to produce accurate models describing fermentation process behavior, since it can be used in fault diagnosis, performance estimation and prediction, scheduling and optimization. In batch and fed-batch bioprocesses, the maximum performance is achieved by optimizing the initial conditions and subsequent profiles of manipulated variables such as feed rates, temperature and pH during the process operation (Shimizu *et al*., 1989; Hilaly *et al*., 1995; Rodrigues and Maciel, 1997).

Since the production of exopolysaccharides by lactic acid bacteria are of commercial interest for their potential application as natural texturizers, viscosifiers and syneresis-lowing agents, the mass production of exopolysaccharides from this bacteria is currently being extensively studied (Shiomi *et al*., 1982; Mitsue *et al*., 1999; Degeest *et al*., 2001; Leroy *et al*., 2002). For case study of exopolysaccharides production by lactic acid bacteria, the model of kefiran productio n by *Lactobacillus kefiranofaciens* was developed (Cheirsilp *et al*., 2001). *L. kefiranofaciens* used lactose as substrate to produce kefiran and lactic acid. Since it is well known that accumulated lactic acid inhibits cell growth of lactic acid bacteria, it is expected that fed-batch culture might soften the inhibition by lactic acid. In case of *L. kefiranofaciens* it was also found that high concentration of lactose and galactose (product of lactose hydrolysis) also inhibit cell growth (Cheirsilp *et al*., 2001).

In the previous study, batch culture of *L. kefiranofaciens* was performed at pH 5.0 30ºC to obtain information on the essential growth, substrate consumption and production of lactic acid and kefiran (Cheirsilp *et al*., 2001). Figure 1 shows the results of kefiran fermentation in batch culture. Kefiran production of *L. kefiranofaciens* which form capsule surrounding the cell is called capsular kefiran and which is excreted to the medium is called broth kefiran. In order to maximize the total production of kefiran, the total amount of both types of kefiran needs to be

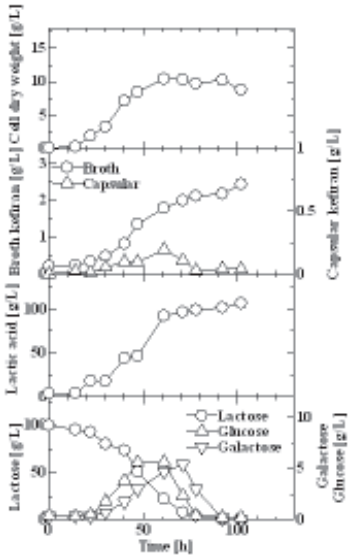

Figure 1. Time courses of kefiran fermentation at pH 5 30ºC.

considered. From Figure 1 it was found that capsular kefiran concentration increased with cell growth and decreased when cell growth stopped. When lactose was used as a carbon source, galactose and glucose accumulated in the medium but their concentrations started to decrease at the end of fermentation.

The present work aims at the maximization of biomass concentration. However, experimental optimization of fed-batch is complicated and costly, so a model for fed-batch culture is needed. Kinetics parameters in the model were estimated by a simplified method. Considering that substrate and product inhibitions were possible, the fed-batch mode was studied by simulation, since the substrate concentration in the culture medium can be controlled by this kind of operation. The different feed techniques were compared: constant feeding, exponential feeding and feedback control of substrate concentration to achieve low level of substrate (lactose and galactose) and lactic acid concentration.

Materials and Methods

Experimental data

L. kefiranofaciens JCM6985, which was used as a kefiran producer in this research, was obtained from Japan Collection of Microorganisms (JCM), RIKEN, Japan. The medium for kefiran production was a modified MRSL broth medium and batch cultures of *L. kefiranofaciens* were done as 3 L of experimental medium in a 7 L jar fermentor (MBF-800, Tokyo Rikakikai Co., Tokyo). The air in the medium was removed by introducing carbon dioxide into the medium before inoculation. The temperature was kept at 30ºC. pH was monitored with an online pH sensor (DPAS, Oriental Yeast Co., Tokyo) and maintained at 5.0 with 10 N NaOH solution. In case of fed-batch culture the initial volume of medium was set at 0.5 L and 300 g L^{-1} of lactose feed stock was fed as defined feeding mode.

Assays

Cell concentration was measured based on the optical density at 660 nm. The relationship between the optical density at 660 nm and the dry-cell concentration was approximated as a linear plot by the least squares method, and used for the estimation of cell concentration. Because capsular kefiran was attached to the cell, the net dry-cell concentration was calculated by subtracting the weight of capsular kefiran from the total dry-cell

concentration. Broth kefiran in the supernatant was precipitated by the addition of the same volume of cold ethanol (-20ºC) as that of the sample and then centrifugated. The precipitate was re-dissolved in distilled water. To remove any remaining un-dissolved materials, the solution was centrifuged and the clear supernatant was again precipitated in the same way. The resulting precipitate was re-dissolved in distilled water. Broth kefiran was then quantified colorometrically by adding 2.5 ml of anthrone reagent (0.8 g of anthrone (Wako), 100 ml of H_2O, and 300 ml of H_2SO_4) to 0.25 ml of the broth kefiran solution. The reaction mixture was incubated for 10 min at 100ºC and cooled to room temperature. The absorbance at 620 nm was measured. The concentration of broth kefiran was calculated using the standard curve of lactose.

Capsular kefiran was extracted from the cells by boiling at 100ºC for 30 min with distilled water. The mixture was centrifuged, the clear supernatant was decanted, and capsular kefiran in the supernatant was measured in the same way as that of broth kefiran.

Glucose concentration was measured with a glucose analyzer (model 2700; YSI Inc., Yellow Springs, Ohio, USA). Lactose and galactose concentrations were measured using a lactose-galactose F-kit (Roche Diagnostics, Tokyo, Japan). DL-Lactic acid concentration was measured with a gas chromatograph (Model G-3000 Hitachi, Tokyo, Japan).

Simulation Model used in this study was adapted from the previous study (Cheirsilp *et al*., 2001). Ordinary differential equations were solved by the Runge-Kutta single-step fourth-order method. The programs were coded in the Visual Basic program Ver. 6.0 (Microsoft Inc., USA).

Results and Discussion

1. Model for batch and fed-batch culture

Optimization studies have been reported in several kinds of fermentation processes (Shioya, 1992) based on the method of searching for the optimal profiles of specific rates. The relationship between specific production rate and specific growth rate was commonly used to determine the optimal profiles of the specific growth rates in many batch and fed-batch cultures (Shimizu *et al*., 1989; Shioya, 1992). A summary of the relationship between specific production rates and specific growth rates at various pH values in this kefiran production process is shown in Figure 2. The time course of specific production rates and specific growth rates during fermentations at various pH values are plotted in the direction of arrows as shown in Figure 2. It was found that both specific rates at various pH decreased during cultivation. In order to optimize kefiran production both specific rates should be kept constant at maximum values. However, it is difficult to maintain both specific rates at constant levels in batch culture due to the accumulation of lactic acid and the decrease of lactose concentration. Therefore, to maximize kefiran production a model describing the effects of pH, and lactose and lactic acid concentrations

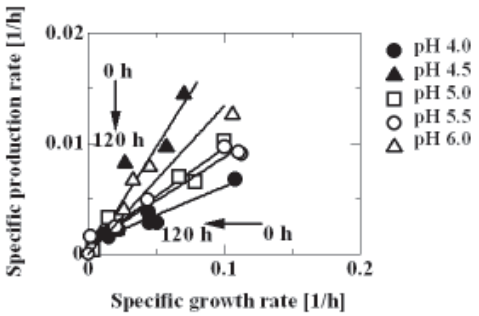

Figure 2. Relationship between the specific growth rate and specific production rate of total kefiran at pH 4, 4.5, 5, 5.5, and 6.

Table 1. A mathematical model of batch and fed-batch culture

Variables	Model of specific rates	Batch	Fed-batch
specific growth rate, μ	$\mu = \mu_m\left(1-\frac{L}{K_{i,L(\mu)}}\right)\frac{S}{K_{m(\mu)}+S+S^2/K_{i,S(\mu)}}$	$\frac{dX}{dt}=\mu X$	$\frac{dX}{dt}=(\mu-F)X$
specific consumption rate, v	$v = v_m\left(1-\frac{L}{K_{i,L(v)}}\right)\frac{S}{K_{m(v)}+S+S^2/K_{i,S(v)}}$	$\frac{dS}{dt}=-vX$	$\frac{dS}{dt}=-vX+F(S_f-S)$
specific product formation rate, ρ	$\rho=\alpha\mu+\beta$	$\frac{dP}{dt}=\rho X$	$\frac{dP}{dt}=\rho X-PF$
net specific production rates of glucose, η_{Glc}	$\eta_{Glc}=\rho_{Glc}S-\frac{v_{Glc}}{S}$	$\frac{dGlc}{dt}=\eta_{Glc}X$	$\frac{dGlc}{dt}=\eta_{Glc}X-GlcF$
net specific production rates of galactose, η_{Gal}	$\eta_{Gal}=\rho_{Gal}S-\frac{v_{Gal}}{S}$	$\frac{dGal}{dt}=\eta_{Gal}X$	$\frac{dGal}{dt}=\eta_{Gal}X-GalF$

***S*: substrate concentration (g L^{-1}), *F*: feed rate ($l^{-1}h^{-1}$), S_f: substrate concentration in feed stock (g L^{-1}), μ_m: maximum specific growth rate (h^{-1}), v_m: maximum specific consumption rate (h^{-1}), $K_{m(\mu)}$: saturation constants of μ (g L^{-1}), $K_{m(v)}$: saturation constants of *v* (g L^{-1}), $K_{i,s(\mu)}$: substrate inhibition parameters of μ(g L^{-1}), $K_{i,s(v)}$: substrate inhibition parameters of *v* (g L^{-1}), *L*: lactic acid concentration (g L^{-1}), $K_{i,L(\mu)}$ and $K_{i,L(v)}$: pH dependence of product inhibition (g L^{-1}), ρ: specific production rates of kefiran and lactic acid (h^{-1}), η_{Glc} and η_{Gal}: kinetic parameters for the production of glucose and galactose (h^{-1}), v_{Glc} and v_{Gal}: kinetics parameters for the consumption of these monosaccharides (h^{-1}). All of specific rates under different pH conditions were investigated. The effect of pH on the specific activities is incorporated in the model, with analogy to the effect of pH on enzyme reaction as**

$$PARA=\frac{PARA_{m,pH}}{1+(k_{PARA1}/[H^+])+k_{PARA2}[H^+]},$$

where *PARA* is the representative parameter for μ_m, v_m, $K_{i,L(\mu)}$, $K_{i,L(v)}$, α, β, ρ_{Gal}, v_{Gal}, ρ_{Glc} and v_{Glc}. $PARA_{m,pH}$,, k_{PARA1} and k_{PARA2} are kinetic parameters describing the effect of pH

on the specific rates is necessary. Especially, the construction of model for fed-batch culture will help us to get useful information how to maintain lactic acid at low concentration. Table 1 shows batch culture model which refer from previous study (Cheirsilp *et al*., 2001) and fed-batch culture model which was developed in this study. The model in Table 1 consisted of specific growth rate, specific consumption rate, specific product formation rate and net specific production rate of glucose and galactose. The substrate and product inhibition was considered in the model. From our literature, no model for exopolysaccharides fermentation, which incorporates broth and capsular polysaccharides production, has been reported thus far. From this point of view, the model for broth and capsular kefiran from Cheirsilp *et al*. (2001) was useful tool to describe the effect of fed-batch culture on exopolysaccharides production.

2. Simplification of kinetic parameter fitting

Figure 3 shows the outline of the algorithm for fitting parameters in the model. Because of model complexity and requirement for many model parameters, a three-step parameter fitting method was employed to simplify the calculation. The algorithm started from the parameter fitting in the function of specific growth rate, μ. By giving the initial values of the parameters of μ, the time courses of cell concentration, X_{cal}, for all the exper-

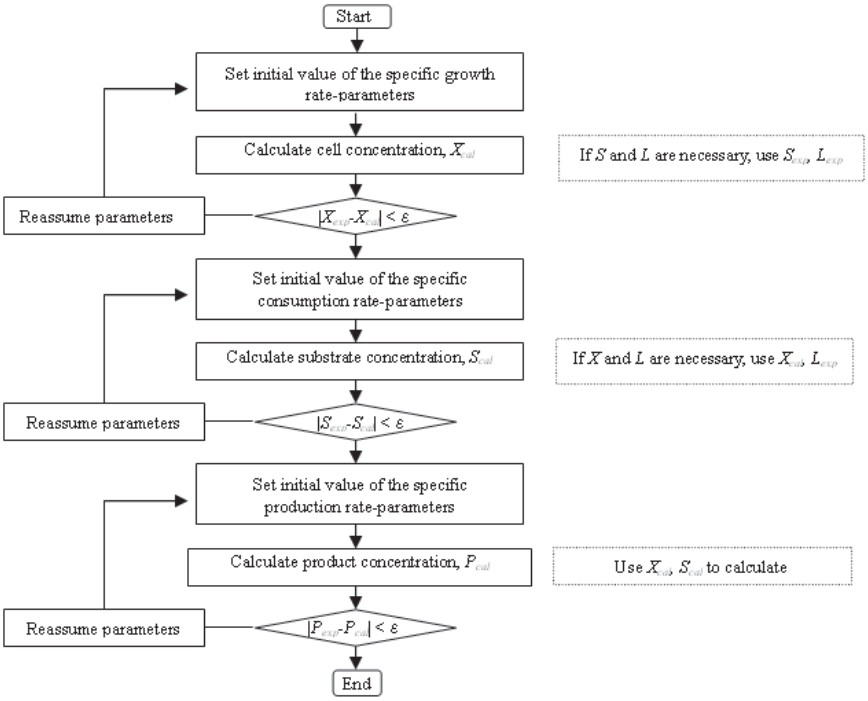

Figure 3. The outline of parameter fitting procedure. *X*: cell concentration, *S*: substrate concentration, *L*: lactic acid concentration, *P*: product concentration. Subscripts of exp and cal are experimental and calculated, respectively.

imental data sets were calculated. In this step, data interpolated from the experimental ones of the substrate, S_{exp}, and the experimental data of lactic acid, L_{exp}, by use of spline functions were used because there was no parameter value of function of specific consumption rate, v, and specific production rate of lactic acid, ρ_L, to calculate these concentrations in the first step. Then, the parameters of μ were relatively corrected by the simplex method to minimize errors between experimental and calculated data of cell concentration throughout the fermentation. After the parameters of μ were determined, the next step was fitting the parameters of v in the same manner using the cell concentration calculated based on the model with the estimated parameters, X_{cal}, and the experimental lactic acid concentration, L_{exp} to calculate for μ and v. When the parameters of v were estimated, summation of errors with respect to S was checked whether it was allowable or not compared with those of the interpolated data (S_{exp}). After the parameters of μ and v were determined, the next step was fitting the parameters involved in formation of lactic acid. These parameters were estimated, and summation of errors with respect to L was checked whether it was allowable or not compared with those of the interpolated data (L_{exp}). If not allowable, estimation of the parameters of μ and v was repeated. The last step was fitting the parameters involved in the formation of kefiran, glucose and galactose using the calculated cell concentration, X_{cal}, the calculated substrate concentration, S_{cal}, and the calculated lactic acid concentration, L_{cal}, to calculate for μ and v. Based on this parameters fitting procedure, all of the determined parameters are shown in Table 2.

3. Constant feeding in fed-batch mode

The constant substrate feeding mode was not controlled by a model and was simulated by the application of various feed rates and initial substrate concentrations. All parameters obtained

Table 2. Parameter values in the expression of pH dependency from simplified fitting method

PARA	$PARA_{m,pH}$	k_{PARA1}	k_{PARA2}
<pH dependent>			
μ_m (h^{-1})	0.218	$+7.75 \times 10^{-7}$	$+3.66 \times 10^{4}$
v_m (h^{-1})	1.08	$+2.66 \times 10^{-7}$	$+2.42 \times 10^{4}$
$K_{i,L(\mu)}$ (g L^{-1})	69.4	$- 1.46 \times 10^{-9}$	$- 6.71 \times 10^{3}$
$K_{i,L(v)}$ (g L^{-1})	136	$+7.20 \times 10^{-7}$	$- 1.78 \times 10^{3}$
α_{total}	0.101	$- 2.21 \times 10^{-7}$	$- 1.24 \times 10^{4}$
β_{total} (h^{-1})	3.02×10^{-2}	$+8.40 \times 10^{-5}$	$+7.72 \times 10^{5}$
α_L	4.98	$- 1.12 \times 10^{-7}$	$- 7.25 \times 10^{3}$
β_L (h^{-1})	1.56	$+7.05 \times 10^{-5}$	$+1.42 \times 10^{6}$
α_{eps}	6.20×10^{-2}	$- 3.65 \times 10^{-7}$	$- 1.50 \times 10^{4}$
β_{eps} (h^{-1})	4.99×10^{-2}	$+6.53 \times 10^{-5}$	$+1.15 \times 10^{6}$
ρ_{GAL} ($g^{-1} L^{-1} h^{-1}$)	4.16×10^{-4}	$+1.93 \times 10^{-6}$	$- 7.37 \times 10^{3}$
v_{GAL} (g L^{-1} h^{-1})	0.285	$+3.45 \times 10^{-6}$	$- 2.41 \times 10^{4}$
ρ_{GLU} ($g^{-1} L^{-1} h^{-1}$)	0.223	- 1.26	$+1.27 \times 10^{10}$
v_{GLU} (g L^{-1} h^{-1})	0.153	$+2.63 \times 10^{-4}$	$- 2.71 \times 10^{6}$
<non-pH dependent>			
$K_{m(\mu)}$ (g L^{-1})	17.9		
$K_{m(v)}$ (g L^{-1})	30.4		
$K_{i,s(\mu)}$ (g L^{-1})	853		
$K_{i,s(v)}$ (g L^{-1})	886		

in Table 2 were used for fed-batch simulation. Figure 4 shows simulation results of constant feed rates at 0.01-0.04 L h^{-1}, initial volume of 500 mL, initial substrate concentration (lactose concentration) of 20 g L^{-1} and substrate concentration in feed stock, S_f, was set at 300 g L^{-1}. In order to investigate the effect of fed-batch on kefiran fermentation, batch mode without feed was also simulated. Since the total substrate of fed-batch at feed rate 0.01 L h^{-1} for 200 h was 600 g, the initial substrate concentration of batch was set at 600 g in volume of 1 L. It was found that high substrate and lactic acid concentrations in batch mode inhibited kefiran production and galactose consumption. Therefore the fed-batch was thought to be useful tool to reduce substrate and product inhibition. At high feed rate, the amount of total kefiran (the multiplied values of kefiran concentration and volume) was high, but the concentrations of lactose, lactic acid and galactose were also high. Though the concentration of lactic acid became low at the end of fermentation due to high dilution rate, lactose and galalctose concentrations still were high. From this view of point the low feed rate at 0.01 L h^{-1} was chosen to be useful for keeping lactose, lactic acid and galactose at low level. Figure 5 shows simulation results of various initial substrate concentrations 20-50 g L^{-1} at constant feed rate of 0.01 L h^{-1}. It was found that substrate concentration profiles at each initial substrate concentration became the same after 60 h of fermentation. Since the production rates of kefiran, lactic acid, and galactose concentrations are the function of substrate concentration (Cheirsilp *et al*., 2001), there was no significant difference in kefiran, lactic acid, and galactose concentrations at various initial substrate concentrations.

4. Exponential feeding profile in fed-batch mode

The exponential feeding is controlled by an equation that depends exponentially on the

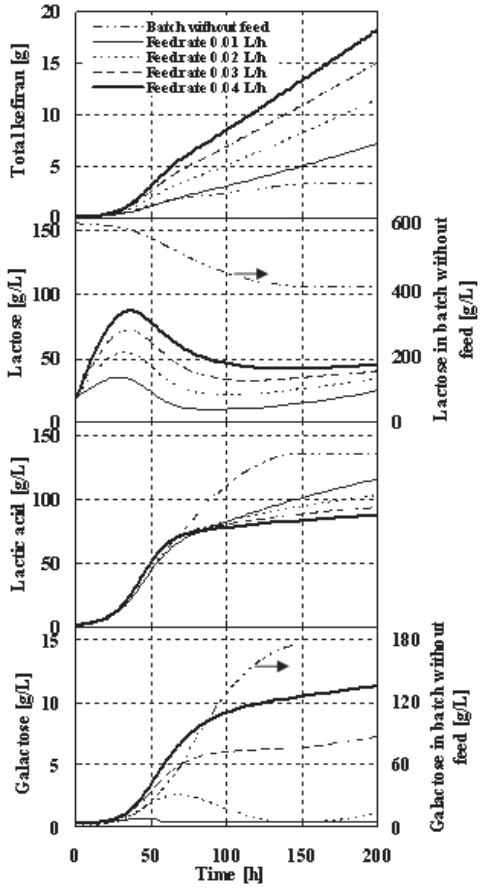

Figure 4. Simulation results of fed-batch cultures at various constant feedings and the initial lactose concentration of 20 g L^{-1} compared to that of batch culture without feed.

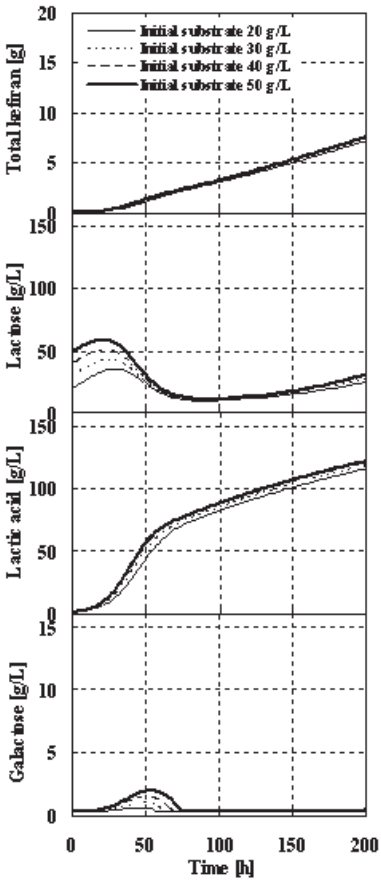

Figure 5. Simulation results of fed-batch cultures at various initial lactose concentrations and constant feeding of 0.01 L h^{-1}.

specific growth rate, and the kinetic model will influence the process performance. Two most obvious exponential feed policies are feeding profiles to obtain constant substrate concentration at its initial concentration and constant specific growth rate at desired value (Da Cunha *et al*., 1998). Calculation of feeding profile corresponding to constant substrate concentration (which specific growth rate, μ, is assumed to be constant) is described by the equation (Lim *et al*., 1977):

$$f = f_0 \exp(\mu t) \qquad (1)$$

where the initial feed rate, f_0 is:

$$f_0 = \frac{\mu X_0 V_0}{Y_{X/S}(S_f - S_0)} \qquad (2)$$

and X_0, S_0 and V_0 are the initial values for cell concentration, substrate concentration and volume, respectively. S_f, is substrate concentration in feed stock and $Y_{X/S}$ is yield of cell concentration to substrate concentration obtained from batch culture at the value of 0.025.

Figure 6 shows the exponential feeding techniques, using four different initial concentrations of substrate when substrate concentration in feed stock, S_f, was set at 300 g L^{-1}, and calculated specific growth rate was used to determine feed rate. This technique showed that more difference

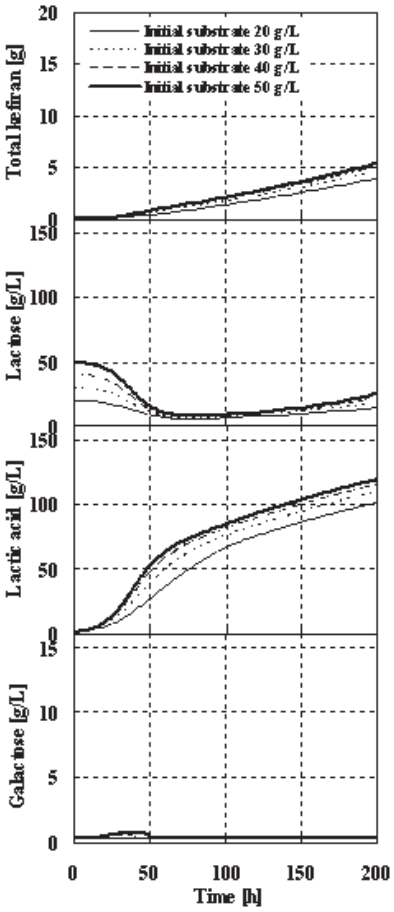

Figure 6. Simulation results of fed-batch cultures with exponential feeding using four different initial concentrations of lactose.

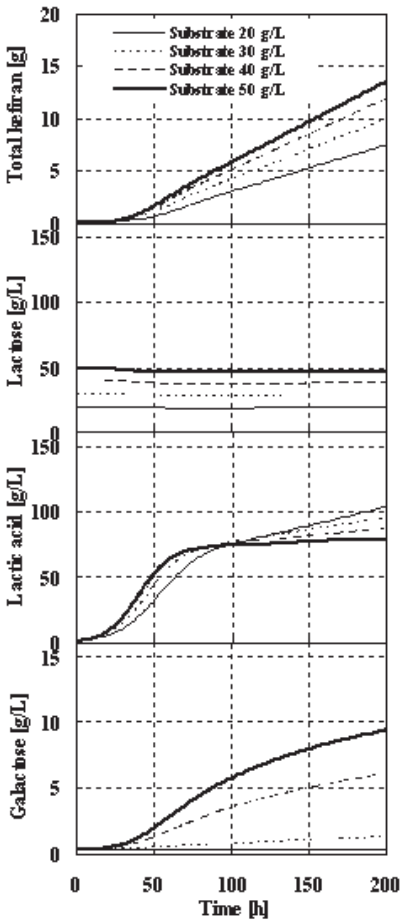

Figure 7. Simulation results of fed-batch cultures with feedback control of substrate concentration feeding using four different concentrations of lactose.

of lactic acid concentration was observed at different initial substrate concentrations. However, total kefiran obtained by this exponential feeding profile was lower than that of constant feeding. This might be due to the fact that production of kefiran was both growth-associated and non-growth-associated product (Leudeking and Piret, 1959). Namely, if feed rate was controlled by specific growth rate, substrate supply for non-growth-associated production will be limited. Also according to slight difference of substrate concentrations in each exponential feeding, only little increase in total kefiran was obtained (Figure 6). Though feeding profile was set to correspond to fermentation at constant substrate concentration, substrate concentrations at each initial substrate concentration in Figure 6 could not be kept constant. This can be explained by t he constraint that the specific growth rate is not only the function of substrate concentration but also the function of product especially lactic acid (Table 1). Therefore, the exponential feeding for constant substrate concentration can be designed only in the period which constant specific growth rate can be obtained (at low concentration of lactic acid).

5. Feedback control of substrate concentration in fed-batch mode

Since the mathematical model allows us to simulate substrate concentration in the medium, calculated substrate concentration is used for feedback control of substrate concentration instead of experimental value. Then, the feed rates to keep substrate concentration in the medium constant were determined as substrate consumption rates. Namely, if feed rate could supply enough substrate to consumption rate by lactic acid bacteria, the substrate concentration in the medium will be constant. Figure 7 shows the simulation of fed-batch, which was controlled at each constant substrate concentration when substrate concentration in feed stock, S_f, was set at 300 g L^{-1}. From simulation results, fed-batches were effective to keep lactic acid at lower level compared to that of other feeding techniques explained above. At 20 g L^{-1} of lactose concentration, lactic acid level was the lowest. And at high lactose concentration, galactose accumulated in the end of fermentation. Therefore, to use galactose effectively, lactose concentration should be controlled constant at 20 g L^{-1}.

6. Experimental validation in fed-batch mode

Since the objective of this study is to maximize kefiran production by minimizing the substrate and product inhibitions, fed-batch simulations using fed-batch model were studied. Among different feed techniques: constant feeding, exponential feeding and feedback control of substrate concentration, it was found that constant feeding at 0.01 L h^{-1} (Figure 4) and feedback control of substrate concentration at 20 g L^{-1} (Figure 7) were effective feeding profiles to achieve low level of both substrate (lactose and galactose) and lactic acid concentrations. Since constant feeding at 0.01 L h^{-1} is more practical and simple than feedback control of substrate concentration at 20 g L^{-1}, constant feeding profile at 0.01 L h^{-1} was chosen to be experimentally done for fed-batch of kefiran fermentation. Figure 8 shows experimental results compared to simulated results of kefiran production in fed-batch mode with constant feeding at 0.01 L h^{-1}. Basically the simulation results showed good agreement with experimental data.

Conclusions

A mathematical model, the use of which enables the prediction of the overall influence of substrate and product inhibition as a function of pH, substrate and product, is thus developed and found to successfully predict the experimental behavior. The model allows us to predict the performance of production under different conditions, such in the fed-batch, and to realize optimum operation. Here, the simulations from fed-batch model indicated that lactose concentration should be controlled at 20 g L^{-1} to soften the lactic acid inhibition and also to stimulate utiliz-

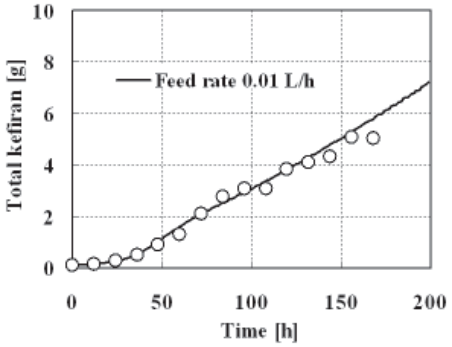

Figure 8. Simulated (lines) and experimental (symbols) results of fed-batch culture with constant feeding of 0.01 L h^{-1}.

ation of galactose in each feeding techniques. And most effective feeding profile for kefiran fermentation according to the simulation results in this study was realized by actual experiment.

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