

Production of Biofloculants Prepared from Wastewater Supernatant of Anaerobic Co-Digestion of Corn Straw and Molasses Wastewater Treatment

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Novel biofloculants (BS-MBF) were prepared using the wastewater supernatant from anaerobic co-digestion of corn straw and molasses wastewater as a nutrient resource. Acetic acid and ethanol were the dominant fermentation products during the anaerobic digestion process and were estimated to be 50.5% and 30.0%, respectively, after 150 d of operation. Equal volumes of biofloculant producing bacteria F2 (*Rhizobium radiobacter*) and F6 (*Bacillus sphaericus*) were mixed to form F⁺, which was inoculated to wastewater supernatant at different times. A maximum flocculation activity of 91.3% was achieved, and 2.32 g/L of purified biofloculant was extracted when a compound medium from 110-d wastewater supernatant was used. The removal efficiencies of heavy metals from simulated electroplating wastewater were tested by using these prepared biofloculants. The optimal conditions for heavy metal removal to BS-MBF were found to be at 374 mg/L at an initial pH of 6.0 and a contact time of 40 min. The adsorption capacities for Cu²⁺ and Zn²⁺ reached more than 90%, while for Cr⁶⁺ it reached approximately 30%. Overall, the study showed for the first time that wastewater supernatant from anaerobic co-digestion of corn straw and molasses wastewater can be used for producing biofloculants, which can be effectively used to remove heavy metals from electroplating wastewater.

Keywords: Biofloculant; Corn straw; Molasses wastewater; Wastewater supernatant; Electroplating wastewater

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INTRODUCTION

Anaerobic digestion (AD) technology has been widely applied to dispose of various kinds of organic waste products, such as agricultural solid wastes, livestock manure, and domestic waste, and has led to renewable energy production, nutrient recycling, and ecological environment protection (Zhang *et al.* 2014; Kamali *et al.* 2016). According to a Chinese renewable energy report, approximately 100,000 large-scale biogas-engineering projects were constructed in 2013 (Wu *et al.* 2015). However, a large amount of biogas slurry produced from these projects is not treated appropriately, which can cause secondary pollutions to soil and water (Bian *et al.* 2014; Wentzel *et al.* 2015). Currently, biogas slurry is used for fertilizer and seed leachate production because it contains abundant amounts of N, P, K, and micronutrients (Garg *et al.* 2005). However, many safety evaluations of ecological environments argue against biogas slurry fertilizers and irrigation techniques.

Therefore, key technologies for harmless treatment and resource recycling of biogas slurry are urgently required.

Biofloculants are a type of biodegradable macromolecular flocculants that have attracted increasing technological attention in water and wastewater treatment because of their lack of secondary pollution and harmless effects on the environment (Salehizadeh and Shojaosadati 2002; Li *et al.* 2009). The major stumbling block for commercial development of biofloculants is the high cost of substrates used for biofloculant production (Yang *et al.* 2012; Guo *et al.* 2013). Although several studies have investigated the biofloculant production using various low-cost substrates, such as agricultural biomass, wastewater, and industrial waste materials, there has been less investigation of biofloculant production from wastewater supernatant during an AD process (Zhao *et al.* 2012). Biogas slurry digested from organic wastes contains various dissolved organic matter, such as acids, alcohols, inorganic salts, and micronutrients, which could be utilized by certain biofloculant-producing microorganisms (Fujita *et al.* 2001; Wang *et al.* 2007; You *et al.* 2008).

Biofloculants are commonly applied to wastewater treatment, especially in heavy metal wastewater treatment, because they are safe to use in the environment. Electroplating wastewater containing complex heavy metals such as Cr^{6+} , Zn^{2+} , and Ni^{2+} , which can damage human health and the ecological environment, is discharged during the electroplating process (Martín-Lara *et al.* 2014; Lu *et al.* 2015).

Corn straw and molasses wastewater from the AD process for biofloculant production has not been reported in previous studies. The present study focused on the production and optimization of biofloculants by *Rhizobium radiobacter* and *Bacillus sphaericus* using wastewater supernatant from anaerobic co-digestion of corn straw and molasses wastewater as substrates. Furthermore, the produced biofloculants were evaluated to determine their ability to treat heavy metal ions contained in electroplating wastewater.

EXPERIMENTAL

Materials

Design and operation of the AD bioreactor

The corn straw was collected from Xiangfang farm in Heilongjiang, China. Molasses wastewater ($\text{COD}_{\text{Cr}} = 78680 \pm 232$ mg/L, $\text{TN} = 1528 \pm 34$ mg/L, $\text{pH} 6.5 \pm 0.1$) was obtained from Shengda Yeast Food Co., Ltd in Heilongjiang, China. A continuous stirred tank reactor (CSTR) with a total volume of 20 L (24 cm diameter \times 50 cm height) was used as a bioreactor, and the mixed fermentation liquor used was stirred 10 min/d with agitation at 100 rpm. Corn straws were cut into 0.5- to 1-cm sections, and the CSTR was operated continuously at an initial total solid concentration (TS) of 10 wt% for 210 d as a batch mode at 35 °C. The biogas slurry used for inoculum was taken from a large-scale biogas engineering plant utilizing cow manure at 35 °C in Hailin farm in Heilongjiang province.

Biofloculant-producing strains and biogas slurry management

Biofloculant-producing bacteria F2 (*Rhizobium radiobacter*) and F6 (*Bacillus sphaericus*) were screened from soils and offered by Heilongjiang Environmental Biotechnology Key Laboratory (China). Equal volumes of F2 and F6 were then mixed to

form compound bioflocculant-producing bacteria (F^+) used for bioflocculant production of wastewater supernatant. The seeding culture medium contained the following sterilized medium at pH 7.0: 10 g/L glucose, 0.5 g/L yeast extract, 0.5 g/L urea, 0.5 g/L K_2HPO_4 , 5 g/L KH_2PO_4 , 0.2 g/L $MgSO_4$, and 0.1 g/L NaCl.

The wastewater supernatant from anaerobic co-digestion of corn straw and molasses wastewater was collected from the CSTR at various fermentation periods and centrifuged at 12,000 g for 5 min. Then, glucose (2 g/L), KH_2PO_4 (2 g/L), and K_2HPO_4 (5 g/L) were mixed into a sterilized medium of wastewater supernatant as a compound culture medium following the procedure given in the authors' previous study (Li *et al.* 2014).

Bioflocculant flocculating activity test for electroplating wastewater

Simulated electroplating wastewater was prepared containing Cr^{6+} (48.5 mg/L), Zn^{2+} (36.8 mg/L), Cu^{2+} (59.5 mg/L), and Ni^{2+} (36.8 mg/L) using deionized water. The pH of the solution was adjusted using HCl and NaOH solutions. To analyze the flocculating activity with respect to heavy metal ion removal from electroplating wastewater, approximately 20 mL of wastewater was added to the bioflocculant at various dosage levels (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, and 5 mL); pH (2, 3, 4, 5, 6, and 7); temperatures (10, 15, 20, 25, 30, 35, 40, and 45 °C); and time (5, 10, 15, 20, 25, 30, 40, 50, 60, 80, and 100 min). The bioflocculants were mixed in simulated electroplating wastewater operating at 25 °C with shaking at 140 rpm, and a settling period of 15 min.

Methods

Analytical methods

The total solids (TS), volatile solids (VS), chemical oxygen demand (COD), total nitrogen (TN), total organic carbon (TOC), and pH were determined using standard methods (APHA 2005). The volatile fatty acid (VFA) and methane contents were measured using gas chromatography (Agilent technology, HP-6890, Wilmington, DE). Biogas yield was monitored on a daily basis by the water displacement method (Park and Li 2012). The reducing sugars were analyzed using the dinitrosalicylic acid (DNS) method (Miller 1959). The surface morphology of corn straw was observed with a scanning electron microscope (SEM, JSM-6390, JEOL, Tokyo, Japan). The heavy metal ions were determined by inductively coupled plasma atomic emission spectrometry (ICP-OES, Optima 5300DV, Perkin Elmer, USA).

Determination of flocculating activity

Flocculating activity and extraction efficiency of the bioflocculants were determined according to a previous study (Liu *et al.* 2015, 2016). Ten milliliters of compound medium of wastewater supernatant were added to a kaolin suspension. At first, approximately 5 g of kaolin clay was suspended in 1000 mL of distilled water and then mixed with 1.5 mL of 10% $CaCl_2$ at pH 7. A control was prepared using the same method without the addition of bioflocculant, and flocculating activity was calculated according to the following formula,

$$\text{Flocculating activity (\%)} = \frac{A - B}{A} \times 100\% \quad (1)$$

where A and B are the OD_{550} (optical density at 550 nm) of the control and sample, respectively.

Adsorption capacity test of bioflocculant

The amount of heavy metal ions adsorbed by compound bioflocculants (q_e , g/g) and their removal efficiency (η , %) were estimated according to following equations,

$$q_e = [(C_o - C_e)V] / m \quad (2)$$

$$\eta = (C_o - C_e) / C_o \quad (3)$$

where C_o and C_e are the initial concentrations of heavy metal ions in simulated electroplating wastewater (g/L); V is the total volume of the wastewater (L); and m is the mass weight of bioflocculant (g) used for adsorption.

RESULTS AND DISCUSSION

Performance of Anaerobic Digestion Process

Variation of chemical composition

The experiments were performed in a continuous stirred tank reactor (CSTR), which was fed with corn straw and molasses wastewater at 35 °C. The total volatile fatty acids (VFAs) values are important for AD operation, and those values were used here as the critical indicators of methanogenesis (Liew *et al.* 2011; Linke *et al.* 2015). The VFAs and changes in biogas production yield, pH, total organic carbon (TOC), and total nitrogen (TN) contents are shown in Fig. 1 and Table 1.

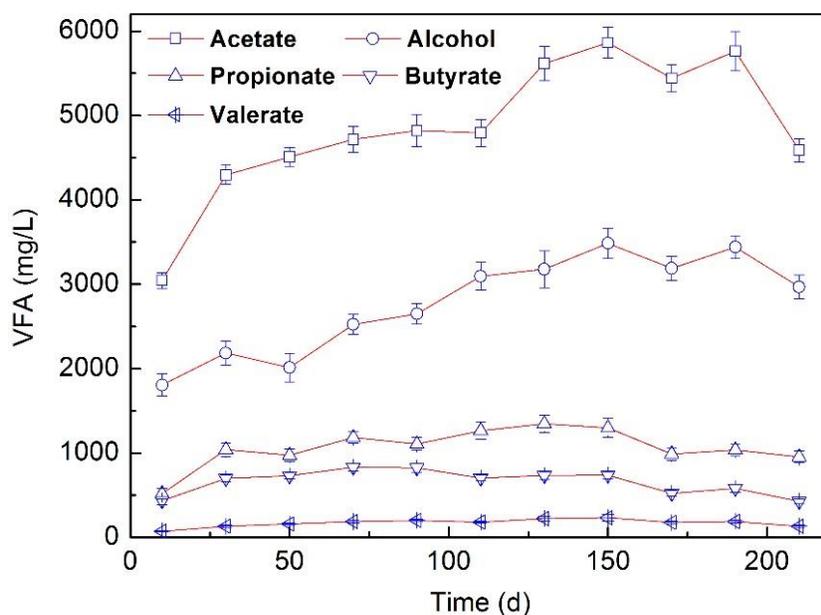


Fig. 1. Variation of VFAs during the co-digestion process

The pH decreased from 7.5 to 6.25 after 30 d of operation. The fermentation products formed during the AD process were alcohol, acetate, propionate, butyrate, and valerate, which all initially exhibited an increasing trend and then started decreasing. All the VFAs showed a similar trend, and increased dramatically during the start-up period of 30 d.

The concentrations of ethanol and acetic acid dominated in the VFAs, which accounted for 30.01% and 50.48% after 150 d of operation, and the biogas production reached 725 mL/L_{reactor}/d after only 10 d of operation. We speculate that the molasses wastewater containing easily degradable organic matter, such as acetate, was beneficial to acidification, and the methanogenesis process sped up the degradation of corn straw, and caused an optimum biogas yield.

The TOC concentration in the fermentation liquor decreased rapidly to 8953 mg/L after 10 d of operation and then increased to 42437 mg/L on the 90th day. The rapid decrease of TOC content was attributed to the adsorption by corn straw and the biogas-producing process. The degradation of corn straw contributed to the TOC content in fermentation liquor. The nitrogen (N) ratio during the anaerobic co-digestion of corn straw and molasses wastewater decreased from 1528 to 853.6 mg/L, which improved the anaerobic bacterial growth and metabolism. Meanwhile, the increase of the carbon-nitrogen (C/N) ratio reduced the risk of ammonia inhibition and was helpful in improving the methane potential during the AD process (Yen and Brune 2007; Procházka *et al.* 2012).

Table 1. Variation of Chemical Composition during AD Process

Operation Time (d)	pH	TOC (mg/L)	TN (mg/L)	Biogas Production (mL/L _{reactor} /d)
10	7.23 ± 0.1	8952.6 ± 226.7	1134.2 ± 56.7	725
30	6.25 ± 0.06	15328.4 ± 296.5	928.7 ± 47.2	860
50	6.28 ± 0.05	36713.7 ± 356.2	853.6 ± 35.3	880
70	6.32 ± 0.06	39581.9 ± 321.5	1023.7 ± 57.6	770
90	6.17 ± 0.04	42436.7 ± 347.1	972.4 ± 33.1	660
110	6.22 ± 0.03	28374.2 ± 213.4	1110.8 ± 41.1	720
130	6.23 ± 0.04	19337.1 ± 166.2	996.1 ± 28.3	680
150	6.37 ± 0.05	14873.6 ± 126.4	824.1 ± 33.8	650
170	6.39 ± 0.06	10248.1 ± 146.9	735.8 ± 23.2	630
190	6.31 ± 0.05	8534.2 ± 129.3	912.7 ± 41.1	580
210	6.43 ± 0.08	7653.7 ± 116.1	820.5 ± 22.4	565

SEM images of corn straw degradation

A scanning electron microscope was used to observe the morphological structure of corn straw residue during various stages of the AD process. As shown in Fig. 2, after 30 d of fermentation, the cell structure of corn straw, such as epidermis cells, parenchyma and vascular bundles, was still clearly observable.

After 90 d of AD performance, the surface morphology of corn straw changed considerably, with the collapse of the regulated cell structure apparent. Then, after 150 d of fermentation, the vascular bundle exhibited fragility and many holes were detected in the surface. After 210 d of fermentation, the parenchyma cells were severely damaged, and a large ring of holes could be seen clearly.

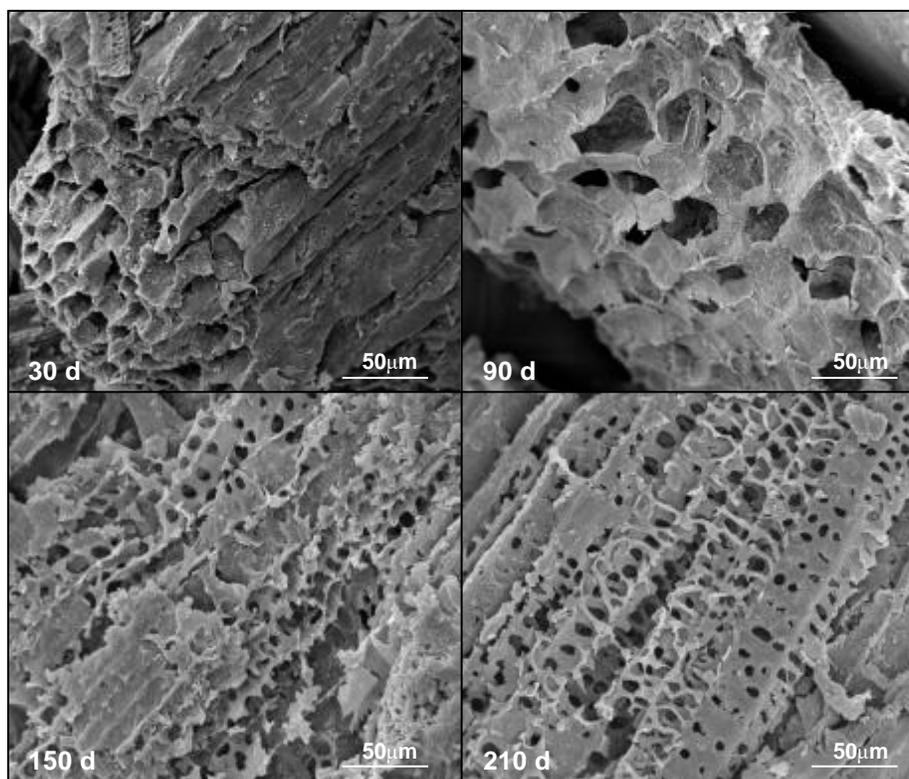


Fig. 2. SEM observation of corn straw degradation during the AD process

Bioflocculant Production using Wastewater Supernatant

Flocculating activity and reducing sugar variations from different AD periods

The wastewater supernatant from the co-digestion of corn straw and molasses wastewater was collected at different times (30th, 50th, 70th, 90th, 110th, 130th, and 150th days) for bioflocculant production. It is known that the composition of the medium plays a key role in biopolymer production and flocculating efficiency (Salehizadeh and Yan 2014). The compound medium from wastewater supernatant was sterilized and inoculated with F^+ at a concentration of 8 wt%. The cultivating conditions were operated at an initial pH of 7.0, a temperature of 30 °C, with shaking at 140 rpm, and a fermentation period of 24 h.

As shown in Fig. 3, the variation of flocculating activity and the reducing sugar contents exhibited a similar trend of increase initial and then decrease. The bioflocculant activity was achieved at the 60.5% level using a 30-d fermentation liquor, and the activity was enhanced at the 8.8%, 10.7%, 22.4%, and 31.2% levels using 50-, 70-, 90- and 110-d fermentation liquors, respectively. Clearly, the maximum bioflocculant activity of 91.3% was obtained with reducing sugar content of 1.56 g/L when a 110-day wastewater supernatant was utilized, in comparison with other periods. It might be inferred that the wastewater supernatant from AD co-digestion of corn straw and molasses wastewater on day 110 were suitable for bioflocculant production, which might be due to its high concentrations of reducing sugar. Furthermore, the corn straw was rich in organic matter, VFAs, alcohol, and some dissolved organic matter from the hydrolysis-acidification process on day 110. Such substances also could be utilized easily by F^+ and were beneficial to biopolymer production. Recently, many researchers have focused on developing cost-effective bioflocculant from wastes. Methanol wastewater has been used as a carbon source for bioflocculant production with optimum flocculant-producing activities of 93.1% (Cao

et al. 2015). Dairy wastewater, when used as a medium for bioflocculant production, could achieve flocculant-producing activities of 95.4% (Wang *et al.* 2007).

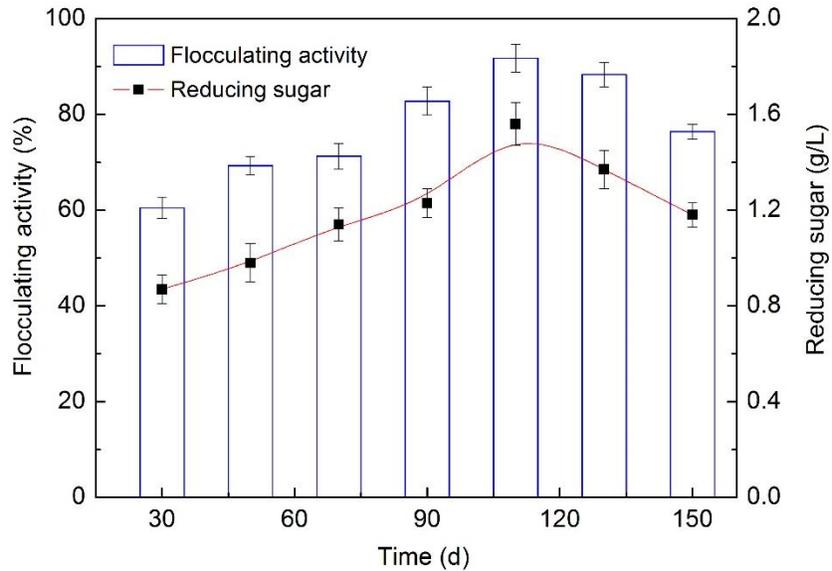


Fig. 3. Comparison of flocculating activities and reducing sugar contents from different periods of the AD process

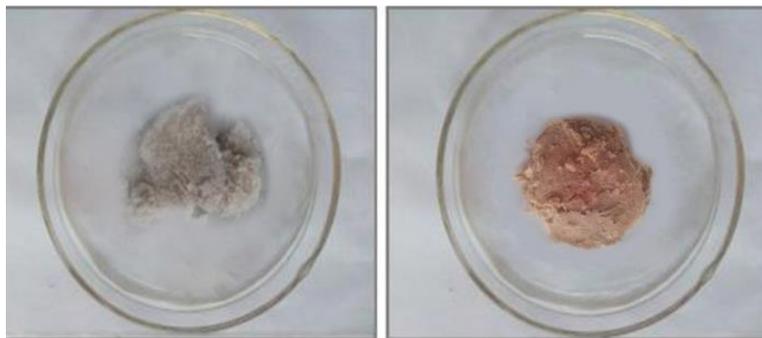
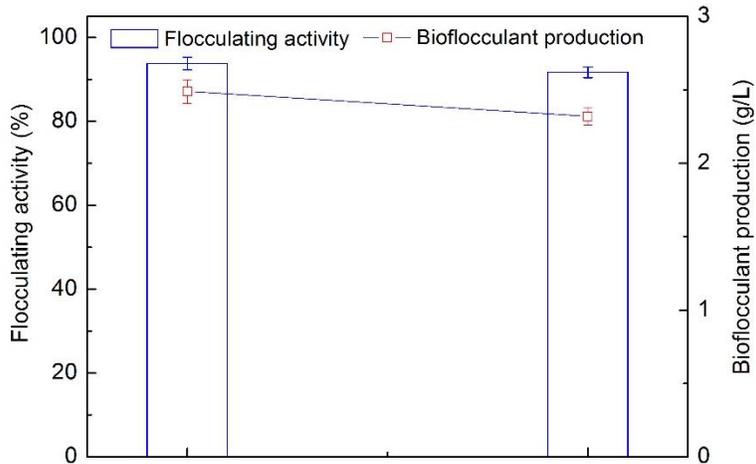


Fig. 4. Comparison of flocculating activity and bioflocculant production between optimum biogas compound medium (BS-MBF) and traditional medium (CK)

Bioflocculant production from optimum biogas compound medium

Figure 4 reveals the yield of bioflocculant obtained by extraction from the optimum biogas compound medium. The highest yield of 2.32 g/L was achieved using 110-day wastewater supernatant from the AD process, while 2.49 g/L was achieved from the traditional mediums (Wang *et al.* 2015). Therefore, it is feasible to use wastewater supernatant from the AD co-digestion of corn straw and molasses wastewater as a low-cost substrate to produce bioflocculants.

Application of MBF in Treating Electroplating Wastewater

Factors impacting removal efficiency of heavy metal ions

A number of heavy metal ions, such as Cr^{6+} , Zn^{2+} , and Ni^{2+} are typically present in electroplating wastewater, which is one of the most harmful industrial wastewaters and is severely toxic to humans. To investigate the removal efficiency of these ions and the adsorption capacity of optimal dosage of bioflocculants, different dosages of bioflocculants were mixed in simulated electroplating wastewater operating at 25 °C with shaking at 140 rpm and an adsorption period of 50 min.

As shown in Fig. 5, the Zn^{2+} and Cu^{2+} ions removal efficiency that could be achieved was more than 90%, while the maximum Cr^{6+} and Ni^{2+} ions removal efficiency was able to reach just 37% and 65%. The adsorption capacity of Cu^{2+} ions decreased strongly from 96.8% to 76.45% when increasing the dosage of bioflocculants from 0.5 mL (62.25 mg/L BS-MBF) to 2.0 mL (249 mg/L BS-MBF) (Fig. 6). The removal efficiency and the cost of bioflocculants were comprehensively evaluated, and 3.0 mL (374 mg/L BS-MBF) was considered as the optimum dosage in the simulated electroplating wastewater treatment.

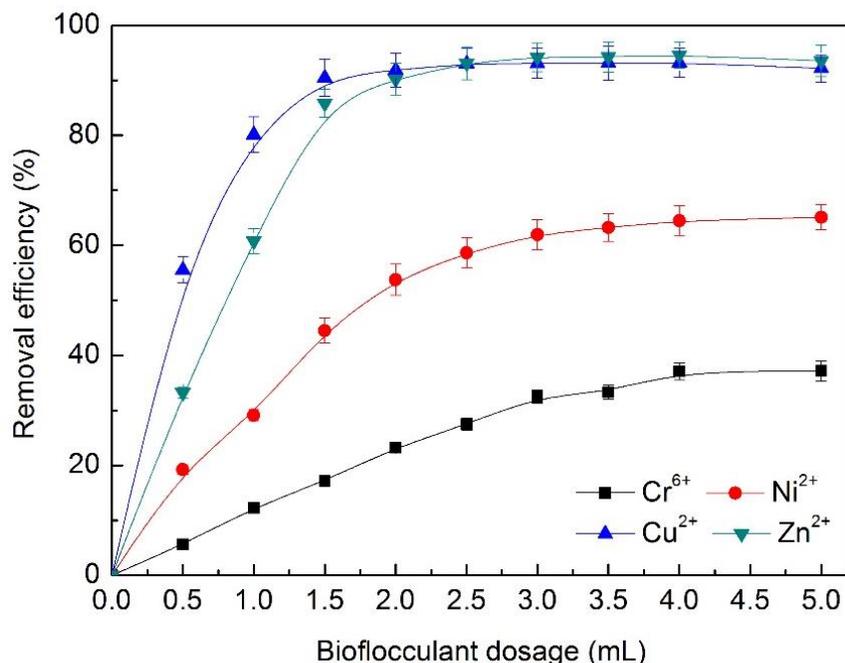


Fig. 5. Effects of bioflocculant dosage on removal efficiency of heavy metal ions

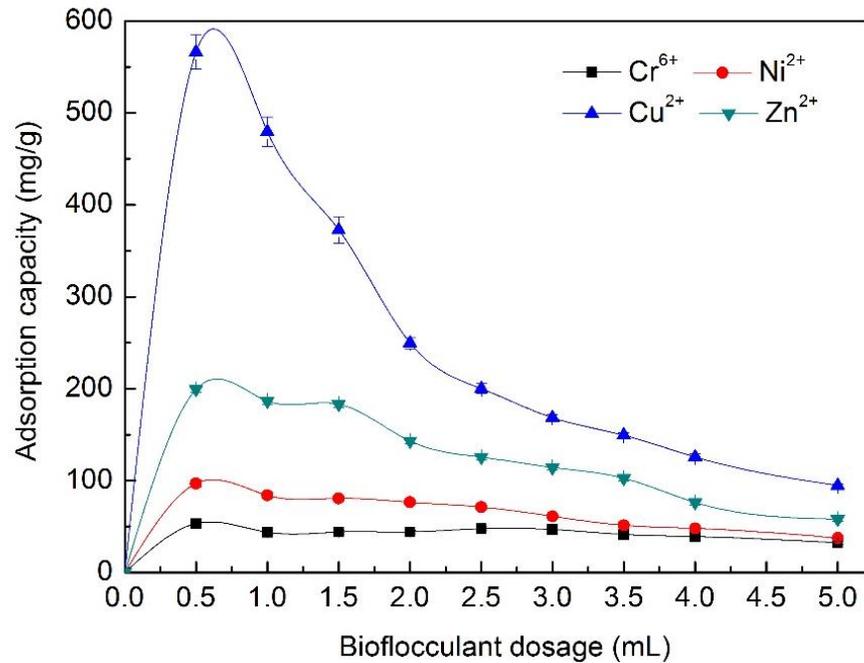


Fig. 6. Effects of bioflocculant dosage on adsorption capacity of heavy metal ions

The influence of pH on the removal efficiency of heavy metal ions was also studied, and 3 mL wastewater supernatant of bioflocculants was added and operated at 25 °C, with shaking at 140 rpm for 50 min. As shown in Fig. 7, the pH had a marked impact on the removal efficiency.

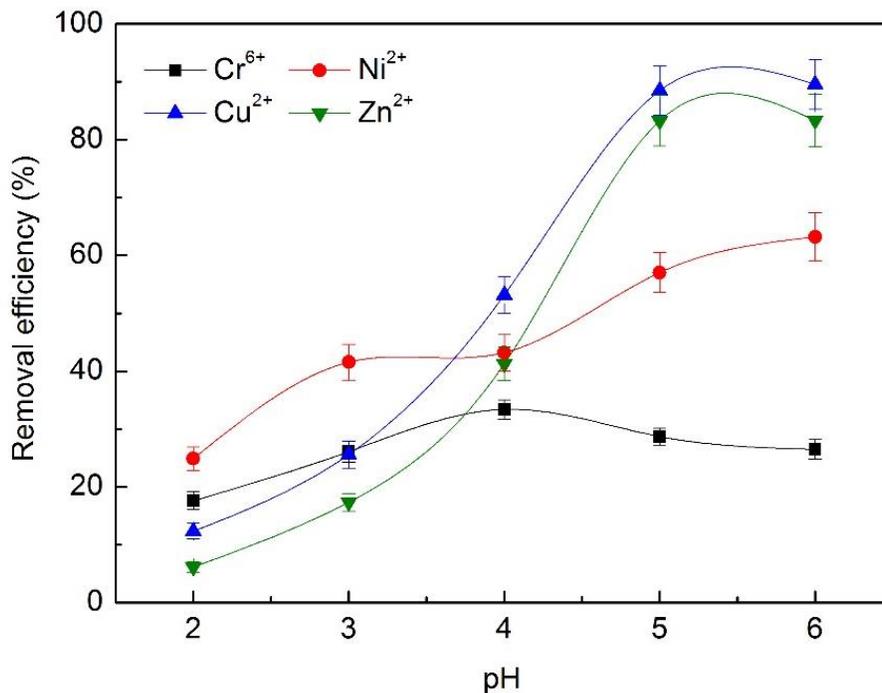


Fig. 7. Effects of pH on removal efficiency of heavy metal ions

The bioflocculants exhibited much less removal capacity for Cr^{6+} ions than the other metal ions, and the maximum removal efficiency of Cr^{6+} reached merely 33.4% at pH 4.0, after which it decreased with further increases in pH. The ionic species present will be affected by pH, even if the valence state remains the same. In the pH range 2.0 to 6.0, HCrO_4^- and Cr_2O_7^- ions are in equilibrium. Chromium exhibited a state of HCrO_4^- at pH 4, and could be removed by electrostatic adsorption from the positive adsorption center of $-\text{NH}_4^+$ and $-\text{OH}_2^+$ of bioflocculants during acid conditions, which would be favorable to the adsorption process (Kumar *et al.* 2008). As the pH increases, the number of negatively charged sites increases and the number of positively charged sites decreases. Further, some enhancement of removal may be attributed to the fact that chromium (VI) is expected to oxidize some of the biopolymers or other organic matter present, and may get reduced to chromium (III) (Harry *et al.* 2008; Hubbe *et al.* 2011). The bioflocculants appeared to exhibit better adsorption capacity for chromium (III). The optimum removal efficiencies of Zn^{2+} and Cu^{2+} by wastewater supernatant bioflocculants at pH 5.0 to 6.0 reached 89% and 83%, while the maximum removal efficiency of Ni^{2+} was 63.1% at pH 6.0. These results demonstrated that wastewater supernatant bioflocculants could have optimum removal efficiency for heavy metals of electroplating wastewater at pH 6.0.

The influence of the contact time of heavy metals with electroplating wastewater on removal efficiency was determined at optimum dosages (374 mg/L BS-MBF) and pH (6.0), as illustrated in Fig. 8.

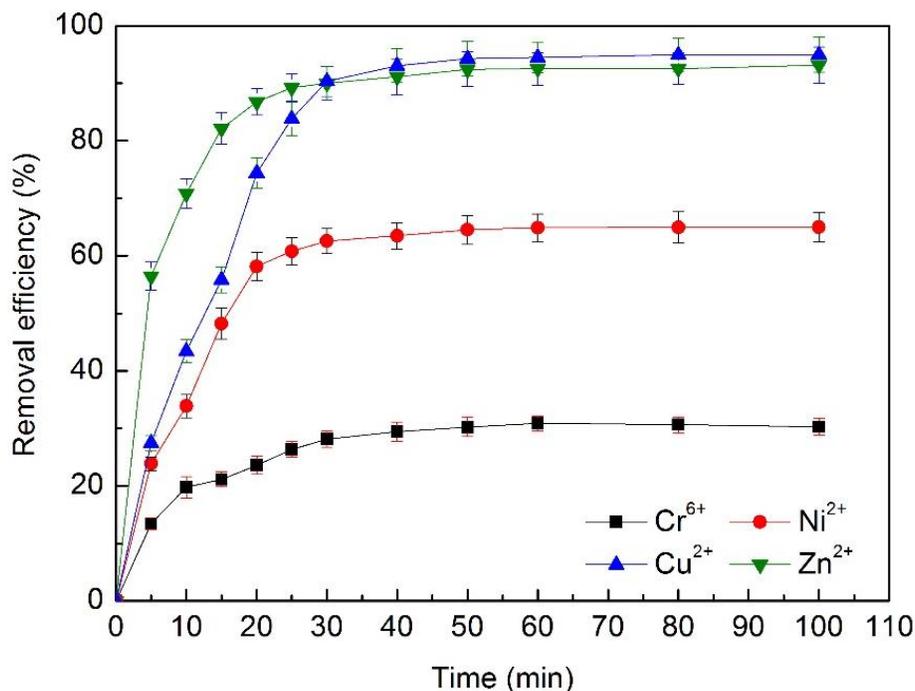


Fig. 8. Effects of adsorption time on removal efficiency of heavy metal ions

There were three stages: fast adsorption, moderate adsorption, and dynamic equilibrium. The removal efficiency of Cr^{6+} , Ni^{2+} , Zn^{2+} , and Cu^{2+} ions increased during the initial rapid-adsorption stage of 20 min, while the adsorption sites of bioflocculant were being gradually replaced, and reached 23.5%, 58.1%, 74.3%, and 86.7%, respectively. Then the removal efficiency continued to increase at a relatively slow speed with contact

time of 40 min, until an equilibrium time was reached. Obviously, the adsorption equilibrium time of Cr^{6+} was 40 min from the electroplating wastewater, and approximately 30 min for Ni^{2+} , Zn^{2+} , and Cu^{2+} : thus 40 min was confirmed as the optimum contact time.

CONCLUSIONS

1. It is feasible to use wastewater supernatant from anaerobic co-digestion of corn straw and molasses wastewater as a nutrient resource for producing bioflocculants.
2. The maximum bioflocculant activity obtained was 91.3% with reducing sugar content of 1.56 g/L, and the highest yield of 2.32 g/L was achieved when 110-day wastewater supernatant was utilized from the AD process.
3. The optimal conditions of heavy metal ions removal from electroplating wastewater for BS-MBF occurred at 374 mg/L, with an initial pH of 6.0, and a contact time of 40 min. The adsorption capacity of Cu^{2+} and Zn^{2+} reached more than 90%, while Cr^{6+} adsorption only reached approximately 30%.

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