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A modelling study on hydrolysis of whey lactose and stability of β-galactosidase

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Abstract−In the present study, the effect of process conditions on whey lactose hydrolysis and enzyme inactivation were investigated. The experiments were carried out in 250 mL of 25 mM phosphate buffer solution by using β-galactosidase produced from *Kluyveromyces marxianus lactis* in a batch reactor system. The degree of lactose hydrolysis (%) and residual enzyme activity (%) against time were investigated versus lactose concentration, enzyme concentration, temperature and pH. The mathematical models were derived from the experimental data to show the effect of process conditions on lactose hydrolysis and residual enzyme activity (in the presence and absence of lactose). At the optimum process conditions obtained (50 g/L of lactose concentration, 1 mL/L of enzyme concentration, 37 °C of temperature and pH 6.5), 81% of lactose was hydrolyzed and enzyme lost its activity by 32%. The activation energy for hydrolysis reaction (E_A) and the enzymatic inactivation energy (E_D) were calculated as 52.7 kJ/mol and 96.7 kJ/mol. Mathematical models at various process conditions have been confirmed with the experimental results.

Key words: Whey Lactose Hydrolysis, β-Galactosidase, Process Conditions, Kinetic Study, Modelling

INTRODUCTION

Lactose is a disaccharide found in milk, whey and other dairy products. It has low solubility and low sweetness compared to its hydrolysis products; glucose and galactose [1-4]. Lactose hydrolysis has been studied due to the several reasons. The main reason is that lactose is scarcely digestible for non-Caucasian people (approx. 70% of world population) [2,5]. Every year, 3.2 million tonnes of lactose, dissolved in whey (contains about 6% solids of which 70% or more is lactose and about 0.7% is proteins), the major by-product of cheese and casein manufacture, is discarded as waste, which causes environmental pollution [6-10]. After the lactose hydrolysis, the biodegradability of whey is increased [8,11,12]. Furthermore, the hydrolysis process increases the solubility and the sweetness, resulting in improvement of sensorial characteristics of foods containing hydrolyzed lactose from milk or whey. For the production of ice cream and other refrigerated dairy products, positive effects on the crystallization would be achieved after lactose hydrolysis [6,11].

In the literature, two methods have been applied for the lactose hydrolysis: acidic and enzymatic hydrolysis [5]. The enzymatic hydrolysis of lactose is more adequate than acidic hydrolysis, because enzymatic hydrolysis does not cause bad flavours, odours and colours [8,11]. Also, this process does not require high temperature. The enzymatic hydrolysis of lactose is carried out by β-galactosidases, which are found in animals, plants and microorganisms. However, in industry, only the enzymes from microorganisms are used for lactose hydrolysis [13].

The hydrolysis of lactose to glucose and galactose by β-galactosidases has been studied by several authors due to the reasons mentioned above [1,3,5-7,11-22]. In the present study, therefore, the effects of process conditions on the whey lactose hydrolysis and the enzyme stability were investigated with respect to processing time, using a batch reactor system. Kinetics of lactose hydrolysis reaction and enzyme inactivation were examined at various process conditions, and mathematical models depending on these process conditions were also developed. Activation energy for hydrolysis reaction (E_A) and enzymatic inactivation energy (E_D) were calculated by using the data obtained from the temperature experiments.

MATERIALS AND METHODS

1. Bioreactor

A Gallenkamp Modular Bioreactor system (Model No: FER-195-010, manufactured by Sanyo Gallenkamp PLC, Loughborough) was used for lactose hydrolysis. The controls of various parameters including impeller speed, pH and temperature were performed by its modules. The 1.0 litre vessel (round bottom design) was constructed of glass and stainless steel with an aspect ratio (height/diameter) of 1.545. The important design details were as follows: operating volume, 0.5 litre; internal diameter, 11 cm; height, 17 cm; number of baffles, 4; baffle height, 13.5 cm; baffle width, 1.5 cm, number of impellers, 1; location of impeller from top plate, 14 cm; location of impeller from bottom plate, 3 cm; type of impeller, Rushton disc turbine; impeller diameter of disc, 4.8 cm; impeller blade width, 1.4 cm; impeller blade length, 1.9 cm; number of blades, 6. The proportionality of diameter of impeller to diameter of tank (D/T) was 0.436.

2. Materials

Spray-dried whey lactose was a gift from Maybi Company. It was obtained from pasteurized raw whey by ultra filtration, demineralization and drying. β-Galactosidase enzyme used is obtained from DSM Food Specialties with a commercial name of Maxilact LX 5000 in a liquid form (produced by *Kluyveromyces marxianus lactis* with a product code: EC 3.2.1.23) and stored at 4 °C in the

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refrigerator. The activity of enzyme is 5000 Neutral Lactase Unit (NLU) per gram enzyme (or 6240 NLU per mL enzyme).

3. Determination of Lactose Concentration

For determination of the residual lactose concentration [23], the samples were taken from the reaction solution at timed intervals. In the samples taken, the hydrolysis reaction was stopped by adding 5 N HCl (50 μL) and they were diluted with distilled water. The glucose amount in these samples was determined by using a glucose test kit obtained from Elitech Company (product code: 02-0541). Then, these values were used for calculation of the remaining lactose amount in the reaction medium. For each sample, the assay was carried out in triplicate and their averages were taken. In this study, all experiments were carried out at least in duplicate and the reproducibility between trials was within ±5%.

4. Determination of β-Galactosidase Activity

To determine the enzyme activity, samples (0.1 mL) were taken from the reaction solution at specific time intervals. By addition of the 2 mL of ONPG (Ortho-nitrophenyl-β-D-galactopyranoside) solution (prepared by solving 300 mg ONPG in 100 mL pH 7.4 100 mM phosphate buffer solution) into these samples, the hydrolysis of ONPG with β-galactosidase was provided. The mixture was kept at 28 °C for 5 min, and then the reaction was stopped by adding 1 M Na_2CO_3 solution. Reference solution was prepared by adding the ONPG and 1 M Na_2CO_3 in the sample which does not contain enzyme. Then, by considering the yellow colour of ONP (ortho-nitrophenol), which is a result of ONPG hydrolysis, the absorbance values of samples against reference were measured at 420 nm [5, 13,19,20]. For each sample, the assay was carried out in triplicate and their averages were taken; and also the experiments were carried out at least in duplicate and the reproducibility between trials was within ±5%. Enzyme activities prior to hydrolysis process were also determined as the initial activities. In calculations, these activities

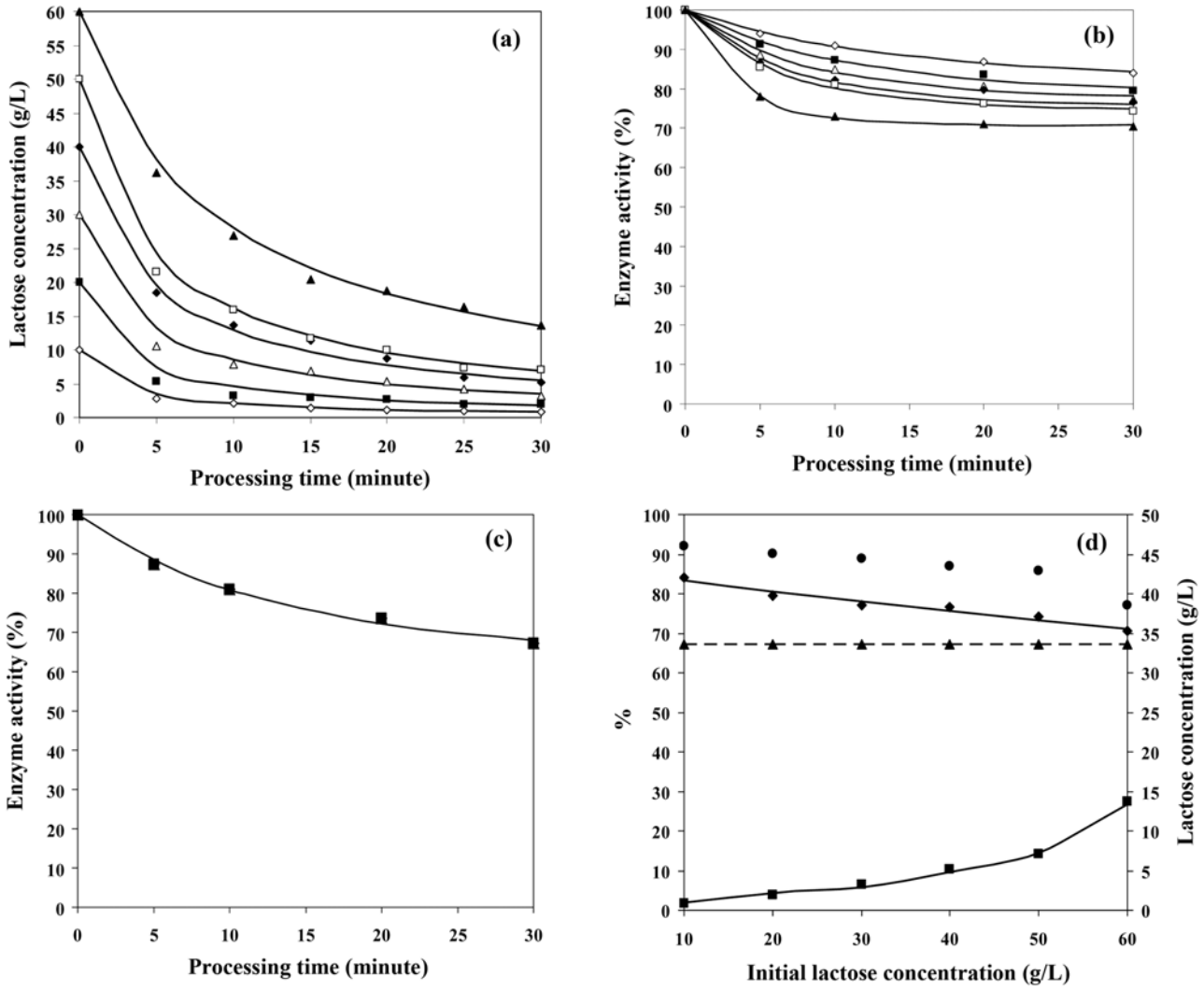

Fig. 1. Effect of initial lactose concentration on lactose hydrolysis and β-galactosidase stability. (a) Residual lactose concentration; (b) Residual enzyme activity (%) in the presence of lactose and (c) residual enzyme activity (%) in the absence of lactose versus processing time; (◇ 10 g/L; ■ 20 g/L; △ 30 g/L; ◆ 40 g/L; □ 50 g/L; ▲ 60 g/L; — models); (d) Residual enzyme activity (%), hydrolysis degree (%) and residual lactose concentration versus initial lactose concentration (◆ enzyme activity (with lactose); ▲ enzyme activity (without lactose); ● hydrolysis degree; ■ lactose concentration; — models) (at conditions of E=3 mL/L, pH= 6.5, T=37 °C, N=300 rpm).

determined as 100% activity. Activities at any operational conditions (A) were then obtained as the percentage values of the initial activities.

5. Experimental Conditions Used for Lactose Hydrolysis

To investigate the hydrolysis of lactose recovered from whey and enzyme activity, experiments were performed at various process variables such as lactose concentration, enzyme concentration, temperature and pH. The experiments, which were carried out at least in duplicate and the reproducibility was within the range of ±5%, were carried out in 250 mL of 25 mM phosphate buffer solution in the presence or absence of the lactose.

6. Computational Work

The software package MATLAB 5.0 was used in the numerical calculations. The parameters were evaluated by the nonlinear least squares method of Marquardt-Levenberg until minimal error was achieved between experimental and calculated values. The residual (SSR) is defined as the sum of the squares of the differences between experimental and calculated data and is given by

$$SSR = \sum_{m=1}^{N_d} (C_m^{obs} - C_m^{cal})^2$$

where m is the observation number and N_d is the total number of observations. The estimated variance of the error (population variance) is calculated by the SSR at its minimum divided by its degrees of freedom:

$$\sigma^2 \approx s^2 = \frac{(SSR)_{min}}{(m-p)}$$

where p is the number of parameters and s^2 is the variance. The standard error, σ(the estimated standard deviation), is calculated by taking the square root of the estimated variance of the error.

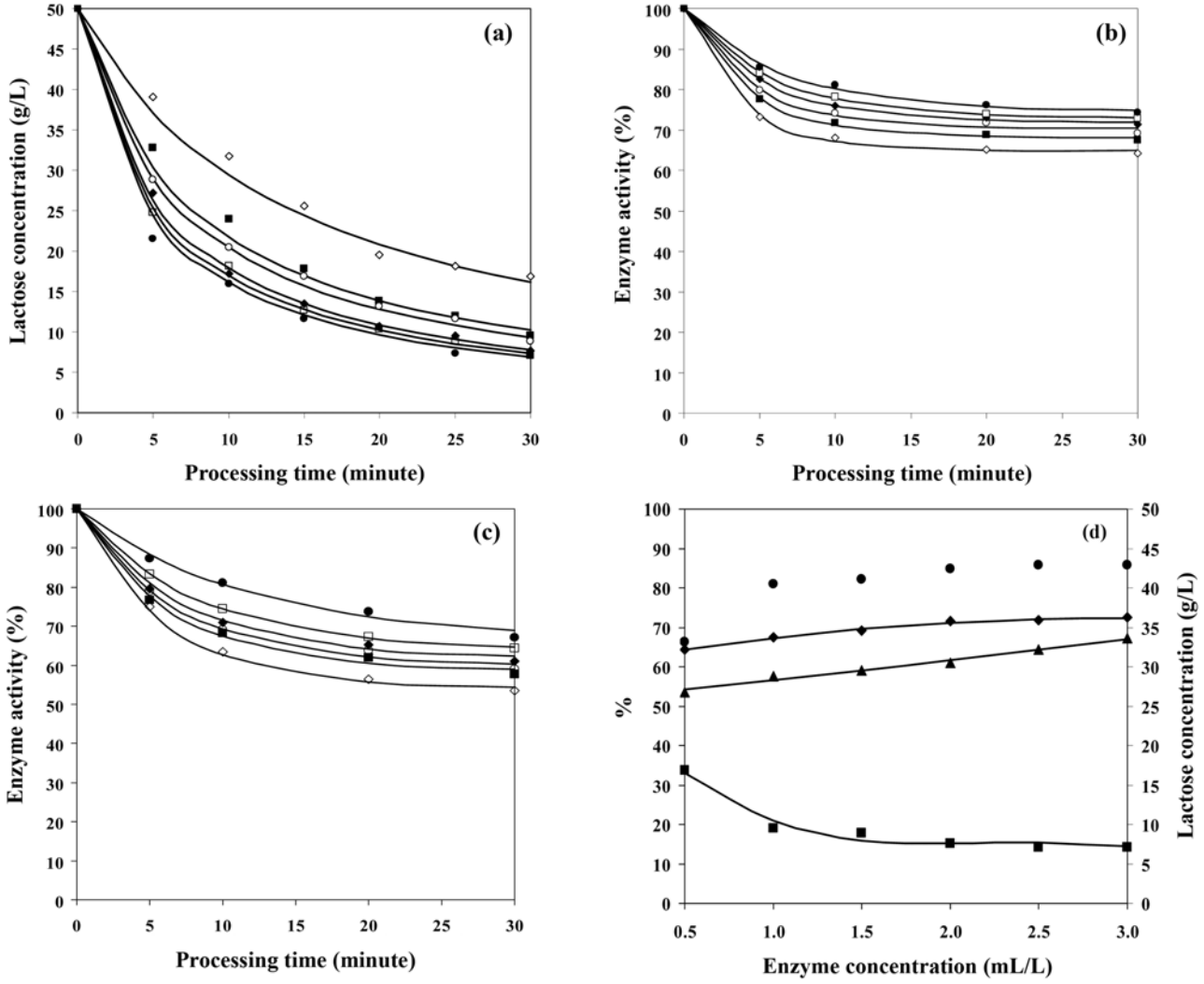

Fig. 2. Effect of enzyme concentration on lactose hydrolysis and β-galactosidase stability. (a) Residual lactose concentration; (b) Residual enzyme activity (%) in the presence of lactose and (c) residual enzyme activity (%) in the absence of lactose versus processing time; (◇ 0.5 mL/L; ■ 1 mL/L; ○ 1.5 mL/L; ◆ 2 mL/L; □ 2.5 mL/L; ● 3 mL/L; — models); (d) Residual enzyme activity (%), hydrolysis degree (%) and residual lactose concentration versus enzyme concentration (◆ enzyme activity (with lactose); ▲ enzyme activity (without lactose); ● hydrolysis degree; ■ lactose concentration; — models) (at conditions of lactose concentration=50 g/L, pH=6.5, T=37 ºC, N=300 rpm).

RESULTS AND DISCUSSION

1. Effect of Lactose Concentration on Hydrolysis and Enzyme Activity

For determination of the effect of lactose concentration on hydrolysis and enzyme stability, the experiments were performed at various lactose concentration values ranging from 10 to 60 g/L for 30 min at process variables: 3 mL enzyme per litre, 37 °C, pH 6.5 and 300 rpm impeller speed. The stability behaviour of β-galactosidase enzyme was also investigated in lactose-free medium at the same process conditions. The results are shown in Fig. 1(a)-(d).

As can be seen from Fig. 1(a)-(c), the residual lactose concentration and residual enzyme activity in the presence and absence of lactose decrease with respect to time for all initial lactose concentrations used, as expected. On the other hand, the hydrolysis degree and the enzyme activity decrease as initial lactose concentration increases (Fig. 1(d)). The hydrolysis degree decreases considerably above 50 g/L initial lactose concentration. At this concentration, the hydrolysis degree was found as 86%. So, the optimum lactose concentration was chosen as 50 g/L as found in the study performed by Szczodrak [7].

As the residual enzyme activity values were compared for the conditions of presence and absence of lactose, it was found that the residual enzyme activity was lower in the absence of lactose. This result indicated that there is a protective effect of substrate molecules upon the enzyme denaturation, such as the negative influences of physical or chemical factors like temperature or pH on the stability could be prevented with the presence of lactose in the reaction medium. The protective effect of the presence of the substrate or substrate like compounds was also stated by Toscano et al. [24]. Besides the protective effect, there is also an inhibitory effect of substrate, as can be seen from Fig. 1(d); the hydrolysis degrees were

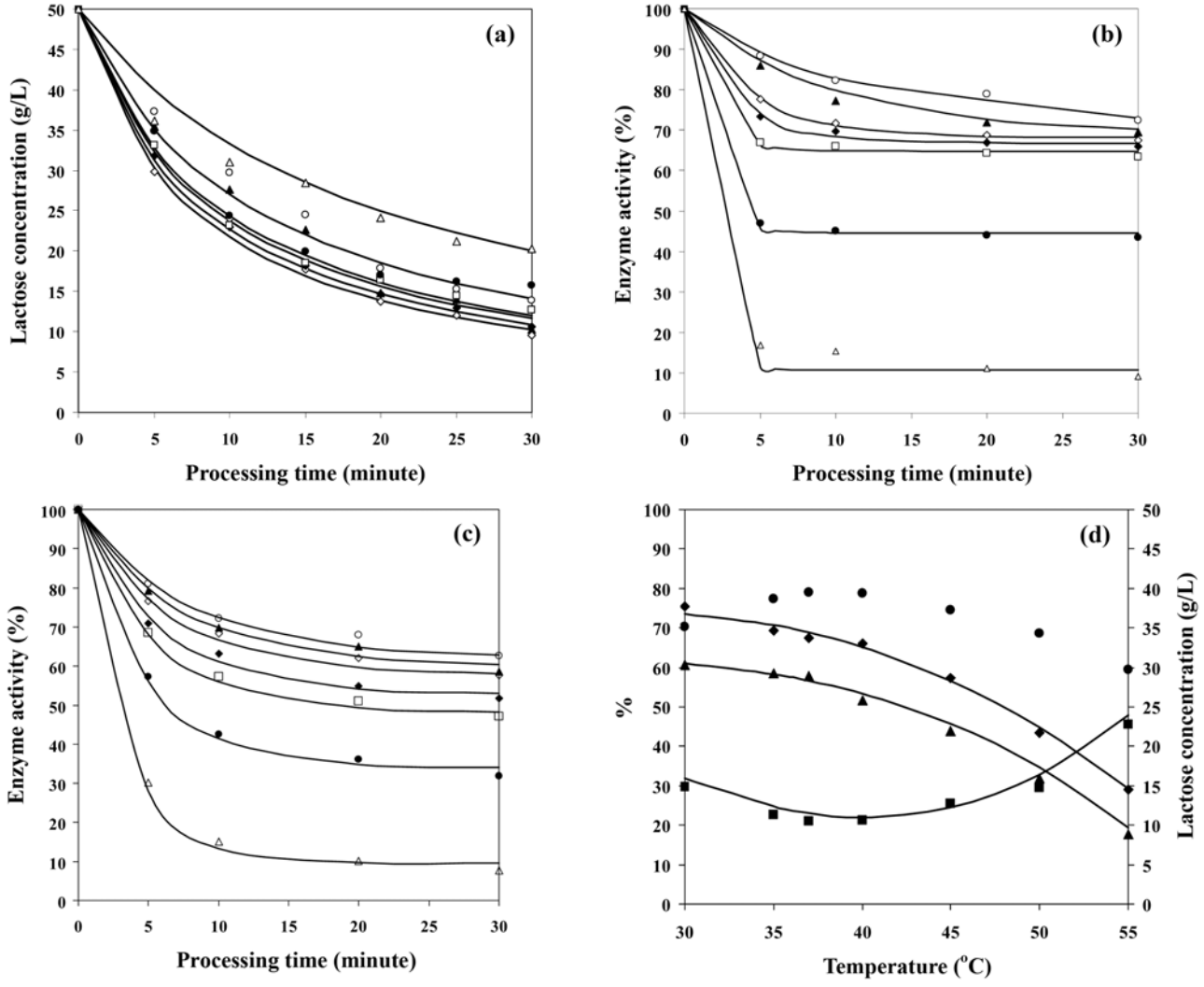

Fig. 3. Effect of temperature on lactose hydrolysis and β-galactosidase stability. (a) Residual lactose concentration; (b) Residual enzyme activity (%) in the presence of lactose and (c) residual enzyme activity (%) in the absence of lactose versus processing time; (○ 30 °C; ▲ 35 °C; ◇ 37 °C; ◆ 40 °C; □ 45 °C; ● 50 °C; △ 55 °C; — models); (d) Residual enzyme activity (%), hydrolysis degree (%) and residual lactose concentration versus temperature (◆ enzyme activity (with lactose); ▲ enzyme activity (without lactose); ● hydrolysis degree; ■ lactose concentration; — models) (at conditions of lactose concentration=50 g/L, pH=6.5, E=1 mL/L, N=300 rpm).

decreased with the increase on substrate concentration. If the inhibition was irreversible, it was expected that the residual enzyme activity values would decrease with the increase on initial substrate concentration.

2. Effect of Enzyme Concentration on Hydrolysis and Enzyme Activity

To investigate the effect of enzyme concentration on hydrolysis process and enzyme activity, experiments were performed at the enzyme concentrations ranging from 0.5 to 3 mL/L at process variables such as 37 ºC, pH 6.5 and 300 rpm impeller speed.

Fig. 2(a)-(c) shows the effect of enzyme concentration on the residual lactose concentration and residual β-galactosidase activity (in the presence and absence of lactose) versus processing time, respectively. The residual lactose concentration and the residual enzyme activity values decreased with respect to time, as expected. It was found that the residual enzyme activity in the absence of lactose was lower than the value obtained in the presence of lactose.

The residual lactose concentration and the residual enzyme activity (in the presence and absence of lactose) versus enzyme concentration profiles at the end of processing time of 30 minutes are presented in Fig. 2(d). By increasing the enzyme concentration from 0.5 to 3.0 mL/L, the hydrolysis degree and residual enzyme activity values increased from 66.3 to 85.8% (approx. 29.4% increased) and from 64.3 to 72.4% (approx. 12.7% increase), respectively. Likewise, by increasing enzyme concentration in the absence of lactose, the residual enzyme activity increased from 53.6 to 67.1% (approx. 25.3% increase). The increase on the enzyme stability with the increase of initial enzyme concentration could be explained with some kind of enzyme-enzyme interaction. Also, the hydrolysis products, which increased as a result of increased enzyme concentration, may have a stabilizing effect on enzyme. Above 1 mL/L enzyme concentration, the hydrolysis rate was not changed significantly. These results indicate that a concentration somewhere in the range of 1-3 mL/L must saturate the lactose molecules with active

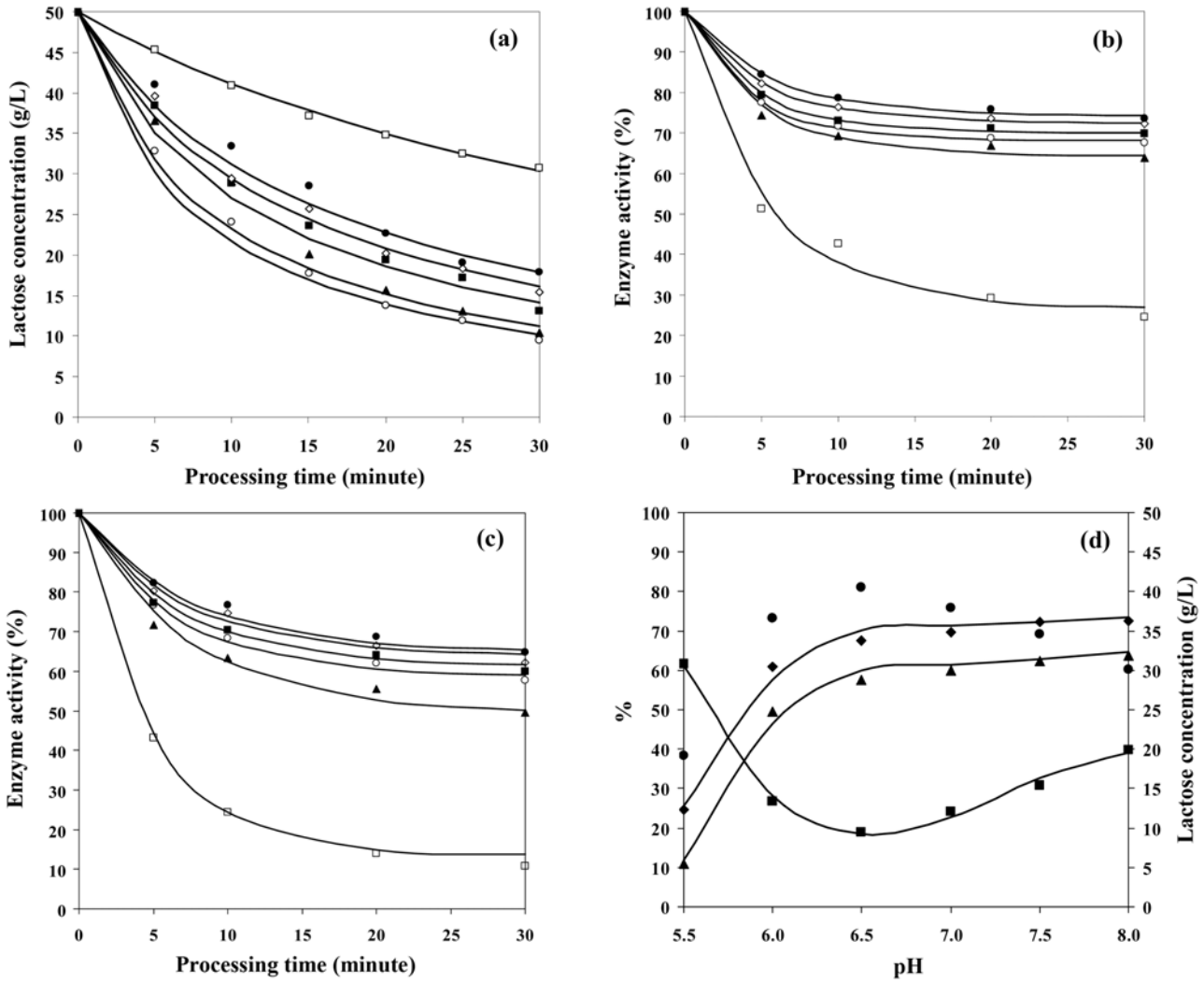

Fig. 4. Effect of pH on lactose hydrolysis and β-galactosidase stability. (a) Residual lactose concentration; (b) Residual enzyme activity (%) in the presence of lactose and (c) residual enzyme activity (%) in the absence of lactose versus processing time; (□ 5.5; ▲ 6; ○ 6.5; ■ 7; ◇ 7.5; ● 8; — models); (d) Residual enzyme activity (%), hydrolysis degree (%) and residual lactose concentration versus pH values (◆ enzyme activity (with lactose); ▲ enzyme activity (without lactose); ● hydrolysis degree; ■ lactose concentration; — models) (at conditions of lactose concentration=50 g/L, T=37 ºC, N=300 rpm, E=1 mL/L).

enzyme molecules. Because of economical reasons, it is desired to use as low enzyme concentrations as possible in order to decrease the costs of the hydrolysis process. Therefore, the optimum enzyme concentration was chosen as 1 mL/L. At the end of 30 minutes of the processing time for 1 mL/L enzyme concentration, the hydrolysis degree was found as 81% and β-galactosidase enzyme in the presence and absence of lactose lost 32% and 42% activities, respectively.

3. Effect of Temperature on Hydrolysis and Enzyme Activity

The effect of temperature on lactose hydrolysis and enzyme activity was investigated in the range of 30-55 °C at process variables (1 mL/L enzyme, pH 6.5 and 300 rpm impeller speed) and the results are given in Fig. 3(a)-(d).

The residual lactose concentration and the residual enzyme activity (in the presence and absence of lactose) decrease with respect to time for all temperatures, as expected (Fig. 3(a)-(c)). As can be seen from Fig. 3(d), the residual lactose concentration with respect to temperature decreases up to 37 °C. Above this temperature, the residual lactose concentration increases as a result of enzyme inactivation. On the other hand, the residual enzyme activity (in the presence and absence of lactose) decreases even further after 40 °C due to inactivation by temperature. In the literature, it has been reported that temperatures over 45 °C cause a significant inactivation of enzyme [5]. It was also found that in the absence of lactose, the residual enzyme activity was lower than the value obtained in the presence of lactose for all temperature values.

For this study, the optimum temperature for lactose hydrolysis with concerning residual enzyme activity was found as 37 °C. At this temperature, the degree of hydrolysis and the residual enzyme activity in the presence and absence of lactose were 81%, 67.6% and 57.7%, respectively, at the end of a processing time of 30 minutes.

4. Effect of pH on Hydrolysis and Enzyme Activity

The aim of this investigation was to establish the effect of pH on hydrolysis and β-galactosidase activity. The experiments were performed at various pH values in the range of 5.5-8.0 for the processing time of 30 minutes at 37 °C, 300 rpm impeller speed and 1 mL enzyme per liter. The results are shown in Fig. 4(a)-(d).

As can be seen from Fig. 4(a)-(c), the residual lactose concentration and the residual enzyme activity (in the presence and absence of lactose) decrease with respect to time for all pH values. In the absence of lactose, the residual enzyme activity for all pH values

Table 1. Lactose conversion ratios ($X_{conv.}$) of various studies compared with the present study

	Lactose	Enzyme	Operating conditions	$X_{conv.}$
Ladero et al. [13]	50 g/L in milk buffer	β-galactosidase from *Escherichia coli*	at 40 °C, 300 rpm, pH 7, at the end of 30 min, in a batch reactor	12% and 7% at enzyme conc. of 7 and 3.5 mg/mL, resp.
Pessela et al. [1]	50 g/L in Novo buffer	Thermophilic β-galactosidase	at 50 °C, pH 6.5, at the end of 30 min, in a batch reactor	15% at enzyme conc. of 60 mg/mL
Di Serio et al. [26]	9.58, 23.6 and 47.54 g/L in phosphate buffer	Immobilised β-galactosidase from *Kluyveromyces marxianus lactis*	at 37 °C, pH 7.0, at the end of 1 h in continuous packed bed tubular reactor	80.2%, 63% and 50% at lactose conc. of 9.58, 23.60, 47.54 g/L, resp.
Roy and Gupta [12]	milk whey lactose and milk	Immobilized β-galactosidase from *Kluyveromyces fragilis* (Lactozym 3000L)	at 30 °C, pH 6.6, at the end of 30 min, in continuous batch mode (CBM) and fluidized bed mode (FBM)	Whey lactose: 94% in FBM 80% in CBM. Milk: 7% in FBM
Szczodrak [7]	whey permeate containing 50 g/L lactose	Immobilized β-galactosidase from *Kluyveromyces fragilis*	at 35 °C, pH 7.0, at the end of 48 h, in batch reactor (BR) and recycling packed bed bioreactor (RPBB)	96% in BR, 82.6%, 87.3% and 90% at flow rate of 2, 0.7 and 0.3 mL/min in RPBB, resp.
Haider and Husain [27]	Skim milk and whey	β-galactosidase from almond (*Amygdalus communis*)	at 50 °C, pH 5.5, at the end of 30 min, in stirred batch reactor	Whey: 64% Milk: 50% at enzyme conc. of 1130 U and 1700 U, resp.
Haider and Husain [28]	Skim milk and whey	Immobilized β-galactosidase from (*Aspergillus oryzae*)	at 32 °C, at the end of 1 h, in stirred batch reactor	Whey: 54% Milk: 45% at enzyme conc. of 400 U and 1000 U, resp.
Santos et al. [5]	50 g/L in milk buffer	β-galactosidase from *Kluyveromyces fragilis* (Lactozym 3000L)	at 40 °C, pH 6.5, at the end of 30 min, in a batch reactor	67%, 52%, 37% and 10% at enzyme conc. of 11.7, 7, 5.7 and 2.3 mg/mL, resp.
Şener et al. [29]	milk	β-galactosidase from *Kluyveromyces marxianus lactis* (Maxilact LX 5000)	at 37 °C, pH 6.7, at the end of 30 min, with or without sonication	90% with sonication, 84% without sonication, at enz. conc. of 1 mL/L
Present study	50 g/L whey lactose in phosphate buffer	β-galactosidase from *Kluyveromyces marxianus lactis* (Maxilact LX 5000)	at 37 °C, pH 6.5, at the end of 30 °min, in a batch reactor	81% at enzyme concentration of 1 mL/L

was lower than the value obtained in the presence of lactose. It is clearly seen from Fig. 4(d) that the residual lactose concentration decreases up to pH 6.5, and above this value the residual lactose concentration begins to increase. Therefore, the optimum pH was chosen as 6.5 for the lactose hydrolysis. In the literature, the optimum pH values are given in the range of 6.5-7.0 depending on the β-galactosidase used and its source [5].

A summary of the performance of lactose hydrolysis process--studied by several authors that employed in several types of reactors by using β-galactosidase obtained from different sources--is given in Table 1.

5. Modeling Study

To predict the kinetics of hydrolysis reaction, the data of residual lactose concentration versus processing time were evaluated for all process conditions, and a second order kinetic expression (Eq. (1)) was produced:

$$\frac{d[C_L]}{dt} = -k[C_L]^2, \ \frac{1}{C_L} = \frac{1}{C_{L0}} + k*t \quad (1)$$

where $[C_L]$ is residual lactose concentration (g/L), $[C_{L0}]$ is lactose concentration (g/L) at t=0 and k is hydrolysis kinetic constant (L/g·min).

On the other hand, to predict the effect of processing time on enzyme stability, the data of residual enzyme activity (in the presence and absence of lactose) with respect to processing time for all process conditions were evaluated; and the inactivation data was fitted to a single-step non-first-order enzyme inactivation kinetic model (Eq. (2)) given by Sadana and Henley [25].

$$A=(100-\alpha_1)\exp(-k_D t)+\alpha_1 \quad (2)$$

where A is residual enzyme activity (%), α_1 is the ratio of the specific activity of the final state to the initial state and k_D is the enzymatic inactivation constant (min^{-1}). The estimated coefficients and statistical values for Eqs. (1) and (2) are given in Table 2.

Moreover, the data of residual lactose concentration and residual enzyme activity versus all process conditions were also evaluated to predict the effect of these conditions on hydrolysis and enzyme stability (in the presence and absence of lactose) at the end of pro-

Table 2. Estimated parameters and statistical data of residual lactose concentration (Eq. (1)) and residual enzyme activity in the presence and absence of lactose (Eq. (2)) versus processing time

	Residual lactose concentration			Residual enzyme activity (with lactose)				Residual enzyme activity (without lactose)			
$[C_{L0}]$	k	σ	R^2	α_1	k_{D1}	σ	R^2	α_1	k_{D2}	σ	R^2
10	0.0375	0.0401	0.9948	82.51	0.0746	0.4487	0.9980	66.17	0.0839	1.0319	0.9974
20	0.0167	0.0289	0.9854	79.21	0.0957	1.0236	0.9936	66.17	0.0839	1.0319	0.9974
30	0.0084	0.0149	0.9862	77.63	0.1218	1.2361	0.9925	66.17	0.0839	1.0319	0.9974
40	0.0052	0.0112	0.9826	75.59	0.1379	0.7239	0.9972	66.17	0.0839	1.0319	0.9974
50	0.0042	0.0059	0.9914	74.64	0.1535	0.8797	0.9972	66.17	0.0839	1.0319	0.9974
60	0.0019	0.0031	0.9867	70.83	0.2771	0.2708	0.9998	66.17	0.0839	1.0319	0.9974
[E]	k	σ	R^2	α_1	k_{D1}	σ	R^2	α_1	k_{D2}	σ	R^2
0.5	0.0014	0.0021	0.9902	64.89	0.2761	0.6883	0.9992	57.06	0.1666	0.7957	0.9992
1.0	0.0029	0.0054	0.9851	68.14	0.2348	0.5628	0.9993	58.69	0.1545	1.4207	0.9973
1.5	0.0029	0.0040	0.9921	70.33	0.2198	0.9916	0.9976	59.87	0.1454	1.3281	0.9975
2.0	0.0036	0.0026	0.9977	71.80	0.1925	0.4609	0.9994	61.37	0.1364	0.9994	0.9984
2.5	0.0039	0.0028	0.9978	72.92	0.1714	0.3526	0.9996	63.67	0.1214	0.3661	0.9998
3.0	0.0042	0.0059	0.9914	74.64	0.1535	0.8798	0.9972	66.17	0.0839	1.0319	0.9974
T	k	σ	R^2	α_1	k_{D1}	σ	R^2	α_1	k_{D2}	σ	R^2
30	0.0017	0.0029	0.9895	72.63	0.0597	1.8025	0.9888	62.01	0.1291	2.2189	0.9919
35	0.0022	0.0051	0.9835	68.95	0.1050	0.5189	0.9993	59.82	0.1387	1.9313	0.9946
37	0.0029	0.0054	0.9851	68.14	0.2348	0.5628	0.9993	58.69	0.1545	1.4207	0.9973
40	0.0024	0.0017	0.9978	66.78	0.3041	0.9537	0.9983	52.69	0.1723	1.7935	0.9968
45	0.0021	0.0049	0.9675	64.74	0.6201	0.9940	0.9979	48.16	0.1899	2.3119	0.9955
50	0.0017	0.0033	0.9790	44.62	0.7983	1.0070	0.9992	34.06	0.2188	3.2324	0.9944
55	0.0010	0.0020	0.9803	10.65	0.9825	1.1651	0.9996	9.53	0.3172	1.8256	0.9991
pH	k	σ	R^2	α_1	k_{D1}	σ	R^2	α_1	k_{D2}	σ	R^2
5.5	0.0004	0.0003	0.9982	26.62	0.1867	3.4281	0.9935	16.54	0.1167	5.1243	0.9918
6.0	0.0023	0.0053	0.9816	64.40	0.2078	1.6762	0.9933	51.21	0.1344	2.3189	0.9948
6.5	0.0029	0.0054	0.9851	68.14	0.2348	0.5628	0.9993	58.69	0.1545	1.4207	0.9973
7.0	0.0017	0.0034	0.9846	70.01	0.2216	0.4911	0.9992	61.08	0.1471	1.6295	0.9960
7.5	0.0014	0.0017	0.9945	72.49	0.2013	0.3757	0.9994	63.62	0.1389	2.3799	0.9899
8.0	0.0012	0.0019	0.9903	74.21	0.1786	0.7303	0.9982	65.64	0.1329	2.9542	0.9823

*k: L/g·min; α_1: none; k_{D1}, k_{D2}: min^{-1}

cessing time of 30 minutes. It was found that third order polynomial model simulated adequately the data of residual lactose concentration for all process conditions. The quadratic model was fitted to the data of the residual enzyme activity for lactose concentration,

Table 3. Estimated parameters and statistical data of residual lactose concentration and residual enzyme activity at the end of the processing time

Mathematical models	Coefficients	Standard error (σ)	R^2 statistic
Initial lactose concentration			
$[C_L]=a_1+b_1[C_{L0}]+c_1[C_{L0}]^2+d_1[C_{L0}]^3$	$a_1=-3.06$ $b_1=0.5164$ $c_1=-0.0166$ $d_1=0.0002$	0.7023	0.9958
$[A]=a_{A1}+b_{A1}[C_{L0}]+c_{A1}[C_{L0}]^2$ (with lactose)	$a_{A1}=79.56$ $b_{A1}=0.0131$ $c_{A1}=-0.0034$	1.1059	0.9807
Enzyme concentration			
$[C_L]=a_2+b_2[E]+c_2[E]^2+d_2[E]^3$	$a_2=27.72$ $b_2=-28.41$ $c_2=13.17$ $d_2=-2.0029$	1.0427	0.9842
$[A]=a_{A2}+b_{A2}[E]+c_{A2}[E]^2$ (with lactose)	$a_{A2}=60.76$ $b_{A2}=7.89$ $c_{A2}=-1.3443$	0.4046	0.9949
$[A]=a_{A2}+b_{A2}[E]+c_{A2}[E]^2$ (without lactose)	$a_{A2}=51.68$ $b_{A2}=4.81$ $c_{A2}=0.0893$	0.8049	0.9916
Temperature			
$[C_L]=a_3+b_3[T]+c_3[T]^2+d_3[T]^3$	$a_3=126.57$ $b_3=-7.31$ $c_3=0.1429$ $d_3=-0.0008$	0.7721	0.9889
$[A]=a_{A3}+b_{A3}[T]+c_{A3}[T]^2$ (with lactose)	$a_{A3}=34.29$ $b_{A3}=3.0451$ $c_{A3}=-0.0570$	1.5147	0.9972
$[A]=a_{A3}+b_{A3}[T]+c_{A3}[T]^2$ (without lactose)	$a_{A3}=14.39$ $b_{A3}=3.3513$ $c_{A3}=-0.0599$	0.8334	0.9991
pH			
$[C_L]=a_4+b_4[pH]+c_4[pH]^2+d_4[pH]^3$	$a_4=2213.62$ $b_4=-922.50$ $c_4=127.52$ $d_4=-5.81$	1.0291	0.9964
$[A]=a_{A4}+b_{A4}[pH]+c_{A4}[pH]^2+d_{A4}[pH]^3$ (with lactose)	$a_{A4}=-4013.32$ $b_{A4}=1716.92$ $c_{A4}=-240.01$ $d_{A4}=11.16$	3.7754	0.9916
$[A]=a_{A4}+b_{A4}[pH]+c_{A4}[pH]^2+d_{A4}[pH]^3$ (without lactose)	$a_{A4}=-4418.58$ $b_{A4}=1886.57$ $c_{A4}=-264.27$ $d_{A4}=12.31$	3.4615	0.9941

a_1: g lactose/L; b_1, a_{A1}: none; c_1, b_{A1} (g lactose/L)$^{-1}$; d_1, c_{A1}: (g lactose/L)$^{-2}$; a_2: g lactose/L; b_2: g lactose/ml; c_2: g lactose·L/ml^2; d_2: g lactose·L^2/ml^3; a_{A2}: none; b_{A2}: (ml enzyme/L)$^{-1}$, c_{A2}: (ml enzyme/L)$^{-2}$; a_3: g lactose/L; b_3: g lactose/L (°C); c_3: g lactose/L (°C^2); d_3: g lactose/L (°C^3); a_{A3}: none; b_{A3}: °C^{-1}; c_{A3}: °C^{-2}; a_4, b_4, c_4, d_4: g lactose/L; a_{A4}, b_{A4}, c_{A4}, d_{A4}: none

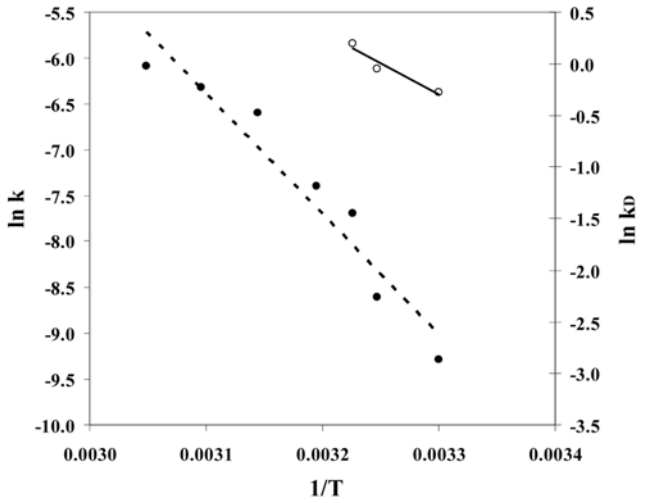

Fig. 5. Arrhenius plot for the determination of the hydrolysis activation and enzymatic inactivation energies (○) kinetic constants (lnk); (●) enzymatic inactivation constants (lnk_D).

enzyme concentration and temperature. On the other hand, a third order polynomial model was fitted to the data of residual enzyme activity for pH. The estimated coefficients and statistical values for these equations are given in Table 3.

6. Calculation of Hydrolysis Activation Energy and Enzymatic Inactivation Energy

For the determination of hydrolysis activation and enzymatic inactivation energies, the data obtained from the temperature experiments were analyzed. For calculation of the hydrolysis activation energy, an Arrhenius relationship (Eq. (3)) was used by concerning the kinetic constants obtained at 30, 35 and 37 °C (Table 2), as hydrolysis degree was not improved after 37 °C due to the existence of enzyme inactivation. Then, the activation energy for hydrolysis was calculated as 52.7 kJ/mol (presented in Fig. 5) with a standard error of 0.0998 and R^2 statistical value of 0.9645.

$$\ln k = -\frac{E_A}{RT} + A_A \quad (3)$$

On the other hand, to calculate the enzymatic inactivation energy, the values of the enzymatic inactivation constants (k_D) given in Table 2 were used and the data (presented in Fig. 5) were successfully fitted to the Arrhenius equation (Eq. (4)). The value of enzymatic inactivation energy was determined as 96.7 kJ/mol with a standard error of 0.3086 and R^2 statistical value of 0.9644.

$$\ln k_D = -\frac{E_D}{RT} + A_D \quad (4)$$

CONCLUSIONS

An evaluation of the experimental data with respect to processing time showed that many process conditions are involved in whey lactose hydrolysis and β-galactosidase inactivation, using a batch reactor system. On the other hand, according to the data obtained from the temperature experiments, the activation energy for whey lactose hydrolysis reaction (E_A) and enzymatic inactivation energy (E_D) were also calculated.

For the enzymatic hydrolysis of the whey lactose process, the optimum process conditions were obtained as 50 g/L of lactose concentration, 1 mL/L of enzyme concentration, 37 °C of temperature and pH 6.5. At these optimum conditions, the hydrolysis degree was 81%, the residual enzyme activity in the presence of lactose was 68% and the residual enzyme activity in the absence of lactose was 58% at the end of processing time of 30 minutes.

Mathematical models were proposed to predict the residual lactose concentration, and the residual enzyme activity at various process conditions was confirmed with the experimental results. It was found that a second order kinetic model and a single-step non-first-order enzyme inactivation kinetic model given by Sadana and Henley [25] accurately represented the data of the residual lactose concentration and the inactivation data for β-galactosidase with respect to time, respectively, for all process conditions applied.

For the final residual lactose concentrations (at the end of processing time of 30 minutes) versus process conditions--initial lactose concentration, enzyme concentration, temperature and pH--a third order polynomial fits sufficiently representing the data. On the other hand, the final data of enzyme inactivation versus process conditions resulted in quadratic fits. But, only the data obtained from pH experiments resulted in third order polynomial fit.

By using the Arrhenius equation, the hydrolysis activation energy was determined as 52.7 kJ/mol and the enzymatic inactivation energy was calculated as 96.7 kJ/mol by using the enzymatic inactivation constants (k_D).

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NOMENCLATURE

$[C_L]$: residual lactose concentration [g L^{-1}]
$[C_{L0}]$: initial lactose concentration [g L^{-1}]
k : hydrolysis kinetic constant [L $g^{-1}min^{-1}$]
t : processing time [min]
A : residual enzyme activity [%]
α_1 : ratio of the specific activity of the final state to the initial state
k_{D1} : enzymatic inactivation constant (min^{-1}) (in the presence of lactose)
k_{D2} : enzymatic inactivation constant (min^{-1}) (in the absence of lactose)
[E] : enzyme concentration [mL L^{-1}]
E_A : activation energy for hydrolysis reaction [kJ mol^{-1}]
E_D : enzymatic inactivation energy [kJ mol^{-1}]
A_A, A_D : frequency factor
R : gas constant [kJ mol^{-1} K^{-1}]
T : temperature [°C]
a, b, c, d : constants

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