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Estimation of fungal biomass using multiphase artificial neural network based dynamic soft sensor

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ABSTRACT

Interest in low cost cellulase production has become a major challenge in recent years for biorefineries. Fed-batch fermentation of *Trichoderma* strains for the production of low cost cellulase is carried out on complex media that has various soluble and insoluble substrates. The lack of direct estimation of biomass in the presence of insoluble substrates is one of the major concerns for controlling bioprocesses in industries. In this paper, a Multiphase Artificial Neural Network (MANN) based dynamic soft sensor is developed to predict the biomass concentration of *Trichoderma* during fed batch fermentation in the presence of insoluble substrates. The soft sensor has three Nonlinear Auto Regressive with eXogenous input (NARX) models to capture the complete dynamics of lag, log and stationary phases of the microbe. At different phases, a particular neural network model is triggered based on the period of operation. Each NARX model estimates biomass concentration using online measurements such as pH, substrate concentration and agitation speed. The predicted output of the proposed model and single ANN model are compared against real-time biomass sensor data. The results demonstrated indicate that the proposed MANN based soft sensor shows good performance with focus on the dynamic behavior of the bioreactor. Also, the developed model recursively predicts the biomass concentration with acceptable deviation with respect to realistic measurement. The results summarized could offer a new methodology in estimating fungal biomass accurately, thereby increasing the productivity of cellulase in industries.

1. Introduction

Estimation of biomass plays a crucial role during fermentation. The process of monitoring biomass continuously helps in determining the state of process, to determine the time to start fed-batch and also to determine the time to harvest the product. Low cost cellulase production using lignocellulosic substances as carbon source has gained interest over recent years and the process predominantly uses filamentous fungi such as *Trichoderma reesei*, for better yield (Domingues et al., 2000). A major challenge during low cost cellulase production is the estimation of fungal biomass in the presence of insoluble substrates. However, control of the batch/fed-batch fermentation is achieved only when estimation of accurate biomass concentration is possible. A method for estimating the concentration of fungal biomass in the presence of insoluble substrates (solka floc cellulose and corn steep solids) during the *Trichoderma reesei* fermentation based on the DNA quantification is described in (Rahumathulla et al., 2009). But monitoring DNA concentration throughout the fermentation is not a suitable method for online monitoring. During microbial cultivations, the specific growth rate is the function of substrate concentration and physical parameters such as pH, temperature and dissolved oxygen. Thus, it is difficult to estimate biomass concentration without considering the changes in the physical parameters. Hence, modeling the bioprocess for the estimation of biomass is a major concern in industries.

Bioprocess models are broadly classified into first principles and empirical models. Monod model is the widely used unstructured model for the estimation of cell growth. But the model is unable to capture the dynamics during lag phase and stationary phase of cell growth. Though, the estimation of cell growth during stationary phase is accomplished by logistic models, they suffer during prediction of growth in lag phase. Structured models, on the other hand use more parameters and hence they are difficult to handle (Lin et al., 2000). Determination of parameters accurately in first principles models require laborious experimentation which make these models unfit for use in the design of control systems (Zelić et al., 2004). Though the estimation techniques based on agitation speed of Dissolved Oxygen (DO)-stat culture (Jeong-Geol et al., 2005), the capacitance of the batch culture (Ferreira et al., 2005), and impedance measurements (Bragós et al., 1999) have been reported, these methods require costlier instrumentation.

In recent years, soft sensors are widely used for the estimation of

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biomass. Soft sensors estimate the unknown state variable by using some other measured variables that influences the unknown state (Bogaerts and Hanus, 2001). Artificial Neural Networks (ANN) based soft sensor have the capability to learn nonlinearity of the process using experimental plant data and thus can be used to estimate the state of bioprocess such as biomass concentration (James et al., 2002; Won and Yoon-Keun, 2006). Rahman et al., 2009 have predicted the enzymatic synthesis yield of dioctyl adipate ester using feed forward neural networks. Trained ANN with good experimental data can be effectively used for estimation and control of process variables (Dochain and Perrier, 1997). On comparison with other empirical models, neural networks are relatively less sensitive to noise and hence can be applied to process control systems with higher level of uncertainty (Zelić et al., 2006). During batch/fed-batch, ANN can be effectively used for estimation of biomass or product, optimization of fed-batch run and online control of bioprocess systems (Hajmeer and Basheer, 2003; Vlassides et al., 2001; Haider et al., 2008). ANNs are suitable for many applications such as nonlinear filtering, prediction of output using input are widely used in the modeling of dynamic systems (Menezes Jr and Barreto, 2008). Non-linear regressive models with exogenous input (NARX) are found to be effective for non-linear system identification, as they have good predictive capability (Narendra and Parthasarathy, 1990; Lin et al., 1997).

The aim of this work is to find a suitable model using ANN for the estimation of biomass. To achieve this, initially a single ANN model is developed and compared with real time experimental data. In order to capture the complete dynamics of microbial strain in lag, log and stationary phases, three NARX models are generated. A program is developed in MATLAB to estimate the biomass concentration by switching among the NARX models whichever is appropriate based on the time of operation. A MANN model using one-step ahead forecasting of the bioreactor dynamics is developed and validated. The performance of the model is compared with single ANN model. Results show that MANN model has better performance criteria than single ANN model.

2. Materials and methods

2.1. Setup overview

The experimental set up (Fig. 1) is a 3.5 L autoclavable bioreactor (Biojenik Engineering, LS035). The Process and Instrumentation Diagram (P&ID) of experimental setup is shown in Fig. 2. The reactor is equipped with acid, base, antifoam and feed pumps. Air is supplied via compressor, Mass Flow Controller (MFC) to the reactor vessel. The bioreactor is equipped with four standard baffles and mechanically sealed stirrer shafts entering from the bottom. A pair of standard 6-bladed Rushton turbines is mounted on the shaft. The agitation rate is controlled by a motor driven from the bottom of the vessel. The vessel is equipped with METTLER TOLEDO probes (Mettler-Toledo India Private Limited, Mumbai, India) for temperature, pH, dissolved oxygen, and foam level. Samples are taken via a sample valve for offline analysis of substrate and product concentrations. The bioreactor can be operated in batch, fed-batch and continuous mode (Lidén, 2002). In this work, the bioreactor is operated in fed-batch mode. The operation of the bioreactor is performed via LabVIEW graphical user interface (LabVIEW for Windows, Version 2014, National Instruments (NI), Austin, Tex). It is preferred because of its data flow and parallel programming nature (Rudolf et al., 2005). The human machine interface panel developed using this platform enhances accessibility to the reactor and also it ensures safety. The bioreactor includes measurement system for temperature, pH and dissolved oxygen. RTD temperature probe is used to acquire the temperature inside the reactor. pH inside the reactor can be measured using the specially designed gel filled probe. The flow rates from acid and base pump of fixed speed can be used as manipulating variables. DO probe is used for the online measurement of dissolved oxygen in the reactor. Variable speed pump is used to feed the substrate inside the reactor in case of fed batch process. To reduce the foam produced during the process, antifoam pump of fixed speed can be operated to inject silicone oil. The bioreactor is also equipped with ABER capacitance probe (Aber Instruments, Aberystwyth, Wales, UK) for online measurement of biomass (Krairak et al., 1999). The signal from DO probe is taken as input through analog input module NI-DAQ 9203 to personal computer (PC). The control signal is given by the PI controller implemented in LabVIEW to analog output module NI-DAQ 9263 which manipulates the stirrer speed in the range 0–1500 rpm. The stirrer speed is kept initially at 150 rpm and is varied to a maximum of 800 rpm during the experiment (Chitra et al., 2018). The substrate concentration is estimated using GOD-POD assay.

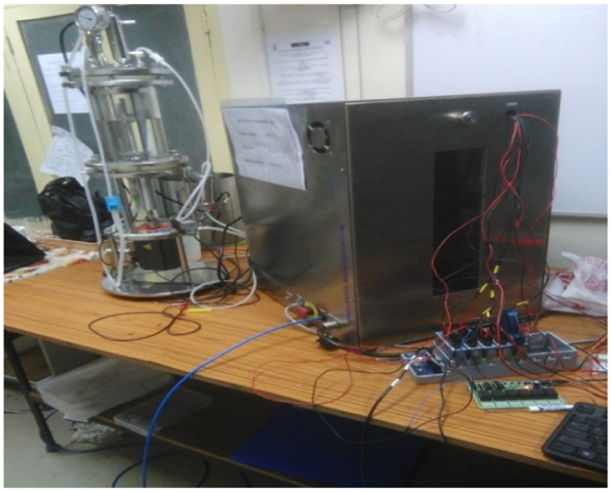

Fig. 1. Bioreactor lab set-up interfaced with NI-DAQ cards.

2.2. Strain and culture conditions

Trichoderma reesei Rut C-30 (NRRL 11460) strain is obtained from AU-KBC Research Centre (MIT Campus, Anna University). For stock cultures preparation, the strain is grown on potato dextrose agar slants at 30 °C. After 5–7 days, *T. reesei* spores are suspended in 0.8% (w/v) NaCl solution containing 0.015% (w/v) Tween 80 and 20% glycerol and stored at −80 °C.

The fungal strain, *T. Reesei* is cultivated in fed-batch mode to test and validate the model. In this study, *T. Reesei* is cultured in the medium containing (g/L) Glucose, 10; wheat bran, 10; Avicel, 6; corn steep liquor, 5; urea, 0.3; KH2PO4, 2.0; MgSO47H2O, 0.4; CaCl2, 0.4 and trace mineral solution, 1 mL. The trace mineral solution consisted of (g/L): FeSO47H2O, 6; ZnSO47H2O, 1.8; MnCl24H2O, 2.5; and CoCl2, 3.6. The initial pH of the medium is 5.5.

In this work, the cultivation of *Trichoderma* strain from lignocellulosic substrate is considered. Hence, the reactor is maintained with temperature of around 30 °C, pH of 5.5 and Dissolved Oxygen (DO) above 35% by varying the agitation rate upto 800 rpm to attain better growth of microbe. During batch/Fed-batch, the variation in pH, agitation speed and substrate concentration with respect to time are recorded.

3. Development of soft sensor

3.1. Single ANN model development

Model identification with input–output measurements is generally used to predict the system behavior without a deep mathematical knowledge (Ljung, 2001; Linder and Enqvist, 2017; Ang et al., 2011). For closer approximations of actual process, a NARX model is generally employed. (Chetouani, 2007). The NARX is a recurrent dynamic

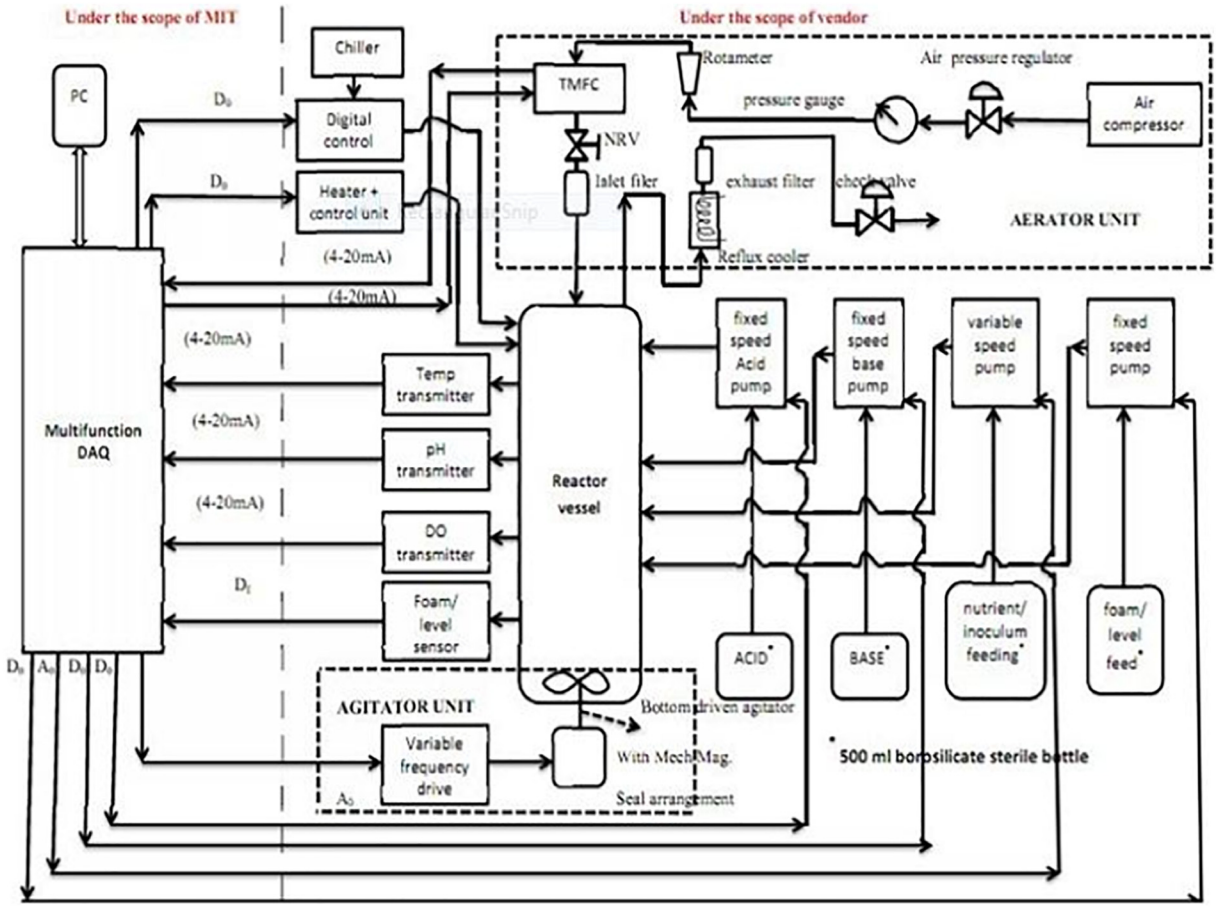

Fig. 2. P&ID of Bioreactor laboratory set-up.

network, with feedback connections enclosing several layers of the network. The defining equation for the NARX model is given in Eq. (1).

$$y(t) = f(y(t-1), y(t-2), y(t-3), \ldots..y(t-ny), u(t-1) \ldots u(t-nu)) + e(t) \quad (1)$$

Where $y(t)$ and $u(t)$ are output and input signal, 'f' is a nonlinear function, ny and nu are the output and input delays of nonlinear model and $e(t)$ is the error term. The next value of the dependent output signal is regressed on previous values of the output signal and previous values of an independent (exogenous) input signal (Ljung, 1987). The NARX dynamic neural network (DNN) used as a soft sensor is represented in Fig. 3.

The successful estimation of process state by soft sensor greatly depends on input output data set. The input variable should be chosen such that it has a direct or indirect relation with the estimation variable. Won and Yoon-Keun, 2006 proposed that estimation of biomass by ANN

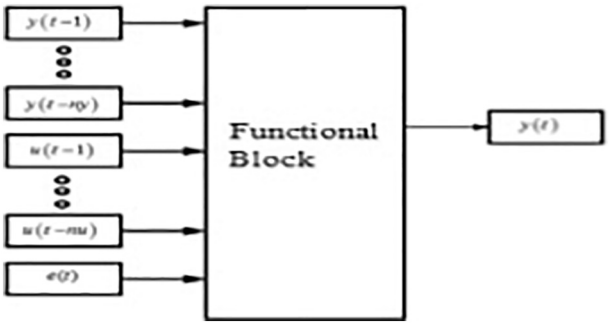

Fig. 3. Structure of NARX dynamic neural network.

can be done from the pH control signal. As, the pH value, agitation speed and substrate concentration inside the bioreactor vessel influence the microbial cell metabolism, it is directly related to the biomass concentration. In this section, a single ANN model is developed for estimation of biomass concentration using the dataset of pH, agitation speed and substrate concentration values starting from the time of inoculation till the end of fed batch process. The experimental data is divided into training, testing and validation in the ratio 70:15:15. The inputs for the NARX model are present values of pH, substrate concentration (S), agitation speed and previous sampling instant biomass concentration, X(k-1) and the output is the estimated biomass concentration, X(k) as shown in Fig. 4.

To obtain best performance from single ANN model, two hidden layers are used and the numbers of hidden neurons in each layer are chosen based on Mean Square Error (MSE). ANN parameters used in the single ANN model development are listed in Table 1.

3.2. Proposed MANN model development

In order to capture the process dynamics completely, a multiphase neural network (MANN) is developed using NARX structure and the simulation is carried out for different phases. The inputs for the NARX model at each phase are the time dependent changes of pH, agitation speed, substrate concentration and the output is the prediction of biomass concentration. The MANN model design emphasis on effective learning of nonlinear relationships between input and output data. The experimental data is divided into training, testing and validation in the ratio 70:15:15. ANN parameters used in the multiphase model development are listed in Table 2. Neural network with varying number of hidden neurons has been trained and Mean Squared Error (MSE)

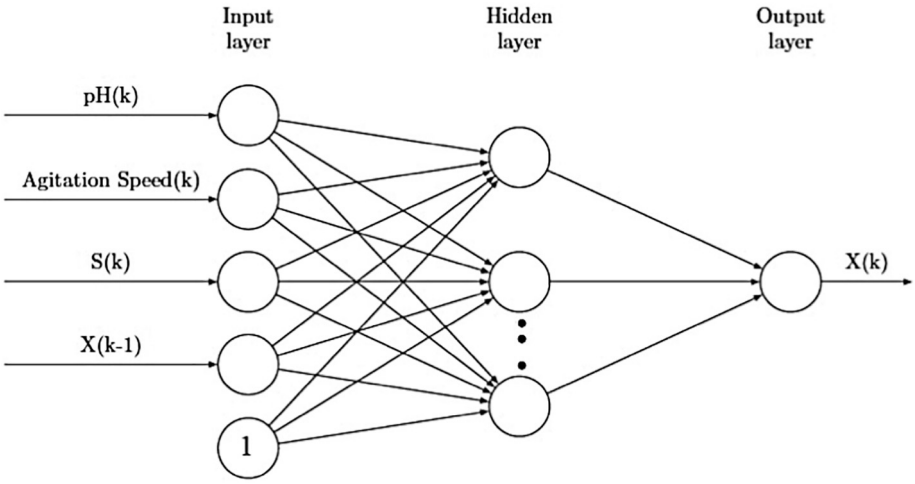

Fig. 4. Single ANN NARX model with various layers.

Table 1
ANN Parameters for single ANN model development.

Parameters	Value
Architecture	Dynamic neural network(NARX)
Training Algorithm	Levenberg-Marquardt algorithm
Number of iterations	1000
Number of hidden layer	2
Number of hidden neurons in first layer	18
Number of hidden neurons in second layer	10
Bias	1
Activation function (hidden layer)	Sigmoid
Activation function (Output)	Linear
Performance Evaluation	Mean Square Error

Table 2
ANN Parameters for MANN model development.

Parameters	Type/Value in Lag, Log and Stationary Phases
Architecture	DNN (NARX)
Training Algorithm	Levenberg-Marquardt algorithm
Number of iterations	1000
Number of hidden layer	1
Number of hidden neurons	15,19,20
Bias	1
Activation function (hidden layer)	Sigmoid
Activation function (Output)	Linear
Performance Evaluation	Mean Square Error

between the training set and neural network is calculated. Network with less MSE has been selected to find optimal hidden neurons. The timing for the switch is based on real time experimental data obtained via ABER capacitance probe. The developed MANN model using NARX neural network structure is represented as shown in Fig. 5.

In Fig. 5, pH(k) represents the present pH value, Agitation speed (k) represents the present agitation speed, S(k) represents the present substrate concentration and X_k and X_{k-1} represents the present and past values of biomass concentration. A switching program enables the selection of appropriate NARX network automatically based on time of operation (T_k) and estimation output of biomass concentration is displayed. Though the proposed MANN model is developed using *Trichoderma reesei* batch cultivation data, it can be used for other strains also. Each of the MANN model inputs is multiplied by a connection weight. Based on the strain cultivated, the weights are updated during training of the model. The products are summed, fed to a transfer function (activation function) to generate a result, and then sent as output.

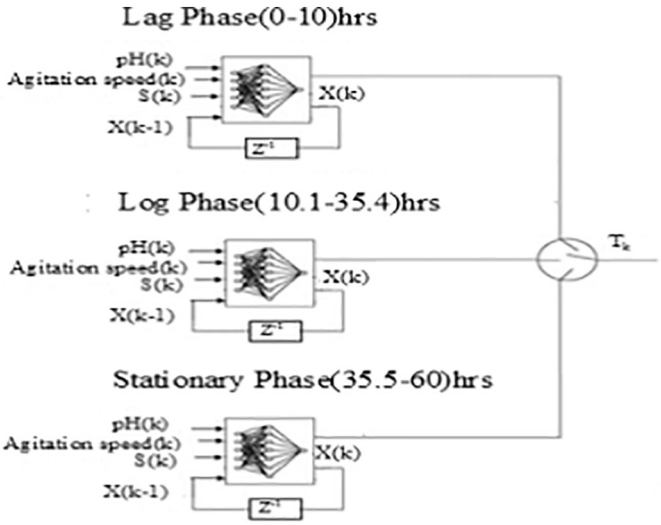

Fig. 5. Proposed MANN model with switching structure.

4. Experimental validation

The capacitance probe has found wide application in the measurement of biomass for the past two decades. The annular dielectric probe when inserted into the bioreactor, gives a capacitance value that can be directly related to the concentration of biomass. The probe is useful particularly during fungal biomass cultivation due to the nonexistence of accurate offline measurements (Carvell et al., 2015). As the probe measures only the viable cells excluding the dead cells and insoluble substrates, it is an optimal choice in our case for process validation. The probe generates a dielectric spectrum at 2 different frequencies given, based on cell size, morphology etc. (Kiviharju et al., 2008; Markx and Davey, 1999; Davey and Kell, 1998). Typical realistic biomass measurement software used with the capacitance probe connected to the laboratory bioreactor is shown in Fig. 6.

In this work, the capacitance probe is utilized in microbial mode and the biomass is measured at frequencies 0.6 MHz and 15 MHz. The 15 MHz reading is used as a form of auto zero and subtracted from the 0.6 MHz. Therefore, the capacitance of background matter is automatically subtracted from the signal. A resolution of 0.1 pF/cm on the instrument typically represents 106 Cells/ml, or 0.5 g per litre. The real-time experimental data is plotted as shown in Fig. 7.

The dynamic NARX networks are trained with different sets of input output data. The response of single NARX neural networks to test inputs

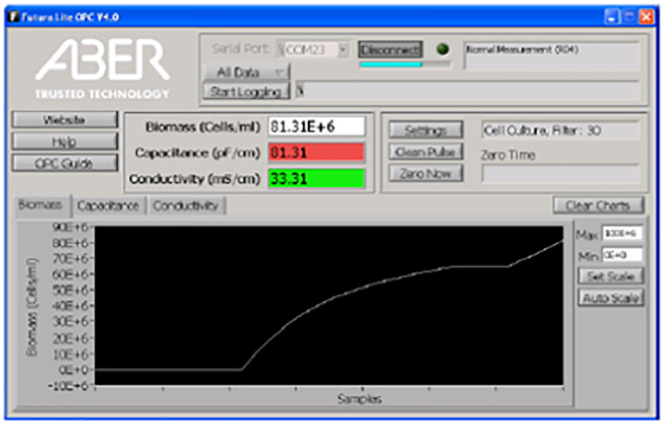

Fig. 6. Data logging software used with capacitance probe.

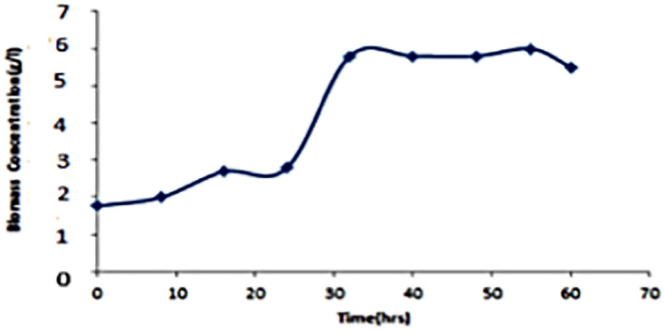

Fig. 7. Real time data of biomass concentration monitored through capacitance probe.

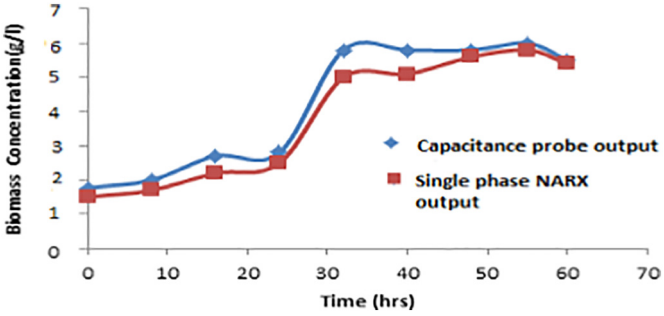

Fig. 8. Single ANN model output in comparison with real time biomass concentration.

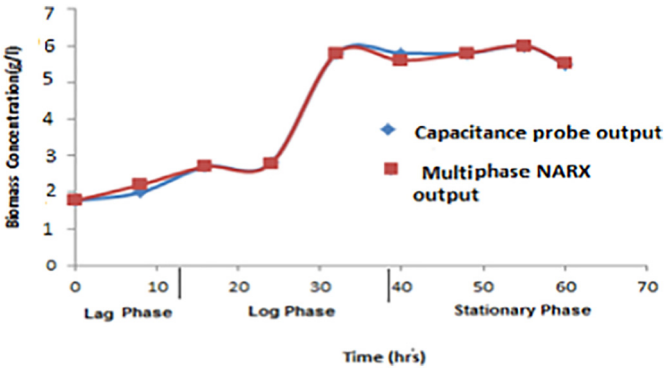

Fig. 9. MANN model output in comparison with real time biomass concentration.

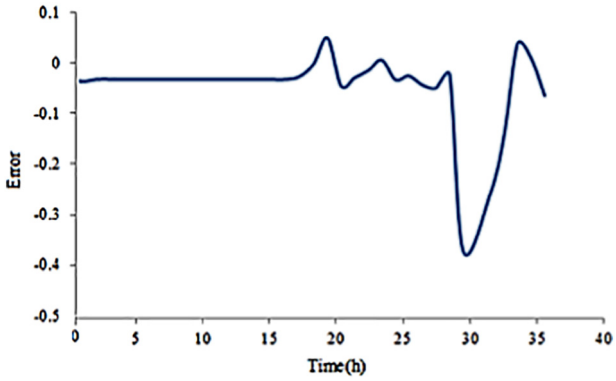

Fig. 10. Error plot for the single ANN.

is shown in Fig. 8 and MANN model to the test inputs to estimate the biomass concentration at different phases is shown in Fig. 9.

From Figs. 8 and 9, it is inferred that the trained dynamic NARX networks can be used in the place of biosensor. The error response for single NARX network is shown in Fig. 10. The Multiphase NARX network predicts the biomass concentration well with less deviation and is shown in Fig. 11. NARX network incorporates time delay effectively and results in better prediction of biomass concentration values based on time series feedback as depicted in Fig. 12 and Fig. 13 respectively.

The performance of NARX networks are analyzed based on the performance criteria, Root Mean Square Error (RMSE) and coefficient of determination (R^2). RMSE is calculated by the Eq. (2).

$$\mathrm{RMSE} = \sqrt{\frac{1}{N}(y_i - t_i)^2} \tag{2}$$

where N is the length of data, yi is the predict value and ti is the target value (Rafsanjani and Samareh, 2016). The comparison of performance metrics for single ANN and Multi phase ANN (MANN) are shown in Table 3. The correlation graphs for single ANN model and proposed MANN model are shown in Figs. 14 and 15 respectively.

It is observed that the proposed Multiphase ANN model (MANN) has a low value of RMSE, a very high value of R^2 and good correlation to real time probe data, which confirms that this dynamic neural network soft sensor performs well in the estimation of biomass concentration.

5. Conclusions

Single ANN and Multiphase ANN (MANN) models are developed using NARX network for the estimation of biomass concentration during fed-batch cultivation of *Trichoderma* in the presence of insoluble substrates. In the proposed MANN model, individual NARX model is developed for different phases within a batch of the process. Both single

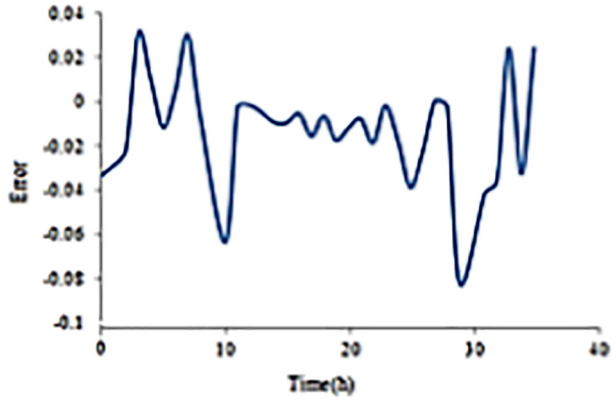

Fig. 11. Error plot for the Multiphase ANN.

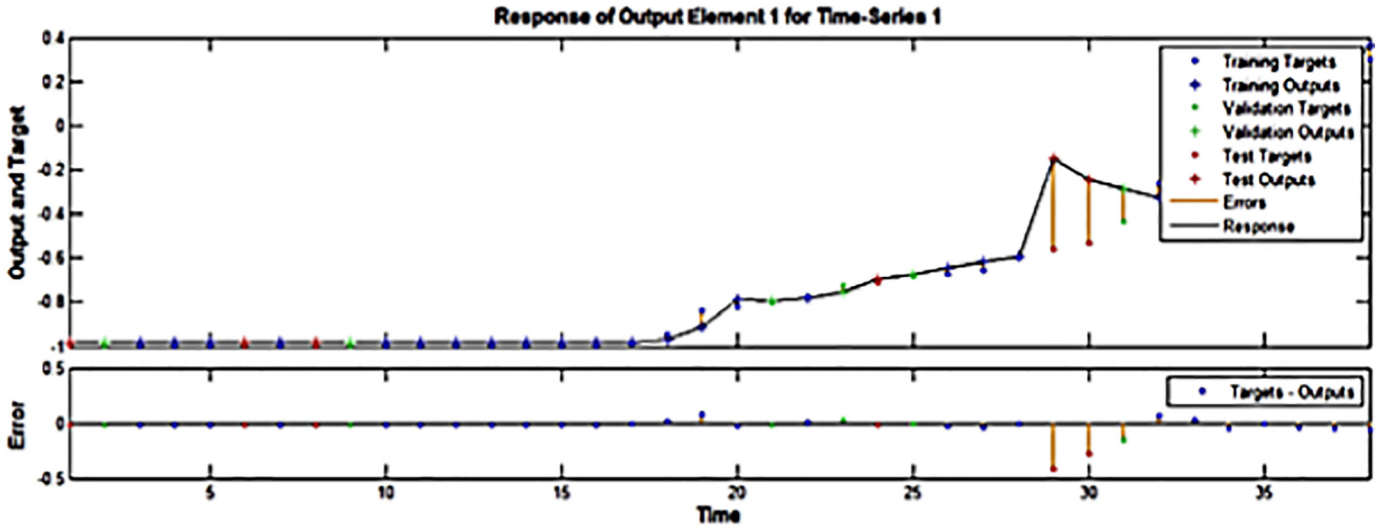

Fig. 12. Time series response of single NARX network.

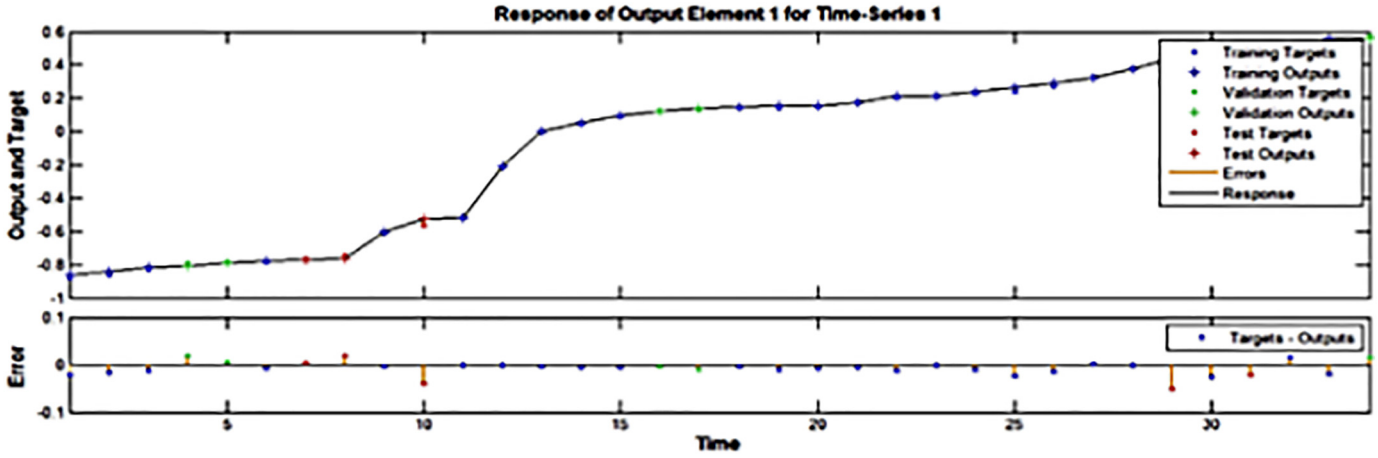

Fig. 13. Time series response of Multi phase NARX network.

Table 3
Performance metrics of single and Multiphase ANN model.

Type of NARX network	RMSE	R^2
Single ANN model	0.01	0.8789
Multiphase ANN (MANN) model	$1.00e^{-5}$	0.9912

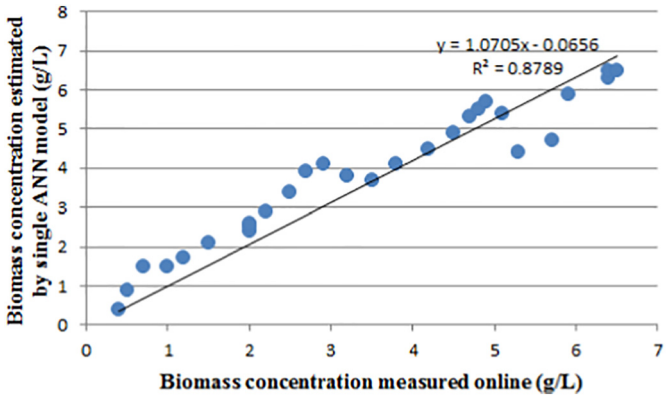

Fig. 14. Correlation between biomass concentrations estimated by the single ANN model and real-time probe data.

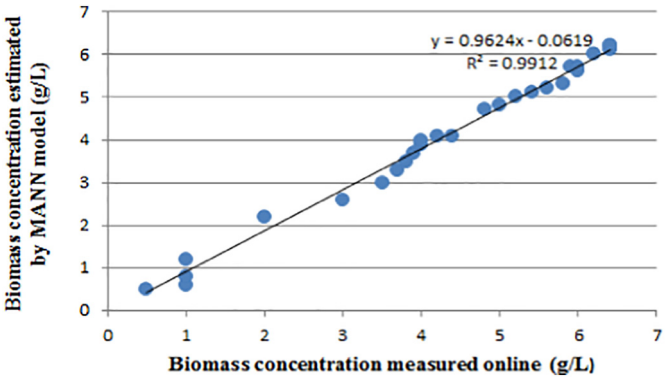

Fig. 15. Correlation between biomass concentrations estimated by the MANN model and real-time probe data.

and MANN models are trained using real time experimental data obtained from the capacitance probe. The results show that the responses of designed MANN model reproduce the underlying nonlinear characteristics of the fed-batch bioreactor accurately. The multiphase method provides a reliable model for dynamic analysis, which is of practical significance particularly in the presence of insoluble substrates and can be effectively used for estimation of biomass concentration.

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