Enzyme Production Modeling Simulation Using Neural Techniques

M. CARAMIHAI, IRINA SEVERIN
University POLITEHNICA Bucharest
Spl. Independentei, 313, sector VI, Bucharest
ROMANIA
m.caramihai@ieee.org, irina.severin@upb.ro,
www.pub.ro

Abstract: - In the present work, growth and cellulase production by the cellulolytic fungus Aspergillus niger in fed-batch culture using an agricultural residue as the substrate have been investigated. The Windows application of Artificial Neural Network (ANN) to the estimation of bioprocess variables is presented. A neural network methodology is discussed, which uses environmental and physiological information available from on-line sensors, to estimate the cellulase production in a fed-batch bioprocess. An efficient optimization algorithm that reduces the number of iterations required for convergence is proposed. Results are presented for different training sets and different training methodologies.

Key-Words: - intelligent techniques, neural network, biological process, enzyme production

1 Introduction

Development of fermentation models is quite important for the improvement of understanding of the processes taking place in the bioreactor, and more specifically in the microorganisms growing in the bioreactor. Quantitative description of the microbial growth kinetics started with the pioneering work of Monod [1]. He related the specific microbial growth rate and the extracellular concentration (S) of the growth-limiting substrate with the well-known hyperbolic expression. Most mathematical models of microbial growth kinetics have been developed on Monod model, which was considered to describe satisfactory, a large range of fermentation processes.

The biological processes are characterized by a large degree of complexity and for a better understanding of the fundamental processes in a fermentation, various approaches including expert systems, fuzzy control, and neural network control have been applied [2], [3].

Microorganisms growing on cellulose must produce extracellular cellulolytic enzymes capable of degrading the polysaccharide to soluble sugars. The degradation products, mainly glucose and cellobiose, are used as carbon and energy sources by microorganism. The cellulose conversion represents a very interesting example of fermentation where growth, enzyme production, and enzyme reaction are closely interdependent. Lignocellulosic biomass, the source of cellulose, is the most abundant renewable resource on earth. Cellulose is readily available from agricultural residues, herbaceous crops, forestry by-products, and pulp and paper industry waste. Extensive research during the past decade has shown that the cellulase production is the key of the cellulose bioconversion. Fungi, bacteria, and plants synthesize cellulases, but research has focused primarily on fungal and bacterial cellulases. The aerobic mesophilic fungus Trichoderma reesei and its mutants have been the most intensely studied sources of cellulases; other fungal cellulase producers include Penicillium sp., Aspergillus sp., Fusarium sp.

In the past decades, enzymatic conversion of cellulose has received attention for renewable production of fuels from cellulosics because of the high selectivity of enzymatic hydrolysis compared to acid hydrolysis. In the enzymatic conversion of cellulose, the cost of producing hydrolytic enzymes (cellulases) constitutes a major portion of total production cost. To improve the economics of the process, research has been focused on several areas including improving the strain performance, selecting an efficient mode of operation, and optimizing the operating conditions. Currently, cellulase enzymes are produced by growing Trichoderma reesei and its mutants, but it is of interest to use other cellulolytic fungi such as, Penicillium and Aspergillus.

The research has been reported the great potential for improving cellulase production using a fed batch mode of fermentation [4]. An entirely fed-batch operation serves the purpose of controlling the specific growth rate on a value bellow the critical growth rate

In the present work, growth and cellulase production by the cellulolytic fungus Aspergillus niger in fed-batch culture using an agricultural residue as the substrate have been investigated. The estimation of bioprocess variables has been realized using Artificial Neural network (ANN).

2 Problem Formulation

2.1. The micro-organism

The cellulolytic fungus Aspergillus niger from the microbial collection of ICECHIM (Institute for Chemical Research) was used in all experiments.

2.2. Fermentation

Fermentations were carried out in a 5L bioreactor using wheat straw as carbon source. The process started as a conventional batch culture with 50g/L substrate. After 48-72 h, when the growth was observed to slow down, specified amounts of substrate (20g/L daily) were added. The nutrient salts were added according to Mandels' medium [5]. The growth temperature was maintained at 28 oC, and the pH was held to 4.5 by adding NH4OH (3M) and H3PO4 (3M). Dissolved oxygen was kept above 20% of the saturation volume for the medium. The bioreactor was inoculated with preinoculum obtained from agitated flask cultures. Antifoam emulsion was used.

2.3. Analysis

Cellulase activity was measured as carboxymethylcellulase (CMC) using 1% carboxymethylcellulose [6]. The activity was expressed as international units (IU), defined as the amount of enzyme required to produce one micromole of glucose per minute in the standard conditions. Soluble sugar content was determined with dinitrosalicylic reagent [7]. Soluble protein was measured by Lowry method [8]. Aliquots of the culture broth were taken out at definite intervals from the fermentor and biomass concentration was determined gravimetrically. The residual substrate was calculated from the difference of total dry weight and mycelium dry weight. The mycelium dry weight was determined by repeated extraction with NaOH and protein analysis according to Lowry.

2.4. Neural Nets Configuration

Neural networks with their inherent parallelism and their ability to learn, has been seen by many authors in the field of system controlling, as an exciting possibility to design adaptive controllers, when the dynamics of the system is deeply nonlinear, complex or unknown. The main advantages of the neural networks, which make them an important tool in order to enhance the capabilities of conventional controllers and to create robust controllers, able to better adapt the controller parameters to different plants and to environmental changes, are:

- Neural networks can approximate any linear or nonlinear mapping between the input and the output of the system.
- They are able to learn in order to perform this approximation.
- Robustness to partially network destruction, noise tolerance, and generalization ability to situations not contained in the training data set.
- Computationally fastness once trained.

The problem of capturing the nonlinearity of the process to be modeled and controlled is to match the nonlinearity of the process with that of the network, by learning, neural networks being nonlinear systems themselves. It was shown in the literature that neural networks can solve complex and difficult control tasks, where traditional control methods fail, neural networks being also able to work in the presence of noise.

In this paper we introduce a class of networks, called Jordan-Elman networks, that use context units with local feedback to provide memory and store the recent past. Since the feedback is usually fixed, they can be placed anywhere in an MLP without changing the feedforward nature of the learning. They should be contrasted with the more general time-lagged recurrent nets, which use cascaded context units with a single adaptable feedback parameter.

Jordan and Elman networks extend the multilayer perceptron with context units, which are processing elements (PEs) that remember past activity. Context units provide the network with the ability to extract temporal information from the data. In the Elman network, the activity of the first hidden PEs is copied to the context units, while the Jordan network copies the output of the network. Networks, which feed the input and the last hidden layer to the context units, are also available.

The proposed architecture is one example of a Jordan-Elman neural network, where the context units are connected to the input layer to provide a memory of the recent input data. The neural net design was made using NeuroSolutions® software package. The memory depth of a context unit is adjusted through its feedback gain, or time constant. The context unit is represented by the IntegratorAxon. NeuroSolutions offers other context units, including the sigmoid and tanh context units, whose outputs saturate at predefined limits.

The context unit remembers the past of its inputs using what has been called a recency gradient, i.e., the unit forgets the past with an exponential decay. This means that events that just happened are stronger than the ones that have occurred further in the past. The context unit controls the forgetting factor through the Time constant. Useful values are between 0 and 1. A value of 1 is useless in the sense that all of the past is factored in. On the other extreme, a value of zero means that only the present time is factored in (i.e., there is no self-recurrent connection). The closer the value is to 1, the longer the memory depth and the slower the forgetting factor.

The setup values for the NN training are the follows:

- Input: 1, Output: 1, Hidden: 3, Exemplars: 51
- Context unit: time 0.8, Integrator axon
- Hidden layer: 1, Transfer: TanhAxon, Learning rule: momentum, step size: 0.1, momentum: 0.7
- Output: similar
- Maximum epochs: 1000, weight update: batch, termination: threshold: 0.01

The neural net configuration is presented in Fig. 1.

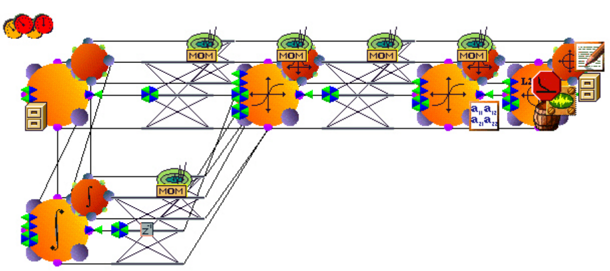

Fig. 1 The Jordan-Elman Neural Network configuration

3 Problem Solution

In order to test the performances of the neural network, the case study is made on the common bioprocess described above, illustrated by the following equation [9]:

$$\mu = \frac{k_1 S}{k_2 + S}\left(1 - e^{\left(1 - \frac{k_3}{S}\right)}\right)$$

where S = substrate concentration [g/L], μ = specific growth rate [h^{-1}] and k_1, k_2, k_3 = constants

The training data set contains 51 examples, also the testing data set. During this simulation, the training values were determined based on model (eq. 1) simulation, meanwhile the test data were determined experimentally. The graphical representation of the training curve is shown in Fig. 2.

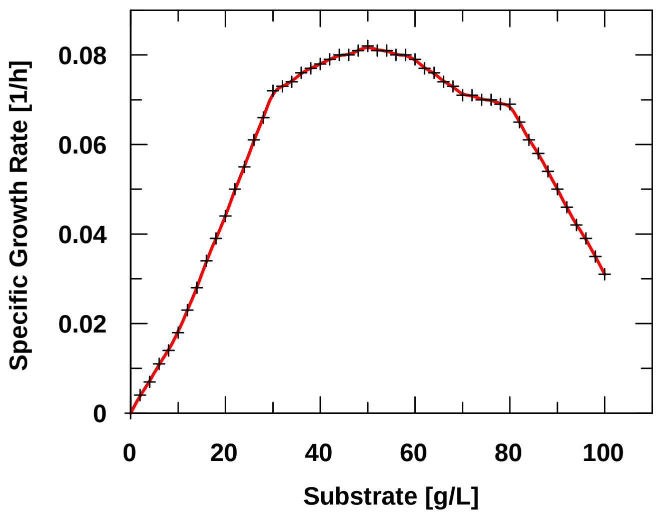

Fig. 2 The training curve for fungal cultivation based on eq. 1

The learning process is described below by the Active Cost function (Fig. 3). As the figure shows, the learning curve has a slow decreasing after a "high" peak. During the learning process, the Active Cost curve till to zero within a 636 epochs interval.

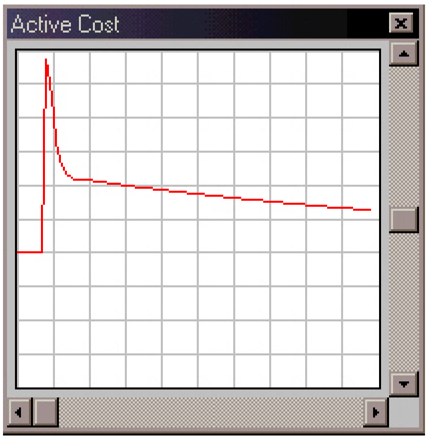

Fig. 3 The graphical representation of the active cost during the training process

After the learning process was finished, the neural net structure was tested using a set of experimental data. The testing data were selected in the same interval ($S \in [0, 100$ g/L]) as the training data, but with a different increment. The comparison between the two curves (Fig. 4) shows a quasi-superposition of the shapes for a substrate concentration locked in a medium interval (35, 75 g/L).

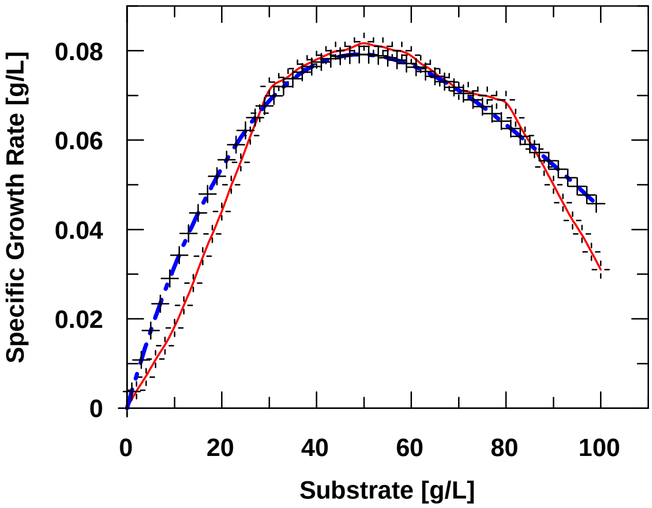

Fig. 4 Comparison between the training and testing curves

Taking into account that eq. 1 fits very well this kind of data, this difference can be explained by the particular structure of the neural network. Despite the multiple advantages of the Jordan-Elman neural structure, it is supposable that other particular configurations could be useful for this type of bioprocess.

4 Conclusion

The results show the potential advantages of enzyme production and fungal cultivation processes using fed-batch cultivation. A relationship between the substrate concentration and specific growth rate has been obtained using a particular neural network structure (i.e. Jordan-Elman). The tests had shown that, in defiance of a close training process, the neural structure has had a good response only for a well-defined substrate concentration interval. A comparison between different neural net structures for this type of bioprocess will be usefully in order to obtain a good fit of real data using intelligent techniques.

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