Investigation of colloidal biogenic sulfur flocculation: Optimization using response surface analysis

Fan Chen¹, Ye Yuan¹, Chuan Chen¹, Youkang Zhao¹, Wenbo Tan¹, Cong Huang¹, Xijun Xu¹, Aijie Wang^{1,2,*}

1. State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China. E-mail: chenfanhit@163.com
2. Key Laboratory of Environmental Biotechnology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, China

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Abstract: The colloidal properties of biogenic elemental sulfur (S^0) cause solid–liquid separation problems, such as poor settling and membrane fouling. In this study, the separation of S^0 from bulk liquids was performed using flocculation. Polyaluminum chloride (PAC), polyacrylamide (PAM) and microbial flocculant (MBF) were compared to investigate their abilities to flocculate S^0 produced during the treatment of sulfate-containing wastewater. A novel approach with response surface methodology (RSM) was employed to evaluate the effects and interactions of flocculant dose, pH and stirring intensity, on the treatment efficiency in terms of the S^0 flocculation and the supernatant turbidity removal. The dose optimization results indicated that the S^0 flocculation efficiency decreased in the following order PAC > MBF > PAM. Optimum S^0 flocculation conditions were observed at pH 4.73, a stirring speed of 129 r/min and a flocculant dose of 2.42 mg PAC/mg S. During optimum flocculation conditions, the S^0 flocculation rate reached 97.53%. Confirmation experiments demonstrated that employing PAC for S^0 flocculation is feasible and RSM is an efficient approach for optimizing the process of S^0 flocculation. The results provide basic parameters and conditions for recovering sulfur during the treatment of sulfate-laden wastewaters.

Keywords: Elemental sulfur; Flocculation; Optimization; Response surface methodology (RSM)

*Corresponding author: E-mail: ajwang@rcees.ac.cn (Aijie Wang)

Introduction

Sulfate-laden wastewaters are produced by pharmaceutical enterprises, pulp and paper manufacturers, petrochemical plants, mineral processes and acid mine drainage resulting from mining activities (Knobel and Lewis, 2002). In anaerobic environments, sulfate can be converted into sulfide, such as H_2S , which is corrosive to metals and toxic to living species (Celis-Garcia et al., 2008). Biological processes for treating sulfate-laden wastewater mainly include two processes, the reduction of sulfate to sulfide by sulfate-reducing bacteria and the oxidation of sulfide to sulfur (S^0) by sulfide oxidation bacteria (Wang et al., 2005). Yuan et al. (2014a) developed an integrated reactor system for the simultaneous removal of COD, sulfate and ammonium (Integrated C-S-N removal system).

The S^0 reclaimed from sulfate-laden wastewaters can be recovered as a renewable resource for sulfuric acid production, fertilizer industries, and as a substrate for bioleaching processes (Celis-Garcia et al., 2008). S^0 -containing effluents are stable suspensions containing biogenic sulfur colloids that are either largely associated with biomass or cannot be isolated from the suspension because their particle size is too small (Schlegel, 1989; Janssen et al., 1994; Sahinkaya et al., 2011; Yuan et al., 2014b). However, the excessive accumulation of biogenic sulfur without efficient isolation may result in pipe blockage (Beristain-Cardoso et al., 2008; Fortuny et al., 2010) and secondary pollution (Hao et al., 2006). Thus, a highly effective biogenic sulfur isolation step is essential for the successful application of the biological process.

Many researchers have studied biogenic sulfur isolation processes based on the surface characteristics and aggregation of biogenic sulfur (Yuan et al., 2014b; Janssen et

al., 1994; Li et al., 2000, 2006). For example, Li et al. (2000) used the sand filtration-extraction-distillation process and Paques (Holland) developed an air flotation process to separate biogenic sulfur (Cao et al., 2002). In comparison with separation processes such as filtration, extraction and flotation, the plain sedimentation of sulfur particles is the cheapest and most attractive method (Janssen et al., 1996), while flocculation and sedimentation can achieve more efficient biogenic sulfur separation (Yuan et al., 2014b).

In the flocculation process, the efficiency is governed by various factors, such as the type and dosage of flocculant, pH, mixing speed and time, temperature and retention time (Wang et al., 2011). A proper optimization of these factors could significantly increase its treatment efficiency. Response surface methodology (RSM) is an efficient way to achieve such an optimization by analyzing and modeling the effects of multiple variables and their responses and finally optimizing the process. This method has been widely used for the optimization of various processes in food chemistry, material science, chemical engineering and biotechnology (Wang et al., 2011). Córdova et al. (2011), Kiran and Thanasekaran (2011), and Özer et al. (2008) studied the biosorption of lead, copper and nickel on *Aspergillus terreus*, *Lyngbya putealis* and *Enteromorpha prolifera*, respectively, using response surface methodology. Zheng et al. (2014) investigated the optimization of the flocculation process using RSM for diethyl phthalate removal with anionic polyacrylamide. Jadhav and Mahajan (2014) successfully applied RSM in water/wastewater treatment using *Coccinia indica* and found that RSM is a highly effective tool for optimizing the flocculation process.

The main objective of this work was to separate S^0 using the flocculation process, which was optimized using RSM. Removal efficiencies of both S^0 and supernatant turbidity were chosen as the dependent output variables. The novel optimization strategy used for S^0 flocculation in this study is expected to provide basic parameters and conditions for recovering sulfur during the treatment of sulfate-laden wastewaters.

1 Materials and methods

1.1 Integrated C-S-N removal system and effluent

The integrated C-S-N removal system modified from Yuan et al. (2014a) was used to produce S^0 -containing effluent. The plexiglass expanded granular sludge blanket reactor was a modified version of the reactor developed by Chen et al. (2008). The reactor was kept at $30 \pm 1^\circ\text{C}$. The compositions of the medium and the micronutrients were described by Yuan et al. (2014a). An internally circulating fluid with reflux ratio of 6:1 was used to suspend granules in the reactor. Adding 1.6 kg TOC/($\text{m}^3 \cdot \text{day}$), 1 kg SO_4^{2-} /($\text{m}^3 \cdot \text{day}$), and 0.6 kg N/($\text{m}^3 \cdot \text{day}$), respectively, resulted in nearly complete conversion of sulfate, nitrate and TOC to S^0 , N_2 and CO_2 . The effluent from the denitrifying sulfide removal unit (**Fig. 1**) was a milky white suspension with a turbidity of 350 ± 25 nephelometric turbidity units (NTU) and a pH of 9.80 ± 0.20 . The zeta potentials of the suspended particles in the S^0 -containing effluent were -19.6 ± 1.4 mV, and the S^0 concentration was between 100 and 120 mg/L.

1.2 Preparation of flocculant

Flocculants are classified as inorganic flocculants, such as polyaluminum chloride (PAC), synthetic organic flocculants, such as polyacrylamide (PAM) derivatives, and natural occurring flocculants, such as microbial flocculant (MBF) and chitosan (Bezawada et al., 2013; Prazeres et al., 2013; Riaño and García-González, 2014). Chemical flocculants are commonly used in water and wastewater treatment industries because of their cost-effectiveness and efficient flocculation abilities (More et al., 2014). Bioflocculants have attracted research and industry interest as alternative flocculants due to their high flocculation performance, ecofriendliness, and biodegradability (Aljuboori et al., 2013, 2014; Bezawada et al., 2013). Therefore, PAC, PAM and MBF were chosen in this study. Moreover, because biogenic sulfur colloids carry a negative charge, cationic

polyacrylamide was used to separate biogenic sulfur. PAC and PAM, purchased from Tianjin Chemical Reagent Co., China, were of analytical reagent grade and used without further purification. MBF was produced by the mixed culture of F2 and F6 with the proportion of 1:1 at the following fermentation conditions: fermentation time of 24 hr, temperature of 30°C, rotation speed of 150 r/min (Zhu et al., 2006). Strain F2 and F6 are *Bacillus sp.*.

1.3 Batch flocculation studies

Batch flocculation experiments were performed in 500 mL beakers containing 300 mL S^0 -containing effluent mixed with known flocculant doses. In a preliminary study, experiments were initiated to determine narrower ranges of flocculant dose, pH and stirring intensity before designing the experimental runs. According to the results of the preliminary experiments for different flocculant, there were significant effects on S^0 flocculation when the dose, pH and stirring intensity changed from 1.3 to 3.3 mg flocculant/mg S, 3 to 9 and 80 to 160 r/min, respectively. Next, dose optimization batch experiments were performed at 25°C with rapid mixing (300 r/min, 3 min), and then slow mixing (120 r/min, 10 min) with 1.3-3.3 mg flocculant/mg S was performed to determine the optimum dose. Then, the suspension was allowed to settle freely for another 30 min. Suspension samples at 2 cm below the water surface were collected to analyze their turbidity and sulfur contents. Coagulation tests with no flocculants were used as controls. Next, the interactions of pH (ranging 3-9) and mixing speed (ranging 80-160 r/min) on the flocculating rate were investigated. The slow mixing rate changed when the effect of the mixing speed was considered. The pH of each solution was initially adjusted using 1 and 0.1 mol/L HCl and NaOH solutions to reach the required pH value before adding the flocculant. All of the experiments were performed in triplicate, and their mean values are reported. The biogenic sulfur flocculation rate (θ , %) was calculated according to the following Eq. (1):

$$\theta = (A_0 - A_1) / A_0 \times 100\% \quad (1)$$

where, A_0 (mg/L) is the total biogenic S^0 concentration in the effluent of denitrifying sulfide removal unit and A_1 (mg/L) is the S^0 concentration in the supernatant after flocculation.

1.4 Experimental design and optimization

The effects of operating parameters were optimized using RSM. Design Expert (version 8.0.1, Stat-Ease, Inc., MN) software was used for statistical data analysis. RSM represents independent process variables using the following quantitative equation:

$$Y = f(A_1, A_2, A_3, \dots, A_n) \quad (2)$$

where, Y is the biogenic sulfur flocculation rate; f is the response function, and $A_1, A_2, A_3, \dots, A_n$ are independent variables.

The response surface is obtained by plotting the expected response; however, the value of f is unknown and can be very complicated. A quadratic model that includes the linear model used to predict the response variable and explore the design surface is shown below (Eq. (3)).

$$Y = b_0 + \sum_{j=1}^k b_j A_j + \sum_{j=1}^k b_{jj} A_j^2 + \sum_i \sum_{<j=2}^k b_{ij} A_i A_j + \varepsilon \quad (3)$$

where, Y is the biogenic S^0 flocculation rate, A_i and A_j are variables, b_0 is the constant coefficient, b_j , b_{jj} and b_{ij} are interaction coefficients of the linear, quadratic and second order terms, respectively, and ε is the error.

In this study, central composite design (CCD) was used for RSM in the experimental design, which is well suited for fitting a quadratic surface and usually works well for process optimization. In this study, one factor design using 5 levels for a quadratic model was used to evaluate the effects of flocculant dose on S^0 flocculation (**Table 1**). pH and stirring intensity of S^0 flocculation were studied using the CCD model with two levels (the minimum and the maximum) when the optimum dose was determined. The

experimental factor levels used in the factorial design are described in **Table 2**. In the experimental design model, pH (3-9) and stirring intensity (80-160 r/min) were used as input variables. In addition, the biogenic S^0 flocculation rate was used as the response of the system. The experimental design matrix derived from the CCD model and the results is shown in **Table 3**.

The quality of the polynomial model fit was expressed using the regression coefficient (R^2) and R_{adj}^2 . The statistical significance was checked using an adequate precision ratio and the F -test.

1.5 Analysis methods

The collected liquor samples were passed through 0.45 μm filters before measuring the sulfate and thiosulfate concentrations using an ion chromatograph (ICS-3000, Dionex, USA) equipped with a conductivity detector and an Ion-Pac AG4A AS4A-SC 4 mm analytical column. Elemental sulfur in the Integrated C-S-N removal system effluent was measured using the sulfite method (Jiang et al., 2009). A JJ-3A six digital electric mixer was used to stir the solution.

2 Results and discussion

2.1 Effect of flocculant dose on biogenic S^0 flocculation

2.1.1 RSM one-factor designs and results

The flocculant dose was varied from 1.3 to 3.3 mg flocculant/mg S, while the other parameters were held constant (temperature 25°C, rapid mixing (300 r/min, 3 min), slow mixing (120 r/min, 10 min) and pH 5). The experimental designs and results are shown in **Table 1**.

The S^0 flocculation and turbidity removal curves were fitted using multiple regressions in Design Expert 8.0.1 based on the experimental value and the RSM one-factor predicted value (**Fig. 2**). It is important to determine whether fitted curving

adequately approximates real values. Graphical and numerical methods are primarily used to validate the models (Jadhav and Mahajan, 2014). In this case, the R^2 value of the biogenic S^0 flocculation curves (PAC, 0.9943; PAM, 0.9671; MBF, 0.9963) and the turbidity removal curves (PAC, 0.9994; PAM, 0.9433; MBF, 0.9741) only indicate that 5.67%-0.06% of the total variation is not explained by these curves. The R_{adj}^2 values of the biogenic S^0 flocculation curves (PAC, 0.9829; PAM, 0.9506; MBF, 0.9925) and turbidity removal curves (PAC, 0.9829; PAM, 0.8866; MBF, 0.9481) are high enough to indicate that these fitting curves were highly significant. **Fig. 3** is the normal probability plot for S^0 flocculation and confirms that the assumptions of normality were satisfied for the experimental data. Adequate precision can be used to measure the signal to noise ratio, and a ratio greater than 4 is considered desirable. Therefore, the ratios of the biogenic S^0 flocculation capacity (PAC, 23.81; PAM, 15.76; MBF, 34.78) and turbidity removal (PAC, 71.12; PAM, 10.23; MBF, 13.60) indicate adequate signals for the models used to navigate the design space.

2.1.2 Biogenic S^0 flocculation and turbidity removal analysis

The effects of flocculant dose on the S^0 flocculation rate by PAC, PAM and MBF are shown in **Fig. 2**, which also shows that PAC and MBF were more effective than PAM for S^0 flocculation. The S^0 flocculation capacity of PAC increased up to 2.30-2.80 mg PAC/mg S, and the S^0 flocculation rate reached 81%-86% before decreasing as the PAC dose increased. The S^0 flocculation capacity of PAM gradually increased to 13%-14% as the PAM dose increased to 2.3-2.8 mg PAM/mg S, and then remained relatively constant. Compared with PAC and PAM, the effects of the MBF dose on the S^0 flocculation rate were obviously different. The S^0 flocculation rate rapidly increased from 1.3 to 1.8 mg MBF/mg S before gradually increasing as the MBF dose increased to 2.3-2.8 mg MBF/mg S, reaching a flocculation rate of 75%-76% S^0 . Above 2.3-2.8 mg MBF/mg S, the S^0 flocculation capacity of MBF remained stable. These results indicated that

excessive flocculant doses resulted in colloidal restabilization, while low doses were insufficient for destabilizing the aggregates in most of the biogenic S^0 colloids in the effluent, and the both excessive and low doses resulted in low flocculation rates.

Turbidity depends on the degree of water purification and is an important index for evaluating the effects of biogenic sulfur flocculation. The turbidity removal showed trends similar to those of S^0 flocculation (**Fig. 2**). The turbidity decreased as the PAC dose increased to 1.3-1.5 mg PAC/mg S and increased when greater PAC doses were used. Maximum turbidity removal occurred at 2.3-2.8 mg PAC/mg S, which corresponded to a turbidity removal of approximately 82%-88%, and then decreased as the PAC dosage increased. The removal of turbidity did not significantly change as the MBF dose increased and fluctuated between approximately 30%-46% (**Fig. 2**). Meanwhile, the removal rate of turbidity fluctuated between approximately 21% and 36% as the PAM dose increased from 1.3 to 3.3 mg PAM/mg S. When the turbidity removal plateaued, the flocculant dose was approximately the same as that during maximum S^0 flocculation.

Fig. 2 shows that optimum S^0 biogenic flocculation points were observed when using PAC, PAM and MBF. The optimum dose could be obtained by parsing the flocculation curves in Design Expert 8.0.1. The predicted optimal doses and experimental validation results are shown in **Table 4**. As shown in **Table 4**, the experimental values of the S^0 flocculation rate reached 86.43%, 13.14% and 77.90% at 2.42 mg PAC/mg S, 2.75 mg PAM/mg S and 2.38 mg MBF/mg S, respectively.

In the flocculation process, the flocculant was used to agglomerate the destabilized colloidal particles into large particles and then precipitates (Wang et al., 2011). The major mechanisms of flocculation of PAC were surface-charge neutralization and bridging (Gregory, 1996). For PAC, surface-charge neutralization occurred because its charge was opposite in sign to S^0 , which result in aggregation caused by specific ion absorption. For PAM, the most important mechanism of flocculation is the polymer bridging, which occurs because segments of a polymer chain get absorbed on various particles, thus linking the

particles together (Tripathy et al., 2001). Usually, PAM has good adsorption effect on the small destabilized flocs because of the carboxylic functional groups and the molecular chains. But due to its microsize and zeta potential (-19.6 ± 1.4 mV), biogenic S^0 exhibits colloidal properties, hampering its adsorption with PAM. The single PAM had low efficiency in S^0 removal because there is little charge on its polymer to aggregate the suspension during the process of flocculation (Zhang et al., 2004) and the adsorption capacity of PAM is not enough to destabilize the colloid system. For MBF, the adsorption bridging and compressing the electric double layer played the leading role in the flocculation process (Wang, et al., 2015). Meanwhile, the net-charge of MBF can promote the S^0 flocculation. As a result, PAC were more effective as flocculating agents for destabilizing and sedimenting colloidal S^0 .

2.2 RSM approach for optimizing the flocculation conditions

2.2.1. Model building and data analysis

In this study, two parameters (pH and mixing speed) were studied using a CCD model with two levels (the minimum and maximum). As shown in **Table 3**, the S^0 flocculation rate by PAC, PAM and MBF fluctuated between 21.5%-96.7%, 3.2%-15.5% and 15.9%-73.1%, respectively, in the tested pH and stirring intensity ranges. Meanwhile, 91.7%-96.7%, 14.8%-15.5% and 69.2%-73.1% S^0 flocculation rates were obtained at pH 6 and at a stirring speed of 120 r/min in the PAC, PAM and MBF case, respectively. These data show that the interactions between pH and the stirring intensity affected S^0 flocculation. Statistical analysis of the experimental data is necessary to establish optimal S^0 flocculation conditions in the range of the studied variables. The experimental results were evaluated, and quadratic models of the PAC and MBF flocculation capacities for S^0 were obtained using Eqs. (4)-(5), respectively.

$$y_1 = 91.32 - 22.77A + 8.98B - 28.71A^2 - 26.56B^2 \quad (4)$$

$$y_2 = 14.67 - 1.92A + 1.87B - 0.67AB - 5.65A^2 - 4.00B^2 \quad (5)$$

$$y_3 = 69.93 - 12.57A + 5.60B - 3.02AB - 21.06A^2 - 24.96B^2 \quad (6)$$

In Eqs. (4)-(5), y_1 , y_2 and y_3 are the S^0 flocculation rates following the addition of different PAC, PAM and MBF doses, respectively, and A and B correspond to the independent variables (pH and stirring intensity).

As shown in **Table 5**, when "Prob > F" is less than 0.0500, the model terms are significant. In PAC case and in MBF case, the values of A , A^2 , and B^2 were significant model terms. In PAM case, A , B , A^2 , B^2 are significant model terms. The normal probability and studentized residual plots are shown in **Fig. 4** for the flocculation of S^0 by PAC, PAM and MBF, respectively. The R^2 value (PAC, 93%; PAM, 96%; MBF, 95%) indicates that the model could explain the majority of the total variations. The experimental values correspond well with the predicted values for PAC, PAM and MBF. The statistical analysis results showed that these quadratic models could be used to navigate the design space.

2.2.2 Mutual parameter effects

A response surface plot was used to determine S^0 flocculation by PAC, PAM and MBF over interactive variables pH and stirring intensity (**Fig. 5**). The two dimensional contour plots are shown in **Fig. 6**. As shown in **Fig. 5**, the flocculation of S^0 increased in the PAC case as the pH increased up to 4.6-4.9 and then decreased as the pH continued to increase. For PAM and MBF, maximum flocculation occurred at pH 5.3-5.6 and 5.0-5.2, respectively, and lower flocculation occurred at higher pH values, potentially due to the negative charge on the biogenic S^0 surface. The amount of positive charge needed to neutralize the negative charge of biogenic S^0 at low pH is greater than that at high pH. Meanwhile, pH is the most important factor that affects the Zeta potential, and the absolute value of the Zeta potential approaches zero as the pH decreases, making the colloid dispersed system unstable (Hunter, 2013). The acidic condition was in favor of the improvement of cationic charge density as well as the extension of the grafting chain

in the solution (Wang et al., 2011). Both the charge neutralization ability and the sweep-floc ability were enhanced in this case. Taking the two factors into account together, acidic condition was appropriate for the biogenic S^0 flocculation.

Fig. 5 also shows the effects of stirring intensity on flocculation. Mixing during the flocculation process provides close encounters between the particles and flocculating agents (Chen et al., 1998). To achieve a high flocculation rate, the effects of high and low mixing speeds on biogenic S^0 flocculation were investigated. As shown in **Fig. 5**, the flocculation resulting from PAC and PAM increased as the mixing speed increased to 120-130 r/min, and then slightly decreased. In the MBF case, the biogenic S^0 flocculation rate sharply increased with increasing stirring intensity. The maximum flocculation rate observed at 120-130 r/min was 70%-75%. When the stirring intensity was between 80 and 120 r/min, the S^0 flocculation results indicated that the flocculation rate increased as the mixing speed increased, which provided more opportunities for contact between the S^0 and flocculants to allow for aggregation and to increase the flocculation rate. However, flocs are easily destroyed when mixing speeds are too fast. Thus, the flocculation rate decreased as the mixing speeds increased beyond 120 r/min.

2.2.3 Optimization analysis and model validation

Optimum factor levels were obtained by analyzing the response surface contour and derivatives of the equation of the above model. According to the results shown in the contour plot (**Fig. 6**), relatively high S^0 flocculation rates of 97.12%, 15.08% and 72.23% were predicted at pH 4.73 and 128.85 r/min in the PAC case, pH 5.45 and 129.96 r/min in the PAM case and pH 5.08 and 125.25 r/min in the MBF case, respectively.

Verification tests were performed using the predicted optimal parameters, and the results are summarized in **Table 6**. As shown in **Table 6**, at pH 4.73 and 129 r/min, the experimental value of the S^0 flocculation rate reached 97.53% when 2.42 mg PAC/mg S was added. At pH 5.45 and 130 r/min, the experimental value of the S^0 flocculation rate

reached 15.41% when 2.75 mg PAC/mg S was added. At pH 5.08 and 125 r/min, the experimental value of the S^0 flocculation rate reached 71.98% when 2.38 mg MBF/mg S was added. The S^0 flocculation rates obtained from experimentation were very similar to those estimated using the quadratic model. Consequently, the RSM approach was successfully applied to model and optimize the biogenic S^0 flocculation process.

3 Conclusions

This study successfully compared the PAC, PAM and MBF performances for biogenic S^0 flocculation and established optimum operating parameters for sulfur recovery. The effects of flocculant dose on biogenic S^0 flocculation indicated optimal doses of 2.42 mg PAC/mg S, 2.75 mg PAM/mg S and 2.38 mg MBF/mg S, with S^0 flocculation rates reaching up to 86.78%, 13.37% and 78.27%, respectively. Based on the RSM approach, which uses CCD for experimental design, and the fitness of the polynomial equation, the optimal S^0 flocculation conditions occurred under conditions of pH 4.73, 129 r/min and 2.42 mg PAC/mg S. Under these conditions, the predicted and actual flocculation efficiencies reached 97.12% and 97.53%, respectively, in the presence of PAC.

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JES-D-15-00867- tables

Table 1 Experimental design and flocculant dose optimization results

Experimental run	Flocculant dose (mg flocculant/mg S)	Biogenic S^0 flocculation rate (%)			Turbidity removal (%)		
		PAC	PAM	MBF	PAC	PAM	MBF
1	2.80 (05.)	81	14	76	82	23	40
2	3.30 (1.0)	75	11	67	70	28	43
3	2.30(0.0)	86	13	75	88	35	48
4	3.30 (1.0)	77	12	69	71	27	44
5	1.30 (-1.0)	63	2	6	45	18	30
6	1.30 (-1.0)	64	3	8	46	22	32
7	1.80 (-0.5)	70	7	64	53	36	46

PAC: polyaluminum chloride; PAM: polyacrylamide; MBF: microbial flocculant.

Table 2 Experimental factor levels used in the factorial design

Factor	Code	Units	Low	Central value	High
pH	A		-1 (3.5)	0 (5.25)	1 (7)
Stirring intensity	B	r/min	-1 (80)	0 (120)	1 (160)

Table 3 Experimental design and biogenic S^0 flocculation parameter results

Run	pH	Stirring intensity (r/min)	Biogenic S^0 flocculation rate (%)			Turbidity removal (%)		
			PAC	PAM	MBF	PAC	PAM	MBF
1	3	80	44.2	5.3	26.7	43.4	14.6	26.3
2	9	80	20.6	3.2	15.9	19.8	10.6	16.1
3	3	160	71.5	9.1	41.5	68.1	16.4	44.9
4	9	160	21.5	4.3	18.6	23.7	12.5	17.8
5	3	120	87.3	10.4	66.2	85.2	18.5	61.4
6	9	120	24.3	5.8	24.5	26.2	13.7	24.3
7	6	80	45.1	6.6	33.4	46.0	16.2	27.8

8	6	160	70.8	12.9	49.5	69.7	20.3	50.2
9	6	120	91.7	14.8	73.1	94.3	34.8	69.3
10	6	120	95.8	15.1	69.2	93.5	33.9	64.7
11	6	120	93.4	14.6	70.8	91.6	35.6	68.5
12	6	120	96.7	15.5	72.4	98.4	37.7	67.8
13	6	120	92.6	15.2	71.2	95.8	36.4	69.4

Table 4 The predicted optimal dose and verification results

Solution	Dose	Biogenic S ⁰ flocculation rate	
		Prediction (%)	Validation (%)
PAC	2.42 mg PAC/mg S	86.78	86.43
PAM	2.75 mg PAM/mg S	13.37	13.14
MBF	2.38 mg MBF/mg S	78.27	77.90

Table 5 ANOVA results of the quadratic models for biogenic S⁰ flocculation

Source	Solution	Model	A-pH	B-Stirring intensity	AB	A ²	B ²
Sum of Squares	PAC	10587.65	3109.927	484.2017	174.24	2276.594	1948.391
	PAM	255.5252	22.04167	20.90667	1.8225	88.27456	44.2667
	MBF	5911.346	947.5267	188.16	36.6025	1224.809	1720.481
df		5	1	1	1	1	1
Mean Square	PAC	2117.531	3109.927	484.2017	174.24	2276.594	1948.391
	PAM	51.10503	22.04167	20.90667	1.8225	88.27456	44.2667
	MBF	1182.269	947.5267	188.16	36.6025	1224.809	1720.481
F Value	PAC	26.44388	38.83698	6.046745	2.175921	28.43026	24.33164
	PAM	33.21049	14.32373	13.58615	1.184348	57.36503	28.76662
	MBF	27.14088	21.75199	4.319513	0.840269	28.11746	39.49639
p-value Prob > F	PAC	0.0002	0.0004	0.0435	0.1837	0.0011	0.0017
	PAM	< 0.0001	0.0069	0.0078	0.3125	0.0001	0.0010
	MBF	0.0002	0.0023	0.0763	0.3898	0.0011	0.0004

Table 6 The predicted optimal parameters and verification results

Solutions		pH	Stirring intensity (r/min)	Biogenic S ⁰ flocculation rate (%)
PAC	Predicted value	4.73	128.85	97.12
	Experimental value	4.73	129	97.53
PAM	Predicted value	5.45	129.96	15.08
	Experimental value	5.45	130	15.41
MBF	Predicted value	5.08	125.25	72.23
	Experimental value	5.08	125	71.98