Bioresource Technology 100 (2009) 4233–4239

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Optimization of flocculation conditions for kaolin suspension using the composite flocculant of MBFGA1 and PAC by response surface methodology

Zhao-Hui Yang *, Jing Huang, Guang-Ming Zeng, Min Ruan, Chang-Sheng Zhou, Lu Li, Zong-Gen Rong

College of Environmental Science and Engineering, Hunan University, Changsha 410082, China

ARTICLE INFO

Article history:
Received 20 August 2008
Received in revised form 15 December 2008
Accepted 15 December 2008
Available online 5 May 2009

Keywords:
Microbial flocculants
Polyaluminium chloride (PAC)
Composite flocculant
Response surface methodology (RSM)

ABSTRACT

The response surface methodology (RSM) was employed to study the treatment of kaolin suspension by the composite flocculant of MBFGA1 and PAC. And the two quadratic models of the five factors were established with the flocculating rate and floc size as the target responses. The optimal flocculating conditions are MBFGA1 99.75 mg/L, PAC 121 mg/L, pH 7.3, $CaCl_2$ 27 mg/L and the top speed of stir 163 rpm, respectively. That was obtained from the compromised results of two desirable responses, flocculating rate as 100% and floc size as 0.7 mm which were deduced from the frequency of responses. By means of Zeta potential measurement and experiment of flocculating process, it could be concluded that PAC has more capability on changing the potential of colloid and MBFGA1 is good at absorption and bridge effect. The composite of two kinds of predominance makes a significant sense on enhancing flocculating rate, reducing flocculent costs and decreasing secondary pollution.

© 2008 Published by Elsevier Ltd.

1. Introduction

Flocculants, especially the type of synthetic chemical, nowadays have been widely used in material separation processes, such as tap water production, wastewater treatment, dehydration of activated sludge, dredging, downstream processing, food and fermentation process (Zhang et al., 1999; Salehizadeh and Shojaosadati, 2001). Despite the effective flocculating performance and low cost of the synthetic chemical flocculants, their popularization has resulted in some health and environmental problems. For example, the remaining aluminium causes the incidence of Alzheimer's disease (Christopher et al., 2006; Arezoo, 2002). Furthermore, the acrylamie monomer is not only neurotoxic and carcinogenic but also nonbiodegradable in nature (Rudén, 2004). On the contrary, bioflocculant is a kind of environment-friendly material with the character of nontoxic and biodegradable (Kurane et al., 1986; Deng et al., 2003). In recent years, the use of bioflocculants has been considered as a solution to the secondary environment pollution caused by the traditional flocculants (Joung et al., 2007).

However, flocculating activity and cultivation cost are always the major limitation of bioflocculants application (Kurane et al., 1994; Li et al., 2003; He et al., 2004; Jang et al., 2001). Consequently, over the past decades, emphasis on this field was mainly placed on screening new strains producing bioflocculants with high flocculating activity and optimization of their culture conditions for a higher yield and lower cost. Simultaneously, in the flocculating process, composite flocculant is another way to reduce the cost. Because this kind of flocculant can reduce the dosage of bioflocculant and can improve the flocculating activity. From another aspect, composite flocculant reduces the risk brought by the synthetic chemical flocculants, since their dosage was decreased to the least.

In present study, the polyaluminium chloride (PAC) was selected to be compounded with MBFGA1. And the response surface methodology (RSM), an effective tool for building a multivariable equation and finding their optimal values (Triveni et al., 2001), was employed to optimize the conditions of flocculating process and to investigate the interactive effects of experimental factors including dosage of MBFGA1, PAC, $CaCl_2$, pH and the top speed of stir. The kaolin suspension was used as a representative colloidal material because kaolin is a well-known and widespread thickening agent. Also the surface characteristics of kaolin are well-understood to allow analysis, and in consequence kaolin has received great interest in recent years (Nasser and James, 2007). The flocculating rate and the floc size were chosen as the response variables, and the optimal conditions were their compromised result.

2. Methods

2.1. Materials

The kaolin was chemically pure grade (Sanpu Chemicals, China), and its suspension was prepared at the concentration of 3 g/L. The upper phase was used for the flocculating experiment after 1 h of stir at 300 rpm and 2 h of gravity settlement. PAC with Al hydrolysis

* Corresponding author. Tel.: +86 0731 8649168; fax: +86 0731 8822829.
E-mail address: yzh@hnu.cn (Z.-H. Yang).

0960-8524/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.biortech.2008.12.033

ratio and Al_2O_3 (Fuyuan Chemicals, China) concentration of 2.4 and 28%, respectively, was prepared at the concentration of 10 g/L. $CaCl_2$, analytically pure grade (Sanpu Chemicals, China), was prepared at the concentration of 3 g/L. And the $CaCl_2$ can provide Ca^{2+} for the flocculating process as a kind of coagulant aid which increases the initial adsorption of biopolymers on suspended particles by decreasing the negative charge on both the polymer and the particle (David and Matthew, 2002; Salehizadeh and Shojaosadati, 2001).

2.2. Bacteria strain and culture conditions

GA1, flocculant-producing strain CCTCC M206017, which was identified as *Paenibacillus polymyxa* by 16S rDNA sequence and its biochemical and physiological characteristics, was screened from the soil collected in the Yuelu Mountain Changsha, China (Yang et al., 2006). The composition of the seed medium was as follows: peptone 10.0 g, beef extract 3.0 g and NaCl 5.0 g in 1 L distilled water with the pH adjusted to 7.0. The steam sterilization lasted for 30 min at the pressure of 0.1 MPa. After the inoculation of the seed medium, the GA1 was cultured on the roundabout shaker at 150 rpm and 30 °C for 24 h.

The fermentation medium consisted of sucrose 40.0 g, yeast extract 4.0 g, K_2HPO_4 5.0 g, KH_2PO_4 2.0 g, NaCl 0.1 g and $MgSO_4$ 0.2 g dissolved in 1 L distilled water with the pH adjusted to 7.0. The steam sterilization lasted 30 min at the pressure of 0.07 MPa. And this cultivated process was divided into two stages. In the first 24 h, the medium was incubated at 30 °C and was shaken at 150 rpm which provided a positive effect for the growth of the bacteria. In the following 48 h, the rotating speed of shaker was decelerated to 100 rpm and the temperature was decreased to 25 °C, since this conditions was favorable to the yield of MBFGA1. After 72 h of cultivation, the fermentation liquid with the effective components, linear molecule of polysaccharide containing lots of hydroxyl and carboxyl (Ruan et al., 2007), of 17.5 g/L was stored in 4 °C, and it will be utilized directly in the flocculation.

2.3. Measurements of flocculating activity

Flocculating activity was evaluated by the flocculating rate (FR) according the Eq. (1) (Kurane et al., 1994),

$$FR = \frac{(b-a)}{b} \times 100\% \tag{1}$$

where a and b are the optical densities (OD) of the sample and control, respectively, at 550 nm. The flocculating object was the kaolin suspension. The beaker, capacity of 800 mL, was injected 400 mL kaolin suspension and then fixed on the floc-tester (ET-720, Lovibond, Germany). After each addition, the reaction was preformed with rapid mixing for 30 s, followed by slow mixing at 40 rpm for 5 min. The OD of the upper phase was measured at 550 nm with a spectrophotometer (7230G, Shanghai, China) when the mixture was sustained for 80 min of gravity settlement procedure.

2.4. Floc size analysis

For Reynolds numbers less than 1.0, viscosity is the predominant force governing the settling process. The settling velocity of spherical particle is given by Eq. (2) (Duncan, 1992),

$$u_p = \frac{g(\rho_p - \rho_w)}{18\mu} d_p^2 \tag{2}$$

where u_p is the floc settling velocity, ρ_p and ρ_w are the density of floc and water, respectively, g is acceleration due to gravity, μ is dynamic viscosity and d_p is diameter of floc. Assuming that flocs are spherical particles with the same density, we can put forward a conclusion that flocs in various diameters will get different settling velocity. According to this theory, a transparent rectangular container (15 × 5 × 100 cm) was applied to separate the flocs huddled together. And a digital camera was placed on the base of the container where the flocs were separated completely, thus assuring the camera to capture each of the flocs clearly. A black background, at a low level of reflection, was also needed to provide good quality images, and a measuring tape with scale in millimeters was set-up on the surface of the container to furnish a direct scale in images. The diameters of flocs were analyzed from the images by software named Image-pro Plus6.0 (Media Cybernetics Inc., USA), and they were sequenced according to their numerical magnitude in each group. The top 50 will be used to calculate the mean value.

2.5. RSM experimental design

The central composite design (CCD), a standard RSM, was selected for the optimization of the factors which made sense on the flocculating effect. In this design, five factors were the dosage of MBFGA1 (x_1), PAC (x_2), $CaCl_2$ (x_4), pH (x_3) and the top speed of stir (x_5), respectively. All factors were controlled at five levels. The response variable (y) that represented flocculating rate or floc size was fitted by a second-order model in the form of quadratic polynomial equation:

$$y = \beta_0 + \sum_{i=1}^{m} \beta_i x_i + \sum_{i<j}^{m} \beta_{ij} x_i x_j + \sum_{i=1}^{m} \beta_{ii} x_i^2 \tag{3}$$

where y is the response variable to be modeled, x_i and x_j are independent variables which determine y, β_0, β_i and β_{ii} are the offset term, the i linear coefficient and the quadratic coefficient, respectively. β_{ij} is the term that reflect the interaction between x_i and x_j (Otto, 1998). The actual design ran by the statistic software, Design-expert 7.1.3 (Stat-Ease Inc., USA), is presented in Table 1.

2.6. Zeta potential analysis

The variation of Zeta potential during the process of flocculation using PAC was measured by Zetasizer 2000 (Malvern Instruments Ltd., Company, England). And the procedure of sampling was carried out at three different time-points: kaolin suspension without additives, after adjusting the pH and after adding the flocculant. Correspondingly, the process using MBFGA1 or composite flocculant would be inserted a point following adding $CaCl_2$.

3. Results and discussion

3.1. Determination of the dose range

The OD_{550} changed with the time at the different dosage of MBFGA1 varying from 43.75 to 262.5 mg/L is shown in Fig. 1a. And the flocculation using PAC (25–150 mg/L) is shown in Fig. 1b. In the MBFGA1 flocculating process, pH of 400 mL kaolin suspension was adjusted to 7.0, and 2 mL $CaCl_2$ of 3 g/L was then

Table 1
Coded levels for five variables framed by CCD.

Factors	Codes	Coded levels				
		−2.38	−1	0	1	2.38
MBFGA1 (mg/L)	x_1	43.75	94.46	131.25	168.04	218.75
PAC (mg/L)	x_2	25	82.96	125	167.04	225
pH	x_3	5.0	6.2	7.0	7.8	9.0
$CaCl_2$ (mg/L)	x_4	0.00	14.49	25	35.51	50
Top speed (rpm)	x_5	100	158	200	242	300

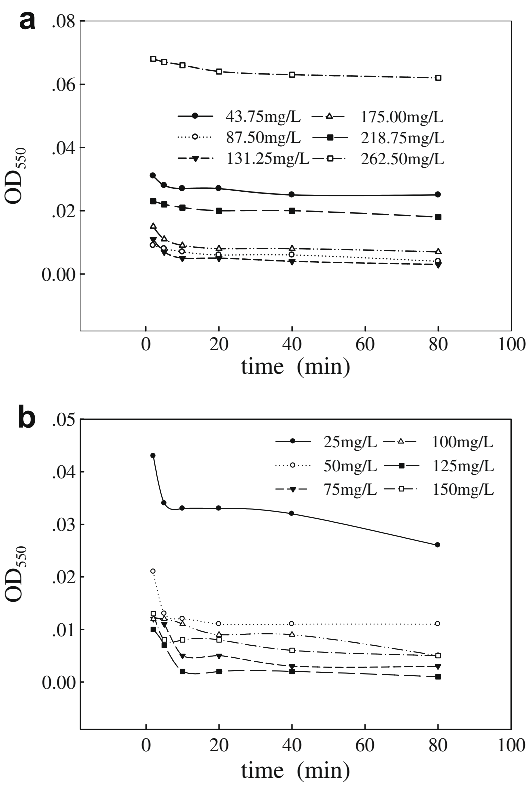

Fig. 1. OD_{550} varied with time at different dosage of (a) MBFGA1 and (b) PAC.

added to it. The pH of PAC flocculating process was also adjusted to 7.0. The time-points for sampling were 2, 5, 10, 20, 40 and 80 min (Sagbo et al., 2008).

Fig. 1a shows that OD_{550} goes to the lowest value when the dosage of MBFGA1 is 131.25 mg/L. According to Eq. (1), it means that MBFGA1 is with the best flocculating activity at 131.25 mg/L. In the same way, PAC gets the best at 125 mg/L (see Fig. 1b). It is well-known that excessive dosage of flocculant leads to the stabilization of the colloidal system again. Therefore, the flocculating rates that varied with the dosage presented a kind of normal distribution approximately and manifested descending trend, when the dosage of MBFGA1 and PAC exceeded the optimal amount. Furthermore, the background value held by the flocculants could influence the OD_{550}, especially at a large amount. Compared with Fig. 1b, most of the curve slopes in Fig. 1a from 2 to 10 min are smaller. In other words, this phenomenon means that flocs produced by MBFGA1 have a higher settling velocity than PAC, because the majority of flocs produced by MBFGA1 had finished settlement process in the first 5 min, which was much shorter than the others. The difference between these two kinds of flocculants attributed to the floc size and density. In the flocculating process of MBF, research has demonstrated that the biopolymers can form a matrix which encapsulates the microbes and aids in the aggregation of particles, and floc formation (David and Matthew, 2002). This mechanism produced a positive effect on the floc size and density. In addition, the proper level of $CaCl_2$ was helpful for the size by means of the absorption bridging action. On the basis of this difference between MBFGA1 and PAC, the size controlling of floc could be achieved by their composite flocculant.

3.2. Experimental results of RSM

The response variables, flocculating rate and floc size, obtained from 50 groups of experiments including parallel control groups are summarized in Table 2.

3.2.1. Data analysis of flocculating rate as the response variable

Following equation represents empirical relationship in the form of quadratic polynomial between the flocculating rate (y_1) and the other five factors ($x_1 - x_5$).

$$\begin{aligned} Y_1 = & -1514.02 + 5.46X_1 + 0.15X_2 + 278.91X_3 + 1.93X_4 \\ & + 0.87X_5 - 9.36 \times 10^{-3}X_1X_2 - 0.51X_1X_3 + 2.43 \\ & \times 10^{-3}X_1X_4 + 1.14 \times 10^{-3}X_1X_5 + 0.43X_2X_3 + 1.93 \\ & \times 10^{-3}X_2X_4 + 6.81 \times 10^{-4}X_2X_5 - 9.30 \times 10^{-3}X_3X_4 \\ & - 3.47 \times 10^{-2}X_3X_5 - 4.86 \times 10^{-3}X_4X_5 - 2.74 \times 10^{-3}X_1^2 \\ & - 8.44 \times 10^{-3}X_2^2 - 15.51X_3^2 - 3.10 \times 10^{-2}X_4^2 - 1.96 \\ & \times 10^{-3}X_5^2 \end{aligned} \tag{4}$$

The statistical testing of this model was performed with the Fisher's statistical method for analysis of variance (ANOVA). The result of ANOVA for flocculating rate, shown in Table 3, indicates that the second-order equation fitted well. Because model F-value of 4.23 was greater than $F_{0.01}(20, 29) = 2.57$, values of 'Prob > F' = 0.0002 less than 0.05, and the total determination coefficient R^2 reached 0.7449.

The significance testing for the coefficient of the equation whose variables are in terms of coded factors is listed in Table 4.

Table 3
ANOVA for flocculating rate.

Item	Sum of squares	Degrees of freedom	Mean square	F-value	Prob > F
Model	94999.15	20	4749.96	4.23	0.0002
Residual	32526.75	29	1121.61	–	–
Lack of fit	32526.73	22	1478.49	4.650×10^5	0.0001
Pure error	0.022	7	3.180×10^{-3}	–	–

R^2 = 0.7449.

Table 2
CCD design and response values.

Run	Coded values					Real values					Response	
	x_1	x_2	x_3	x_4	x_5	x_1 (mg/L)	x_2 (mg/L)	x_3	x_4 (mg/L)	x_5 (rpm)	Flocculating rate (%)	Floc size (mm)
1	1	−1	−1	1	1	168.04	82.96	6.2	35.51	242	88.36	0.57
2	−1	−1	1	1	1	94.46	82.96	7.8	35.51	242	54.63	0.28
3	0	0	0	0	−2.38	131.25	125	7.0	25	100	98.48	0.91
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
48	0	0	0	0	0	131.25	125	7.0	25	200	99.36	0.75
49	1	−1	1	−1	1	168.04	82.96	7.8	14.49	242	84.05	1.03
50	1	1	1	−1	−1	168.04	167.04	7.8	14.49	158	98.48	0.82

Table 4
Significance of quadratic model coefficient of flocculating rate.

Independent variables	Regression coefficients	Degrees of freedom	Standard error	Prob > F
x_3	34.23	1	5.09	<0.0001
x_1x_2	−14.47	1	5.92	0.0208
x_1x_3	−15.83	1	5.92	0.0122
x_2x_3	15.07	1	5.92	0.0165
x_2^2	−14.92	1	4.49	0.0024
x_3^2	−10.97	1	4.49	0.0210

And the transformation between the coded factor and actual factor is obtained from Eq. (5),

$$x_i^* = \frac{x_i - M}{H} \quad (5)$$

where x_i^* and x_i are coded factor and actual factor, respectively. M is the median of the variable range in experiment, and H is the radius of it (Otto, 1998). Values of 'Prob > F' less than 0.05 indicate model terms are significant. In the linear terms, pH was significant and unique, and it played a decisive role in the flocculating process. Because the effective components of MBFGA1 were linear molecules which were always in the curliness shape except in the alkaline environment that could stimulate the molecules spread in linear shape by acting on the absorption sites with negative charge. And the linear shape could provide more absorption space volume for the particles. Compared with PAC, obviously, the dosage of MBFGA1 had a higher significant effect on the flocculating rate. This behavior sprang from the reason that the PAC had a wider flocculating dosage range than MBFGA1. Among the higher order effects, the quadratic terms of PAC and pH were significant. The interaction terms with significant effect are shown in Fig. 2a–c, respectively.

Fig. 2a shows the change of flocculating rate with the dosage of PAC and MBFGA1 varying within the experimental ranges, while the pH, dosage of $CaCl_2$ and the top speed of stir are kept at central level. The figure of the curve and the curvature of the contour on the bottom indicate that PAC has obvious quadratic effect on the flocculating rate in composite process, and this agrees with the previous study using PAC only. And it is predicted that, at a low level of PAC, flocculating rate enhances as MBFGA1 added and gets to the peak ultimately. However this target obviously becomes easier when the concentration of PAC is kept at central level. This phenomenon suggested that PAC was a proper flocculant which could produce a positive effect on the flocculating rate at low level of MBFGA1 for compounding. Fig. 2b shows that, when the top speed of stir, dosage of PAC and $CaCl_2$ are at central level, the flocculating rate can get to an anticipant value at a small quantity of MBFGA1 when pH is higher than 7.4 approximately, which indicates that the activity of MBFGA1 depends upon an alkaline environment. Fig. 2c also indicates the importance of a higher pH for flocculating process when the other three factors are kept at central level.

3.2.2. Data analysis of floc size as the response variable

Eq. (6) represents empirical relationship in the form of quadratic polynomial between the floc size (y_2) and the other five factors ($x_1 - x_5$).

$$\begin{aligned} Y_2 = &-3.89 - 4.94 \times 10^{-3}X_1 + 2.43 \times 10^{-2}X_2 + 0.66X_3 \\ &+ 2.53 \times 10^{-2}X_4 + 7.11 \times 10^{-3}X_5 - 1.15 \times 10^{-5}X_1X_2 \\ &+ 1.39 \times 10^{-3}X_1X_3 + 1.14 \times 10^{-4}X_1X_4 - 6.38 \times 10^{-6}X_1X_5 \\ &- 2.22 \times 10^{-3}X_2X_3 + 4.45 \times 10^{-5}X_2X_4 - 1.12 \times 10^{-5}X_2X_5 \\ &- 5.33 \times 10^{-3}X_3X_4 - 5.61 \times 10^{-4}X_3X_5 - 5.61 \times 10^{-5}X_4X_5 \\ &- 1.08 \times 10^{-5}X_1^2 - 2.89 \times 10^{-5}X_2^2 - 1.94 \times 10^{-2}X_3^2 \\ &- 1.78 \times 10^{-6}X_4^2 - 1.60 \times 10^{-6}X_5^2 \end{aligned} \quad (6)$$

The result of ANOVA for floc size, shown in Table 5, indicates that the second-order equation fitted well. Because model F-value of 5.91 was greater than $F_{0.01}(20, 29) = 2.57$, values of 'Prob > F' < 0.0001 less than 0.05 and the total determination coefficient R^2 reached 0.8029.

The significance testing for the coefficient of Eq. (6) in which variables are in terms of coded factors is listed in Table 6. In the lin-

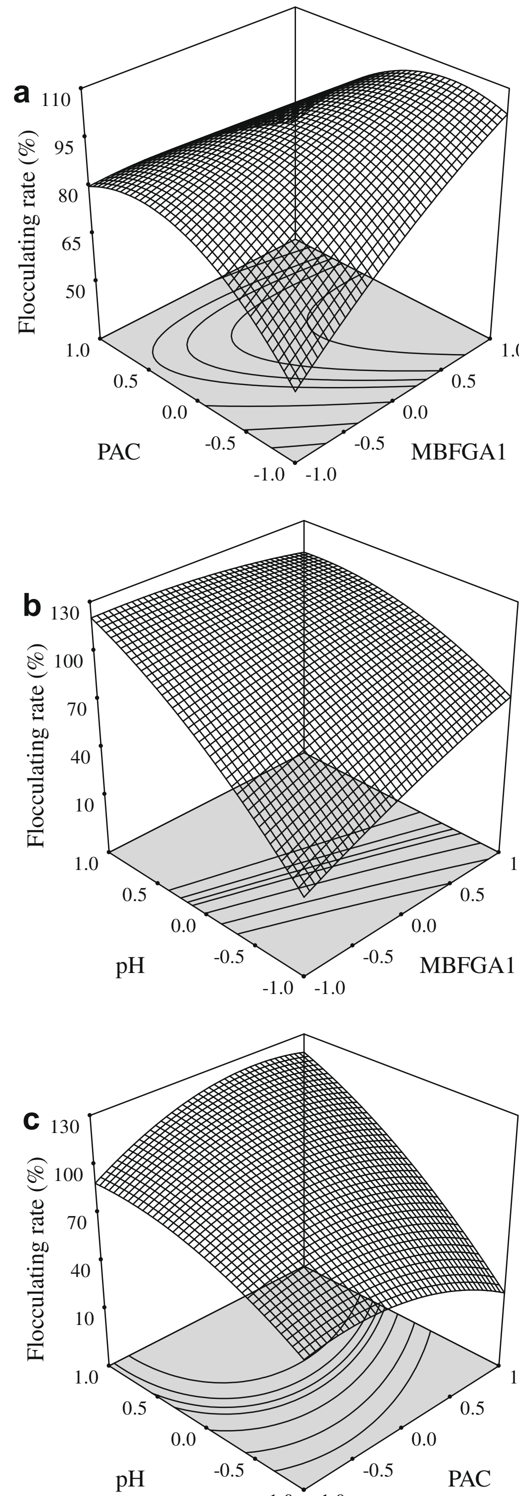

Fig. 2. Surface graphs of flocculating rate showing the effect of variables: (a) MBFGA1–PAC, (b) MBFGA1–pH, and (c) PAC–pH.

Table 5
ANOVA for floc size.

Item	Sum of squares	Degrees of freedom	Mean square	*F*-value	Prob > *F*
Model	1.17	20	0.058	5.91	<0.0001
Residual	0.29	29	9.870×10^{-3}	–	–
Lack of fit	0.26	22	0.012	2.62	0.0969
Pure error	0.031	7	4.429×10^{-3}	–	–

$R^2 = 0.8029$.

Table 6
Significance of quadratic model coefficient of floc size.

Independent variables	Regression coefficients	Degrees of freedom	Standard error	Prob > *F*
x_1	0.078	1	0.015	<0.0001
x_2	−0.044	1	0.015	0.0069
x_3	0.041	1	0.015	0.0106
x_5	−0.046	1	0.015	0.0048
x_1x_3	+0.043	1	0.018	0.0204
x_1x_4	+0.044	1	0.018	0.0180
x_2x_3	−0.078	1	0.018	0.0001
x_3x_4	−0.047	1	0.018	0.0119
x_2^2	−0.051	1	0.013	0.0006

ear terms, PAC, pH and the top speed of stir were significant, especially for MBFGA1 attributing to the absorption bridging action, its flocculating mechanism, which could influence the size and density of flocs. Further more, the top speed of stir could be used as a rough measure of mixing effectiveness, based on the reasoning that a higher speed creates greater turbulence, and greater turbulence leads to better mixing (George et al., 2003). However, the time that forming the flocs needed were different in each kinds of flocculating conditions, and a greater turbulence environment had a more negative impact on the flocs forming. Therefore, the top speed of stir was significant. In the quadratic terms, PAC was significant and unique for the reason that excessive dosage of PAC led to the stabilization of colloid suspension which could disaggregate the flocs again. The interaction terms with significant effects are shown in Fig. 3a–d, respectively.

Fig. 3a shows that the floc size is enhanced with the advance of MBFGA1 and pH, while the dosage of $CaCl_2$, PAC and the top speed of stir are kept at central level, and this indicates that MBFGA1 makes an obviously positive effect on floc size in alkaline environment. Fig. 3b shows that the floc size varies with pH and PAC when the top speed of stir, dosage of MBFGA1 and $CaCl_2$ are at central level. At low level of pH, the influence of PAC on floc size was inconspicuous, however, with the advance of pH, PAC shown a negative effect on floc size gradually, and this might rise from the comprehensive effect between MBFGA1 and PAC. On the one hand, since the advance of pH was helpful for the activity of MBFGA1, the floc

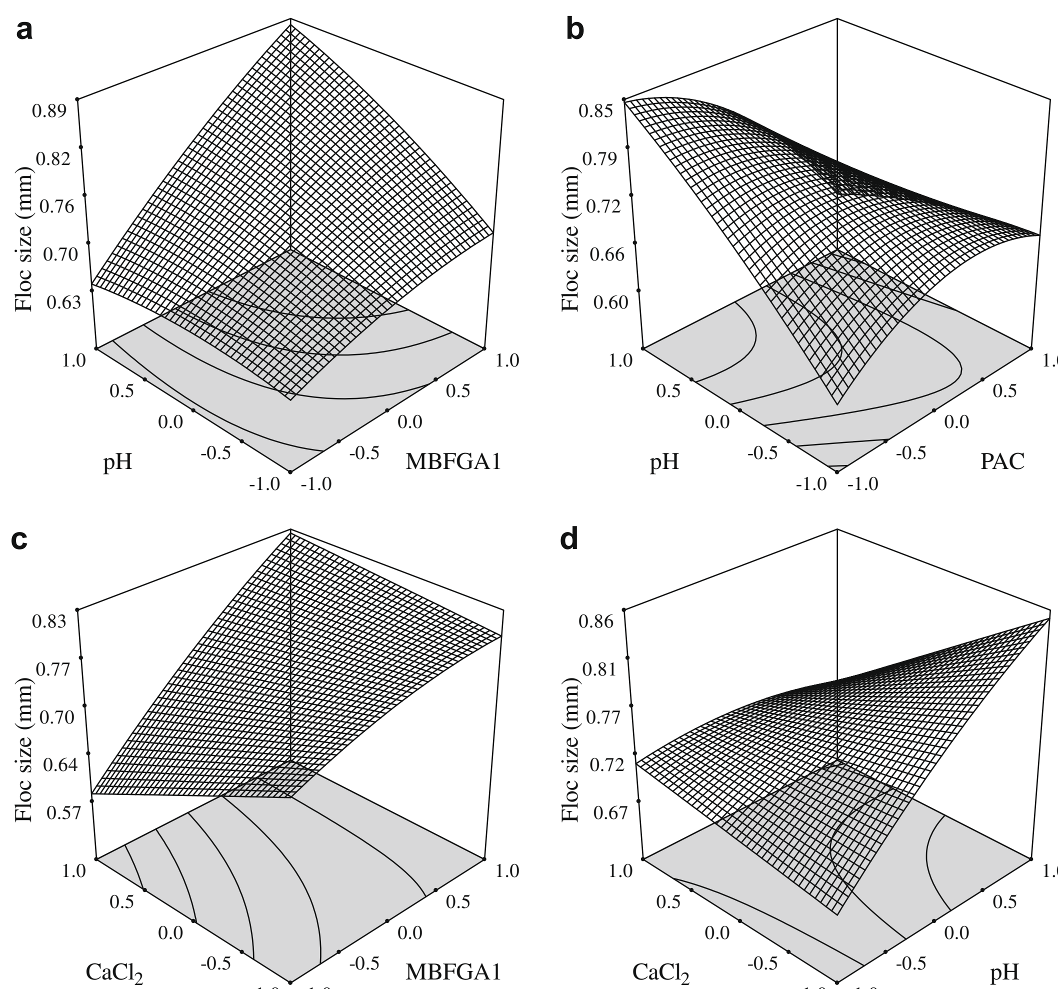

Fig. 3. Surface graphs of floc size showing the effect of variables: (a) MBFGA1–pH, (b) PAC–pH, (c) MBFGA1–$CaCl_2$, and (d) pH–$CaCl_2$.

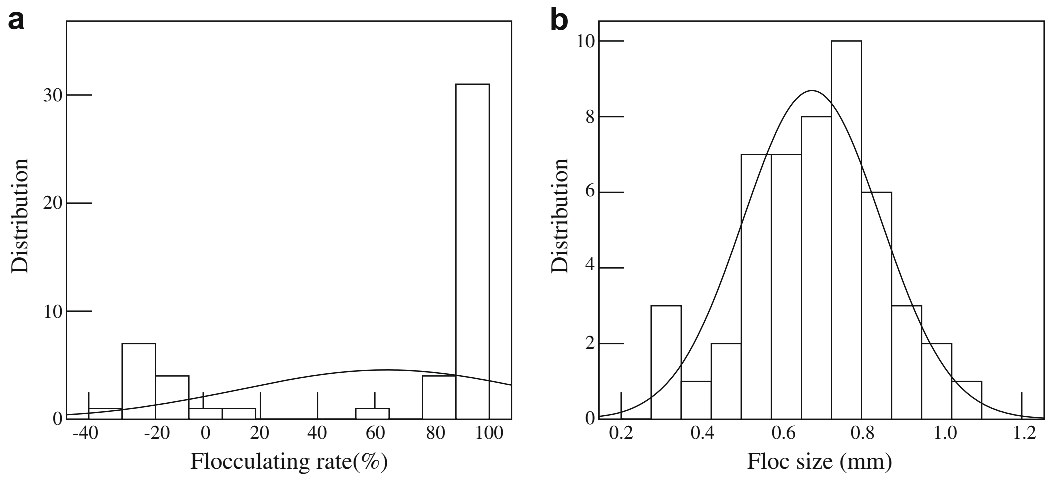

Fig. 4. Distribution of (a) flocculating rate and (b) floc size from the CCD experiment.

size was enhanced at the low level of PAC. On the other hand, the ratio of small flocs increased with the advance of PAC, thus the floc size was decreased. Fig. 3c provides more evidence that MBFGA1 can improve the size when there is plenty of $CaCl_2$ as the additive for flocculating process. However, Fig. 3d indicates that $CaCl_2$ is not always a positive effect on the floc size. Though $CaCl_2$ provided sufficient Ca^{2+} which could destroy the stability of colloid suspension by compressing twin electrical layer and stimulate the bridging action, excessive $CaCl_2$ debased the size at a certain extent. Because excessive Ca^{2+} might occupy the absorption sites with negative charge on MBFGA1 molecules, then it could hinder the absorption bridging action which could increase the floc size. In general, researchers always paid attention to the positive effect of metal ions on the flocculating activity of MBF. But this work revealed another effect of Ca^{2+} from the aspect of floc size, which is significant for the process of settlement and dehydration.

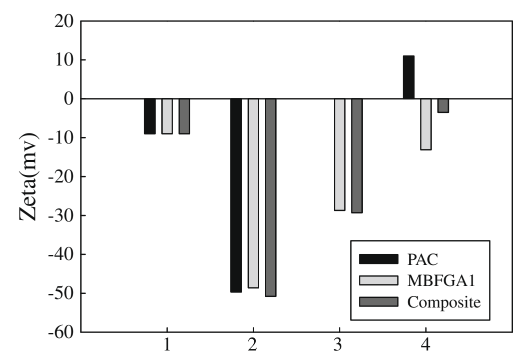

Fig. 5. Contrast of Zeta potential between the flocculants in different time point ((1) kaolin suspension without additives, (2) after adjusting the pH, (3) after adding $CaCl_2$, and (4) after adding the flocculant).

3.3. Optimal flocculent condition

The numerical range of the two kinds of responses, flocculating rate and floc size, was divided into 12 parts, respectively, and their distribution frequency was accounted using the statistics software in each part. Results are shown in Fig. 4a and b.

There is a phenomenon shown in the figures that a larger floc size does not always represent higher flocculating rate. Many reasons explain this phenomenon, on the one hand, the large size goes against the forming of netting mechanism during the slow mixing process, which is the precondition for the sufficient particle collection, though it has a good performance on the sedimentation of flocs; on the other hand, the small size plays an inverse role on the sedimentation and leads to float again, which influences the flocculating rate and sludge dehydration while the system encountered low level of turbulence. Therefore, the optimal floc size, as shown in Fig. 4b, was fixed at 0.7 mm.

According to the target value of the two individual responses, 100% and 0.7 mm, the optimal condition calculated from the regression equations were MBFGA1 99.75 mg/L, PAC 121 mg/L, pH 7.3, $CaCl_2$ 27 mg/L and the top speed of stir 163 rpm, respectively. The flocculating rate of verification test operated under optimal condition is greater than 98%.

3.4. Zeta potential analysis base on different flocculants

The result of Zeta potential testing during the process of flocculation is shown in Fig. 5. The numbers on *x*-axis represent the different time-points for sampling, and the bars in different color reveal the extent of the variation by using different flocculants. Comparing with the bars, it is obvious that the Zeta potential of kaolin particles has the maximum variation after PAC had been added, which indicates that, among the tested flocculants, PAC has the strongest ability to modify the Zeta potential of kaolin particles. This result attributes to the higher positive charge of PAC (Gao et al., 2005). Simultaneously, Ca^{2+} can also modify it by decreasing the negative charge on both the polymer and particle (Levy et al., 1992). And in this field, researchers always use this kind of bivalent cation for destroying the stability between the colloids (Salehizadeh and Shojaosadati, 2001). However, Fig. 5 shows that Ca^{2+} is not as efficient as PAC. This limitation might induce that the particles were not fully captured, especially for the smaller one. And the flocculating phenomenon did revel this during the flocculation using MBFGA1. Furthermore, the previous study in this work indicated that excessive Ca^{2+} may reduce the floc size by occupying the absorption sites on MBFGA1 molecules and hindering the bridging action. Therefore, the adding of PAC produced a significant effect on the flocculating process of MBFGA1. On the one hand, low dose of PAC can adequately destroy the stability of kaolin suspension which could provide a more unstable colloid suspension for the following absorption bridging and netting action; on the other hand, the decreasing of Ca^{2+} can reduce their negative influence on the floc size.

4. Conclusions

The optimal flocculating conditions are MBFGA1 99.75 mg/L, PAC 121 mg/L, pH 7.3, $CaCl_2$ 27 mg/L and the top speed of stir 163 rpm, respectively. In addition, the larger floc size did not always represent the higher flocculating rate, floc size of 0.7 mm was set as another target value in the calculation step for optimal conditions.

The dosage of MBFGA1 revealed obvious effect on the floc size which ensured the implement of netting mechanism effectively. Meanwhile, PAC has the strongest ability to modify the Zeta potential of kaolin particles. Therefore, the composite flocculant holding these two advantages obtained a favorable flocculating effect.

Acknowledgement

This project was supported by Grant Number 50478053 from National Natural Science Foundation of China.

References

Arezoo, C., 2002. The potential role of aluminium in Alzheimer's disease. Nephrol. Dial. Transplant. 17 (Suppl. 2), 17–20.
Christopher, E., Olga, K., Deborah, J., Stanislav, S., Anthony, P., Peter, C., 2006. Non-invasive therapy to reduce the body burden of aluminium in Alzheimer's disease. J. Alzheimer's Dis. 10, 17–24.
David, C.S., Matthew, J.H., 2002. Examination of three theories for mechanisms of cation-induced bioflocculation. Water Res. 36, 527–538.
Deng, S.B., Bai, R.B., Hu, X.M., Luo, Q., 2003. Characteristics of a bioflocculant produced by *Bacillus mucilaginosus* and its use in starch wastewater treatment. Appl. Microbiol. Biotechnol. 60, 588–593.
Duncan, J.S., 1992. Colloid and Surface Chemistry, fourth ed. Butterworth–Heinemann, Woburn.
Gao, B.Y., Chu, Y.B., Yue, Q.Y., Wang, B.J., Wang, S.G., 2005. Characterization and coagulation of a polyaluminum chloride (PAC) coagulant with high Al_{13} content. J. Environ. Manage. 76, 143–147.
George, T., Franklin, L.B., David, S., 2003. Wastewater Engineering, fourth ed. Matcalf & Eddy Inc., New York.
He, N., Li, Y., Chen, J., 2004. Production of a novel polygalacturonic acid bioflocculant REA-11 by *Corynebacterium glutamicum*. Bioresour. Technol. 94, 99–105.
Jang, J.H., Ike, M., Kim, S.M., Fujita, M., 2001. Production of a novel bioflocculant by fed-batch culture of *Citrobacter* sp. Biotechnol. Lett. 23, 593–597.
Joung, H.Y., Sung, J.K., Se, H.A., Hong, K.L., 2007. Characterization of a novel bioflocculant, p-KG03, from a marine dinoflagellate, *Gyrodinium impudicum* KG03. Bioresour. Technol. 98, 361–367.
Kurane, R., Takeda, K., Suzuki, T., 1986. Screening for and characteristics of microbial flocculants. Agric. Biol. Chem. 50, 2301–2307.
Kurane, R., Hatamochi, K., Kakuno, T., Kiyohara, M., Hirano, M., Taniguchi, Y., 1994. Production of a bioflocculant by *Rhodococcus erythropolis* S-1 grown on alcohols. Biosci. Biotechnol. Biochem. 58, 428–429.
Levy, N., Magdasi, S., Bar-Or, Y., 1992. Physico-chemical aspects in flocculation of bentonite suspensions by a cyanobacterial. Water Res. 26, 249–254.
Li, Y., He, N., Guan, H., Du, G., Chen, J., 2003. A polygalacturonic acid bioflocculant REA-11 produced by *Corynebacterium glutamicum*: a proposed biosynthetic pathway and experimental confirmation. Appl. Microbiol. Biotechnol. 63, 200–206.
Nasser, M.S., James, A.E., 2007. Effect of polyacrylamide polymers on floc size and rheological behaviour of kaolinite suspensions. Colloids Surf. A 301, 311–322.
Otto, M., 1998. Otto: Chemometrics. WILEY-VCH Verlag Gmbh, Weinheim.
Ruan, M., Yang, Z.H., Zeng, G.M., Xiong, L.J., Tao, R., Wang, R.J., Liu, Y.S., 2007. Flocculating capability and mechanism of bioflocculant produced by *Paenibacillus polymyxa* GA1. Environ. Sci.-China 28, 2336–2341.
Rudén, C., 2004. Acrylamide and cancer risk-expert risk assessments and the public debate. Food Chem. Toxicol. 42, 335–349.
Sagbo, O., Sun, Y., Hao, A., Gu, P., 2008. Effect of PAC addition on MBR process for drinking water treatment. Sep. Purif. Technol. 58, 320–327.
Salehizadeh, H., Shojaosadati, S.A., 2001. Extracellular biopolymeric flocculants: recent trends and biotechnological importance. Biotechnol. Adv. 19, 371–385.
Triveni, R., Shamala, T.R., Rastogi, N.K., 2001. Optimised production and utilization of exopolysaccharide from *Agobacterium radiobacter*. Process. Biochem. 36, 787–795.
Yang, Z.H., Tao, R., Zeng, G.M., Xiao, Y., Deng, E.J., 2006. Culture medium and grading culture techniques for bioflocculant production by *Paenibacillus polymyxa* GA1. Environ. Sci.-China 27, 1444–1449.
Zhang, T., Lin, Z., Zhu, H.L., 1999. Microbial flocculant and its application in environmental protection. Environ. Sci.-China 11, 1–12.