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Removal of Total Nitrogen from Tannery Wastewater by Bioflocculant

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The total nitrogen in tannery wastewater was treated by prepared bioflocculant at different conditions. The most suitable conditions for total nitrogen removal was attained at a normal temperature, pH of 8, flocculant of 1.0 g/L when the shaking speed was 200 r/min and the reaction time was 30 min. Through testing saccharide and protein in the bioflocculant, it was found that the bioflocculant contained saccharides and proteins. After detecting the content of saccharide and protein before and after reacted, it was found that the content of saccharides and proteins in the bioflocculant decreased after treating total nitrogen which meant the saccharides and the proteins in the bioflocculant played flocculation effect in the removal of total nitrogen from tannery wastewater, and the saccharides played main effect. It is expected that the results will contribute to the industry treatment of total nitrogen removal in tannery effluent by bioflocculant.

Key words: Tannery wastewater, Total nitrogen, Bioflocculant.

At present, the removal of total nitrogen in tannery wastewater is needed to be treated urgently because it will cause the deterioration of environmental quality. The total nitrogen in tannery wastewater comes mainly from two aspects. One is from the transformation of organic nitrogen in leather itself; the other is from the processes adding ammonium such as de-liming, bating, pickling, tanning and dyeing (Xue, 2009). As the total nitrogen content in tannery wastewater varies widely with the changed processes in leather industry, it is very important to look for an economic and reasonable method to remove total nitrogen in the tannery wastewater.

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The method using bioflocculant to remove total nitrogen in the tannery wastewater has become a new research direction and researchers pay more and more attention to the study of bioflocculant. As we know, the bioflocculant is a kind of polymers possessing flocculation activity which comes from the microbes at special culture conditions. It is for environmental protection which can easily eliminate the secondary pollution and has the unique effect for a wide range of biodegradation (Levy,1992; Vijayalakshmi, 2003). Recently, there have been a few reports about treatment of tannery wastewater with the bioflocculant. Chai et al. isolated two species of bacteria which produced bioflocculant and used them to treat the tannery effluent (Chai, 2000). Qin used microorganism as flocculant to treat leather effluent and found it had good effect for

the removal of COD, SS, Cr^{3+} in leather effluent (Qin, 2006). Wang et al. selected four species of bacteria producing bioflocculant and found the color, turbidity, COD in the tannery wastewater was significantly reduced using the composite bioflocculant (Wang, 2009). According to the literatures, it was found that *Bacillus cereus* has the ability to produce bioflocculant (Zhang, 2008) and can removal ammonia in tannery wastewater (Zhao, 2012). However, there is no report about removal of total nitrogen in tannery wastewater with bioflocculant. In the experiments, the *Bacillus cereus* will be used to prepare bioflocculant and the bioflocculant will be used to remove total nitrogen in tannery wastewater.

MATERIALS AND METHODS

Materials

Yeast extract, peptone and bovine serum albumin were biochemical level and all purchased from Beijing AoBoXing LEIVERSEEN BIO-TECH Co. Ltd (China). The tannery wastewater was from a tannery in Sichuan (China). All other reagents used in this study were research grade.

Bacillus cereus

The bacteria strain *Bacillus cereus* in the experiments was conserved in our laboratory. Before used, it was cultured in Luria broth containing nitrogen to domesticate it and adapt for the tannery wastewater.

Preparation of bioflocculant

First, the culture medium was prepared with: $NaNO_3$ 2 g, KCl 0.5 g, K_2HPO_4 1 g, $MgSO_4$ 0.5 g, $FeSO_4$ 0.01 g, sucrose 30 g and water 1L. The pH of the culture medium was nature and the medium was sterilized at 121! (0.105MPa) for 20 min before used. Then, the domesticated *Bacillus cereus* was inoculated to the sterilized conical flasks containing 100 mL of the culture medium. Next, the flasks were incubated at 35 ! while shaking at 180r/min. After 24h, the cultured cells were centrifuged at 8000r/min (7020g) for 10min at 4 !. The supernatants were added with ethanol pre-cooled at 4 ! which was 3 times the volume of the supernatants. After the mixture was placed at 4 ! for 6h, it was centrifuged at 10000r/min for 10min. Then, the precipitate was collected and washed with distilled water. After that, it was centrifuged at 8000r/min for 10min. Through dialyzed for 3 times repeatedly, the centrifuged precipitate was freeze-dried in vacuum and thus the bioflocculant was prepared.

Factors influencing the removal of total nitrogen

pH

Tannery wastewater samples were adjusted to different pH values (6.0-10.0) using 0.1mol/L NaOH and 0.1mol/L HCl solution. Next, 50mL of wastewater containing 350mg/L of total nitrogen with different pH values were poured to conical flasks and added with 0.4g/L of the prepared bioflocculant. After that, the flasks were placed in a magnetic stirring device (SH-3A, Ziguang Instrument Co., Beijing, People's Republic of China) with the stirring speed of 200r/min for 2min and then 40r/min for 10min at normal temperature (25!). After placed for 20min, the samples were filtered and the total nitrogen in each sample was determined through an Automatic Kjeldahl nitrogen analyzer (Kjeltec 2200, FOSS Co., Sweden). In addition, the wastewater reacted through stirring but untreated by the bioflocculant at each pH was used as a control. The calculational formula for the removal rate of the total nitrogen was as follows:

$$F = (A_1 - A_2)/S$$

Where F meant the removal rate of the total nitrogen in tannery wastewater; A_1 meant the content of the total nitrogen in tannery wastewater which was treated by the bioflocculant; A_2 meant the content of the total nitrogen for the control which was not treated by the bioflocculant; S meant the content of the total nitrogen before reacted. In addition, the removal rate of the total nitrogen for the following procedures was the same as this formula.

Temperature

A total of 0.2g/L bioflocculant were added to 50mL tannery wastewater containing 350mg/L of total nitrogen (pH=8.0). Next, the flasks containing the wastewater were respectively stirred at 20, 30, 40, 50 and 60! for 2min with the stirring speed of 200r/min and then for 10min with 40r/min. After placed for 20min, the samples were filtered and the total nitrogen in each sample was determined. In addition, the wastewater untreated by the bioflocculant at each temperature was used as a control.

Amount of bioflocculant

The prepared bioflocculant was added to conical flasks containing 50mL tannery wastewater (pH=8.0) to give samples with different amount of bioflocculant (0.1, 0.2, 0.5, 1.0, 2.0 g/L). The samples were then placed in the magnetic stirring device with the stirring speed of 200r/min for 2min and then 40r/min for 10min at normal temperature (25!). After placed for 20min, the samples were filtered and the total nitrogen in each sample was determined. In addition, the wastewater untreated by the flocculant with different amount of bioflocculant was used as a control.

Stirring speed

A total of 1.0g/L flocculants were added to 50mL of tannery wastewater containing 350mg/L of total nitrogen (pH=8.0). Next, the flasks containing the wastewater were stirred at normal temperature (25!) for 30min with the stirring speed of 50, 100, 150, 200 and 250 r/min, respectively. After placed for 20min, the samples were filtered and the total nitrogen in each sample was determined. In addition, the wastewater untreated by the bioflocculant at each stirring speed was used as a control.

Reaction time

A total of 1.0g/L bioflocculant were added to 50mL tannery wastewater containing 350mg/L of total nitrogen (pH=8.0). Next, the flasks containing the wastewater were stirred at normal temperature (25!) with the stirring speed of 200r/min for 3, 6, 9, 12, 15, 30 and 60min, respectively. After placed for 20min, the samples were filtered and the total nitrogen in each sample was determined. In addition, the wastewater untreated by the bioflocculant at each reaction time was used as a control.

Preparation of the bioflocculant solution

The bioflocculant solution sample before reacted was prepared through adding it to distilled water and stirred at 200r/min for 30min at 25! to be mixed uniformly. And the bioflocculant solution sample after reacted was prepared through adding it to tannery wastewater containing total nitrogen (pH=8) and stirred at 200r/min for 30min at 25! to be mixed uniformly.

Structure analysis of the bioflocculant (Shih, 2001; Hu, 2007)

Identification of saccharide in the bioflocculant

The method of Molish reaction is most commonly used for the identification of saccharide. The reaction will be positive if there is saccharide whether free or combined existed. The detailed procedure was as follows. First, 5 grams of á-naphthol was dissolved in 50mL 95% (w/w) of ethanol to prepare á-naphthol reagent. Then, 1mL of bioflocculant solution before reacted and 2 drops of á-naphthol reagent were added to a test tube. After mixed uniformly, 1mL of concentrated sulfuric acid (H_2SO_4) was slowly added to the tube along the tube wall carefully. Then, it was observed between two lays including concentrated H_2SO_4 and sample to detect whether there was purple ring appeared or not.

Identification of protein in the bioflocculant

Biuret reaction in this experiment was used to identify whether there has protein in the flocculant or not. First, 2mL of NaOH solution was added to get alkaline environment. Then, Biuret reagent was prepared using 0.15g of $CuSO_4$ and 0.6g of potassium sodium tartrate dissolved in 50mL of distilled water, and the solution was added 30mL 10%(w/w) of NaOH solution and 0.1g of KI and then diluted to a final volume of 100mL. Next, 2mL of samples and 10 drops of Biuret reagent were mixed and placed to detect the change of the color. If the solution turned purple, it showed there was protein existed.

Observation of the bioflocculant by FT-IR

The prepared bioflocculant was observed by FT-IR (Fourier transform infrared spectrum) (Equinox 55, Bruker Co., Germany) to detect the components in the bioflocculant.

Analysis for content of saccharide and protein in bioflocculant (Fujita, 2001; Zheng, 2004)

Analysis for content of saccharide in bioflocculant

The saccharides content was determined as follows. First, 1 mg/mL of glucose solution was made using 50 mg glucose dissolved in 50mL of volumetric flask. After different volumes (1-9mL) of the glucose solution (1 mg/mL) were pipetted into 50mL volumetric flask to prepare standard glucose solution, 1 mL of the standard glucose solution and 1mL 5% (w/w) of phenol solution were added vertically to each test tube (20mL). Then, 5mL of concentrated sulfuric acid was added to each tube quickly. After 5min, tubes were placed in a boiling water bath for 15min. When the tubes were cooled to room temperature, the extinction of the samples in a 2cm cell at 490nm was measured

against the blank (2mL of distilled water) using a UV/VIS spectrophotometer (Hong Qiao High-tech Instrument Co., Shanghai, People's Republic of China). An extinction-concentration calibration curve of glucose was then plotted. The extinction of saccharides in the bioflocculant solution sample before and after reacted was analyzed according to the measuring procedure as described above, after which the saccharides content was determined using the calibration curve.

Analysis for content of protein in bioflocculant

The proteins content was determined as follows. First, 1 mg/mL of serum albumin solution was made using 100 mg bovine serum albumin dissolved in 100mL of volumetric flask. After different volumes (0, 0.2, 0.4, 0.6, 0.8 and 1.0mL) of the serum albumin solution (1 mg/mL) and corresponding distilled water (1.0, 0.8, 0.6, 0.4, 0.2, 0 mL) were pipetted into respective test tubes. Then, the Biuret reagent was added to each tube for color reaction. After placed for 30min, the extinction of the samples in a 2cm cell at 540nm was measured using the UV/VIS spectrophotometer. An extinction-concentration calibration curve of serum albumin was then plotted. The extinction of proteins in the bioflocculant solution sample before and after reacted was analyzed according to the measuring procedure as described above, after which the proteins content was determined using the calibration curve.

RESULTS AND DISCUSSION

pH

As can be seen from Figure 1, the removal rate of total nitrogen in tannery wastewater was highest (30.43%) when pH was 8 which meant slightly alkaline environment was propitious to play the flocculation effect for the prepared bioflocculant. When pH was 7, the removal rate of total nitrogen was lowest (20.10%). It showed that the same bioflocculant might have different flocculation ability at different pH because the change of pH altered the state of charge for the bioflocculant and its capacity for the neutralization of charge; also, the surface properties and the ability of molecular adsorption for the flocculated substances were changed. Thus, pH of 8 was selected as the optimum pH to remove total nitrogen from tannery wastewater with the bioflocculant in the following experiments.

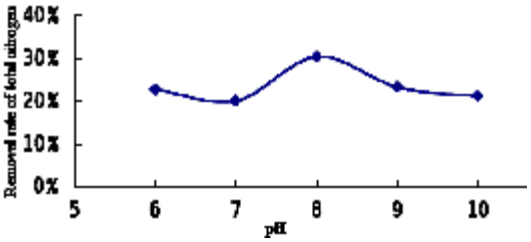

Fig. 1. Removal rate of total nitrogen at different pH

Temperature

to 29.2% as the increase of temperature from 20 to 50! and then decreased a little to 28.6% at 60! which meant the change for the total nitrogen removal was not large from 20 to 60! although there was a maximum value at 50! (Figure 2). It indicated that the bioflocculant could adapt to the tannery wastewater containing total nitrogen when temperature was from 20 to 60!. To economize electrical source, the normal temperature was selected as the reaction temperature to remove total nitrogen from tannery wastewater in the following experiments. When treating practical wastewater, the normal temperature could also be chosen.

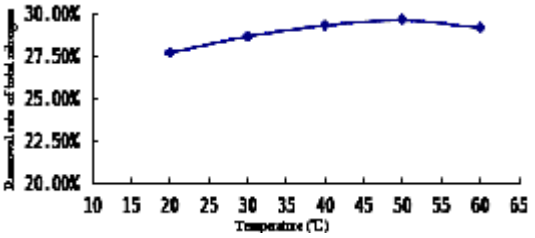

Fig. 2. Removal rate of total nitrogen at different temperature

Amount of flocculant

As shown in Figure 3, the removal rate of total nitrogen increased as the increase of bioflocculant from 0 to 1.0g/L and the effect was all better when the bioflocculant was from 0.2 to 2.0g/L. The highest removal rate (32.56%) was observed at 1.0g/L. When the bioflocculant was more than 1.0g/L, the removal rate of total nitrogen decreased slightly. This was because the treated object was surrounded by excessive polymers viz. bioflocculant and lost the possibility of bridging

combination with other particles. In addition, the flocculation balance achieved at a certain concentration of bioflocculant; when the concentration was more or less than it, the flocculation effect became poor especially deteriorated (Dermlim, 1999).

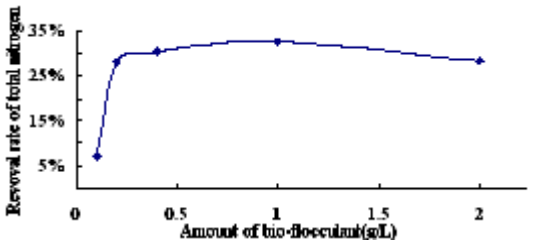

Fig. 3. Removal rate of total nitrogen with different amount of bioflocculant

Stirring speed

As shown in Figure 4, the removal rate of total nitrogen increased as the increase of stirring speed from 50 to 200r/min. When the stirring speed was 200r/min, the removal rate of total nitrogen reached highest of 34.79%. When the stirring speed was more than 200r/min, the removal rate of total nitrogen decreased. This was because appropriate stirring speed was propitious to the flocculant playing its effect and helping the adsorption of bioflocculant and the treated object, which thus advanced the removal rate of total nitrogen in tannery wastewater; however, the bioflocculant might appear agglutination phenomenon when stirring speed was too high which caused the removal efficiency reduced

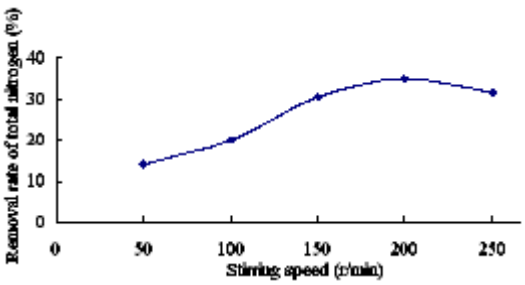

Fig. 4. Removal rate of total nitrogen with different stirring speed

Reaction time

As can be seen from Figure 5, the removal rate of total nitrogen increased as the increase of reaction time from 3 to 60min. However, the removal rate of total nitrogen for 30 and 60min was nearly the same, respectively 34.79 and 35.07%. It showed that the removal rate of total nitrogen increased with the increase of the adsorption ability for the bioflocculant and the treated object when the reaction time was increased appropriately; however, the removal rate of total nitrogen was changeless even decreased as the adsorbed compounds on the surface of the flocculant exceeded its saturated adsorption capacity when the reaction time was too long (Tao, 2005). Thus, 30min could be selected as the optimum reaction time to remove total nitrogen in tannery wastewater considering the cost.

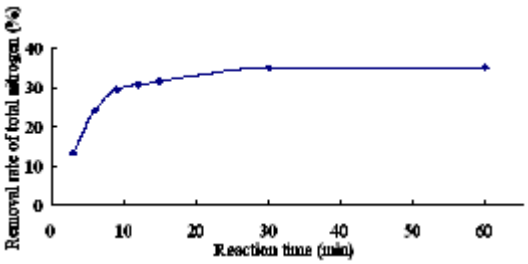

Fig. 5. Removal rate of total nitrogen with different reaction time

Structure analysis of the bioflocculant

In the experiment of Molish reaction, there was a purple ring between the concentrated H_2SO_4 and the bioflocculant sample in the test tube. The reason was that the saccharides in the bioflocculant sample reacted and generated furfural or its derivatives through dehydrated by the concentrated H_2SO_4, the derivatives then reacted with á-naphthol and generated purple substances (Zheng, 2004). It indicated that the bioflocculant sample reacted positively in the Molish reaction and contained saccharides.

In the experiment of Biuret reaction, the solution in the test tube became purple which meant there were proteins in the bioflocculant because the Biuret reagent turned from blue to violet in the presence of proteins, blue to pink when combined short-chain polypeptides (Zheng, 2004).

Therefore, the bioflocculant contained saccharides and proteins through the experiments of Molish reaction and Biuret reaction.

Analysis by FT-IR

The FT-IR showed that there was a peak of stretching vibration for primary amino group (-

NH-) which was one of the main groups in protein near the wavelength of 3426.3nm and a peak of bending vibration for the primary amino group (-NH-) at the wavelength of 1632.6nm; in addition, there was a peak of bending vibration for the carboxyl (-CO-) at 1071.2nm, and vibration peaks for -C-C-, -C-H- at 861.2, 580.7nm respectively (Figure 6) which were the obvious groups in saccharide. Through the analysis of FT-IR, it indicated that there was protein and saccharide presented in the bioflocculant possibly.

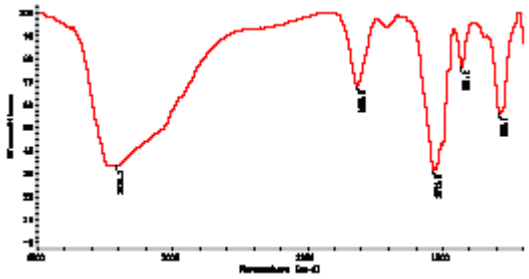

Fig. 6. Fourier transform infrared spectrum for the prepared bioflocculant

Analysis for content of saccharide and protein in bioflocculant

As shown in Table 1, the saccharide content in the bioflocculant after treating the total nitrogen decreased 0.3780 g/L, compared with the bioflocculant before reacting. And the protein content in bioflocculant after treating the total nitrogen decreased 0.0055 g/L compared with the bioflocculant before reacting. It indicated that the lost saccharide and protein in the bioflocculant were consumed in the reaction for removal of total nitrogen as the bioflocculant was not dissolved in water, which thus meant the saccharide and the protein in the bioflocculant played flocculation effect in the removal of total nitrogen from tannery wastewater, and the saccharide might play main effect because the lost content was bigger.

Table 1 The content of saccharide and protein in bioflocculant

	Content before reacted (g/L)	Content after reacted (g/L)	Decreased content after reacted (g/L)
saccharide	6.6250	6.2470	0.3780
protein	0.1370	0.1315	0.0055

CONCLUSION

This study represents the prepared bioflocculant in this experiment had the ability to remove total nitrogen in tannery wastewater and the bioflocculant contains saccharides and proteins. The saccharide and the protein in the bioflocculant played flocculation effect in the removal of total nitrogen from tannery wastewater, and the saccharide played main effect. It is expected that the results will contribute to the industry treatment of total nitrogen removal in tannery wastewater by bioflocculant.

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