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Research note

Application of Artificial Neural Network in Deoxygenation of Water by Glucoseoxidase Immobilized in Calcium Alginate-MnO_2 Composite

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ABSTRACT

A three-layer artificial neural network (ANN) model was developed to predict the remained DO (deoxygenation) in water after DO removal with an enzymatic granular biocatalyst (GB) based on the experimental data obtained in a laboratory stirring batch reactor study. In enzymatic method for removing dissolved oxygen of water, glucose oxidase accelerates the reaction between O_2 and glucose. Therefore, oxygen is removed. The effects of operational parameters, such as initial pH, initial glucose concentration, and temperature, on DO removal were investigated. On the basis of batch reactor test results, the optimum value of operating temperature, glucose concentration, and pH were found to be 30 ^oC, 80 mM, and 7, respectively. The less dissolved oxygen in water there is, the more prevention of corrosion will occur. In optimum operating condition, the concentration of DO reached zero. After back-propagation training, the ANN model was able to predict the remaining DO with a tangent sigmoid function (tansig) at hidden layer with 7 neurons and a linear transfer function (purelin) at the output layer. The linear regression between the network outputs and the corresponding target was proven to be satisfactory with a correlation coefficient of 0.995 for three model variables used in this study.

1. Introduction

Water deoxygenation, as a necessary step for preventing problems such as corrosion caused by dissolved oxygen (DO), is a requirement in many industries [1-3]. Methods for deoxygenation of water that have been used till now can be classified in two main groups, i.e., physical and chemical methods. Physical methods include thermal degassing, vacuum degassing, nitrogen bubbling and

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deoxygenating through membrane modules, while chemical methods are involved with utilization of chemical agents such as hydrazine, sodium sulfite, and hydrogen [4-6]. Electrochemical method is also proposed for deoxygenation of water [7]. For this objective, a biological method has been proposed recently. In this method, DO removal is conducted by oxidation of glucose as a biological material by DO with a low reaction rate [8].

Oxidoreductases (Enzyme Commission [EC] primary class 1) catalyze the oxidation reaction of one chemical species (a reducing agent or electron donor) with the concurrent reduction of another (an oxidizing agent or electron acceptor) in the form $A^{-} + B \rightarrow A + B^{-}$ [9].

Oxidoreductases can act in a wide range of both organic substrates including alcohols, amines and ketones and inorganic substrates including small anions such as sulfite and metals such as mercury[9]. Glucose oxidase(GOD) is one of the categories that has attracted attention because of its robustness and stability [10]. GOD is a flavoprotein which catalyses the oxidation reaction of β-D-glucose to D-glucono- δ-lactone and H_2O_2 using molecular oxygen as an electron acceptor[11]. This reaction can be divided into a reductive and an oxidative step. In the reductive half reaction, GOD catalyzes the oxidation of β-D -glucose to D -glucono-δ-lactone, which is non-enzymatically hydrolyzed to gluconic acid [11].

In the oxidative half reaction, the reduced GOD is reoxidized by oxygen to yield H_2O_2. It catalyzes glucose and O_2 reaction into H_2O_2 and gluconic acid [11]. Because of high cost of enzyme production, its stability and reusability in a process is an obligation that can be achieved by enzyme immobilization [12]. Immobilization of an enzyme on a stable and insoluble support leads to heterogeneous processes, which are attractive on an industrial scale [8]. Common techniques of immobilization include covalent binding, entrapment, adsorption ionic binding, affinity binding, and cross linking [13]. Although adsorption method, caused by interaction between enzyme and support, is limited because of tendency of enzyme to desorb from the support, its simplicity and reversibility have led to its extensively utilization over the recent few decades [12]. Various supports, such as Al_2O_3, SiO_3, and gold(Au) nano-particles, have been used for GOD immobilization [14-21]; mesoporous MnO_2 particles have been used successfully for GOD immobilization and DO removal[8]. The ability of MnO_2 particles for hydrogen peroxide decomposition has made it considerably significant for enzymatic deoxygenation.

In this paper, biological deoxygenation of water with granular biocatalysts (GB), which are prepared by immobilization of GOD on MnO_2 mesoporous particles, and utilization of calcium alginate as a binder will be introduced. Experiments are done for studying influencing parameters behavior on GB activity, and the optimum level of each parameter was determined. Artificial neural network (ANN) was also used for modeling obtained data and predicting removal of DO by GB in several conditions. Inspired by psychology of human brain and nervous system, ANN has neurons, synaptic weights, and activation function and has mechanism of self-learning, which can be taught to learn correlative patterns between variables and can be used subsequently to predict output from new inputs and is able to make correlation between memorization and information [22-

25]. These features have led ANN to be utilized in so many fields such as process control, system classification, and communication [26]. The most significant characteristic of modeling based on ANNs is that the material description of involved process is not required [27, 28]. When data of a complex system enter an ANN, it can create a suitable model; even the data are incomplete [23]. Correlation of a set of input data to a set of output data is the main purpose of ANN that is conducted by training with input and output data [25, 26].

On the basis of obtained experimental data, a three-layer ANN model was proposed using a back propagation algorithm to predict DO removal by GB and optimize the reaction condition as a novelty. In addition, an optimization study of determining the optimal network structure was conducted. Finally, outputs obtained from the ANN modeling were compared with experimental data; the advantages and further developments were discussed, too.

2. Theoretical Framework

2.1. Artificial neural network

ANNs as predictive computational models, which simulate neural system of human brain, have attracted great interest during the last decades. In this work, multilayer feed-forward ANN with one hidden layer has been used. All the neurons are connected with different weights (w_{ij}). For each neuron, the weighted input values are summed and a threshold value (b_j) is added. Then, a non-linear transfer function (f()) is applied to this linear combination of inputs to produce the output neuron (O_j) (see Figure 1):

$$O_j = f(\sum w_{ij}.x_i + b_l) \qquad (1)$$

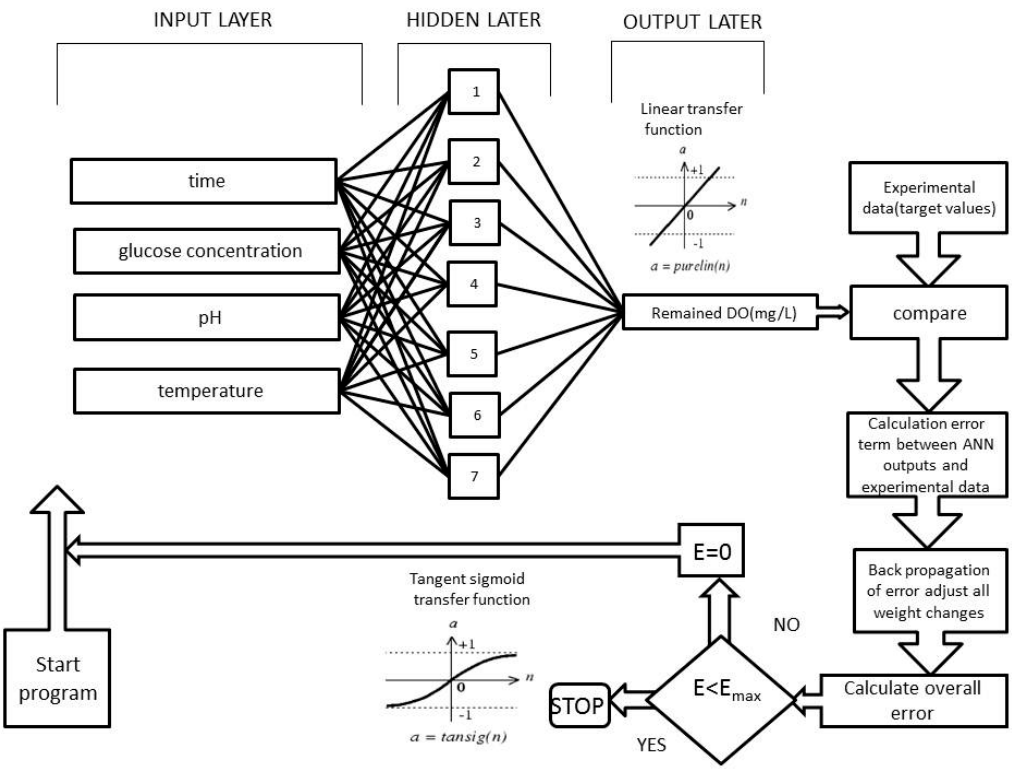

Figure 1. Optimal ANN structure with a flowchart of the BP algorithm for the prediction of the remaining DO.

The output of one neuron provides the input to neurons in the next layer; for all data sets, sigmoid transfer in the hidden layer and a linear transfer function in the output node were used. The ANN was trained using back propagation algorithm to adjust its weights to present a training dataset and minimize the network performance function, which is commonly mean square error (MSE) that means the average squared error between the network output values and target values for these outputs, shown by Eq. 2:

$$E = \frac{1}{n}\sum_{1}^{n} t_i - t_o \tag{2}$$

where n is the number of training data, and t_i and t_o are target output and calculated output for training i, respectively. Once the training phase of the model has been successfully accomplished, the performance of the trained model has to be validated by an independent testing set. All calculations were carried out with Matlab mathematical software with ANN toolbox. Time, pH, glucose concentration, and temperature were used as inputs of ANN. Obtained experimental data were randomly split between training, validation, and test sets. About 70 percent of data were used for training, 15 percent for validation, and 15 percent for testing. All samples were normalized in the 0-1 range; therefore, all the data (xi) are converted to normalized values (x_{norm}) as follows [27]:

$$x_{norm} = \frac{x_i - x_{\min}}{x_{\max} - x_{\min}} \tag{3}$$

There is no universal method to determine the number of hidden neurons; a trial and error process was applied to identify optimum network.

In this paper, the ANN toolbox in MATLAB was used to compute predicting process. For this purpose, a network trained by the Levenberg-Marquardt back-propagation algorithm was selected. LMBP was proved to be the most efficient training algorithm among all [23-29]. A nonlinear hyperbolic sigmoid and a linear activation functions were used in the hidden and output layers, respectively.

3. Experimental

3.1. Materials

Glucoseoxidase (EC 1.1.3.4, from Aspergillus niger, 800 U/g) and sodium alginate were obtained from Sigma Aldrich. $KMnO_4$, $MnCl_2.2H_2O$, β-D-glucose, calcium chloride, and CCl_4 were purchased from Merck.

3.2. Preparation and characterization of mesoporous MnO_2 particle

First, 30 ml of 1M $MnCl_2.2H_2O$ solution was added into 80 ml CCl_4; after separating phases and achieving obvious CCL_4/H_2O interface, 40 ml of 0.5 M $KMnO_4$ solution reached the interface drop by drop. Then, the whole reaction system stayed in static state for 48 hours. During this time, the rest of the reactants reacted together slowly and were converted to brown MnO_2. After collection of MnO_2, it was washed with deionized water and pure ethanol for three times in order to eliminate impurities. Finally, MnO_2 was heated at 150^{o}C in air for 12 hours [8].

FT-IR, SEM, and BET analyses proved the production of suitable MnO_2 particles for GOD adsorption [8].

3.3. Preparation of GB

Then, 1 g of prepared MnO_2 was dispersed in 20 mL of 80 U/mL GOx solution in distilled water and incubated at 30 ^{o}C (120 rpm). Immobilized enzyme was put into 1 % w/w sodium alginate solution and agitated for 10 min to get uniform suspension of immobilized particles in sodium alginate.

To achieve a suitable biocatalyst for bioreactor usage, the supplied sodium alginate was put into calcium chloride 2 % w/w solution drop by drop through silicon tube to get granular biocatalysts (GB)[30].

3.4. Assay of GOx and GB activity

The measurement of free GOD and granular biocatalyst activity was carried out by monitoring DO concentration decrease in 20 mM glucose solution in an exactly filled stirred batch reactor at 100 rpm and during 1 min, using DO meter sensor (Extech, DO600-k, USA). One unit of free enzyme activity is the amount of enzyme which can catalyze 1 µmol O_2 per min at 25 ^oC, while, for granular biocatalyst, it is the amount that can catalyze 0.5 µmol O_2 in the same condition [8].

4. Results and discussion

4.1. Mechanism of enzymatic deoxygenation

Consumption of DO during oxidation of glucose has been defined as the enzymatic deoxygenation. Glucose oxidase (GOD), as an oxidoreductase enzyme, is used widely to catalyze the oxidation of β-D-glucose by transforming electrons to O_2 and generating H_2O_2 and Gluconic-acid (Eq. 4). β-D-glucose is oxidized to δ-glucono-1-5-lactone which is hydrolyzed to D-Gluconic acid simultaneously [11].

$$\beta - D - glucose + O_2 \rightarrow D - gluconic\,acid + H_2O_2 \tag{4}$$

The presence of hydrogen peroxide is destructive, causes more corrosion, and accelerates this phenomenon. Hence, degradation of H_2O_2 is done by MnO_2 (Eq. 5).

$$H_2O_2 \xrightarrow{catalyst} \frac{1}{2} O_2 + H_2O \tag{5}$$

In other words, MnO_2 has two roles in this study: as enzyme support and H_2O_2 scavenger. D-Gluconic acid, as another product of the above-mentioned reaction, has a passive role [31].

4.2. Effect of operating parameters on the GB activity

In biological DO removal, operating parameters including temperature, pH, and concentration of glucose as substrate have significant effects. Commonly, operating temperature has to be chosen in a range in which denaturation of enzymes does not occur. Thus, the optimal level of temperature was determined (specified) by a set of experiments. As it is shown in Table 1, 30 ℃ is achieved to be the so-called optimum temperature, which is in agreement with literature [11].

Table 1
Effect of temperature on deoxygenation rate in the batch reactor (time=10min).

T (^oC)	Remained DO (mg/L)	Reaction rate (mg/L.min)
20	0.58	0.84
30	0.03	0.80
40	1.11	0.63
50	1.25	0.53

The other factor influencing GB activity in this DO removal process is the concentration of glucose. Therefore, a set of experiments was done in a batch reactor. The results are shown in Table 2; 80 mM glucose led to the highest activity of GB and the most effective DO removal. As for the reasons of this observation, low diffusion rate of substrate and mass transfer limitations at lower concentrations of glucose or production of inhibitor at higher concentrations could be proposed. The pH of the surrounding reaction as another influencing factor was investigated in experiments. It was proved that, in pH of 7, GB had the highest activity and could remove more DO. The results are shown in Table 3.

Table 2
Effect of glucose concentration on remaining DO(mg/L) at various reaction times while deoxygenation is conducted in T=30 ℃ and pH of 7.

Time (min)	Glucose conc. (mM)							
	0.5	4	20	40	65.5	80	100	120
0	9	9	9	9	9	9	9	9
1	8.5	8.43	7.61	7.4	7.23	6.73	6.62	6.4
2	8.49	8.19	6.67	6.64	6.1	5.26	5.69	5.37
3	8.47	7.9	5.8	5.53	4.85	3.87	4.7	4.6
4	8.43	7.58	5.04	4.95	3.6	2.92	3.95	3.95
5	8.39	7.28	4.39	4.03	2.7	2.07	3.32	3.38
6	8.33	6.94	3.74	3.28	2.06	1.44	2.8	2.93
7	8.25	6.61	3.23	2.83	1.45	1.01	2.36	2.5
8	8.18	6.3	2.72	2.36	1.02	0.7	1.92	2.13
9	8.09	5.98	2.27	1.97	0.78	0.51	1.64	1.8
10	7.91	5.7	1.9	1.63	0.58	0.43	1.36	1.55

Table 3
Effect of pH on remaining Do(mg/L) at various reaction times while deoxygenation is conducted in T=30 ℃ and [G]=80 mM.

Time (min)	pH				
	4	5	7	9	10
0	8.80	8.80	8.80	8.80	8.80
1	8.60	7.87	7.23	7.63	8.11
2	8.41	6.94	6.1	6.87	7.68
3	8.28	5.96	4.85	6.16	7.33
4	8.05	5.03	3.60	5.61	7.00
5	7.79	4.13	2.7	5.09	6.68
6	7.45	3.33	2.06	4.64	6.43
7	7.01	2.63	1.45	4.20	6.19
8	6.54	2.02	1.02	3.78	5.88
9	6.23	1.59	0.78	3.45	5.66
10	5.58	1.28	0.58	3.15	5.45

4.3. ANN modelling

The input values of feed-forward neural network include temperature (over 20-50 ^{o}C range), duration of deoxygenation (over 0-10 min), concentration of glucose (over 0.5-120 mM range), and pH (over 4-10 range). The remaining DO concentration was defined as experimental response or output target, which was between 0-9 mg/L. After normalization of all input data in the range of 0-1, they were introduced into ANN. The dataset was divided into three subsets: training, cross-validation, and testing including 70 %, 15 %, and 15 % of the all data, respectively. Each subset has certain effect on the training procedure of the network. The training subset is used to generate errors during the training process and, then, update the weights. The cross-validation subset ensures that the network does not become over-trained. The training stops when the mean square error (MSE) in the cross-validation set starts to increase. The test subset, which remains unused during the training, is utilized after the training to examine the generalization capabilities of the network and to compare with the other errors (training error and cross-validation error) [24]. Then, ANN topology, including specification of number of neurons in hidden layer, was optimized according to minimum prediction error of the neural network. Therefore, to determine the optimum number of hidden nodes, different topologies were examined in which the number of neurons varied from 2 to 10. Because of the effect of weights and biased initial guessing on network output, it is advisable to generate several neural networks meeting the criteria. Therefore, each topology is repeated 5 times. Figure 2 illustrates the MSE as a performance function of network (Eq. 2) versus number of neurons in hidden layer.

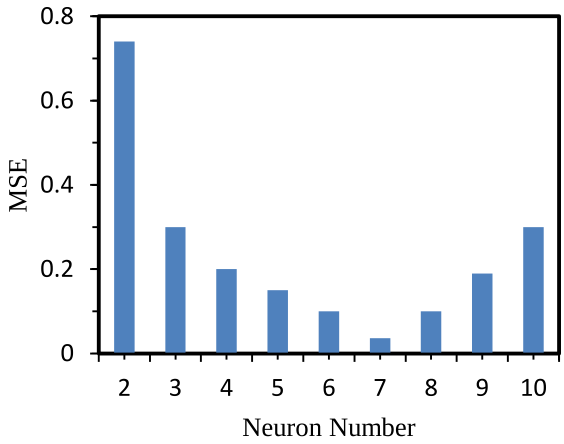

Figure 2. Variation of MSE versus number of neurons in hidden layer.

When there are 7 neurons in the hidden layer, it is clear that the minimum network square error occurs. Therefore, a three-layer feed-forward back-propagation neural network with 7 nodes in hidden layer was generated. The input layer weights (ILW), input layer biases (ILB), hidden layer weights (HLW), and hidden layer biases (HLB) of obtained optimum model are given in Tables 4 and 5, respectively.

Table 4
Matrix of weights between input and hidden layers in optimized ANN model.

Neuron	N_1	N_2	N_3	N_4	N_5	N_6	N_7
Weight of connections from time	0.192	-0.006	-0.759	0.163	0.424	-0.219	-0.325
Weight of connections from glucose concentration	-1.090	0.319	-0.127	-0.588	0.491	1.942	0.750
Weight of connections from pH	-0.974	-0.114	-0.290	0.002	1.046	-1.670	1.302
Weight of connections from T	0.544	1.453	0.164	0.215	-0.227	-0.627	0.001
Bias in hidden layer	0.508	-2.959	-0.264	-0.309	0.521	0.298	-1.639

Table 5
Matrix of weights between hidden and output layers.

Neuron	N_1	N_2	N_3	N_4	N_5	N_6	N_7
Weights	-7.375	-7.119	-1.798	18.246	-7.585	4.871	5.945
Bias in output layer	7.099						

The obtained network was evaluated by comparing it with an independent experimental data set (test set). Figure 3 indicates the experimental results (test set) versus predicted outputs in corresponding points. As is clear, the dataset is distributed around X=Y line in a narrow area, and R^2 (correlation coefficient) is 0.995. Therefore, there is excellent agreement between the neural network and experimental data.

Hence, this model could be quite accurate and reliable in predicting the amount of DO removal by the enzymatic process. The effects of simultaneous variation of temperature-time, pH-time, and glucose concentration-time are shown in Figures 4-6. In each figure, two variations out of four are constant. The shown contour maps determine the point at which DO removal is in optimum state. These maps confirm the reliability of the obtained neural network.

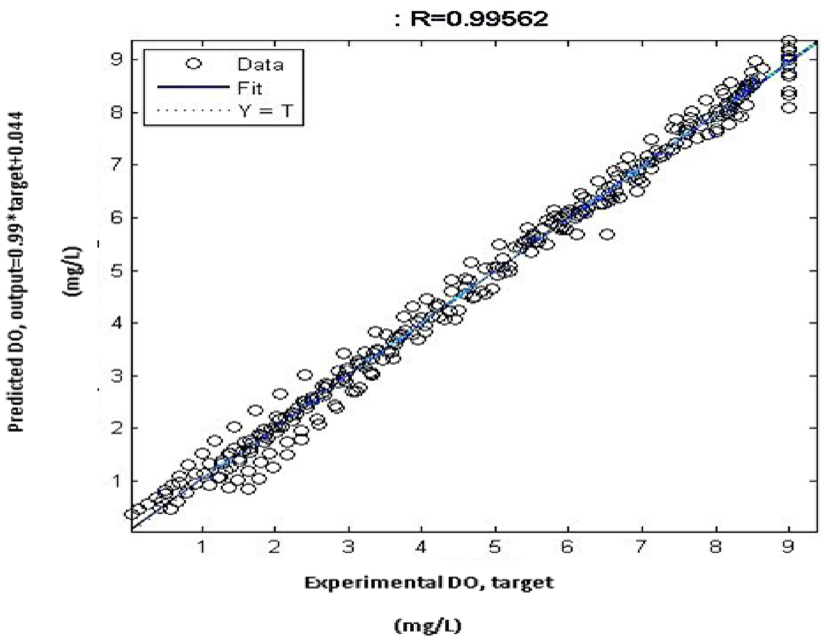

Figure 3. Predicted remained DO(mg/L) versus experimental data.

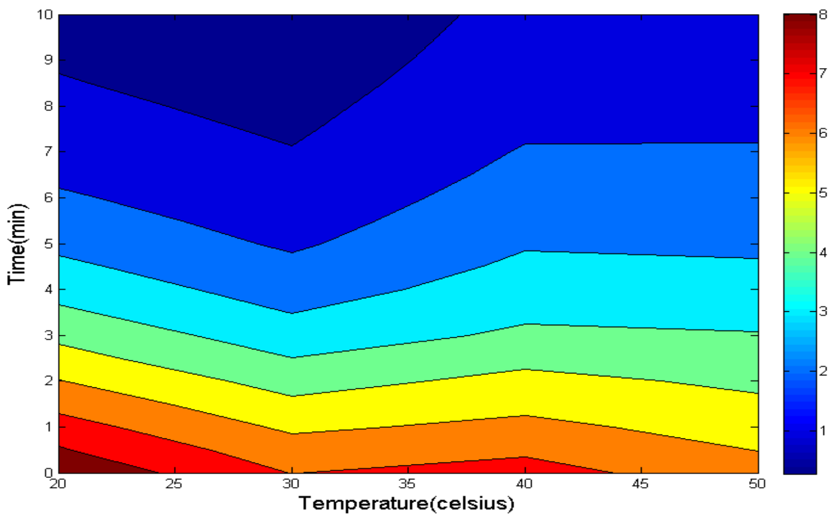

Figure 4. Impact of temperature and time on remaining DO(mg/L) estimated with ANN model.

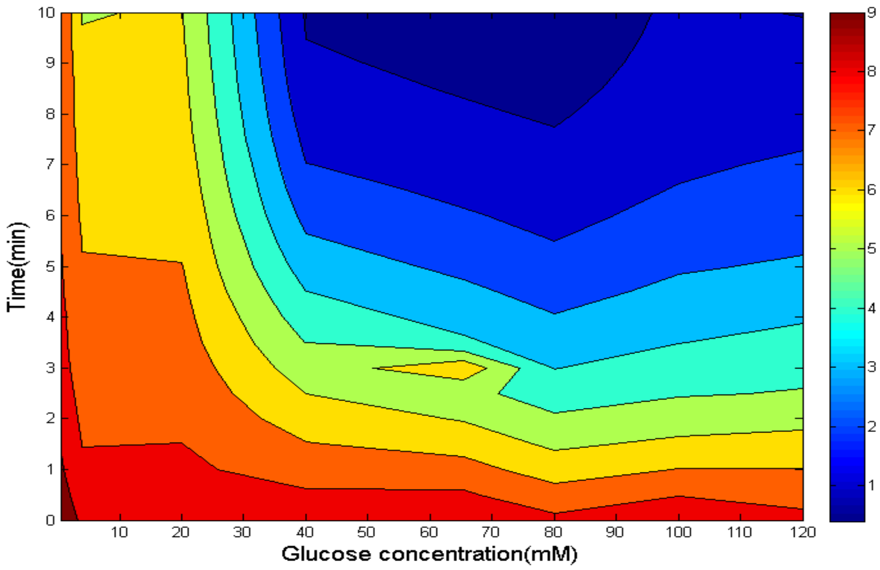

Figure 5. Impact of glucose concentration and time on remaining DO(mg/L) estimated with ANN model.

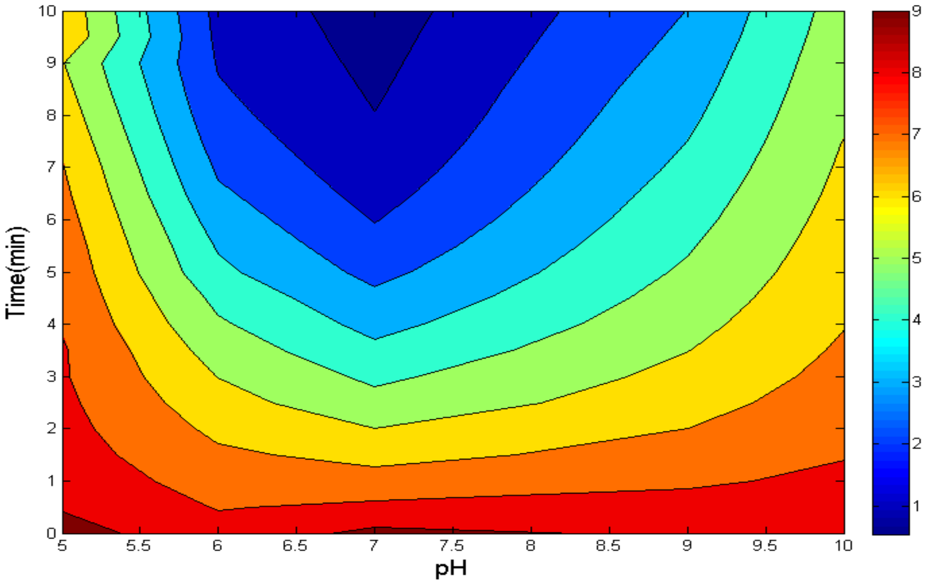

Figure 6. Impact of pH and time on remaining DO(mg/L) estimated with ANN model.

5. Conclusions

Enzymatic granular biocatalyst (GB) based on Glucoseoxidase/calcium alginate was synthesized successfully and was used for dissolved oxygen removal of water. The effect of various operational parameters on dissolved oxygen removal was investigated and optimized. According to the dissolved oxygen removal experiments in stirring batch reactor, optimal operating temperature, initial glucose concentration, and initial pH were determined to be 30 ^{o}C, 80 Mm, and 7, respectively, causing the oxygen dissolved in water to become zero. Based on batch dissolved oxygen removal tests, an important purpose was to obtain an artificial neural network model as an applicable mathematical model to make a reliable prediction about dissolved oxygen removal with granular biocatalyst. A three-layer artificial neural network with a tangent sigmoid transfer function (tansig) at a hidden layer and a linear transfer function (purelin) at output layer was proposed to predict the remaining dissolved oxygen after removal. Back-propagation algorithm was used and the optimal neuron number of 7 was determined at hidden layer with MSE of 0.036. Accordingly, considering seven neurons in the hidden layer, the difference of modelled dissolved oxygen from those achieved in experiments reaches the minimum amount, which is 0.036.

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Nomenclature

Abbreviation

DO	dissolved oxygen [ppm].
GOD	glucose oxidase.
ANN	artificial neural network.
GB	granular biocatalyst.
MSE	mean square error.
ILW	input layer weights.
ILB	input layer bias.
HLW	hidden layer weight.
HLB	hidden layer bias.
FT-IR	Fourier transform spectroscopy.
BET	Brunauer Emmett-teller.
SEM	scanning electron microscopy.

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