Preparation and characterization of composite bioflocculants in comparison with dual-coagulants for the treatment of kaolin suspension

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HIGHLIGHTS

- The modification of bioflocculants by cations or inorganic coagulants was proposed.
- Composite bioflocculants (PACICBF) were prepared from CBF and PACI.
- PACICBF was more efficient than dual-coagulants for lower kaolin concentrations.

GRAPHICAL ABSTRACT

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ABSTRACT

In this study, the preparation of composite bioflocculants from compound bioflocculant (CBF) and polyaluminum chloride (PACI) was investigated under different PACI/CBF ratios (v/v), basicities ($[\text{OH}]/[\text{Al}]$ ratios) and polymerization approaches: the composite polymerization (PACICBF) and the co-polymerization (PCBFACI). Fourier transform infrared (FTIR), Al-feron spectrophotometry, scanning electron microscope (SEM) and zeta potentials studies were performed as well to characterize the composite bioflocculants. Finally, coagulation performance and floc properties of the composite bioflocculant in comparison with dual-coagulants were studied for treatment of kaolin suspension with different concentrations. From the results, interactions took place between Al species and CBF molecules, which lead to the formation of composite species. Significant improvement of coagulation efficiency was achieved by composite bioflocculants at PACI/CBF ratio ranging from 0.05 to 0.25 and basicity ranging from 1.1 to 1.7. PACICBF appeared to be more efficient than PCBFACI owing to their higher Al_b content. Furthermore, composite bioflocculants was more preferred than dual-coagulants for treatment of kaolin suspension with lower concentrations ($<50 \text{ mg/L}$).

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1. Introduction

In general, bioflocculants are extracellular biopolymers containing polysaccharides, glycoproteins, proteins and lipids [1]. Many microorganisms, including algae, bacteria, yeast and fungi have been found to produce bioflocculants [2–5]. The studies and appli-

cations of bioflocculants have attracted great attention because of their significant advantages, such as biodegradability, inherently benign and safety to human beings [6]. However, as bioflocculants carry highly negative charges [2,7], little or no flocculation occurs when they are present alone [8,9]. The low flocculating activity and costly production of bioflocculants result in limited practical applications [10].

Many efforts have been made to overcome these limitations. Most prior research focused on screening for microorganisms, culture conditions and the combined use of bioflocculants with

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inorganic coagulants (dual-coagulants). Recently, Ma et al. proposed a compound bioflocculant (CBF) with higher flocculating activity by co-culturing two strains isolated from soil samples [11]. Bo et al. studied the coagulation performance and floc properties of CBF combined with aluminum sulfate in the treatment of synthetic kaolin-humic water [12]. Zhao et al. studied the coagulation performance and floc characteristics of CBF combined with titanium tetrachloride or aluminum sulfate [13]. And Yang et al. employed the response surface methodology to study the optimum flocculating conditions using a bioflocculant MBFGA1 combined with polyaluminum chloride and CaCl_2 [14]. However, additional equipment was still required for handling dual-coagulants, which derived from separate reagents. Since flocculating activity and floc properties are significantly improved by dual-coagulants, the modification of bioflocculants by inorganic coagulants will be worthy of study. There has been no previous report of the composite bioflocculants as one unique reagent.

Additionally, floc properties including floc size, floc growth rate, fractal dimension (D_f), floc strength and recovery factors are important parameters in coagulation process [15]. A higher floc growth rate and larger floc size are usually preferred as they imply a faster coagulation process [16]. Since it is inevitable for flocs to break into small particles during the separation process with high shear force, fractal dimension (D_f), floc strength and recovery factors are considered to be crucial as well [17]. Flocs with a larger value of strength factor are better to resist rupture. Likewise, flocs with larger recovery factors have better recovery capacity after breakage. And D_f increased when flocs are stronger and more compact. But little is known about the floc properties of composite bioflocculant.

In this study, the preparation of composite bioflocculants was proposed by using compound bioflocculant (CBF) and polyaluminum chloride (PACl) under different PACl/CBF ratios (v/v), basicities ($[\text{OH}]/[\text{Al}]$ ratio) and polymerization approaches: co-polymerization (hydroxylation of the mixture consisting of AlCl_3 and CBF, PCBFACl) and composite polymerization (hydroxylated PACl combined with CBF, PAClCBF). The characterizations of composite bioflocculants were investigated using Fourier transform infrared (FTIR), Al-ferron spectrophotometry, scanning electron microscope (SEM) and zeta potentials. A jar test was conducted by treating kaolin suspension to study the coagulation performance and floc properties of composite bioflocculants in comparison with dual-coagulants.

2. Methods

2.1. Materials

Poyaluminum chloride (PACl, $\text{Al}_2\text{O}_3\%$ = 10.0%, basicity = 1.7) and polyferric sulfate (PFS, $\text{Fe}\%$ = 11%) were obtained from Shandong Zhongke Tianze Purification Materials Corporation, China. Cations were prepared by their chloride salts (analytically grade). Compound bioflocculant (CBF), produced by mixed culture of *Rhizobium radiobacter* F2 and *Bacillus sphaericus* F6, was obtained from State Key Lab of Urban Water Resource and Environment, Harbin Institute of Technology, China. CBF was mainly composed of polysaccharide (90.6%) and protein (9.3%). Zeta potential of CBF was ca. -46.0 mV, and its molecular weight was about 10^5 – 10^6 Da [11].

2.2. Procedure for the preparation of composite bioflocculants

Inorganic polymer flocculants and cations were examined for the preparation of composite bioflocculants. The modification of CBF was carried out by adding an appropriate amount of each

cation or inorganic polymer flocculant into CBF (denoted as PAClCBF, PFS-CBF, AlCl_3 -CBF, FeCl_3 -CBF, CaCl_2 -CBF, MgCl_2 -CBF). PACl was then selected for further investigation. Composite bioflocculants were prepared under different PACl/CBF ratios (v/v, varying from 0.025 to 0.375), basicities ($[\text{OH}]/[\text{Al}]$, varying from 0.6 to 2.2) and polymerization approaches: the composite polymerization (PAClCBF) and the co-polymerization techniques (PCBFACl) [18].

In the composite polymerization, the appropriate amount of AlCl_3 solution was slowly titrated by NaOH, creating an intermediate PACl solution, and then added into a predetermined amount of CBF. In the co-polymerization, the appropriate amounts of AlCl_3 solution was initially added into a predetermined amount of CBF. Afterwards, the desired amount of NaOH solution was slowly added in order to achieve the target basicity. Other reaction conditions were predetermined as the optimum conditions (reaction temperature of 60°C and time of 2 h) through our preliminary experiments (data not shown).

2.3. Jar test

Coagulation experiments were performed by jar test (JTY-6 Laboratory Stirrer) at room temperature. The dosage of coagulants expressed in mg/L referred to the dry weight of coagulants. Kaolin suspension was prepared by dispersing kaolin (average diameter 2–4 μm) in deionized water which contained 5.0 mmol/L NaHCO_3 . The pH was not adjusted after the addition of coagulants for the buffering capacity of suspension (the final pH was around 8.0). The samples were stirred rapidly at 200 rpm for 1 min, followed by slow stirring at 40 rpm for 15 min and sedimentation for 30 min [12]. After settling, about 10 mL of sample was withdrawn 1 cm below the liquid surface for analytical determinations. Coagulation performance of the composite bioflocculant in comparison with dual-coagulants were carried out at different kaolin concentrations (varying from 25 to 5000 mg/l). Dual-coagulants, PACl–CBF (PACl dosed firstly) and CBF–PACl (CBF dosed firstly), were applied by dosing PACl and CBF in reverse order according to Bo et al. [12].

Coagulation efficiency was expressed as flocculating rate. The measurement of flocculating rate was based on a decrease in OD_{550} of kaolin suspension according to the method of Kurane et al. with several modifications [19]. The absorbance of the supernatant and the blank control without bioflocculants was measured at 550 nm (as OD_{550} and $\text{OD}_{550,\text{blank}}$, respectively) with a spectrophotometer (Hach DR-5000). The flocculating rate was defined and calculated as follows:

$$\text{flocculating rate}(\%) = \frac{\text{OD}_{550,\text{blank}} - \text{OD}_{550}}{\text{OD}_{550,\text{blank}}} \times 100 \quad (1)$$

2.4. Characterization

Composite bioflocculants was dried by a freeze dryer (Boyikang FD-1A-50). Dried powders were characterized by Fourier transform infrared (FTIR, Bruker Optics Tensor 27) and scanning electron microscope (SEM, Hitachi S-3000). Zeta potentials were determined using a zeta potential analyzer (Malvern Zetasizer 2000).

The Al(III) species distribution was measured by a timed complexation spectroscopy method with a spectrophotometer (Hach DR-5000). Visible light absorbance was measured as a function of time at a wavelength of 370 nm to qualify the amount of metal complex formed. The Al(III) species reacting with ferron in 1 min was considered to be Al(III) monomeric species (denoted as Al_a), and the Al(III) species reacting with ferron over the next 2 h was considered to be Al(III) polymeric species (denoted as Al_b), and

the unreactive Al(III) species in 2 h was considered to be Al(III) colloidal and precipitated species (denoted as Al_c) [20].

Floc formation, breakage and recovery during the coagulation process were conducted using a jar tester. Coagulation process consisted of 1 min of rapidly stirring (200 rpm), 15 min of slow stirring (40 rpm) for floc formation, 1 min of rapidly stirred (200 rpm) for breakage, and 15 min of slow stirring (40 rpm) for recovery [12]. Dynamic floc size was determined using a laser diffraction instrument (Malvern Mastersizer 2000) as the coagulation processed. The suspension was monitored by drawing water through the optical unit of the Mastersizer and back into the jar by a peristaltic pump, which was located downstream of the instrument to prevent disturbing the flocs prior to measurement. Size measurements were taken every 0.5 min and the median volumetric diameter ($d_{0.5}$) was used to denote the floc size. The floc growth rate was denoted as the slope of rapid growth region [16]. The breakage and recovery of flocs were studied using the strength and recovery factors [21]:

$$\text{growth rate} = \frac{\Delta \text{size}}{\Delta \text{time}} \quad (\mu\text{m}/\text{min}) \quad (2)$$

$$\text{strength factor} = \frac{d_2}{d_1} \times 100 \quad (3)$$

$$\text{recovery factor} = \frac{d_3 - d_2}{d_1 - d_2} \times 100 \quad (4)$$

where d_1 is the average floc size of the steady phase before breakage, d_2 is the floc size after breakage and d_3 is the floc size of the new steady phase after regrowth.

Fractal dimension (D_f) of flocs can be determined from the negative slope of a plot of log scatter intensity I as a function of log scatter vector Q :

$$I \propto Q^{-D_f} \quad (5)$$

The vector was defined as the difference between the incident and scattered wave vectors of the radiation beam in the medium, which was given by:

$$Q = \frac{4\pi n}{\lambda} \sin\left(\frac{\theta}{2}\right) \quad (6)$$

where n , θ , and λ were the refractive index of the medium, the scattered angle and the wavelength of radiation in vacuum, respectively [22].

3. Results and discussion

3.1. Determination of kaolin concentration

In order to determine the concentration of kaolin suspension, turbidity and OD₅₅₀ were employed by different studies. Fig. 1 shows the development of turbidity (NTU) and OD₅₅₀ as a function of the kaolin concentration and the calibration curve of OD₅₅₀ and turbidity. OD₅₅₀ was found to be a linear function of turbidity when kaolin concentration was lower than 500 mg/L. Therefore, kaolin suspension with different concentrations were measured in terms of OD₅₅₀.

3.2. Selection of cations

Presence of cations could stimulate the coagulation process by neutralizing residual negative charges of both bioflocculants and suspended particles [6,23]. This effect was highly dependent on both the concentration and valence of cations [24]. It was different when bioflocculant was modified by cations. As can be seen from

Fig. 1. Development of turbidity (NTU) and OD₅₅₀ as a function of the kaolin concentration and the calibration curve of OD₅₅₀ and turbidity.

Fig. 2. Flocculating rate of composite bioflocculants with various cations.

Fig. 2, little or no flocculation occurred when CBF was modified by PFS, MgCl₂ and CaCl₂. This reduction of coagulation performance was partially due to the solids formation during modification, which also suggested the existence of strong interactions between CBF molecules and several cations (e.g. electrostatic interactions and H-bonding) [25]. The composite bioflocculant prepared from CBF and PACI was found to be a relatively clear solution. Specifically, significant improvement of coagulation efficiency was obtained by using PACICBF. Therefore, inorganic coagulant PACI was selected for the preparation of composite bioflocculants.

3.3. Preparation of composite bioflocculants

3.3.1. Effect of PACI/CBF ratios

The effect of different PACI/CBF ratios on the flocculating rate was shown in Fig. 3. At the PACI/CBF ratio of 0.025, the flocculating rate was less than that of PACI. Zeta potential were still negative at lower PACI/CBF ratios, leading to insufficient charge neutralization. The inter-particle repulsion was strong resulting in poor coagulation performance. This indicated that charge neutralization was one important mechanism by composite bioflocculants. Under the PACI/CBF ratios ranging from 0.05 to 0.25, flocculating rate was significantly increase with increasing PACI/CBF ratio. Synergistic effects were observed as composite bioflocculants were more

Fig. 3. Effect of PACI/CBF ratio (v/v) on flocculating rate of composite bioflocculants.

efficient than PACI. However, when PACI/CBF ratio increased to 0.375, the flocculating rate slightly decreased. Therefore, an optimal amount of PACI (i.e. PACI/CBF ratio of 0.25) could be introduced to CBF.

3.3.2. Effect of basicity

A series of composite bioflocculants were prepared under different polymerization approaches. Fig. 4 shows the effect of basicity on flocculating rate of PACICBF in comparison with PCBFACI. Flocculating rate increased with increasing basicity, except for a slight decrease when basicity reached 2.2. Composite bioflocculants with the highest basicity became unstable as the stratification occurred within a few hours. It was preferred to prepare composite bioflocculants with basicity ranging from 1.1 to 1.7. Although composite bioflocculants prepared with the composite polymerization method (i.e. PACICBF) are more efficient than those prepared with the co-polymerization method (i.e. PCBFACI), this difference was not obvious when basicity was in the range from 1.1 to 1.7. It was indicated that coagulation performance was closely related to Al

species as basicity increased, while other mechanisms could be involved as well. Based on the above described results, PACI (basicity = 1.7) was directly used for the preparation of composite bioflocculants at PACI/CBF ratio of 0.25.

3.4. Characterization of composite bioflocculants

3.4.1. Al species distribution

Al species distribution of PACICBF and PCBFACI are shown in Fig. 5. Similar trends were observed for both kinds of composite bioflocculants. The Al_a content decreased, while the Al_c content increased along with an increase in basicity. The Al_b content increased and reached the highest at basicity of 1.7, then decreased. $Al(III)$ polymeric species (Al_b) was commonly recognized as the most active species for charge neutralization and particle bridging actions in coagulation process [26]. A production of high content of Al_b have been the main aim of the production of PACI [27]. It was also crucial to produce high content of Al_b for the preparation of composite bioflocculants.

Despite the fact that Al species distribution was similar, there was still an alternation regarding the polymerization approach. More Al_b content and less Al_a content were obtained in PACICBF than those in PCBFACI. During the composite polymerization, freshly prepared PACI solution was reacted with CBF, polymeric species (Al_b) initially existed in the mixture. In the case of copolymerization, Al^{3+} solution and CBF were polymerized together. Monomeric species (Al_a) were the main hydrolysis products, and Al_b does not exist in the original Al^{3+} solution. It seemed that the presence of CBF tended to hinder the formation of Al_b during polymerization. This finding provides an explanation for the effect of basicity on flocculating rate.

3.4.2. SEM

Fig. 6 illustrates SEM images of PACICBF and PCBFACI with different basicities. Changing basicity noticeably affects the morphology of samples. A smooth surface gradually became to be irregular. Distinguishable cubes and amorphous aggregates were formed as basicity increased. It can be suggested that these irregular formations consisted mainly of Al species [18]. Composite bioflocculants could consist of Al polymerized species, CBF molecules and

Fig. 4. Effect of basicity on flocculating rate of PACICBF and PCBFACI.

Fig. 5. Al species distribution of PACICBF and PCBFACI with different basicities.

probably composite species. However, it remains difficult to evaluate the effect of different polymerization approaches on the morphology of samples from the visual comparison of SEM images of PACICBF and PCBFACI.

3.4.3. FTIR

Fig. 7 illustrates the FTIR spectra of CBF, PACI and composite biofloculants with different basicities. A weak C–H asymmetric stretching vibration band was observed at 2926 cm^{-1} . The weak band near 1429 cm^{-1} was characteristic of the bending vibration of CH_3 and the scissor vibration of CH_2 , which indicated the presence of carboxyl groups. The strong peak observed around 1085 cm^{-1} was assigned to the C–O stretching vibration. These bands could be an indication of polysaccharide and its derivatives in CBF [9,28]. The major changes of CBF after the modification by PACI occurred at 3400 cm^{-1} and 1634 cm^{-1} , which were assigned to OH stretching from the hydroxyl group and the Al–O bond vibrations. When basicity was higher than 1.7, the Al–OH–Al stretching vibrations and Al–OH transformation vibrations were apparently observed at 1100 cm^{-1} and 613 cm^{-1} . These bands were expected in the structure of PACI as well [29]. Specifically, the bands at the region $2970\text{--}2840\text{ cm}^{-1}$ and $2380\text{--}2285\text{ cm}^{-1}$ disappeared. These alternations could be indicative of interaction between PACI and CBF molecules, which result in the formation of composite species. Regarding the changes occurring in the spectra of PCBFACI, it could

be noticed that they were similar as in the case of PACICBF. It seemed that the different polymerization approaches did not influence the nature of the bonds in the composite biofloculants, which agreed with the conclusion by N.D. Tzoupanos and A.I. Zouboulis in the preparation of composite coagulants from PACI and polyelectrolyte [18].

3.5. Comparative study of composite biofloculants and dual-coagulants

3.5.1. Coagulation efficiency

Fig. 8 displays the coagulation efficiency of PACICBF, in comparison with two dual-coagulants (PACI–CBF and CBF–PACI). Distinct difference in coagulation efficiency was obtained for the treatment of kaolin suspension with different concentrations. When kaolin concentration was lower than 50 mg/L , PACICBF gave better coagulation efficiencies than others, while CBF–PACI performed better when kaolin concentration was higher than 100 mg/L . Composite biofloculants and dual-coagulants could be applied according to different water qualities.

For all coagulants, coagulation efficiency was influenced by the kaolin concentration. At lower kaolin concentrations, the distance between suspended kaolin particles were relatively larger. As a consequence, the collision probability of particles was reduced. PACICBF was expected to span the gap between particles through charge neutralization and bridging mechanisms together. At higher kaolin concentrations, the distance between kaolin particles were smaller. CBF–PACI increased the effective collision diameter of particles through an adsorption bridging mechanism. Moreover, the optimal dosage increased with the increasing kaolin concentration, which was consistent with adsorption and charge neutralization mechanisms [30].

3.5.2. Zeta potential

CBF was known to have high negative charges (-46.0 mV). Zeta potential was greatly changed when CBF was modified by highly positive charged PACI [31]. As can be seen from Fig. 9, zeta potential increased with the increase of coagulant dosage. When coagulant dosage was in the range of $1.3\text{--}3.9\text{ mg/L}$, PACICBF exhibited the highest impact on the colloids surface charge. Charge reversal occurred with PACICBF at the dosage of 6.5 mg/L , whereas the zeta potential was 0.5 mV . The modification of CBF by PACI significantly increased the charge neutralization ability of composite biofloculants. Kaolin particles could be destabilized through charge neutralization. In addition, PACI–CBF exhibited the weakest effect on the colloids surface charge, while CBF–PACI exhibited the strongest

Fig. 6. SEM images of PACICBF and PCBFACI.

Fig. 7. FT-IR spectra of PACI, CBF, PACICBF and PCBFACI.

Fig. 8. Comparative flocculating rate of PACICBF, PACI-CBF and CBF-PACI.

impact when coagulant dosage was in the range of 5.2–6.5 mg/L. This phenomenon is consistent with the observation by Bo et al., when using CBF-aluminum sulfate dual-coagulants in treating kaolin-humic acid solution [12].

3.5.3. Floc formation, breakage and recovery

Floc formation, breakage and recovery are of great practical importance. The change in floc size during coagulation process are shown in Fig. 10. Flocs rapidly grew and flocs formation soon began after coagulants addition. During breakage process, an obvious drop in floc size was observed. Then flocs began to recovery and reached a new steady state as the slow stirring was reintroduced.

As shown in Table 1, floc size, growth rate, strength factor, recovery factor and D_f were adopted to investigate the floc

properties. CBF-PACI exhibited the lowest strength factor as Al species reacted with both kaolin particles and CBF. This agreed with the observation of Bo et al. who found that flocs formed by CBF-aluminum sulfate were weaker [12]. However, in contrast to the observation of Bo et al., relatively small flocs were formed by PACI-CBF. This difference could be ascribed to the inorganic polymerized flocculants PACI, which involved both charge neutralization and adsorption bridging mechanisms during coagulation. The bridging ability of CBF could be limited when PACI was dosed first.

In the case of composite bioflocculant, composite species were formed by reaction of CBF with Al species. The interaction between kaolin particles and composite species could be complex. The difference of D_f was small, which indicated no significant change in floc structure between composite bioflocculants and dual-coagulants. PACICBF exhibited higher growth rate, strength and

Fig. 9. Zeta potentials of PACICBF, PACI-CBF and CBF-PACI as a function of coagulant dosage.

Fig. 10. Floc formation, breakage and recovery of PACICBF, PACI-CBF and CBF-PACI.

Table 1

Floc properties of PACICBF, PACI-CBF and CBF-PACI.

Coagulant	Floc size	Growth rate	Strength factor	Recovery factor	D_f
PACICBF	627.5	226.2	55.2	7.8	2.44
PACI-CBF	551.6	140.3	54.6	6.5	2.46
CBF-PACI	637.7	217.2	51.9	7.1	2.48

recovery factors compared with dual-coagulants. Recovery factors of all coagulants were quite low, which indicated that sweep flocculation could contribute the coagulation process for sweep flocs were irreversible after breakage [32]. According to the foregoing results, composite bioflocculants cause coagulation of particles by charge neutralization, sweep flocculation and adsorption bridging. No matter which mechanism would be the dominant of coagulation, it can be assumed that bridging eventually occurred after the particles have adsorbed onto the chains of composite bioflocculants.

4. Conclusions

The preparation of the composite bioflocculant (PACICBF) from compound bioflocculant (CBF) and polyaluminum chloride (PACI) is accomplished. Based on the experimental results, PACI is preferred for the modification of CBF. Composite species form from

Al species and CBF molecules. PACICBF are more efficient than dual-coagulants for treatment of kaolin suspension with lower concentrations (<50 mg/L). Flocs formed by PACICBF are faster, stronger and more recoverable in comparison with dual-coagulants. It can be concluded that charge neutralization, adsorption bridging and sweep flocculation are involved in the coagulation process with PACICBF. Based on these results, the further studies and applications of composite bioflocculants seem to be promising.

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