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TECHNICAL NOTE

Optimizing of Iron Bioleaching from a Contaminated Kaolin Clay by the Use of Artificial Neural Network

M. Pazouki [a,*], Y. Ganjkhanlou [a], A. A. Tofigh [a], M.R. Hosseini [b], E. Aghaie [b], M. Ranjbar [b]

[a] *Materials and Energy Research Center, Karaj, Iran*
[b] *Department of Mining Engineering, Engineering Faculty, Shahid Bahonar University, Kerman, Iran*

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A B S T R A C T

In this research, bioleaching of Iron from highly contaminated kaolin sample with *Aspergillus niger* was optimized. In order to study the effect of initial pH, sucrose and spore concentration on Iron, oxalic and citric acid concentration, more than twenty experiments were performed. The resulted data were utilized to train, validate and test the two layer artificial neural network (ANN). In order to minimize the over fitting, Bayesian regularization and early stopping methods with back propagation technique were utilized as training algorithm of ANN. Good validation for prediction of Iron removal percentage was resulted due to the inhibition of over-fitting problems with selection of appropriate ANN topology and training algorithm. The results showed that optimized condition of initial pH, sucrose and spore concentration to achieve high Iron removal (about 65%) should be 6, 60 g/l and 3.5×10^{7} spore/l, respectively.

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1. INTRODUCTION[1]

Iron oxides are class of undesirable impurities in kaolin mineral that decrease the brightness and quality of kaolin. Many methods such as magnetic separation, froth flotation, applications of potent reductants (for instance dithionite and hydrazine), selective flocculation, size separation by hydrocyclones, and leaching were utilized for the removal of iron oxides from kaolin and subsequently improving the clay quality. But, the efficiency of Iron removal in the mentioned methods is low [1]. Bioleaching is one of the most promising methods that were applied for iron removal from the kaolin. In comparison with the aforementioned methods, bioleaching has higher Iron removal efficiency, lesser environmental problems, lower energy and operating cost. In fact, microorganism can accelerate aluminosilicate mineral weathering reactions in direct contact with their surfaces, by producing organic and inorganic acids, creating metal-complex ligands, changing the redox condition or mediating the formation of secondary mineral phases. Several statistical methods have been applied to optimize this process [1, 2].

Artificial neural network (ANN) is an efficient tool to predict various nonlinear relations among experimental parameters, optimization, classification, control, etc. [3-7]. For optimization, the first step involves the design of the network and then the selection of initial weights and biases and finally using the best algorithm to change the weights and biases during the learning process to find the best weights and biases in order to produce desirable outputs from the input pattern [5, 7]. Feed forward ANN with back-propagation training algorithm is one of the most established ANNs for optimization issue [3, 4]. In this method, in every interval, current weights and biases have been utilized for computation of output from the input pattern and in the second step weights and biases are altered with a backward algorithm .

There are many algorithms such as gradient descent, conjugate gradient descent, quasi-Newton method, gradient descent with momentum, resilient back-propagation, variable learning rate back-propagation and Levenberg-Marquardt methods for training of the ANN. In all the algorithms mentioned above, an error function (usually mean square error (MSE) or sum square error (SSE)) is selected and then, the error function is minimized by changing the weights and biases step by step and numerically. Among the above mentioned algorithms, Levenberg-Marquardt method is

*Corresponding author: Email-*mpazouki@merc.ac.ir*

one of the fastest and most efficient algorithms for ANN training. In this method, the approximated Hessian matrix (second derivative) and Jacobean matrix is applied for altering the weights and biases as follows:

$$H=J^TJ,\ g=J^Te,\ X_{k+1}=X_k-[H-\mu I]^{-1}g \tag{1}$$

Here J is the Jacobean matrix containing the first derivatives of the network errors with respect to the weights and biases, X is the weight or bias, g represents the gradient of performance function, e is error (true output-network output), I is Identity Matrix, JT is the transposed matrix of J, H is the Hessian matrix and the µ is a scalar value between 0 and 1 [8-11].

Due to the high capability of ANN for learning the complex function, usually, it could fit very complex and unsmooth functions to the output data which were resulted in over fitting problem. In over fitted ANN, the error on the training set is driven to a very small value, but when new data is presented to the network, the error is large.

The efficient way to avoid over-fitting is to use lots of training data [4, 12]. But this is impossible in some cases due to the increasing time and cost of experiments. Other important methods for improving the network generalization and avoiding over-fitting are Model selection, Jittering, Early stopping, Weight decay, Bayesian learning [13] and Combining networks. In this work, a combination of Bayesian learning and Early-Stopping was used to avoid the over-fitting problem. Bayesian learning resembles to the Levenberg-Marquardt method and differs only in the error function (Eq. (2)) which contains new term that consists of the mean of the sum of squares of the network weights and biases [13]:

$$e_{BR}=\gamma MSE+ (1-\gamma)MSE \tag{2}$$

In the above equation, e_{BR} denotes error function of Bayesian learning algorithm, γ is a parameter known as performance ratio which is calculated from the weight and biases by statistical techniques and MSE is the mean square error. Using this error function will cause the network to have smaller weights and biases, and this will force the network response to be smoother and less likely to overfit.

The aim of this work is to investigate the effect of initial pH, sucrose and spore concentration on the oxalic acid, citric acid and bioleached Iron concentration and also to determine the optimal initial pH, sucrose and spore concentration for the maximum production of oxalic and citric acid, and consequently improving the bioleaching of Iron from kaolin clay.

2. EXPERIMENTAL

2.1. Kaolin sample Kaolin sample with 2.19% Iron contamination and particle size of 90% below 5.74 µm was provided by Mehrkhak Company, Tehran, Iran.

2.2. Microorganisms and culture media The fungus strain were originally isolated from pistachio shell on potato dextrose agar (PDA) and Czapek Dox agar (CZ) by streak method. It was later identified as *Aspergillus niger* according to the method of Klich [14].

A solid media (malt extract, 30 g/l; meat peptone, 3 g/l and agar, 15 g/l, at pH 5.6) was employed for the growth and maintenance of the microorganism at 30 °C. A synthetic media [15] containing NH_4NO_3, 450 mg/l; KH_2PO_4, 100 mg/l; $MgSO_4{\cdot}7H_2O$, 300 mg/l; $FeSO_4{\cdot}7H_2O$, 0.1 mg/l; $ZnSO_4{\cdot}7H_2O$, 0.25 mg/l, and sucrose at five different levels [1] was employed as culture media.

2.3. Bioleaching Of Mineral Samples A 2^3 Central Composite Design with 15 runs, and six replications of the center points was selected to determine the initial pH, sucrose and spore concentration for the maximum production of oxalic and citric acid, and consequently improvement of Iron removal from kaolin clay. Citric and oxalic acids can be produced by *Aspergillus niger* growing on media containing sucrose or glucose and these acids have the capability of Iron complexing and reducing [1]. In addition, another 6 experiments were done to obtain more details about the process. The condition and final results of these extra tests beside the maximum and minimum values for the input parameters are listed in Table 1.

Fungal spores were suspended from a 7-day agar slant in a sterile solution (0.1% Tween80, and 0.9% NaCl) and enumerated by a microscope. Bioleaching experiments were carried out in 500-ml Erlenmeyer flasks containing 100 ml of culture media inoculated at five different concentrations [1], and incubated at 30 °C, and 160 rpm on a rotary shaker. Amount of 3g of kaolin was added to the culture media in the beginning of the cultivation. All experiments were performed in duplicate, and the average of the results reported, were with 2% deviation.

2.4. Methods of Analysis In order to determine the kaolin composition, and specially its Iron contents, XRF analysis was done by ARL 8410 instrument, tube anode: Rh, and 60 kV. Also, to determine the kaolin particle size, particle size analysis was made by Fritsch Particle Sizer “Analysette 22”. Results showed that 90% of the clay particle size was below 5.74 µm.

To register the changes in pH, dissolved Iron, sucrose, and oxalic and citric acid concentrations, sampling was done in determined intervals. Then, the solid phase containing kaolin and biomass was separated from leaching medium by centrifuging at 1000 rpm.

TABLE 1. Conditions of six extra tests beside the maximum and minimum values for input parameters.

Run no.	Spore (spore/l)	Initial pH	Sugar (g/l)	Responses		
				Iron concentration (mg/l)	Citric acid (g/l)	Oxalic acid (g/l)
1	4.00E+08	4.5	50	244.9	4.8	3.6
2	4.00E+08	5	50	250	4.5	3.38
3	5.00E+08	6	50	313.8	5.5	3.38
4	4.50E+08	5	40	301	5.1	3.6
5	3.00E+08	3.5	140	244.9	10.3	7.65
6	3.50E+08	4	120	219.4	9.2	6.53
max	5.00E+08	6.02	150.5	---	---	---
min	1.4E+6	0.98	40	---	---	---

The pH value of the liquor was measured by Metrohm pH meter model 744. The dissolved iron concentration was measured by the *o*-phenanthroline method [16]. To quantify the concentration of the organic acids exerted to the media, pyridine acetic anhydride method [17] was applied to determine the citric acid concentration, and manganometry method [16] to measure the oxalic acid concentration. To determine the total sugar contents, the spent media were hydrolyzed, and analyzed by colorimetry using the Nelson and Somogyi method [18, 19].

2.5. Neural Network Topology The data were gathered in order to get sufficient input data to train, validate and test the ANN and thus investigating the effect of initial pH, sucrose and spore concentration. 60% of data were used for training, 20% for validating the ANN and 20% for testing the trained ANN. Two-layer feed forward ANN was employed for the training and three neurons were used in both hidden and output layer. The input pattern consisted of initial pH, sucrose and spore concentration and the output data consisted of oxalic acid, citric acid, and Iron concentration (Figure 1).

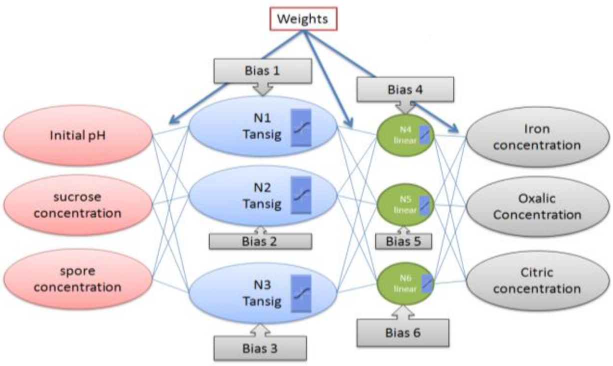

Figure 1. Neural network topology

Tan-sigmoid (tansig) function (Eq. (3)) was selected as the hidden layer transfer function, and a linear function was selected for output layer transfer function due to their ability to learn complex nonlinear relation between input patterns and output data.

$$Tansig\ (N) = [2/(1+e^{-2N})]-1 \tag{3}$$

Before the training, validating and testing the designed ANN, all of input data preprocessed and converted to values in the range of 0 to 1 to improve the generalization of network. In order to inhibit the over-fitting problem, Bayesian regularization training method in combination with early stopping were applied in the current work.

3. RESULTS AND DISCUSSION

Figure 2 depicts the reductions of mean squared error (MSE) for train, validation and test data versus training iteration (Epoch). It can be seen that before epoch 5, MSE of validation data was decreased, but afterward, it was enhanced. Consequently, after epoch 5, the ANN was over-fitted. The training was stopped in epoch 5 (MSE=3983) in order to inhibit over-fitting formation.

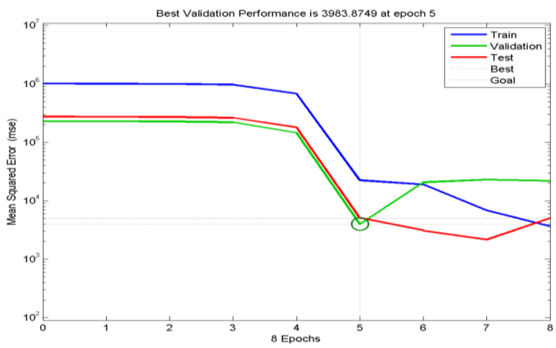

Figure 2. The reductions of mean squared error (MSE) for train, test and validation data versus training iteration (Epoch)

Figure 3 shows the generalization of the trained ANN in training, validation and tested points and also shows the best linear fit for the data. With respect to the obtained results, it can be concluded that the predicted values of ANN for Iron concentration shows good correlation (R Value) with the real experimental values for all of validation data, training data and test data. Also, it can be seen that the error of data for all data have normal distribution. Moreover, in comparison to the statistical method [1], the accuracy of ANN is higher.

3.1. Effect of parameters on Iron removal Filled contours in Figure 4 show the effects of initial pH, sucrose and spore concentration on Iron removal percentage. All of the contours were plotted in optimum values of other parameters. Using these contours, the effect of input parameters of ANN on output parameters can be predicted.

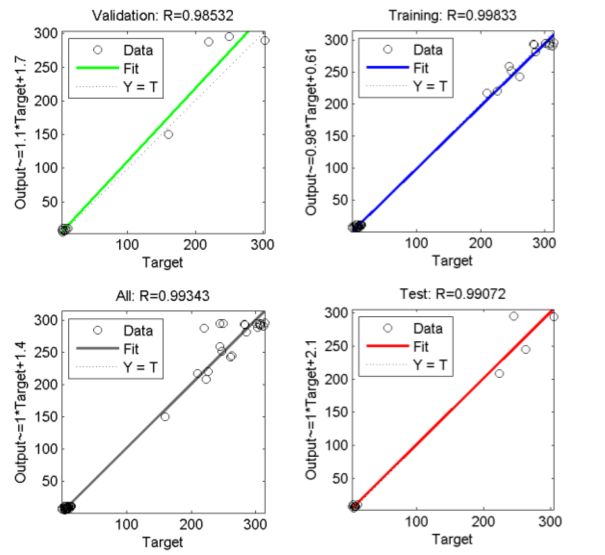

Figure 3. Correlation between the predicted value of Iron concentration with experimental value for validation, test and train data

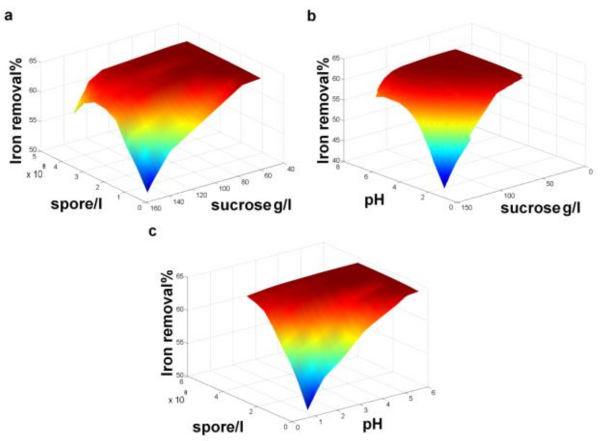

Figure 4. 3D plot of Iron removal as function of initial pH, sucrose and spore concentration.

As it is presented in Figure 4a, the extent of Iron removal reduces by increasing the amount of sucrose in the leaching medium when the spore concentration of the medium is low. However, for higher spore concentration, from 2×10^8 up to 3×10^8 spore/l, changing the sucrose concentration has not significant effect on the response surface. Following this point, again the Iron removal decreases by increasing the sucrose concentration after experiencing a plateau from 50 to 120 g/l of sucrose concentration.

Also, concerning the concentration of fungi spores, it is obvious from the picture that when the medium is low in sucrose, changing the spore contents does not influence the Iron elimination. However, for the higher sucrose concentration, the response experiences a different trend. For example, at the highest level of sucrose concentration, started from 150.5 in the 0.014×10^8 spore/l, the removal percentage has an upward trend until reaches to an optimum point in 2.5×10^8 spore/l of spore concentration. Afterward, there is a downward trend in Iron removal, and it reaches its lowest record (56%) in 4.379×10^8 spore/l. Generally, regarding the both effective criteria, the highest removal percentage of iron from kaolin was registered in 50~60 g/l, and 3~4 $\times10^8$ spore/l of sucrose and spore concentration, respectively. On the contrary, the lowest amount of response factor gained in 150.5 g/l, and 0.014×10^8 spore/l of sucrose and spore concentration, respectively.

Figure 4b illustrates the influence of sucrose concentration and initial pH and their interaction on the Iron removal. From the initial pH point of view, there is not any variation at low sucrose concentration. Nevertheless, at higher sucrose contents, a slight growth in Iron removal from pH=1.4~3 can be seen, but any further reduction in the initial pH results in a sharp fall in the response. If the sucrose concentration is considered, when the medium initial pH is set to lesser quantities, increasing the sucrose concentration to 100 g/l does not change the response, though after this point, a dramatic decline can be observed. For upper initial pH, the response variable remains relatively stable as the sucrose quantity increases. The best result (61%), according to the both sucrose concentration and initial pH is where they are set to 50~60 g/l and 5~6, respectively.

The interaction between pH and spore concentration is depicted in Figure 4c. It can be concluded from the figure that the lowest Iron removal takes place when the both parameters, are set at their lowest levels (0.98, and 0.014×10^8 spore/l). In contrast, the best Iron removal occurs at the maximum amount of the both factors. Moreover, although the Iron removal is not affected by initial pH at greater concentrations of fungi spore, it clearly goes up as a result of pH increase, when the spore content is low. Also, the same trend is observed by changing the spore concentration.

3.2. Effect of parameters on oxalic acid concentration The 3D plots shown in Figure 5, explains the effects of initial pH, sucrose and spore concentration on the oxalic acid concentration. Plotted in optimum value of other parameters, these contours predict the effect of input parameters of ANN on the output variable.

It can be inferred from Figure 5a that, at high concentrations of spore (4.379×10^8 spore/l), the initial pH does not influence the oxalic acid exertion, but regarding the lower spore concentrations, began from low concentration of acid in pH=0.98, the oxalic acid produced by *Aspergillus niger* in the presence of kaolin clay rises to 8 g/l, before it levels out at this concentration. The same behavior can be observed for the spore effect on oxalic acid concentration at different levels of initial pH as well. At pH=0.98, as the spore concentration increases from 0.014×10^8 to $\sim3\times10^8$, a slight rise is seen in oxalic acid production from 5.7 to 6.2 g/l. Further increase in the medium spore content leads to a dramatic growth in acid concentration (8 g/l). Considering the two factors together, the greatest oxalic acid concentration is gained where the pH and spore concentration are at their highest level.

Influence of sucrose concentration and initial pH and their interaction on oxalic acid exertion to the leaching medium is shown in Figure 5b. As it is clear from this figure, there is a minimum point in the response surface, where the initial pH and sucrose concentration are at 0.98 and 150.5 g/l. Any decrease in the sucrose content in low initial pH or any increase in pH values at high concentrations of sucrose results in a sharp climb in the oxalic acid concentration; otherwise, oxalic acid is at its maximum concentration and changes a little.

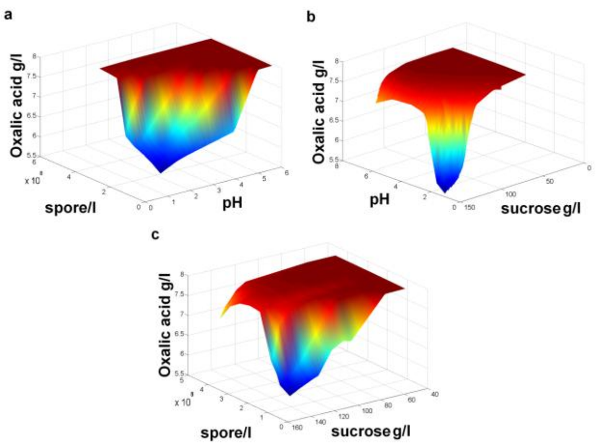

Figure 5. 3D plot of Oxalic acid concentration as a function of initial pH, sucrose and spore concentration.

Figure 5c gives a picture of interaction between sucrose and spore concentration. It can be deduced from this diagram that when the sucrose concentration is high, oxalic acid generation increases constantly by increasing the spore level until it peaks to a 7.6 g/l of acid concentration at 2.3×10^8 spore/l. By changing this optimum level, the sucrose concentration does not enhance the acid production any more, but when the spore concentration is near to the optimum, acid concentration increases gradually and reaches a constant concentration at 8 g/l. However, in lower spore concentrations, reducing the sucrose concentration profoundly increases the oxalic acid concentration. Generally, considering the both factors mentioned, the optimum condition occurs at 50~60 g/l and $3\sim4\times10^8$ spore/l of sucrose and spore concentration.

3.3. Effect Of Parameters On Citric Acid Concentration The diagrams shown in Figure 6, illustrates the effects of initial pH, sucrose and spore concentration on the citric acid production. These contours predict the effect of input parameters of ANN on output parameters in optimum values of other parameters. Looking at Figure 6a which presents the effects of sucrose and pH on citric acid concentration, it can be understood that being at a maximum level, citric acid is not influenced by the variations in initial pH between 49.5 and 100 g/l of sucrose concentration, before it slumps to 7.3 g/l of acid concentration when the sucrose contents of the medium goes up to 150.5 g/l. Also, at this amount of sucrose, increasing the initial pH of the leaching medium from 0.98 to 2.5, leads to a quick rise in acid production to 10.0 g/l that continues moderately until reaches its maximum quantity at pH=3.5~4. In summary, maximum acid concentration, takes place in a point where sucrose and pH are at 50~60 g/l and 4~6, respectively.

Figure 6b shows how the citric acid concentration is affected by sucrose and spore concentration. Although in low spore concentrations, every little decrease in sucrose content of the medium positively influences the acid production, at higher spore concentrations, this influence becomes lesser so that at its optimum level (0.014×10^8 spore/l) at 10.7 g/l of citric acid, changing the sucrose content does not affect the acid concentration. For the greater spore quantities, acid concentration rapidly reaches a plateau in consequence of lessening the sucrose concentration from 150 to 140 g/l. Moreover, the maximum concentration of citric acid happens in 4×10^8 spore/l and 50~60 g/l of spore and sucrose concentration.

The last diagram in Figure 6c represents the interaction between initial pH and spore concentration. In the first look, a steady rise in the citric acid concentration is distinguished as a result of increasing the initial pH from 0.98 to 4.1 at the small amounts of spore concentration. Nevertheless, for the spore quantities higher than 3×10^8 spore/l; the response surface levels off at a maximum acid concentration as the pH become more than 3. On the other hand, this figure implies that at small initial pH, shifting the spore concentration of the medium from the 0.014×10^8 spore/l to higher levels, substantially augments the acid exertion by the fungi; while, at higher initial pH, the spore population effect becomes weaker. Lastly, it can be concluded that the best condition for these two parameters is at 4~6 and $3\sim3.7\times10^8$ spore/l of initial pH, and spore concentration, respectively.

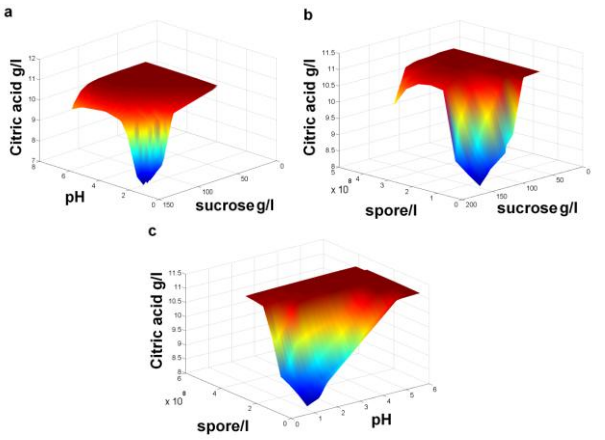

Figure 6. 3D plot of citric acid concentration as a function of initial pH, sucrose and spore concentration.

With Iron removal from the kaolin by the bioleaching process the quality of the kaolin (especially its brightness) can be improved. In the current work, bioleaching of Iron from highly contaminated kaolin sample with *Aspergillus niger* (a microorganism) was optimized. The processed kaolin is quite attractive for many applications including porcelain, paper coating, paint, plastics, bricks, rubber, pharmaceuticals, pesticides, and fertilizers due to its enhanced brightness.

4. CONCLUSION

For the purpose of studying the effect of the initial pH, sucrose and spore concentration on the Iron removal percent, citric and oxalic acid production in the kaolin bioleaching process, more than twenty experiments were carried out. Then, the resulted data were used to train, validate and test the ANN. Consequently, following conclusions can be drawn from the proposed model.

1. ANN is a practical tool for modeling and optimization of the bioleaching of Iron.
2. Optimized conditions of initial pH, sucrose and spore concentration were determined to be 4~6, 50~60 g/l and 3~4×10^8, respectively.
3. Bayesian regularization method in combination with early stopping of network during training can inhibit the over-fitting problem better than the Bayesian regularization or early stopping alone.
4. The optimized value for the Iron removal percentage is approximately about 65%.

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[a] *Materials and Energy Research Center, Karaj, Iran*
[b] *Department of Mining Engineering, Engineering Faculty, Shahid Bahonar University, Kerman, Iran*

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چکیده

در این کار میزان استخراج آهن با فرایند فروشویی زیستی یک نمونه کائولن دارای ناخالصی بالای آهن توسط *Aspergillus niger* بهینه سازی شد. بیش از ۲۰ آزمایش برای مطالعه اثر pH اولیه، غلظت ساکارز و اسپور بر روی غلظت آهن، اسید اگزالیک و سیتریک انجام شد. داده های بدست آمده از این آزمایشات برای آموزش، تایید و آزمایش یک شبکه عصبی دو لایه ای مورد استفاده قرار گرفت. برای جلوگیری از بیش برازش، الگوریتم انتظام بیزی و توقف زودتر با تکنیک پس انتشار بکار گرفته شد. بدلیل جلوگیری از بیش برازش با استفاده از طراحی توپولوژی شبکه و انتخاب الگوریتم آموزش مناسب، تطابق خوبی بین داده های پیش بینی میزان حل شدن آهن با داده های تجربی بدست آمد. نتایج نشان داد که شرایط بهینه برای دستیابی به بیشترین میزان خارج کردن آهن (حدود ٪۶۵) برای pH اولیه، غلظت ساکارز و اسپور بترتیب برابر با ٦، ٦٠ گرم بر لیتر و $۳/۵\times۱۰^{۷}$ اسپور بر لیتر می‌باشند

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