

# Hydrolysis of Lactose: Estimation of Kinetic Parameters Using Artificial Neural Networks

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Received: June 6, 2013 / Published: October 20, 2013.

**Abstract:** The analysis of any kinetic process involves the development of a mathematical model with predictive purposes. Generally, those models have characteristic parameters that should be estimated experimentally. A typical example is Michaelis-Menten model for enzymatic hydrolysis. Even though conventional kinetic models are very useful, they are only valid under certain experimental conditions. Besides, frequently large standard errors of estimated parameters are found due to the error of experimental determinations and/or insufficient number of assays. In this work, we developed an artificial neural network (ANN) to predict the performance of enzyme reactors at various operational conditions. The net was trained with experimental data obtained under different hydrolysis conditions of lactose solutions or cheese whey and different initial concentrations of enzymes or substrates. In all the experiments, commercial  $\beta$ -galactosidase either free or immobilized in a chitosan support was used. The neural network developed in this study had an average absolute relative error of less than 5% even using few experimental data, which suggests that this tool provides an accurate prediction method for lactose hydrolysis.

**Key words:** Cheese whey,  $\beta$ -galactosidase, lactose hydrolysis, artificial neural network.

## 1. Introduction

The disposal of whey permeate, a by-product of cheese manufacture, remains a significant environmental problem for the dairy industry. Whey permeate has high biological oxygen demand (BOD) due mainly to its lactose content (3%-5%) and determines that it should be treated prior to its discharge [1]. The most important cost-effective utilizations of cheese whey is valorization technologies, such as recovery of protein and lactose from whey or spray drying, and direct utilization of whey as animal feed [2-4].

Lactose can be recovered alongside the whey protein, however, its worldwide demand is much lower than the actual lactose availability [5]. Lactose

transformation to value added products, such as sweetening syrups, galacto-oligosaccharides or ethanol, constitutes a feasible strategy to further utilize the commodity. The hydrolysis of lactose is a key step in any conversion process and it can be accomplished chemically or enzymatically using  $\beta$ -galactosidase.

There are basically two different ways to use  $\beta$ -galactosidase. The soluble enzyme is normally used in batch processes while the immobilized form is used in batch or continuous operation. Despite the high cost of enzyme attachment, immobilized  $\beta$ -galactosidase systems remain more economically feasible than free enzyme systems, as these processes may be performed continuously and offer the possibility of reutilizing the enzyme [6, 7]. The choice of lactose hydrolysis in batch and continuous mode depends primarily on the enzymatic characteristics and the economics encompassing the production, storage and reusability [8].

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Various types of supports and techniques have been used for  $\beta$ -galactosidase immobilizations but the covalent immobilization shows the best industrial applications. Recently, the covalently bound of  $\beta$ -galactosidase beads prepared from different sources (Artemisia seed gum, chitosan, and carrageenan) was done in the presence of glutaraldehyde and the effects of various preparation conditions on the activity of the immobilized  $\beta$ -galactosidase were studied. The results of these works showed that this methodology resulted in an increase in enzyme stability [9-11].

The kinetic and yield characterization of the reaction system is very important. The process's conditions should be adequately controlled so that the conversion rates and yields are optimized. The effects of substrate and enzyme concentrations on the conversion efficiency have been extensively studied and modeled. Enzyme immobilization has been proven to increase the process efficiency and inhibition effects have been related to the presence of hydrolysis by-products and whey proteins. The reaction kinetics of the enzymatic hydrolysis of lactose to glucose and galactose has traditionally been characterized using the Michaelis-Menten model with competitive inhibition by product [12, 13].

$$\frac{ds}{dt} = -E k_2 \frac{s}{k_m \left( 1 + \frac{s_0 - s}{k_i} \right) + s} \quad (1)$$

where,  $s$  (mM) is the residual substrate concentration (lactose),  $s_0$  the initial substrate concentration,  $E$  (mg protein/mL) the enzyme concentration as dairy protein,  $k_2$  specific enzymatic rate constant,  $k_m$  saturation constant and  $k_i$  inhibition constant.

Although the kinetics parameters of this model, namely  $k_m$ ,  $k_2$  and  $k_i$ , have physical meaning and can be obtained from the experimental data using a regression procedure, the ability of this approach to simultaneously characterize the effect of several factors is limited. Artificial neural networks (ANNs) are gaining popularity in food applications as powerful and efficient tools to model complex

processes that exhibit non linear behaviors [14].

Because of their learning ability, fault tolerance and high computational speed, they can analyze and model the effect of several conditions at the same time. The suitable control of industrial effluents has become very important to solve environmental issues. Better control of treatment plants may be achieved by the use of a robust model to predict certain key parameters based on past observations. Models based on artificial neural networks have been successfully used in wastewater treatment systems and are very effective at capturing the nonlinear relationships existing between variables (multi-input/multi-output) in complex systems [4].

The aim of this study was to use this ability of ANN to predict the performance of enzyme reactors at various operational conditions using an artificial neural network approach. For this purpose, the net was trained with experimental data obtained under different hydrolysis conditions of lactose solutions or whey, and different initial concentrations of enzymes or substrates. In all the experiments, the enzyme  $\beta$ -galactosidase was used either free or covalently immobilized onto chitosan beds using glutaraldehyde as activating agent.

## 2. Materials and Methods

### 2.1 Hydrolysis of Lactose

Lactose solutions of 2.5%-10.0% (w/v) were prepared by dissolving lactose (reagent grade, Merck, USA) in a phosphate buffer pH 6.85 and used as substrate. The effect of whey proteins on the conversion efficiency was assessed by using two additional reaction media, whey permeate and whey permeate with proteins.

The enzyme  $\beta$ -galactosidase from *Kluyveromyces fragilis* (Lactozym 3,000 L, Novo Nordisk A/S, Denmark) with an initial activity of 3,000 LAU/mL was used in this study. A microbial lactase unit (LAU) is defined as the quantity of enzyme that will liberate 1 mol of glucose/min at 37 °C and pH 6.7, using

lactose 4.7% (w/w) as substrate. The enzyme was used either free or covalently bound to chitosan beads. The beads were prepared by dissolving crab shell chitosan (Sigma Co., USA) in an aqueous solution of acetic acid (2.5% w/v). The chitosan solution was added using a peristaltic pump into a stirred coagulation solution of NaOH (1 M) and sodium tri-polyphosphate (1.5% w/v). The resulting beads, which had an average diameter of 2.1 mm, were held in the coagulating solution for 30 min. Once the chitosan spheres were produced, the cross linking was carried out in a glutaraldehyde solution (2% w/v) for 12 h. In order to achieve the covalent immobilization of the enzyme, the activated beads were placed in a  $\beta$ -galactosidase solution (2% w/v) also for another 12 h [15].

The enzyme (free or immobilized) and the substrate (or the reaction media) were placed in a batch reactor. The lactose conversion was followed by monitoring the glucose concentration using a kit for enzymatic glucose determination (Wiener Lab, Rosario, Santa Fe, Argentina) followed by absorbance measurement at 505 nm in a spectrophotometer (Spectronic Genesys 5, Milton Roy, Rochester, NY, USA). All experiments were carried out in triplicate.

280 experimental data sets were obtained using different combinations of the following conditions (Table 1)

## 2.2 Artificial Neural Network

ANN models were constructed by interconnecting many nonlinear computational elements, known as neurons or nodes, operating in parallel, ordering into different layers and interconnected respect to a given topology. This arranging in patterns is similar to biological networks [16]. ANN has the ability to learn the solution of the problem from a set of examples, and to provide a smooth and accurate interpolation for new data [17]. For model identification, it is necessary to select real data coming from the process. Those data must include

**Table 1** Assay conditions used for obtaining the experimental data.

Conditions	Ranges
Substrate conc. (mM)	0.07, 0.15, 0.22 and 0.24
Enzyme conc. (mg/L)	0.8-3
Enzyme condition	Free or immobilized
Reaction media	Lactose sn, whey permeate, whey permeate with proteins
Protein concentration	0-protein free; 1-with protein 0.5% (w/v)

enough information to represent the different normal operation conditions of the process.

Artificial neural networks are mathematical model predicting certain key parameters based on past observations in complex systems [4]. There are many types of artificial neural network and in this study, a cascade-forward back-propagation model using three layers was adopted to model the performance of enzyme reactors.

Once the experimental data was obtained, a back propagation three-layer neural network model was developed. The input layer had six elements (Fig. 1). The optimal number of neurons ( $n$ ) in the hidden layer was determined following a trial and error procedure. Neurons were progressively added to the hidden layer until a good correlation between the experimental and output values were attained, based on the  $R^2$  value. The output layer had only one element, the residual lactose concentration.

All the tested networks used hyperbolic tangent transfer functions in the hidden layers and a linear transfer function in the output layer.

After the neural network, configuration was optimized, the training was carried out in batch mode during at most 200 epochs, using the Levenberg-Marquardt optimization procedure.

The experimental dataset was divided into two groups by random selection, the first group (150 observations) was used for network training and the remaining data (130 observations) were used for validation.

The minimum number of training data required to obtain an adequate estimation of the network (150

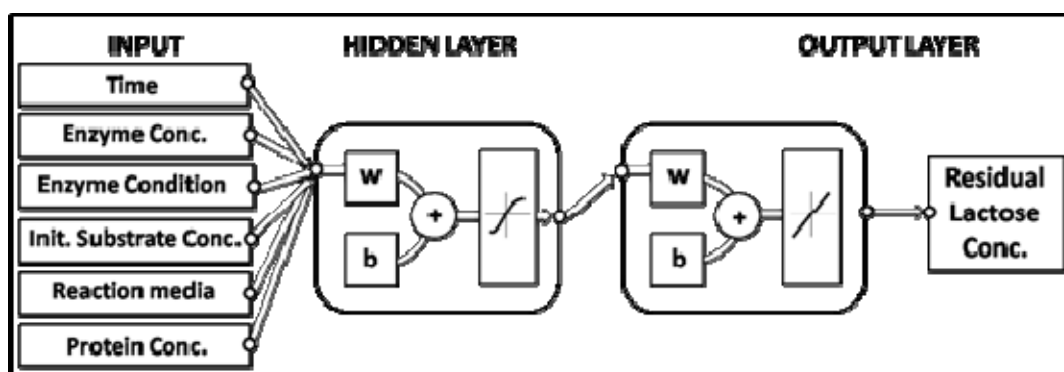


Fig 1 Schematic view of the artificial network used in this work ( $w$ : connection weights,  $b$ : threshold values).

observations) was determined using as a selection measure the mean or average error. Values below 10% ensured that enough data was used during the learning period (Fig. 2). The network architecture as well as its training, validation and simulation were programmed using MatLab<sup>®</sup> 6.1 (Mathworks, Inc., Natick, Massachusetts, USA).

### 3. Results and Discussion

#### 3.1 Hydrolysis of Lactose

The enzyme *b*-galactosidase from *Kluyveromyces fragilis* was used either free or covalently bound to chitosan beads to obtain the experimental data sets A and B, respectively (Table 2). To immobilize the enzyme, previously spheres chitosan activated with glutaraldehyde were used. The immobilization process allowed obtaining biocatalysts with high activity, stability and strength. The enzyme *b*-galactosidase from *Kluyveromyces fragilis* was used either free or covalently bound to chitosan beads to obtain the experimental results set. Previously spheres chitosan activated with glutaraldehyde were used to immobilize the enzyme [18, 19].

Compared to other techniques, this method have the following advantages: enzymes does not leak or detach from the carrier, the biocatalyst can easily interact with the substrate, since being on the surface of the carrier [20].

The use of immobilization technology is of significant importance from economic point of view

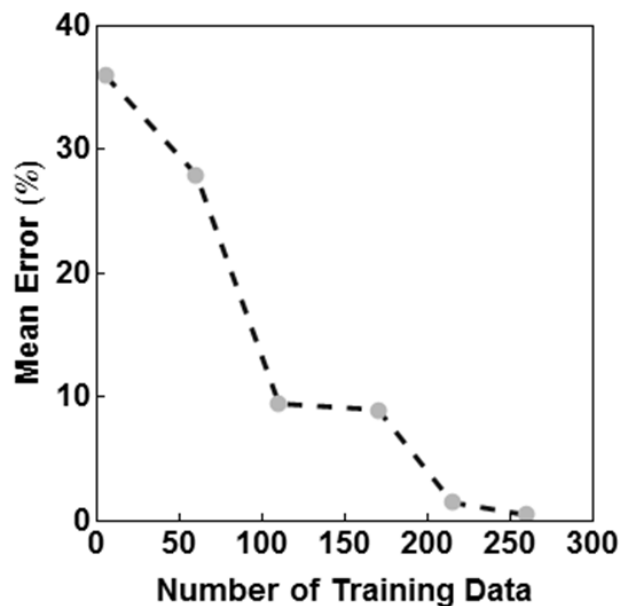


Fig. 2 Selection of the number of training data.

Table 2 Predictive ability of the optimized neural network.

Initial lactose conc.	Enzyme conc.	Enzyme condition	Time (min)	Residual lactose conc. (mM)	
				Observed	Predicted
0.146 mM	2.5 mg/L	Immobilized	0	0.146	0.147
			2	0.137	0.137
			4	0.132	0.128
			6	0.124	0.122
			8	0.117	0.118
			10	0.111	0.113
0.219	0.16	Free	0.5	0.213	0.212
			1	0.207	0.204
			1.5	0.199	0.196
			2	0.187	0.191
			3	0.177	0.182
			4	0.163	0.173
			4.5	0.161	0.167

since it makes reutilization of the enzyme and continuous operation possible [21].

### 3.2 Artificial Neural Network

The criterion used to select the optimal network architecture was based on using the smallest number of hidden neurons that would ensure good generalization and consequently good predictions [22]. It was found that one of the most precise network structures and that also consumes less computer time to use experimental data, were formed in three layers (an input layer, a hidden layer and an output one). Finally, the number of hidden neurons that satisfied this requirement was 13: six neurons in the first layer, six neurons in the second and one neuron in the third one (Fig. 3). Transference functions used were sigmoid in all cases. Although, other functions tested prediction errors were not satisfactory. Levenberg-Marquardt algorithm was used for optimization.

The training procedure was monitored in terms of the mean squared error. This learning stage was stopped when the above mentioned measure reached a constant value. This was performed in order to avoid, or at least minimized, network over-fitting. After this step was accomplished, the connection weights ( $w$ ) and threshold values (bias- $b$ ) of the network were recorded. These values were used to predict the conversion efficiency of data obtained under conditions not considered during the training (Fig. 4).

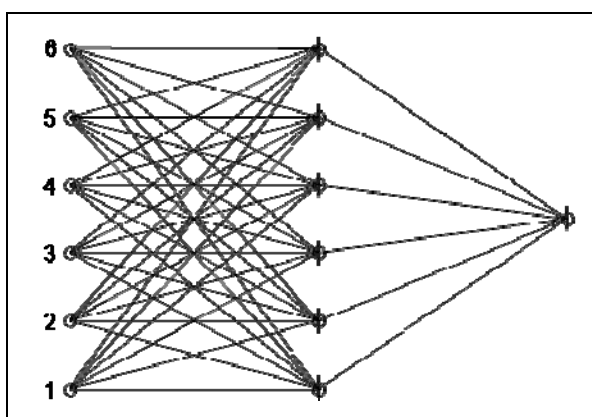


Fig. 3 Neural network structure.

Two examples of the predictive ability of the neural network are shown in Fig. 5. These results were compared with the data sets A and B obtained using free and an immobilized enzyme, respectively. Additional information about each data set is listed on the Table 2.

The neural network was trained with different sets of randomly chosen 150 observations, which permits to predict the conversion efficiency of the totality of the data sets obtained (280). The observations and predictions for all of them are presented in the Fig. 6. Parity plot shows that there is a good correlation between all the experimental residual lactose concentration data and the values predicted using the neural network. Kinetic values for this data are given in Table 3.

In catalytic kinetic studies, where experimental data is used to estimate the kinetic model parameters, ANN may have several benefit, this points have been well demonstrated in the open literature [23-25]. The main advantages of using ANN are that they can be efficiently used to approximate highly nonlinear functions, can deal with large data sets and can be trained for multi-input and multi-output variables.

## 4. Conclusions

In dairy industry, cheese whey is a waste, which causes several economical and environmental problems. Hydrolysis of lactose present in whey converts the effluence into very useful sweet syrup, which can be used in the dairy, confectionery, baking and drinks industries. Numerous immobilization systems for lactose hydrolysis have been investigated, but only few of them were scaled up with success and even few applied at an industrial or pilot scale. It is mainly because the materials and methods used for enzyme immobilization are either too expensive or difficult to use in industrial scale [20, 21]. This behavior shows the importance of developing and implementing a model to predict the system performance.

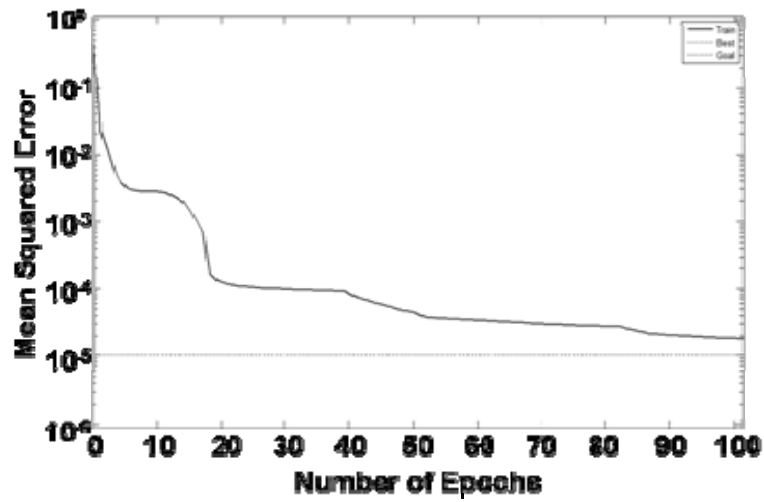


Fig. 4 Mean squared error during the training of the network.

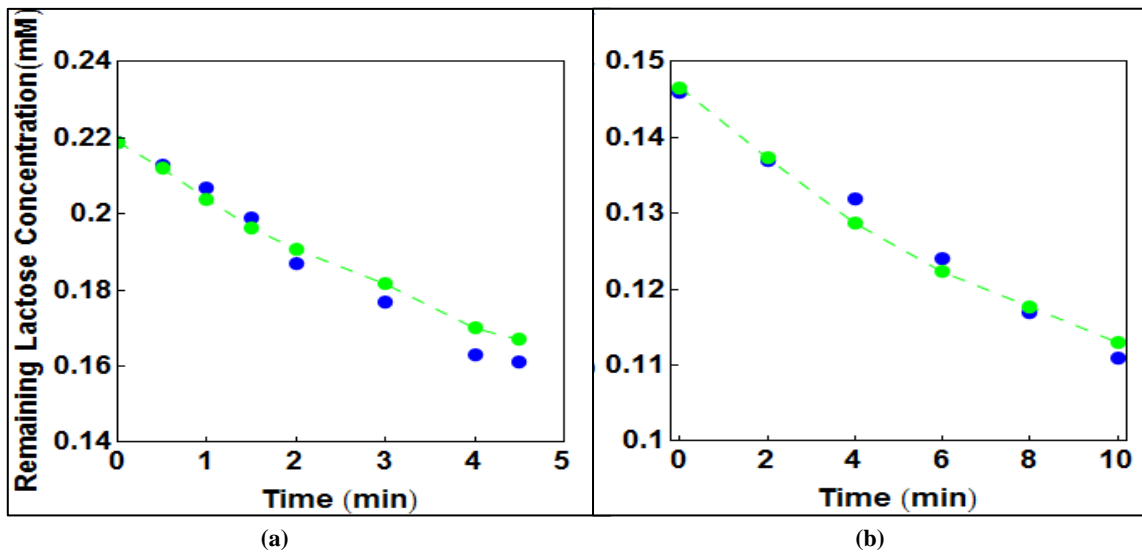


Fig. 5 Examples of the predictive ability of the neural network (blue: observed data; green: predicted data).

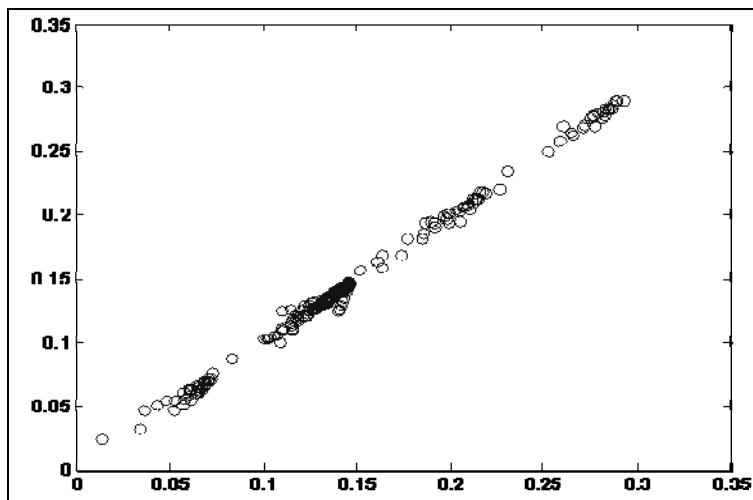


Fig. 6 Parity plot of measured values against predicted values (ideally the straight line should have slope = 1).

**Table 3** Kinetic constant predicted with the neuronal network.

Kinetic constant	Free enzyme	Immobilized enzyme
$k_m$ (mmol/L)	38.93	233.64
$k_2$ (mmol/(L min mg))	3.815	0.973
$k_i$ (mmol/L)	79.207	404.97

Advanced design and control of biotechnology systems is based upon developing and using high-fidelity models. Parameter estimation is a key step in the development of these models and is based upon minimizing an error function given by the sum of the squares of the difference between the observed data and the model predictions [23]. In this respect, ANNs are an attractive alternative because they can perform correlations without requiring mechanistic assumptions of how the output depends on the inputs. The literature showed the potential used of ANNs in kinetic studies [24-26].

In this work, the neural-network-based models showed good performance to predict lactose hydrolysis kinetics under several conditions. The network had an average absolute relative error of less than 5%, which suggests that this tool provides an accurate prediction method for lactose hydrolysis. Additionally, the ANN technique enabled us to investigate the effects of the operational factors simultaneously instead of evaluating their effects separately. The superiority of the ANNs over conventional methods for the prediction on complex relationship, might be attributed to the capability of the ANNs to capture the nonlinear features and generalize the structure if whole data set [27].

Finally, we conclude that ANNs allow the modelling of complex real systems in a relatively simple manner without the need of a mathematical model or a prediction equation associated to the physical problem. The use of artificial neural networks technique in lactose hydrolysis can predict the results of process with high precision before it is implemented as practical. This advantage can save the process cost and runtime [28-31].

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