Dynamic sensing of intra-cellular variables in an imperfectly mixed bioreactor by a recurrent neural network

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Received 25 January 1999; accepted 10 December 1999

On-line measurement of intra-cellular variables in a bioreactor is difficult through methods using instrumentation. However, close monitoring of these variables is essential in recombinant fermentations, which are sensitive to disturbances and spatial heterogeneity. Based on an earlier study, a recurrent neural network of the Elman type was applied to a fed-batch fermentation for β-galactosidase by a temperature-sensitive strain of Escherichia coli. Simulated data of four intra-cellular concentrations in an imperfectly mixed bioreactor were used to train the network, and its predictive capability was tested with unseen data of the rDNA and β-galactosidase concentrations. Over a wide range of mixing intensity and a fermentation period longer than normally required, the Elman network’s performance was better than that reported for modified feed-forward networks and it was unaffected by the hydrodynamics. This type of neural network may thus be employed as an on-line soft sensor for large scale recombinant fermentations.

The on-line measurement of many important variables pertinent to microbial reactions in a fermentation vessel (bioreactor) is difficult and expensive. Even a basic variable such as the cell mass concentration is often monitored by withdrawing samples and measuring the optical density off-line; on-line methods have not been successful because of many limitations. It is even more difficult to follow in situ the variations of intra-cellular variables.

Nevertheless, intra-cellular variables are important because the chemical and biological reactions that generate the desired product(s) occur inside living cells. Not always are the products secreted out. Even if they are, their synthesis within the cells is influenced by other intra-cellular variables such as DNA, RNA and enzyme concentrations. The limitations of off-line analyses are more severe for intra-cellular components; typically, a spectrophotometric measurement for the optical density takes less than 30 min while the estimations of RNA, DNA and protein concentrations may take 6 to 8 h.

The problems of on-line measurements happen to be particularly serious in situations where accurate monitoring is vital for optimum performance. These are fermentations utilizing microorganisms that have been genetically altered so as to be able to synthesize proteins which the natural (wild type) organism cannot. A description of how this is done through recombinant DNA (rDNA) technology is available in Imanaka and Ryu et al. From the perspective of measurement, it is important to recognize that recombinant fermentations are extremely sensitive to disturbances and fluid mixing in the bioreactor’s nonidealities whose presence increases with the scale of operation.

A solution to the limitations of techniques using instrumentation, especially for large bioreactors, is to use ‘soft sensors’ to generate on-line data of intra-cellular variables. These are ‘intelligent’ software which are trained with on-line as well as off-line data until they are able to predict the off-line variables during the process even for situations not experienced before. Classical methods of soft sensing include Kalman filters and adaptive (linear and nonlinear) estimators. However, these are not truly intelligent sensors of the type available in knowledge-based systems, artificial neural networks and fuzzy systems. Sometimes it is beneficial to combine two of these methods so that the weaknesses of one may be offset by the advantages of the other, as in fuzzy neural networks.

Artificial neural networks (ANN) have emerged as a versatile and powerful tool for bioreactor state estimation and control. Even though applications to biological and chemical reactors began recently, their rapid growth is already documented. Since many therapeutically important but expensive proteins are produced by recombinant microorganisms, and the processes are difficult to model and control, much of the current work with ANNs for cellular processes has been in this area. Many of these studies have been
for ‘clean’ systems, i.e. without the noise and mixing imperfections prevalent on a practically useful scale. ANNs will, however, be really useful in these nonideal fermentations because it is difficult to formulate process models that are accurate, versatile and simple enough to permit easy automation. In particular, imperfect mixing in the bioreactor has a strong effect on the process\(^5\). Since the best performance may require a time-dependent rate of mixing\(^4\) and it is impractical to have probes whose optimum locations change with time, neural networks offer a viable alternative.

The present study analyzes the suitability of a recurrent ANN for on-line estimations of intracellular rDNA and protein concentrations in a fermentation which is inherently time-dependent. The process involves the production of β-galactosidase by genetically altered *Escherichia coli* bacteria in a fed-batch fermentation. Details about the organism and the kinetics have been reported by Betenbaugh *et al.*\(^{15}\). In the next section the kinetics and the reactor model are described briefly, followed by the choice of the ANN architecture. This particular fermentation problem was selected because the complexity of the kinetics and hydrodynamics provides a stringent test for the ANN.

**Description of the Problem**

The *E. coli* culture in the fermentation problem studied here has a critical temperature, \(T_c\); below this temperature the fermentation rate is extremely low and therefore not viable, while above \(T_c\) the rate multiplies uncontrollably. Since neither extreme is desirable, a common solution is to alternate for short periods between the temperature zones. While this strategy does result in stable operation in an ideal situation\(^5,16\), it has three problems. First, to maximize productivity it is necessary to optimize the cycling periods; in an unsteady state fed-batch fermentation the optimum values have to be updated continually. Secondly, it is not easy to devise a fast, stable, on-line controller for such operations. Thirdly, since recombinant fermentations are sensitive to disturbances\(^5,8,17\), the process may digress from one temperature zone to the other at unintended times.

These difficulties may be alleviated if (a) non-invasive on-line methods of estimation are available and (b) the periods of super-critical operation are minimized. For the first solution, ANNs have been shown to be able to mimic, predict and control bioreactor performance\(^{12,13,18}\). However, relatively few of these works\(^5,17\) have focussed on intracellular variables in an unsteady state. Moreover, most studies are with small reactors which have been idealized to be perfectly homogeneous. So, in this work simulated data have been used to study ANN performance for a nonideal bioreactor; the validity of this method has been demonstrated earlier\(^9\). On the question of minimizing super-critical operation, it has been shown\(^{20}\) that sub-critical fermentation is sustainable if fluid mixing in the bioreactor is maintained at a controlled degree of imperfection. These two features have been incorporated in the models presented below. Modeling is done at two levels: (i) within the cells, where the transformations take place and (ii) for the fluid as a whole, incorporating overall kinetics and transport.

**Cell model and kinetics**—Two kinds of cells are normally present in a recombinant system: naturally occurring (wild type) cells and genetically modified (recombinant) cells. Only the latter can synthesize the recombinant product (β-galactosidase). However, recombinant cells are unstable and slowly revert to wild type cells. All fermentations therefore try to minimize, and if possible, prevent this decay. As shown before\(^5\), deliberate imperfect mixing arrests the instabilities of recombinant cells, thereby enabling low temperature (sub-critical) fermentation over a practically useful length of time.

Nielsen *et al.*\(^{16}\) model contains the essential features of the metabolism of recombinant fermentations without being too complex. They visualized each cell to be composed of four lumped compartments, designated A, G, P and E (Fig. 1).

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![Structured model of a bacterial cell according to Nielsen et al.](image-url)
Compartment A contains mRNA, tRNA and ribosomes, P contains the plasmid DNA, and the recombinant protein (β-galactosidase) is assigned to E. The rest of the cell, comprising mainly genetic and structural material, has been lumped into the G compartment. P and E are of primary interest since they contain the key recombinant variables. Naturally occurring cells contain only A and G. During fermentation, cells grow, decay and also generate β-galactosidase; these processes utilize externally supplied substrate. As Fig. 1 shows, there is transfer of substrate into the cells and mass flows among the compartments. The kinetics of this metabolic machinery is described by the equations given below.

\[(a) \text{ Recombinant cells}\]
\[
\frac{d\rho_i}{dt} = \frac{1}{D} \begin{bmatrix} 1 & 1 & 1 \end{bmatrix} \begin{bmatrix} \rho_i \\ \mu_i \\ \tau \end{bmatrix}, \quad \ldots (1)
\]
\[
\frac{d\rho_0}{dt} = 0 \begin{bmatrix} 0 & 0 & 0 \end{bmatrix} \begin{bmatrix} \rho_0 \\ \mu_0 \\ \tau \end{bmatrix} + \begin{bmatrix} \mu_0 \rho_0 \\ \mu_0 \rho_0 \\ \mu_0 \rho_0 \end{bmatrix} \ldots (2)
\]
and \(\tau = D\). As explained later in section 2.2, \(D\) is one of three dilution rates definable for a model of an imperfectly mixed reactor. In Eq. (2), \(x_n\) is the 'normal' plasmid concentration, whose value for the present system is given in Table 1. The specific growth rate is:
\[
\mu_1 = \gamma_n \mu_n - (1 - \gamma_2)(\mu_0 + \mu_1 + \mu_2)
\]
\[
(3)
\]
(b) Wild type cells
\[
\begin{bmatrix} \frac{dx_n}{d\tau} \\ \frac{dx_0}{d\tau} \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ 0 & 0 \end{bmatrix} \begin{bmatrix} \mu_n x_n \\ \mu_0 x_0 \end{bmatrix} \ldots (4)
\]

\begin{table}[h]
\centering
\begin{tabular}{ccc}
\hline
Parameter & Units & Value \\
\hline
\(k\) & h\(^{-1}\) & 0.834 \\
\(k_i\) & h\(^{-1}\) & 6.67 \\
\(k_g\) & h\(^{-1}\) & 1.57 \\
\(k_x\) & h\(^{-1}\) & 1.70x10\(^3\) \\
\(K\) & g l\(^{-1}\) & 0.175 \\
\(K_1\) & g l\(^{-1}\) & 0.07 \\
\(K_2\) & g l\(^{-1}\) & 0.032 \\
\(K_3\) & g l\(^{-1}\) & 0.076 \\
\(x_n\) & g l\(^{-1}\) & 4.32x10\(^4\) \\
\(x_0\) & g l\(^{-1}\) & 0.6 \\
\(\gamma_n\) & -- & 0.535 \\
\(\gamma_2\) & -- & 0.850 \\
\(\theta\) & -- & 0.001 \\
\(\omega_0\) & -- & 1.0 \\
\hline
\end{tabular}
\caption{Values of the parameters in Eqs (1)-(20) and (5)-(6).}
\end{table}

Similar to Eqs (2) and (3), we may write:
\[
\begin{bmatrix} \frac{dr_n}{d\tau} \\ \frac{dr_0}{d\tau} \end{bmatrix} = \begin{bmatrix} k_1 & k_2 \\ k_2 & k_3 \end{bmatrix} \begin{bmatrix} r_n \\ r_0 \end{bmatrix} + \begin{bmatrix} \mu_n r_n \\ \mu_0 r_0 \end{bmatrix} \ldots (5)
\]
and \(\mu_1 = \gamma_n \mu_n - (1 - \gamma_2)\mu_0 \ldots (6)

In these equations, the superscript + denotes recombinant cells, − denotes non-recombinant cells, and the subscript j equals 1 or 2, depending on which mixing region (see the next section) is being analysed.

The overall specific growth rate of biomass in each region is the weighted sum of the growth rates for the two kinds of cells:
\[
\mu_j = \gamma_j \mu_n + (1 - \gamma_j)\mu_0 \ldots (7)
\]
Conservation equations relate the concentrations of intra-cellular components to the overall concentrations of natural and recombinant cells.
\[
x_n^j + x_0^j + x_0^j + x_r^j = x_j^j; \quad j = 1 \text{ or } 2 \ldots (8)
\]
\[
x_n^j + x_0^j = x_j^j; \quad j = 1 \text{ or } 2 \ldots (9)
\]
The intra-cellular substrate concentrations, \(\sigma_j^1\) and \(\sigma_j^2\), may also be related to the concentrations, \(S_j\), in the medium. This, however, is not straightforward like Eqs (8) and (9). The method has been described earlier, and it leads to
\[
\sigma_j^1 = \sigma_j^2 \frac{kK_jS_j}{k_1(S_j + K)}; \quad j = 1 \text{ or } 2 \ldots (10)
\]

**Bioreactor model**— Chemical and biological reactors may be operated in any of three ways: batch, continuous and fed-batch. In batch operation there is...
no inflow or outflow, whereas in continuous operation there are equal rates of inflow and outflow. Fed-batch fermentation has inflow but no outflow; this feature distinguishes fed-batch from the other two in one important aspect—the volume of the fluid mixture in the reactor does not remain constant.

Fed-batch fermentation has many benefits for recombinant systems\textsuperscript{13,20,22} and has therefore been adopted in this study. It is shown schematically in Fig. 2a. In an ideal bioreactor the fluid is spatially uniform. Nonideal mixing is modeled by a combination of ideal reactors\textsuperscript{23}. The basis of most models is the classical result that a chain of well-mixed reactors tends toward a plug flow reactor (with no mixing at all) as the number of reactors increases. A direct application of this concept would imply adjusting the number of perfectly mixed reactors to simulate a given degree of mixing. This, however, leads to models which are too complex for either validation or fast on-line response. Tanner and coworkers\textsuperscript{24} circumvented this problem by not considering the reactors in the model to be perfectly mixed. As a result, a combination of just two reactors with internal recycle could simulate any degree of mixing. Their original model for batch operation was modified recently\textsuperscript{20} for fed-batch fermentation (Fig. 2b).

One conceptual reactor represents the zone into which the substrate enters and the remainder of the fluid is the second zone. It makes sense to consider that the faster the internal circulation rate the better is the mixing. This idea is expressed by two dilution rates:

\[ \frac{dS_i}{dt} = \frac{Y_{sx}}{\mu x} \left[ \mu_i x_e^i (1-\theta) + \Delta_i x_i^e - \Delta_i x_i^a \right] - x_i \]  

\[ \frac{dx_i^e}{dt} = \frac{Y_{sx}}{\mu x} \left[ \mu_i x_e^i (1-\theta) + \Delta_i x_i^e - \Delta_i x_i^a \right] - x_i^e \]  

\[ \frac{dx_i^a}{dt} = \frac{Y_{sx}}{\mu x} \left[ \mu_i x_e^i (1-\theta) + (\Delta_i x_i^e - \Delta_i x_i^a) \omega \right] - x_i^a \]  

\[ \frac{dS_i}{dt} = \frac{Y_{sx}}{\mu x} \left[ \mu_i x_e^i (1-\theta) + (\Delta_i x_i^e - \Delta_i x_i^a) \omega \right] - x_i \]  

\[ \frac{dS_i}{dt} = (1+\omega)s_i + \frac{Y_{sx}}{\mu x} (\Delta_i s_i - \Delta_i s_a) - s_i \]  

\[ \frac{dS_i}{dt} = \frac{Y_{sx}}{\mu x} (\Delta_i s_i - \Delta_i s_a) \]  

Eqs (12)-(17) incorporate the optimum dilution rate for fed-batch fermentations\textsuperscript{13,21}, \( D = \mu x / Y_{sx} \), where \( x \) is the total cell mass concentration:

\[ x = x_i^e + x_i^a + x_i^e + x_i^a \]  

From a mass balance for \( V_2 \) it can be shown that \( \Delta_i - \Delta_i = \omega D \)  

The overall specific growth rate is the weighted sum of the growth rates in the two regions:

\[ \mu = \left( x_i \mu_i + \omega x_i \mu_j \right) / \left( x_i + \omega x_i \right) \]  

The individual specific growth rates, \( \mu_i \), \( \mu_j \), and \( \mu_k \), with \( j=1 \) or 2, are computed according to Eqs (3), (6) and (7). Eqs (1)-(20) were solved for \( D_i, D_2 \) and \( D \) in the range 0.01 to 4.0 h\textsuperscript{-1} to generate data for neural simulations. Larger dilution rates were not required as almost perfect mixing had been reached. The values
of all parameters were the same as in Nielsen et al.\textsuperscript{16} and Patnaik\textsuperscript{20}; these are summarised in Table 1.

### Choice of Network Topology

Although the feed-forward neural network is the most widely used for bioreactor problems, it has weaknesses for time-dependent problems because it provides only a static mapping between input and output variables\textsuperscript{11}. Thus, it is unsuitable for fed-batch fermentations.

Modifications have been proposed to enable a feed-forward ANN to function for dynamic problems. One way is to provide input data at intervals of time and output data one or more time steps ahead. However, this method works well only if the process characteristics do not change appreciably, which is not true of run-away fermentations. An alternate approach is to add a dynamic element to a static ANN. DiMassimo and coworkers\textsuperscript{18} did this by passing the outputs through a first order transfer function with time delay. Glassey \textit{et al.}\textsuperscript{12} preferred a first order low pass filter.

In a strict sense, none of these modifications changes the feed-forward architecture. Moreover, such a contrived method of dynamic simulation by a static network can lead to significant overparameterization\textsuperscript{11}. Recurrent ANNs have inherent dynamics and are therefore suitable for fed-batch fermentations. This consideration has led to a shift in emphasis from feed-forward to recurrent ANNs. Chen and Weigand\textsuperscript{25} demonstrated the superior applicability of a recurrent ANN for baker’s yeast fermentation. Tsai and Chang\textsuperscript{26} combined the two kinds of networks to detect liquid flow malfunctions. For β-galactosidase fermentation in a perfectly homogeneous bioreactor, Patnaik\textsuperscript{17} showed that a recurrent ANN of the Elman\textsuperscript{27} type could faithfully portray the transient intra-cellular dynamics even under disturbed conditions. Therefore, to extend that work to an imperfectly mixed large bioreactor, the same topology was maintained (Fig. 3).

### Application and Discussion

Patnaik\textsuperscript{20} showed that a controlled finite degree of fluid mixing is favourable to a recombinant fermentation whereas perfect mixing is not. This implies that the incomplete mixing in large bioreactors may be usefully exploited. To account for wide ranges of mixing, the dilution rates were varied from 0.01 h\textsuperscript{-1} (predominantly segregated flow for $D_1$, $D_2$ and cell starvation for $D$) to 4.0 h\textsuperscript{-1} (nearly complete mixing and/or substrate inhibition). For compatibility with previous work\textsuperscript{16,20}, the duration of fermentation was maintained at 30 h and the values of the parameters and the initial conditions were unchanged.

As a substitute for ‘live’ data, numerical data were generated by solving Eqs (1)-(20). They were partitioned into two sets, one for training the ANN and the other to evaluate its performance. The usefulness of this approach has been substantiated by previous studies\textsuperscript{5,9,28}, which considered inflow disturbances but not imperfect mixing. Two issues need to be addressed at the start of training. The first is: how large should the training and the test data sets be? The second is: what strategy should be applied to derive the best network configuration? While there are heuristics\textsuperscript{29} and the Vapnik-Chervonenkis\textsuperscript{30} dimension for static feed-forward networks, there is little guidance for dynamic ANNs. Different norms have been suggested for data clustering. Although straightforward, random clustering may not provide efficient training and carries the risk of the training data being biased differently from those used for network testing\textsuperscript{9,29}. An alternate method\textsuperscript{11}, adopted here, is to partition the data set in different ways and continue testing the network ‘until all exemplars in the original, unpartitioned training set have been used for testing exactly once’.

A fundamental problem not often considered in data collection is the sampling frequency. Although a
constant sampling interval is easy to implement and thus commonly employed\textsuperscript{11,12,31}, it has a weakness for a process with strong nonlinearities. Too large a sampling period generates too few data and may miss some crucial features in a rapidly changing region. Too small an interval provides unnecessary weightage to the slowly changing time slices. So, in this study the model was solved for small time steps (0.01 h) and the data for ANN validation were sampled at intervals proportional to the gradients of the concentration profiles. To obtain data at the same points in time for both rDNA and β-galactosidase, the latter times were taken as the reference values (since the primary objective is to generate the protein) and rDNA concentrations at instants which did not occur in the gradient-based algorithm were computed by interpolation\textsuperscript{12,18}.

The structural design of a recurrent ANN involves specifying the number of hidden layers and the number of neurons in each layer which are recurrent and those which are not. Based on previous studies of β-galactosidase\textsuperscript{17} and other reacting systems\textsuperscript{7,12,32}, one hidden layer was preferred; this choice is also supported by Hornik et al.'s\textsuperscript{33} result that one or two hidden layers are sufficient to represent any real-valued function. The heuristic rules to choose the starting number of hidden neurons for a feed-forward ANN\textsuperscript{26} cannot be applied to a recurrent ANN because they do not take account of the internal feedback of information. So the direct approach of DiMassimo et al.\textsuperscript{18} was employed; neurons are added one at a time until the accuracy of prediction (measured, for example, by the mean squared error) stabilizes or deteriorates (due to over-learning). Following the training method suggested by Montague and Morris\textsuperscript{11}, the final Elman network had a 6-10-2 topology. The input neurons correspond to the four intra-cellular components (Fig. 1) and the concentration and flow rate of the substrate. The output neurons correspond to the intra-cellular rDNA and β-galactosidase concentrations. Six of the hidden neurons were recurrent; thus, each input neuron received signal feedback from the hidden layer, as observed also with a somewhat different experimental arrangement for the same fermentation\textsuperscript{17}.

Representative results are shown in Figs 4 to 6 for the test data at $D=0.1 \text{ h}^{-1}$. Since the actual concentration profiles have been reported elsewhere\textsuperscript{20}, the performance of the ANN has been represented by the deviations of the predicted values from the 'true' values, similar to earlier studies\textsuperscript{17,28}. In Fig. 4 the region receiving the substrate (region-1) and the other region-2 are both moderately mixed to the same degree. In Fig. 5 region-1 is highly segregated and region-2 well-mixed, while Fig. 6 represents the reverse case. In all three cases the ANN has been able to predict the bioreactor behaviour with better than 85% accuracy (i.e. the largest error is smaller than 15%). Similar errors were also obtained for β-
galactosidase production in a perfectly mixed vessel with inflow disturbances; this indicates that the ability of an Elman neural network to portray the fermentation behaviour is not impaired by nonidealties. The accuracy is better than that reported by Glassey et al. for another recombinant system. Thus the observation of Chen and Weigand that a recurrent ANN is superior to a feed-forward network with appended dynamics (in the form of data filters and transfer functions) for cell mass concentration in a conventional fermentation is also true for intracellular concentrations in a complex recombinant system. Results for large dilution rates are not shown because the error distributions were similar to that in Fig. 6. Dilution rates smaller than 0.1 h\(^{-1}\) are not practically useful since the fluid in the bioreactor is too poorly mixed (for \(D_1\) and \(D_2\)) and/or productivity is low because of insufficient substrate (for \(D\)).

To test whether the prediction errors are correlated, Pearson’s product moment correlations were calculated for different combinations of \(D, D_1\) and \(D_2\). In addition to the combinations corresponding to Figs 4 to 6, the values for a large inflow rate are also presented in Table 2 to assess whether the kinetic dependence of the rates of cell growth and production formation on the substrate concentration availability has an effect on the ANN’s performance. All correlation coefficients are much smaller than one and all P-values are much larger than zero, indicating that the errors are uncorrelated. (The obvious exceptions are the self-relationships, which have to be unity for the correlation coefficients and zero for the P-values.) Table 2 thus provides quantitative support to the inferences from Figs 4 to 6 that the Elman network’s ability to learn the underlying process characteristics is unaffected by cellular metabolism and the hydrodynamics.

An earlier study of this fermentation in a perfectly mixed reactor subject to disturbances in the feed stream also indicated the suitability of an Elman ANN. Combined with the present results, it may be inferred that such a network is appropriate for large bioreactors, where both spatial variations in the broth and disturbances occur. By accommodating the time-varying nonideal features within the network

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**Table 2 — Pearson’s product moment correlations**

<table>
<thead>
<tr>
<th>Dilution rates(^a)</th>
<th>Variable</th>
<th>Correlation coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>time</td>
<td>DNA</td>
</tr>
<tr>
<td>(D=0.1)</td>
<td></td>
<td>time</td>
<td>DNA</td>
</tr>
<tr>
<td>(D_1=0.3)</td>
<td></td>
<td>1.000</td>
<td>0.0176</td>
</tr>
<tr>
<td>(D_2=0.3)</td>
<td>DNA</td>
<td>0.0176</td>
<td>1.000</td>
</tr>
<tr>
<td>(D_1=0.5)</td>
<td>time</td>
<td>1.000</td>
<td>-0.183</td>
</tr>
<tr>
<td>(D_2=0.1)</td>
<td>DNA</td>
<td>-0.183</td>
<td>1.000</td>
</tr>
<tr>
<td>(D_1=0.1)</td>
<td>time</td>
<td>1.000</td>
<td>0.0011</td>
</tr>
<tr>
<td>(D_2=0.5)</td>
<td>DNA</td>
<td>0.0011</td>
<td>1.000</td>
</tr>
<tr>
<td>(D=1.0)</td>
<td></td>
<td>time</td>
<td>DNA</td>
</tr>
<tr>
<td>(D_1=0.3)</td>
<td></td>
<td>1.000</td>
<td>0.0548</td>
</tr>
<tr>
<td>(D_2=0.3)</td>
<td>DNA</td>
<td>0.0548</td>
<td>1.000</td>
</tr>
<tr>
<td>(D_1=0.5)</td>
<td>time</td>
<td>1.000</td>
<td>-0.108</td>
</tr>
<tr>
<td>(D_2=0.1)</td>
<td>DNA</td>
<td>-0.108</td>
<td>1.000</td>
</tr>
<tr>
<td>(D_1=0.1)</td>
<td>time</td>
<td>1.000</td>
<td>-0.0792</td>
</tr>
<tr>
<td>(D_2=0.5)</td>
<td>DNA</td>
<td>-0.0792</td>
<td>1.000</td>
</tr>
</tbody>
</table>

\(^a\)Values of all dilution rates are in reciprocal hours.
structure, a recurrent ANN (such as of the Elman type) performs better than a feed-forward ANN with added nonlinear components.\(^{12,21,25}\)

**Nomenclature**

\[
\begin{align*}
D &= \text{overall dilution rate, } 1/h \\
D_i &= \text{axial dispersion coefficient, } \text{cm}^2/h \\
D_k &= \text{internal dilution rate for } j\text{-th region, } 1/h \\
k &= \text{forward rate constant for enzyme-mediated substrate transport across cell wall, } 1/h \\
k^1, k^2, k^3, k^4 &= \text{reaction rate constants, } 1/h \\
K &= \text{equilibrium constant for enzyme-mediated substrate transport across cell wall, g/l} \\
K^1, K^2, K^p &= \text{equilibrium constants, g/l} \\
L &= \text{characteristic length for bioreactor, cm} \\
Q &= \text{substrate feed rate, } 1/h \\
Q_i, Q_{i+1} &= \text{internal flow rate from region(1) to region(2), } 1/h \\
Q_{i+1, j} &= \text{rate of change of } A\text{-compartments in } j\text{-th region, } 1/h \\
Q_{i+1, j}^1, Q_{i+1, j}^2 &= \text{rates of change of } G\text{-compartments in } j\text{-th region, } 1/h \\
Q_{i+1, j}^3 &= \text{rate of change of } P\text{-compartments in } j\text{-th region, } 1/h \\
Q_{i+1, j}^4 &= \text{rate of change of } E\text{-compartments in } j\text{-th region, } 1/h \\
S &= \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_{i+1} &= \text{substrate concentration in j-th region, g/l} \\
S_{i+1}^1, S_{i+1}^2 &= \text{concentration of plasmid-free cells in } j\text{-th region, } g/l \\
S_{i+1}^3, S_{i+1}^4 &= \text{concentration of plasmid-containing cells in } j\text{-th region, } g/l \\
S_{i+1}^5 &= \text{concentration of plasmid-hating cells in } j\text{-th region, g/l} \\
S_{i+1}^6 &= \text{concentration of plasmid-free cells in j-th region, g/l} \\
S_{i+1}^7 &= \text{concentration of plasmid-free cells in j-th region, g/l} \\
S_{i+1}^8 &= \text{concentration of plasmid-containing cells in j-th region, g/l} \\
S_{i+1}^9 &= \text{concentration of E-compartment in } j\text{-th region, g/l} \\
S_{i+1}^{10} &= \text{concentration of E-compartment in } j\text{-th region, g/l} \\
S_{i+1}^{11} &= \text{yield coefficient for biomass from substrate, g/g} \\
\alpha &= \text{intra-cellular substrate concentration in } j\text{-th region, g/L} \\
\alpha^j &= \text{plasmid loss probability, dimensionless} \\
\beta &= \text{plasmid-containing cells} \\
\gamma &= \text{plasmid-free cells} \\
\tau &= \text{dimensionless} \\
\delta &= \text{dimensionless} \\

\text{Superscript} \\
+ &= \text{plasmid-containing cells} \\
- &= \text{plasmid-free cells} \\

\text{References} \\