CHEMICAL ENGINEERING TRANSACTIONS
VOL. 65, 2018
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Eliseo Ranzi, Mario Costa

ISBN 978-88-95608-62-4; **ISSN** 2283-9216
DOI: 10.3303/CET1865074

Comparison Study of Hematite Bioflotation by *R. erythropolis* and its Biosurfactant: Experiments and Neural Network Modeling

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The relevance of Biotechnology applied to mineral processing has been increasing during the last decade. Initially, microorganisms as bacteria and yeast were used as collectors due to their hydrophobic properties. However, the complexity of their cell wall makes the understanding of the adsorption mechanisms involved during the microbial cell-mineral interactions difficult. Thus, more simple substances such as their metabolic products or extracellular polymeric substances (EPS) started to be extracted and used. One of these substances, biosurfactants, has shown a great potential as collectors and also as frothers. This work presents a comparative study between *R. erythropolis* bacteria and its biosurfactant in the flotation of hematite. The objective was to study the floatability efficiency of the biorreagents using artificial neural network. The experimental conditions varied from 3 – 11 to pH, 0 – 150 mg/L to concentration. Recovery of hematite analysis (%) was performed to evaluate the efficiency of the process. A database was constructed with the information of the experiments, dividing them into groups of training (65%) and test (35%). The models were obtained using toolbox of the MATLAB R2017a. In the developed neural network, the data obtained by the different conditions were used as neurons in the input layer and the percentage of hematite recovered was used as the only neuron in the output layer. The performance of the neural network was evaluated by the correlation coefficient (R^2) and the error index (SSE). The model developed from the neural network was satisfactory, since the R^2 value was close to 1 and the error index values were close to 0. In addition, the angular and linear coefficients of the adjustment lines were respectively close to 1 and 0, confirming the good fit between the data tested and the developed model.

1. Introduction

The flotation is a mineral separation process based on the differences in the surface properties of the minerals and gangue (Kelly and Spottiswood, 1990). This process is able to separate minerals due to their hydrophobic properties; among several factors affecting the performance of flotation, the most important are particle size, pulp density, and mainly, the pH of the pulp. The last one is also the most important factor with respect to bioflotation. In bioflotation microbial cells and their by-products are used as collectors, depressors and also as frothers, their use is justified by environmental and technological aspects as mentioned by several authors (Merma et al., 2013; Mesquita et al., 2003; Natarajan 2006; Rao and Subramanian, 2007). According to these authors the use of these bioreagents can improve the mineral recovery. The microbial cells adhesion onto mineral surface is strongly related to attractive and repulsive forces between the bacterial wall and the mineral surface. Thus, the separation of a desirable mineral depends on the selectivity of a bacterial strain for the mineral surface, as seen in Lopez et al. (2015) and in Merma et al. (2017). Another possibility is the use of biosurfactant. These molecules are capable to reduce the surface tension. For instance, for water it can reduce it from 72 mN/m to 28 mN/m, due to amphiphilic molecule, with hydrophilic and hydrophobic groups. The application of these biosurfactants in mineral flotation is recent; however, literature presents some works such as those by Szymanska and Sadowski (2010), who reported a promising use of biosurfactant produced

Please cite this article as: Gutierrez Merma A., Castaneda Olivera C.A., Torem M.L., Santos B.F., 2018, Comparison study of hematite bioflotation by r. erythropolis and its biosurfactant: experiments and neural network modeling, Chemical Engineering Transactions, 65, 439-444 DOI: 10.3303/CET1865074

by *Pseudomonas aerugiosa* for flotation of hematite, and by Sanwani et al. (2017), where the recovery of copper from pyritic copper using a biosurfactant-producing mixotrophic bacterium is reported.
In order to ensure a large research of processes, mathematical modeling can be applied. Artificial neural network (ANN) models based software allows simulating the variable predictions based on input data. ANN is an attractive tool for non-linear modeling and process optimization. The operations consist of a large number of simple elements called neurons. Each neuron receives information through input variables and calculates the output, internally weight and bias are adjusted by the training algorithm. Neural models have been used in minerals processing as reported by Zhang et al. (2017) and Bhunia et al. (2015).
This work aims to compare the hematite recovery (%) between microbial biomass (*R. erythropolis*) and its biosurfactant, to develop a neural model based on input variables (pH and concentrations), as well as to determine optimal conditions through response surface analysis.

2. Materials and methods

2.1 Sample preparation

A pure quartz sample and a hematite sample (92% Fe_2O_3) were provided by a local supplier (Belo Horizonte, Minas Gerais State) to be used in this study. The samples were crushed and screened to ≤ 3 mm. Then, the samples were dry-ground in a porcelain mortar and wet-screened. The desired size fraction (>75 < 106 µm) was used for flotation studies. Then, the quartz sample was washed with a KOH (0.1 M) solution to remove the impurities present on the surface and then the sample was washed several times with double-distilled water until the pH suspension achieved the initial pH. Finally, the quartz and hematite samples were dried and stored in a desiccator.

2.2 Microorganisms, media, growth and biosurfactant extraction

The *Rhodococcus erythropolis* strain was supplied by The Chemical, Biological and Agricultural Pluridisciplinary Research Center (CPQBA) and was developed in a TSB solid medium. Stocks of the bacteria were prepared frequently using this medium in Petri plates and they were stored in a freezer at 12 °C. Afterwards, the bacterial cells were separated from the culture by centrifugation at 3500 g for 8 min, followed by re-suspending twice with deionized water. The previous is the procedure to obtain a bacterial concentrate; the crude biosurfactant extraction followed the procedure from Moreau et al. (2003). In this case, the centrifuged cells were re-suspended in ethanol (500 ml for 1 L of broth), and autoclaved at 1 bar and 121° C for 20 minutes. The insoluble part was removed by centrifugation (3500 g), and then the soluble part was dried at 45°C for 24 h. After this time, this material was dissolved in water, the insoluble part was separated from the soluble part by centrifugation (3500 g) and filtration (25 µm). The final solution is the crude biosurfactant solution and the biosurfactant concentration was determined by dry-weight measurement.

2.3 Microflotation experiments

The floatability of the minerals was evaluated in a modified Hallimond tube. One gram of the mineral was added to a 0.16 L total volume solution of known bioreagent (bacterial cells and biosurfactant). The mineral was conditioned with the bioreagent solution at a desired value of pH inside the Hallimond tube under constant stirring for 5 minutes. Finally, the microflotation tests were carried out using an air flow of 15 ml/min for 2 min. The settled and floated fractions were carefully separated, washed, dried and weighed. The floatability was then calculated as the ratio of floated and non-floated mineral amounts to the total weighed sample.

2.4 Development of neural networks model

Artificial Neural Networks (ANN) modelling performs a nonlinear mapping between inputs and outputs without the necessity of requiring minimal prior knowledge of the system, an alternative method for the generation of process models. The employed ANN is the feed forward multilayer perceptron with three independent layers (input, hidden and output). Input neurons receive the input variable (pH and biosurfactant concentrations-mg/L) values and store the scaled input values. Meanwhile hematite recovery (%) was the only output layer variable. The quantity of neurons in the hidden layer was defined by the smallest error criterion and the constant effective number of parameters as well. The activation functions in the hidden layer were logistic (logsig) and hyperbolic tangent sigmoid transfer functions (tansig) and in the output layer a linear transfer function was used (purelin).
The training was developed in MATLAB R2016a using some algorithms to optimize parameters (weights and bias) such as the gradient descent backpropagation with adaptive learning rate (traingdx), the Levenberg Marquardt based backpropagation algorithm (trainlm) and in conjunction with Bayesian regularization (trainbr).

The quality of the training was assessed by error criteria from the total sum squared error (SSE), comparing the observed and predicted values of the network (Equation 01).

$$SSE = \sum_{i=1}^{n}(Y_{observed} - Y_{predicted}) \quad (1)$$

The coefficient of determination (R^2) is one measure of how well a model can predict the data, as seen Equation 02.

$$R^2 = 1 - \frac{\sum_{i=1}^{n}(Y_{observed} - Y_{predicted})^2}{\sum_{i=1}^{n}(Y_{observed} - Y_{mean})^2} \quad (2)$$

3. Results and discussion

The procedures for the preparation and standardization of the inoculum ensured reliable fermentation experiments, making sure that viable cells reached the same final concentrations in the different fermentation cultures.

3.1 Flotation using bacteria as collector

The floatability of hematite as a function of bacterial concentration and pH is shown in Fig. 1. As expected, hematite floatability increase with bacterial concentration. According to Olivera et al. (2016) this effect is observed until a bacterial concentration of 200 mg/L. beyond that value the floatability is reduced, which is also observed by Yang et al. (2007). The pH is also a relevant factor in bioflotation because of the activation of the functional groups of the bacteria and because of the mineral surface modification as a function of pH. In this case the highest hematite floatability (37%) was achieved at pH 7, moreover at acid and basic conditions the floatability values were low.

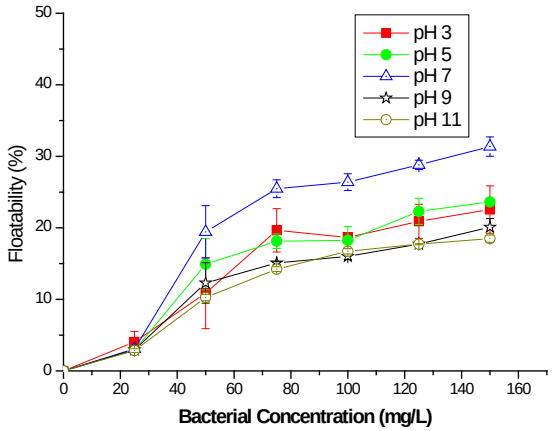

Figure 1: Floatability of hematite using the Rhodococcus erythropolis bacteria as collector. Particle size: (<106 µm, >75 µm; [NaCl]: 10^{-3} mol/L.

3.2 Flotation using biosurfactant as collect

Figure 2 shows the hematite floatability using the crude biosurfactant extracted from the *R. erythropolis* by hot ethanol. As in the previous experiments a higher biosurfactant concentration produced higher floatability values. The most contrasting difference, regarding bioflotation with the bacteria, is that the higher hematite floatability is reached at pH 3, whereas using bacteria the higher recovery was attained at pH 7, achieving values about 98% and 37 %, respectively. Moreover, the biosurfactant presents good response in the floatability of hematite at acid conditions, low response at neutral pH, and even lower at basic conditions; while the bacteria present very low values of floatability at acid conditions and a little higher at neutral pH. It is also possible to observe that lower concentration of biosurfactant (50 mg/L) is necessary, in comparison to bacteria (150 mg/L), in order to attain the highest value of floatability.

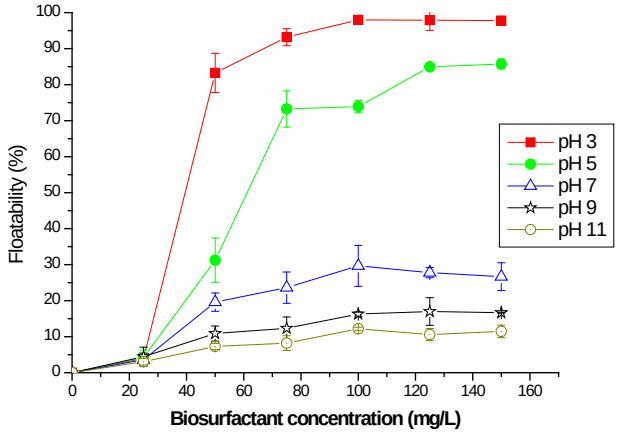

Figure 2: Floatability of hematite using the crude biosurfactantextracted from the Rhodococcus erythropolis bacteria as collector. Particle size: (<106 µm, >75 µm); [NaCl]: 10-3 mol/L.

3.3 Neural network model

The experimental data was collected during the study in duplicate and the average between them was used to train the model. Dataset was randomly split into sets of training data (65%) and testing data (35%) and normalized in the range [-1,1]. Since there was no theoretical principle in choosing the proper network topology, the number of hidden neurons was determined using cross validation technique to obtain the best one. The numbers of neurons in the hidden layer were varied from 2 to 8 (Table 1) and the network was trained and tested after each addition of neuron. All the scenarios of simulation were proposed with two input neurons corresponding to pH and biosurfactant concentration (mg/L). The output layer was hematite recovery (%), activated by *purelin* function.

Table 1: Results of the neural model with varying number of neurons in the hidden layer

Number of hidden neurons	Activation function	Training algorithm	SSE	R^2
2	Tansig	traingdx	3.330	0.955
3	Tansig	trainlm	3.120	0.958
4	***Tansig***	***trainbr***	***0.280***	***0.997***
4	Logsig	trainbr	0.368	0.996
5	tansig	trainbr	0.347	0.996
4	tansig	traingdx	1.300	0.983
4	tansig	trainlm	0.287	0.997
5	logsig	trainlm	0.355	0.996

The best scenario shows the topology with 4 neurons in hidden layer, *tansig* as activation function, *trainbr* as training algorithm can be seen in Figure 3. The value of the SSE for the dataset was 0.280 with R^2 of 0.99 (linear regression Figure 4). Despite several topologies showing satisfactory performance, the effective number of parameters of the chosen model was 14, the lowest value among the possible models discarding overfitting.

The results were plotted as the recovery graph (%) shown in Figure 4. As shown in this Figure, predicted data from model are in accordance to observed data, the model is capable to predict the highest and lowest hematite recovery properly. Several studies carried out earlier by other researchers also show that ANN based models such as Arumugasamy and Selvarajoo (2015). They developed a neural model to predict weight loss of biomass in the pyrolysis process. The neural network and topology consisted of 2 input neurons, 20 hidden neurons and 1 output neuron achieving R^2 values of 0.99. Comparing their model with the one presented here, it is possible to realize that the model has many more effective parameters, 81 against 17.

With respect to neural models applied to bioflotation of hematite from biosurfactant produced by *R. erythropolis* nothing was reported, proving the significance of the matter.

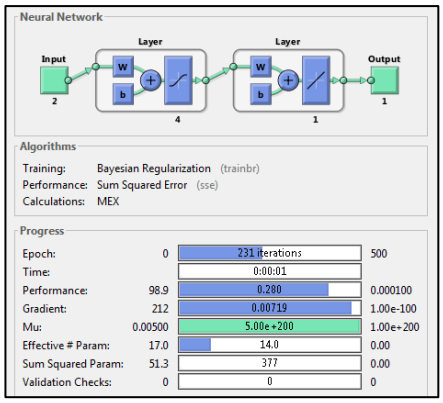

Figure 3: Training with the best topology: 2-4-1, activation functions: tansig/purelin and training algorithm: trainbr.

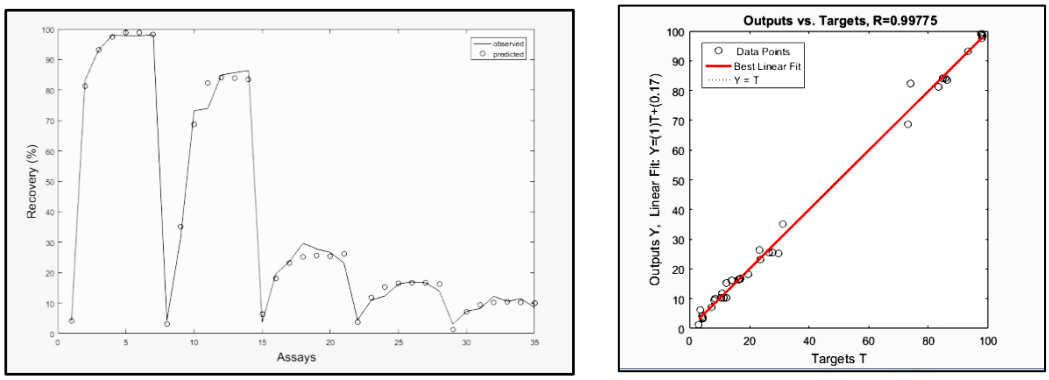

Figure 4: Curve of observed versus predicted data and linear regression of test data.

Figure 5 shows the response surface for hematite recovery (%) predicted by the neural model when it happens a variation in pH and biosurfactant concentration (mg/L).

As shown in Figure 5, analysis from neural model suggests optimum value for recovery of hematite is pH between 3 and 5 and biosurfactant concentration between 50 and 100 mg/L. Even the model indicating the beginning of optimal region, the neural model is considered proper in order to optimize the experimental conditions because reached recovery values near to 100 % (possible maximum experimental value).

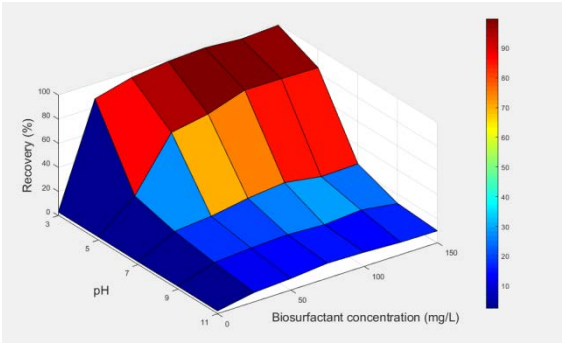

Figure 5: Response surface of the hematite recovery (%) when the biosurfactant was used.

4. Conclusions

In the current work microbial biomass (*Rhodococcus erythropolis* bacteria) and its biosurfactant were tested as bioreagent to recovery hematite. The biosurfactant was able to recover more significant quantities (near to 100 %) in comparison with the biomass (around 37%), which evidences their potential application in mineral bioflotation. The use of a neural model is an important tool for interpolation between experimental data. With this strategy the model performance was assessed through R^2 and error index, 0.99 and 0.280 respectively. To our knowledge this is the first report on the use of neural model for hematite bioflotation. The use of the model as an optimization tool would have a huge impact aiming at an industrial scale.

Acknowledgments

The authors acknowledge CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), ITV-VALE, CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and FAPERJ (Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro) for their financial support.

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