Research Article

Colloidal Influence Factor of Bioflocculant in Coagulation of Chromium and Nitrobenzene

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The aim of this study is to investigate the flocculation capacity of activated sludge flocculant in chromium ion and nitrobenzene solutions. Besides comparing with activated carbon, we changed some potential factors which may influence the flocculation, such as solution pH value, reaction time, flocculant dosage, and solution initial concentration, and tested the flocculation capacity of this bioflocculant for both chromium and nitrobenzene. In addition, the flocculation of activated sludge after acidic or alkali modification for double solutions was also estimated. Compared with activated carbon, the activated sludge flocculant showed a good flocculation capacity for nitrobenzene, but poor flocculation for chromium following the initial concentration increase. The flocculation for nitrobenzene or chromium increased at initial stage and decreased gradually following the primary dosage of flocculant increase. The flocculation for nitrobenzene increased at the primary stage and decreased after peak, while the flocculation for chromium increased following the pH increase of both solutions. Although the flocculation for nitrobenzene decreased, the flocculation for chromium increased obviously, when we prolonged the reaction time. The flocculant showed a gradual increasing trend in double kinds of solution when they were heating. The flocculation for both nitrobenzene and chromium decreased after acid modification of activated sludge flocculant, while the flocculation increased after basic modification. The present research provided further theoretical basis of the biosorption of heavy metal waste water and nitrobenzene organic toxicants.

1. Introduction

With the rapid development of chemical industry, mining, and metallurgy, emissions of industrial wastewater usually contains an excess of heavy metals [1] and organic toxicants, such as nitrobenzene, which have threatened the environment and human health seriously [2]. The biological flocculation method has been paid more attention due to its efficient and low cost in treating wastewater [3, 4]. The flocculation of some living or dead biomass has been explored in heavy metals and organic compounds [5] by many researchers, such as activated sludge, bacteria, fungi, or algae [6]. Activated sludge is not only for green environmental protection, but also for circular economy in wastewater treatment field. It can decompose organic substances in industrial wastewater by the biochemical metabolic reactions of aerobic microbial substances, which is related to the flocculation, coagulation, and precipitation of activated sludge [7]. In addition, activated sludge can effectively remove colloidal and dissolved substances in waste water and purify the wastewater [3, 8, 9].

Starch is the most widely used synthetic flocculant with a long molecular chain structure. Its amide group (CONH₂) can adsorb many substances to form hydrogen bonds [10]. There have been experiments in which alumina hydrate was added into partially hydrolyzed starch solution, and the anions of the polymerization were adsorbed on the cations of alumina and the viscosity was increased rapidly or colonially [6, 11, 12]. This enables it to bridge the adsorbed particles, connect several or even dozens of particles together, form flocs, accelerate the descent of particles, and make it the most ideal flocculant. The main reasons for improving
floculation efficiency by modifying starch are as follows [13]: firstly, because of its polar gene-amide group, it is easy to adsorb on the surface of sediment particles by its hydrogen-binding effect. Secondly, because of its long molecular chains and huge adsorption surface area in water, long chains of large orders of magnitude have good floculation effect [14]. They can bridge the particles by using long chains to form large particles of floculants and accelerate sedimentation. Thirdly, with the aid of floculation-coagulation aid of starch, double ionization compression may occur during the mud coagulation process of water purification, which reduces the stability of particle aggregation [15]. Under the action of molecular gravity, the simple anions of dispersed phase can be replaced by polymer anion groups. Fourthly, the chemical interaction between macromolecule and suspended matter in natural water, or between ions of hydrolytic coagulant added before it, may be a complexation reaction. Finally, because the molecular chains are fixed on the surface of different particles, a polymer bridge is formed between the solid particles [16].

Ionic biomacromolecular floculants have the following characteristics compared with traditional organic macromolecular floculants because they carry various anionic and cationic functional groups: the floculation performance is less affected by the fluctuation of water pH value and salt [17, 18]; the clarification performance is mainly obtained by charge neutralization. The function of this kind of floculant is mainly to flocculate colloids with positive and negative charges. It has the functions of turbidity removal and decolorization. It is suitable for water treatment with high content of organic colloids [19, 20]. In the present study, we prepared activated sludge floculant by industrial heavy water and evaluated its floculation properties for nitrobenzene and heavy metals such as chromium ion. After the comparison of floculation with activated carbon, we changed some factor which can influence the floculation potentially, such as primary concentration of chromium and nitrobenzene, pH value of solution, reaction time, floculant dosage, or solution temperature, and evaluated the floculation properties of activated sludge floculant [5–8]. In addition, we modified the floculant with acid or alkali in order to optimize the floculation ability.

2. Materials and Methods

2.1. The Experimental Equipment and Reagents

(1) The main equipment includes a centrifuge (Beckman 6700, USA), constant temperature water tank, UV spectrophotometer (Model-T6, PGENERAL Ltd., Beijing, China), atomic absorption spectrophotometer (4530 Jingke Ltd., Shanghai, China), and electronic scales. The conventional experimental instrument included various ranges of beaker, pipette, volumetric flask, round bottom flask, and glass rod.

(2) The main reagents are activated sludge of wastewater treatment plant, ammonium sulfamate, N-(1-naphthyl)ethylenediamine, acetone, potassium dichromate, diphenyl hydrazine, nitrobenzene, ethanol, sodium hydroxide, zinc powder, hydrochloric acid, sulfuric acid, nitrate, copper sulfate, sodium nitrite, and sodium chloride.

2.2. Preparing Activated Sludge Biological Floculant. We made the activated sludge, which has been cultured for some time, standing for 20 minutes and washed twice by distilled water. The supernatant was removed after centrifugation of the sludge at 3000 r/min centrifuge. The remaining material is the activated sludge biological floculant.

2.3. Preparation of Biological Floculant from Activated Sludge. The activated sludge was washed with tap water and air-dried at room temperature. After pulverization and screening by a 0.5 mm sieve, the material was mixed well by deionized water and stored for 24 hours. After removal of the suspended matter and soluble material, the sludge was dried at 105°C into powder. We mixed the activated sludge powder (100 g weighted) with phosphoric acid solution or sodium hydroxide solution (1000 mL, 1 mol/L) and stirred for 1 hour in a beaker. After standing, the supernatant was removed from the mixture, which was then dried at 105°C. Next, the sludge was heated at 180°C for 1 hour and washed by deionized water (75°C). After removing the free phosphoric acid and drying at 105°C, the acid- or base-modified activated sludge was prepared finally [21].

2.4. Preparing Different Concentrations of Nitrobenzene Solution and Chromium Ion Solution. A little ether was mixed with nitrobenzene (100 mg) in a 100 mL volumetric flask. After dissolution, the volumetric flask was filled with benzene. The concentration of initial nitrobenzene solution was 1000 mg/L. Different concentrations of nitrobenzene solution was gained by dilution to the initial nitrobenzene solution with benzene.

2.5. Batch Experiment of Colloidal Stability of Chromium Adsorbed by Biofloculants. Biofloculants was diluted to 60 mg/L by the addition of distilled water for floculation of chromium and nitrobenzene. The effects of floculation conditions (temperature (°C), pH, floculation time (hour), biofloculant dosage (mg/L), solutional zeta potential (mg/L), and floculant dosage) were studied. The pH was adjusted to approximately 3.0-11.0 by 0.1 M HCl and 0.1 M NaOH. Likewise, the effect of temperature was incubated at desired temperatures.

After setting up of fixed parameters, tubes containing the mixture of the biofloculant solution with chromium ion and nitrobenzene were shaken in an orbital shaker (Model-HZQ-X100, HDL APPARATUS Ltd., China) and stirred at a constant speed of 250 rpm. The initial nitrobenzene concentration was chosen with 2 mg/L. Zeta potential detection value of samples was determined at the liquid surface height of the beaker. The zeta potential of the solution without biofloculant was set as the
control. Change efficiency of colloid stability of the flocculant was calculated by Equation (1) [22, 23]:

\[
\text{Change efficiency of colloid stability (\%)} = \left(\frac{|A - B|}{|A|}\right) \times 100, \tag{1}
\]

where \(A\) is value of the sample and \(B\) is value of the control which is determined by an atomic absorption spectrometer.

2.6. ANOVA Analysis of Colloidal Stability of Chromium Adsorbed by Biofloculants. Statistical design of the experiments and data analysis was used by The Design-Expert Software (version 8.3) [24]. The central composite design (CCD) and response surface methodology (RSM) were chosen and applied to optimize the five factors: temperature, \(pH\), flocculation time, flocculant dosage, and chromium ion concentration (see supplementary data S2).

3. Experimental Protocols

3.1. Comparison of Flocculation between Activated Sludge Flocculant and Carbon. In the present studies, we compared the flocculation of activated sludge and carbon in different concentration solutions of nitrobenzene or chromium ion.

1000 mL nitrobenzene solution of different concentrations (500 mg/L, 300 mg/L, 100 mg/L, 50 mg/L, 30 mg/L, 10 mg/L, 1 mg/L, and 0.5 mg/L; 8 kinds of concentration) were mixed with 100 g activated sludge. The suspension were agitated in a beaker for 1 h at 25°C and left to stand for 72 hours. The nitrobenzene concentrations were determined by high-performance liquid chromatography and the removal rates were calculated.

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We mixed 1000 mL chromium ion solutions of different concentrations (500 mg/L, 300 mg/L, 100 mg/L, 50 mg/L, 30 mg/L, 10 mg/L, 1 mg/L, and 0.5 mg/L; 8 kinds of concentration) with 100 g activated sludge. All the suspensions were agitated in the beaker for 1 hour at 25°C. After standing for 72 hours, the chromium ion concentrations were determined by atomic absorption spectroscopy and the removal rates were calculated.

3.2. Flocculation of Activated Sludge Flocculant after Changes of Experimental Conditions. In the following experiments, we changed some experimental conditions, such as activated sludge dosage, solutions’ \(pH\) value, suspension standing time, and solution temperature, in order to demonstrate whether the conditions could affect the flocculation of activated sludge (refer to supplementary data S3 for details of the experiment).

3.3. Flocculation Performance of Modified Activated Sludge Flocculant. In the following research, we changed the \(pH\) value of activated sludge for acid or basic modification and determined the flocculation in nitrobenzene or chromium ion solutions.

The activated sludge (100 g) after acid modification, \(pH\) value of 2, 3, 4, 5, and 6, were mixed with 1000 mL solutions of nitrobenzene (10 mg/L) or chromium ion (100 mg/L). The suspensions were stirred for 1 hour at 25°C and left to stand for 72 hours. Finally, the remaining concentration of nitrobenzene or chromium ion were determined and the removal rates were calculated.

The activated sludge (100 g) after basic modification, \(pH\) value of 8, 9, 10, 11, and 12, were mixed with 1000 mL solutions of nitrobenzene (10 mg/L) or chromium ion (100 mg/L). The suspensions were stirred for 1 hour at 25°C and left to stand for 72 hours. Then, we determined the remaining concentration of nitrobenzene or chromium ion by chromatography or spectroscopy and calculate the removal rates.

3.4. High-Performance Liquid Chromatography

(1) Instruments and reagents

Instrument and reagents include the following: Waters 600 high-performance liquid chromatography (Waters Inc., USA), Waters 2487 Ultraviolet Detector (Waters Inc., USA), methanol (chromatographic purity), distilled water, glacial acetic acid, and nitrobenzene.

(2) Chromatographic conditions (see supplementary data and Figures S1 and S2)

3.5. Analytical Methods. Flocculation&removal rate = \(((A - B)/A) \times 100\%\), where \(A\) was the concentration of nitrobenzene or chromium ion solution before flocculation, while \(B\) was the concentration after flocculation by carbon or activated sludge.

Zeta potential measurement of colloids and flocculant solution was conducted by a zeta potential analyzer (Malvern Nano ZS, Malvern, England).

Fourier-transform infrared spectrometer (Nicolet 6700, ThermoFisher, USA) for space atmospheric component detecting of colloids and floculants.

4. Results and Discussion

4.1. Comparison of Flocculation between Activated Sludge Flocculant and Carbon. With the increase of initial concentration of nitrobenzene, the activated sludge and carbon
provided an increased flocculation when the concentration was lower than 100 μg/mL. However, with the increasing of concentration, the sludge showed a gradual decreasing flocculation, while the flocculation of carbon increased when the concentration was higher than 300 μg/mL. In general, the sludge showed a predominant flocculation than carbon in the nitrobenzene solution (Figure 1(a)). In the chromium solution, the sludge showed a decreased elimination rate of chromium, while the elimination rate increase was shown in activated carbon. In general, the activated carbon showed a predominant flocculation than activated sludge (Figure 1(b)). When the amount of activated sludge was relatively small, the area used for adsorption was relatively small. Because the adsorption site cannot be provided, the removal rate decreased in the chromium solution. On the contrary, the removal rate of activated sludge increased gradually, which proved that the amount of flocculant to remove certain chromium was less. The macromolecule flocculant can provide enough coordination through modification and molecular conformation change.

4.2. Flocculation of Activated Sludge Flocculant after Changes of Experimental Conditions. In the present experiment, we tried to determine the optimal dosage of activated sludge which could absorb the nitrobenzene or chromium at most. Six weights of dosage were tested in the nitrobenzene or chromium solution. The dosage of the flocculant on the flocculation efficiency did not show a single trend in the present experiment. Nearly 80 percent of nitrobenzene was absorbed by 30 g activated sludge. Too much flocculant (more than 100 g) just provided less than 50 percent removal rate (Figure 2(a)). In the chromium ion solution, the absorption peak was shown at a weight of 50 g activated flocculant. Unlike high dose of nitrobenzene, nearly 80 percent of chromium was absorbed by more than 100 g sludges (Figure 2(b)).

The difference of solution pH value showed a totally different flocculation of sludge in nitrobenzene or chromium ion solution. An acid or weak circumstance could provide more than 80 percent removal rate in nitrobenzene, while a sharp decline of flocculation was shown with very alkaline in solution. The flocculant showed a proximal 100 percent absorption rate when the pH value was 5 (Figure 2(c)). In the chromium solution, the sludge also showed an obvious increasing flocculation with the pH ranging from 2 to 8. The flocculation of sludge approached the maximum even if the pH value raised continuously (Figure 2(d)). The chromium ions existed in an alkaline state as precipitate or complexes, which could not represent the biological removal flocculant. Therefore, the flocculant showed a greater flocculation property in acidic condition.

In the present research, we mixed the sludge with nitrobenzene or chromium solution and was left standing for 72 hours usually. However, we hypothesized that different

![Figure 1: (a) Flocculation of activated carbon or flocculant in nitrobenzene. (b) Flocculation of activated carbon or flocculant in chromium.](image_url)
standing time or reaction time may affect the flocculation and gave some tests. The sludge showed a total difference in absorption with the time increasing. A gradual flocculation decline was detected after standing within 10 hours, while severe decrease was shown after 12 hours in nitrobenzene solution (Figure 3(a)). In the chromium solution, most ion, nearly 80 percent, was absorbed by sludge within 8 hours, while a platform in flocculation was observed after 12 hours (Figure 3(b)). The interpretation of this phenomenon was that the elimination rate changed following the change of its initial concentration.

In temperature-flocculation tests, the sludge showed a similar trend in both solutions of nitrobenzene and chromium ions with the temperature increasing. From room temperature to 40°C, an obvious incline of absorption was observed in the double solutions. When the temperature was above 40°C, saturation phenomena were shown, with proximal 80 percent in nitrobenzene and 90 percent in chromium of removal rate (Figures 3(c) and 3(d)). The increased temperature could aggravate the thermal motion of molecules, which is in contact with the activated sludge more frequently. Hence, the activated sludge may overcome the barriers to chemical flocculation activation energy and enhance the diffusion rate of particles, which led to an increased elimination rate finally.

4.3. Flocculation Performance of Modified Activated Sludge Flocculant. Finally, we modified the activated sludge with acid or baseline and tested the flocculation in the nitrobenzene and chromium solution. Interestingly, the acidic modified sludge showed a similar flocculation capacity in the double solutions. When the sludge was very acidic (pH value was below 4), nearly 80 percent of nitrobenzene and 85 percent of chromium was absorbed by the flocculant (Figures 4(a) and 4(b)). Although the peak rate was shown at the pH of 2, there was no serious difference of the rate with the pH ranging from 2 to 4. Considering the economy or efficiency, pH value of 4 is more profitable in dealing with too many samples due to its investment reduction in information technology for strong acid.

The basic modified activated sludge showed an increased trend in the elimination of nitrobenzene and chromium ion following the increased pH value. Differently, the flocculation of basic modified sludge was a little more predominant in nitrobenzene than in chromium (Figures 4(c) and 4(d)). Although the peak absorption was shown at the pH of 12, proximal 60 percent of nitrobenzene or chromium was absorbed by modified sludge at the pH of 10. Considering the economy or efficiency, pH value of 10 is more reasonable in dealing with mixture. Nevertheless, basic modified sludge generally showed a weaker flocculation than acidic modification in both nitrobenzene and chromium solution.

4.3.1. Effects of Flocculant Dosage, Temperature, pH, and Flocculation Time on Colloidal Stability of Chromium and Nitrobenzene Adsorbed by Flocculants. The experiment assignment and the collected response data are shown in Table S1 and Table S2. As shown in Table S3, the linear terms for flocculant dosage (C) and flocculation time (D) had significant effects on the change efficiency of colloid stability (F value <0.05), but temperature (A) and pH (B) had no significant effects on the change efficiency of colloid stability. The relationship between temperature in the range of 10–40°C and change efficiency of colloid stability was studied. Figure 5 shows that the highest change efficiency of colloid stability was achieved at 17.58°C. With the
temperature rising from 10 to 17.58°C, change efficiency of colloid stability increased from 69.87% to 71.81% and decreased to 60.72% with temperature rising to 30°C.

Table S3 and Figure 4 (above) shows that the influence of pH on flocculation was not significant, the change efficiency of colloid stability just increased by 1%, with pH raising from 5 to 9. As shown in Figure 5 (below), zeta potential measurement of flocculant illustrated that it was mainly negatively charged in alkaline condition and in acidic condition. Flocculant has been reported to have different electric states at different pH; it is positively charged in acidic condition (pH below 4) and negatively charged in neutral and alkaline conditions (pH above 4). It could be concluded that electrostatic repulsion effect was not the main factor affecting the change efficiency of colloid stability.

For these reasons, we chose the most conventional condition viz pH 6.5-8 and temperature 15-25°C in the optimized experimental condition [25]. According to the experimental results, the main factors affecting the change efficiency of colloid stability are flocculant dosage (C) and flocculation time (D). The optimization of the operating conditions was conducted by the quadratic models of the experimental design. Figure 5 illustrates that all the selected optimum solutions had a desired prediction of the change efficiency of colloid stability with the maximum experimental data. By triplicates of the experiments, the validated experimental results of the change efficiency of colloid stability and the values predicted by the models were shown in Figure 5. Therefore, the model was considered to fit the experimental data very well.
4.4. Zeta Potential and Fourier-Transform Infrared Spectrometer Analysis of Colloids and Flocculant Solution. Infrared radiation (IR) spectrum was investigated. The IR spectrum of flocculant exhibited a hydroxyl stretching peak at 3700 cm⁻¹ and a carboxyl band at 1750, 1500, and 1400 cm⁻¹ as presented in Figure 6. The C-O-H stretching vibration displayed adsorption peak at 700 cm⁻¹. 1750, 1500, and 1400 and 700 cm⁻¹ indicated the deformation vibration of C-O-H and the bending vibration of Cr-O, respectively. Oxygen-containing groups above [26], especially the strong polar groups such as -OH, are abundant on the surface of the bioflocculant, which makes the materials water soluble. Shock and displacement of the absorption peak showed that the dispersion is good and the polarity is enhanced, and the adsorption of chromium by bioflocculant is promoted [27]. The migration and change of the infrared peak show that the existence of flocculant may destroy the stability of the colloid, which may be caused by adsorption, but it may also be caused by the role of net catching.

Zeta potential measurement of flocculant (Figure 6) indicated that it was negatively charged in both alkaline solution and acidic solution; however, zeta potential of colloids after flocculation was electrically neutral (2 < pH < 7) and was decreased by bioflocculant in alkaline (10 < pH < 7) (Figure 6).

5. Conclusions

In activated sludge wastewater treatment process, the degradation of pollutants is divided into two stages: the first stage is that sludge flocs adsorb organic pollutants from wastewater outside the cell wall of bacteria (adsorption stage); the second stage is that bacteria transport pollutants to the substances and energy required for cell metabolism (degradation stage) through active transportation. Physical effect plays a decisive role, and extracellular polymer (EPS) is the main component of sludge flocs, accounting for 50% to 90% of the total organic matter of activated sludge [22]. EPS has a significant impact on the physical and chemical properties of sludge flocs, such as floc structure, surface charge, flocculation, sedimentation, dewatering, and adsorption properties. Many domestic scholars and abroad have discussed the influence of sludge EPS adsorption, but they mainly focus on the adsorption of heavy metals by activated sludge EPS [23]. Studies on extracting EPS from aerobic granular sludge by cation exchange resin found that the presence of EPS significantly increased the bacterial adsorption performance. Colloidal stability is directly related to the flocculation efficiency of pollutants. As the research shows, bridging, netting and compressing double electric layer can make the colloid lose stability, so that the pollutants can be removed from the water by flocculation or air flotation.
Previous studies have focused on one pollutant, which can be easily explained by the principle and model of flocculation. However, when two pollutants exist, especially organic pollutants and inorganic ions, the colloidal stability and destabilization process become very complex. This paper studies the influence of different conditions on the system. However, it must be acknowledged that the methods used in this paper, such as zeta potential and Fourier infrared scanning, are

![Graphs and images showing the effects of various conditions on colloidal stability and flocculation.](a) (b) (c) (d)
not enough to explain the molecular role in this complex system. Therefore, further research is needed.

Little research has been done on the adsorption of organic pollutants by sludge EPS; the analysis of experimental results obtained the following conclusions:

(1) Compared with activated carbon, the activated sludge showed a greater flocculation capacity on heavy metals or organics

(2) The appropriate pH modification, acidic or basic, could increase the elimination rate, while the acid modification is more effective

(3) Activated sludge process should strictly follow the reduction, recycling, and innocuity principles

Data Availability
The data used to support the findings of this study are included within the article.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

Supplementary Materials
SI-1: chromatographic conditions and standard curve. SI-2: ANOVA analysis of colloidal stability of chromium adsorbed by biofloculants. SI-3: flocculation of activated sludge flocculant after changes of experimental conditions. SI-4: transcription analyses procedure. Table S1: independent variables and their levels in the experimental design. Table S2: experimental design and results of the central composite design. Table S3: ANOVA analysis for responses Y (change efficiency of colloid stability (%)). (Supplementary Materials)

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