On-line Control of a Fed-Batch Fermentation by using SOM Based Multiple Local Linear Models

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Abstract

In this paper, we present the theoretical development and application of Self-Organizing Map (SOM) based multiple local models (MLLM) for on-line control of fed-batch fermentation process. The recalling and generalization of the model was carried out using simulated data. The prediction of the MLLM was evaluated based on the normalized root mean square error and the result is found to be satisfactory. A feed forward control law was derived for a biomass tracking problem and incorporated with the proposed model to control the fed-batch yeast cultivation. The result observed from the simulation and experimental study illustrates the effectiveness of the model for on-line bioprocess control application.

1. Introduction

The control of a fermentation process along a predetermined trajectory requires on-line monitoring of state variables. However, the lack of reliable on-line sensors for measurement of key state variables makes this a very difficult task. This need is acutely experienced in fermentation and other biological systems as these are nonlinear, complex and time varying in nature. In this area, neural networks as a global model has drawn much attention and it has been applied successfully for prediction and modeling of bioprocesses [1]-[3]. However, global models have shown some difficulties in cases when the dynamical system characteristics vary considerably over the operating regime and in effectively bringing the issue of time varying parameters (or nonlinearity) into the design. On the other hand, these difficulties are addressed by local modeling that are derived based on neighboring samples in the operating space to characterize some operating point or similar feature [4]-[6].

A Self-Organizing Map (SOM) has been used in a number of local modeling applications to divide operating regimes into different local regions [7]-[11]. In this application, the role of the SOM is to discover patterns in the metabolic state of the cells (high dimensional space) and to divide the fermentation process in sub-regions of physiological conditions that are represented by the weights of each neuron (lower dimensional space). The associated Multiple Local Linear Models (MLLM) specific to each operating regime is represented by neurons based on the featured input-output data. These models are used to estimate the state of the process under specific operating condition. In addition, using SOM as a local model infrastructure mitigates the discontinuity problem that occurs in most of operating regime based modeling techniques [8], [12].

In this paper, we present the use of SOM based Multiple local modeling for online control implementation in a biomass tracking problem for a fed-batch fermentation. The cultivation of *Saccharomyces cerevisiae* was selected as the system of study. The results demonstrate that the proposed strategy is capable of predicting state variables with acceptable limits of error, within a reasonable boundary outside its training domain and showed a promising result for online control implementation.

2. Theoretical development

2.1 Process description

The fermentation process selected for this study is the cultivation of *Saccharomyces cerevisiae*, which is a widely studied bioprocess in system identification and control implementation. *Saccharomyces cerevisiae* converts glucose to ethanol. Glucose degradation proceeds via two metabolic pathways, oxidative and reductive. The utilization of glucose to produce ethanol is quite complex and it is this feature that makes it a representative process for studying nonlinear dynamical behavior. Ethanol is formed as the product of reductive energy metabolism and can only be utilized oxidatively. Glucose is used preferentially over ethanol and because of the involvement of a limited respiratory capacity; the ethanol is consumed only subject to this capacity and not from glucose repression of respiration. The respiratory capacity of the cells governs glucose or ethanol metabolism in growing cells and product formation, and represents a bottleneck for oxidative substrate utilization. At low enough substrate fluxes (sub-critical) pure oxidative metabolism is observed. Under these circumstances, glucose is consumed preferentially over ethanol to satisfy the bottleneck conditions. If glucose flux exceeds the respiration bottleneck (supra-critical substrate flux), a partition of glucose flux occurs. Since ethanol utilization can be a purely oxidative process only, ethanol utilization is observed to have lower priority than glucose utilization. Consequently, growth on ethanol as the sole substrate is definitely limited by the respiratory capacity.

Clearly, ethanol fermentation process exhibits a range of physiological states depending on the operating condition in the reactor. Changes in the feed concentration and flow rate, aeration and mixing, will affect the amount of cell mass or ethanol produced. Consequently, controlling the process along a predetermined trajectory is difficult. We have explored a wide range of process conditions that can occur in an industrial environment. Our objective was to capture the spectrum of metabolic states that are manifested, and use this *a priori* knowledge for designing a realistic controller. We have used the SOM to cluster similar metabolic states together. The nonlinear dynamics has been approximated by local linear models and a feed forward control strategy have been designed on this schema and implemented in a real time yeast fermentation process.

2.2 SOM based multiple local linear models

We assume at the outset, that the system under consideration is described by the general state dynamics

$$\begin{aligned} \dot{\mathbf{x}} &= \mathbf{f}(\mathbf{x},\mathbf{u}) \\ \mathbf{y} &= \mathbf{h}(\mathbf{x},\mathbf{u}) \end{aligned} \tag{1}$$

Where $\mathbf{x} \in \Re^n$ is state variable, $\mathbf{u} = \Re^m$ is the control input and $\mathbf{y} = \Re^p$ is the output variable. The theory of operating regime based modeling allows us to approximate the underlying dynamics $\boldsymbol{f}$ as,

$$\widetilde{\boldsymbol{f}} \approx \bigcup_{i=1,\cdots,N} \boldsymbol{f}_i \tag{2}$$

Where N is the number of operating regions and $\boldsymbol{f_i}$ is the local model representing the i^{th} operating region. After the operating regimes are divided by the SOM, the underlying dynamics of the process is approximated by N local models. These N local linear models for fed batch yeast fermentation can be described by,

$$\begin{aligned} \mathbf{x}(t+1) &= \boldsymbol{f}_i\big(\mathbf{x}(t), u(t)\big) \\ &= \boldsymbol{A}_i^T \mathbf{x}(t) + \boldsymbol{c}_i^T u(t) + \boldsymbol{b}_i^T \end{aligned} \tag{3}$$

Where, $\mathbf{x} = \begin{bmatrix} x & s & e & c_L \end{bmatrix}^T$ is the vector of state variables, u is the control input variable (dilution rate $D = u = F/V$ = feed flow rate / reactor volume), $\boldsymbol{A_i}$ is 4×4 matrix of unknown parameters, and $\boldsymbol{b_i}$ and $\boldsymbol{c_i}$ are the 4×1 vector of unknown parameters for i^{th} operating regime. Each map unit of the SOM is extended with a local model to learn the mapping $\mathbf{x}(t+1) = \boldsymbol{f}_i\big(\mathbf{x}(t), u(t)\big)$ in a supervised way. Accordingly, the local model coefficients $\begin{bmatrix} \boldsymbol{A}_i^T & \boldsymbol{c}_i^T & \boldsymbol{b}_i \end{bmatrix}$ for each map unit are computed directly from the desired signal, that is, the codebook vector of the best matching unit and the input-output samples by a least square fit within a *Voronoi* region centered at the current winning map unit. This is represented schematically in Fig. 1.

The design procedure for SOM based multiple local linear modeling is as follows:

i. A training data generated for possible different initial condition presented to the SOM and the winning map unit corresponding to the input space was

determined. This process gives the winner-input pairs.

ii. The least square fit was used to find the local linear model coefficients for the winning map unit (also called the best matching unit or BMU), i^0, where desired output vector $\mathbf{r}_{i^0,j} \in \Re^M$ as

$$\mathbf{r}_{i^0,j} = \begin{bmatrix} \mathbf{A}_{i^0}^T & \mathbf{c}_{i^0}^T & \mathbf{b}_{i^0}^T \end{bmatrix} \begin{bmatrix} \mathbf{x}(j)_{i^0} \\ u(j)_{i^0} \\ \mathbf{I} \end{bmatrix} \quad \text{for } \forall_j \in M \qquad (4)$$

Where $\begin{bmatrix} \mathbf{A}_{i^0}^T & \mathbf{c}_{i^0}^T & \mathbf{b}_{i^0}^T \end{bmatrix}$ is the sought linear model coefficients, M is the size of the data involved in the winning map unit i^o.

iii. In testing, a testing data set is taken and fed to the SOM, once the winning map unit is determined, select appropriate local model from the list of associated models and apply it to obtain the estimated output $\hat{\mathbf{x}}(t+1)$ from the following equation.

$$\hat{\mathbf{x}}(t+1) = \boldsymbol{A}_i^T \mathbf{x}(t) + \boldsymbol{c}_i^T u(t) + \boldsymbol{b}_i^T \qquad (5)$$

For iterated prediction (in our case for concentration profile prediction), the generated sample is fed back to the input (delayed by one sample) and the procedure repeated as many times as needed.

Our proposed modeling methodology is summarized as follows: first, the input-output joint space is decomposed into a set of operating regimes that are assumed to cover the full operating space. Next, for each operating regime we choose a simple linear model to capture the dynamics of the region. Consequently, the overall dynamics of the process is approximated by a concatenation of local linear models.

2.3 Data generation and training of SOM

Many different models have appeared in the literature for describing yeast fermentation. Among the various models that have appeared in the literature, the Sonnleitner-Kappeli [13] model describes the aerobic growth of *Saccharomyces cerevisiae* on the premise of respiratory capacity. This model has been previously used by us to simulate fed batch yeast cultivation for control application. The result of those studies showed that our experimental data was best fitted by the Sonnleitner-Kappeli model [14]. For the work in this paper, we used the following range values of initial conditions for generating data: 0.25-0.625 g/L biomass, 10.8-0.625 g/L glucose, 0.23 g/L ethanol and 96% dissolved oxygen. The feed into the reactor is assumed sterile and the glucose concentration in the feed is 21.6 g/L. We have used different operating conditions, within the selected range of the initial conditions, in such a way that the

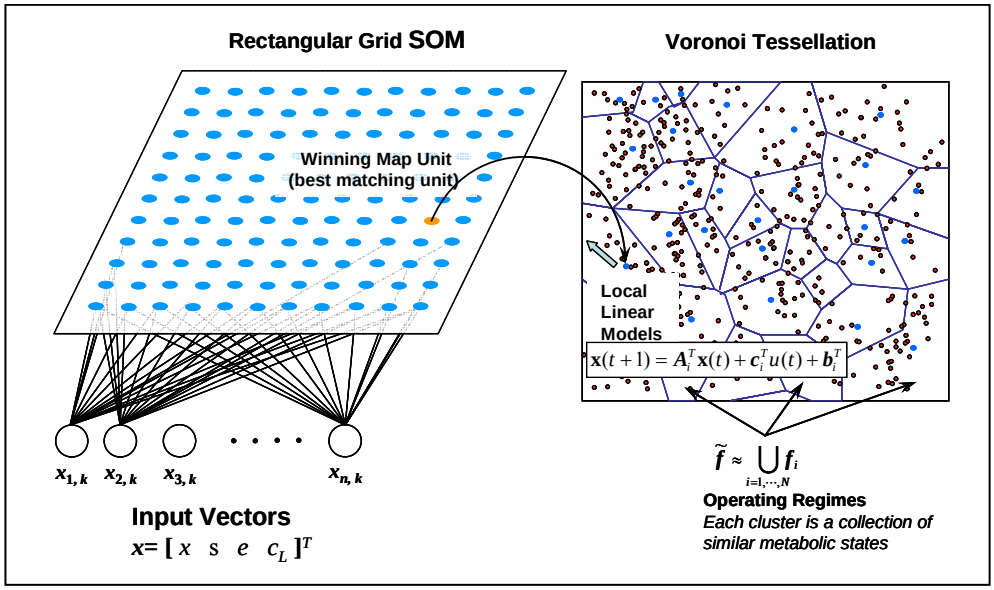

Fig. 1 Representation of the SOM based local linear model of the ethanol fermentation process

metabolic states of the cells were different. In addition, the constraints for the simulation were defined by the physical limitation of the bioreactor. For example, the minimum volume for batch cultures was 4 L and the maximum working volume of the bioreactor for fed-batch processes was 12 L. The data was generated with a constant sampling interval of 6 min for a maximum of 12 h operation. The reactor was operated initially (first 4 h) in the batch mode with an initial volume of 4 L. Then the operation was shifted to the fed-batch mode, during that a fresh substrate was fed into the reactor based on the metabolic requirement and the control objective. A MATLAB program was written to generate the training data for the given initial conditions.

Training of the SOM was accomplished using the Kohonen learning process [15]. The input vector for the SOM consists of biomass (x), substrate (s), ethanol (e) and dissolved oxygen (c_L) concentration and the dilution rate (D). With each input vector the Kohonen learning algorithm adaptively discretizes the continuous input space into a set of K disjoint Voronoi cells. The response of SOM to an input vector is determined by the reference vector w_i^0 of the neuron that produces the best match to the input. Then the k^{th} adaptation of the weights is done in the following manner,

$$w_{i,k+1} = \begin{cases} w_{i,k} + \eta_k \Lambda_{i,k}\left(c_k - w_{i,k}\right), & i = i^0 \\ w_{i,k} & \text{otherwise} \end{cases} \qquad (6)$$

Where η_k is the learning rate and $\Lambda_{i^0,i,k}$ denotes the topological neighborhood function centered on the winning neuron i^0 defined as

$$\Lambda_{i^0,i,k} = \exp\left(\frac{\left\|r_i - r_{i^0}\right\|^2}{2\sigma_k^2}\right) \qquad (7)$$

Where $\left\|r_i - r_{i^0}\right\|$ represents the Euclidean distance in the output space between the i^{th} neuron and the winning neuron and σ_k is the effective width of the topological neighborhood, which is shrinking with time.

2.4 Application for bioprocess control: The control Strategy

The control problem is formulated to control the cell concentration along a linearly increasing profile given by $x = c_1 t + c_0$, where c_0 and c_1 are constants, and t is the fermentation time. Since the rate of change of biomass in fed-batch mode is given by $\frac{dx}{dt} = \mu x - Dx$. To implement this control strategy, the feed forward control law is very simply:

$$D = u = \mu - \frac{c}{x} \qquad (8)$$

Where x is the biomass concentration and μ is specific growth rate that depends on all the states. The state variables required to calculate the current value of the process input u, the substrate-feeding rate, are estimated by using the SOM based multiple local linear models. During online implementation, the fermentation process behaves differently from the simulations used to train the SOM because of variability of process conditions, and the physiology and metabolic condition of the yeast cells. Therefore to select the appropriate operating regime and corresponding local model, the required information that describing the current state of process is acquired from the dissolved oxygen concentration measurement which reflects the metabolic state of the process continuously and reliably. The block diagram for the proposed control strategy is given in Fig. 2.

3. Materials and methods

An ethanol fermentation experiment using *S. cerevisiae* was performed to evaluate the online performance of the proposed control strategy. A 15 L reactor (B-Braun Biostat C, Germany) was used for cultivating the yeast cells. The experiment was designed to achieve a predefined control objective of tracking a linear biomass trajectory with a slope of 0.3.

3.1 Inoculum preparation

For the cultivation of the seed culture, 400 ml (10% of initial reactor volume) of YPMG media was prepared in 1 L Erlenmeyer flask and the pH adjusted to be 5.0. The composition of the YPMG media was: yeast extract 0.3%, malt extract 0.3%, peptone 1.5%, glucose 3%, KH_2PO_4 0.03% and $MgSO_4$ 0.01%. The

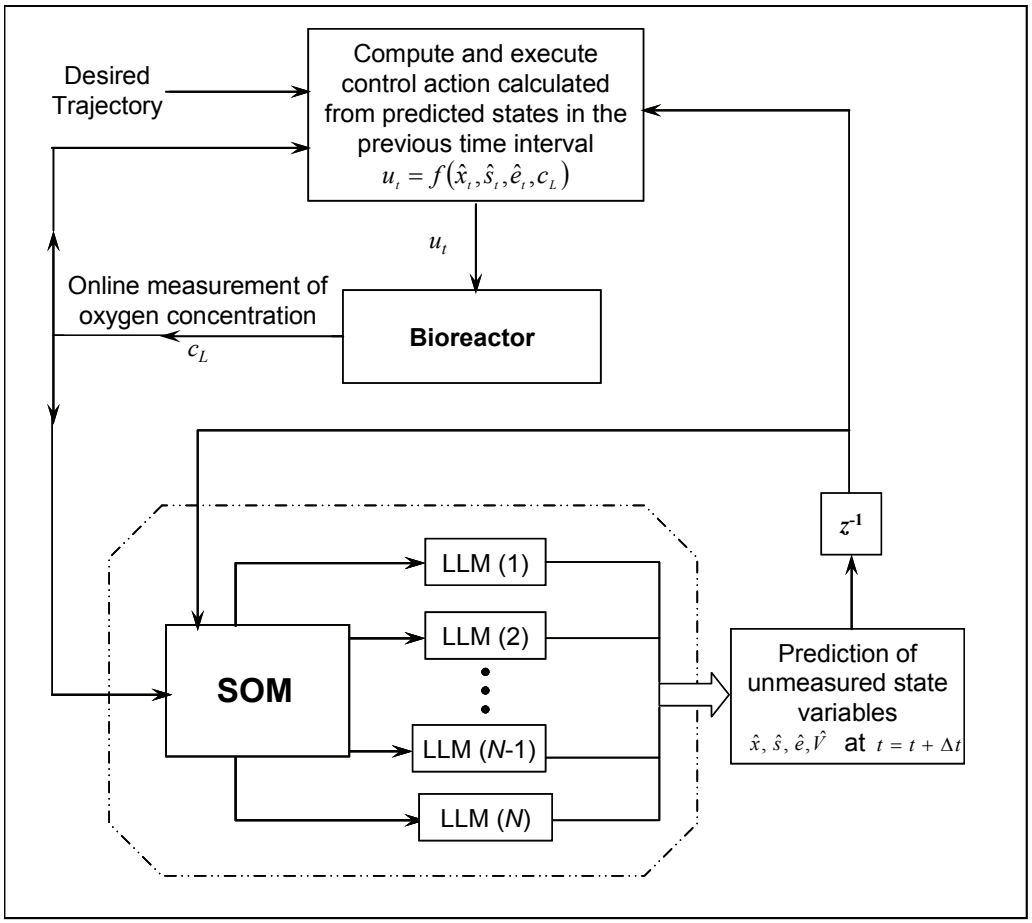

Fig. 2 Block diagram for application of SOM based models to control yeast fermentation by using a feed forward control law

prepared media was autoclaved at 121 ^{o}C for 20 min then it was inoculated from a one day old liquid culture of *Saccharomyces cerevisiae* (Y1012) strain. The seed culture was incubated at 30 °C and agitated at 150 rpm in an orbital shaker (Orbitek, Sygenics, India) for 10 hr. Then it was harvested and used to inoculate the 15 L reactor.

3.2 Bioreactor operation

A volume of 3.60 L of production media (glucose, yeast extract 1%, peptone 1%) was prepared in the 15 L reactor and sterilized at 121^{o}C for 30 min and the pH was adjusted to 5 by the addition of 2N HCl solution prior to the inoculation. The reactor was inoculated with the 400 ml of seed culture so that the initial reactor volume was 4 L. At time t = 0, a sample was drawn from the reactor and initial conditions measured was found to be 0.372 g/L biomass, 12.0 g/L glucose, 0.23 g/L ethanol and 96% dissolved oxygen. The feed into the reactor was sterilize and had a concentration of 21.6g/L glucose. Samples were taken at every 30 min interval. The batch phase was completed after 4 h and the fed-batch mode was started by feeding medium into the reactor using a peristaltic pump. To run SOM-MLLM controller, a Visual C++ *mex* program was written for interfacing the D/A and A/D cards (Data Translation, USA). The control action was computed at every 6 min and implemented through the DT2815 D/A card as a flow rate using the peristaltic pump. The dissolved oxygen value was acquired using the DT2814 A/D card. The fed-batch phase continued until the maximum working volume of the test run was reached. In this study, the total fermentation time was 9 h and the final volume of the reactor at the end of the operation

was 12 L. The fermentation was carried out at 30^{o}C, pH 5, and agitation speed of 300 rpm.

4. Results and discussions

Two SOM's were trained simultaneously, one for the batch and the other for the fed-batch phase of the process. This modification was required to incorporate the dilution rate, which is the input variable for the fed batch system. Training of the network was carried out by using a public domain SOM-toolbox [16]. The training parameters were selected automatically by using the toolbox during the training process. The dimension of the trained SOM's were obtained as 20×9 for batch and 22×9 for fed-batch phase. Thus, from the trained SOM, a total of 180 local linear models for batch phase and 198 local linear models for fed batch phase were identified to represent the overall plant dynamics.

4.1 Recalling and generalization performance of model

The recalling and generalization performance of the proposed modeling architecture was studied by using a simulated data. The Normalized Root Mean Square Errors (NRMSE) gained for six different initial conditions and one-step prediction task is shown in Table 1. To evaluate the performance of the SOM for recall, conditions I – III were used and to evaluate its performance for generalization, conditions IV – VI were used. Conditions I – III have the initial glucose and biomass concentration used for training but conditions IV – VI have initial values that are different from those used for training. Accordingly, the model prediction performance was studied for data different from the training set. From the result shown in Table 1, we observe that the NRMSE value for biomass, glucose and ethanol profile prediction is accurate up to 10^{-4}, 10^{-3}, and 10^{-2}, respectively. In addition, the NRMSE values obtained for conditions IV – VI shows a comparable performance for the prediction of states outside the domain of the training conditions.

4.2 On-line control implementation

The observed experimental result and the predicted profile for the on-line control implementation of the proposed architecture is given in Figs. 3 and 4. Figure 3 shows the performance of the SOM–MLLM as a predictor of the state variables for a simulation carried out with initial conditions III (see Table 1). Hence, it is demonstrated that the predictions of the SOM–MLLM is acceptable. The approximation of the nonlinear dynamics by the MLLM in the *N*-different metabolic regimes proves that it is possible to collect experimental data from multiple experiments with different conditions in this fashion without sacrificing the information content.

Table 1: Recall and Generalization Performance of the SOM based MLLM

	Recall				Generalization		
	Initial Condition		NRMSE		Initial Condition		NRMSE
	Variable	Value (g/l)			Variable	Value (g/l)	
I	x	0.25	0.000046	**IV**	x	0.3	0.000571
	s	10.8	0.009236		s	12	0.001261
	e	0.23	0.007492		e	0.23	0.022102
II	x	0.375	0.000399	**V**	x	0.4	0.000768
	s	14.4	0.009306		s	15	0.017000
	e	0.23	0.006394		e	0.3	0.070829
III	x	0.5	0.000351	**VI**	x	0.55	0.001144
	s	18	0.003829		s	18	0.008875
	e	0.23	0.012009		e	0.23	0.012154

Figure 4 shows that results of the SOM-MLLM feed forward controller in maintaining the process along the *desired* linear trajectory. The predictions of the SOM-MLLM were used as in the case of the simulation experiment with one difference. The online measurement of dissolved oxygen concentration (c_L) was used to replace the corresponding predicted value. The simple procedure shifts the identification of the winning unit of the SOM sufficiently, so that the real time fermentation process is tracked accurately. The robustness is clearly demonstrated because the online dissolved oxygen (c_L) contains noise that associated with the electrode used for measurement. The slope of the biomass trajectory obtained from experiment is calculated as 0.269 that is close to the desired value of 0.3. The observed deviation on the state variables may be ascribed to a number of reasons. There will be approximation errors in using the MLLM. Unlike in the case of the simulation experiment, here the process conditions will change depending on the actual condition in the reactor. Local metabolic regions can form within the reactor due to imperfect mixing that cause local mass transfer variations. The model used for generating and training the SOM does not account for these variations. However, considering that there will always be plant-model mismatch, the performance of the feed forward controller employing the SOM-MLLM is satisfactory. The main advantage of this approach is that data from different experiments can be pooled together. As new data is acquired, that SOM can be re-trained and the new metabolic information will become incorporated within leading to improved controller performance.

5. Conclusions

In this study we have combined the superior clustering capability of SOM with the simplicity of local linear modeling for bioprocess application. The control performance achieved using local linear models was studied for different process conditions. The ability of the MLLM to track the changing dynamics of the process behavior around the training domain is verified. The result observed shows that the model is capable of predicting the unmeasured state variables with reasonable limits of errors outside the training domain. In this paper, we have applied the proposed architecture to control fed-batch yeast cultivation. We have demonstrated that the performance of the model in state prediction and control implementation, applying the SOM based multiple local linear models for online implementation of bioprocess control provides assured benefits in alleviating problems associated with the dynamics of bioprocess system.

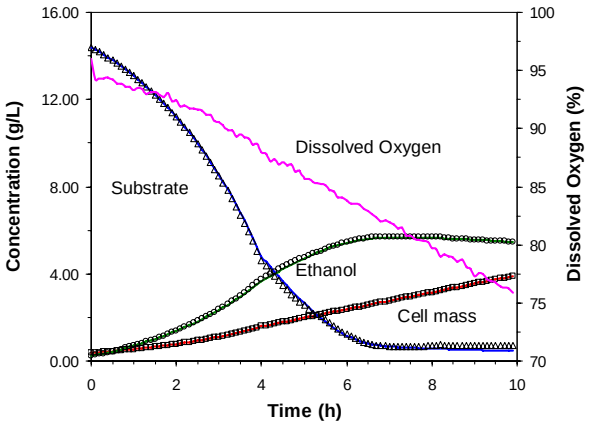

Fig. 3 A comparison of the prediction of the SOM–MLLM during recall. The result for initial condition III is shown here. —— Simulated from the model. All symbols are prediction of SOM–MLLM (Δ substrate ○ ethanol □ cell mass)

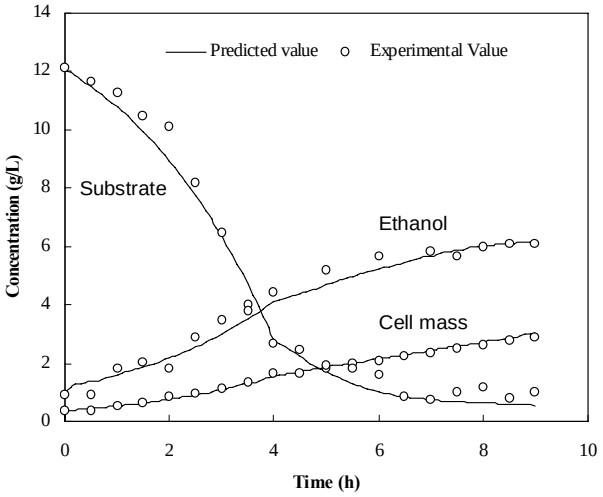

Fig. 4 On-line control implementation of SOM–MLLM feed forward controller for *S. cerevisiae* fermentation process in a 15 L reactor. The desired trajectory is to control the cell mass concentration along the profile $x = 0.3 \times \text{time}$

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