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Modeling of the simulated moving-bed reactor for the enzyme-catalyzed production of lactosucrose

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Abstract

The enzyme-catalyzed production of lactosucrose in a simulated moving-bed reactor is investigated. A numerical model is derived and verified by data obtained from simulated moving-bed reactor experiments. Based on the derived model, parameter studies and optimization are carried out. It is found that along with the flow rate settings, substrate feed and enzyme concentration and thermal deactivation of enzyme strongly influenced the product yield. Simulation showed that despite of parallel and consecutive side reaction, the maximum lactosucrose yield can reach 69%, which represents a yield increase of 36% relative to the equilibrium yield.

Keywords: Enzyme; Modeling; Simulated moving bed; Optimization; Simulation; Chromatographic reactor; Oligosaccharide

1. Introduction

The integration of a reaction process together with a chromatographic separation in a simulated moving-bed reactor (SMBR) was already studied in various publications during the last decades. It was proven both experimentally (Ray and Carr, 1995; Kawase et al., 1996) and numerically (Ray et al., 1994; Fricke et al., 1999) that the simultaneous reaction and separation in a SMBR can overcome the equilibrium yield in reversible reaction systems. This is of special interest in the field of bioreactions (Cen and Tsao, 1993); enzyme catalyzed processes are basically reversible and therefore equilibrium limited, and accumulation of products may reversibly or irreversibly inhibit the reaction. Consequently, product removal is expected to increase the productivity.

The first studies dealing with the application of the SMBR technology for the field of biocatalysis can be subdivided by the reactor setup: one, in which the SMBR apparatus consists of separated reaction and separation zones (Hashimoto et al., 1983), and the other setup in which the reaction and separation take place in situ (Ganetsos et al., 1990; Barker et al., 1992). The latter setup is employed in this study.

Studies carried out on the SMBR for enzyme-catalyzed reactions were either of mainly experimental (Kawase et al., 2001) or numerical nature (Ching and Lu, 1997; Meurer et al., 1997). In the case that both experimental and numerical studies were carried out (Azevedo and Rodrigues, 2001), the reaction system, though being convenient for scientific investigations, were comparatively uncomplicated compared to the majority of industrial reaction systems occurring in the biotechnology field. The absence of necessary experimentally verified numerical models for the design and optimization of SMB reactors in the field of biocatalysis might be one of the reasons why, to the authors' knowledge, the technology has not been realized so far in biotechnology industries. This fact implicates that verified models are a prerequisite for the successful development of bioreaction processes using the simulated moving-bed technology.

For this purpose, the production of an oligosaccharide, lactosucrose (*O*-*β*-D-galactopyranosyl-(1 → 4)-*O*-α-D-glucopyranosyl-(1 → 2)-*β*-D-fructofuranoside), using *β*-fructofuranosidase is investigated. In the industrial process, lactosucrose is produced in a batch reaction process in an

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enzymatic transfer reaction from sucrose and lactose using β-fructofuranosidase from *Arthrobacter* sp. K-1 (Fujita et al., 1990a) as follows:

$$\text{Sucrose} + \text{Lactose} \rightleftarrows \text{Lactosucrose} + \text{Glucose} \quad (1)$$

At the same time, a parallel reaction and a consecutive reaction occur, namely the hydrolysis of sucrose and lactosucrose.

$$\text{Sucrose} \xrightarrow{H_2O} \text{Fructose} + \text{Glucose} \quad (2)$$

$$\text{Lactosucrose} \xrightarrow{H_2O} \text{Fructose} + \text{Lactose} \quad (3)$$

The authors have already reported in a previous publication (Kawase et al., 2001) that the lactosucrose production under product separation conditions will lead to a higher product yield, although, in contrast to reaction systems without side reactions, a product yield of unity cannot be obtained. The lactosucrose production process was transferred to the SMBR process and experimentally optimized by adjusting the enzyme concentration. The increase of product yield, however, was comparatively small, which indicated that further process optimization is required. On the other side, process optimization by experiments is usually a quite time-intensive undertaking, and its extent increases overproportional with the number of parameters which are to be optimized. Therefore, a numerical model, which allows optimization by simulation is a prerequisite for successful process design.

2. Experimental

The SMBR used in the series of experiments consisted of 12 water-jacketed columns (I.D. 1.2 cm × 19 cm). The columns were connected in series by a rotary valve and packed with Amberlite CR1310Na. The reactor was subdivided into four zones by the inlets and outlets as shown in Fig. 1. Mass flow controllers and gear pumps were used for feeding the desorbent and sugar solutions. Enzyme solution was injected by a syringe pump. The raffinate and extract were withdrawn from the reactor by peristaltic pumps. All experiments were carried out at 50 °C with a column shift interval of 10 min. The desorbent was distilled water which included 0.1 mol/m^3 NaCl or 0.1 mol/m^3 NaOH in order to prevent ion exchange of the resin during reaction.

Sugar feed concentration and enzyme feed concentration at the SMBR inlet were calculated from the respective flow rates and compositions in the two feed solutions, assuming no reaction.

β-fructofuranosidase (E.C. 3.2.1.26) from *Arthrobacter* sp. K-1 and lactosucrose of at least 99 mol% purity were kindly provided by Ensuiko Sugar Refining Company, Yokohama, Japan. All other chemicals and saccharides were purchased from commercial sources and were of reagent grade.

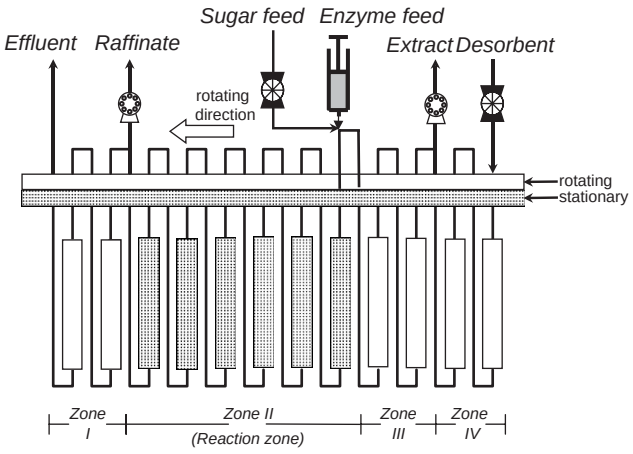

Fig. 1. Simulated moving-bed reactor setup.

The resin used in all SMBR experiments was Amberlite CR-1310Na (Rohm and Haas Japan, Tokyo, Japan).

Each SMBR experiment was carried out until the reactor reached an apparent steady state, i.e., the extract and raffinate concentrations averaged over a subsequent switching interval were constant. The extract and raffinate concentrations were determined by sampling the outgoing streams during one switching interval at the respective withdrawal ports. The concentration profile within the reactor was obtained by withdrawing the solution remaining in the lines between the inlets and outlets of all columns at the end of each experimental run. The samples were subsequently boiled at 100 °C for 10 min to stop the reaction by enzyme denaturalization and analyzed by HPLC using the following setup: mobile phase, acetonitrile/water, 70:30 v/v; TSKgel Amid-80 column (Tosoh Corporation, Tokyo, Japan), 4.6 × 250 mm; Shodex RI-71 differential refractometer (Showa Denko K.K., Tokyo, Japan).

The enzyme activity was determined according to Fujita et al. (1990b), where one unit U of enzyme activity is defined as the amount of enzyme required to transfer 1 μmol of fructose per min under the following conditions: 0.2 cm^3 of a diluted enzyme solution and 0.2 cm^3 of a mixture of 20% sucrose and 40% xylose in a phosphate buffer (pH 6.84) are incubated for 10 min at 40 °C, and the reaction was stopped by heating at 100 °C for another 10 min. The fructose and glucose concentration is measured with an F-kit (glucose/fructose, Roche Diagnostics, Basel, Switzerland).

We also investigated the possibility of thermal deactivation of β-fructofuranosidase. Though the deactivation of fructofuranosidase was not evident at the previous kinetic study (Pilgrim et al., 2001), results later shown in this study suggested that it might be deactivated under the applied conditions. Enzyme deactivation experiments were carried out in a 200-cm^3 Erlenmeyer flask heated to 50 °C, 60 °C or 70 °C in a constant-temperature bath. In addition to a temperature controller, the temperature was monitored by a thermometer inserted into the flask. 100 cm^3 of 0.1 mol/m^3

NaOH solution was stirred until the respective temperature was reached, and approximately 1000 U of enzyme were injected. Samples were withdrawn every 10 min and cooled in an ice bath until enzyme activity determination. The residual activity was determined by the method described before.

3. Model description

3.1. Simulated moving bed

In this study, a linear driving force model (Hashimoto et al., 1983; Kawase et al., 1996, 1999) was used to calculate the saccharides concentrations, using linear isotherms of the Henry type. Since breakthrough experiments showed that enzyme is not adsorbed on the resin under the given conditions, the reaction is assumed to take place in the liquid phase. The following equations can be formulated:

$$\text{liquid phase: } \varepsilon_b \frac{\partial C_k}{\partial t} = -v_n \frac{\partial C_k}{\partial z} - K_f a_{v,k}(C_k - C_k^*) + \varepsilon_b r_k; \quad (4)$$

$$\text{solid phase: } (1-\varepsilon_b)\frac{\partial C_k^*}{\partial t} m_k = K_f a_{v,k}(C_k - C_k^*). \quad (5)$$

The boundary conditions are as follows:

$$C_{k,\mathrm{IV\ in}} = 0 \quad \text{at the desorbent inlet,} \quad (6)$$

$$C_{k,\mathrm{IV\ out}} = C_{k,\mathrm{III\ in}} \quad \text{at the extract port,} \quad (7)$$

$$v_{\mathrm{Feed}} C_{k,\mathrm{Feed}} + v_{\mathrm{III}} C_{k,\mathrm{III\ out}} = v_{\mathrm{II}} C_{k,\mathrm{II\ in}} \quad \text{at the feed port,} \quad (8)$$

$$C_{k,\mathrm{II\ out}} = C_{k,\mathrm{I\ in}} \quad \text{at the raffinate port.} \quad (9)$$

Table 1
Henry coefficients and volumetric mass transfer coefficients

Component	$m[-]$		$K_f a_v[\mathrm{min}^{-1}]$	
	35 °C	50 °C	35 °C	50 °C
Sucrose	0.33	0.32	0.59	0.72
Lactose	0.34	0.34	0.79	0.91
Lactosucrose	0.25	0.25	0.36	0.63
Glucose	0.49	0.49	1.28	1.62
Fructose	0.62	0.58	1.13	1.46

The bed porosity ε_b is 0.46 at 50 °C, which was determined by tracer experiments. Henry coefficients m_k and volumetric mass transfer coefficients $K_f a_{v,k}$ were determined in breakthrough experiments using a chromatographic batch column of the same geometry as the SMBR reactor columns. The values are listed in the Table 1.

The differential equations were converted to finite difference equations by the explicit Euler's method and solved numerically. The shifting of inlets and outlets was emulated by shifting the concentration profile after each shifting time interval.

3.2. Reaction rate equations

The detailed reaction mechanism, reaction kinetics and kinetic parameters were already reported in a previous publication (Pilgrim et al., 2001). The reaction term for the transfer reaction and the respective hydrolysis reactions was determined as follows:

$$\text{transfer reaction: } r_{\mathrm{tr}} = \frac{k_{\mathrm{tr}} \cdot ([S][L] - [LS][G]/K_r)[E_0]}{1 + [S]/K_s^S + [S][L]/K_s^S K_m^L + [LS][G]/K_s^{LS} K_m^G + [LS]/K_s^{LS} + [LS][L]/K_s^{LS} K_i^L + [S][G]/K_s^S K_i^G}; \quad (10)$$

$$\text{sucrose hydrolysis: } r_h^S = \frac{k_h^S [S][E_0]}{1 + [S]/K_s^S + [S][L]/K_s^S K_m^L + [LS][G]/K_s^{LS} K_m^G + [LS]/K_s^{LS} + [LS][L]/K_s^{LS} K_i^L + [S][G]/K_s^S K_i^G}; \quad (11)$$

$$\text{lactosucrose hydrolysis: } r_h^{LS} = \frac{k_h^{LS} [LS][E_0]}{1 + [S]/K_s^S + [S][L]/K_s^S K_m^L + [LS][G]/K_s^{LS} K_m^G + [LS]/K_s^{LS} + [LS][L]/K_s^{LS} K_i^L + [S][G]/K_s^S K_i^G}. \quad (12)$$

These equations, together with the stoichiometry (Eqs. (1)–(3)), allow calculating the reaction rate r_k of component k for a given composition.

3.3. Enzyme deactivation

The enzyme deactivation process is usually of complex nature, and efforts were made to derive models which consider different deactivation mechanisms (Aymard and Belarbi, 2000) or the effect of substrate concentration (e.g. Lejeune et al., 2001). However, not only that investigating and deriving a detailed model for the used enzyme is beyond the scope of this work; in many cases, a first-order deactivation model represents the enzyme deactivation kinetics adequately (Sadana, 1993). In this model, the enzyme deactivation is treated as a one step–two states process, i.e., the active form of the enzyme is denaturated by a unimolecular irreversible reaction into a deactivated form. The balance equation for enzyme is formulated as follows:

$$\varepsilon_b \frac{\partial[E_0]}{\partial t} = -v_n \frac{\partial[E_0]}{\partial z} - \varepsilon_b k_d [E_0]. \quad (13)$$

The deactivation constant k_d was determined by fitting the experimental data of the activity change that were gained in batch deactivation experiments at different temperatures.

4. Results and discussion

4.1. Model verification

4.1.1. Effect of enzyme concentration

SMBR experiments were carried out in order to verify the numerical model. The flow rates were determined in such a way that lactosucrose and glucose were separated in zones II and III by using the so-called β-parameter criterion established by Hashimoto et al. (1993). The β-parameter of component k in zone n is defined as follows:

$$\beta_{k,n} = \varepsilon_b u_n / [(1-\varepsilon_b) u_s m_k]. \quad (14)$$

In this approach, the simulated moving-bed is assumed to behave as a true moving bed with a superficial velocity $u_n = v_n - \varepsilon_b u_s$ and a resin velocity of u_s. For a β-value less than unity, the component k will migrate with the apparent resin flow in the simulated moving bed, and vice versa.

The valve switching time was set to 10 min. The enzyme concentration in the enzyme solution was varied from 1 to 100 MU/m^3 in the experiments. Furthermore, numerical simulation was carried out by numerically solving Eqs. (4)–(13).

Fig. 2 shows the obtained lactosucrose yield. The symbols represent the experimental results. As far as the flow rates and reactor dimensions are fixed, the lactosucrose yield reaches a maximum at a certain optimum enzyme concentration. The experimental results show a maximum lactosucrose yield of 58% around the optimum enzyme concentration of

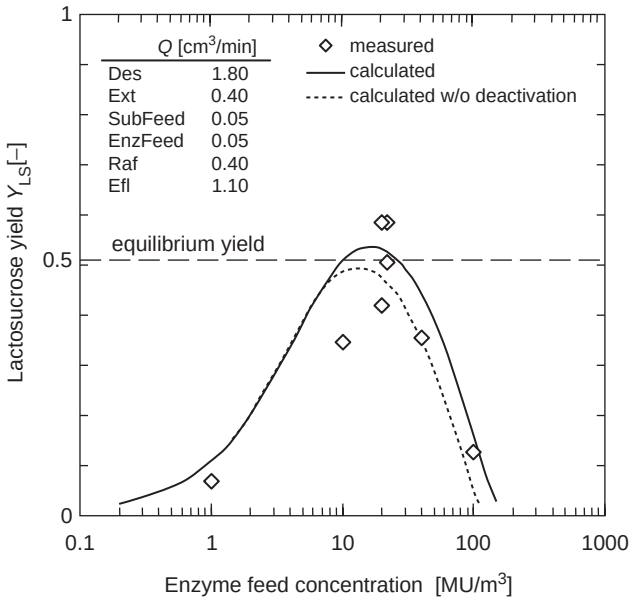

Fig. 2. Effect of enzyme concentration in enzyme feed. Operation conditions: $C_{S,SubFeed} = 500\,mol/m^3$, $C_{L,SubFeed} = 530\,mol/m^3$, $T = 50\,°C$.

20 MU/m^3, which is higher than the equilibrium yield of the main reaction. This indicates the enhancement of the bioreaction by the simultaneous chromatographic separation.

The calculated results are represented by the thick line. In spite of the complexity of the reaction system, the calculation could represent the experiment fairly. The maximum of the calculated line coincides with that of the experimental data.

In order to demonstrate the significance of the enzyme deactivation, the lactosucrose yield was calculated neglecting the deactivation (broken line). In that case, the lactosucrose yield calculated by simulation does not exceed the equilibrium yield. Therefore, the enzyme deactivation has to be included into the numerical model for the simulated moving-bed reactor, though it is insignificant in the case of a batch reaction. A detailed discussion of this effect will be done later.

4.1.2. Effect of flow rate settings in zone II and zone III

Subsequently, the effect of flow rates is investigated. The enzyme and sugar feed concentrations, $C_{SubFeed}$ and $C_{EnzFeed}$, and flow rates, $Q_{SubFeed}$ and $Q_{EnzFeed}$, were kept constant. In addition, the flow rates in zone I and zone IV were not changed either. The flow rates Q_{II} in zone II and Q_{III} ($=Q_{II} - Q_{Feed}$) in zone III were varied, and the lactosucrose yield determined both experimentally and numerically.

In Fig. 3, the lactosucrose yield is plotted against the ratio of u_{II} to u_s. The symbols show the experimental results while the thick line represents the calculated values. The vertical lines indicate the u_{II}/u_s value for which the β-parameter for component k in zone II is unity, i.e., the component migrates neither with the liquid flow nor with the apparent resin flow.

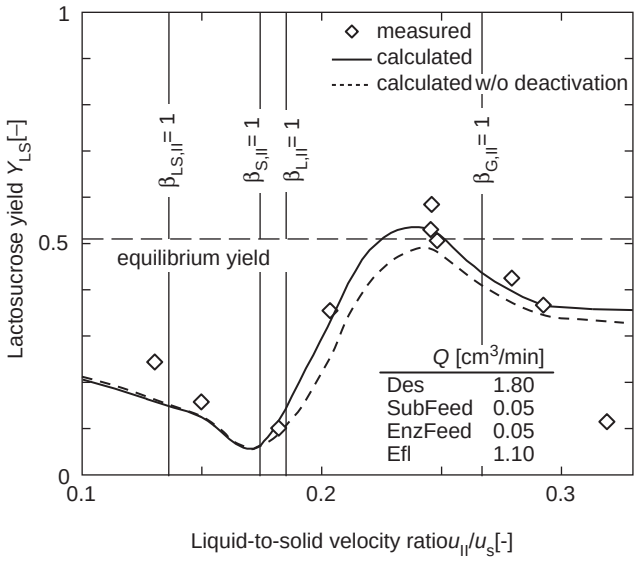

Fig. 3. Effect of flow rate settings in zone II and zone III. Operation conditions: $C_{S,SubFeed} = 500\,mol/m^3$, $C_{L,SubFeed} = 530\,mol/m^3$, $C_{EnzFeed} = 22\,MU/m^3$, $T_{Shift} = 10.0$ min, $T = 50\,°C$.

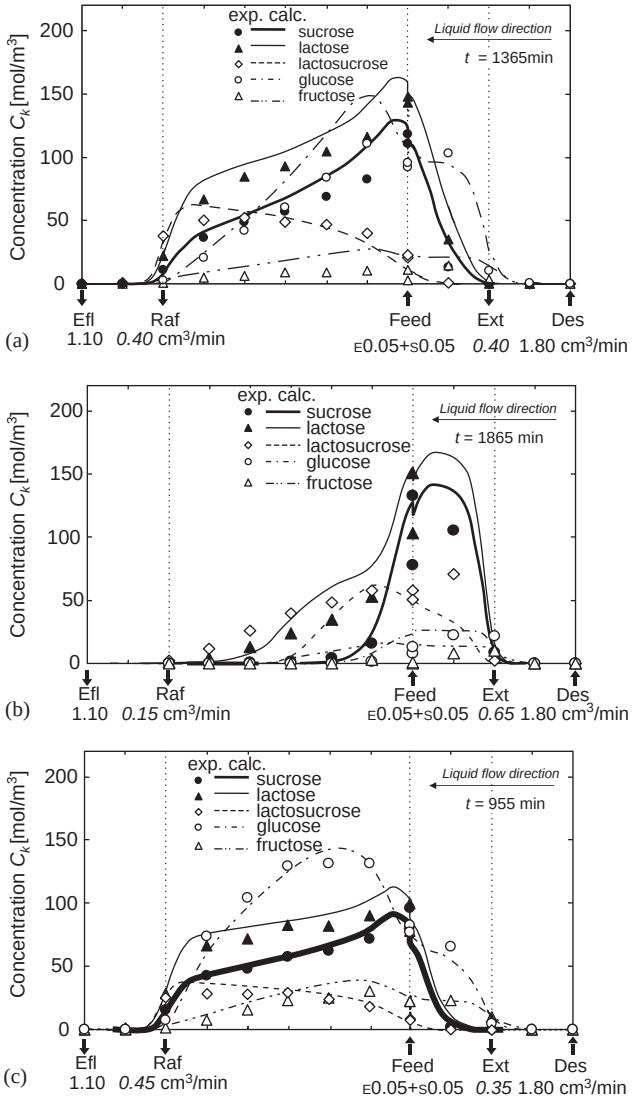

Fig. 4. Axial reactor concentration profile. Operation conditions: $C_{S,SubFeed} = 500\,mol/m^3$, $C_{L,SubFeed} = 530\,mol/m^3$, $C_{EnzFeed} = 22\,MU/m^3$, $T_{Shift} = 10.0$ min, $T = 50\,°C$.

Therefore, e.g. in the region being on the left side of each line, the respective component migrates with the apparent resin flow in the reactor. In the region right of $\beta_L = 1$, the reaction occurs in zone II, while in the region left of $\beta_S = 1$, the reaction mainly takes places in zone III. In the latter case, as enzyme is not adsorbed, only a small amount of enzyme exists in zone III.

Fig. 3 shows both a maximum and a minimum of lactosucrose yield. The maximum exists in the region in which glucose is removed from the reaction zone (zone II). Fig. 4(a) shows the experimental data of the concentration profile for the run with the highest lactosucrose yield. The experimental lactosucrose yield of 58% is higher than the equilibrium yield. The simulation results are also shown in Fig. 4(a). The simulation results are in good agreement with the experimental data. Although there is a certain deviation, it resulted from fluctuations of the experimental flow rates. The concentration profile was measured at a moment when the reactor was shut down in the middle of a switching interval. Deviation of the flow rates from the measured average flow rates, which were used in the numerical simulation, led to the concentration deviation from the simulation results.

When the flow rates in zones II and III are reduced, i.e., u_{II}/u_S becomes smaller, the lactosucrose yield decreases and reaches a minimum. This is because the substrates, sucrose and lactose, start to migrate in the direction of the apparent resin flow and therefore are removed from zone II in which the enzyme is present as shown in Fig. 4(b). Also in this case, the calculated results express the experimental data fairly.

On the other side, when the ratio of u_{II}/u_S is higher than the optimum, the yield also decreases. This can be expected, as glucose is not removed from the reaction zone. In this case, the chromatographic separation does not apply anymore, and the SMBR is reduced to a tubular reactor. Fig. 4(c) exemplifies this situation.

As seen in Fig. 3, the lactosucrose yield can reach a higher yield than the equilibrium yield for both experiments and simulation. However, when neglecting the enzyme deactivation term in the numerical model, the calculated results become worse as shown by a broken line in Fig. 3. Hence, the integration of the enzyme deactivation model is necessary to describe the behavior of the SMBR sufficiently.

4.2. Numerical study and optimization

4.2.1. Effect of feed concentration

Based on the derived and verified model, numerical simulation was carried out for further improving the lactosucrose yield. Fig. 5 shows the influence of the substrate

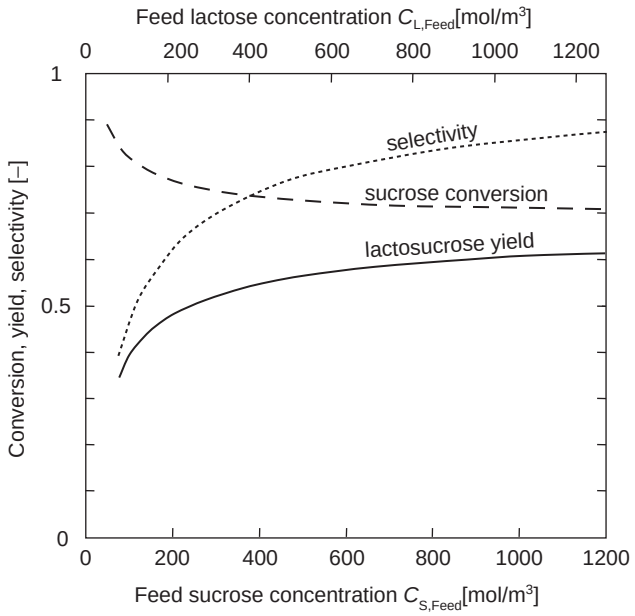

Fig. 5. Effect of substrate concentration. $Q_{Des} = 1.80\,cm^3/min$, $Q_{Ext} = 0.40\,cm^3/min$, $Q_{Feed} = 0.10\,cm^3/min$, $Q_{Raf} = 0.40\,cm^3/min$, $C_{Enz}/C_{S,Feed} = 0.044\,MU/mol$, $T_{Shift} = 10.0$ min, $T = 50\,°C$.

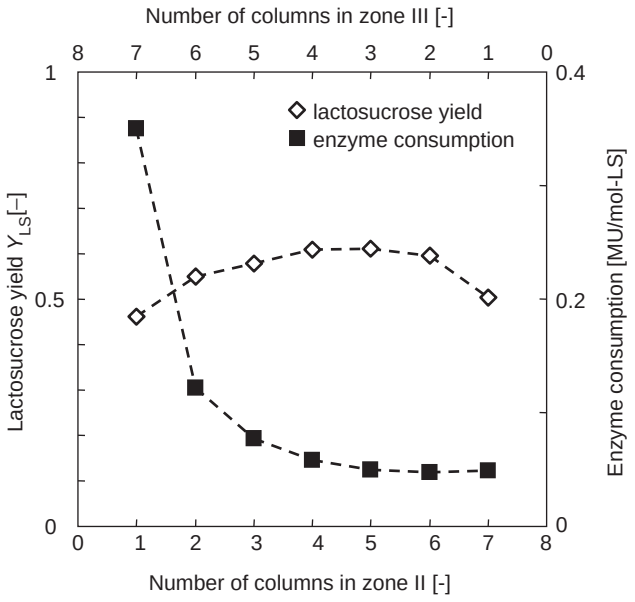

Fig. 6. Effect of position of feed in zone II/III on yield and enzyme consumption. $Q_{Des} = 1.80\,cm^3/min$, $Q_{Feed} = 0.10\,cm^3/min$, $Q_{Efl} = 1.10\,cm^3/min$, $C_{S,Feed} = 1000\,mol/m^3$, $C_{L,Feed} = 1000\,mol/m^3$, $T_{Shift} = 10.0$ min, $T = 50\,°C$.

concentration in the feed on the yield. The sucrose concentration was varied from 10–1000 mol/m^3. The ratios of C_L/C_S and C_{Enz}/C_S in the feed solutions and the other conditions were kept constant during this study.

In the case that the feed concentration is reduced below 300 mol/m^3, the lactosucrose yield becomes smaller than the equilibrium yield. On the other side, when increasing the substrate concentration in the feed, the lactosucrose yield also increases monotonically. This behavior is similar to the relationship between the yield and the initial reactant concentration in a batch reactor (Kawase et al., 2001), although the yield in the SMB reactor is higher than that in a batch reactor. From a chemical reaction engineering point of view, such a behavior is expected, because in the case of higher reactant concentrations, the transfer reaction becomes predominant over the side reactions because of its higher order.

4.2.2. Position of feed in zone II/III

In the previous investigation, the column arrangement of the SMBR was 2-6-2-2 for zones I–IV, respectively, the reaction zone being the longest zone. This is usually desirable because a longer zone will lead to a longer residence time, thus reducing the required amount of enzyme to catalyze a certain amount of substrate during this time. However, when the total number of columns is limited by the apparatus specification, an increase in the number of columns in zone II automatically leads to a decrease in zone III.

In the following simulation, the feed position in the simulated moving bed was varied, so that n columns were placed in zone II and $8 - n$ columns in zone III, respectively. The substrate concentration was set to the concentration applied in the industry, 1000 mol/m^3. The feed flow rate Q_{Feed} was kept constant. The SMBR was then optimized by adjusting the enzyme concentration and the extract and raffinate flow rates. The flow rates in zone I and IV, however, were kept constant.

Fig. 6 shows the result of the numerical investigation. When increasing the number of columns in the reaction zone, the maximum attainable yield also increases to a maximum of 61% for five columns in zone II. When increasing the number of columns further, the yield starts to decrease again. On the other side, the enzyme requirement decreases down to a minimum for six columns in zone II, but increases slightly again when the number is raised up to seven columns in zone III, which can be led back to an insufficient column number in zone III. Therefore, the optimum setup is chosen to be 2-6-2-2 for zones I–IV, respectively.

4.2.3. Multi-parameter optimization

In the previous numerical study, one or more of the process parameters $C_{Feed}, C_{Enz}, Q_{Feed}, Q_{Ext}, Q_{Raf}$, were kept constant. This kind of optimization corresponds to the method of lines, which does not guarantee that the true optimum point of the reactor operation is obtained. Therefore, a multi-parameter optimization based on the Newton's method was carried out.

The substrate feed concentration was set to 1000 mol/m^3. The enzyme feed concentration was set to the concentration of the undiluted crude enzyme solution, 1206 MU/m^3. Furthermore, the flow rate in zone IV, i.e., Q_{Des}, was also kept constant to the previously determined value of 1.8 cm^3/min. Flow rates of enzyme and sugar feed solutions, extract and

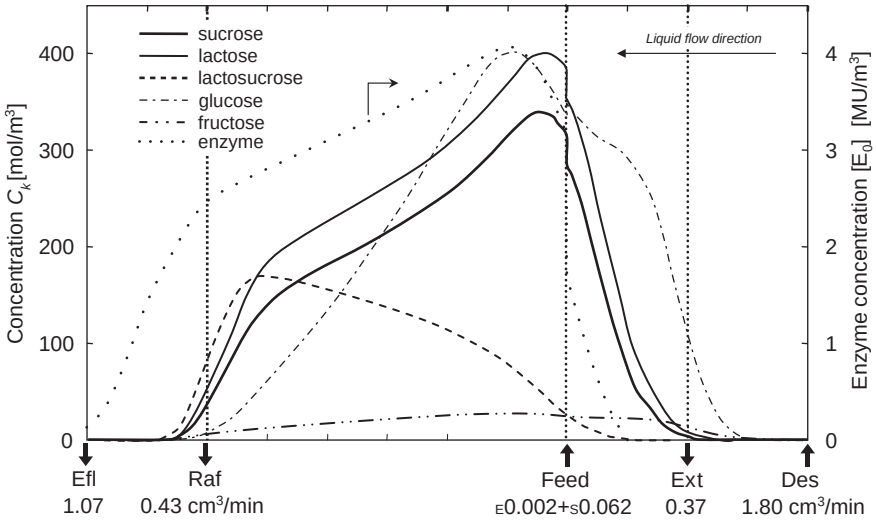

Fig. 7. Reactor profile for SMBR under optimized conditions. $C_{S,SubFeed} = 1000\,mol/m^3$, $C_{L,SubFeed} = 1000\,mol/m^3$, $C_{EnzFeed} = 1206\,MU/m^3$, $T_{Shift} = 10.0$ min, $T = 50\,°C$, $t = 3005$ min.

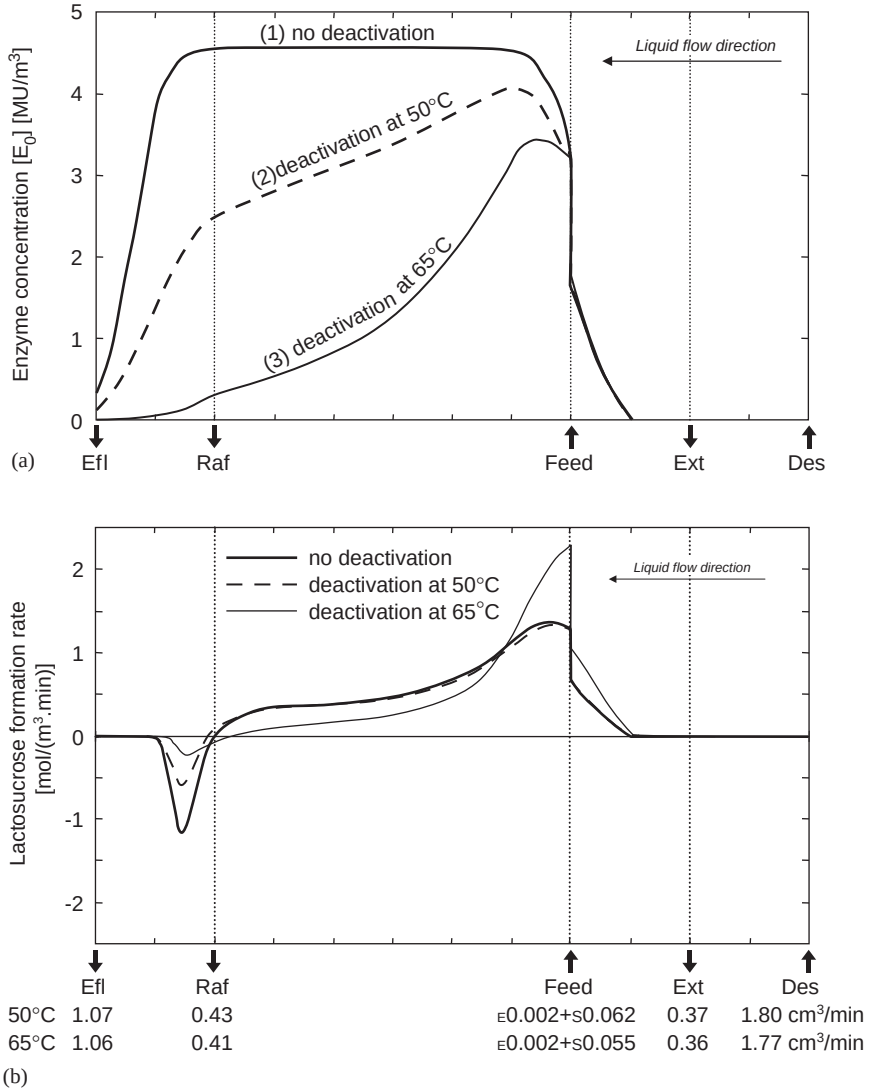

Fig. 8. Effect of enzyme deactivation. (a) Axial enzyme concentration profile; (b) axial lactosucrose formation rate profile.

raffinate were determined by the optimization. Their converged values were $Q_{\mathrm{SubFeed}} = 0.062\,\mathrm{cm}^3/\mathrm{min}$, $Q_{\mathrm{EnzFeed}} = 0.002\,\mathrm{cm}^3/\mathrm{min}$, $Q_{\mathrm{Ext}} = 0.37\,\mathrm{cm}^3/\mathrm{min}$, and $Q_{\mathrm{Raf}} = 0.43\,\mathrm{cm}^3/\mathrm{min}$. The reactor concentration profile for these optimum operation conditions is shown in Fig. 7. In this case, the optimum lactosucrose yield reached 65%.

4.2.4. Enzyme deactivation effect

As already discussed, a model for the SMBR process which includes enzyme deactivation showed a higher optimum lactosucrose yield than a model without this deactivation. Fig. 8(a) shows the calculated enzyme concentration profile, averaged over one shift interval. Line 1 and line 2 represent the model including and excluding enzyme deactivation, respectively. When neglecting the enzyme deactivation, the enzyme concentration is constant in zone II, although tails resulted from column switching and averaging. On the other side, when including the enzyme deactivation, the enzyme concentration decreases toward the direction of raffinate port.

Furthermore, the net lactosucrose production rate for each position in the reactor was calculated from the concentration and enzyme profile and shown in Fig. 8(b). Production and consumption zones exist inside the reactor (Kawase et al., 2001). Though the reaction rates calculated by the both models are comparatively identical in the production region, they differ greatly in the lactosucrose consumption region around the raffinate port, as the enzyme concentration is greatly different. In this region, the substrate lactose, which is also a strong inhibitor of the lactosucrose hydrolysis reaction, is separated from lactosucrose. This separation, in this case, has an effect on the SMBR performance because it accelerates the consecutive lactosucrose hydrolysis reaction.

However, in the case where enzyme deactivation occurs in the SMBR, the enzyme activity around the raffinate port is lower, as shown in Fig. 8(a). Therefore, the hydrolysis reaction is suppressed, which explains that the enzyme deactivation SMBR model leads to a higher lactosucrose yield.

4.2.5. Optimization of temperature

The deactivation of enzyme has a positive impact on the process performance. Therefore, a further investigation on the influence of the temperature is carried out by numerical simulation.

The kinetic deactivation constant k_{d} in Eq. (13) depends on the temperature. Enzyme deactivation experiments, which were carried out under the same conditions as in the SMBR experiments showed that k_{d} can be expressed by the Arrhenius equation as shown in Fig. 9.

The transfer and hydrolysis reaction parameters for a temperature higher than 50 °C was extrapolated from data at 35 and 50 °C (Pilgrim et al., 2001). The Henry and volumetric mass transfer coefficients were also extrapolated in the same way. The expansion of the packed bed was assumed linear, and the expansion coefficient determined from experiments.

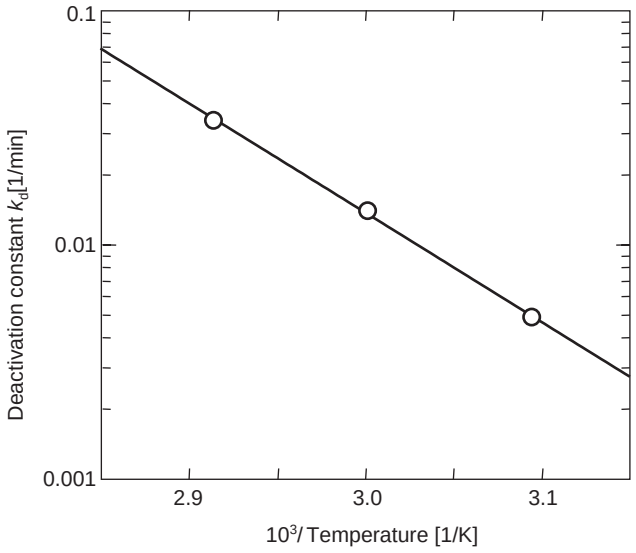

Fig. 9. Effect of temperature on enzyme deactivation rate.

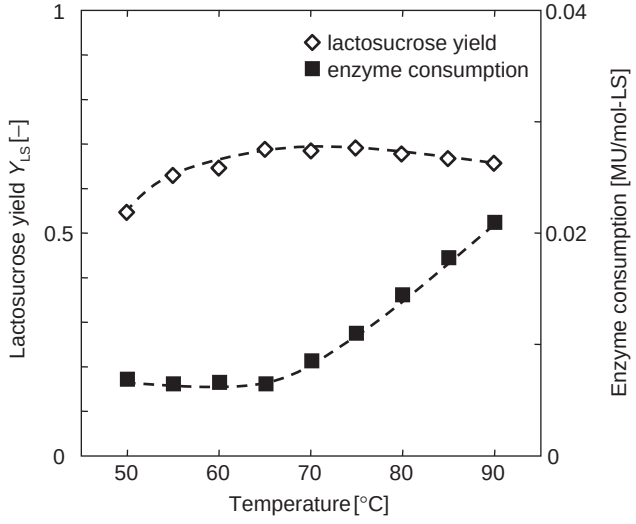

Fig. 10. Effect of reactor temperature on yield and enzyme consumption.

The bed porosity ε_{b} was constant during the observed temperature range. The flow rate in zone IV was set in that way so that the β-parameter for fructose, the strongest adsorbed component, was constant. All other flow rates then were optimized as described before in order to obtain an optimum lactosucrose yield for a certain reactor temperature.

The results of the numerical study are shown in Fig. 10. When increasing the reactor temperature, the lactosucrose yield increases in the simulation to 69% at 65 °C. For temperatures higher than 65 °C, the lactosucrose yield slowly decreases again. The enzyme requirement, on the other side, first remains on a certain plateau when increasing the temperature. Then, for temperatures higher than 65 °C, the enzyme requirement increases rapidly. This shows that the temperature has to be included in the optimization process for the

present reactor system in order to achieve a high yield and to reduce the enzyme consumption.

5. Conclusions

In this study, an appropriate model for the lactosucrose production process in a simulated moving-bed reactor is developed. The model is verified and evaluated using experimental data, which were gained from reaction experiments using a simulated moving-bed reactor.

The effect of various process parameters is investigated. Besides flow rate settings, substrate feed, enzyme concentration, and temperature had the strongest influence on the product yield. Temperature influenced not only the adsorption isotherms and sugar reaction rates but also the thermal deactivation rate of enzyme. At an optimum temperature, the unfavorable side reaction around the product withdrawal port was suppressed by enzyme deactivation while the main reaction rate kept a sufficiently high reaction rate around the sugar feed port.

By optimizing the flow rates, substrate and enzyme concentration, and the reactor temperature, the process could be enhanced. The maximum predicted lactosucrose yield in the simulation was 69% at 65 °C, which represents increase of 36% relative to the equilibrium yield. This result indicates that by the application of the simulated moving-bed reactor, a higher yield can be attained even for a complex reaction system, which includes parallel and consecutive side reactions.

Notation

C	concentration, mol/m^3
k	reaction rate constants
K	enzyme complex equilibrium constant, mol/m^3
$K_f a_v$	volumetric mass transfer coefficient, min^{-1}
L	column length, m
m	Henry coefficient, dimensionless
Q	volumetric flow rate, m^3/min
r	reaction rate, mol/(m^3 min)
t	time, min
T	temperature, °C
T_{Shift}	shifting time interval, min
u	superficial velocity in equivalent true moving bed, cm/min
v	superficial velocity, cm/min
Y	yield, dimensionless
z	length, cm

Greek Letters

β	β-parameter
ε_b	bed porosity

Superscripts

*	liquid phase concentration in equilibrium with stationary phase
LS	lactosucrose hydrolysis
S	sucrose hydrolysis

Subscripts

d	deactivation
Des	desorbent
Efl	effluent
Enz	enzyme
EnzFeed	enzyme feed, before mixing of feed streams
Ext	extract
F	fructose
Feed	substrate and enzyme feed
G	glucose
L	lactose
LS	lactosucrose
n (I, II, III, IV)	zone number
Raf	raffinate
S	sucrose
SubFeed	substrate feed, before mixing of feed streams
tr	transfer reaction

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