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Optimization of Recycle Use of Residual Ferrous Medium for Hydrogen Sulfide Bioremoval

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One of the most potential approaches for hydrogen peroxide removal is reaction with ferric iron to produce elemental sulfur and ferrous iron followed with the oxidization of ferrous to ferric for recycling by the chemoautotrophy microorganism, *Acidithiobacillus ferrooxidans*. In the whole process there is no waste pollution produced. However, there are still a few large scale of this process because the recirculatory operation is not well established. In this work, the ferrous media after H_2S removal was repeatedly used for *Acidithiobacillus ferrooxidans* cultivation by external nutrition (NH_4OH, K_2HPO_4 and $MgSO_4 \cdot 7H_2O$) supplement according to response surface methodology experiments. The recycle process was continuously operated for 40 days (six rounds) with a relatively stable oxidation rate of Fe^{2+} by supplementing the nutrient. In conclusion, it was a reliable method that the residual ferrous sulfate medium was recycled for *Acidithiobacillus ferrooxidans* cultivation with nutrition addition.

Keywords: *Acidithiobacillus ferrooxidans*, Ferric iron, Hydrogen sulphide removal, Response surface methodology.

INTRODUCTION

Hydrogen sulphide (H_2S) is a highly undesirable compound and is harmful to human health. H_2S contained in biogas, natural gas or other gas stream can be extremely corrosive for the gas-pipeline system, also the amount of H_2S released into air is under strictly control for human health and environmental issue[1,2]. At present, H_2S is generally removed by physico-chemical techniques including alkanolamine treatment and the Claus process[3]. However, these processes triggered another kind of environmental pollution and a large amount of waste because of those relatively large quantities of chemical, catalyst and waste disposal[4]. Therefore, it is necessary to develop an environmental friendly process to remove H_2S under mild conditions, which is acceptable on large-scale application. Removal of H_2S through biological processes under ambient temperature and atmospheric pressure demand low cost of heat, pressurization and the energy, which could reduce the cost to minimum[1,5].

One of the successful processes is Shell-Paques process for natural gas desulfurization. In this process H_2S is absorbed into sodium carbonate/bicarbonate solution and then it is treated in the bioreactor where H_2S is mostly converted biologically to elemental sulfur. The bioreactor is supplied with nutriental stream and air. And the same, a continuous bleed stream is operated to avoid accumulation of sulfate. It is achieved that H_2S is less than 4 ppmv in effluent when treated natural gas containing 2000 ppmv H_2S[3,6].

Another promising alternative for H_2S removal is achieved in a two-stage chemical biological process[7]. In the first stage, the ferric sulfate solution reacts with the H_2S in an absorber. The H_2S is oxidized to elemental sulfur, while the ferric sulfate is reduced to ferrous sulfate. Elemental sulfur produced is removed by solid-liquid separation and the ferrous sulfate solution is cyclically sent to the second stage. In the second stage, the immobilized chemoautotrophy aerobic bacteria, *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) oxidizes ferrous iron to ferric iron again in a bioreactor[8,9]. *A. ferrooxidans* derives energy for its metabolic functions with the results of ferrous iron oxidation, while the CO_2 in air is fixed as its carbon source and the NH_4^+ is used as nitrogen source[10]. The reaction of H_2S with ferric sulfate is relatively fast and complete so that there remains low concentration of H_2S. During the first stage, the pH of the ferrous sulfate solution slightly decreases which would lead to the unfavorable growth of the bacteria. Therefore, the increased acidity is neutralized using alkali[1,11]. Additionally, precipitate, namely jarosite, produces during the growth of *A. ferrooxidans,* especially when the bioreactor is operated

continuously over a long period. Jarosite consists of $XFe_3(SO_4)_2(OH)_6$, where X could be K^+ (potassium jarosite), Na^+ (natro jarosite), NH_4^+ (ammonio jarosite), or H_3O^+ (hydronium jarosite)[12,13]. The formation of jarosite represents the consumption of ions in media, which is responsible for the *A. ferrooxidans* growth, with results of performance loss caused by obstructing the support pores and restricting the transport of media[13]. Although, the removal of H_2S using ferric solution is fast and complete, there are a few large scale of this process because the recirculatory operation is not well established[1]. Response surface methodology (RSM) which has been successfully applied in many optimizations of medium is used as an effective method for the analysis of complex composition to establish the continues system for *A. ferrooxidans* cultivation and H_2S removal[14,15]. In the present work, the ferrous solution after removal of H_2S was optimized using RSM to supplement the nutrition and the recirculatory process was operated for 40 days with a relatively stable oxidation rate of Fe^{2+}.

EXPERIMENTAL

Microorganism and media: Strain *A. ferrooxidans* Z1 was kindly provided by Prof. Jinshen Di (Hebei University of Technology, China). The initial media which *A. ferrooxidans* was grown in contains (g/L): $FeSO_4·7H_2O$ 44.68, $(NH_4)_2SO_4$ 3.0, $MgSO_4·7H_2O$ 0.5, KCl 0.1, K_2HPO_4 0.5 and $Ca(NO_3)_2$ 0.01. The initial pH of the culture was adjusted to 1.5 with 1 mol/L H_2SO_4.

In this article, fresh media represent the initial media while the refreshed media represent the ferrous solutions after removal of H_2S.

Bioreactor experiments: The flask culture of *A. ferrooxidans* was performed in 300 mL Erlenmeyer flask containing 100 mL of the medium with 10 % (v/v) inoculum on a rotary shaker at 150 rpm and 30 ºC. All the batch culture was repeated 3 times.

The bioreactor batch culture was performed with 15 % (v/v) inoculum to make the cell immobilized on the support. When most of ferrous ion was oxidated, 85 % medium was replaced by fresh medium. To make sure the formation of biofilm, the batch culture was performed for 3 times.

Operation of the bioreactor: The biological oxidation of ferrous ion by immobilized *A. ferrooxidans* was performed in a packed-bed bioreactor that was made of plexiglass (polymethyl methacrylate plastics). The main part of bioreactor was a biocatalyst bed of 60 mm diameter and 360 mm height with a total operating volume 1.05 L. The bioreactor was filled with the polyurethane foam particles as supports shaping of 10 mm × 10 mm × 3 mm, on which the *A. ferrooxidans* cells were immobilized. The density of particles was 20 kg/m^3. The inlets for fresh medium and fresh air were at the bottom of the column and the outlets for effluent and exhaust air were at the top of the column. A reservoir was provided to collect the effluent, as shown in Fig. 1.

The reactor was maintained at 30 ºC using an external jacket. The reactor was aerated at 120 L/min using an air pump and the flow rates for the media was 0.3-0.5 L/h using a peristaltic pump according to the Fe^{2+} concentration in outlet. Under this condition, the concentration of Fe^{3+} in outlet was 8.5 g/L.

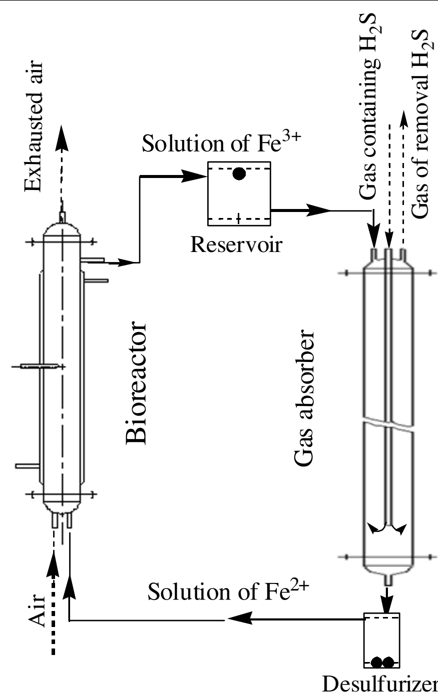

Fig. 1. Flow chart process proposed for H_2S removal and Fe^{2+} recycling

Operation of the H_2S absorber: The H_2S removal was performed in a bubble column reactor which was made of plexiglass, with a diameter of 150 mm, height of 1850 mm and a total operating volume was 2.0 L. The inlet for gas was at the bottom of the reactor with a cannulation and the outlet for cleaned gas was at the top, on the contrary, the inlet for the ferric solution was at the top of the reactor and the outlet for ferrous solution was at the bottom, as shown in Fig. 1. The flow rate of ferric solution was 5 L/h and the inlet gas was 6 L/h. At those flow rates the H_2S concentration of the effluent gas was less than 20 ppmv. The reaction was done at room temperature.

To facilitate the research and ensure the reduction of Fe^{3+}, gas with high H_2S concentration was used in this experiment. The gas was from China Petroleum & Chemical Corporation Tianjin Branch, containing H_2S 30 % (v/v), the rest was some gaseous hydrocarbon.

Repeat use of ferrous media: The solution of ferrous ion effusing from the bottom of H_2S absorber contains a lot of sulfur which was the product from the reaction of ferric ion and H_2S. The sulfur was removed by centrifugation after standing for one day without any agitation. Then the ferrous solution was used as the medium after supplementing 25 % (v/v) NH_4OH, K_2HPO_4 and $MgSO_4·7H_2O$ to complement the N, P resources that were consumed during the cell growth of last round and the formation of jarosite and adjusting pH to 1.5 with KOH solution[8,12].

Experimental design and evaluation: A Box-Behnken factorial design with 3 factors and 3 levels was used for making a 3D response surface. The three independent variables studied were NH_4OH, K_2HPO_4 and $MgSO_4·7H_2O$, respectively. The range and levels of the 3 variables were listed in Table-1. Selection of the levels was according to the results obtained in our previous work. The experimental sequence was randomized to minimize the effects of the uncontrolled factors.

TABLE-1
BOX-BEHNKEN EXPERIMENTS DESIGN WITH EXPERIMENTAL VALUES OF Fe^{2+} OXIDATION RATE

Std order	Run order	Pt type	Blocks	X_1 ammonia (g/L)	X_2 KH_2PO_4 (g/L)	X_3 $MgSO_4·7H_2O$ (g/L)	Y oxidation rate of Fe^{2+} (g/L/h)
6	1	2	1	1 (2.5)	0 (0.2)	-1 (0.1)	0.306
1	2	2	1	-1 (0.5)	-1 (0.1)	0 (0.2)	0.282
9	3	2	1	0 (1.5)	-1 (0.1)	-1 (0.1)	0.304
5	4	2	1	-1 (0.5)	0 (0.2)	-1 (0.1)	0.286
14	5	0	1	0 (1.5)	0 (0.2)	0 (0.2)	0.315
15	6	0	1	0 (1.5)	0 (0.2)	0 (0.2)	0.315
13	7	0	1	0 (1.5)	0 (0.2)	0 (0.2)	0.316
12	8	2	1	0 (1.5)	1 (0.3)	1 (0.3)	0.293
2	9	2	1	1 (2.5)	-1 (0.1)	0 (0.2)	0.292
10	10	2	1	0 (1.5)	1 (0.3)	-1 (0.1)	0.281
3	11	2	1	-1 (0.5)	1 (0.3)	0 (0.2)	0.276
11	12	2	1	0 (1.5)	-1 (0.1)	1 (0.3)	0.283
7	13	2	1	-1 (0.5)	0 (0.2)	1 (0.3)	0.278
8	14	2	1	1 (2.5)	0 (0.2)	1 (0.3)	0.292
4	15	2	1	1 (2.5)	1 (0.3)	0 (0.2)	0.297

A mathematical model, describing the relationships between the process index (the oxidation rate of Fe^{2+}) and the variables (supplement amounts of nutrient) in a second-order equation, was developed. The final oxidation rate of Fe^{2+} was multiply regressed with respect to the amounts of nutrient supplement by the least squares method as eqn. 1:

$$Y = a_0 + a_1X_1 + a_2X_2 + a_3X_3 + a_4X_1^2 + a_5X_2^2 + a_6X_3^2 + a_7X_1X_2 + a_8X_1X_3 + a_9X_2X_3 \quad (1)$$

where, Y is the predicted response variable; a_0, a_1, ….., a_9 are constant regression coefficients of the model and X_1, X_2 and X_3 represent the independent variables in the form of coded values.

Statistical analysis: Data from the Box-Behnken factorial design as shown in Table-1 were used for determining the regression coefficients of the second-order multiple regression model. The accuracy and general ability of above polynomial model was evaluated by the coefficient of determination (R^2)[16]. MINITAB release 15 software was used for regression analysis and analysis of variance (ANOVA). Response surfaces methodology were developed using the fitted quadratic polynomial equation obtained from regression analysis, holding one of the independent variables at a constant value corresponding to the stationary point and changing the other two variables. Confirmatory experiments were carried out to validate the equation using flask culture.

Analytical methods: Concentrations of ferrous ion and total iron were determined by titration against 0.017 mol/L potassium dichromate in the presence of N-phenylanthranilic acid as indicator[17]. Ferric ion was calculated by subtracting ferrous iron from the total iron. H_2S concentration at the outlet of absorber was measured by H_2S analyzer (PHSJ-4A, Shanghai Leici, China). The pH of the media was measured at room temperature with a pH meter (Metrohm, model 691). The biofilm of support particles was observed with a Scan electron microscope (XL30W/TMP, Philips).

The Fe^{2+} oxidation rate of flask culture was defined as expression (2):

$$\frac{(Fe_0^{2+} - Fe_1^{2+})}{t} \quad (2)$$

where, Fe_0^{2+} is the initial concentration of Fe^{2+}, Fe_1^{2+} is the remaining concentration of Fe^{2+} after biocatalysis, t is the culture period.

The Fe^{2+} oxidation rate of bioreactor was defined as expression (3):

$$\frac{(Fe_{inlet}^{2+} - Fe_{outlet}^{2+}) \times L}{V} \quad (3)$$

where, Fe^{2+}_{inlet} is the Fe^{2+} concentration at inlet of bioreactor, Fe^{2+}_{outlet} is the remaining concentration of Fe^{2+} at outlet of bioreactor, L is the flow rate of media and V represents the available volume of bioreactor (700 mL).

RESULTS AND DISCUSSION

Biofilm formation in bioreactor: The natural tendency of *A. ferrooxidans* to grow on solid surfaces makes it prone to absorption on the support[18-20]. The batch culture was performed for 3 times in the bioreactor to ensure that the *A. ferrooxidans* cells were immobilized on the support, then the bioreactor was run continually under the conditions described above without any inoculation. Several support particles were taken out to check the biofilm formed on the surface of support by scan electron microscope. It is clearly observed from Fig. 2 (B) that the biofilm was formed on the surface of support, since the surface was covered with a layer of precipitate while the surface of fresh support is smooth, as shown in Fig. 2(A).

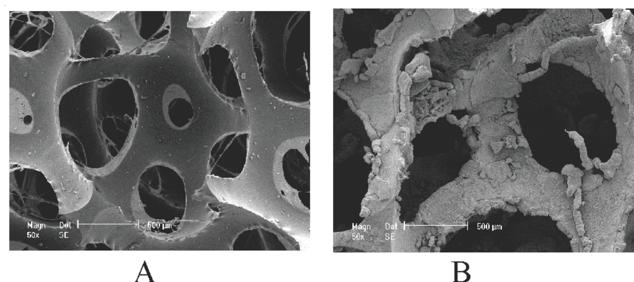

Fig. 2. Electron micrograph pictures of support particles A, the fresh support; B, the support in bioreactor

In our previous research, it was found that jarosite, ranged from 0.5-10 g/L, had no interference either on cell growth or on oxidation of Fe^{2+} in flask culture (data not shown). However, the presence of abundant jarosite mass-formed will obstruct the flow of media and air in bioreactor leading to decrease the oxidation rate of Fe^{2+}. The formation of jarosite during

A. ferrooxidans growth is related to the pH. The appropriate pH is effective on reducing the deposit of jarosite[13]. As shown in Fig. 3, the pH of media showed an significant effect on the jarosit formation and Fe^{2+} oxidation[21]. However, there was less precipitate when the initial pH of media was 1.5, also a high oxidation rate of Fe^{2+} similar with that of pH 2.0 is obtained. Thus, the initial pH of media for the bioreactor, as well as the refreshed medium, was adjusted to 1.5.

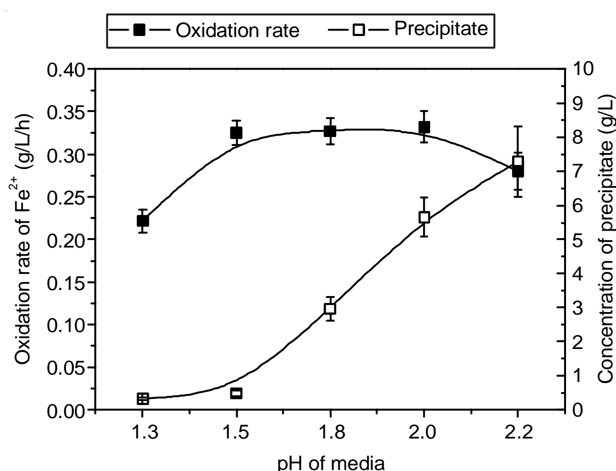

Fig. 3. Effects of pH on the Fe^{2+} oxidation rate and precipitate production

Repeat use of ferrous media: The liquid flowed out from outlet of absorber was turbid because of the formation of solid sulfur. Nonetheless, it became clear after removing the sulfur by centrifugation. Then, different amounts of 25 % (v/v) NH_4OH, K_2HPO_4 and $MgSO_4{\cdot}7H_2O$, designated as X_1, X_2 and X_3 in this design, were added according to the design listed in Table-1, meanwhile the pH of the solution were adjusted to 1.5 with KOH. Shaker culture was performed to compare the Fe^{2+} oxidation rate in various refreshed media. The centre point in the design was repeated 3 times to estimate the error. Results were shown in Table-1, the Fe^{2+} oxidation rate ranged from 0.276 to 0.316 g/L/h and run numbers 11 and 7 had the minimum and maximum oxidation rate, respectively. However, the difference among each result was not very obvious. It was probably due to that *A. ferrooxidans* was capable of storing a portion of the energy obtained from oxidizing ferrous iron in the fresh media for subsequent use[22].

Interpretation of the regression analysis: The response surface regression results namely T and P values along with the constant and coefficients were listed in Table-2. The value of the constant was determined to be 0.2189 and the values of T and P that were used to determine the significance of the regression coefficients and the smallest level of significance leading to rejection of null hypothesis[15], respectively, were 17.963 and 0.000. The effects of all linear terms, X_1, X_2 and X_3, were found to be significant ($p = 0.001$, 0.004 and 0.017, respectively, less than 0.05). Thus there was a linear relation of them with the Fe^{2+} oxidation rate. Likewise, the effects of three quadratic terms were also significant. However, only one of the interaction terms, $X_2 \times X_3$ that is $K_2HPO_4{\times}MgSO_4{\cdot}7H_2O$ ($p = 0.002$), was found to be significant. Finally, the polynomial model for Fe^{2+} oxidation rate (Y) was prepared as shown in eqn. 4:

TABLE-2
ESTIMATED REGRESSION COEFFICIENTS FOR EXPERIMENTAL Fe^{2+} OXIDATION RATE

Term coefficient	Coefficient	Standard error coefficient	T-Value	*p*-Value
Constant	0.21894	0.012188	17.963	0.000
X_1	0.04813	0.006110	7.876	0.001
X_2	0.35292	0.070193	5.028	0.004
X_3	0.24542	0.070193	3.496	0.017
X_1^2	-0.01417	0.001489	-9.516	0.000
X_2^2	-1.44167	0.148873	-9.684	0.000
X_3^2	-1.06667	0.148873	-7.165	0.001
X_1*X_2	0.02750	0.014303	1.923	0.113
X_1*X_3	-0.01500	0.014303	-1.049	0.342
X_2*X_3	0.82500	0.143033	5.768	0.002

S = 0.00286 R^2 = 98.49 % R^2(adj) = 95.76 %.

$$Y = 0.2189 + 0.0481 X_1 + 0.3529 X_2 + 0.2454 X_3 - 0.0142 X_1^2 - 1.4417 X_2^2 - 1.0667 X_3^2 + 0.8250 X_2X_3 \quad (4)$$

A positive sign of the coefficient represents a synergistic effect, while a negative sign indicates an antagonistic effect. Thus, all linear variables, NH_4OH, K_2HPO_4 and $MgSO_4{\cdot}7H_2O$ and the interaction term $K_2HPO_4{\times}MgSO_4{\cdot}7H_2O$ have positive effects on the oxidation rate of Fe^{2+}. That means that the Fe^{2+} oxidation rate increases with the increase of these factors. On the contrary, the increase of all three quadratic terms will result in the decrease of Fe^{2+} oxidation rate. The standard deviation (0.0029) between the experimental and predicted results indicates that eqn. 4 adequately represents the actual relationship between the response and the significant variables. Moreover, the analysis of coefficient of determination, R^2 (98.49 %) and R^2 (adjusted) (95.76 %) imply a high dependence and correlation between the observed and the predicted values of the response. It also indicates that 98.49 % of the total variance could be explained by this model.

Analysis of variance (ANOVA) is a statistical technique that subdivides the total variation in a set of data into component parts associated with specific sources of variation to test hypotheses on the parameters of the model[15]. The F statistics value (36.15) for all regressions is high indicating that most of the variation in the response could be explained by the regression equation. And the *p*-value (0.001) is lower than 0.05, which indicates that the model is statistically significant[23].

Analysis of response surface: The response surface and the surface plot were produced using the software of MINITAB, as shown in Fig. 4, to investigate the individual and interactive effects of these factors on the Fe^{2+} oxidation rate. It was obvious that the supplement amounts of NH_4OH, K_2HPO_4 and $MgSO_4{\cdot}7H_2O$ was responsible for the Fe^{2+} oxidation rate. It changed with the variation of supplement amounts of ammonia, K_2HPO_4 and $MgSO_4{\cdot}7H_2O$. Under certain condition (supplement 25 % (v/v) NH_4OH = 1.7929 g/L, K_2HPO_4 = 0.1909 g/L and $MgSO_4{\cdot}7H_2O$ = 0.1768 g/L), a maximal contour (Y = 0.3171 g/L/h) could be predicted.

In order to verify the prediction of the model, confirmatory experiments were carried out using flask culture with the optimal supplement amounts. The average of Fe^{2+} oxidation rate was 0.316 ± 0.008 g/L/h, which was figured well within the estimated value of the model equation. Thus, the model developed is considered accurate and reliable for predicting

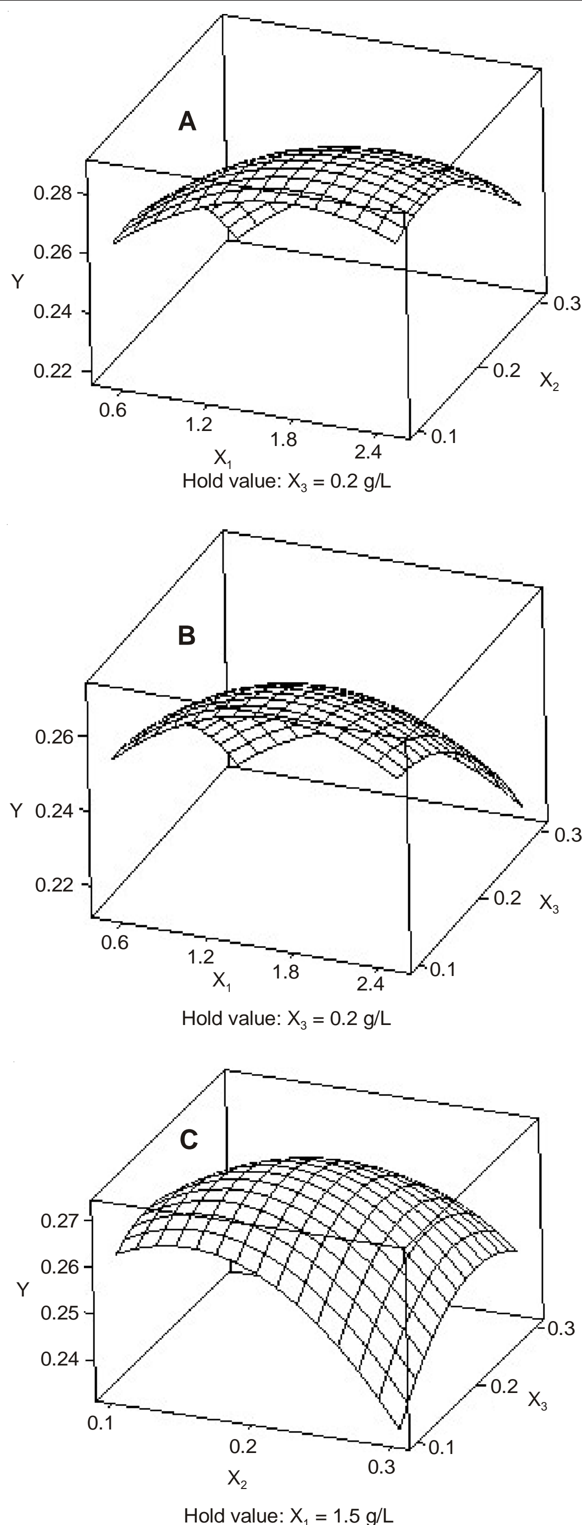

Fig. 4. Surface plots of combined effects of supplement of nutrition on the Fe^{2+} oxidation rate

the supplement amount of nutrition for the recycle use of media after removal of H_2S.

Continued operation of bioreactor: The repeat use of media after removal of H_2S was performed in the bioreactor using the calculated optimal supplementing composition for six times, the results were shown in Fig. 5. However, the Fe^{2+} oxidation rate (3.156 g/L/h) of repeatedly used media for the first times was lower than that of the fresh media (3.346 g/L/h).

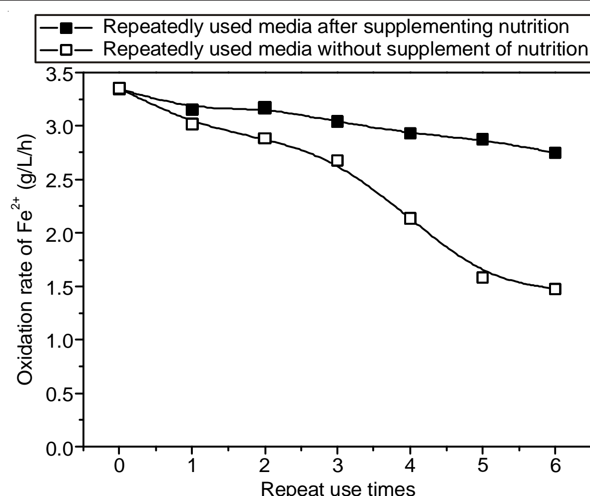

Fig. 5. Fe^{2+} oxidation rate of bioreactor with repeatedly used media 0, fresh meida; 1, 2, 3, 4, 5, 6 repeat use times for refreshed media

It was because that the conversion ratio of Fe^{3+} to Fe^{2+} was 75 % when reacted with H_2S to ensure the removal of H_2S. Additionally the Fe^{2+} conversion ratio was 95 % in bioreactor, thus the concentration of Fe^{2+} in repeatedly used media was 6.5 g/L which was lower than that of fresh media (9 g/L).

Luckily, compared with the fresh media the efficiency of bioreactor with repeatedly used media after supplementing some nutrition showed a relatively stability for 40 days operation. But, it fell to 2.746 g/L/h when using the repeatedly used media for the sixth times because the formation of jarosite which affected the flow of media and air. However, for each repeat use times the Fe^{2+} oxidation rate of repeatedly used media after supplementing nutrition were all higher than those without supplement of nutrition. Thus, the optimal supplement composition calculated by the method of response surface could be considered to provide a possible solution for the continued operation process of bioreactor with immobilized *A. ferrooxidans* after removal of H_2S.

Conclusion

Response surface methodology was proved to be a powerful tool for media optimization of many cells, which could overcome the limitations of classic empirical methods to study the combined effects of culture media compositions[8,22]. In this study, an RSM model was produced to optimize the nutrition supplement amounts of ferrous media for recycle after H_2S removal. With the optimal supplement amounts, the value of Fe^{2+} oxidation rate (0.316 g/L/h) of repeatedly used media was almost equivalent to that of the fresh media (0.325 g/L/h) in flask cultivation. Validation experiments were carried out in the bioreactor. The results showed that the Fe^{2+} oxidation rate of bioreactor supplied with the repeatedly used media which was supplemented some nutrition showed a relatively stable. Therefore, this work provides a possible solution for the continued process with immobilized *A. ferrooxidans* for H_2S removal.

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