Operating Temperature Profile for Immobilized Enzyme Lactose Hydrolysis Reactor Operating at Constant Conversion

مسار درجة الحرارة التشغيلية لمفاعل تميؤ سكر اللاكتوز بواسطة إنزيم محمل اللازم للحصول على مستوى تحويل ثابت للسكر

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Abstract

In this work, reactor performance of lactose hydrolysis by immobilized β-galactosidase (lactase) from thermophilic and mesophilic sources are studied. For continuous flow immobilized reactors, the two ideal models are the plug flow (PFR) and the continuous stirred tank reactors (CSTR) were considered. Reactor performance is expressed in terms of the operating temperature profile required achieving a constant outlet lactose conversion. The reactor design equation for lactose hydrolysis using immobilized β-galactosidase is based on Michaels-Menten kinetics with competitive product inhibition by galactose. Also, this study takes into consideration the enzyme lactase deactivation during the continuous reactor operation. An increasing operating temperature profiles are obtained for different operating time periods, reactor residence times, enzyme concentrations and the desired lactose conversions. Almost constant lactose conversion can be achieved at relatively large residence time and high enzyme concentration. The temperature rise with time increases at higher degree of conversion, low enzyme concentration, and a relatively low residence time.

في هذا العمل تم دراسة أداء مفاعل تميوء سكر اللاكتوز بواسطة إنزيم β-جلاكتوسيديز المحمل لمفاعلي الخزان ذو الخلط المستمر ومفاعل السريان الأنبوبي المستمر. فقد تم تمثيل أداء المفاعل من خلال مسار درجة الحرارة التشغيلية اللازم للحصول على مستوى تحويل ثابت لسكر اللاكتوز. معادلة تصميم المفاعل لتحويل سكر اللاكتوز باستخدام الإنزيم المحمل β -جلاكتوسيديز (اللاكتيز) تعتمد على حر كيات ميكالز- منتن مع وجود تثبيط تنافسي من منتج سكر الجلاكتوز. أيضا فإن هذة الدراسة تأخذ بعين الاعتبار عملية تناقص نشاط فاعلية إنزيم اللاكتيز خلال عملية تشغيل المفاعل المستمر. وقد تم الحصول على مسارات لدرجات حرارة تشغيلية متصاعدة خلال فترات زمن تشغيلية مختلفة، و زمن مكوث مختلف في المفاعل، و تركيز أنزيم مختلف، و قيم مختلفة لمستويات تحويل ثابتة للسكر. فقد تم الحصول تقريباً على مستوى تحويل ثابت للسكر عند قيم مرتفعة من زمن المكوث في المفاعل، و قيم تركيز أنزيم عالية. من الملاحظ أن قيمة درجات الحرارة اللازمة للتشغيل تزداد لقيم التحويل العالية للسكر، وتركيز منخفض للأنزيم، وتقريبا عند زمن مكوث منخفض في المفاعل.

Keywords: Lactose Hydrolysis; Continuous Immobilized β-galactosidase Reactor; Operating Temperature Profile.

1. Introduction

Lactose, the sugar of milk, is a disaccharide of D-glucose and D-galactose. It is the main carbohydrate present in milk and whey at a concentration between 50 and 100 g/L [1], depending on the source of milk. The presence of lactose in dairy products is undesirable for several reasons. In particular, almost 70% of world population cannot consume dairy products because of lactose intolerance due to the shortage of β-galactosidase in humans. The enzyme β-galactosidase is commonly known as lactase. The other reason is the environmental pollution caused by the disposal of dairy wastes with a high content of lactose, as is the case for the disposal of large quantities of whey. Because of these mentioned reasons, enzymatic hydrolysis of lactose has received much attention in recent years [1, 2, 3, 4, 5]. Hydrolysis of lactose to D-glucose and D-galactose is usually carried out using the enzyme β-galactosidase. Another important aspect related to the application of β-galactosidase enzyme to the hydolysis is that the resulting mixture of sugars is sweeter than lactose. Actually, the resulting syrup of glucose and galactose is four times sweeter than the lactose [1, 3].

β-galactosidase enzyme is produced by microbial sources (bacteria, yeast, and fungi). Usually, mesophilic sources are employed for the production of lactase enzyme, which are quite stable for moderate temperature range. For the hydrolysis of lactose, enzymes obtained from the mesophilic sources such as *Kluyveromyces* yeasts and *Aspergillus niger* fungi are commonly used [3, 4, 6]. But low stability of these enzymes at higher temperatures poses a technical problem for their application [1]. The use of thermophilic enzymes, such as the one obtained from the thermophilic microorganism *Thermus sp*. strain T2 [1], allows for higher operational temperatures up to 90 ^{0}C, which allows the hydroysis to be performed with no problems related to microbial contamination and enzyme stability.

There is a considerable work on the kinetics of lactose hydrolysis using β-galactosidase especially from the mesophilic sources [2, 3, 4, 5, 6]. However, few studies related to the kinetics of lactose by thermophilic sources are available in the literature [1]. The kinetic model widely used to describe lactose hydrolysis using immobilized β-galactosidase is basically Michaels-Menten with competitive product inhibition by galactose.

For continuous immobilized enzyme reactors, such as packed bed or stirred tank reactors, the reactor operating policy should be adjusted with time because of enzyme deactivation. In particular, when lactose flows through a batch of slowly deactivating lactase enzyme, the lactose conversion drops progressively with time because of enzyme deactivation. In order to keep constant the outlet conversion during the reactor operation, the reactor operating policy should be adjusted accordingly. This can be accomplished either by replacing the enzyme with a fresh amount when the conversion drops below an unacceptable level, or by increasing the reactor residence time or adjusting the reactor operating temperature to keep constant the outlet conversion during the reactor operating time period.

The most important controllable variable influencing the rate of hydrolysis reaction and the activity of lactase is the temperature. At higher temperatures the deactivation rate of lactase increases while the enzyme loses its activity because of denaturation. In general, enzyme deactivation is temperature sensitive; in particular at higher temperatures the enzyme is essentially thermally unstable. However, at a lower temperature enzyme deactivation is negligible and the rate of lactose hydrolysis is also very low. Therefore, a great deal of enzyme loading is required to get a sufficiently high hydrolysis reaction rate. When the enzyme deactivates slowly with time in comparison with the hydrolysis reaction, then psuedo-steady state conditions can be assumed for the operation of such continuous immobilized reactors. And since temperature can be effectively manipulated, high reactor performance could be achieved using a well-programmed temperature policy [7, 8, 9]. Then, the problem of how to operate the reactor is reduced to finding the progression of temperature along this operating time. The pseudo-steady state assumption is based on Michaels-Menten kinetics with competitive product inhibition by galactose, and on the fact that the operating time period is much longer than the residence time of the hydrolysis reaction. Also, a simple model for the

immobilized lactose hydrolysis reactor excluding the effect of mass transfer will be used. This model is based on the works of Yang and Okos [5] and Ladero et al., [1], where they experimentally assured this type of model for the hydrolysis of lactose. Accordingly, a design equation of the algebraic type for each reactor can be derived. Since each reactor type is described by its design equation that is used basically to evaluate its performance. Then, for a given reactor type parameters e.g. specified outlet conversion, specified reactor residence time, reactor operating time period, enzyme concentration and initial lactose concentration should be studied. The following question is then asked: What is the reactor performance in this case? The answer to this question is simply to solve the design equation for the feasible operating temperature at each specified time. Accordingly, the reactor performance at constant outlet conversion is then reduced to finding the progression of temperature with time, namely the temperature profile during this reactor operating time period. Note that the reactor design equation is a nonlinear algebraic equation. So, simply by providing all the required specified parameters, which are related to the reactor operational conditions, the feasible temperatures along that operating time period can be determined.

This work deals with reactor performance of lactose hydrolysis using immobilized lactase from thermophilic and mesophilic sources required achieving a constant outlet lactose conversion. Reactor performance at different operating time periods, reactor residence times, enzyme concentrations and lactose conversions is determined.

2. Mathematical Model

2.1. Kinetics of lactose hydrolysis by β-galactosidase

The kinetics of lactose hydrolysis by β-galactosidase is described by the widely used Michaelis-Menten kinetics with competitive product inhibition by galactose [4, 5]. For the immobilized thermophilic enzyme, the enzyme lactase forms with lactose an enzyme-lactose complex. This complex releases simultaneously the products of hydrolysis glucose and galactose. The reaction steps can be described as follows:

$$E + S \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} ES \overset{k_2}{\rightarrow} E + P + G \tag{1}$$

$$EP \underset{k_{-3}}{\overset{k_3}{\Leftrightarrow}} E + P \tag{2}$$

Where *S*, *P*, *G*, and *E* are lactose, galactose, glucose, and free enzyme, respectively; *ES* and *EP* are complexes of lactose-enzyme and galactose-enzyme respectively; $k_1,...,k_3$ are reaction rate constants. In this mechanism, inhibition is present because of galactose forming the *EP* complex in a subsequent step with the free enzyme, thus assuring that the *EP* complex is formed in that way only. This assumption will lead to the fact that immobilized thermophilic β-galactosidase have the following kinetic rate expression [1]:

$$r = -\frac{dC_s}{dt} = \frac{k_2 C_E C_s}{C_s + k_m(1 + \frac{C_p}{k_i})} \tag{3}$$

Where *r* is the reaction rate per unit volume, C_E is the active enzyme concentration at the reaction time *t*, k_m is the Michaelis constant $k_m = (k_{-1} + k_2)/k_1$, and $k_i = k_3 / k_{-3}$ is the inhibition constant. Each one of these rate kinetic constants depends on the temperature according to Arrhenius law $k = k_0 e^{-E_a/RT}$. Values of the last group of kinetic parameters were taken from the published work of Ladero et al., [1] for the temperature range of 50-80 ^{0}C and are listed in Table 1.

For the immobilized mesophilic β-galactosidase, the product of hydroysis glucose is released first from the enzyme-substrate complex, *ES*, leaving a galactose-enzyme complex *EP*

as an intermediate. This complex then dissociates to release the galactose [5]. Accordingly, this sequential release of the products can be described by the following hydrolysis mechanism:

$$E + S \underset{k_{-1}}{\overset{k_1}{\Leftrightarrow}} ES \overset{k_2}{\rightarrow} EP + G \tag{4}$$

$$EP \underset{k_{-3}}{\overset{k_3}{\Leftrightarrow}} E + P \tag{5}$$

and the following reaction rate expression is used to describe the hydrolysis reaction [5]:

$$r = -\frac{dC_s}{dt} = \frac{k_2 C_E C_s}{(1 + k_p)C_s + k_m(1 + \frac{C_p}{k_i})} \tag{6}$$

Where the rate constant $k_p = k_2 / k_3$, $k_i = k_3 / k_{-3}$, and $k_m = (k_{-1} + k_2)/k_1 = k_{m1} + k_{m2}$. Values for the reaction rate constants were taken from the work of Yang and Okos [5] for the temperature range of 25-60 ^{0}C, and are listed in Table 2. When the *EP* complex dissociates very rapidly, that is when $k_3 >> k_2$, then $k_p \cong 0$, and the rate expression reduces to that of the thermophilic enzyme.

2.2. Lactase deactivation

Enzyme deactivation is a dynamic process where the enzyme activity decreases with time. The deactivation of lactase (β-galactosidase), which is mainly thermal, can be described by a first order irreversible reaction [1, 5]. Accordingly, the following rate equation is used to describe the process of lactase deactivation:

$$\frac{dC_E}{dt} = -k_d C_E \qquad \text{at t=0 } C_E = C_{E0} \tag{7}$$

Where the active free enzyme concentration at any time *t* is C_E, and k_d is the enzyme deactivation rate constant. Note that k_d is a characteristic parameter for each type of enzyme, and depends on temperature according to the Arrhenius law $k_d = k_{d0} e^{-E_a / RT}$.

For the hydrolysis of lactose using lactase, the deactivation reaction of the thermophilic enzyme is verified experimentally to be first order for the temperature range in question until 80 ^{0}C [1], while for the mesophilic the range ends up with 60 ^{0}C [5]. For the thermophilic enzyme, the deactivation constant k_d is determined using the experimental data of Ladero et al., [1] for the temperature range of 50-80 ^{0}C and is given in Table 1. The correlation coefficient of fitting is greater than 99%. And for the mesophilic enzyme the k_d correlation of Yang and Okos [5] for the temperature range of 40-60 ^{0}C is used in this work and it is also listed in Table 2. It should be noted that, the process of enzyme deactivation can work as the limiting factor that will eventually end the hydrolysis reaction. Hence, for a smooth operation, when the enzyme activity drops to a very low value a fresh batch of enzyme must replace the deactivated one.

2.3. Immobilized enzyme reactors

For continuous flow immobilized reactors, the two ideal models are the plug flow reactor (PFR) and the continuous stirred tank reactor (CSTR). In immobilized enzyme reactors, the enzyme is retained in the reactor by immobilizing the enzyme on the surface of or inside of an insoluble matrix, which is held in the reactor. For slowly deactivating enzymes, when the enzyme lactase deactivates slowly with time in comparison with the hydrolysis reaction, then psuedo-steady state conditions can be assumed for the operation of such continuous immobilized reactors. The pseudo-steady state assumption is based on Michaels-Menten kinetics with competitive product inhibition by galactose, and on the fact that the operating

time period is much longer than the residence time of the reactor. Also, a simple model for the immobilized lactose hydrolysis reactor excluding the effect of mass transfer will be used. This model was validated experimentally by Yang and Okos [5] and Ladero et al., [1] for a given set of operating conditions. These experimental conditions for the hydrolysis of lactose using β-galactosidase depend on the amount of enzyme concentration, lactose flow rate and other operating conditions. Yang and Okos [5] experiments were based on the enzyme lactase obtained from *Aspergillus niger* (Lactase LP, Wallerstein Co.). According to their work, this enzyme was immobilized on phenol-formaldehyde resin and the immobilization was done by adsorption and then cross-linking with glutaraldehyde. The immobilized lactase particles were packed in a water jacketed reactor of dimensions (1.5 x 30 cm). While Ladero et al., [1] used as lactase enzyme, the enzyme isolated from the thermophilic microorganism *Thermus sp*. strain T2. This enzyme was immobilized on a support activated with trishydroxymethylphosphine (THP). For more details regarding lactase immobilization, from *Aspergillus niger* and *Thermus sp*. strain T2, and reactor operation see the works of Yang and Okos [5] and Ladero et al., [1].

For an ideal plug flow immobilized enzyme reactor, PFR, in the presence of lactase deactivation and with no mass transfer effects, the following design equation can be used to describe its performance [10]:

$$\tau = C_{s0}\int_0^x \frac{dx}{-r} \tag{8}$$

Where τ is the reactor residence time, C_{s0} is the lactose initial concentration and x is the conversion of lactose defined as:

$$x = \frac{(C_{so} - C_s)}{C_{s0}} = \frac{C_p}{C_{s0}} = \frac{C_G}{C_{s0}} \tag{9}$$

After inserting the rate expression of the lactose hydrolysis reaction in Eq. (8) and performing the integration with respect to C_s, the following algebraic design equation is obtained:

$$\tau\, C_{E0}\, exp(-k_d t) = Bx - A ln(1-x) \tag{10}$$

On the other hand, for the continuous stirred tank immobilized enzyme reactor, CSTR, the reactor is assumed to be well mixed, and all properties are assumed to be uniform within the reactor. The following performance equation with no mass transfer limitations is used [10]:

$$\tau = \frac{C_{so} - C_s}{-r} \tag{11}$$

Using the reaction rate expression for lactose hydrolysis in the above equation, we get the following:

$$\tau\, C_{E0}\, exp\left(-k_d t\right) = Bx + \frac{Ax}{(1-x)} \tag{12}$$

Where

$$A = \frac{k_m}{k_2}\left(1 + \frac{C_{so}}{k_i}\right) \tag{13}$$

$$B = \begin{cases} \dfrac{C_{so}}{k_2}\left(1 + k_p - \dfrac{k_m}{k_i}\right) & \text{for mesophilic lactase} \\ \dfrac{C_{so}}{k_2}\left(1 - \dfrac{k_m}{k_i}\right) & \text{for thermophilic lactase} \end{cases} \tag{14}$$

3. Reactor Operation

Simulating the reactor operation is possible by solving the nonlinear algebraic design equation, one for each reactor, along a time period which is called the reactor operating time period. For each type of enzyme, the length of this time period is determined from the bounds on $1/k_d$, which is the deactivation time constant τ_D. Since the enzyme deactivates slowly with time in comparison with the hydrolysis reaction, then for practical reactor operation τ_D must be very large in comparison with the reactor residence time τ. Accordingly, the length of the reactor operating time period is determined from the bounds on τ_D, keeping in mind that temperature can affect that period too, because of k_d dependency on temperature. Since enzyme deactivation is represented by a first order homogeneous differential equation (Eq. (7)). The residual enzyme activity, *a*, at time t and given temperature, i.e., a constant k_d, can be determined from the following equation:

$$a = \frac{C_E}{C_{E0}} = exp(-k_d t) = exp(-t/\tau_d) \tag{15}$$

Note that, after a time length of reactor operation equal to the deactivation time constant (i.e., for t=1 τ_D), the residual enzyme activity, *a*, is 36.8%, and after 4 τ_D the activity is 2%, while at t=5 τ_D the activity is 0.7%. As it can be seen that after a time period of 4 τ_D to 5 τ_D, the enzyme is almost completely exhausted and should be replaced with a fresh batch of immobilized enzyme. Accordingly, the reactor operating time period should be at least equal to 4 τ_D.

As it can be seen from the Arrhenius law that temperature affects the deactivation constant k_d, which means that lower values of τ_D are estimated at higher temperatures. This in turn shrinks the length of the operating period of the hydrolysis reaction. It is obvious that a very low value of k_d can give an almost infinite operating period ($\tau_D \approx \infty$). Lowering the temperature can make this condition but it tends to decrease the rate of the hydrolysis reaction of lactose. Therefore, the selection of reactor operating time period is dictated by the bounds on temperature that limits the values of k_d and thus τ_D. For the thermophilic enzyme, the allowable temperature of 60 ^{0}C gives a value of τ_D of almost 17 days. While at the maximum allowable temperature of 80 ^{0}C the estimated time constant τ_D is approximately 0.53 days (12.8 hr). It should be mentioned that the limit is more on the maximum allowable temperature, because the linearity of the deactivation reaction is violated at the upper temperature bound. For the mesophilic enzyme at the higher temperature of 60 ^{0}C a value of τ_D= 6.6 days is obtained. While at 45 ^{0}C a value of τ_D= 350 days is estimated, a temperature lesser than that will mean an almost infinite operating time period, and a relatively low reaction rate. For variable temperature operation the effect of temperature on k_d and thus τ_D is more significant. Accordingly, the length of the operating time period must be selected with care over the operating temperature range in question, keeping in mind that τ_D is changing with temperature.

As it can be seen from the above discussion that lactase deactivation is temperature sensitive, in particular at higher temperatures. At higher temperatures, the deactivation rate constant of lactase enzyme increases while the enzyme loses its activity because of denaturation. As a result lactose conversion drops progressively with time. Then the problem of how to operate the reactor reduces to finding the progression of temperature with time i.e., temperature profile. Thus, in order to operate the reactor at a particular conversion level during the operating period, the reactor operating temperature should be adjusted accordingly. Note that E_a/R=7830 K and 4200 K for the main hydrolysis reaction for both mechanisms. While

for the deactivation reaction $E_a / R = 2.012 \text{x} 10^4$ K for thermophilic and $2.812 \text{x} 10^4$ K for mesophilic enzymes respectively. This gives a ratio of the activation energies of the main hydrolysis reaction to that of the enzyme deactivation (for thermo- and mesophlic enzymes) less than 1. Accordingly, the reactor operating policy must be a rising temperature with time so as to keep the specified reactor conversion constant [10]. When the conversion level can no longer be maintained at a particular operating time, then the reactor operation must also be adjusted. If this operating time is too short in comparison with the operating period, then we adjust the conversion level and operate the reactor at the feasible temperature reached for the rest of the operating period selected. If the conversion level can no longer be adjusted because of almost complete loss of enzyme activity, then the reactor is shut down and the enzyme is replaced with fresh amount of lactase accordingly.

For a given reactor type, reactor capacity and lactose flow rate i.e., a given reactor residence time τ . And specified outlet conversion x_0, reactor operating time period t_f, initial enzyme concentration C_E, and initial lactose concentration C_{s0}, the reactor performance at constant outlet conversion can be determined. Reactor performance is translated into finding the progression of temperature with time, namely the temperature profile during this reactor operating time period.

We now present the steps of the reactor performance procedure for the determination of the temperature profile as follows:

(1) At time zero, solve the reactor design equation (Eq. (10) or (12)) to get the initial operating temperature T_o.

(2) Increase the operating time by an increment. With each new time value, solve the design equation to get the feasible temperature. Step 2 is called constant conversion operation. Repeat step 2 if possible until the operating time period is covered.

(3) If the design equation is no longer solvable for temperature i.e. no real feasible solution is found, then switch to variable conversion operation and go to step 4. No solution means that the reactor cannot be run at the specified outlet conversion at this particular time.

(4) For the rest of the operating period, run the reactor under variable conversion i.e. isothermal operation at the maximum feasible temperature reached at step 2. Stop the run if the operating time is covered or the enzyme activity drops to a very low value.

4. Numerical Examples, Results and Discussion

Reactor performance of lactose hydrolysis by immobilized β-galactosidase for the two types of enzymes thermophilic and mesophilic is determined. This translates into finding the temperature profiles required to achieve a constant conversion at different operating periods, residence times, enzyme concentrations and lactose conversions. The initial lactose concentration of 0.146 mole/L is used in this work. This value is a typical concentration of lactose content in cow's milk [1]. By using the operating conditions suggested by Yang and Okos [5] and Ladero et al., [1] will ensure that no mass transfer limitations exist. These operating conditions used in their experimental works are related to lactose feed flow rate, agitation speeds and enzyme loading concentrations. In the case of the mesophilic enzyme used by Yang and Okos [5], the enzyme loading concentrations were (10-100mg/g resin), and feed flow rates were between 0.1 and 15 mL/min using an immobilized packed bed reactor of dimensions (1.5x 30 cm). For long term operation, a typical value of lactose flow rate of 0.5 mL/min is used [5]. By using the above operating conditions a range of (570-1800 mg/L) of active immobilized enzyme concentrations can be estimated. Loadings of thermophilic enzyme of (0.06-2.8 mg/g resin [1]) under the same operating conditions will yield an enzyme concentration between 14-132 mg/L. The lower and upper bounds on the operating temperature are 40 °C and 65 °C for the mesophilic enzyme, and 50 °C and 80 °C for the thermophilic

enzyme respectively. As discussed earlier, for pseudo-steady state hypothesis to be valid, the reactor residence time, τ, is of the order of hours which is less than that for enzyme deactivation which is of the order of days in magnitude. Accordingly, reactor residence times of 45, 60, 75 and 90 min and different operating periods of 4, 8, 12, and 16 days for the mesophilic enzyme and 2, 4, 6 and 8 days for the thermophilic enzyme are considered. Also, different enzyme concentrations and lactose conversions are used.

For both types of reactors and enzymes, mesophilic and thermophilic, the temperature profiles are determined by solving Eqs. (10) and (12) using POLYMATH software. Temperature, lactose conversion and enzyme activity profiles are shown in figs. 1-a through 4-c at reactor residence time of 60 min and different operating time periods. As it can be seen from the figures, constant conversion can not always be achieved through out the whole operating periods. The effect of residence time on the temperature profile is determined at residence times of 45, 60, 75 and 90 min, and for operating period of 16 and 8 days for mesophilic and thermophilic enzymes respectively (Figs. 5-a through 6-b). To show the effect of enzyme concentration on the temperature profile, this temperature profile is plotted at different enzyme concentrations for operating periods of 16 and 8 days for mesophilic and thermophilic enzymes (Figs. 7-a through 8-b). Also, the effect of required reactor outlet lactose conversion on the temperature profile is shown in figs. 9-a, 9-b, 10-a, and 10-b for mesophilic and thermophilic enzymes respectively.

It is clear from the results that an increasing temperature profile is required to obtain a constant lactose conversion for both types of enzymes and immobilized reactors. This gradual increase in temperature is used to compensate for the loss of enzyme activity. And this type of operation is expected since the ratio of activation energy of the main hydrolysis reaction to that of the enzyme deactivation (for thermo- and mesophlic enzymes) is less than 1 [10]. Accordingly, the temperature must be gradually increased to maintain the outlet lactose conversion constant.

At a given enzyme concentration (Figs. 5-a, 5-b, 6-a, and 6-b), it is not always possible to obtain a constant conversion during the whole reactor operating period for both types of reactors and enzymes respectively. This depends on the length of the operating period, reactor residence time, enzyme type and the required degree of conversion. Using a large residence time of 90 min and enzyme concentration of 1300 mg/L and 120 mg/L for mesophilic and thermophilic enzymes respectively, it is possible for mesophilic enzyme to obtain a constant conversion in all the operating periods studied, till the end of the period. While for thermophilic enzyme it is not possible to obtain a constant conversion. For mesophilic enzyme, operating the reactor at 60 min residence time, a constant conversion of 0.4 for CSTR and 0.6 for PFR, can be achieved only in operating time less than 9.5 days (228.5 hrs) and 3.4 days (81 hrs) respectively (figs. 5-a and 5-b). After this time, we operate the reactor at constant temperature of 51.7 °C for CSTR and 55.9 °C for PFR respectively which will result in almost linear decreasing conversion profiles (Figs. 1-b and 2-b). While operating the reactor at low residence time of 45 min, it is not possible to obtain a constant conversion of 0.4 for CSTR and 0.6 for PFR after operating time of 1.9 days (46 hrs) and 0.8 days (19.2 hrs) respectively. The maximum feasible temperature we can work with is 57.8 °C and 62.5 °C respectively. Similarly, for thermophilic enzyme at a concentration of 120 mg/L and τ of 60 min a constant conversion of 0.4 for PFR can be achieved only in operating time less than 2.7 days (63.6 hrs) and 3.3 days (79.4 hrs) for CSTR at conversion of 0.3 (Figs. 6-a and 6-b). The maximum feasible temperatures are 63.6 °C and 62.5 °C respectively (See also figs. 3-a and 4-a).

As it can be seen from figs. 1-c, 2-c, 3-c, and 4-c that the enzyme profiles can be divided into two distinct regions. The first one corresponds to the increasing temperature profile (constant conversion operation) followed by the constant temperature profile (falling conversion operation). The effect of enzyme concentration on the operating temperature and conversion profiles is shown in figs. 7-a, 7-b, 8-a, 8-b, 9-a, and 9-b. It is clear that constant

conversions can be achieved at higher enzyme concentrations. The effect of reactor residence time on the operating temperature, conversion and enzyme activity profiles is shown in figs. 5-a, 5-b, 6-a, and 6-b. It is clear that the temperature rise with time is small, almost isothermal operation, when operating the reactor at relatively high residence time such as 90 min. The temperature rise is significant when operating the reactor at relatively low residence time of 45 min. As it can be seen from figs. 9-a, 9-b, 10-a, and 10-b that operating the reactor at high degree of conversions results into higher temperature profiles.

List of Symbols

A, B	Parameters of reactor design equation
a	Residual enzyme activity
C_E	Concentration of active enzyme **(**mg/L)
C_{E0}	Initial concentration of active enzyme **(**mg/L)
C_G	Galactose concentration (mol/L)
C_s	Lactose concentration (mol/L)
C_{s0}	Initial Lactose concentration (mol/L)
E	Enzyme
E_a	Activation energy (kJ/mol)
EP	Enzyme galactose complex
ES	Enzyme lactose complex
G	Glucose
$k_1, k_{-1}, k_2, k_{-2}, k_3, k_{-3}$	Specific rate constants
k_d	Enzyme deactivation rate constant (min^{-1})
k_i	Inhibition constant (mol/L)
k_m	Michaelis-Menten constant (mol/L)
k_{m1}, k_{m2}	Component constants of k_m (mol/L)
k_p	Reaction constant
P	Galactose
R	Ideal gas constant (kJ/mol K)
T	Temperature (K)
T_0	Initial temperature (K)
t	Time (days)
t_f	Reactor operating period (days)
x	Lactose conversion

x_0	Initial lactose conversion
Greek Symbols	
τ	Reactor residence time (h)
τ_D	Deactivation time constant (min)

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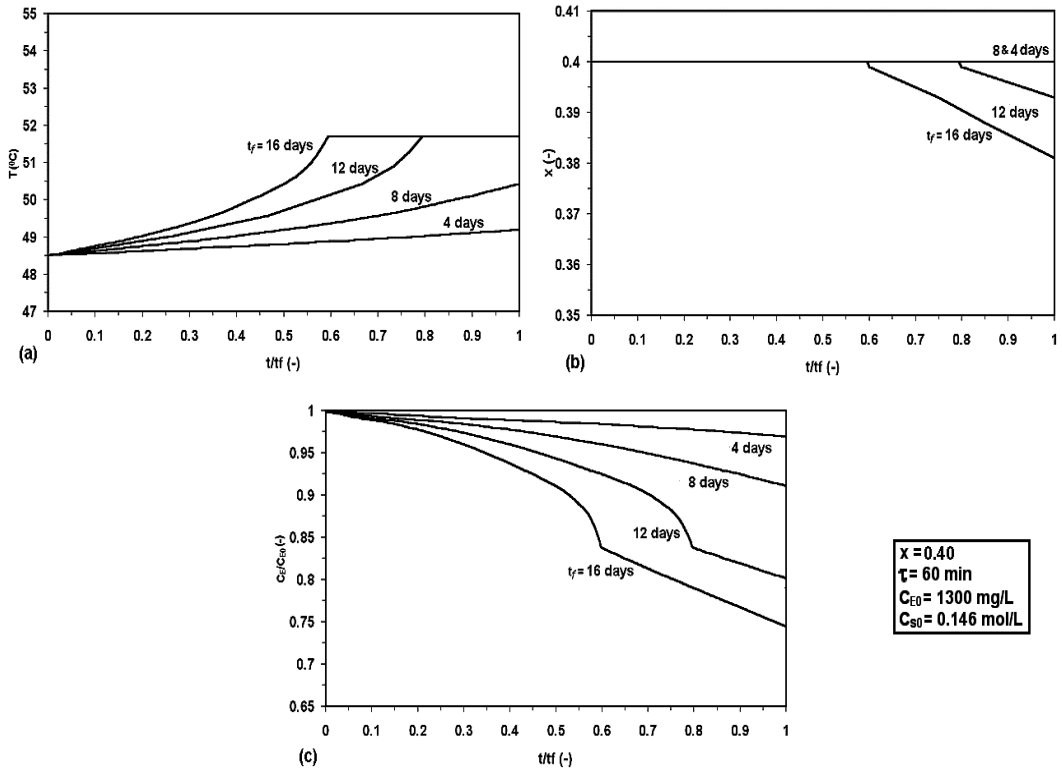

Fig. 1: (a) Temperature profiles, (b) Conversion profiles, (c) Activity profiles of an immobilized mesophilic lactose hydrolysis CSTR at different operating periods, residence time of 60 min and enzyme concentration of 1300 mg/L.

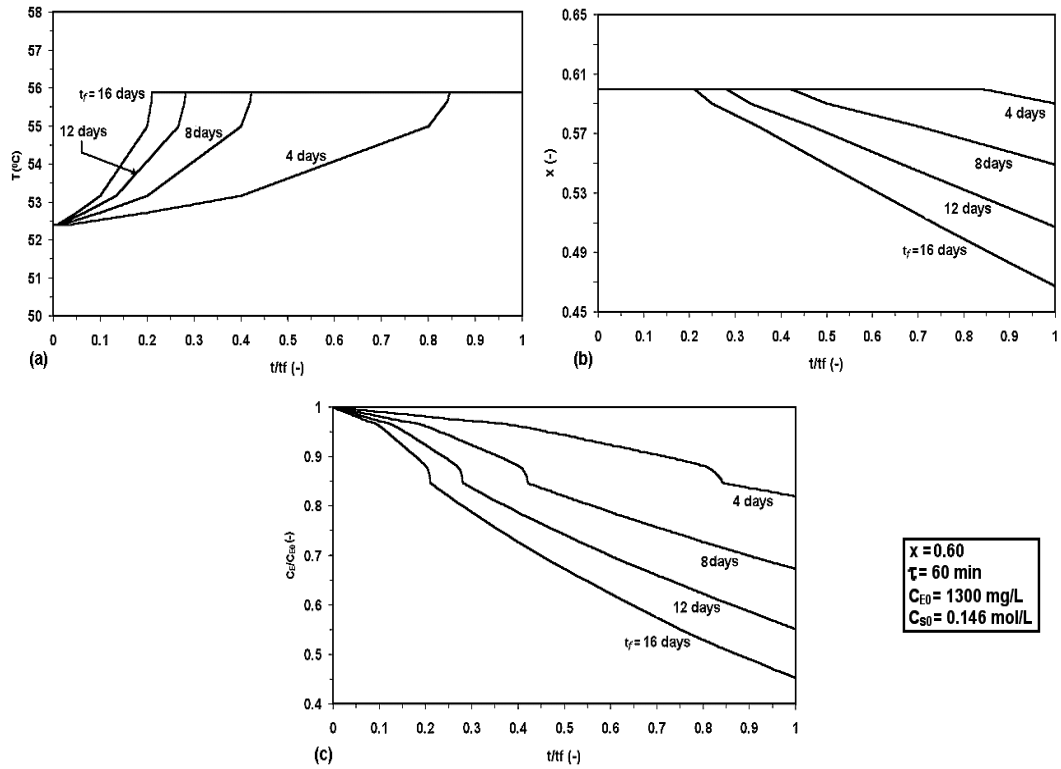

Fig. 2: (a) Temperature profiles, (b) Conversion profiles, (c) Activity profiles of an immobilized mesophilic lactose hydrolysis PFR at different operating periods, residence time of 60 min and enzyme concentration of 1300 mg/L.

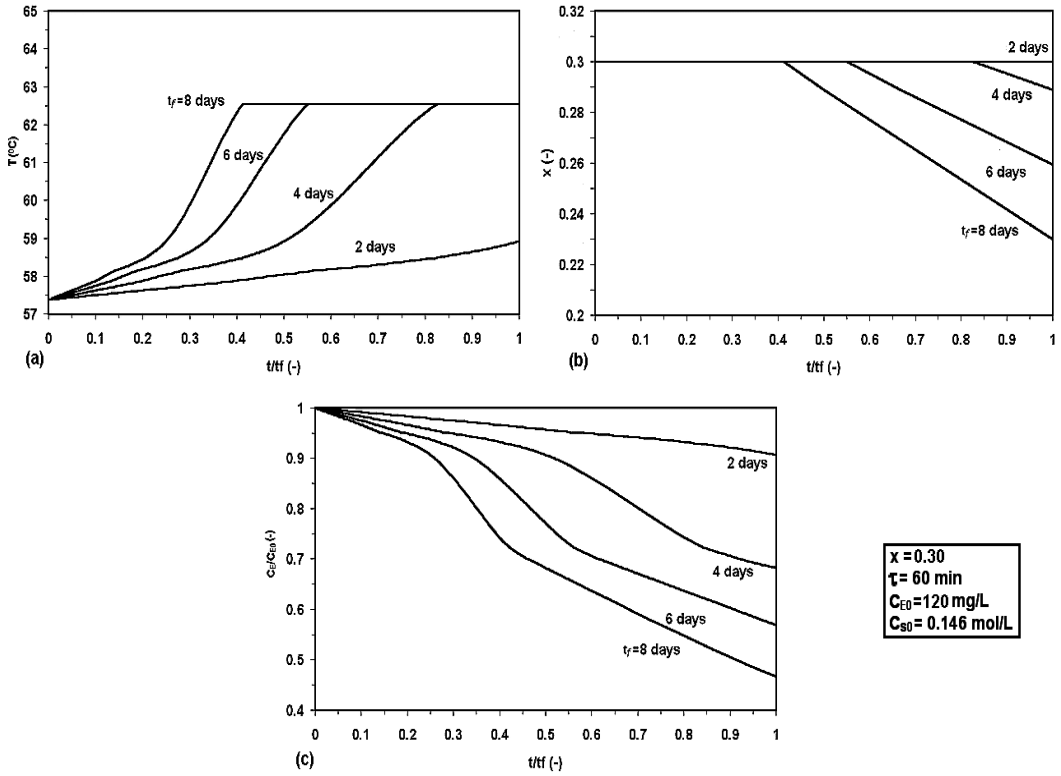

Fig. 3: (a) Temperature profiles, (b) Conversion profiles, (c) Activity profiles of an immobilized thermophilic lactose hydrolysis CSTR at different operating periods, residence time of 60 min and enzyme concentration of 120 mg/L.

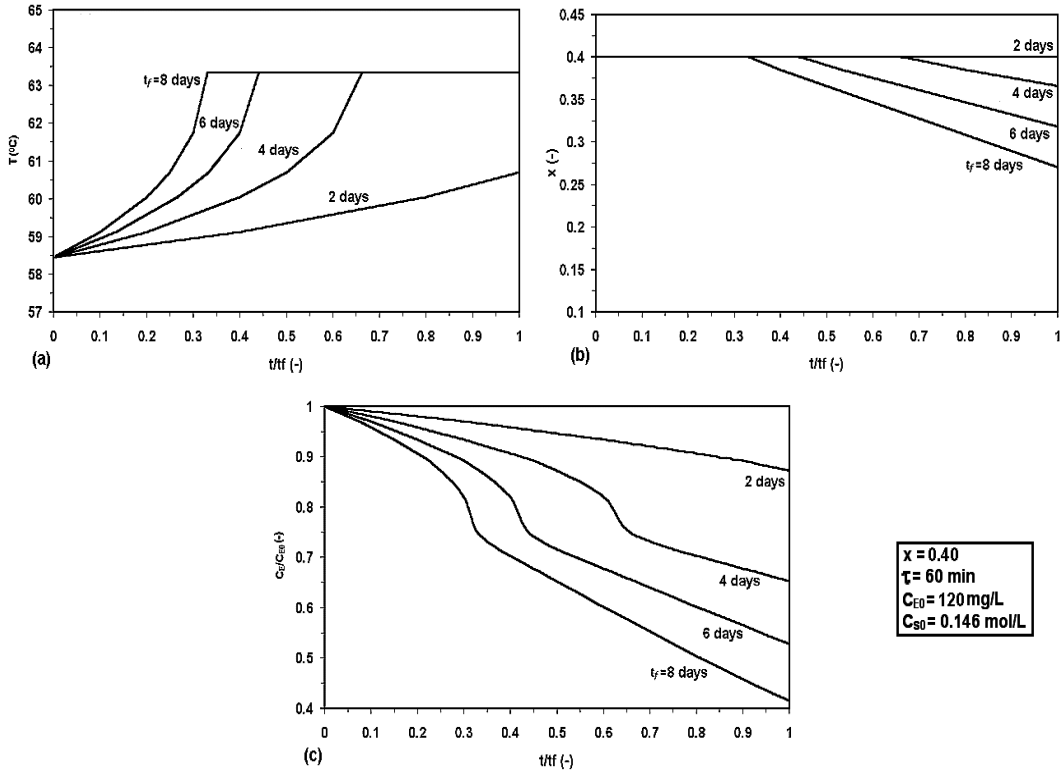

Fig. 4: (a) Temperature profiles, (b) Conversion profiles, (c) Activity profiles of an immobilized thermophilic lactose hydrolysis PFR at different operating periods, residence time of 60 min and enzyme concentration of 120 mg/L.

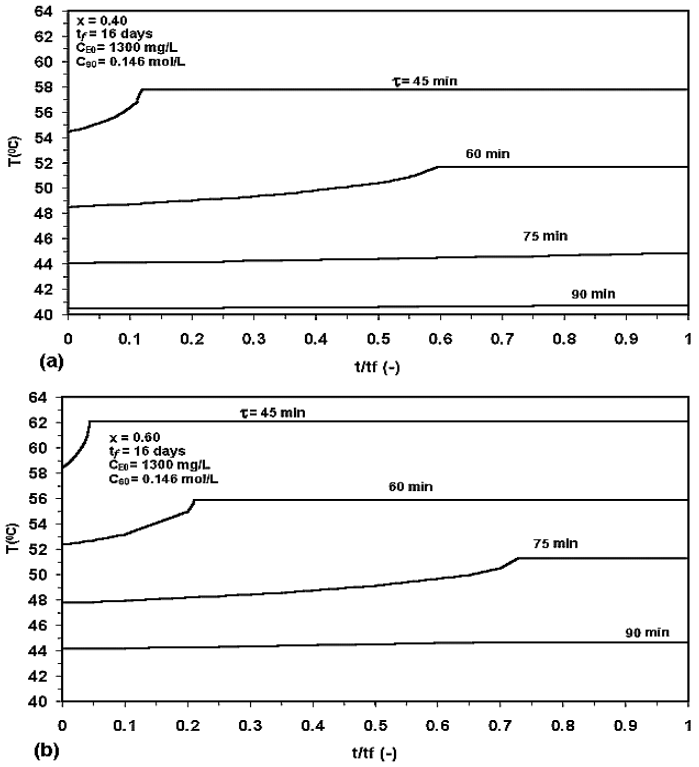

Fig. 5: Temperature profiles of an immobilized mesophilic lactose hydrolysis (a) CSTR, (b) PFR at different residence times and operating period of 16 days at enzyme concentration of 1300mg/L.

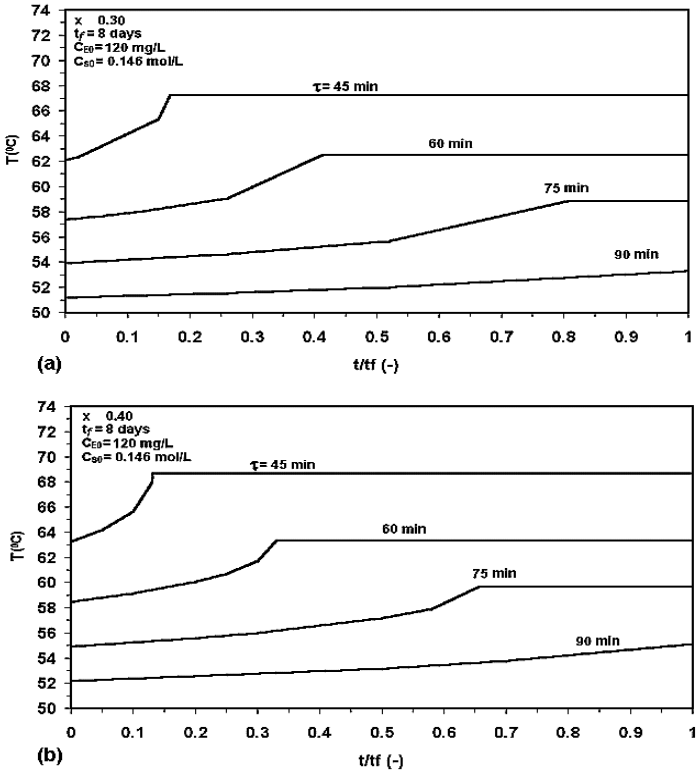

Fig. 6: Temperature profiles of an immobilized thermophilic lactose hydrolysis (a) CSTR, (b) PFR at different residence times and operating period of 8 days at enzyme concentration of 120mg/L.

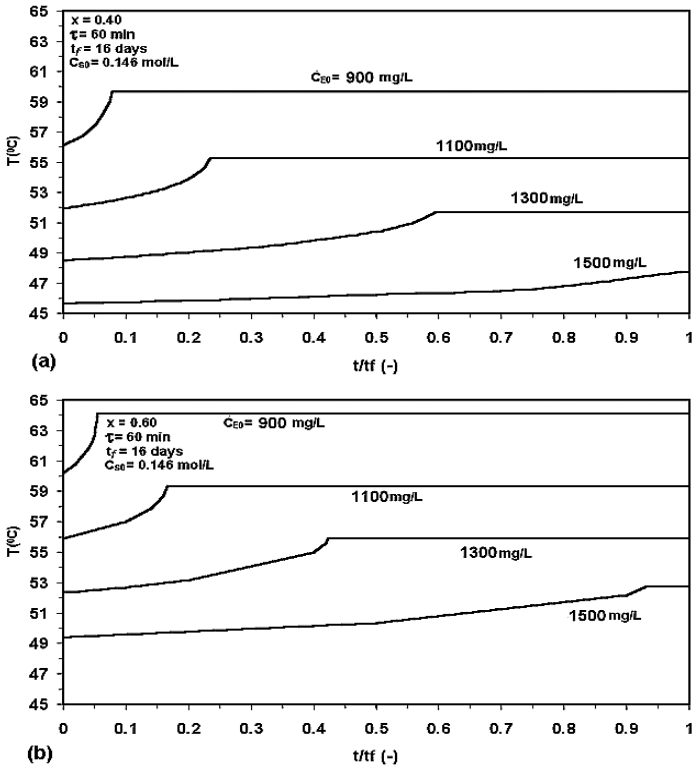

Fig. 7: Temperature profiles of an immobilized mesophilic lactose hydrolysis (a) CSTR, (b) PFR at different enzyme concentration and operating period of 16 days at a residence time of 60 min.

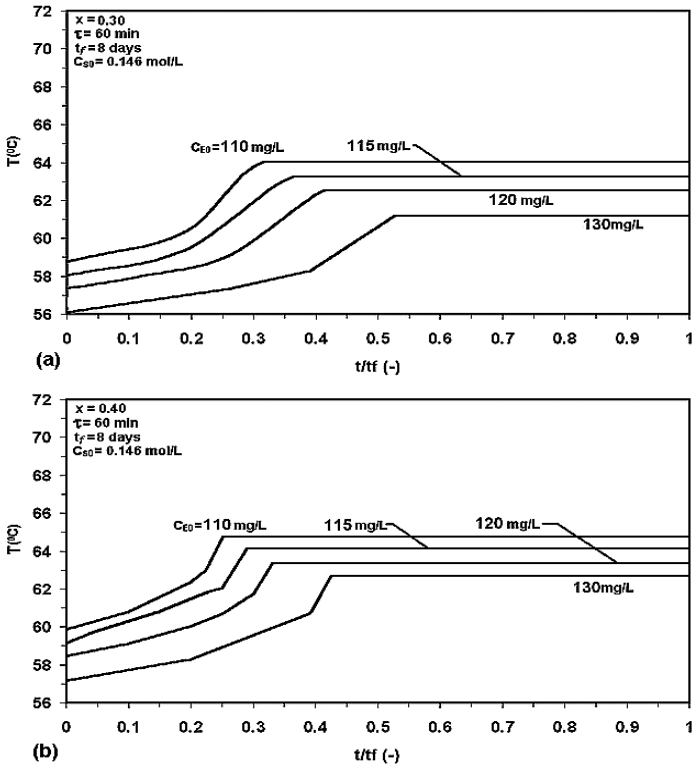

Fig. 8: Temperature profiles of an immobilized thermophilic lactose hydrolysis (a) CSTR, (b) PFR at different enzyme concentrations and operating period of 8 days at a residence time of 60 min.

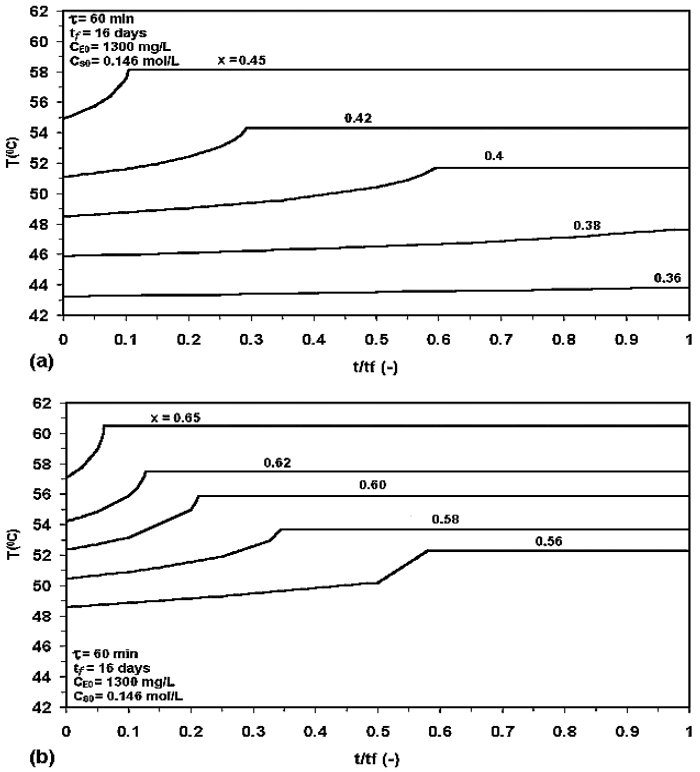

Fig. 9: Temperature profiles of an immobilized mesophilic lactose hydrolysis (a) CSTR, (b) PFR at different initial conversions and operating period of 16 days at a residence time of 60 min.

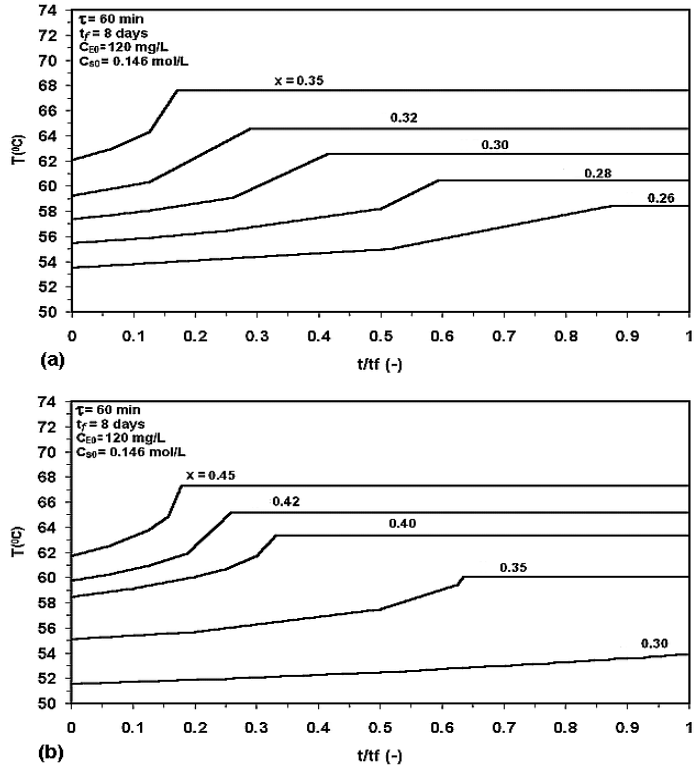

Fig. 10: Temperature profiles of an immobilized thermophilic lactose hydrolysis (a) CSTR, (b) PFR at different initial conversions and operating period of 8 days at a residence time of 60 min.

Table 1: k_0 and E_a / R for reaction rate constants for thermophilic β-galactosidase

Parameter	Unit	k_0	E_a / R (K)
k_2	$mole.mg^{-1}.min^{-1}$	3.21×10^5	7830
k_m	$mole/L$	5.58×10^8	7851
k_i	$mole/L$	5.88×10^7	7505
k_d	min^{-1}	7.27×10^{21}	2.012×10^4

Table 2: k_0 and E_a / R for reaction rate constants for mesophilic β-galactosidase

Parameter	Unit	k_0	E_a / R (K)
k_2	$mole/L..mL.mg^{-1}.min^{-1}$	2.57×10^5	4.2×10^3
k_{m1}	$mole/L$	3.02×10^{-2}	1×10^2
k_{m2}	$mole/L$	1.34×10^{-3}	-1.12×10^3
k_i	$mole/L$	1.20×10^{-4}	2.01×10^2
k_p	-	4.57	-7.25×10^2
k_d	min^{-1}	4.4×10^{32}	2.81×10^4