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Modeling of the simultaneous hydrolysis–ultrafiltration of whey permeate by a thermostable β-galactosidase from *Aspergillus niger*

Dimitris G. Hatzinikolaou [a], Efstathios Katsifas [a], Diomi Mamma [b], Amalia D. Karagouni [a], Paul Christakopoulos [b], Dimitris Kekos [b,*]

[a] *Laboratory of Microbiology, Sector of Botany, Department of Biology, National and Kapodistrian University of Athens, 15781 Zografou, Attica, Hellas, Greece*

[b] *Biotechnology Laboratory, Department of Chemical Engineering, National Technical University of Athens, 15780 Zografou, Attica, Hellas, Greece*

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Abstract

A wild type strain of *Aspergillus niger*, denoted as BTL, produced elevated levels of β-galactosidase when grown in a low cost medium that contained wheat bran as the sole carbon and energy source. The enzyme was collected, concentrated and partially purified from the culture supernatant. Its kinetic and stability properties were thoroughly examined towards its potential use for the hydrolysis of acid whey permeate lactose. The β-galactosidase of *A. niger* BTL showed increased pH and thermal stability, with activation energy for the first order deactivation constant equal to 180 kJ/mol at pH 3.5. Lactose hydrolysis by the enzyme was described by Michaelis–Menten kinetics with competitive inhibition only from galactose. An integrated process, concerning the simultaneous hydrolysis–ultrafiltration of whey lactose that incorporated the specific kinetic properties of the β-galactosidase was developed and modeled. The model proved very successful in predicting the behavior of a continuous laboratory hydrolysis–ultrafiltration set up, specifically designed for that purpose. The validated model was finally used in a number of computer simulations in order to investigate the effect of the various process parameters on the overall system performance.

Keywords: β-Galactosidase; Whey; Hydrolysis; Ultrafiltration; Process integration; Kinetic parameters; Modeling

1. Introduction

Whey is the major byproduct waste of the dairy industry. It is characterized by its elevated BOD values, in the range of 30–60 kg/m^3, mainly resulting from its high lactose content, which usually accounts between 4 and 8% on a weight per volume basis. Milk proteins, the second major constituent of whey, at a concentration of 1% (w/v), are almost universally recovered by the dairy companies via ultrafiltration and sold as a concentrate [1]. The resulting whey permeate though, still represents a major disposal problem due to the presence of lactose that remains in it. Increased production rates in the latter decades have exposed the problem of whey utilization, since lactose is one of the most difficultly fermented sugars and represents a major pollutant for aquatic environments [2].

A number of applications for whey permeate have been developed in an effort to overcome the problem of its disposal. In order to be economically attractive, these applications must go beyond the traditional use of concentrated whey as an additive for animal feedstock. One alternative is the use of whey as the basic medium for various fermentation processes including the production of ethanol, methane, yeast protein, xanthan gum [3] or organic acids such as lactate, propionate or acetate [1,4]. Nevertheless, since the number of lactose fermenting microorganisms is limited and the use of genetically engineered microorganisms for lactose bioconversion [5] is still far from industrial use, the efficient hydrolysis of whey lactose remains one of the major factors that determine the overall process economics.

Hydrolysis of lactose can be performed either by acids or by enzymatic treatment with β-galactosidase. Acid hydrol-

* Corresponding author. Tel.: +30 210 772 3205; fax: +30 210 772 3161.
E-mail address: kekos@chemeng.ntua.gr (D. Kekos).

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Nomenclature

D	steady state dilution rate ($=F_1/V=F_4/V$) (h^{-1})
[E]	enzyme concentration (nkat/L)
E_{eff}	enzyme efficiency (mmol/nkat)
[E_i]	enzyme concentration in stream i (nkat/L)
[E_0]	initial enzyme concentration (nkat/L)
E_a	activation energy for the thermal deactivation constant (kJ/mol)
F_1	reactor feed flowrate (L/h)
F_2	recycle stream flowrate (L/h)
F_3	ultrafiltration retentate stream flowrate (L/h)
F_4	ultrafiltration permeate stream flowrate (L/h)
[GA]	galactose concentration (mM)
[GA_i]	galactose concentration in stream i (mM)
[GL]	glucose concentration (mM)
[GL_i]	glucose concentration in stream i (mM)
k	rate constant in lactose hydrolysis model (mmol/(nkat h))
k_d	first order thermal deactivation constant (h^{-1})
K_I	galactose inhibition constant (mM)
K_m	Michaelis–Menten constant (mM)
[LA]	lactose concentration (mM)
[LA_i]	lactose concentration (mM)
[LA_0]	initial lactose concentration (mM)
P	volumetric productivity (mmol/(L h))
r_d	rate of first order enzyme deactivation (nkat/(L h))
r_{GA}	rate of galactose production (mM/h)
r_{GL}	rate of glucose production (mM/h)
r_{LA}	rate of lactose production (mM/h)
t	time (h)
T	temperature (°C)
V	working volume of the enzyme reactor vessel (L)

ysis of whey lactose has been employed in the production of a hydrolysate that served as a carbon source for ethanol production by non-lactose fermenting microorganisms [6]. However, the acid hydrolysis process, in general, requires the use of high acid concentrations and elevated temperatures. In addition, the hydrolysis products, glucose and galactose, are not produced at quantities equimolar to the initial lactose concentration, since a number of undesired byproducts are also produced [7].

Enzymatic hydrolysis of either whey or pure lactose has been accomplished in various reactor configurations. Since the cost of the enzyme is the most important factor that determines process economy, only continuous systems that involve the reuse of a single batch of enzyme can be considered. Thus, enzymatic lactose hydrolysis has been studied by using either immobilized enzyme reactors [7–10] or enzyme membrane reactors [11]. However, the majority of these studies are mainly focused on the evaluation of the kinetic models applied for lactose hydrolysis or the immobilization techniques [8,9] and not on the evaluation of the overall process characteristics.

Membrane bioreactors for lactose or whey hydrolysis, practically involve the use of a thermostated stirring vessel that provides the space and time for hydrolysis, the content of which is continuously circulated towards an ultrafiltration module. The main advantage of this configuration, compared to immobilized enzyme systems, is the possibility to work with free enzyme in homogenous solutions with its substrate, thus fully exploiting its native kinetic properties. The major disadvantages of this configuration are the increased risk of microbial contamination, especially during prolonged operation times at ambient temperatures, and the clogging of the ultrafiltration membranes with milk proteins. These drawbacks can, at least partially, be alleviated by operating the system at relatively high temperatures and by using deproteinated substrates such as whey permeate.

An efficient and economically feasible process for the hydrolysis of whey lactose, strongly depends on the production cost of the enzyme catalyst in combination with favorable kinetic and stability properties. More specifically, the enzyme should be produced at high concentrations, by a GRAS (Generally Recognized As Safe) microorganism using a low cost medium. In addition, it should be recovered from the extracellular medium, a fact that results at reduced recovery cost. Commercial sources for β-galactosidases used in lactose hydrolysis are mainly the yeast *Kluyveromyces* species and fungal *Aspergillus* species. The *Aspergillus* enzymes are preferred for the hydrolysis of whey lactose due to their acidic pH optima and higher thermal stabilities [12].

In the framework presented above, we describe the enhanced production of an extracellular thermostable β-galactosidase from a wild type strain of *Aspergillus niger* using a low-cost culture medium that contains wheat bran as a sole carbon and energy source. The kinetic and stability properties of a partially purified enzyme preparation, obtained during optimum growth conditions, were evaluated towards whey lactose hydrolysis. The kinetic data were incorporated in a complete mathematical model that describes the continuous hydrolysis–ultrafiltration of whey lactose at various process temperatures. A laboratory set up for the continuous hydrolysis–ultrafiltration process was constructed in order to experimentally evaluate the predicting ability of the proposed model, which was finally successfully used in a number of computer simulations for the evaluation of the various overall process characteristics.

2. Materials and methods

2.1. Microorganism and growth conditions

A wild type strain of *A. niger* (strain BTL) isolated from corn [13] was used for the production of β-galactosidase.

The fungus was maintained on potato–dextrose–agar (PDA) slants at 4 °C. The microorganism was grown in 3 L Erlenmeyer flasks (750 mL working volume) in a low-cost medium, consisted only of 10 g/L $(NH_4)H_2PO_4$ and wheat bran (St. George Mills S.A., Pireas, Greece). The concentration and particle size of wheat bran was optimized with respect to β-galactosidase production. The initial pH of the medium was adjusted to 4 with a 2N HCl solution. Sterilized medium flasks (121 °C, 15 min) were directly inoculated with a spore suspension from PDA slants and incubated in an orbital shaker at 29 °C and 250 rpm. The course of growth was assessed by monitoring the activity of β-galactosidase, excreted in the culture supernatant.

2.2. Preparation of the enzyme

When extracellular β-galactosidase activity leveled off, the culture supernatant was collected by centrifugation at 10,000 × *g* for 20 min (10 °C). The enzyme sample was concentrated by ammonium sulfate precipitation (35–95% saturation), resuspended in deionized water and desalted using a PD-10 gel filtration column (Pharmacia™). The desalted enzyme sample was finally lyophilized (overnight, 10 mTorr, −48 °C) and used as the enzyme source in all subsequent experiments.

2.3. Analytical methods

β-Galactosidase activity was determined using *o*-nitrophenyl-β-D-galactopyranoside (oNPG) as substrate. Fifty microliters of properly diluted enzyme sample were added to 950 μL of 2.5 mM oNPG solution in 100 mM citrate–phosphate buffer, pH 3.5. Following 10 min incubation in a water bath at 60 °C, 1 mL of 10% (w/v) $NaCO_3$ solution was added and the absorbance of the final mixture was measured at 410 nm in order to determine the amount of released *o*-nitro-phenol (oNP). A boiled (15 min) enzyme sample was used as blank. As an activity unit we used nkat, defined as the amount of enzyme required for the hydrolysis of 1 nmol of substrate per second.

Glucose concentration was determined using a commercially available kit (Biosis S.A., Athens, Greece) that employed the Glucose Oxidase–Peroxidase (GOX–PER) method. Reducing sugars were determined by the 3,5-dinitrosalycilic acid (DNS) method [14]. For the latter, a separate reference curve was constructed for glucose, galactose and lactose.

Sugar analysis, for selected samples, was additionally performed using an HPLC system (HP 1050 Series) equipped with a RI detector (HP 1047A). A silica-NH_2 column was used as a stationary phase and acetonitrile:water (75:25) at a flowrate of 1 mL/min was employed for elution.

Protein concentration was determined with the dye-binding method of Bradford [15].

2.4. Effect of pH and temperature on enzyme activity and stability

The effect of pH and temperature on enzyme activity was determined using the natural substrate of the enzyme (lactose) at an initial concentration of 100 mM. Optimum pH was determined at 60 °C using a buffer range from pH 2 to 7, while optimum temperature was determined at pH 3.5 within a temperature range from 35 to 80 °C. In all experiments, the reaction rate was calculated by measuring the glucose concentration after 10 min of reaction time.

For stability measurements, a properly diluted enzyme sample was incubated in a thermostated water bath, at various pH (100 mM citrate–phosphate buffer) and temperatures. Aliquots, of 50 μL were removed at specific time intervals and assayed for their residual enzyme activity (oNPG).

2.5. Whey pretreatment

De-proteinated whey permeate was kindly provided by the Greek dairy industry MEVGAL S.A. (Nea Sindos, Greece). In order to be used in the ultrafiltration unit, whey had to be previously clarified for the removal of any suspended insoluble materials. Clarification involved continuous centrifugation followed by filtration on a Millipore system using a 0.1 μm porous size membrane. The clarified whey had a final lactose concentration of 112 mM, only trace amounts of glucose and galactose (less than 0.1 mM) and a protein content of less than 0.1 g/L.

2.6. Kinetic experiments

All kinetic experiments were conducted at pH 3.5 (100 mM citrate–phosphate buffer) in screw cap glass test tubes that served as batch enzyme reactors. Whey permeate, pretreated as described above, was diluted with citrate–phosphate buffer (pH 3.5) to yield the appropriate buffer molarity (100 mM) and desired initial lactose concentration. All reaction tubes were prepared in an ice bath, and upon enzyme addition, were placed in a water bath thermostated at the temperature of the reaction. The reaction rate was followed by determining the glucose concentration (GOX–PER method) in reaction aliquots (20 μL), withdrawn every 3 or 4 min from the reaction mixtures. For a number of samples, at the highest lactose concentration (112 mM) and extended reaction times, HPLC analysis was performed for probable detection of oligosaccharide formation.

3. Results and discussion

3.1. Enzyme production

Wheat bran, a low cost agricultural byproduct, that has been reported to induce the expression of galactosidases in various microbial species [16,17], has been also shown to induce β-galactosidase production by the fungus *A. niger* BTL

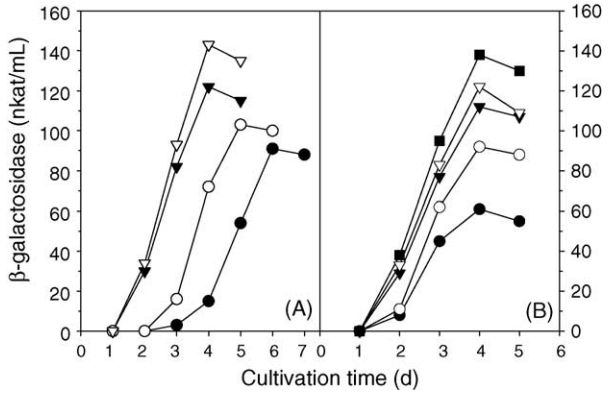

Fig. 1. (A) Effect of wheat bran (40 g/L) particle size (PS) on β-galactosidase production by *A. niger* BTL. (●) 850 > PS > 600 μm; (◯) 600 > PS > 300 μm; (▼) 300 > PS > 150 μm; (▽) 150 > PS > 75 μm. (B) Effect of wheat bran concentration (particle size: 150 > PS > 75 μm) on β-galactosidase production by *A. niger* BTL. (●) 30 g/L; (▽) 35 g/L; (■) 40 g/L; (▼) 45 g/L; (◯) 50 g/L.

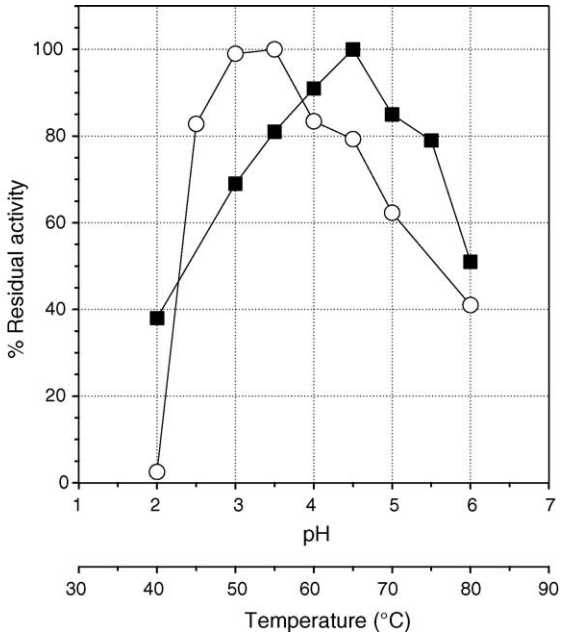

Fig. 2. Optimum pH (◯) and temperature (■) for the activity of the partially purified β-galactosidase of *A. niger* BTL (reaction time 10 min).

when used as the sole carbon and energy source [13]. Initially, the wheat bran particle size distribution was examined with respect to enzyme production. The results, depicted in Fig. 1A, show that the size of wheat bran particles significantly influenced β-galactosidase production. More specifically, the smaller the particle size, the highest the maximum enzyme activity. In addition, the productivity of β-galactosidase production was significantly increased, since, in the cultures with low wheat bran particle size, maximum extracellular enzyme levels were obtained at shorter cultivation times. This fact can easily be explained, since wheat bran serves as the only carbon and energy source in the culture, and the high specific surface area provided by the small particle size, facilitates its utilization by the microorganism. Wheat bran concentration also affected β-galactosidase production (Fig. 1B). Optimum enzyme levels were obtained at a wheat bran concentration of 40 g/L.

The optimum culture conditions with respect to β-galactosidase production by *A. niger* BTL were employed for the production of the biocatalyst for whey lactose hydrolysis. Two liters Erlenmeyer flask cultures were used (750 mL working volume). At the end of the 4th day, the supernatant was collected and the biocatalyst was recovered in the form of lyophilized powder, as described under Section 2. The whole procedure resulted in the removal of about 50% of the total extracellular protein with the simultaneous recovery of 80% of the β-galactosidase activity (enrichment 1.5 times). The enzyme preparation after lyophilization was highly water-soluble, possessed high β-galactosidase activity (190 nkat/mg protein) and could be readily used for the hydrolysis of whey lactose.

3.2. Activity and stability properties

The optimum pH for β-galactosidase activity was determined at 60 °C, using an initial lactose concentration of 100 mM. The reaction time was kept to a minimum (10 min) to avoid possible enzyme inactivation during prolonged incubation times. As with other fungal β-galactosidases [18,19], the enzyme had acidic pH optima between 3 and 3.5 (Fig. 2). The optimum activity temperature, determined at pH 3.5, was 65 °C, with more than 80% of the residual activity retained between 55 and 75 °C (Fig. 2). The above temperature–activity optima are among the highest reported for β-galactosidases from mesophilic microorganisms [18,20,21].

The pH stability of the β-galactosidase from *A. niger* BTL was determined within the range of pH values from 2 to 9. The enzyme was incubated at 25 °C at the appropriate buffers and the residual activity was determined at specific time intervals. The results are depicted in Fig. 3A. At this temperature (25 °C), the enzyme preparation was extremely stable between pH 3 and 6, showing no detectable activity loss even after 5 days of incubation at these pH values. Temperature stability was examined in a similar manner, at two different pH values, namely 3.5 and 5 (Fig. 3B). β-Galactosidase was very stable up to 55 °C, at both pH values tested. Above this temperature, the enzyme gradually lost its activity, although at pH 5 it was much more stable than pH 3.5. These stability properties rank β-galactosidase from *A. niger* BTL as one of the most stable mesophilic fungal enzymes [18,22] that is even more stable than yeast β-galactosidases immobilized at various supports [23–25]. Thermal denaturation of the β-galactosidase at the optimum pH for the activity (pH 3.5), clearly follows first order deactivation kinetics at all temperatures examined (Fig. 4). The values of the deactivation constants, k_d, were successfully fitted into an Arrhenius plot

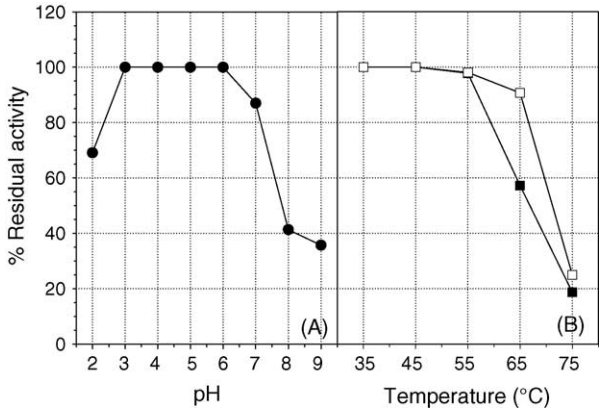

Fig. 3. (A) pH stability of the β-galactosidase from *A. niger* BTL. Residual activity was determined after 120 h incubation at the indicated pH values and 25 °C. (B) Thermal stability of the β-galactosidase of *A. niger* BTL. The enzyme was incubated for 2 h at the indicated temperature at pH 3.5 (■) and 5 (□).

(Fig. 4, insert) yielding the following equation:

$$k_d = 9.5142 \times 10^{26} \exp\left(\frac{-21677.4}{T + 273}\right) \quad (1)$$

that results in an activation energy, E_a, equal to 180.3 kJ/mol (43.1 kcal/mol). This value for the activation energy is the lowest reported for mesophilic enzymes in solution, and very close to the E_a values obtained for the same enzymes in immobilized form [22].

The enhanced stability properties, of the β-galactosidase of *A. niger* BTL, combined with its acidic pH activity optima, suggest that the enzyme is an ideal candidate for the hydrolysis of lactose in acid whey permeate.

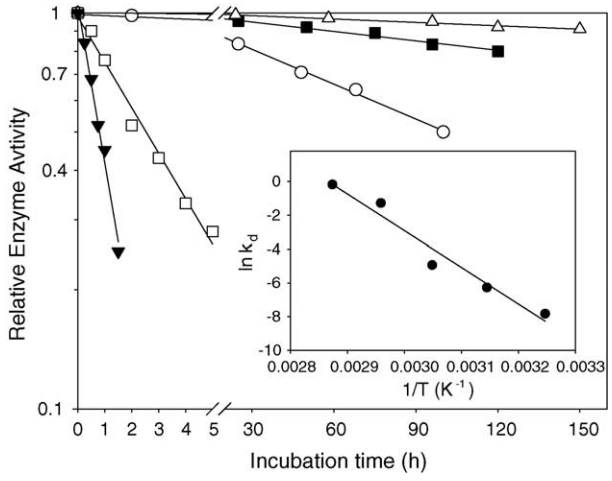

Fig. 4. Thermal deactivation of the β-glucosidase of *A. niger* BTL at pH 3.5. (△) 35 °C; (■) 45 °C; (◯) 55 °C; (□) 65 °C; (▼) 75 °C. Insert: Arrhenius plot for the determination of the activation energy for the first order deactivation constant, k_d (h^{-1}).

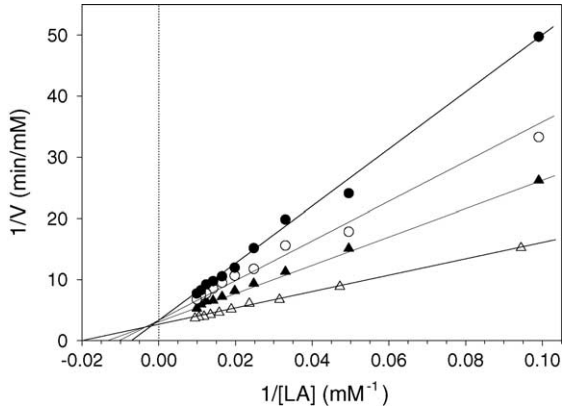

Fig. 5. Lineweaver–Burk plot for the determination of the inhibition pattern of β-galactosidase by galactose, during whey lactose hydrolysis at pH 3.5 and 55 °C. Galactose concentration (mM): (△) 0; (▲) 5; (◯) 10; (●) 15.

3.3. Kinetics of lactose hydrolysis

The kinetic model that describes the hydrolysis of whey lactose, in free solution, was initially determined at pH 3.5 and 55 °C. A classic approach was followed, using a number of initial reaction rate experimental sets (reaction time 10 min), covering a range of substrate and product(s) concentrations. The different substrate concentrations were obtained by diluting whey to a lactose concentration range from 10 to 112 mM (the latter is the lactose concentration of undiluted whey). Within the above range of substrate concentrations, no inhibition from lactose was observed. In addition, HPLC analysis on the reaction products, did not show any production of significant amounts of galactooligosaccharides, even at the highest lactose concentrations used.

Product inhibition is a very common feature shared by many β-galactosidases [7,8,24,26]. The enzyme from *A. niger* BTL was not inhibited by glucose up to a concentration of 150 mM (data not shown). In contrast, galactose proved to be a strong inhibitor. As revealed from the corresponding Lineweaver–Burk plot (Fig. 5) galactose was a competitive inhibitor of the β-galactosidase of *A. niger* BTL. According to the above results, the Michaelis–Menten rate equation with competitive inhibition from galactose, was used to describe the kinetics of whey lactose hydrolysis by the β-galactosidase of *A. niger* BTL (Eq. (2)).

$$r_{GL} = r_{GA} = -r_{LA} = \frac{k[E][LA]}{[LA] + K_m[1 + ([GA]/K_I)]} \quad (2)$$

The values of the three kinetic parameters, k, K_m and K_I, in Eq. (2) were determined using whey lactose, at five different temperatures, namely 35, 45, 55, 65 and 75 °C. The experimental procedure employed for each temperature was the following: A number of initial reaction rate (10 min) experiments at different galactose concentrations within a range of whey dilutions were initially performed, in order to obtain good estimates for K_m and K_I from the corresponding Lineweaver–Burk plots. Thereafter, four batch hydrol-

Table 1
Effect of temperature (at pH 3.5) on the values of the kinetic constants for the whey lactose hydrolysis model by the β-galactosidase of *A. niger* BTL

Temperature	K_m (mM)	K_I (mM)	k (mmol/(nkat h))
35	31.8	6.56	0.000792
45	39.2	6.98	0.001390
55	61.4	8.92	0.002166
65	89.2	12.7	0.002635
75	106.4	13.8	0.001981

$$K_m = 2.3861 \times 10^6 \exp\left(\frac{-3471.78}{T+273}\right), \quad R^2 = 0.981 \qquad (3)$$

$$K_I = 8.4596 \times 10^3 \exp\left(\frac{-2228.64}{T+273}\right), \quad R^2 = 0.943 \qquad (4)$$

$$k = 0.0101 - 6.8993 \times 10^{-4}T + 1.5881 \times 10^{-5}T^2 - 1.0842 \times 10^{-7}T^3, \quad R^2 = 0.999 \qquad (5)$$

ysis experiments, at two initial lactose concentrations (112 and 56 mM) and two initial enzyme concentrations (50,000 and 100,000 nkat/L) were performed where lactose, glucose and galactose concentrations were determined at various times during the reaction progress. Using the non-linear least squares regression routines of SigmaPlot Ver. 7 (SPSS Inc.) with the values of K_m and K_I determined above as initial estimates, the data were fitted into an integrated form of Eq. (2) that incorporated the first order thermal deactivation kinetics of the enzyme, according to Eq. (1). The mean values (from the four extended hydrolysis experiments) of the kinetic constants of Eq. (2) determined that way are summarized in Table 1. These values were used in order to derive, through linear regression, Eqs. (3)–(5) (Table 1), that describe the temperature dependence of the kinetic constants in the whey lactose hydrolysis model. While for K_m and K_I the data were successfully fitted to Arrhenius type equations, the corresponding fit for k, could not be achieved ($R^2 < 0.85$) for the complete range of temperatures used. Consequently, a cubic polynomial equation was chosen to describe the temperature dependence for this constant.

3.4. Model development

The construction of the model that describes the hydrolysis–ultrafiltration of whey lactose was based on the generalized experimental set-up depicted in Fig. 6. The set-up is comprised of a mixing vessel (practically a batch enzyme reactor) of working volume V, in which whey is fed at a constant flowrate F_1. A pump drives a portion of the reactor content towards the ultrafiltration unit at a flowrate F_2, the retentate of which (F_3) is returned to the mixing vessel. The permeate of the ultrafiltration unit, is the net product of the complete hydrolysis–ultrafiltration process (F_4). The basic assumptions that will be used during the construction of the mathematical equations that describe the system are the following:

- The dead volume of the piping and the ultrafiltration unit is negligible compared to the volume of the mixing vessel, that is, the mixing vessel is the only part of the system that provides time and space for the hydrolysis reaction.

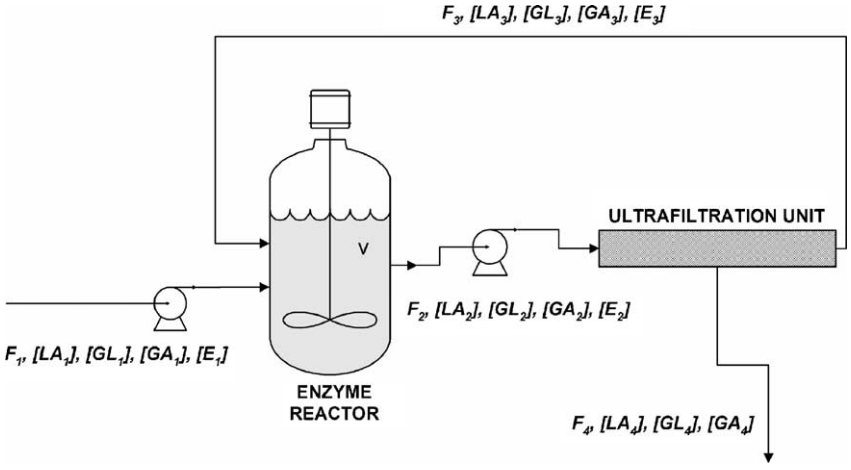

Fig. 6. Generalized experimental set-up for the continuous hydrolysis–ultrafiltration of whey lactose.

- The passage of the reaction mixture through the ultrafiltration unit has no effect on the concentration of the low molecular weight compounds involved, that is:

$$[LA_2] = [LA_3] = [LA_4], \quad [GL_2] = [GL_3] = [GL_4],$$
$$\text{and} \quad [GA_2] = [GA_3] = [GA_4].$$

- There are no enzyme losses in the ultrafiltration unit.
- Enzyme deactivation in the system follows first order kinetics, where k_d (h^{-1}) is the corresponding deactivation constant, that is a function of the process temperature, according to Eq. (1).
- The hydrolysis of lactose by β-galactosidase follows Michaelis–Menten kinetics with competitive inhibition only from galactose (Eq. (2)), with temperature dependence of the kinetic constants according to Table 1.

In order to fully exploit the process characteristics, modeling of the transient mode was selected. The process commences ($t = t_0$) with the mixing vessel filled with a whey volume V, of initial lactose concentration $[LA_0]$, where a certain amount of β-galactosidase at a concentration $[E_0]$ has been added. Following the above assumptions and initial conditions, the system of first order linear differential equations that describe the complete hydrolysis–ultrafiltration unit can be derived.

3.4.1. Volume change—system dilution rate D

The differential mass balance in the mixing vessel yields:

$$\frac{dV}{dt} = F_1 + F_3 - F_2 \Rightarrow \frac{dV}{dt} = F_1 - F_4 \tag{6a}$$

Eq. (6a) implies that in order to achieve constant volume in the mixing vessel, flows F_1 and F_4 should be equal, thus defining the dilution rate, D, of the system as:

$$D = \frac{F_1}{V} = \frac{F_4}{V} \tag{6}$$

3.4.2. Enzyme concentration

The differential mass balance on enzyme concentration (β-galactosidase) in the constant volume mixing vessel is the following:

$$V\frac{d[E_2]}{dt} = F_3[E_3] + F_1[E_1] - F_2[E_2] + Vr_d \tag{7a}$$

Since we consider that there are no enzyme losses in the ultrafiltration unit:

$$F_3[E_3] = F_2[E_2] \tag{7b}$$

Substituting Eqs. (6) and (7b) into Eq. (7a) and simplifying, we come up with the final differential equation that describes the change of enzyme concentration in the reactor:

$$\frac{d[E_2]}{dt} = D[E_1] - k_d[E_2] \tag{7}$$

with initial condition: at $t = 0 \ldots [E_2] = [E_0]$.

3.4.3. Lactose concentration

The mass balance for lactose in the reaction vessel can be written as:

$$V\frac{d[LA_2]}{dt} = F_3[LA_3] + F_1[LA_1] - F_2[LA_2] + Vr_{LA} \tag{8a}$$

By incorporating Eq. (6) and taking into account that $[LA_2] = [LA_3]$ as well as that $F_2 = F_3 + F_4$, Eq. (8a) is transformed into:

$$\frac{d[LA_2]}{dt} = D([LA_1] - [LA_2]) + r_{LA} \tag{8b}$$

which takes its final form through the substitution of the corresponding kinetic model for β-galactosidase as expressed through Eq. (2):

$$\frac{d[LA_2]}{dt} = D([LA_1] - [LA_2]) - \frac{k[E_2][LA_2]}{[LA_2] + K_m[1 + ([GA_2]/K_I)]} \tag{8}$$

with initial condition: at $t = 0 \ldots [LA_2] = [LA_0]$.

3.4.4. Concentration of glucose and galactose

An analysis similar to lactose, yields the differential equations that describe the concentration change of glucose and galactose in the enzyme reactor:

$$\frac{d[GL_2]}{dt} = D([GL_1] - [GL_2]) + \frac{k[E_2][LA_2]}{[LA_2] + K_m[1 + ([GA_2]/K_I)]} \tag{9}$$

with initial condition: at $t = 0 \ldots [GL_2] = 0$.

$$\frac{d[GA_2]}{dt} = D([GA_1] - [GA_2]) + \frac{k[E_2][LA_2]}{[LA_2] + K_m[1 + ([GA_2]/K_I)]} \tag{10}$$

with initial condition: at $t = 0 \ldots [GA_2] = 0$.

Eqs. (7)–(10) describe the transient kinetics for the general continuous hydrolysis–ultrafiltration process for whey lactose. The above system of equations can be simultaneously integrated for a variety of experimental conditions in order to evaluate the effect of the various process parameters into the overall system performance at various temperatures. The mathematical software package MATHCAD™ (Ver. 6.0) was successfully implemented for that purpose.

3.5. Experimental evaluation of the proposed model

In order to experimentally evaluate the validity of the proposed model, we designed the laboratory layout for the continuous hydrolysis–ultrafiltration of whey that is depicted in Fig. 7. It is comprised of a double wall glass vessel, with 1.2 L working volume. The vessel content is kept at a constant temperature, through the circulation of water between the vessel

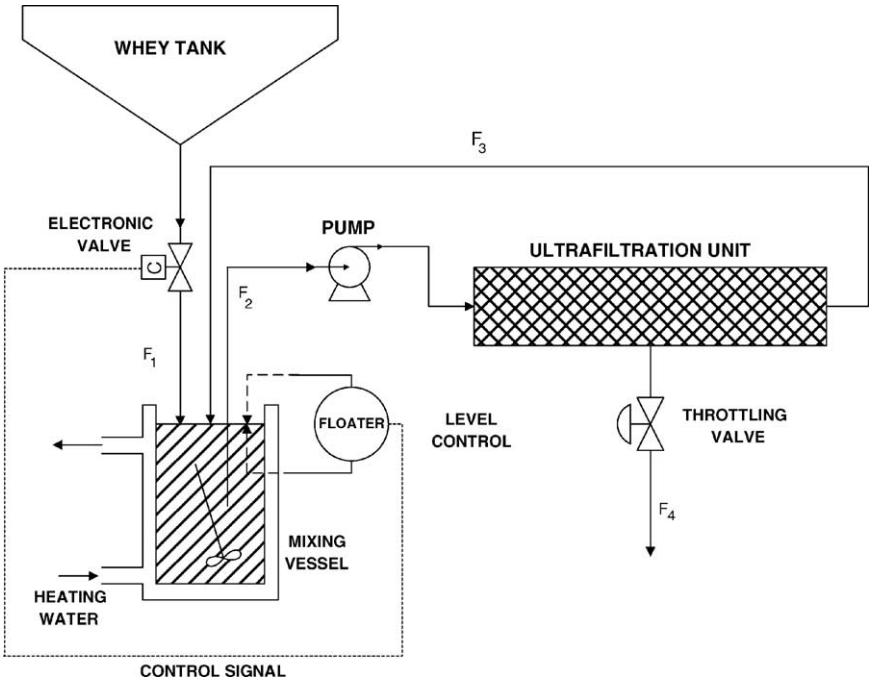

Fig. 7. Laboratory layout for the experimental validation of the proposed model for the simultaneous hydrolysis–ultrafiltration of whey. The volumetric flowrates F_i's correspond to those of Fig. 6.

walls. A peristaltic recirculation pump drives whey from the vessel to the Ultrafiltration Unit (Millipore, Minitan System), consisting of a four membrane (15 cm × 8 cm each) module with a molecular weight cut-off of 30 kDa. The latter was chosen based on the molecular weight of the β-galactosidase from *A. niger* BTL which was determined equal to 120 kDa [27]. The flowrate of the permeate stream (F_4) is initially controlled by a throttling valve. In order to compensate for the permeate losses, and thus achieving steady state (with respect to the mixing vessel volume) conditions for the system volume ($F_1 = F_4$), an electromagnetic level controller (floater) has been incorporated on top of the mixing vessel, automatically controlling the electric valve that regulates the gravitational feed of the vessel with whey.

Using the above laboratory layout, we performed the experimental evaluation of the mathematical model for the continuous hydrolysis–ultrafiltration of whey that we developed in the previous paragraphs. More specifically, we conducted three continuous hydrolysis–ultrafiltration experiments that lasted approximately 60 h each. For every experiment, the mixing vessel was initially filled with 1.18 L of pretreated whey (initial lactose concentration of 112 mM). The process was initiated by starting the recirculation pump upon addition of 20 mL of concentrated enzyme solution, to yield the desired $[E_0]$ in the final working volume (1.2 L). In addition, no enzyme was added in the feed stream ($[E_1] = 0$). The dilution rate of the system ($D = F_4/V = F_1/V$) was kept constant by maintaining the level of permeate (product) flowrate (F_4) through manual manipulation (on a 24 h basis) of either the throttling valve on the untrafiltration unit or the recirculation flowrate F_2. More specifically, the permeate volume was measured at 15–30 min intervals and checked if it corresponded to the desired dilution rate. Increases in the permeate flowrate (when needed) where implemented by either releasing the throttling valve or increasing the revolutions of the recirculating pump and vice versa. The pretreatment of the whey used, combined with the relatively high molecular weight cut-off of the ultrafiltration membrane (both prevented extensive membrane clogging) did not allow for deviations in the dilution rate greater than ±8% of the set value. Glucose concentration in the product ($[GL_4] = [GL_2]$) was determined at specific time intervals during operation. The process parameters chosen for the three experiments were the following:

- Experiment 1: $T = 35\,^{\circ}C$, $[E_0] = 200$ nkat/mL, $D = 0.2\,h^{-1}$ ($F_1 = F_4 = 0.24$ L/h).
- Experiment 2: $T = 55\,^{\circ}C$, $[E_0] = 60$ nkat/mL, $D = 0.4\,h^{-1}$ ($F_1 = F_4 = 0.48$ L/h).
- Experiment 3: $T = 55\,^{\circ}C$, $[E_0] = 50$ nkat/mL, $D = 0.75\,h^{-1}$ ($F_1 = F_4 = 0.90$ L/h).

Fig. 8 shows the experimentally determined values of glucose concentration in the hydrolyzed product in comparison with the values predicted by the model. It is obvious, that model predictions correlate excellently with the experimental values, a fact that fully verifies the validity of the model. A small deviation from the predicted values observed during the low dilution rate experiment at elevated temperature (Experiment 2), is, most probably, due to an error introduced from a small degree of evaporation in the open mixing vessel (process temperature 55 °C), since the evaporation losses

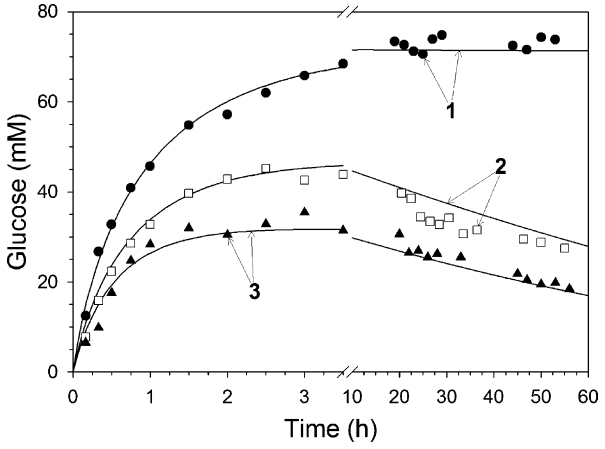

Fig. 8. Comparison between experimentally determined values and model predictions for glucose concentration in the product stream (F_4), during evaluation of the model for the continuous hydrolysis–ultrafiltration of whey lactose. (1) $T = 35\,^{\circ}C$, $[E_0] = 200$ nkat/mL, $D = 0.2\,h^{-1}$. (2) $T = 55\,^{\circ}C$, $[E_0] = 60$ nkat/mL, $D = 0.4\,h^{-1}$. (3) $T = 55\,^{\circ}C$, $[E_0] = 50$ nkat/mL, $D = 0.75\,h^{-1}$. Solid lines: model predictions, symbols: experimental results.

were compensated by the control mechanism with whey addition.

3.6. Sensitivity analysis

Having established an experimentally validated model for the continuous hydrolysis–ultrafiltration of whey lactose, by the β-galactosidase of *A.niger* BTL, a series of simulation experiments, can be performed in order to examine the sensitivity of the system to the various process parameters at steady state. Thus, the derivatives in Eqs. (7)–(10) were set to zero, and the model was solved as a system of non-linear equations with all kinetic constants being functions of temperature, through Eqs. (1) and (3)–(5).

Initially, it was chosen to examine the combined effect of dilution rate and process temperature. A lactose concentration in the feed, equal to 112 mM and an enzyme load (initial and steady state enzyme concentration in the mixing vessel, $[E_2] = [E_0]$) of 100 nkat/mL, was employed. At all temperatures, an increase of the dilution rate had a negative effect on the obtained degree of hydrolysis (Fig. 9A) and a concomi-

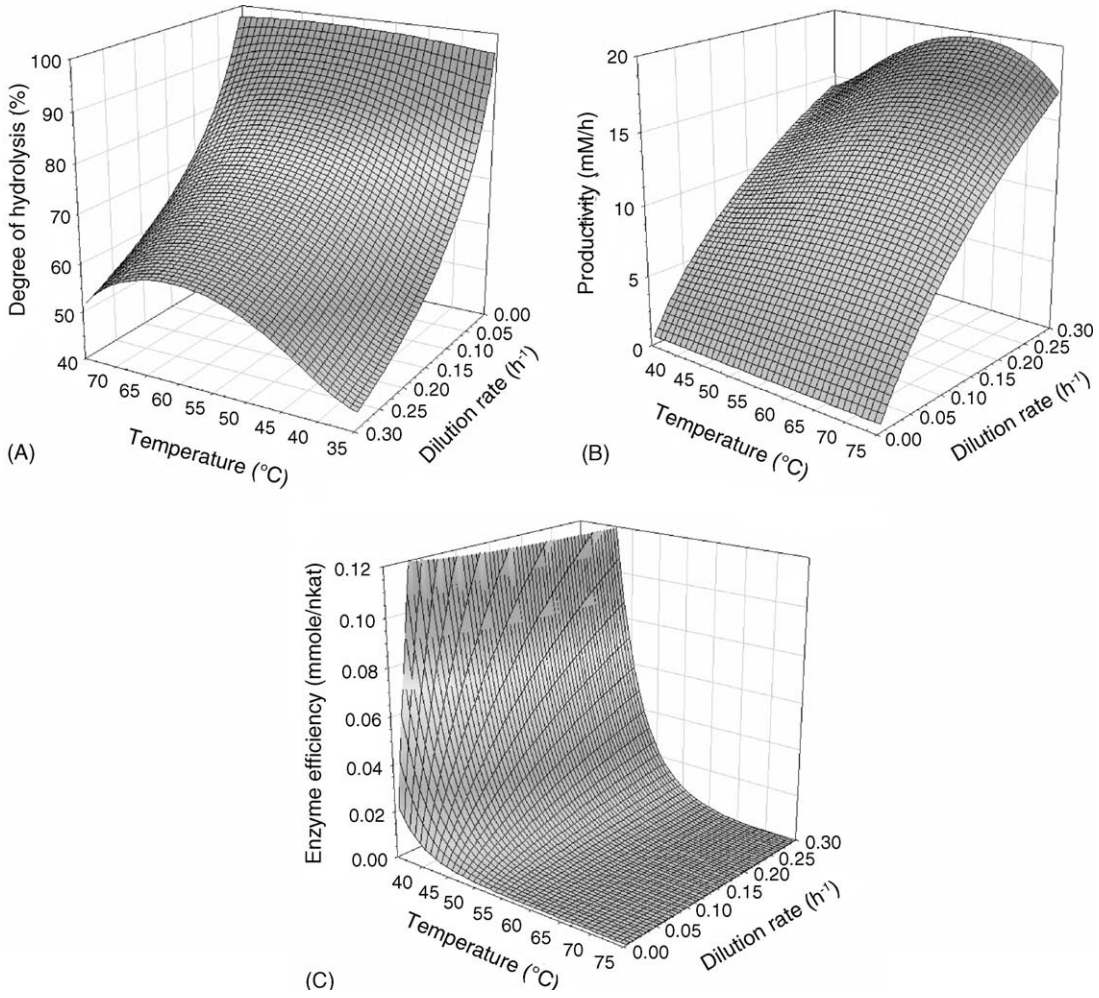

Fig. 9. Combined effect of temperature and dilution rate on the degree of hydrolysis (A), productivity (B) and enzyme efficiency (C) during the continuous hydrolysis–ultrafiltration of whey permeate lactose by the β-galactosidase of *A. niger* BTL. $[LA_0] = [LA_1] = 112$ mM, $[E_0] = [E_2] = 100$ nkat/mL.

tant positive effect on the volumetric productivity, defined as $D[GL_4] = D[GA_4]$ (Fig. 9B). This feature has been also verified in other continuous enzyme reactor systems for lactose hydrolysis [11,28,29]. Temperature optima (62 °C), with respect to the degree of hydrolysis, are found near the optimum temperature for enzyme activity, at all dilution rates. Again, these optima correspond to volumetric productivity minima. The effect of temperature though, is becoming less significant as the dilution rate decreases. As implied from the model equations, in order to achieve steady state conditions, a certain enzyme concentration, $[E_1]$, should be fed in the system to compensate for activity losses and maintain a constant enzyme concentration in the mixing vessel, $[E_2]$, especially at high process temperatures. Since enzyme production, is a major cost factor for the overall process, in Fig. 9C, we additionally investigated the combined effect of dilution rate and temperature on enzyme efficiency, E_{eff}, defined as the mmol of glucose (or galactose) produced per nkat enzyme consumed in the feed stream ($E_{eff} = [GA_4]/[E_1] = [GL_4]/[E_1]$). The corresponding results are quite interesting, since they suggest, that in case enzyme efficiency is of major concern, the process should be run at relatively low dilution rates, and at temperatures far below the optimum one for enzyme activity.

Fig. 10 shows the combined effect of temperature and enzyme load in the process performance characteristics for a dilution rate of 0.15 h^{-1}. The effect of enzyme load (the steady state concentration of enzyme in the mixing vessel, $[E_2] = [E_0]$) on the obtained degree of hydrolysis reveals a saturation profile, with an initial sharp increase and an apparent leveling off at high enzyme concentrations (Fig. 10A). A similar profile was obtained for the volumetric productivity, which was also an increasing function of enzyme load (Fig. 10B). These results, combined with the fact that enzyme efficiency is favored at lower enzyme loads (Fig. 10C) clearly advise, that the chosen steady state enzyme concentration should not necessarily be kept at as high levels as possible, but rather at the lowest possible levels for the desirable degree of hydrolysis and volumetric productivity.

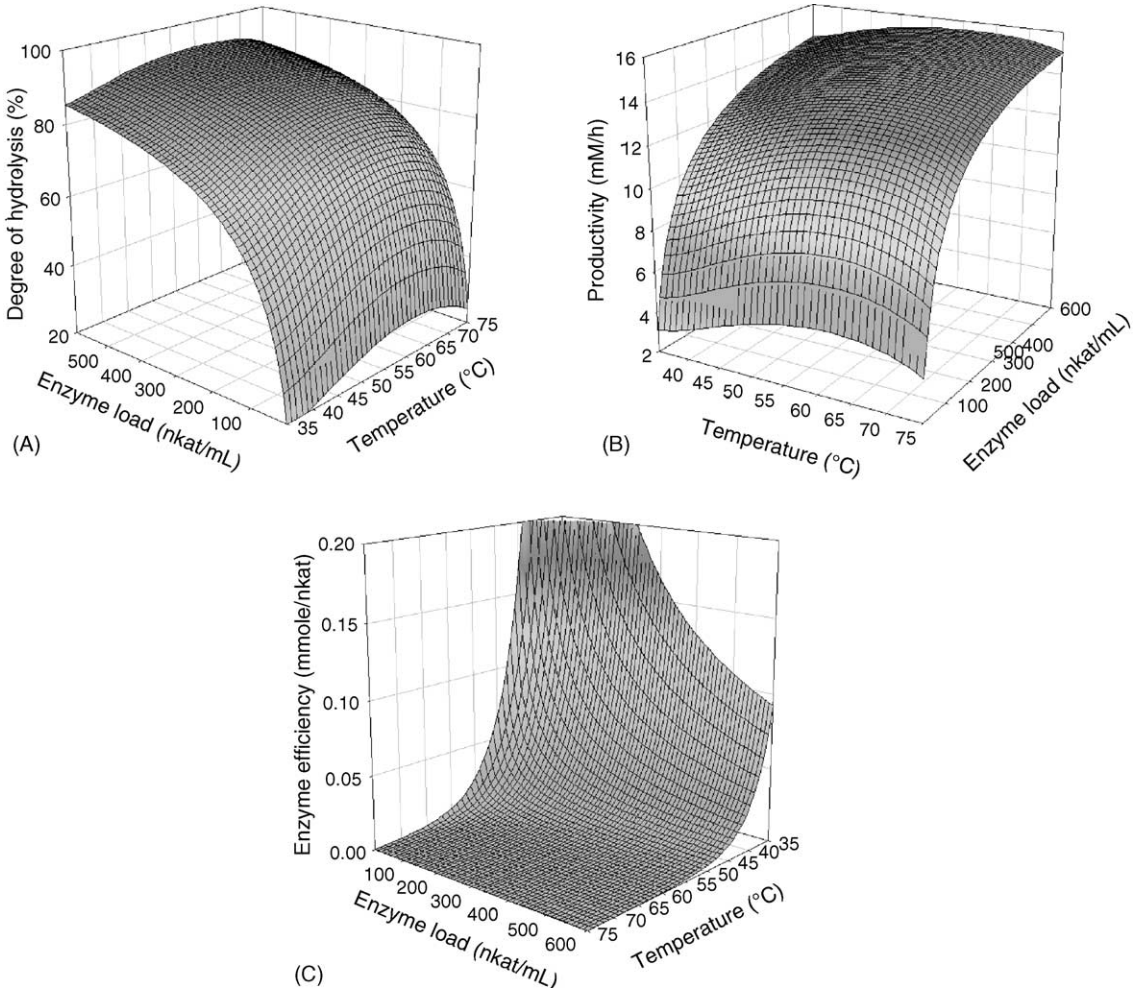

Fig. 10. Combined effect of temperature and enzyme load on the degree of hydrolysis (A), productivity (B) and enzyme efficiency (C) during the continuous hydrolysis–ultrafiltration of whey permeate lactose by the β-galactosidase of *A. niger* BTL. $[LA_0] = [LA_1] = 112$ mM, $D = 0.15\ h^{-1}$.

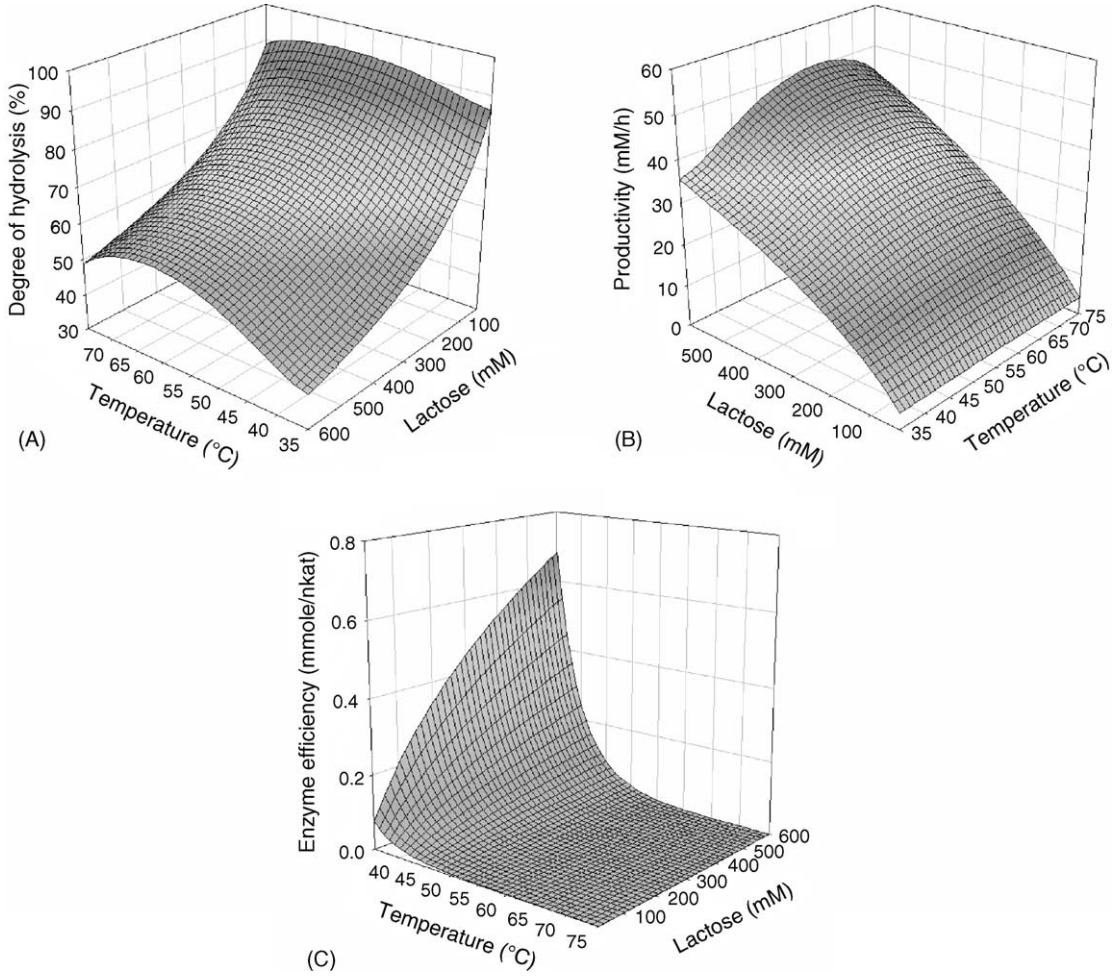

Fig. 11. Combined effect of temperature and lactose concentration on the degree of hydrolysis (A), productivity (B) and enzyme efficiency (C) during the continuous hydrolysis–ultrafiltration of whey permeate lactose by the β-galactosidase of *A. niger* BTL. $[E_0] = [E_2] = 200$ nkat/mL, $D = 0.15\,h^{-1}$.

Finally, since concentrated whey can be easily made available through a number of dairy industrial processes, in Fig. 11, we examined the combined effect of lactose concentration in the feed stream and temperature. The simulation was performed at a dilution rate of $0.15\,h^{-1}$ and an enzyme load of 200 nkat/mL. Increasing lactose concentration in the feed, results in higher reaction rates that lead to increased volumetric productivities and enzyme efficiencies. The obtained degree of hydrolysis though, is negatively affected, since galactose accumulation at high lactose concentrations gradually inhibits the enzyme reaction.

4. Conclusion

A complete mathematical model for the simultaneous hydrolysis–ultrafiltration of whey permeate lactose was developed. It incorporated the specific kinetic characteristics of a thermostable β-galactosidase, produced at elevated levels from a wild type of *A. niger* strain, grown on wheat bran as a sole carbon and energy source. Model predictions were very accurate in describing both the transient and steady state behavior of a continuous whey permeate hydrolysis–ultrafiltration laboratory set up. The model was successfully used for in silico evaluations on the effect of the various process parameters such as dilution rate, operation temperature, enzyme load and lactose concentration on the performance characteristics of the system, providing a useful tool that can be used in combination with the corresponding cost analysis data on the determination of the optimum operating conditions of such a process.

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