Hybrid neural simulation of a fed-batch bioreactor for a nonideal recombinant fermentation

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Abstract Fermentations employing genetically modified microbes under industrial conditions are difficult to monitor on line or to describe by simple, good mathematical models. So, a practically convenient approach is to combine mathematical models of some aspects with artificial neural networks of those aspects which are difficult to measure or model. Such hybrid models have been applied earlier to laboratory-scale bioreactors. In the present work, a model based on laboratory data for the synthesis of recombinant β-galactosidase was corrupted by adding imperfect mixing and noise in the feed stream to generate data mimicking a real nonideal operation. These data were used to train a recurrent Elman neural network and a hybrid neural network, and it was seen that a hybrid network provides more accurate estimates of both extra-cellular and intra-cellular variables. The benefit is enhanced by the hybrid network’s superiority being more pronounced for the intra-cellular recombinant protein, β-galactosidase, which is the main product of interest.

\[ D \text{ overall dilution rate, } 1/\text{h} \]
\[ D_{2} \text{ axial dispersion coefficient, cm}^2/\text{h} \]
\[ D_i \text{ internal dilution rate for } i\text{-th region, } 1/\text{h} \]
\[ k_1, k_2, k_3, k_4 \text{ reaction rate constants, } 1/\text{h} \]
\[ K, K_i, K_2, K_p \text{ equilibrium constants, g/l} \]
\[ Q \text{ characteristic length for bioreactor, cm} \]
\[ Q_i \text{ substrate feed rate, } 1/\text{h} \]
\[ Q_2 \text{ internal flow rate from region (1) to region (2), } 1/\text{h} \]
\[ Q_{ij} \text{ rates of change of } A\text{-compartments in } j\text{-th region, } 1/\text{h} \]
\[ r_{ij}^+, r_{ij}^- \text{ rates of change of } G\text{-compartments in } j\text{-th region, } 1/\text{h} \]
\[ r_{ij}^+ \text{ rate of change of } P\text{-compartent in } j\text{-th region, } 1/\text{h} \]
\[ r_{ij}^+ \text{ rate of change of } E\text{-compartent in } j\text{-th region, } 1/\text{h} \]
\[ S_f \text{ substrate concentration in the feed stream, g/l} \]
\[ S_0 \text{ initial substrate concentration in bioreactor, g/l} \]
\[ S_j \text{ substrate concentration in } j\text{-th region, g/l} \]
\[ s_j \text{ } \]
\[ t \text{ time, h} \]
\[ u \text{ fluid velocity, cm/h} \]
\[ V \text{ total volume of the broth, l} \]
\[ V_j \text{ volume of broth in } j\text{-th region, l} \]
\[ v_1 \text{ dimensionless} \]
\[ X \text{ overall biomass concentration in bioreactor, g/l} \]
\[ X/S_0 \text{ dimensionless} \]
\[ x_j^+ \text{ concentration of plasmid-bearing cells in } j\text{-th region, g/l} \]
\[ x_j^- \text{ concentration of plasmid-free cells in } j\text{-th region, g/l} \]
\[ x_j^+ \text{ } \]
\[ x_j^- \text{ } \]
\[ x_j^+ + x_j^- \text{, dimensionless} \]
\[ x_j^+ \text{ } \]
\[ x_j^- \text{ } \]
\[ x_{Aj}^+, x_{Aj}^- \text{ concentrations of } A\text{-compartments in } j\text{-th region, g/g} \]
\[ x_{Gj}^+, x_{Gj}^- \text{ concentrations of } G\text{-compartments in } j\text{-th region, g/g} \]
\[ x_{Pj}^+ \text{ concentration of } P\text{-compartent in } j\text{-th region, g/g} \]
\[ x_{Ej}^+ \text{ concentration of } E\text{-compartent in } j\text{-th region, g/g} \]
\[ x_{Ej}^- \text{ } \]
\[ Y_{xs} \text{ yield coefficient for biomass from substrate, g/g} \]
\[ Q_j/V_{1s} \text{, } 1/\text{h} \]
\[ \Delta_j \text{ mass fraction of recombinant cells, dimensionless} \]
\[ \gamma_{11}, \gamma_{22} \text{ stoichiometric coefficients for intra-cellular reactions, dimensionless} \]
\[ \omega \text{ overall specific growth of biomass, } 1/\text{h} \]
\[ \mu_i \text{ specific growth of cells in } i\text{-th region, } 1/\text{h} \]
\[ \mu_j \text{ specific growth rate in } j\text{-th region, } 1/\text{h} \]
\[ \sigma_j^+, \sigma_j^- \text{ intra-cellular substrate concentration in } j\text{-th region, g/l} \]
\[ \theta \text{ } \]
\[ \tau \text{ } \]
\[ tD \text{, dimensionless} \]
\[ + \text{ plasmid-containing cells} \]
\[ - \text{ plasmid-free cells} \]

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Introduction
Microbial fermentations carried out under conditions resembling actual industrial operation are difficult to model in a simple and adequate way. Many reasons contribute to the difficulties. First, cellular metabolism comprises many complex and interacting reactions, not all of which can be precisely defined or kinetically modeled. Secondly, these reactions interact not only among themselves but also with the extra-cellular environment through the inward transport of nutrients and sometimes the outward transport of products. The interactions between intra-cellular and extra-cellular variables creates a third difficulty; external disturbances affect cellular metabolism in ways that are not fully understood. Fourthly, even though intra-cellular reactions generate the products of interest, these are difficult to measure on-line, and off-line measurements can be too slow to sense a disturbance or an abnormality sufficiently early [1].

These problems are particularly severe when genetically modified (recombinant) microorganisms are used. A recombinant cell contains an externally introduced plasmid, which has a gene that codes for a desired product. Normal (or wild-type) cells do not have this plasmid, and hence they cannot synthesize this product. Since the plasmid plays a useful role in cellular metabolism, it might appear that the more the number of plasmids present in a cell, the better it is. However, increasing the number of copies of a plasmid also increases the metabolic load on the cell, thereby reducing its stability and growth rate. Cells with few plasmids have high stability and fast growth but are poor synthesizers of the recombinant protein [2]. Therefore it is preferable to have a distribution of plasmid copy numbers among the cells in a bioreactor [3, 4] so that the inhibitory effects of high plasmid content and high protein concentrations are balanced against rapid cell growth at low copy numbers.

Since a distribution in plasmid copy number is a natural feature of the heterogeneity of large bioreactors, the benefits of the distribution may be realized by suitably controlling it instead of the conventional approach of trying to achieve uniformity. This however requires fast on-line measurements of key variables, which is difficult because of instrumental limitations [1] and because the sensitivities of the concentrations in a recombinant fermentation may vary by several orders of magnitude with time and among the variables at a given time [5]. Two features that influence the performance and the sensitivities are macromixing in the broth and the inflow of disturbances. Recent work has shown that, contrary to classical bioreactor theory, neither perfect mixing nor the complete absence of disturbances is best for a recombinant fermentation. Just as for the plasmid copy number, there are optimal degrees of macromixing [6] and filtering of noise [7] to maximize protein synthesis rates.

The optimum values of plasmid copy number distribution, macromixing intensity, filtering of noise, and other operating variables also vary with time in batch and fed-batch operation because the processes are inherently time-dependent. Owing to these complexities, the limitations of measurement techniques [1] and the need to maintain high protein activities for an economically viable process, there has been a shift toward noninstrumental methods for continuous estimations of variables which are difficult to measure and/or whose dynamics are difficult to model.

Artificial neural networks (ANNs) have been shown to be very useful in portraying bioreactor behavior in both real and simulated industrial conditions. Many important applications have been discussed in two recent reviews [8, 9] and are therefore not elaborated here. The applications include bulk drugs such as penicillin, secondary metabolites, amino acids, as well as microbial growth per se. In all cases, neural networks were superior to mathematical models and nonparametric methods such as the extended Kalman filter.

A major reason for the success of ANNs in complex microbial processes is their ability to function without a model for the process. ANNs have other advantages, too [10]. They learn continually from the data, update themselves, are robust to measurement errors and disturbances, and can predict imminent problems to enable timely corrective action.

Ironically, the lack of dependence of neural networks on process models also introduces difficulties in many real applications. Because ANNs function as ‘black-box’ simulators, the data used for training them have a profound effect on their performance. While an inadequately or improperly trained network does not learn enough about a process, excessive training makes the network learn spurious features which impair its performance [10]. Like the proper choice of training data, the design of an ANN in the absence of phenomenological information is also difficult. The topology of an ANN has four principal features: the number of neural layers, the numbers of neurons in each layer, the inter-connections among the neurons, and the transformation functions employed by the neurons. While it is known that at most two hidden layers are sufficient to approximate any real-valued function [11], there are only heuristic rules for the other features and they apply to simple topologies.

Since design, data selection, and training (followed by validation) are bottlenecks in developing ANNs for realistic microbial fermentations and mathematical models are feasible only for some aspects of a fermentation, it is reasonable to combine a partial model with a neural representation of features which are difficult to model. This concept has generated hybrid neural networks (HNNs). The simplest HNN has the structure shown in Fig. 1.

![Artificial neural network](image)

**Fig. 1.** Hybrid neural network structure: x=inputs, z=internal outputs, y=processed usable outputs