Chemical Engineering Journal 191 (2012) 269–277

Contents lists available at SciVerse ScienceDirect

Chemical Engineering Journal

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

Influence of composite flocculant of PAC and MBFGA1 on residual aluminum species distribution

Jing Huang, Zhao-Hui Yang*, Guang-Ming Zeng, Min Ruan, Hai-Yin Xu, Wan-Chao Gao, Yuan-Ling Luo, Hua-Ming Xie

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

ARTICLE INFO

Article history:
Received 22 November 2011
Received in revised form 6 March 2012
Accepted 7 March 2012

Keywords:
Microbial flocculant
Poly aluminum chloride (PAC)
Residual aluminum species
Fluorescence spectrophotometry
Optimization

ABSTRACT

In general, majority of biomaterials have shown good biosorption capacities towards certain of metal ions. In this work, microbial flocculant GA1 (MBFGA1) compound with PAC was used to control the residual aluminum in flocculation water treatment. The fluorescence spectrophotometry combined with different pretreatment methods was employed to divide the species of aluminum and determine their concentration. The results indicated that non-labile monomeric aluminum was the dominant specie in residual aluminum and addition of MBFGA1 could efficiently eliminate them. Two quadratic polynomial models with the response variables of flocculating rate and labile monomeric aluminum concentration were established by response surface methodology, respectively. The optimal flocculating conditions were MBFGA1 at 109.37 mg/L, PAC at 81.87 mg/L, initial pH at 8.5, time duration of stir 72.5 min and ambient temperature at 24.3 °C. Fourier transform-infrared spectra and environmental scanning electron microscope analysis indicated that MBFGA1 holded a large number of functional groups, which had strong capacity on binding aluminum and reducing its residual level during coagulation–flocculation.

1. Introduction

Some aluminum-based compounds and polymers such as alum, aluminum chloride and poly aluminum chloride (PAC) are widely employed as reactants for coagulation–flocculation in water treatment to reduce turbidity, color, organic matter and microorganism levels by forming aggregates and flocs from finely divided particles or dissolved substances [1]. However, it was reported that aluminum coagulants could complex with residual natural organic matter (NOM) during water treatment and notably increase the residual level of aluminum [2,3]. Despite its high efficiency in water treatment, the residual aluminum in drinking water supplied from the plant reduced efficiency of disinfection and decreased carrying capacity of water distribution systems due to post precipitation on the wall of water supply network [4,5]. Furthermore, it has been reported in numerous studies that the aluminum substances remained in the outlet water was always associated with various health problems such as Alzheimer's disease [6].

In response, many countries and regions have promulgated various regulations to control the maximum concentration level of residual aluminum in water treatment at 0.2 mg/L as guided by World Health Organization (WHO). Although this value is not based on any assessment of risks to health, it provides a compromise between the use of aluminum-based salts in water treatment and discoloration of distributed water [7]. Fortunately, under appropriate operating conditions, concentrations of aluminum at 0.2 mg/L or less are achievable in large water treatment plants. But small-scale treatment might experience some difficulties in attaining this level, because small size of the coagulation–flocculation device provides little buffering for fluctuation in operation, which would have a negative effect on the removal of residual aluminum [8]. Consequently, a positive method should be developed to reduce the residual aluminum concentration, besides controlling the operating conditions.

MBFGA1 is a kind of microbial flocculant harvested from the fermentation liquid of *Paenibacillus polymyxa* GA1. In our previous studies, we had optimized its coagulation–flocculation conditions when it was composited with PAC to enhance flocculating activity and reduce costs [9]. In addition, MBFGA1 belongs to a kind of extracellular polymeric substances (EPS) that are complex mixtures of macromolecular polyelectrolytes with variable molecular mass and structural properties including polysaccharides, proteins and nucleic acids [10]. Depending on the chemical composition and structure, EPS exhibit ability for complex-binding with metal ions, and this is also a fundamental extracellular mechanism for protection of the cells [11,12]. Therefore, it is interesting to investigate the influence of composite flocculant of MBFGA1 and PAC on residual aluminum in water.

* Corresponding author. Tel.: +86 0731 88649168; fax: +86 0731 88822829.
E-mail addresses: yzh@hnu.edu.cn, gavinhj@yahoo.cn (Z.-H. Yang).

1385-8947/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.cej.2012.03.015

Nomenclature

a	optical density of the sample at 550 nm
Al_a	total monomeric aluminum
Al_i	labile monomeric aluminum
Al_o	non-labile monomeric aluminum
Al_r	acid-soluble aluminum
Al_T	total aluminum
b	optical density of the control at 550 nm
BSE	backscatter electron
CCD	central composite design
EDS	energy dispersive spectrometer
EPS	extracellular polymeric substances
ESEM	environmental scanning electron microscope
FR	flocculating rate (%)
FT-IR	Fourier transform-infrared spectrometer
MBFGA1	microbial flocculant GA1
NOM	natural organic matter
OD	optical density
PAC	poly aluminum chloride
RCF	relative centrifugal force (g)
RSM	response surface methodology
SE	secondary electron
WHO	World Health Organization
x_1	initial pH
x_2	dosage of MBFGA1 (mg/L)
x_3	dosage of PAC (mg/L)
x_4	time duration of stir (min)
x_5	ambient temperature (°C)
x_i, x_j	independent variables
y_1	response variable of flocculating rate
y_2	response variable of Al_i concentration
β_0	offset term
β_i	i linear coefficient
β_{ii}	i quadratic coefficient
β_{ij}	interaction term between x_i and x_j

Aluminum exists in natural solutions with various chemical species, which are found to be the most important indicator for its toxicity. For example, the organically bound aluminum is less toxic than the inorganic aluminum, and polynuclear aluminum, such as $Al_{13}O_4(OH)_{24}(H_2O)_{12}{}^{7+}$, is the most toxic form for its unstable chemical properties [13–16]. According to the popular classification method [17,18], in this study, aluminum was divided into five operationally defined species: total Al (Al_T), total monomeric Al (Al_a), labile monomeric Al (Al_i), non-labile monomeric Al (Al_o) and acid-soluble Al (Al_r), by respective pretreatment based on the fluorescence spectrophotometry [19]. For one thing this classification provided further information on the relationship between MBFGA1 and the toxicity of residual aluminum; for another it contributed to a better understanding of the effect of MBFGA1 on the distribution of aluminum species. Moreover, the response surface methodology (RSM), an effective tool for building a multivariable equation and determination of their optimal values [20], was employed to optimize the conditions of coflocculation process and to investigate the interactive effects of experimental factors. Meanwhile, the flocculating rate and concentration of Al_i, considered as a representative form in harmfulness, were defined as the response variables, and the optimal conditions were their compromised results.

2. Materials and methods

2.1. Bacterium strain and fermentation

GA1, flocculant-producing strain CCTCC M206017 which was identified as *P. polymyxa* by 16S rDNA sequence and its biochemical and physiological characteristics, was originally isolated from the soil sample collected in the Yuelu Mountain of Changsha, China. 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 adjusted to pH 7.0. 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 adjusted to pH 7.0. After 72 h of cultivation, the fermentation liquid with the effective components of 17.5 g/L was stored in 4 °C, and it will be utilized directly in further flocculation [21].

2.2. Flocculation experiment

Kaolin was chemically pure grade (Damao Chemicals, China) and its suspension was prepared at the concentration of 3 g/L with average particle size and Zeta potential at 23.75 μm and −11.4 mV, respectively (Zetasizer 2000 Malvern Instruments). The upper phase was used for the flocculating experiment after 1 h of stir at 300 rpm and 2 h of gravity settlement.

Three kinds of flocculants, PAC, MBFGA1 and their composition, were employed in the flocculation experiment to explore the influence of MBFGA1 on residual aluminum species distribution. The optimum dosage of PAC and MBFGA1 were 125.00 mg/L and 131.25 mg/L, respectively, according to previous study [9]. In order to achieve accurate and comparable results, the composite flocculant was consisted of 125.00 mg/L PAC and 131.25 mg/L MBFGA1. Furthermore, the flocculating rates (FRs), calculated by Eq. (1) in which a and b are the optical densities (ODs) of the sample and control, respectively, at 550 nm [22,23], were maintained beyond 98%.

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

In general, most of the cell surfaces are anionic [24], and many studies showed that soluble metal ions in the environment could be captured by cell wall because of negative charged groups attached within its fabric [25]. Therefore, cells of GA1 existing in the fermentation liquid may help to reduce the residual aluminum. In order to distinguish that effect made by the molecule of MBFGA1 from the cell of GA1, the flocculation experiment was divided into two steps. Firstly, cells in fermentation liquid were isolated by spinning at a relative centrifugal force (RCF) of 2000 for 30 min at 4 °C, and the supernatant was applied to the composite flocculant in the flocculation. Secondly, the isolated cells were dispersed in the sterile water with the same volume of the fermentation liquid, and then this cell solution was added in the upper phase of the kaolin suspension flocculated in the first step.

2.3. Measurements of aluminum species

The water sample was pretreated according to the process showed in Fig. 1. In the filtration step, the mixed cellulose esters membrane (MF-Millipore, Millipore Corp., USA) with 0.45 μm micropore was employed to remove colloidal and particulate materials. In the digestion step, the water sample was acidified to pH 1.0 for 24 h by addition of concentrated nitric acid. And in the measurement step, methods of 8-HQ and morin were applied to determine the concentration of Al_T, Al_a and Al_i, respectively [20]. Last, $[Al_o]$ was obtained by difference as $[Al_a] - [Al_i]$; $[Al_r]$ was obtained by difference as $[Al_T] - [Al_a]$.

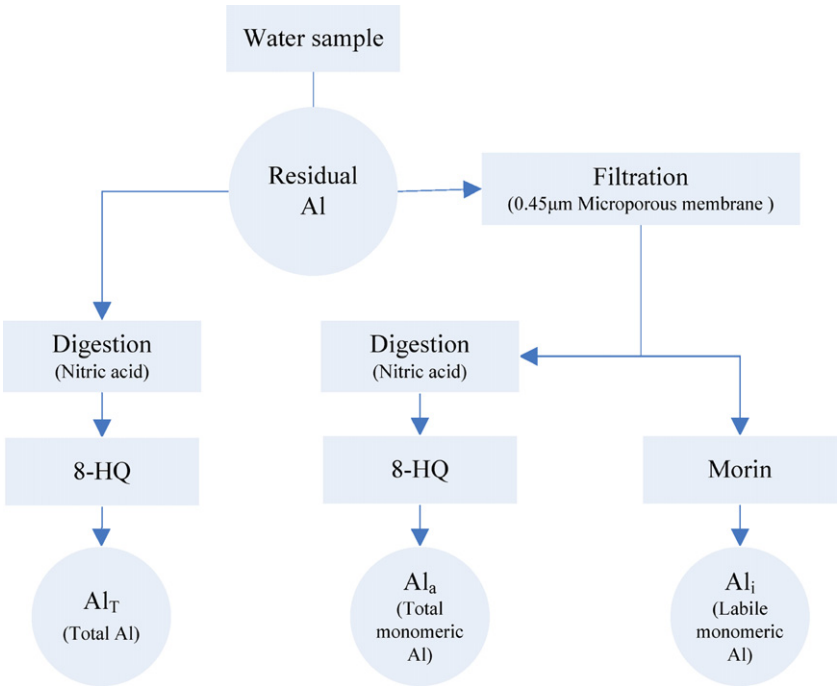

Fig. 1. Pretreatment process for determination of different aluminum species.

2.4. RSM experimental design

The central composite design (CCD), a standard RSM, was selected for the optimization of the factors which affected the flocculating activity. In this design, five factors were initial pH (x_1), dosage of MBFGA1 (x_2) and PAC (x_3), time duration of stir (x_4) and ambient temperature (x_5), respectively. All factors were controlled at five levels. The response variable (y) that represented flocculating rate or concentration of Al_i was fitted by a second-order model in the form of quadratic polynomial equation:

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

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 reflects the interaction between x_i and x_j [26]. The actual design ran by the statistic software, Design-expert 7.1.3 (Stat-Ease Inc, USA), is presented in Table 1.

2.5. Characterization of aluminum biosorption

The crude product of MBFGA1 was extracted from the centrifuged fermentation liquid by precipitation with double volumes of ice-cold acetone at 4 °C for 24 h, washed with 95% (v/v) ethanol and vacuum dried to remove residual acetone. To eliminate the interference brought by the salts and other small molecules, crude MBFGA1 was re-dissolved into deionized water with water bath heating, and was dialyzed against ultrapure water using dialysis tubing (MD34, Solarbio, PRC). After 72 h of dialysis, the internal fluid was lyophilized, and the dried fraction was used as purified MBFGA1 for the further analysis.

Since MBFGA1 is a kind of extracellular polymers and difficult to dissolve in water at room temperature, the biosorption experiments were conducted by agitating 0.5 g of purified MBFGA1 with 200 ml of $AlCl_3$ solution at pH 4.0. A magnetic stirrer was employed to stir the mixed solution at 200 rpm for 2 h to ensure that the adsorption reached equilibrium quickly. The undissolved material, as the role of sorbent, was collected by centrifugation, and followed by vacuum freeze-drying.

In order to qualitatively analyze the major roles of residual aluminum uptake, Fourier transform-infrared spectrometer (Nexus 870, Nicolet, USA) was used to detect vibration frequency changes in the functional groups on MBFGA1 before and after exposed to $AlCl_3$ solution.

The surface morphology of original and aluminum adsorbed MBFGA1 were also studied using an environmental scanning electron microscope (ESEM) (Quanta 200 FEG, FEI, USA) in low vacuum mode at an acceleration potential of 20 kV. Furthermore, microanalysis of the aluminum adsorbed MBFGA1 was carried out with an energy dispersive spectrometer (EDS) equipped on the Quanta 200.

3. Results and discussion

3.1. Distribution of the residual aluminum species

The concentrations of residual aluminum in different species were determined after coagulation–flocculation using different flocculants and treatment methods. The results are shown in Fig. 2. According to concentration comparison in the group of Al_T, PAC alone leads to the highest residual aluminum level of 9.012 mg/L. However, when PAC was composited with MBFGA1, this value decreased to 5.327 mg/L at the same dosage of PAC addition. Obviously, MBFGA1 exhibited a strong ability to eliminate the residual aluminum mainly originated from the aluminum-based components of PAC. Correspondingly, residual aluminum in the sample of kaolin suspension without the application of PAC still reached up to 7.076 mg/L, which indicated that turbidity could elevate the probability of residual aluminum, especially when the kaolin belongs to a kind of hydrated aluminum silicate [27]. For that reason, the flocculating rates in this part were maintained beyond 98% and minimized the influence brought by the suspended kaolin. In addition, compared with the basic standard of 0.2 mg/L, concentrations of Al_T generally improved more than one order of magnitude except

Table 1
Coded levels for 5 variables framed by CCD.

Factors	Codes	Coded levels				
		−2.38	−1	0	1	2.38
Initial pH	x_1	7.00	7.87	8.50	9.13	10.00
MBFGA1 (mg/L)	x_2	50.00	78.98	100.00	121.02	150.00
PAC (mg/L)	x_3	50.00	78.98	100.00	121.02	150.00
Time (min)	x_4	0.0	52.2	90.0	127.8	180.0
Temperature (°C)	x_5	10.0	18.7	25.0	31.3	40.0

the sample using MBFGA1 only. This phenomenon could be due to the coagulation–flocculation jar test in small-scale, which had a negative effect on the removing of residual aluminum.

Comparing Al_r with Al_a, it is obvious that Al_a dominates the species of residual aluminum because Al_r mainly included particulates and extremely non-labile organic complexes [17], and most of them were removed from water by the coagulation–flocculation. Moreover, the significantly increased Al_r of the kaolin suspension also demonstrated that the coagulation–flocculation process was an overwhelming factor on the decreasing of residual Al_r. Accordingly the negative value of Al_r removing rate might derive from the fluctuation of flocculating rates between PAC and PAC + MBFGA1.

Similarly, comparing Al_i with Al_o, it is obvious that Al_o dominates the species of residual aluminum in Al_a and Al_i removing rate reaches the maximum value as 55.4% compared with the others. Because Al_i was mainly composed of aquo aluminum as well as inorganic complexes of aluminum such as Al^{3+}, Al–OH, Al–F and Al–SO_4, which were chemically labile forms and could easily adsorb on the functional groups of organic substances by means of chelation. Besides, the electrostatic charge might be another important factor affecting the adsorption behavior of Al_i due to their strong polarity [28]. Altogether, Al_o, including monomeric alumino-organic complexes, is the dominant species of the residual aluminum after the coagulation–flocculation.

According to the values in the group of Al_T, Al_a and Al_o, it is obvious that the concentration of residual aluminum decreased successively, which suggested that both MBFGA1 and GA1 cells have certain metal-binding capacity to eliminate the residual aluminum. However, it is interesting that the value in the group of Al_i increased to 0.214 mg/L after the upper phase of the kaolin suspension had been treated by GA1 bacteria liquid. For one thing it was almost impossible to introduce fresh aluminum species by the addition of cell solution; for another it was generally agreed that the microbial cell walls were mainly responsible for the metal biosorption [29,30], thus the residual aluminum, especially in the chemically labile form of Al_i, was liable to interact with the active binding cites on the cell walls and turned into another forms. In addition, morin (3,5,7,2′,4′-pentahydroxy flavone) is a phenolic compound derived from hydroxyl substitution on the flavone chromophore and it is only weakly fluorescent in nature [31]. By fluorescence microscope analysis (BX61, Olympus, Japan), as shown in Fig. 3, florescence intensity is increased at cell wall and cytoplasm of GA1 cells when they are treated with morin. This increase indicates that morin may chelate with other non-paramagnetic ions [32] and form fluorescent complexes. Consequently, the increased fluorescence intensity is independent of the residual aluminum. Considering the maximum removing rate of Al_i, this phenomenon indirectly demonstrates that MBFGA1 has a strong metal-binding capacity to eliminate Al_i, which makes sense for reducing the toxicity of residual aluminum.

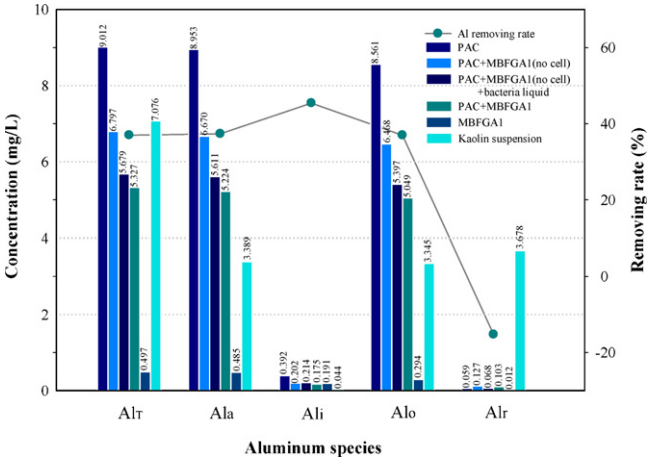

Fig. 2. Comparison of residual aluminum concentrations and removing rates in different species after finishing the coagulation–flocculation process using different flocculants and treatment methods. (The number on the bar represents the mean value of concentration derive from parallel test. The straight line represents the variation of removing rates which were calculated by the difference of residual aluminum concentration between PAC and PAC + MBFGA1 in 5 kinds of species, respectively.)

3.2. Experimental results of RSM

The response variables of flocculating rate and Al_i concentration were obtained from 50 groups of experiments which were summarized in Table 2.

3.2.1. Data analysis of flocculating rate as the response variable

Eq. (3) represents empirical relationship in the form of quadratic polynomial between the flocculating rate (y_1) and the other 5 factors (x_1–x_5).

$$y_1 = 99.16 - 0.47x_1 - 1.18x_2 + 0.65x_3 - 4.55x_4 + 3.86x_5 - 0.61x_1x_2 + 0.15x_1x_3 - 0.40x_1x_4 + 0.88x_1x_5 - 0.40x_2x_3 - 0.71x_2x_4 + 1.07x_2x_5 + 2.13x_3x_4 - 1.43x_3x_5 + 5.05x_4x_5 - 1.11x_1^2 - 0.50x_2^2 - 0.61x_3^2 - 2.07x_4^2 - 0.91x_5^2 \quad (3)$$

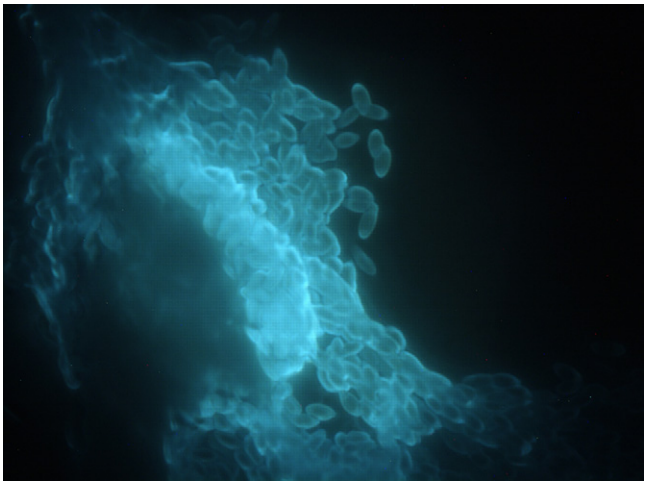

Fig. 3. Fluorescence micrograph of GA1 cells by 100× oil objective lens.

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	x_2 (mg/L)	x_3 (mg/L)	x_4 (min)	x_5 (°C)	Flocculating rate (%)	Al_i (mg/L)
1	−1	1	1	−1	1	7.87	121.02	121.02	52.2	31.3	90.83	0.444
2	1	−1	1	−1	1	9.13	78.98	121.02	52.2	31.3	98.27	0.450
3	2.38	0	0	0	0	10.00	100.00	100.00	90.0	25.0	95.58	0.330
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
48	0	0	0	0	0	8.50	100.00	100.00	90.0	25.0	99.40	0.154
49	−1	1	−1	1	−1	7.87	121.02	78.98	127.8	18.7	71.71	0.427
50	1	1	−1	−1	−1	9.13	121.02	78.98	52.2	18.7	99.69	0.226

The statistical testing of this model was performed with Fisher's statistical method for analysis of variance (ANOVA). The results indicated that the second-order equation fitted well, for model F-value of 8.12 was greater than $F_{0.01}(20, 29) = 2.57$, value of 'Prob > F' < 0.0001 was less than 0.05, and the total determination coefficient R^2 reached 0.85. The significance testing for the coefficient in Eq. (3) are listed in Table 3.

In the linear terms, time duration of stir and ambient temperature were significant. Undoubtedly, sustained stir for a long time could destroy the structure of formed flocs and release kaolin particles back to the suspension. For ambient temperature, Patience's study [33] had demonstrated that temperature induced the change of conformation and polymer structure type, and it played an important role in flocculation of kaolin dispersions. Among the higher order effects, the quadratic term of time duration was significant. Moreover, it was different from the previous study [9] that initial pH was not a significant factor to determine the flocculating rate anymore. This phenomenon might result from the compressed variation range of initial pH value, which ensured the flocculating effect and the comparability of residual aluminum. The interaction terms with significant effect are shown in Fig. 4(a) and (b), respectively.

Fig. 4(a) shows the change of flocculating rate with the variation of time duration of stir and ambient temperature in the experimental ranges, while initial pH, dosage of MBFGA1 and PAC are kept at central level. The curved surface and the curvature of contour on the bottom indicate that, at low temperature, the value of flocculating rate decreases sharply with increasing the time duration. While this phenomenon is not significant at high temperature, which might attribute to the decreased turbulent shear stress one of the critical factors affecting the flocculating rate [34]. In addition, the curved surface dropping with the decreased temperature also demonstrates that the variation of turbulent shear stress does exist especially for a long duration.

Fig. 4(b) shows the change of flocculating rate with the variation of time duration of stir and dosage of PAC in the experimental ranges, while initial pH, dosage of MBFGA1 and ambient temperature are kept at central level. This figure also indicates that time duration of stir is always an important factor to influence the flocculating rate. While, different from general flocculation experiments, the effect of the flocculant dosage is not significant, especially for a short duration. This might attribute to the compressed variation range of PAC dosage for the sake of the comparability of residual aluminum.

3.2.2. Data analysis of Al_i concentration as the response variable

Eq. (4) represents empirical relationship in the form of quadratic polynomial between the Al_i concentration (y_2) and the other 5 factors (x_1–x_5).

$$\begin{aligned} y_2 = {} & 0.16 + 5.17 \times 10^{-3}x_1 - 0.012x_2 + 0.033x_3 + 0.014x_4 \\ & - 3.81 \times 10^{-3}x_5 - 6.19 \times 10^{-3}x_1x_2 - 1.85 \times 10^{-3}x_1x_3 \\ & - 7.92 \times 10^{-3}x_1x_4 + 5.83 \times 10^{-3}x_1x_5 + 1.54 \times 10^{-3}x_2x_3 \\ & + 0.019x_2x_4 + 0.015x_2x_5 - 0.035x_3x_4 + 9.09 \times 10^{-3}x_3x_5 \\ & - 0.022x_4x_5 + 0.029x_1^2 + 0.029x_2^2 + 0.029x_3^2 + 0.047x_4^2 \\ & + 0.053x_5^2 \end{aligned} \quad (4)$$

The ANOVA results indicated that the second-order equation fitted well, for model F-value of 5.24 was greater than $F_{0.01}(20, 29) = 2.57$, value of 'Prob > F' < 0.0001 was less than 0.05, and R^2 reached 0.78. The significance testing for the coefficient in Eq. (4) are listed in Table 4.

In the linear terms, dosage of PAC was significant and unique, which played a decisive role in residual Al_i concentration because majority of residual aluminum originated from this part. Among the higher order effects, all of the quadratic terms were significant. One possible reason is that initial pH, time duration of stir and dosage of MBFGA1 and PAC might affect the variation of flocculating rate hence the level of residual aluminum. In addition, the significant quadratic effect of ambient temperature might be related to potential biosorption of MBFGA1. On the one hand the increased temperature could accelerate the diffusion rate of metal ions from bulk solution to the surface of biosorbent, hence increase the adsorption during the same time [35]; on the other hand, this increase was only reasonably in a rather narrow temperature range, for high temperatures could change the structure of the biomass and destroy the active sites to some extent [36]. The interaction terms with significant effect are shown in Fig. 4(c). According to the contour lines on the bottom, it is obvious that both time duration of stir and dosage of PAC have quadratic effects on the residual Al_i. Furthermore, they had similar interactions that one of the quadratic effects would be weaken when the other was kept at higher level. For stir, long time of stir had a negative effect on flocculating rate

Table 3
Significance of quadratic model coefficient of flocculating rate.

Independent variables	Regression coefficients	Degrees of freedom	Standard error	Prob > F
x_4	−4.55	1	0.66	<0.0001
x_5	3.86	1	0.66	<0.0001
x_3x_4	2.13	1	0.77	0.0094
x_4x_5	5.05	1	0.77	<0.0001
x_4^2	−2.07	1	0.58	0.0013

Table 4
Significance of quadratic model coefficient of Al_i.

Independent variables	Regression coefficients	Degrees of freedom	Standard error	Prob > *F*
x_3	0.033	1	9.75×10^{-3}	0.0022
x_3x_4	−0.035	1	0.011	0.0042
x_1^2	0.029	1	8.61×10^{-3}	0.0022
x_2^2	0.029	1	8.61×10^{-3}	0.0020
x_3^2	0.029	1	8.61×10^{-3}	0.0021
x_4^2	0.047	1	8.61×10^{-3}	<0.0001
x_5^2	0.053	1	8.61×10^{-3}	<0.0001

and then raised the residual level of Al_i. Nevertheless, it was helpful for the sufficient biosorption of the residual aluminum. Similarly, high concentration of PAC could elevate the residual aluminum level directly, worse still when PAC concentration exceeded the optimal amount, it leaded to the re-stabilization of the colloidal system [37]. However, insufficient PAC could not sustain the flocculation process. Consequently, there is an equilibrium point between flocculating rate and Al_i concentration.

According to the target value of the two individual responses, flocculating rate 100% and Al_i concentration 0 mg/L, the optimal condition calculated from the regression equations were MBFGA1 at 109.37 mg/L, PAC at 81.87 mg/L, initial pH at 8.5, time duration of stir for 72.5 min and ambient temperature at 24.3 °C. The values of flocculating rate and Al_i concentration in verification test operated under optimal condition were greater than 99% and lower than 0.136 mg/L, respectively.

3.3. Fourier transform-infrared spectra analysis

The functional groups involved in aluminum biosorption by MBFGA1 were elucidated using FT-IR spectroscopy. Fig. 5 shows the FT-IR comparison between virgin and aluminum loaded MBFGA1 in the range of 4000–400 cm^{-1}. The spectra of virgin MBFGA1 (solid line) displayed a number of absorption peaks indicating the complex nature of the biomass examined. The broad bands in the range of 3600–3200 cm^{-1} were attributed to the stretching vibrations of O—H and N—H groups. The absorption bands at 2935, 1651, 1268, 1134 and 1022 cm^{-1} represented the stretching vibrations of C—H, C═O, C—N, C—O—C and C—O, respectively. The absorption bands at 1459 and 1365 cm^{-1} represented the asymmetric and symmetric bending of $–CH_2–$ and $–CH_3$. The latter absorption band at 926 cm^{-1} was likely attributed to the glycosidic ring vibration. In conclusion, the main functional groups present on MBFGA1 were carboxyl,

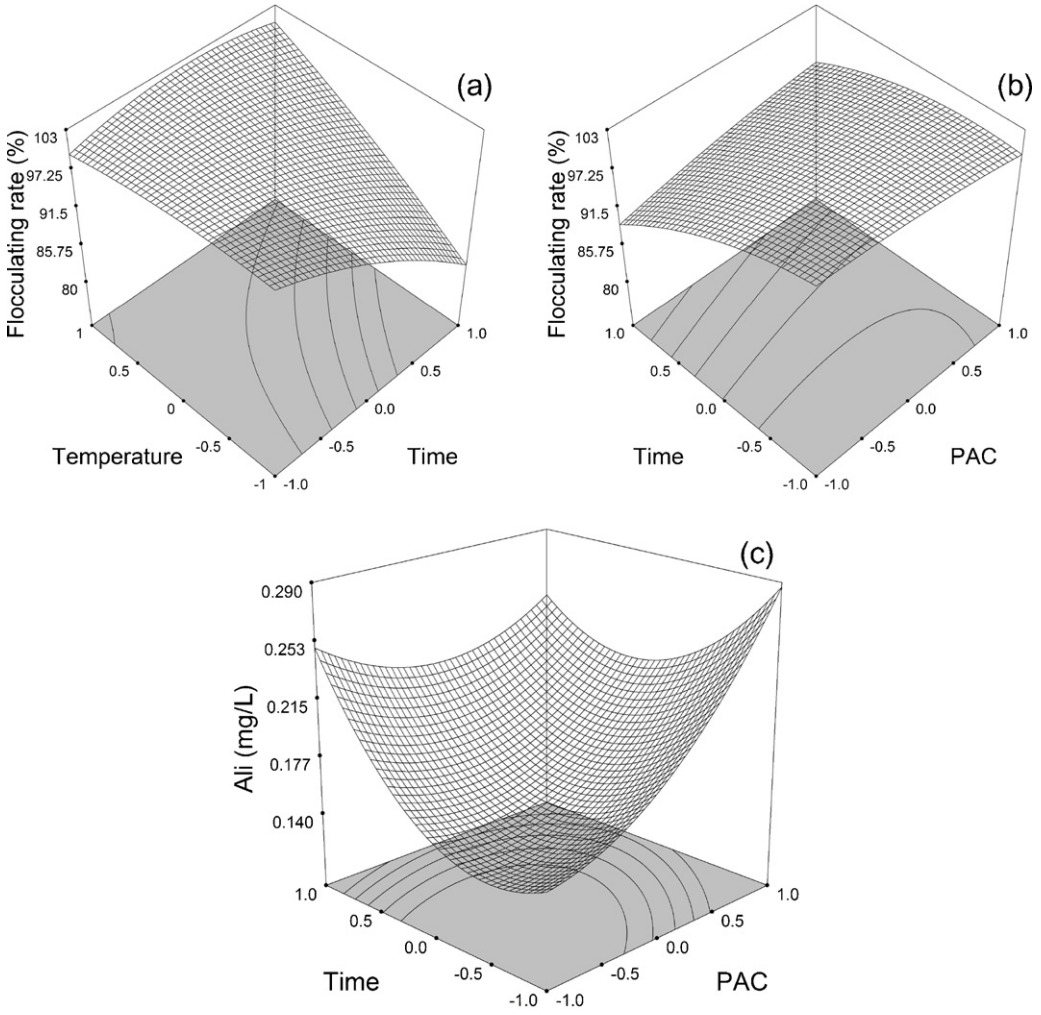

Fig. 4. Surface graphs of flocculating rate and Al_i concentration showing the effect of variables: (a) temperature–time, (b) time–PAC and (c) time–PAC, respectively.

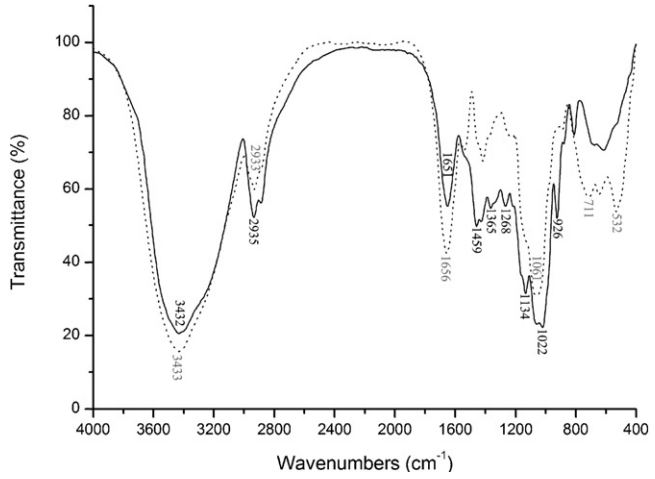

Fig. 5. Fourier transform-infrared spectra of virgin MBFGA1 (solid line) and aluminum loaded MBFGA1 (dotted line). (The values in black and gray represent the wavenumbers of absorption peak, transmittance percent less than 60%, in virgin and aluminum loaded MBFGA1, respectively.)

amine and hydroxyl, which always played vital roles in biosorption of metal cations [38]. According to the spectra of aluminum loaded MBFGA1 (dotted line), the wavenumber and intensity of some absorption peaks were shifted or changed obviously, suggesting the functional groups in the binding of aluminum. In addition, in order to eliminate the interference brought by aluminum precipitation, the biosorption pH was adjusted to 4.0 which might also influence the structure of MBFGA1. For example, the vanishing of the peaks at 1134 and 926 cm^{-1} might derive from the hydrolysis of glycosidic, which could explain why the intensity of O—H and C=O increased. In general, increasing the pH increased the overall negative charge on the surface of biomass until all the relevant functional groups were deprotonated, which favored the electrochemical attraction and adsorption of cations [30]. However, the new generated absorption peaks on the spectra of dotted line in the range of 800–400 cm^{-1} demonstrated its strong capacity on binding of aluminum at low level of pH, which might attribute to carboxylic groups available for the mechanism of ion exchange [39].

3.4. Environmental scanning electron microscope analysis

Fig. 6 shows ESEM micrographs of virgin and aluminum loaded MBFGA1 at different scan modes, respectively. In secondary electron (SE) mode, virgin MBFGA1 prepared by vacuum freeze drying had the flaky texture and smooth surface morphology. On contrast, aluminum loaded MBFGA1 became fibrous texture and rough surface morphology. In backscatter electron (BSE) mode, strongly related to the atomic number, the aluminum loaded MBFGA1 clearly showed the distribution of metal atoms in the form of highlighted spot, which might due to aluminum uptake and crystalloid deposition.

Furthermore, EDS analysis also provided the evidence that the aluminum had been bound on the surface of MBFGA1. As shown

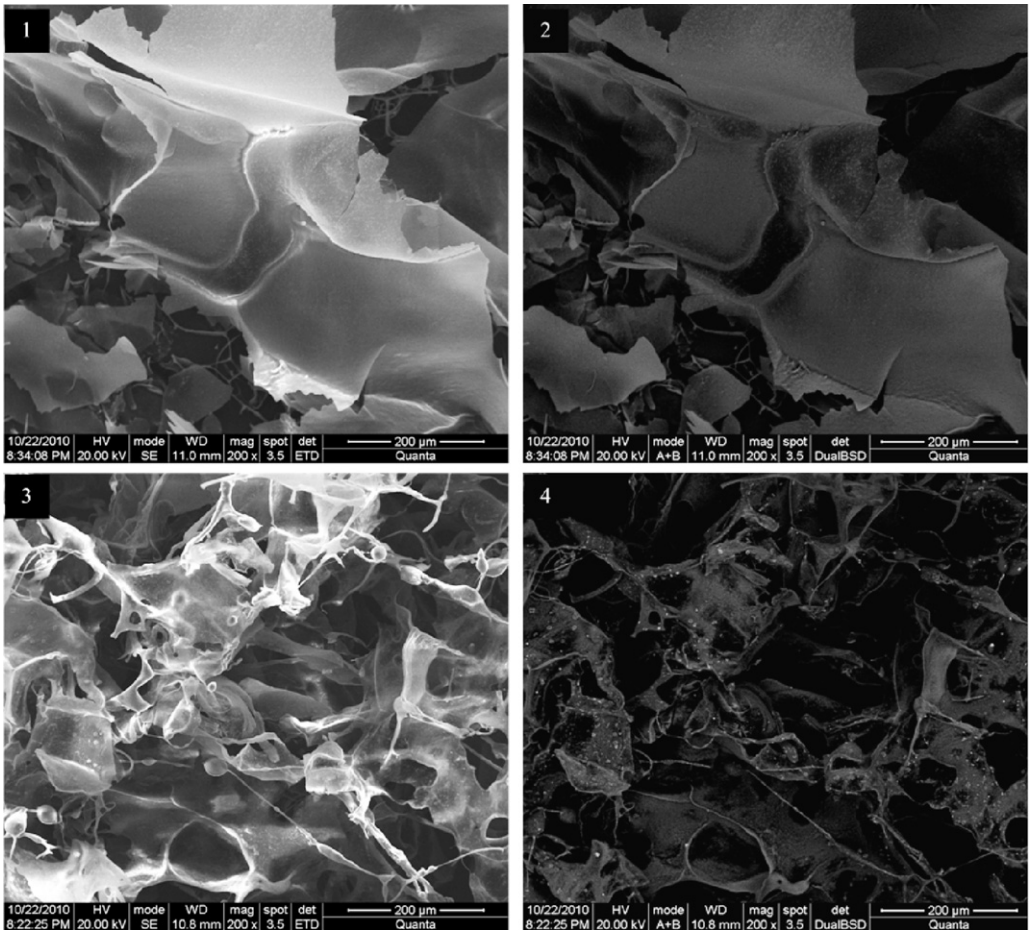

Fig. 6. Environmental scanning electron microscope graphs: (1) SE mode of virgin MBFG1 at 200×, (2) BSE mode of virgin MBFG1 at 200×, (3) SE mode of Al loaded MBFG1 at 200× and (4) BSE mode of Al loaded MBFG1 at 200×.

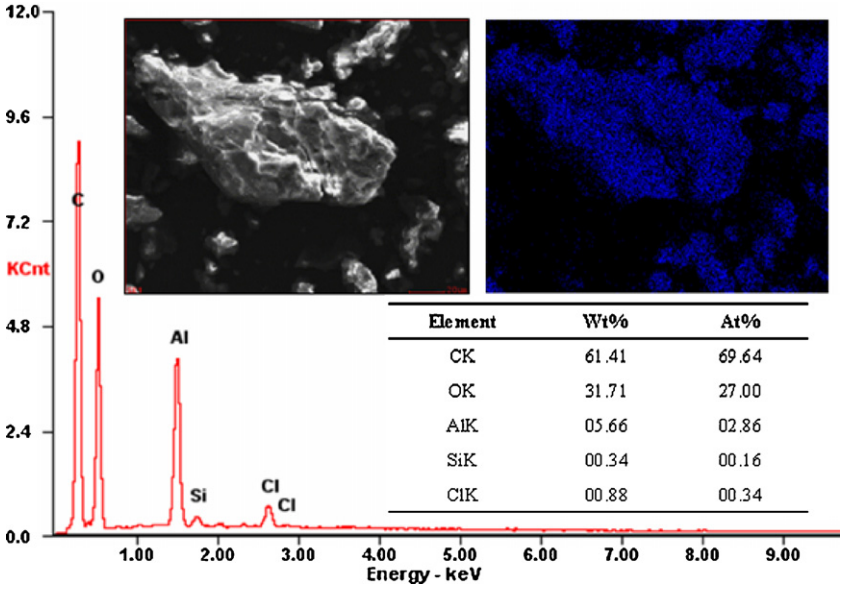

Fig. 7. Energy dispersive spectrometer analysis of aluminum loaded MBFGA1. (For interpretation of the references to color in the text, the reader is referred to the web version of the article.)

in Fig. 7, the shadow, in blue, visualized the aluminum atoms distribution which almost coincided with the shape and position of aluminum loaded MBFGA1 fragments exhibited on the left. Meanwhile, the table and curve show the aluminum content of 5.66% in the detection area. These clearly demonstrated a strong coordination linkage between the aluminum and the functional groups on MBFGA1.

4. Conclusions

Application of PAC significantly elevated the residual aluminum in the outlet water after small-scale treatment of coagulation–flocculation. According to the classification method, Al_a was the dominant species in residual total aluminum and Al_o was the dominant species in residual monomeric aluminum. The removing of the species in Al_r mainly depended on flocculating efficiency. The biosorption behavior of MBFGA1 had a positive effect on removing of monomeric aluminum in particular the form of Al_i, which was meaningful for reducing the toxicity of residual aluminum.

According to response surface methonolody, the optimal operation conditions calculated from the regression equations were MBFGA1 at 109.37 mg/L, PAC at 81.87 mg/L, initial pH at 8.5, time duration of stir for 72.5 min and ambient temperature at 24.3 °C, which maintained flocculating rate greater than 99% and Al_i concentration lower than 0.136 mg/L.

FT-IR spectroscopy and ESEM further demonstrated that the main functional groups present on MBFGA1 were carboxyl, amine and hydroxyl, which played vital roles in biosorption of residual aluminum by means of surface complex reaction or ion exchange.

Acknowledgments

This project was supported by National Natural Science Foundation of China (grant numbers 30970105 and 51078131) and Scholarship Award for Excellent Doctoral Student granted by Ministry of Education. The authors also acknowledge Ms. Zhou Y. from Hong Kong Baptist University for her critical reading of the manuscript.

References

[1] T. George, L.B. Franklin, S. David, Wastewater Engineering, 4th edition, Matcalf and Eddy, New York, 2003.
[2] W.D. Wang, H.W. Yang, X.C. Wang, J. Jiang, W.P. Zhu, Effects of fulvic acid and humic acid on aluminum speciation in drinking water, J. Environ. Sci. 22 (2010) 211–217.
[3] Z.L. Yang, B.Y. Gao, Q.Y. Yue, Y. Wang, Effect of pH on the coagulation performance of Al-based coagulants and residual aluminum speciation during the treatment of humic acid-kaolin synthetic water, J. Hazard. Mater. 178 (2010) 596–603.
[4] K. Meea, C. Hong, S. Yuko, K. Tasuku, M. Yasumoto, Rapid and economical indicator for evaluating arsenic removal with minimum aluminum residual during coagulation process, Water Res. 37 (2003) 4599–4604.
[5] K. Steve, E. Marc, Solubility controls on aluminum in drinking water at relatively low and high pH, Water Res. 36 (2002) 4356–4368.
[6] P.F. Trond, Aluminum as a risk factor in Alzheimer's disease with emphasis on drinking water, Brain Res. Bull. 55 (2001) 187–196.
[7] P.T. Srinivasan, T. Viraraghavan, K.S. Subramanian, Aluminum in drinking water: an overview, Water SA 25 (1999) 47–55.
[8] World Health Organization, Guidelines for Drinking-water Quality, 3rd edition, WHO Press, Geneva, 2008.
[9] Z.H. Yang, J. Huang, G.M. Zeng, M. Ruan, C.S. Zhou, L. Li, Z.G. Rong, Optimization of flocculation conditions for kaolin suspension using the composite flocculant of MBFGA1 and PAC by response surface methodology, Bioresour. Technol. 100 (2009) 4233–4239.
[10] M. Ruan, Z.H. Yang, G.M. Zeng, L.J. Xiong, R. Tao, R.J. Wang, Y.S. Liu, Flocculating capability and mechanism of bioflocculant produced by *Paenibacillus polymyxa* GA1, China Environ. Sci. 28 (2007) 2336–2341.
[11] O. Anselm, C. Jon, Spectroscopic study of extracellular polymeric substances from *Bacillus subtilis*: aqueous chemistry and adsorption effects, Biomacromolecules 5 (2004) 1219–1230.
[12] M.J. Prachi, A.J. Asha, In vivo studies to elucidate the role of extracellular polymeric substances from *Azotobacter* in immobilization of heavy metals, Environ. Sci. Technol. 43 (2009) 5884–5889.
[13] E. Gauthier, I. Fortier, F. Courchesne, P. Pepin, J. Mortimer, D. Gauvreau, Aluminum forms in drinking water and risk of Alzheimer's disease, Environ. Res. 84 (2000) 234–246.
[14] R.A. Yokel, M.S. Golub, Research Issues in Aluminum Toxicity, Taylor and Francis, Washington, 1997.
[15] M. Peter, K. Jana, Complexation efficiency of differently fixed 8-hydroxyquinoline and salicylic acid ligand groups for labile aluminum species determination in soils—comparison of two methods, Anal. Chim. Acta 573 (2006) 474–481.
[16] W.G. Robert, C.P. Richard, The bioavailability and toxicity of aluminum in aquatic environments, Crit. Rev. Environ. Sci. Technol. 29 (1999) 315–450.
[17] C.T. Driscoll, A procedure for the fractionation of aqueous aluminum in dilute acidic waters, Int. J. Environ. Anal. Chem. 16 (1984) 267–283.
[18] E.V. Hullebusch, V. Deluchat, P.M. Chazal, M. Baudu, Environmental impact of two successive chemical treatments in a small shallow eutrophied lake: Part I. Case of aluminium sulphate, Environ. Pollut. 120 (2002) 617–626.

[19] H.Z. Lian, Y.F. Kang, Y. Arkin, S.P. Bi, D.N. Li, S.Z. Mei, X.J. Wu, X.C. Tao, Y.J. Chen, L.M. Dai, N. Gan, L.C. Tian, Fractionation of aluminum in natural waters by fluorometry based on the competitive complexation, Anal. Chim. Acta 511 (2004) 25–31.
[20] R. Triveni, T.R. Shamala, N.K. Rastogi, Optimized production and utilization of exopolysaccharide from *Agrobacterium radiobacter*, Process Biochem. 36 (2001) 787–795.
[21] Z.H. Yang, R. Tao, G.M. Zeng, Y. Xiao, E.J. Deng, Culture medium and grading culture technics for bioflocculant production by *Paenibacillus polymyxa* GA1, China Environ. Sci. 27 (2006) 1444–1449.
[22] C.S. David, J.H. Matthew, Examination of three theories for mechanisms of cation-induced bioflocculation, Water Res. 36 (2002) 527–538.
[23] H. Salehizadeh, S.A. Shojaosadati, Extracellular biopolymeric flocculants: recent trends and biotechnological importance, Biotechnol. Adv. 19 (2001) 371–385.
[24] A.M. James, The electrical properties and topochemistry of bacterial cells, Adv. Colloid Interface Sci. 15 (1982) 171–221.
[25] L. Wang, H. Chua, Q. Zhou, P.K. Wong, S.N. Sin, W.L. Lo, P.H. Yu, Role of cell surface components on Cu^{2+} adsorption by *Pseudomonas putida* 5-x isolated from electroplating effluent, Water Res. 37 (2003) 561–568.
[26] M. Otto, Chemometrics, Wiley-VCH Verlag, Weinheim, 1998.
[27] L.M. Marcelo, I.P. Diego, R.C. Nádia, B.C. Sibele, Synthesis of mordenite using kaolin as Si and Al source, Appl. Clay Sci. 41 (2008) 99–104.
[28] F. Zheng, B. Hu, Novel bimodal porous N-(2-aminoethyl)-3-aminopropyltrimethoxysilane-silica monolithic capillary microextraction and its application to the fractionation of aluminum in rainwater and fruit juice by electrothermal vaporization inductively coupled plasma mass spectrometry, Spectrochim. Acta, Part B 63 (2008) 9–18.
[29] B. Volesky, Z.R. Holan, Biosorption of heavy metals, Biotechnol. Prog. 11 (1995) 235–250.
[30] K. Vijayaraghavan, Y.S. Yun, Bacterial biosorbents and biosorption, Biotechnol. Adv. 26 (2008) 266–291.
[31] S.M.Z. Al-Kindy, F.O. Suliman, S.B. Salama, A sequential injection method for the determination of aluminum in drinking water using fluorescence enhancement of the aluminum–morin complex in micellar media, Microchem. J. 74 (2003) 173–179.
[32] A.V. Victor, H. Alfred, An aluminum–morin fluorescence assay for the visualization and determination of aluminum in cultured cells of *Nicotiana tabacum* L. cv. BY-2, Plant Sci. 122 (1997) 35–42.
[33] M. Patience, A.M. Jonas, R. John, Temperature influence of nonionic polyethylene oxide and anionic polyacrylamide on flocculation and dewatering behavior of kaolinite dispersions, J. Colloid Interface Sci. 271 (2004) 145–156.
[34] Y. Yuan, R.F. Ramin, Strength and breakage of activated sludge flocs, Powder Technol. 199 (2010) 111–119.
[35] S. Al-Ashed, Z. Duvnjak, Adsorption of copper and chromium by *Aspergillus carbonarius*, Biotechnol. Prog. 11 (1995) 638–642.
[36] J.P.V. Vítor, M.S.B. Cidália, A.R.B. Rui, Influence of pH, ionic strength and temperature on lead biosorption by Gelidium and agar extraction algal waste, Process Biochem. 40 (2005) 3267–3275.
[37] B.A. Bolto, Chapter 5: Coagulation and flocculation with organic polyelectrolytes, Interface Sci. Technol. 10 (2006) 63–88.
[38] D. Park, Y.S. Yun, J.M. Park, The past, present and future trends of biosorption, Biotechnol. Bioprocess Eng. 15 (2010) 86–102.
[39] M.M. Figueira, B.H. Volesky, J. Mathieu, Instrumental analysis study of iron species biosorption by *Sargassum* biomass, Environ. Sci. Technol. 33 (1999) 1840–1846.