Application of a modified biological flocculant in total nitrogen treatment of leather wastewater

Yizhuo Zhang, Qinhuan Yang, Hongxia Gao, Yang Zhao, Xuan Tang, Changqing Zhao and Chunyu Fang

ABSTRACT

Leather wastewater harms the ecological environment and human health. In this study, a modified bio-flocculant was prepared to facilitate treatment of leather wastewater. A bio-flocculant produced by Bacillus cereus was combined with amphoteric starch and modified using a cerium ammonium nitrate initiator. Single factor optimization and orthogonal optimization were used to determine the optimal preparation conditions as follows: amphoteric starch-to-flocculant ratio = 22:30; reaction temperature = 64 °C; initiator dosage = 2.00%; reaction time = 15 min; stirring speed = 600 rpm; and flocculation system pH = 8.0. At a dosage of 1 g/L added to simulated leather industry wastewater, the flocculation efficiency (98.17%) and the total nitrogen removal efficiency (100.00%) of modified bio-flocculant was superior to that achieved by 1 g/L of unmodified bio-flocculant (72.16% and 50.00%, respectively), amphoteric starch (8.50% and 0.00%) and polyacrylamide (95.55% and 75.00%). Analysis of natural and flocculated precipitates in the wastewater showed that the modified bio-flocculant significantly changed several characteristics of the flocculated particles; in addition, it promoted the removal of nitrogenous substances in the process of denitrification. These changes helped explain the material’s flocculating ability. The results confirmed that the modified bio-flocculant was an effective additive for treating leather wastewater.

Key words | amphoteric starch, bio-flocculant, graft copolymerization, orthogonal optimization, tannery wastewater, total nitrogen removal

HIGHLIGHTS

- A modified biological flocculant was successfully prepared by using biological flocculant and amphoteric starch.
- The modified biological flocculant has an excellent treatment effect on leather wastewater.
- The flocculation rate of leather wastewater can reach 98.17%, and the removal rate of total nitrogen can reach 100%.
GRAPHICAL ABSTRACT

INTRODUCTION

Globally, the leather industry is an important light industry. Countries such as the United States, Australia, Brazil and Italy export a large amount of raw leather to obtain enormous economic benefits. Likewise, the processing (e.g., tanning, fabrication) of imported leather and re-exportation of finished products has contributed significantly to the economy of many developing countries. China is the world’s largest importer of leather, and the leather industry has promoted the development of the Chinese economy. As a consequence, leather industry wastewater emissions are increasing (Masood & Malik 2014). Leather industry wastewater has a high pollution potential and is difficult to treat because it contains a high concentration of nitrogen (both inorganic and organic) as well as a high concentration of colored substances (Bharagava et al. 2014). To prevent eutrophication of receiving waters by discharged wastewater, many countries have implemented strict regulations to control nitrogen emissions. In 2000, the European Union adopted the Water Framework Directive, which set a limit of 15 mg/L for total nitrogen (TN) emissions from wastewater. In 1970, Japan promulgated the Law on Prevention of Pollution of Water Quality, which stipulated that the TN emission standard for wastewater was 60 mg/L (Niu et al. 2013). The Ministry of Environmental Protection of China issued The Discharge Standards of Water Pollutants for Leather and Fur Processing Industry in 2013, which stipulated that the emission limit of TN in leather industry wastewater was 70 mg/L (Chen & Zhang 2014).

The traditional treatment processes for leather wastewater include adsorption, precipitation, oxidation, biofilm and a combination of processes. However, these methods cause secondary pollution, have high treatment cost, and cause some health and environmental problems (Saxena et al. 2020). Flocculation is a widely used method in industrial wastewater treatment. At present, flocculants are classified into four types, including inorganic, organic, biological and compound. The types and characteristics of these flocculants are shown in Table 1 (Supplementary Materials).

As aids to wastewater treatment, bio-flocculants are studied widely because they have good water-solubility, produce a strong flocculation effect and generate no secondary pollution after treating wastewater. Although there is some reported usage of bio-flocculants to treat industrial wastewater, there are no other reports of bio-flocculants being used to treat TN in leather industry wastewater. In the early stage, our research group optimized the medium of Bacillus cereus, it was found that the optimal medium increased the yield of the bio-flocculant produced by Bacillus cereus by 189.70% and the flocculation activity by 58.87%, in order to achieve better flocculation effect, the flocculant must be modified to improve the treatment capacity of leather industry wastewater (Zhao et al. 2018a, 2018b).

The main ingredient in a bio-flocculant is polysaccharide, a nonionic compound that cannot neutralize the nitrogen in leather wastewater. However, if a bio-flocculant can be modified to become an amphoteric polymer with high molecular weight, it will have dual anionic and cationic characteristics. A bio-flocculant modified in this way cannot only neutralize the nitrogenous pollutants in leather industry wastewater, but also enhance the bridging effect among colloidal particles, so as to further improve the removal of nitrogen (Zhao et al. 2018a, 2018b).

Amphoteric starch is a low cost, nontoxic compound with high molecular weight. Under the action of an appropriate initiator, amphoteric starch and long-chain macromolecules on the graft copolymers of the bio-flocculant can be used to prepare an ideal modified bio-flocculant; with such a material, the TN in leather industry wastewater can be treated comprehensively (Zauro & Vishalakshi 2017). Song (2008) improved the stability of a modified bio-flocculant by graft
copolymerization of bio-flocculant and cationic polyacrylamide (PAM). Min et al. (2012) modified carboxymethyl chitosan flocculant using liquid ferric based on a bio-flocculant and carboxymethyl chitosan solution to obtain modified carboxymethyl chitosan flocculant, which was suitable for removing fluorine ions in wastewater. However, there are no reports on the use of amphoteric starch to modify bio-flocculants to treat leather industry wastewater.

The objectives of this study were to (a) use amphoteric starch to modify a bio-flocculant produced from Bacillus cereus, (b) optimize the modification process, and (c) assess the performance of the modified bio-flocculant in treating leather industry wastewater. The reaction time, reaction temperature, reactant amount, stirring speed, initiator content and flocculation system pH for the modified flocculant were examined. The flocculation effect of the modified flocculant was compared with that for unmodified bio-flocculant, amphoteric starch and PAM; the flocculation efficiency and TN removal were also evaluated. Fourier transform infrared (FTIR) spectroscopy was used to characterize the precipitate obtained in the flocculation process and to explore the changes that occurred to the precipitates in the process. This study provides basic data for the practical application of a modified bio-flocculant to treat leather industry wastewater.

EXPERIMENTAL

Materials

Biochemical-grade peptone was obtained from the Beijing Double Spin Microbial Medium Factory (China). In addition, amphoteric starch was obtained from the Xiangcheng Amylase Factory of Henan Province (China). All other reagents used were of research-grade quality.

Culturing the flocculant-producing bacteria and collecting the bio-flocculant

Preparation of seed liquid medium

The seed liquid media was prepared using NaCl (10.00 g), peptone (10.00 g), yeast extract (5.00 g) and distilled water (1.0 L). Then, 100 mL seed liquid medium was added into a 250 mL conical bottle and the medium was sterilized at 121 °C (0.105 MPa) for 20 min before use. The Bacillus cereus was inoculated into the seed liquid medium on the bechtop. Then, the bottles were put in a shaker maintained at 35 °C and operated at 180 rpm to culture the media for 36 h.

Preparation of culture medium

Culture medium was prepared using mannitol (40.00 g), peptone (4.00 g) and potassium hydrogen phosphate (0.75 g) and distilled water (1.0 L). Then, 120 mL culture medium was added into a 250 mL conical flask, and sterilized at 121 °C (0.105 MPa) for 20 min before use. The 5.00% Bacillus cereus seed liquid was inoculated into the culture medium on the bechtop. Then, the bottles were put in a shaker maintained at 35 °C and operated at 180 rpm to culture the mixture for 48 h (Zhao et al. 2020).

Collection of bio-flocculant

After culturing, the culture medium was centrifuged at 5,000 rpm for 10 min. Following centrifugation, the clarified supernatant was collected and combined with three times the volume of pre-cooled anhydrous ethanol. The mixture was placed in a refrigerator at 4 °C and let stand for 4–6 d. Then, the supernatant-ethanol mixture was centrifuged at 6,000 rpm for 10 min. The precipitate was collected. Then, the supernatant was cooled in a refrigerator and dried in a vacuum freeze-dryer (SCIENTZ-10ND model, Ningbo Xinzhi Biological Technology Co., Ltd, Zhejiang, China) to produce white fluffy powdery bio-flocculant particles.

Optimization of modified bio-flocculant preparation

Single factor optimization of preparation conditions

Influence of the amphoteric starch-to-flocculant ratio. In a 150 mL beaker, 50 mL mixtures at various ratios of amphoteric starch (0.1 g/L) to flocculant (1.0 g/L) were created (1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1). Each mixture was added 5.00% ammonium cerium nitrate initiator (0.02 mol/L) (Zou 2016). Each mixture was placed in an electrically heated magnetic stirring pot (DF-101S model, Yuhua Equipment Factory Co. Ltd, Gongyi, China), and the reaction was carried out at a rotation speed of 240 rpm and constant temperature of 60 °C for 11 min (Zhou et al. 2018). After the reaction, the flocculation effect was evaluated by measuring the flocculation efficiency using the method of An et al. (2017).

Influence of the initiator amount. At the optimum ratio of reactants, the cerium ammonium nitrate initiator was added at various ratios (2.00%, 2.50%, 3.00%, 5.00% and 10.00%) to 50 mL mixture. The subsequent steps were the same as described under Influence of the amphoteric starch-to-flocculant ratio.
**Influence of temperature.** At the optimum ratio of reactants and the initiator amount, the mixture was reacted at different temperatures (50, 55, 60, 65 and 70 °C). The subsequent steps were the same as described under *Influence of the amphoteric starch-to-floculant ratio*.

**Influence of the reaction time.** At the optimum ratio of reactants, the initiator amount and the temperature, the mixture was reacted at different reaction times (11 min, 13 min, 15 min, 17 min, and 19 min). The subsequent steps were the same as described under *Influence of the amphoteric starch-to-floculant ratio*.

**Influence of the flocculation system pH.** At the optimum ratio of reactants, the initiator amount, the temperature and the reaction time, the preparation of modified bio-floculant was completed by the same method. After kaolin solution was added, the pH of each group was adjusted to 4.0, 5.0, 6.0, 7.0 and 8.0, and the flocculation efficiency was measured.

**Influence of stirring speed.** At the optimum ratio of reactants, the initiator amount, the temperature the reaction time and the flocculation system pH, the mixture was reacted at different stirring speed (240, 360, 480, 600 and 720 rpm). The subsequent steps were the same as described under *Influence of the amphoteric starch-to-floculant ratio*.

**Confirmation of single-factor optimization.** The modified bio-floculant was prepared under the optimal conditions determined by single factor optimization, and the flocculation efficiency was measured.

**Orthogonal optimization of preparation conditions**

The six bio-floculant modification preparation variables (reaction time, reaction temperature, amphoteric starch-to-floculent ratio, stirring speed, initiator quantity and the flocculation system pH) were optimized collectively using orthogonal optimization. Five gradient values near the single factor optimization value were selected as the level, and an L_{25} (5^{6}) orthogonal optimization table was designed to determine the optimal value.

**Experimental treatment of leather industry wastewater using modified bio-floculant**

**Preparation of simulated leather industry wastewater**

The preparation of simulated leather wastewater followed the method of Ma (2008). Due to the addition of fur, gelatin, fatliquoring agent, dye, ammonium sulfate and other additives in the preparation process, the prepared leather wastewater had a deep color and a pungent smell. After preparation, the resulting wastewater had a chemical oxygen demand of 3.542.0 mg/L and a TN concentration of 1.142.8 mg/L.

**Determination of optimal dosage of flocculants**

The modified bio-floculant and PAM were added in various dosages (0.3 g/L to 1.2 g/L, in steps of 0.1 g/L) to the simulated leather industry wastewater to determine the optimal dosage.

**Leather industry wastewater treatment**

Unmodified bio-floculant, amphoteric starch and PAM were selected as control treatments against which to compare the flocculation ability and nitrogen removal ability of the modified bio-floculant during treatment of leather industry wastewater. The following procedure was used. First, to 50 mL simulated wastewater was added either 1.0 g/L modified bio-floculant, 1.0 g/L unmodified bio-floculant, 1.0 g/L amphoteric starch or 1.0 g/L PAM (Patil et al. 2011; Menkiti et al. 2018). Each mixture was stirred at 200 rpm for 5 min, then stirred at 100 rpm for 5 min. The flocculation efficiency was measured after letting the mixture stand for 50 min (using blank leather wastewater as the control group). Following treatment, an automatic Kjeldahl nitrogen apparatus (Kjeltec 8400 model, Foss Analytical AB, Hoganas, Sweden) was used to determine the TN concentration in the treated sample. In addition, the denitrification processes of the bio-floculant and modified bio-floculant were analyzed.

**Characterization of flocculating precipitates**

The natural precipitate from the simulated wastewater and the precipitate resulting from treatment of the wastewater using the modified bio-floculant were dried at 50 °C for 30 min and mixed with 1:100 potassium bromide tablet. The mass was ground and crushed and the resulting sample was prepared for characterization using an infrared spectrometer (NICOLET 6700 model, Thermo Fisher Scientific Inc., Waltham, MA, USA).
RESULTS AND DISCUSSION

Single factor optimization of bio-flocculant preparation conditions

Influence of reactant ratio

Amphoteric starch was selected as the modifier to react with bio-flocculant, and the effect of the reactant ratio on the flocculation performance is shown in Figure 1.

As shown in Figure 1, as the ratio of amphoteric starch to bio-flocculant gradually increased from 1:9, the flocculation efficiency also increased and reached a maximum (85.32%) when the mixing ratio was 5:5. As the relative proportion of starch continued to increase, the flocculation performance decreased, reaching only 7.95% at the starch-to-flocculant ratio of 9:1. When the relative proportion of added bio-flocculant was low, a large number of free groups were excited in the solution and there were few substrates that could achieve group graft copolymerization. Therefore, the resulting graft rate of anionic and cationic groups was low and this affected the flocculation efficiency. The combination of equal proportions of starch and bio-flocculant (5:5, v/v) was selected as the optimal ratio for these two reactants.

Influence of the initiator amount

Cerium ammonium nitrate was selected as the initiator in this experiment because the cerium salt redox initiator operates under mild reaction conditions, cerium ammonium nitrate decomposes easily when heated, and the redox reaction occurs with organic alcohols, aldehydes, acids and other functional groups to generate free radicals, which lead to graft copolymerization of monomers (Qi 2014). The effect of initiator amount on flocculation efficiency is shown in Figure 2.

As shown in Figure 2, the amount of initiator had a significant influence on flocculation efficiency. When the initiator dosage increased from 1.00%, the flocculation efficiency increased to its maximum of approximately 80% when the dosage reached 2.50%. Higher dosages of initiator reduced the flocculation efficiency. This effect may be explained by noting that the correct amount of initiator, together with reduced groups in a redox reaction, generated a large amount of free radicals in solution, which in turn caused graft copolymerization. However, when the dosage of initiator became excessive, its role changed and exerted an inhibitory effect, thus reducing flocculation efficiency. Therefore, 2.5% was selected as the optimal dosage of initiator.

Influence of the reaction temperature

The effect of the reaction temperature is shown in Figure 3.

As shown in Figure 3, temperature greatly influenced flocculation efficiency. When the preparation temperature increased to 65 °C, the subsequent flocculation efficiency reached its peak (≈85%). Because polysaccharide was the main ingredient of the bio-flocculant, increased temperature enhanced flocculation activity. Furthermore, because the thermal stability of polysaccharide is higher than that of protein, the bio-flocculant still achieved a certain flocculation efficiency even when high temperature caused structural failure of the protein. On the other hand, the flocculation process is usually an exothermic process in which the particles in the flocculation system have strong irregular movement. Therefore, the particles are easily captured by the long-chain polymer of the modified bio-flocculant; this,
combined with the kinetic factors, enables large flocs to settle rapidly (Fukasawa & Adachi 2006).

When the preparation temperature exceeded 65 °C, the flocculation efficiency declined because the increased temperature also increased the repulsive force of the double electric layer between particles. In addition, increased temperature caused molecular aging of the bio-flocculant, which weakened the flocculation capacity (Xiao et al. 2008). Accordingly, 65 °C was selected as the optimal reaction temperature.

Influence of the reaction time

If the reaction time was too short, the effect of the initiator was insufficient to stimulate free radicals, and the reaction was limited. As reaction time increased, the collision frequency between flocculant and starch particles increased, and the reaction effect was enhanced (Zhou et al. 2018). The effect of reaction time on flocculation efficiency is shown in Figure 4.

As shown in Figure 4, the flocculation efficiency was the highest (∼82%) at a reaction time of 15 min. After 15 min, the flocculation efficiency decreased, suggesting that 15 min was the shortest time needed to complete the bio-flocculant modification reaction. This reaction helped the modified bio-flocculant form bridges among particles. The flocculated particles cleared the liquid by gravity sedimentation and separation (Zhang & Wang 2018). However, the flocculation effect did not increase indefinitely with the extension of reaction time of the bio-flocculant modification process. Instead, excessively long reaction time led to a great decline in the subsequent flocculation efficiency. This may have occurred because the structure of the active group on the graft copolymer disintegrated during very long reaction time. For these reasons, 15 min was selected as the optimal reaction time.

Influence of flocculation system pH

Flocculation experiments were conducted at pH 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0. The pH of the flocculation system greatly influenced the flocculation efficiency achieved by the bio-flocculant (Figure 5). When particles with opposite electrical charges existed simultaneously in the flocculation system, the flocculation process was efficient due to the mutual attraction of the oppositely charged particles.

As shown in Figure 5, flocculation efficiency increased as the process pH changed from acidic to alkaline and reached a maximum of 94.69% at pH 8.0. The reason for this effect was that the Kaolin particles have a positive charge, while functional groups such as the carboxyl group and hydroxyl group on the bio-flocculant can ionize negative charge under the action of an initiator, effectively promoting the flocculation process (Zeng et al. 2019). When the pH...
value was higher than 8.0, the adsorption effect of bio-flocculant on the particles became weaker and the flocculation efficiency decreased. Therefore, the flocculation system pH of 8.0 was selected as the optimal value.

**Influence of stirring speed**

A certain speed of mixing of the reaction system was required to ensure that particles collided with each other and to increase the rate of graft modification, thus enhancing the ability of the modified bio-flocculant to improve flocculation efficiency. The flocculation efficiency of the modified bio-flocculant formed at different stirring speeds is shown in Figure 6.

Figure 6 shows that mixing speed greatly influenced the reaction process. When the stirring speed increased from 240 to 600 rpm, the flocculation efficiency steadily increased, reaching a maximum of approximately 84% at 600 rpm. This occurred because the effect of the initiator (which generated an ample quantity of free radicals), combined with rapid stirring that provided sufficient particle mixing, prompted a flocculation reaction. However, when the stirring speed increased beyond 600 rpm, the circulation flow of the liquid became too strong, and the shear force of the water caused solute particles to break and return to solution, resulting in a lower flocculation efficiency. Thus, 600 rpm was determined to be the optimal mixing speed.

**Single-factor optimization**

Through the single factor experiments, the optimal preparation conditions to modify bio-flocculant were as follows: ratio (v/v) of amphoteric starch to bio-flocculant = 1:1; reaction temperature = 65 °C; dosage of cerium ammonium nitrate initiator = 2.50%; reaction time = 15 min; reaction stirring speed = 600; and treatment system pH = 8.0.

This combination of operating variables was used to prepare modified bio-flocculant. The resulting flocculation efficiency achieved by the bio-flocculant was 91.83%.

**Orthogonal optimization of preparation conditions**

Orthogonal optimization was also used to determine the optimal values of bio-flocculant preparation variables. The results of the L_{25} (5^5) orthogonal optimization shown in Table 2 (Supplementary Materials) indicate that the operating variables had an effect on flocculation performance in the following order: flocculation system pH > initiator dosage > reaction time > mixing speed > reaction temperature > reactant starch-to-flocculant ratio.

The results listed in Table 2 also showed that the orthogonal optimization value of the reaction temperature, reaction time, reactant starch-to-flocculant ratio, initiator dosage, stirring speed and flocculation system pH was A_{6}B_{3}C_{7}D_{2}E_{4}F_{4}. In other words, the optimal values of operating variables to modify bio-flocculant were: reaction temperature = 64 °C; reaction time = 15 min; reactant starch-to-flocculant ratio = 22:30; initiator dosage = 2.00%; stirring speed = 600 rpm; and flocculation system pH = 8.0.

Bio-flocculant modified under this set of optimized variables was produced. The flocculation efficiency achieved by the bio-flocculant was 92.06% (compared to 91.83% achieved by the bio-flocculant modified using the operating values determined by single-factor optimization).

**Treatment of simulated leather industry wastewater with modified bio-flocculant**

The flocculation efficiency of bio-flocculant modified using the variables determined by orthogonal optimization was compared with that of unmodified bio-flocculant, amphoteric starch and PAM. The first step in the comparison was to determine the optimal dosage of flocculant to use.

**Optimal dosage of flocculants**

Equivalent dosages from 0.3 g/L to 1.2 g/L were used for modified bio-flocculant and PAM and were added individually to the simulated wastewater. The results of flocculants are shown in Figure 7. As the flocculant dosage of both flocculants increased, the flocculation efficiency also increased. This occurred because after adding the flocculants, the charge voltage on particles in the wastewater was reduced.
Comparison of modified bio-flocculant performance against other flocculants

The results of comparative flocculation experiments are shown in Table 3 (Supplementary Materials) and Figure 8. Results in Table 3 show that the flocculation efficiency of amphoteric starch was only 8.50%. This poor performance was because amphoteric starch solution had no effective flocculation components. By comparison, the flocculation efficiency of unmodified bio-flocculant was 72.16%, that of modified bio-flocculant was 98.17% and that of PAM was 95.55%. The flocculation efficiency of modified bio-flocculant was greater than that of PAM because the basic composition of leather industry wastewater is derived from biological tissue. Following the addition of flocculant, some suspended substances in this wastewater form macromolecular compounds and precipitate. Modified bio-flocculant also can be targeted to adsorb organic particles.

The results presented in Table 3 also show that modified bio-flocculant achieved high TN removal efficiency that was as high as 100.00%. In the polymer chain with amphoteric starch and an ample supply of free radicals after the graft copolymerization reaction, the modified bio-flocculant had large molecular weight and contained many ionic groups. Furthermore, the modified bio-flocculant had a good neutralization ability for organic nitrogen and inorganic nitrogen. In addition, as shown in Figure 8, the efficiency of removing TN by bio-flocculant and modified bio-flocculant increased with time. Because the TN in leather wastewater mainly includes organic nitrogen and ammonia nitrogen, these substances may combine with other substances in the wastewater system. When modified bio-flocculant with flocculating activity as the main component was added, flocculation occurred rapidly, and the wastewater system could not provide the conditions and raw materials required for the redox of nitrogen element. Therefore, it was difficult to convert

and particle surface repulsion decreased such that the particles condensed with the high polymer flocculants and started to settle (Chen et al. 2019). At the flocculant dosage of 0.7 g/L, the flocculation efficiency achieved by modified bio-flocculant was 91.69% while that achieved by PAM was only 88.62%. At the flocculant dosage of 1.0 g/L, the flocculation efficiency achieved by modified bio-flocculant was 98.12% while that achieved by PAM was only 95.55%. In fact, when the PAM dosage was 1.2 g/L, it achieved a flocculation efficiency of only 97.39%, the flocculation rate was lower than modified bio-flocculant. This phenomenon indicates that the amount of modified bio-flocculant was lower than PAM when the flocculation rate was high or higher. Moreover, since the initial production cost of the modified bio-flocculant was $5.64 per kilogram, at the same time, the cost of PAM was $3.05 per kilogram, although the laboratory production cost of the modified bio-flocculant was slightly higher than PAM, the production cost will be lower than PAM with the progress of industrial production. Therefore, the modified bio-flocculant was more suitable for treating the leather wastewater which characterized by ionic compounds from the perspective of flocculation effect, cost and ecology.

However, at flocculant dosages exceeding 1.0 g/L, the flocculation efficiency increased only slightly. This diminished effect occurred because the suspended particles in the wastewater already had been almost completely absorbed and precipitated by flocculation. Excessive addition of flocculant will increase the concentration of residual ions in the wastewater and increase the treatment cost. Accordingly, 1.0 g/L was selected as the optimal dosage of bio-flocculant for treating leather industry wastewater (Chen et al. 2019).
different valence nitrogen elements among NH₄⁺, NO₃⁻ and so on. The variation in TN removal could also be due to the colloidal particles having both positive charge and negative charge. Which charged area became the attraction point for the amphoteric functional group of bio-floculant and formed flocs, combined with the action of coulomb gravity, achieved the purpose of floculating wastewater particles.

In addition, through the investigation of flocculation mechanism, it was found that a variety of flocculation methods occurred in the treatment of leather wastewater by the modified bio-floculant. Mainly includes adsorption and bridging, chemical interaction, charge neutralization, sweep-floc coagulation. The results showed that the modified bio-floculant combined with a variety of flocculation ways in the treatment of leather wastewater which enhanced the flocculation ability (Zhang et al. 2020).

Characterization of floculated precipitates

The FTIR analysis results are shown in Figure 9 (Supplementary Materials). The new strong absorption peak at 3,746 cm⁻¹ is the stretching vibration peak of –OH. This peak occurred because a large number of –OH groups in the amphoteric starch are grafted onto the surface of the flocculant. The new weaker characteristic peak at 2,924 cm⁻¹ resulted from the C–H stretching vibration, and is the characteristic peak of sugars. At 1,645 cm⁻¹ is the stretching vibration peak of C=O in the carboxyl group. This new peak is sharp and strong, indicating the occurrence of flocculation of polysaccharide. The new peak at 1,029 cm⁻¹ during the flocculation process is a strong absorption peak of aromatic substances. The peak at 875 cm⁻¹ may reflect a link between monosaccharides. Therefore, FTIR analysis indicated that the floculatated precipitate mainly contained sugars and a large number of groups such as –OH and –COOH.

The change in the characteristics of the sediment before and after flocculation was due to the aggregation of wastewater particles–floc–wastewater particles during the flocculation process. Therefore, through the analysis of the groups changes in the precipitate, it can be judged that the chemical reaction occurred when the modified bio-floculant treated the leather wastewater. Overall, the modified bio-floculant played an active role in the treatment of the simulated leather industry wastewater.

CONCLUSIONS

A bio-floculant produced by Bacillus cereus and combined with amphoteric starch was selected for chemical modification to yield a bio-floculant that had comprehensive flocculating ability when treating leather industry wastewater. First, the optimal conditions for modifying the bio-floculant were obtained by single factor optimization as well as orthogonal optimization. The optimal bio-floculant modification conditions were: reactant starch-to-floculant ratio = 22:50 (v/v); reaction temperature = 64 °C; initiator dosage = 2.00%; reaction time = 15 min; stirring speed = 600 rpm; and treatment system pH = 8.0. Based on cost considerations, the optimal dosage of the resulting modified bio-floculant in the treatment of leather industry wastewater was determined to be 1.0 g/L.

It can be seen from the experimental results of wastewater treatment, modified bio-floculant prepared under the optimal conditions causes a strong electro-neutralization reaction in leather industry wastewater due to the generation of free radicals and graft polymerization that give the material high molecular weight. The modified bio-floculant achieved flocculation efficiency (approximately 98%) that was superior to that of unmodified bio-floculant, amphoteric starch and PAM. In addition, the modified bio-floculant removed organic nitrogen and inorganic nitrogenous substances with excellent flocculation capacity, to achieve 100% TN removal efficiency.

The modified bio-floculant can play an active significant role in leather industry wastewater treatment. Given the increasing importance of environmental protection globally, the modified bio-floculant has enormous industrial application value with good prospects for full-scale treatment of leather industry wastewater.

ACKNOWLEDGEMENTS

This work was financially supported by the Science and Technology Department of Sichuan Province (Item No. 2020YFG0161) and the Cooperation Project between Wuliangye Group Co., Ltd and Sichuan University of Science and Engineering (Item No. CXY2019ZR008).

AUTHOR CONTRIBUTIONS

Y.Z. (M.E. student) conducted the experiments and wrote the manuscript.
Q.H. (Associate Professor) conducted the analysis of part of the experimental data.
H.X. (Associate Professor) conducted the analysis of part of the experimental data.
Y. (M.E. student) participated in part of the experiment.
CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

An, X. J., Kang, Y. & Qin, L. 2017 Purification of Chinese herbal extract with chitosan hydrochloride: flocculation of single impurity and flocculation mechanism. Korean J. Chem. Eng. 34, 1756–1762. doi:10.1007/s11814-017-0055-8.

Bharagava, R. N., Saxena, G., Mulla, S. I. & Devendra, K. P. 2018 Characterization and identification of recalcitrant organic pollutants in tannery wastewater and its phytotoxicity evaluation for environmental safety. Arch. Environ. Contam. Toxicol. 75 (2), 259–272. doi:10.1007/s00244-017-0490-x.

Chen, Z. G. & Zhang, Z. J. 2014 Interpretation of water pollutant emission standards for leather and fur processing industry. China Leather. 43 (9), 38–42. doi:10.15356/j.cnki.issn1001-6813.2014-009-011.

Chen, L., Sun, Y., Sun, W., Sha, K. J., Xu, Y. & Zheng, H. 2019 Efficient cationic flocculant MHCS-g-PAM-DAC synthesized by UV-induced polymerization for algae removal. Sep. Purif. Technol. 210, 10–19. doi:10.1016/j.seppur.2018.07.090.

Fukasawa, T. & Adachi, Y. 2006 Effect of floc structure on the rate of brownian coagulation. J. Colloid Interf. Sci. 304, 115–118. doi:10.1016/j.jcis.2006.08.020.

Ma, X. Y. 2008 Toxicity Research for Anaerobic Micro-Organisms Against the Chemical Substances in Tannery Wastewater. PhD Thesis, Shanxi University of Science and Technology, Xi’an, China.

Masood, F. & Malik, A. 2014 Environmental Concerns of the Tanning Industry. Environmental Deterioration and Human Health. Springer, Dordrecht, The Netherlands.

Menkiti, M. C., Okoani, A. O. & Ejimofor, M. I. 2018 Adsorptive study of coagulation treatment of paint wastewater using novel brachystegia eurycoma extract. Appl. Water Sci. 8, 189. doi:10.1007/s13201-018-0836-1.

Min, X. B., Chai, L. Y., Miao, Y., Li, Q. Z., Peng, B., Wang, Q. W., Yang, Z. H. & Yin, Y. N. 2012 A Modified Carboxymethyl Chitosan Composite Flocculant and its Preparation and Application. Chinese Invention Patent, Central South University, China.

Niu, Y., Cheng, B. Z., Ding, Z. W. & Chen, Y. F. 2013 Study on the treatment method of fatliquoring wastewater for leather dyeing. China Leather. 42 (1), 5-8 + 18. doi:10.13536/j.cnki.issn1001-6813.2013.01.011.

Patil, S. V., Patil, C. D., Salunke, B. K., Salunke, R. B., Bathe, G. A. & Patil, D. M. 2011 Studies on characterization of Bio-flocculant exopolysaccharide of azotobacter indicus and its potential for wastewater treatment. Appl. Biochem. Biotechnol. 163 (4), 465–472. doi:10.1007/s12010-010-9054-5.

Qi, M. W. 2014 Study on Surface Grafting Reaction and Material Properties of Carbon Fiber Induced by Cerium Salt. MD Thesis, Harbin Institute of Technology, Harbin, China.

Saxena, G., Purchase, D. & Bharagava, R. N. 2020 Environmental hazards and toxicity profile of organic and inorganic pollutants of tannery wastewater and bioremediation approaches. In: Bioremediation of Industrial Waste for Environmental Safety. Springer, Singapore.

Song, Y. B. 2008 Modification of Concentrated Biological Flocculant. MD Thesis, Harbin Institute of Technology, Harbin, China.

Xiao, F., Ma, J., Yi, P. & Huang, J. C. H. 2008 Effects of low temperature on coagulation of kaolinite suspensions. Water Res. 42, 2983–2992. doi:10.1016/j.watres.2008.04.013.

Zauro, S. A. & Vishalakshi, B. 2017 Amphotheric gellan gum-based terpolymer montmorillonite composite: synthesis, swelling, and dye adsorption studies. Int. J. Ind. Chem. 8 (3), 345–362. doi:10.1007/s40090-017-0126-z.

Zeng, T., Hu, X. Q., Wu, H., Yang, J. W. & Zhang, H. B. 2019 Microwave assisted synthesis and characterization of a novel bio-based flocculant from dextran and chitosan. Int. J. Biol. Macromol. 131, 760–768. doi:10.1016/j.ijbiomac.2019.03.116.

Zhang, H. & Wang, H. R. 2018 Preparation and application of cationic keratin and polyaluminum sulfate complex flocculant. China Leather. 47 (4), 1–8. doi:10.15356/j.cnki.issn1001-6813.2018-004-001.

Zhang, Y. Z., Yang, Q. H. & Zhao, C. Q. 2020 Flocculation mechanism of modified biofloculant for leather wastewater treatment. J. Soc. Leath. Tech. Ch. 104 (4), 184–191.

Zhao, C. Q., Shu, L., Yang, Q. H. & Chen, W. Y. 2018a Exploring the mechanisms of the interaction between a specific biofloculant and tannery wastewater. J. Soc. Leath. Tech. Ch. 102 (2), 75–80.

Zhao, C. Q., She, C. & Yang, Q. H. 2018b Medium optimisation for biofloculant produced from Bacillus Cereus. J. Soc. Leath. Tech. Ch. 101 (3), 129–134.

Zhao, C. Q., Zhang, Y. Z., Yang, Q. H. & Yang, Y. 2020 Culture conditions optimisation of Bacillus subtilis for producing biofloculant. J. Soc. Leath. Tech. Ch. 104 (3), 151–156.

Zhou, H. J., Zhou, L. & Yang, X. Y. 2018 Optimization of preparing a high yield and high cationic degree starch graft copolymer as environmentally friendly flocculant: through response surface methodology. Int. J. Biol. Macromol. 118 (B), 1431–1437. doi:10.1016/j.ijbiomac.2018.06.155.

Zou, Y. Q. 2016 Synthesis and Application of Starch Modified Efficient Flocculant. MD Thesis, Northwest University, Xi’an, China.

First received 25 November 2020; accepted in revised form 9 May 2021. Available online 20 May 2021