Milk Lactose Hydrolysis in a Batch Reactor: Optimisation of Process Parameters, Kinetics of Hydrolysis and Enzyme Inactivation

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The present investigation describes the effects of the process parameters on enzymatic hydrolysis of milk lactose and enzyme stability. The lactose hydrolysis reactions were carried out in 250 mL of milk by using a commercial β-galactosidase produced from *Kluyveromyces marxianus lactis*. The residual lactose mass concentration and residual enzyme activity (%) against time were investigated vs. process variables such as temperature, impeller speed and enzyme concentration. Optimum conditions for hydrolysis were obtained as T = 37 °C, n = 300 rpm, σ = 1 mL L^{-1} enzyme concentration and 30 min of processing time. The lactose hydrolysis process resulted in 84 % of hydrolysis degree and 52 % of residual enzyme activity at the optimum experimental conditions obtained. After evaluation of the data, it was found that the kinetics of hydrolysis and enzyme inactivation could be represented by a first order kinetic model and a single-step non-first-order enzyme inactivation kinetic model for all process conditions applied. Also, to illustrate the effect of process variables on hydrolysis and enzyme stability, some modelling studies were performed. The activation energy for hydrolysis reaction (E_A) was calculated as 50.685 kJ mol^{-1}.

Key words:
Lactose hydrolysis, milk, β-galactosidase, modelling, process variables

Introduction

Lactose is a disaccharide that is found in milk and milk products. It can also be found in a variety of other foods. Although milk is highly nutritious, many people avoid its consumption because of gastrointestinal distress caused by the presence of lactose in milk. Hydrolysis of the lactose in milk can overcome this problem. Beside the reduced lactose content, hydrolysis of lactose in milk and whey results in several changes in their physical and chemical properties which are of interest to the dairy industry. These changes include prevention of lactose crystallization, increased sweetness and more readily fermentable sugars.[1–4] Lactose hydrolysis in milk and other dairy products is achieved by acidic or enzymatic processes. Unlike acidic hydrolysis, enzymatic hydrolysis provides milder process conditions and little or no undesirable side reactions or products. Therefore, the use of enzymes allows selective hydrolysis and produces potentially safer and more defined material. β-galactosidases (EC. 3.2.1.23) are used industrially to obtain the hydrolyzates of lactose from milk and milk whey for utilization in bakery products, ice creams, animal feed and as a sugar source for several fermentation products.[3–7]

The performance of lactose hydrolysis reaction by β-galactosidases has been studied by several authors.[1–15] Before designing a successful hydrolysis system, information is required describing phenomena which affect the kinetics of lactose hydrolysis by β-galactosidases such as temperature, pH, enzyme volume concentration, etc.

In the present study, the kinetics of hydrolysis reaction of lactose in milk was investigated using a stirred batch reactor system at various process conditions. Mathematical models representing the residual lactose mass concentration and the residual enzyme activity were developed according to the data obtained from the hydrolysis experiments.

Materials and methods

Materials

Milk containing approximately 50 g lactose per litre was obtained from a local supplier. β-galactosidase enzyme used was obtained from DSM Food Specialties with a commercial name of Maxilact LX 5000 (product code: EC 3.2.1.23) in liquid form and stored at 4 °C in the refrigerator. The source of this enzyme was *Kluyveromyces marxianus lactis*. The activity of enzyme was 5000 Natural Lactase Unit (NLU) per gram enzyme (1 NLU: mass of commercial enzyme which produces 1 μmol of

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ONP (ortho-nitrophenol) from an ONPG solution under standard conditions).

Determination of lactose mass concentration

For determination of the residual lactose mass concentration in the reaction solution,[16] the samples were taken at timed intervals. The samples taken were diluted with 5 mL distilled water which contained 50 μL HCl to inactivate the enzyme. The glucose amount in these samples was determined by using a glucose test kit obtained from Elitech Company (product code: 02-0541). These values were used for calculation of the remaining lactose amount in the reaction medium.

The same procedure was also followed for determination of the initial lactose mass concentration in milk as mentioned above. To determine the initial lactose mass concentration in milk for each opened milk box, 2 mL L^{-1} of excess enzyme was added in 50 mL of milk sample for complete lactose hydrolysis.

Determination of β-galactosidase activity

To determine the enzyme activity % in the reaction medium, milk samples were taken from reactor at specific time intervals and by addition of 2 mL ONPG (ortho-nitrophenyl-β-D-galactopyranoside) solution (prepared by dissolving 300 mg ONPG in 100 mL pH 7.3 100 mmol L^{-1} phosphate buffer solution) into these samples the hydrolysis of ONPG with β-galactosidase was provided at 28 °C for 5 min. After 5 min, the reaction was stopped by adding 1 mL of 1 mol L^{-1} Na_2CO_3 solution to the mixture. Reference solution was prepared by adding the ONPG and 1 mol L^{-1} Na_2CO_3 in the milk sample that did not contain enzyme. By considering the yellow colour of ONP (ortho-nitrophenol) which is a result of ONPG hydrolysis, the absorbance values of samples against the reference were measured at 420 nm.[6,14,15] Enzyme activities prior to the hydrolysis process were determined and used as the control. This control value was then considered as a 100 % activity. Activity at any operational conditions (A) was then given in terms of percentage values of the control. At least three measurements were made for each condition and the data given are an average of these results.

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.

Results and discussion

Effect of temperature on hydrolysis and enzyme activity

The effect of temperature was investigated depending on the processing time as the temperature is a critical parameter for enzyme stability (causing denaturated enzyme) and thus for the hydrolysis process. The experiments were performed at various temperatures ranging from 20 to 55 °C, at constant process variables of 300 rpm impeller speed, pH 6.7, and 0.2 mL enzyme per litre of milk. The data of residual lactose mass concentration and residual enzyme activity against the processing time at various temperatures are presented in Figs. 1 and 2. In addition, at the end of the processing time of 30 min, the data of residual enzyme activity (%), hydrolysis degree (%) and residual lactose mass concentration with respect to temperature are shown in Fig. 3.

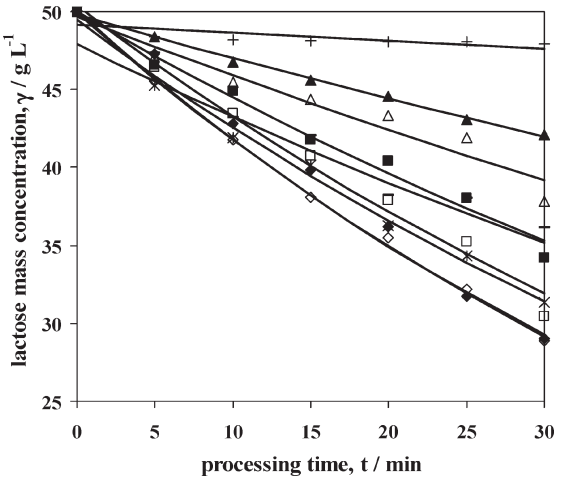
The residual lactose concentrations and residual enzyme activity decrease with respect to time for all temperature values, as expected. However, with increasing temperature, the residual lactose mass concentration decreases up to 37 °C, because the rate of reaction and thus the hydrolysis degree increases. Above 40 °C, the hydrolysis degree decreases. This decrease can be explained with enzyme inactivation. The residual enzyme activity decreases while the temperature increases (Fig. 2).

For this study, the optimum temperature for lactose hydrolysis was found at 37 °C. At this temperature, 42 % of the lactose was hydrolyzed and β-galactosidase enzyme lost 71 % of its activity at the end of 30 min processing time.

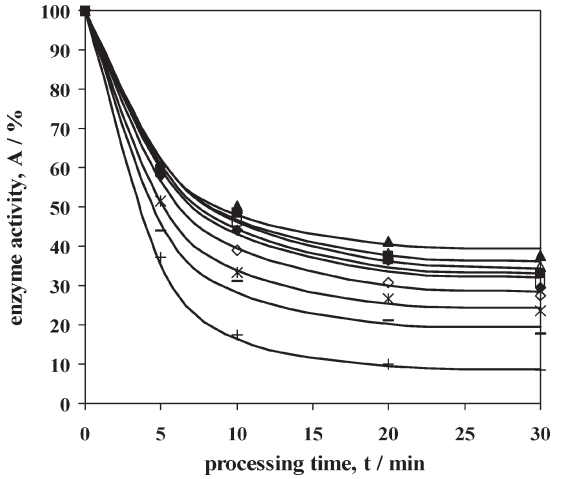
To predict the kinetics of the hydrolysis reaction, the data of residual lactose mass concentration vs. processing time were evaluated for all temperature values and a first order kinetic expression (eq. (1)) produced;

$$\frac{\mathrm{d}\gamma_{\mathrm{L}}}{\mathrm{d}t} = -k\gamma_{\mathrm{L}}, \quad \gamma_{\mathrm{L}} = \gamma_{\mathrm{L}_0} \exp(-k \cdot t) \qquad (1)$$

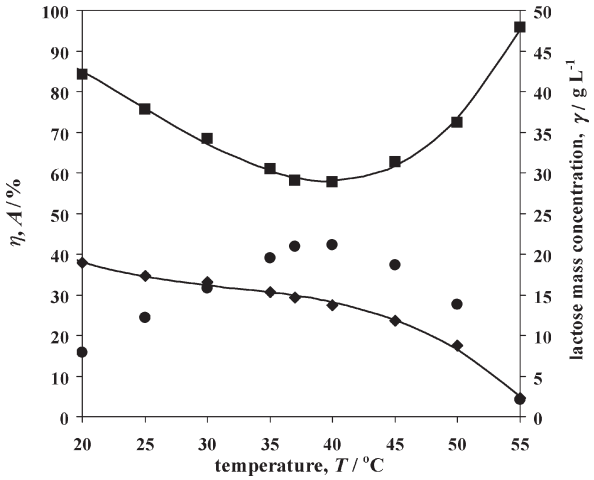
On the other hand, to predict the effect of processing time on enzyme stability, the data of residual enzyme activity with respect to processing time for all temperature values were evaluated and the inactivation data was fitted to a single-step non-first-order enzyme inactivation kinetic model given by Sadana and Henley (1987)[17] (eq. (2)). The



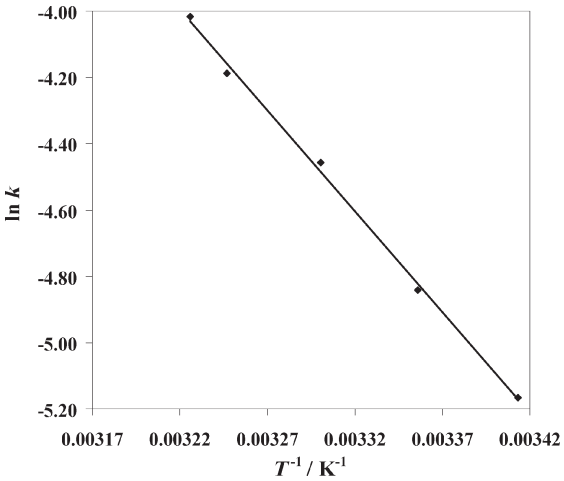
F i g . 1 – *Residual lactose mass concentration values vs. processing time at various temperatures (pH 6.7, n = 300 rpm, σ = 0.2 mL L^{-1}) (▲) 20 °C; (△) 25 °C; (■) 30 °C; (□) 35 °C; (◆) 37 °C; (◇) 40 °C; (*) 45 °C; (–) 50 °C; (+) 55 °C; (–) models*



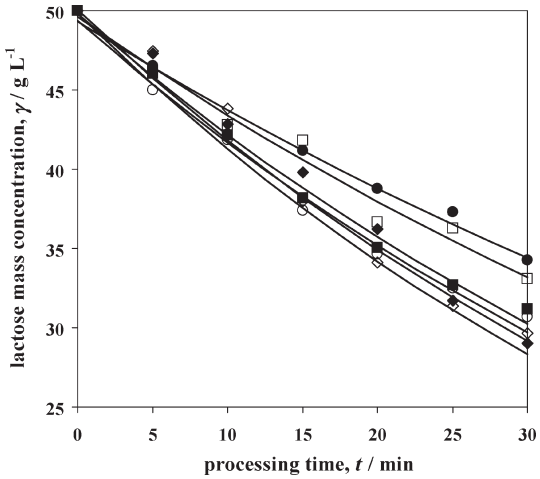
F i g . 2 – *Residual enzyme activity (%) values in milk vs. processing time at various temperatures (pH 6.7, n = 300 rpm, σ = 0.2 mL L^{-1}) (▲) 20 °C; (△) 25 °C; (■) 30 °C; (□) 35 °C; (◆) 37 °C; (◇) 40 °C; (*) 45 °C; (–) 50 °C; (+) 55 °C; (–) models*



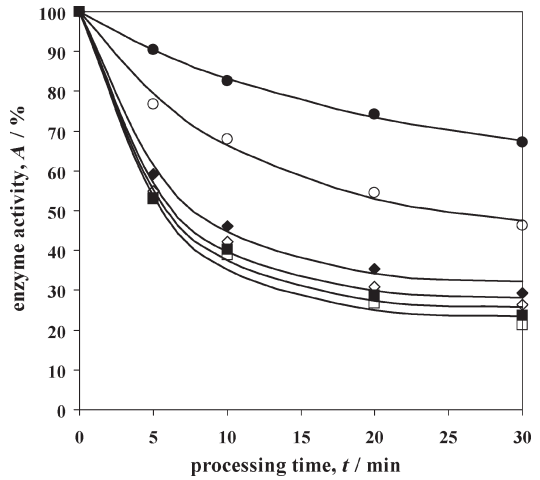
F i g . 3 – *Residual enzyme activity (%), hydrolysis degree (%) and residual lactose concentration values vs. temperature (pH 6.7, n = 300 rpm, σ = 0.2 mL L^{-1}) (◆) enzyme activity; (●) hydrolysis degree; (■) lactose mass concentration; (–) models*



F i g . 4 – *Arrhenius plot for the determination of the hydrolysis activation energy*



F i g . 5 – *Residual lactose mass concentration values vs. processing time at various impeller speeds (pH 6.7, T = 37 ^{0}C, σ = 0.2 mL L^{-1}) (●) 100 rpm; (○) 200 rpm; (◆) 300 rpm; (◇) 400 rpm; (■) 500 rpm; (□) 600 rpm; (–) models*



F i g . 6 – *Residual enzyme activity (%) values in milk vs. processing time at various impeller speeds (pH 6.7, T = 37 ^{0}C, σ = 0.2 mL L^{-1}) (●) 100 rpm; (○) 200 rpm; (◆) 300 rpm; (◇) 400 rpm; (■) 500 rpm; (□) 600 rpm; (–) models*

Table 1 – *Estimated parameters and statistical data of residual lactose mass concentration and residual enzyme activity vs. processing time for various temperature values (pH 6.7, n = 300 rpm, σ = 0.2 mL L^{-1})*

Residual lactose concentration					Residual enzyme activity			
T	γ_{L_0}	k	e	R^2	α_1	k_D	e	R^2
20	49.80	0.0057	0.1832	0.9982	39.28	0.1956	2.0766	0.9974
25	49.67	0.0079	0.9843	0.9727	35.96	0.1779	2.4465	0.9967
30	49.98	0.0116	0.7340	0.9921	33.94	0.1688	2.4152	0.9969
35	50.38	0.0152	0.8801	0.9928	32.65	0.1749	2.2770	0.9974
37	51.04	0.0182	0.8764	0.9947	31.69	0.1786	2.2788	0.9975
40	49.94	0.0178	0.3054	0.9993	28.29	0.1856	1.0152	0.9994
45	49.45	0.0151	0.6018	0.9964	24.22	0.2051	0.8243	0.9997
50	47.94	0.0103	1.4325	0.9609	19.39	0.2223	2.3683	0.9981
55	49.13	0.0010	0.5355	0.7418	8.79	0.2472	3.1437	0.9968

* T – temperature, °C; γ_{L_0} – lactose mass concentration at t = 0, g L^{-1} lactose; k – kinetic coefficient of hydrolysis reaction, min^{-1}; e – standard error; α_1 – ratio of specific activity of final state to initial state, %; k_D – degradation coefficient, min^{-1}

estimated coefficients and statistical values for eqs. (1) and (2) are given in Table 1.

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

Moreover, to predict the effect of temperature on hydrolysis and enzyme stability at the end of 30 min processing time, the data of residual lactose mass concentration and residual enzyme activity vs. temperature were also evaluated, and it was found that a polynomial expression simulated both the data of residual lactose mass concentration (eq. (3) given in Table 2) and residual enzyme activity (eq. (4) given in Table 2). The estimated coefficients and statistical values for eqs. (3) and (4) are given in Table 2.

Calculation of hydrolysis activation energy

For the determination of hydrolysis activation energy, the data obtained from the experiments carried out at various temperatures were analyzed. For calculation of the hydrolysis activation energy, Arrhenius relationship (eq. (5)) was used by considering the kinetic coefficients obtained at 20 – 37 °C (Table 1) 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 50.685 kJ mol^{-1} (presented in Fig. 4) with the standard error of 0.027 and R^2 statistical value of 0.9988.

$$\ln k = -\frac{E_A}{RT} + A_A \qquad (5)$$

Table 2 – *Estimated parameters and statistical data of residual lactose mass concentration and residual enzyme activity at various temperatures (pH 6.7, n = 300 rpm, σ = 0.2 mL L^{-1})*

Mathematical models	Coefficients*	Standard error (e)	R^2 statistics
$\gamma_L = a + b\,T + c\,T^2 + d\,T^3$ (eq. (3))	a = 33.1011 b = 2.1901 c = –0.1169 d = 0.0015	0.4681	0.9984
$A = a_A + b_A\,T + c_A\,T^2 + d_A\,T^3$ (eq. (4))	a_A = 87.9798 b_A = –4.6594 c_A = 0.1372 d_A = –0.0014	0.7308	0.9984

* γ_L – residual lactose mass concentration at any temperature, g L^{-1}; T – temperature °C; a – g L^{-1} lactose; b – g L^{-1} °C^{-1}; c – g L^{-1} °C^{-2}; d – g L^{-1} °C^{-3}; A – residual enzyme activity at any temperature, %; a_A – dimensionless; b_A – °C^{-1}; c_A – °C^{-2}; d_A – °C^{-3}; e – standard error

Effect of impeller speed on hydrolysis and enzyme activity

The effects of various impeller speeds on the enzyme activity and hydrolysis process were investigated versus processing time at impeller speeds in the range of 100–600 rpm at constant process variables such as temperature 37 °C, pH 6.7 and 0.2 mL L^{-1} enzyme.

The data of residual lactose mass concentration and residual enzyme activity against the processing time at various impeller speeds are shown in Figs. 5 and 6. Also, at the end of the processing time of 30 min, the data of residual enzyme activity (%), hydrolysis degree (%) and residual lactose mass concentration with respect to impeller speed are shown in Fig. 7.

By increasing impeller speed, the residual lactose mass concentration slightly decreases up to 300 rpm. Above 300 rpm, residual lactose mass concentration slightly increases. On the other hand, the residual enzyme activity decreases as the impeller speed increases. This could be due to the forces generated in the process fluid by increasing the impeller speed, which causes enzyme denaturation. Therefore, the optimum impeller speed for lactose hydrolysis and enzyme stability was chosen as 300 rpm.

After evaluation of the data of residual lactose concentration vs. processing time for all impeller speed values, again the data produced a first order kinetic expression (eq. (1)). On the other hand, the data of residual enzyme activity with respect to time again resulted in a single-step non-first-order enzyme inactivation kinetic model given by Sadana and Henley (1987)[17] (eq. (2)). The estimated coefficients and the statistical values for eqs. (1) and (2) are given in Table 3.

To predict the effect of impeller speed on hydrolysis and enzyme stability at the end of 30 min of processing time, the data of residual lactose mass concentration and residual enzyme activity vs. impeller speed were evaluated; and produced a quadratic expression for the residual lactose mass concentration (eq. (6) given in Table 4) and as well as for the inactivation data of residual enzyme activity (eq. (7) given in Table 4). The estimated coefficients and statistical values for eqs. (5) and (6) are given in Table 4.

Table 3 – *Estimated parameters and statistical data of residual lactose mass concentration and residual enzyme activity vs. processing time for various impeller speed values (pH 6.7, T = 37 °C, σ = 0.2 mL L^{-1})*

Residual lactose concentration					Residual enzyme activity			
n	γ_{L_0}	k	e	R^2	α_1	k_D	e	R^2
100	49.31	0.0120	0.8232	0.9903	59.59	0.0538	0.6746	0.9989
200	49.35	0.0169	0.7294	0.9955	43.82	0.0909	2.0768	0.9963
300	51.04	0.0182	0.8764	0.9947	31.69	0.1786	2.2788	0.9975
400	51.08	0.0184	1.1085	0.9919	27.88	0.1811	2.2669	0.9978
500	49.79	0.0166	0.6061	0.9969	25.46	0.1830	2.5423	0.9974
600	49.63	0.0134	0.9414	0.9896	23.15	0.1849	2.0747	0.9984

* n – impeller speed, rpm; γ_{L_0} – lactose mass concentration at $t = 0$, g L^{-1} lactose; k – kinetic coefficient of hydrolysis reaction, min^{-1}; e – standard error; α_1 – ratio of specific activity of final state to initial state, %; k_D – degradation coefficient, min^{-1}

Table 4 – *Estimated parameters and statistical data of residual lactose mass concentration and residual enzyme activity at various impeller speeds (pH 6.7, T = 37 °C, σ = 0.2 mL L^{-1})*

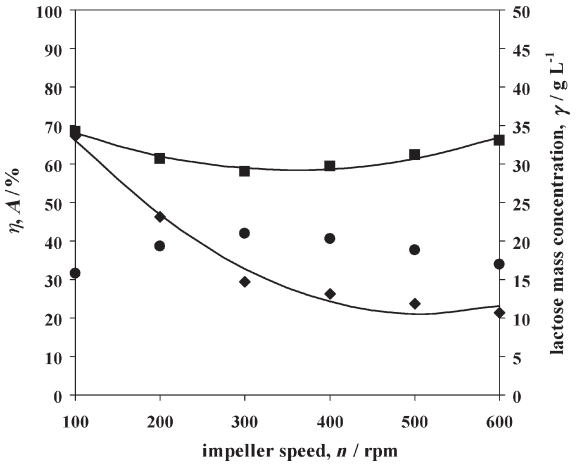
Mathematical models	Coefficients*	Standard error (e)	R^2 statistics
$\gamma_L = a + b\,n + c\,n^2$ (eq. (6))	a = 38.3660 b = –0.0511 c = 0.0001	0.5276	0.9789
$A = a_A + b_A\,n + c_A\,n^2$ (eq. (7))	a_A = 90.7110 b_A = –0.2732 c_A = 0.0003	3.0659	0.9911

* γ_L – residual lactose mass concentration at any impeller speed, g L^{-1}; n – impeller speed, rpm; a – g L^{-1}; b – g L^{-1} rpm^{-1}; c – g L^{-1} rpm^{-2}; A – residual enzyme activity at any impeller speed, %; a_A – dimensionless; b_A – rpm^{-1}; c_A – rpm^{-2}; e – standard error

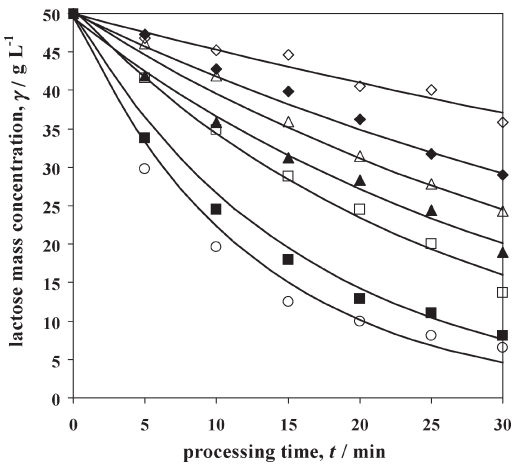
Effect of enzyme concentration on hydrolysis and enzyme activity

To investigate the effect of enzyme volume concentration on lactose hydrolysis, the enzyme preparation was used in the range of 0.1–2 mL L^{-1} at pH 6.7, operating temperature of 37 °C and impeller speed of 300 rpm. The results are shown in Figs. 8 – 10.

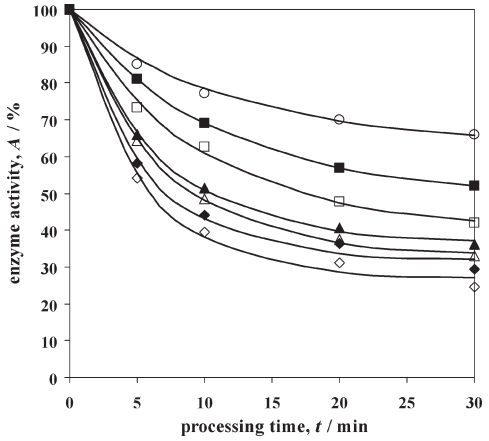
By increasing the enzyme volume concentration, hydrolysis degree and residual enzyme activity increased. At concentrations above 1 mL L^{-1} enzyme, the lactose hydrolysis rate did not change significantly. These results indicate that a concentration somewhere in the range of 1–2 mL L^{-1} enzyme must saturate the lactose molecules with active enzyme molecules. Therefore, the optimum en-



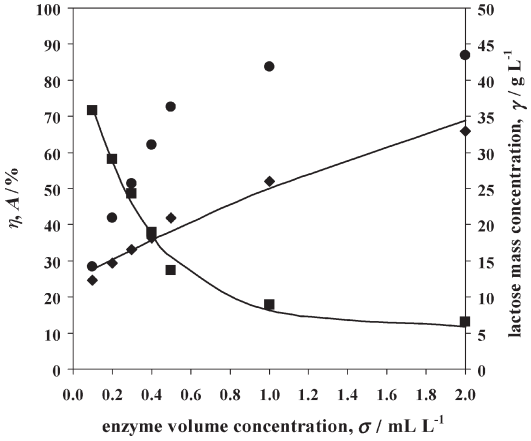
F i g . 7 – *Residual enzyme activity (%), hydrolysis degree (%) and residual lactose mass concentration values vs. impeller speed (pH 6.7, T = 37 ^{0}C, σ = 0.2 mL L^{-1}) (◆) enzyme activity; (●) hydrolysis degree; (■) lactose mass concentration; (–) models*



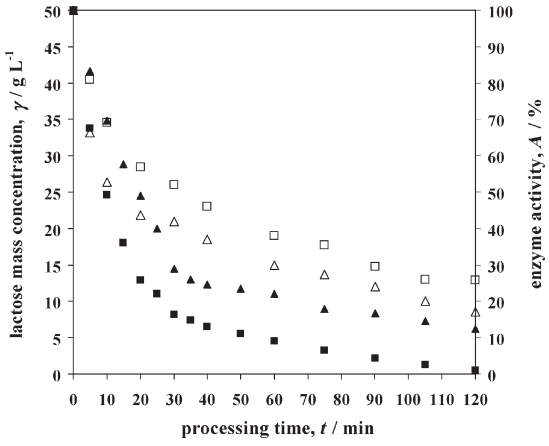
F i g . 8 – *Residual lactose mass concentrations vs. processing time at various enzyme volume concentrations (pH 6.7, T = 37 ^{0}C, n = 300 rpm) (◇) σ = 0.1 mL L^{-1}; (◆) 0.2 mL L^{-1}; (△) 0.3 mL L^{-1}; (▲) 0.4 mL L^{-1}; (□) 0.5 mL L^{-1}; (■) 1 mL L^{-1}; (○) 2 mL L^{-1}; (–) models*



F i g . 9 – *Residual enzyme activity (%) values in milk vs. processing time at various enzyme volume concentrations (pH 6.7, T = 37 ^{0}C, n = 300 rpm) (◇) σ = 0.1 mL L^{-1}; (◆) 0.2 mL L^{-1}; (△) 0.3 mL L^{-1}; (▲) 0.4 mL L^{-1}; (□) 0.5 mL L^{-1}; (■) 1 mL L^{-1}; (○) 2 mL L^{-1}; (–) models*



F i g . 10 – *Residual enzyme activity (%), hydrolysis degree (%) and residual lactose mass concentration values vs. enzyme volume concentration (pH 6.7, T = 37 °C, n = 300 rpm) (◆) enzyme activity; (●) hydrolysis degree; (■) lactose mass concentration; (–) models*



F i g . 11 – *Residual lactose mass concentrations and residual enzyme activity values vs. processing time at enzyme volume concentrations of 0.5 and 1 mL L^{-1} (pH 6.7, T = 37 °C, n = 300 rpm) (■) lactose mass conc. for 1 mL L^{-1} enzyme; (▲) lactose mass conc. for 0.5 mL L^{-1} enzyme; (□) enzyme activity for 1 mL L^{-1} enzyme; (△) enzyme activity for 0.5 mL L^{-1} enzyme*

zyme concentration was chosen as 1 mL L^{-1}. At this enzyme volume concentration, 84 % of lactose was hydrolyzed, and β-galactosidase enzyme lost 48 % of its activity at the end of 30 min processing time.

After evaluation of the experimental data, to represent the relationship between the residual lactose concentration and processing time at all enzyme concentrations, a first order kinetic expression (eq. (1)) was used. The data of residual β-galactosidase activity vs. processing time clearly follows a single-step non-first-order enzyme inactivation kinetic model given by Sadana and Henley (1987)[17] (eq. (2)), at all enzyme volume concentrations examined. The estimated coefficients and statistical values for eqs. (1) and (2) are given in Table 5.

To predict the effect of enzyme volume concentration on the residual lactose mass concentration at the end of 30 min of processing time, a simple exponential function given by Komolprasert and Ofoli (1991)[18] (eq. (8) given in Table 6) was used. On the other hand, a quadratic equation (eq. (9) given in Table 6) was fitted to the data of the residual enzyme activity vs. enzyme volume concentration. The estimated coefficients and statistical values for eqs. (7) and (8) are given in Table 6.

From the economic viewpoint, it is desired to attain as low enzyme volume concentrations as possible in order to decrease the costs of hydrolysis process. Obviously, the same conversions could be achieved with lower enzyme concentrations in a longer period. Thus, two hydrolysis experiments were performed by using 0.5 mL L^{-1} and 1 mL L^{-1} enzyme concentration for 120 min of processing time. The results show that, the same hydrolysis degree with 1 mL L^{-1} enzyme concentration that achieved in 30 min was obtained in 120 min by using 0.5 mL L^{-1} enzyme volume concentration (see Fig. 11). However, in this case, the use of lower enzyme volume concentration is not economical as

Table 5 – *Estimated parameters and statistical data of residual lactose mass concentration and residual enzyme activity vs. processing time for various enzyme volume concentrations (pH 6.7, T = 37 °C, n = 300 rpm)*

Residual lactose concentration					Residual enzyme activity			
σ	γ_{L_0}	k	e	R^2	α_1	k_D	e	R^2
0.1	50.03	0.0099	1.0995	0.9777	26.87	0.1868	2.3672	0.9977
0.2	51.04	0.0182	0.8764	0.9947	31.69	0.1786	2.2788	0.9975
0.3	51.27	0.0239	1.0154	0.9953	33.16	0.1489	0.8297	0.9996
0.4	49.38	0.0299	1.0309	0.9960	36.38	0.1477	0.9207	0.9995
0.5	50.46	0.0383	1.2066	0.9962	40.07	0.1054	1.6889	0.9979
1	48.83	0.0654	1.2748	0.9969	48.85	0.0929	0.0791	0.9999
2	48.50	0.0844	2.1268	0.9923	63.16	0.0879	1.2327	0.9957

* σ – enzyme volume concentration, mL L^{-1}; γ_{L_0} – lactose mass concentration at $t = 0$, g L^{-1} lactose; k – kinetic coefficient of hydrolysis reaction, min^{-1}; e – standard error; α_1 – ratio of specific activity of final state to initial state, %; k_D – degradation coefficient, min^{-1}

Table 6 – *Estimated parameters and statistical data of residual lactose mass concentration and residual enzyme activity at various enzyme volume concentrations (pH 6.7, T = 37 °C, n = 300 rpm)*

Mathematical models	Coefficients*	Standard error (e)	R^2 statistics
$\gamma_L = a \exp(-b\,\sigma) + c$ (eq. (8))	a = 40.8853 b = 2.8371 c = 5.5374	1.1267	0.9965
$A = a_A + b_A\,\sigma + c_A\,\sigma^2$ (eq. (9))	a_A = 24.7702 b_A = 28.4616 c_A = –3.2322	2.7151	0.9942

* γ_L – residual lactose mass concentration at any enzyme volume concentration, g L^{-1}; σ – enzyme volume concentration mL L^{-1}; a – g L^{-1}; b – L mL^{-1}; c – g L^{-1}; A – residual enzyme activity at any enzyme volume concentration, %; a_A – dimensionless; b_A – L mL^{-1}; c_A – L^2 mL^{-2}; e – standard error

Table 7 – *Lactose conversion ratios of various studies compared with the present study*

Data from literature	Lactose	Enzyme	Operating conditions	Lactose conversion ratio
Ladero *et al.,* (2001)[2]	50 g L^{-1} 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 g L^{-1}, resp.
Pessela *et al.,* (2003)[3]	50 g L^{-1} 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 g L^{-1}
Di Serio *et al.,* (2003)[20]	9.58, 23.6 and 47.54 g L^{-1} 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^{-1}, resp.
Roy and Gupta, (2003)[13]	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
Santos *et al.,* (1998)[6]	50 g L^{-1} 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 g L^{-1}, resp.
Şener *et al.,* (2006)[19]	milk	β-galactosidase from *Kluyveromyces marxianus lactis* (Maxilact LX 5000)	at 37 °C, pH 6.7, at the end of 30 min, with sonication	90 % with sonication at enz. conc. of 1 mL L^{-1}
Present study	milk	β-galactosidase from *Kluyveromyces marxianus lactis* (Maxilact LX 5000)	at 37 °C, pH 6.7, at the end of 30 min, in a batch reactor	84 % at enzyme concentration of 1 mL L^{-1}

the energy and labour costs beyond the enzyme cost due to the required processing time, which was 4 times higher. As it can be clearly seen from Fig. 11, the hydrolysis rates were sharply decreased after 30 min processing time, which indicates that the optimum processing time for lactose hydrolysis is about 30 min at the chosen experimental conditions. On the other hand, overall hydrolysis for 1 mL L^{-1} enzyme volume concentration completed at 120 min of processing time.

A summary of 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 7. The value of lactose conversion ratio obtained at the end of 30 min in the present study was considerably higher than the results proposed on literature for a batch reactor.

Conclusions

In this study, the effect of temperature, impeller speed, enzyme volume concentration and processing time on lactose hydrolysis and enzyme stability were investigated by using commercially available β-galactosidase enzyme produced from *Kluyveromyces marxianus lactis*. At optimum process conditions; $T = 37\ ^0$C, $n = 300$ rpm and $\sigma = 1$ mL L^{-1} enzyme concentration; 84 % of hydrolysis was achieved, and enzyme lost its activity by 48 % in 30 min.

For all process conditions applied, a first order kinetic model and a single-step non-first-order enzyme inactivation kinetic model given by Sadana and Henley (1987)[17] accurately represented the data of the residual lactose mass concentration and the inactivation data for β-galactosidase with respect to time, respectively. Mathematical models proposed to predict the residual lactose mass concentration and the residual enzyme activity at various process conditions have been confirmed with the experimental results.

From the studies at various temperature values, the optimum temperature value for lactose hydrolysis was found at 37 °C. The modelling studies for the residual lactose mass concentration and residual enzyme activity depending on temperature at the end of 30 min of processing time resulted in polynomial fit. The hydrolysis activation energy using Arrhenius equation was also determined as 50.685 kJ mol^{-1} by using the hydrolysis kinetic coefficients (k).

The optimum impeller speed for lactose hydrolysis was found as 300 rpm from the studies at vari-

ous impeller speeds. For the residual lactose mass concentration and residual enzyme activity at the end of processing time of t = 30 min vs. impeller speed, quadratic fits sufficiently represented the data.

The optimum enzyme volume concentration for lactose hydrolysis was found as σ = 1 mL L^{-1}. The data of residual lactose mass concentration and residual enzyme activity vs. enzyme volume concentration resulted in a simple exponential function given by Komolprasert and Ofoli (1991)[18] and quadratic fit, respectively, at the end of 30 min.

Finally, the process conditions mentioned above have a significant role to play in the lactose hydrolysis process. The stability behaviour observed was different for each process condition. Thus, the operational parameters should be optimized for the enzyme used to obtain higher degree of hydrolysis as these parameters could cause inactivation or activation of the enzyme during the hydrolysis process.

Nomenclature

k – hydrolysis kinetic coefficient, 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, dimensionless
k_D – degradation coefficient, min^{-1}
σ – enzyme volume concentration, mL L^{-1}
E_A – activation energy for hydrolysis reaction, kJ mol^{-1}
T – temperature, °C, K
n – impeller speed, rpm
γ_L – residual lactose mass concentration, g L^{-1}
γ_{L_0} – initial lactose mass concentration, g L^{-1}
e – standard error
η – hydrolysis degree, %

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