17th European Symposium on Computer Aided Process Engineering – ESCAPE17
V. Plesu and P.S. Agachi (Editors)

Mathematical Modeling of Single Cell Protein and Ethanol Production by *Kluyveromyces cicerisporus* Fermentation on Whey

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Abstract

Phenomenological and empirical nonlinear models were built for describing experimental data of *Kluyveromyces cicerisporus* batch fermentation on whey, at different temperature and pH levels. The phenomenological model is based on cell death; substrate consumption due to product formation, cell growth and maintenance; substrate/product inhibition and growth-associated/nongrowth-associated product formation. The parameters estimation of the phenomenological model was carried out using the maximum likelihood estimation method. The empirical nonlinear neural network model identification employed traditional and the dynamic simulation analysis as the validation procedures.

Keywords: Batch fermentation; Cheese whey; Bioconversion

1. Introduction

Cheese whey is the liquid remaining following the precipitation and removal of milk casein during cheese-making. It represents an important environmental problem because of the high volumes produced and its high organic matter content. Bioconversion of whey lactose to single cell protein, ethanol or methane reduces more than 75% of the organic content, as required by the

pollution laws, which are forcing dairy industries to find alternative processes; in addition, owing to the energy crisis, alcohol fermentation of organic residues has been under growing investigation. The utilization of cheese whey powder, under different pH levels, in order to produce ethanol, was investigated by Kargi *et al.* [1]. The experiments showed that the maximization of product formation was obtained at pH=5. A mathematical model was developed for predicting single cell protein production from cheese whey, using *Kluyveromyces fragilis* under different retention times, mixing speeds and air flow rates [2]. Lee *et al.* [3] applied response surface analysis to optimize the factors affecting the growth (temperature and pH) of *Ganoderma lucidum* mycelium, using cheese whey as substrate. The method of least-squares was used to estimate the parameters of a quadratic polynomial, representing mycelial concentration as a function of temperature and pH. As can be observed from literature, there is a lack concerning modeling investigations of single cell protein and ethanol production by *Kluyveromyces cicerisporus* under pH and temperature varying conditions. The main objective of this paper is building phenomenological and empirical nonlinear models, employing the maximum likelihood estimation method [4] and the identification/validation methodology proposed by Vega *et al*. [5] for empirical models identification, through the use of experimental data.

2. Materials and Methods

Kluyveromyces cicerisporus was isolated from crude milk. Stock cultures were mensal cultivated in lactose (2%), peptone (1%) and agar-agar (3%). The inoculation was done in 500 cm^3 Erlenmeyer flasks, cultured for 12-24 h, depending on the experiment. The fermentation of whey, containing 5.4% of lactose, being aerated with 2.8 dm^3 air/ dm^3 medium/min and stirred at 200 rpm, was carried out in a 2 dm^3 batch fermentor, under different temperatures and pH levels, in triplicate. Cellular quantification was done in a Neubauer camera, using dilutions that produced 200-300 cells/0.1 mm^3. The standard error (S.E.) in the biomass determination was estimated at 4% from replicated measurements. The determination of lactose content in whey was done using the picric acid method [6], which presents a S.E. of 3%. A potentiometer was used for determining pH measurements. The ethanol concentration was measured by gas chromatography (S.E. < 2%).

3. Mathematical Modeling

For phenomenological modeling purposes, the specific growth rate (Eq. 1) was described as a function of an apparent Michaelis constant, which is proportional to biomass concentration. Medium constituents (substrate and product) inhibited the microorganism growth. The cell growth model also accounted for cell death, Eq. 2. The model considered substrate consumption for cell growth,

maintenance and product formation, Eq. 3. The product formation kinetics combined growth-associated and nongrowth-associated contributions, Eq. 4. The model parameters were estimated using the maximum likelihood principle, which presents rapid convergence due to the similarity to a Gauss-Newton iteration method [7] and to a step-limiting procedure introduced by Law and Bailey [8].

$$\mu = \frac{\mu_{\max} S}{k_s X + S} \frac{k_{is}}{k_{is} + S} \frac{k_{iP}}{k_{iP} + P} \tag{1}$$

$$\frac{dX}{dt} = \mu X - k_d X \tag{2}$$

$$\frac{dS}{dt} = -\frac{\mu X - k_d X}{Y_{XS}} - \frac{1}{Y_{PS}} \left\{ \left[alfa\left(\mu X - k_d X\right) + betaX \right] \frac{Y_{PS}}{Y_{XS}} \right\} - \frac{msX}{Y_{XS}} \tag{3}$$

$$\frac{dP}{dt} = \left[alfa\left(\mu X - k_d X\right) + betaX \right] \frac{Y_{PS}}{Y_{XS}} \tag{4}$$

Literature unveils that the neural network (NN) approach has proved to be a useful tool and is the most popular framework for empirical model development. It is well known that the construction of an efficient NN is a function of many factors. The amount and appropriateness of the available training data is an important factor. In addition, the optimal NN structure is not easy to pre-specify; the optimization of the NN weights can result in contrasting generalization characteristics and alternative convergence criteria for training can also result in different solutions. All these steps represent very challenging theoretical and practical problems, for a general theory is not available. NNs were validated in terms of the traditional methods [9-10] and in terms of their complex static and dynamic behavior, using dynamic simulation analysis methodology [5].

4. Results & discussions

The maximum likelihood principle provided an estimation of phenomenological model parameters from experimental data (Table 1) and also an estimate of the uncertainties in the estimated parameters (variance-covariance matrix), providing the significance of the parameters. Table 1 presents the mean value of the parameters using a confidence interval (CI) of 95%.

Experimental investigation showed that optimal bioconversion conditions were $5 \leq pH \leq 7$ and 30ºC-35°C.

A three layer feedforward neural network, using hyperbolic tangent and linear activation functions at the hidden and output layers, respectively, was employed. Past and actual time, lactose, ethanol and cell concentrations were the input data for predicting future lactose, ethanol and cell concentrations. In accordance with standard cross-validation procedures [9-10], a hidden layer with an optimal number of neurons (3 neurons) was selected. In order to build NN empirical models, two independent data (training and validation sets), containing different data sets, were used. A total of six neural models were built for representing each pH and temperature experimental conditions (Fig. 1). Spurious solutions were obtained in all NNs trained with incomplete data set, no matter the values used to initialize the NN parameters. The empirical model dynamic patterns were similar to the one shown by the bioreactor (Fig. 1).
For generalized empirical model building purposes, a three layer feedforward neural network, using hyperbolic tangent and linear activation functions at the hidden and output layers, respectively, was selected. The architecture comprised, as input information, past and actual pH, temperature, time, lactose, ethanol and cell concentrations and, as output data, future lactose, ethanol and cell concentrations.

Table 1 – Kinetic values (mean ± 95% CI)

	pH=4 T=30°C	pH=5 T=30°C	pH=5 T=35°C	pH=6 T=30°C	pH=7 T=30°C
$\mu_{máx}$ [h^{-1}]	0.71 ± 0.16	1.01 ± 0.26	0.82 ± 0.17	0.72 ± 0.10	0.80 ± 0.12
k_S [g/l]	20.45 ± 4.27	20.0 ± 6.04	20.15 ± 1.93	19.12 ± 2.20	19.49 ± 1.84
Y_{XS} [g/g]	0.26 ± 0.05	0.33 ± 0.08	0.31 ± 0.03	0.31 ± 0.03	0.36 ± 0.03
Y_{PS} [g/g]	0.34 ± 0.05	0.31 ± 0.08	0.34 ± 0.04	0.34 ± 0.04	0.32 ± 0.03
ms [h^{-1}]	$9.75.10^{-6}$ ± $3.03.10^{-6}$	0.00001± $2.83.10^{-6}$	$9.95.10^{-6}$ ± $1.22.10^{-6}$	$9.53.10^{-6}$ ± $1.35.10^{-6}$	$1.06.10^{-5}$ ± $1.45.10^{-6}$
k_d [h^{-1}]	0.03 ± $6.20.10^{-3}$	0.02± $8.43.10^{-3}$	0.02 ± $2.04.10^{-3}$	0.02 ± 0.02	0.02 ± 1.23. 10^{-3}
alfa [-]	$4.56.10^{-6}$ ± $1.70.10^{-6}$	0.0001 ± $1.82.10^{-5}$	0.0001 ± $1.11.10^{-5}$	$9.78.10^{-6}$ ± $2.53.10^{-6}$	$7.6.10^{-6}$ ± $1.33.10^{-6}$
beta [-]	0.25 ± 0.06	0.18 ± 0.05	0.50 ± 0.04	0.25 ± 0.02	0.18± 0.01
k_{is} [g/l]	68.66 ± 12.82	70.0 ± 9.67	70.07 ± 6.61	70.73 ± 8.66	63.91 ± 6.41
k_{iP} [g/l]	10.16 ± 2.46	10.0 ± 3.47	10.06 ± 0.85	9.00 ± 0.68	9.95 ± 0.86

It can be observed from the dynamic simulations of Fig. 2, showing substrate, ethanol and cell concentrations as the parameters (pH and temperature) undergo changes, that the general neural model was able to predict successfully the optimal operation range, also indicating that at 40°C and pH=5 the bioconversion is minimum. Anomalous NN models were avoided by using 126 experimental data points, covering the full temperature and pH ranges.

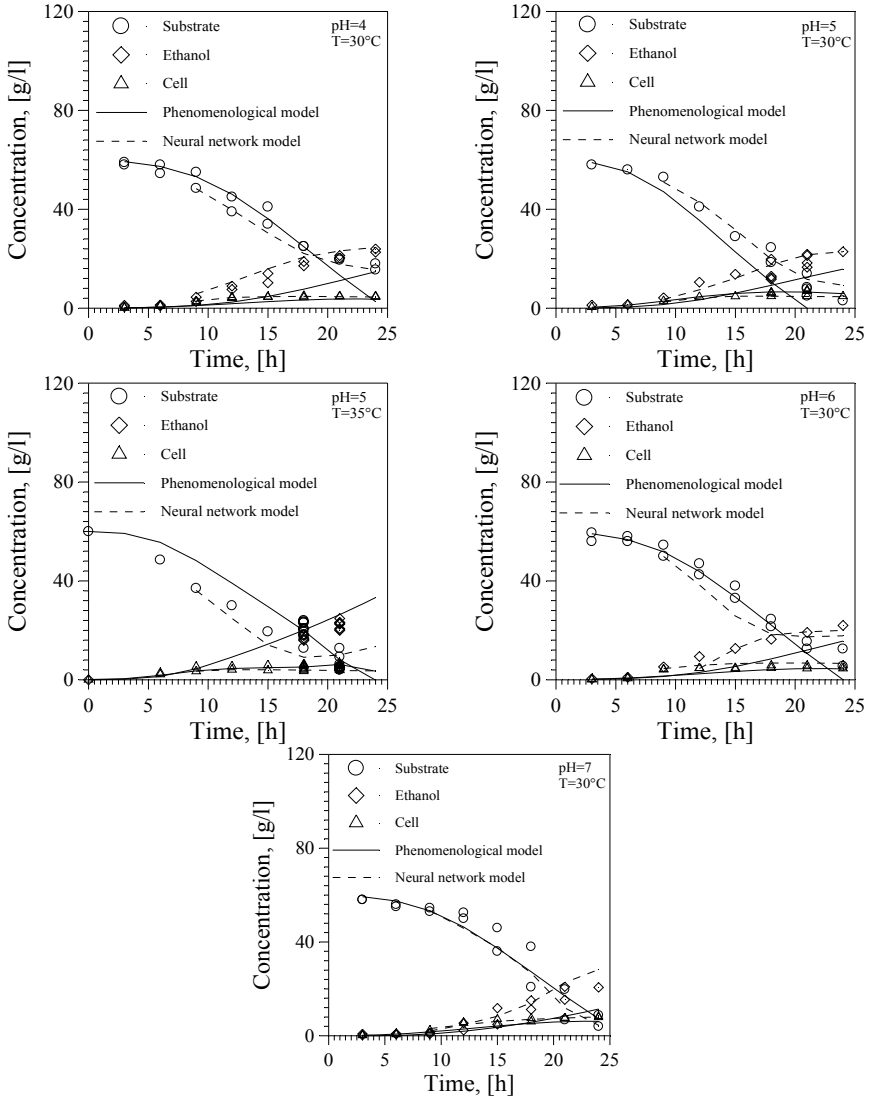

Figure 1 – Model predictions and experimental data. ○ Substrate. ◇ Ethanol. △ Cell. – Phenomenological model. - - Neural network model.

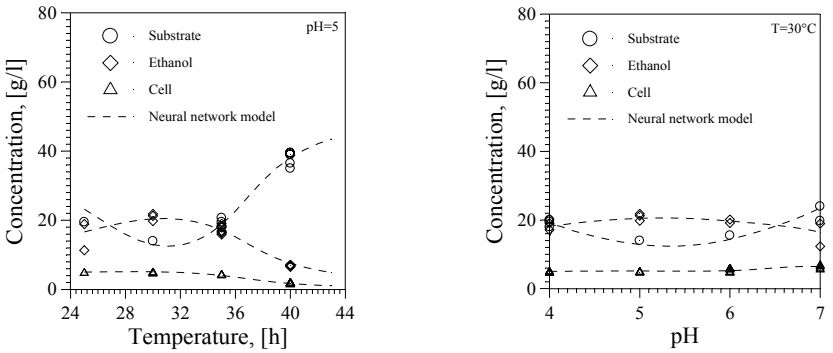

Figure 2 – Generalized neural network model. ○ Substrate. ◇ Ethanol. △ Cell. – Phenomenological model. - - Neural network model.

5. Conclusions

The maximum likelihood method and the dynamic analysis validation technique were used in order to build confident phenomenological and empirical models. The generalized neural model should be the preferred modeling technique for is able to predict substrate, ethanol and cell concentrations under varying pH and temperature conditions. However, empirical models are unsafe for extrapolation purposes, being most appropriate, for this case, the use of a phenomenological model incorporating on the specific growth rate, for example, both pH and temperature effects, a next step of the authors research.

Acknowledgements

We are indebted to CNPq/Brazil for supporting this research.

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