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Preparation of Biological Flocculant and Its Application in Environment Research

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The new biological flocculant Fe-CTS was prepared in order to enhance the solubility, stability and flocculation performance of chitosan flocculant. At the best CTS and Fe mass ratio of 2, the Fe-CTS structure had good flocculation effect and good enmeshment function on pollutants. With the dosage of 10 ppm, the sedimentation rate of simulated wastewater reached 95 %. The prepared flocculants were used for the ethanol wastewater treatment too and this new bio-flocculant Fe-CTS was shown to be an effective way to diminish the content of inhibitory compounds and could be used for the ethanol wastewater treatment. In order to expand the biological flocculation agent application scope, the new modified chitosan flocculant was used in the detoxification of the acid hydrolyzate of lignocelluloses materials for ethanol production. The new bio-flocculant was proved to have stronger adsorption effect on the acid-soluble lignin which had a serious impact on the subsequent fermentation process and difficult to remove.

Keywords: Biological flocculant, Wastewater treatment, Detoxification.

INTRODUCTION

As is known, biological flocculants are harmless to the environment and humans, indicating their potential to replace the existing chemical flocculants. Various bio-flocculants have been used in wastewater treatment[1,2]. Biological flocculants at present can be divided into natural polymer flocculants and microbial flocculants[3]. Natural polymer compounds contain a variety of active groups, such as hydroxide radical and phenolic hydroxy group, which always show lively chemical properties. Natural polymer flocculants have many advantages compared with other synthetic polymers flocculants and inorganic flocculants. They are low cost, easy to get, non-toxic and effective for certain wastewater treatment. So the use of natural polymer flocculant has been emphasized by some researchers. Microbial flocculants are defined using biological technology, through the biological fermentation, extraction and refine to get a kind of highly effective and non-toxic water treatment agent. Compared to microbial flocculants, natural polymer flocculants have certain advantages, such as less chance of mutating, more effective and low requirements of the environment.

At present the preparation methods of biological flocculant can be roughly divided into two kinds, one is chemical synthesis method; and other is biological synthesis method[4]. Chemical synthesis method is simple, low cost, with wide raw material source and easy to realize industrialization. Biological synthesis is slightly complicated and with high cost. Biological synthesis is now still in the laboratory preparation stage. Chemical synthesis methods mainly include the following parts: direct extraction[5], copolymerization[6], chemical cross-linking[7] and molecular modification[8].

Many studies showed that flocculating agent could be directly extracted from the raw material. For example, biological flocculant was extracted from seeds after drying, crushing, extraction and centrifugal steps[5]. Direct extraction method is simple, convenient and feasible, but directs extraction of natural high molecular compound performance is limited. Sometimes the chemical modification is needed to make its active group increase greatly. In the preparation of starch derivatives and cellulose derivatives as biological water treatment agents, the copolymerization is often used. The side chain group with flocculating ability can be grafted on the starch or cellulose polymer. The graft copolymerization method includes chemical trigger graft copolymerization (catalyst and catalysis), radiation method and mechanical methods. Crosslinking effect refers to the formation of chemical bond between molecules. Crosslinking reaction can combine molecules and provide more active group. Molecular modification method includes etherification, methylation, alkylation, acid esterification and phosphate esterification by introducing various functional groups to improve its physicochemical properties and enlarge its application scope.

Chitosan is prepared by chitin, with the removal of acetyl [9,10]. Because these kinds of materials are molecules containing amide group and hydroxyl group, so have the flocculation, adsorption and other functions. In particular the modification of chitosan has attracted great attention in the world. The artificial modification of chitosan can act through the character of chelator. The linear molecular chain of the chitosan contains a number of hydroxy (-OH) and amino ($-NH_2$) which are equatorial bonds seen from conformation. This particular structure provides a certain radius for the chelation of some metal ions in a certain pH. And after the chelation of chitosan and the metal ions the structure itself has not changed, which ensures chitosan can exert the flocculation characteristics of straight-chain organic macromolecules. As the involvement of metal ions, the flocculant reticular formation and the ability of pollutants capture can be increased. Therefore, through the modification, chitosan has the potential to enhance the solubility, stability and performance of flocculation.

In this research, after the new modified chitosan flocculant was prepared, the structure model of the modified chitosan flocculant was discussed. And the synthetic flocculant was used in the simulated wastewater treatment. The simulated wastewater was kaolin solution to measure the adsorptive property of different flocculant.

Ethanol manufacture from different raw materials generates large volumes of high strength wastewater that is of serious environmental concern[11,12]. These wastes are with high concentration, high temperature, little pH and big viscosity. So the prepared flocculants were used for the treatment of ethanol wastewater.

In order to expand the biological flocculation agent application scope, the new modified chitosan flocculant was used in the detoxification of the acid hydrolyzate of lignocelluloses materials for ethanol production. And the detoxification rate was represented by the concetraion change of acid-soluble lignin in the solution.

EXPERIMENTAL

The distillery wastewater, liquid saccharifying enzyme, *Saccharomyces cerevisiae* and corn residue, obtained from a local grain distillery company, were used in this investigation. The *S. cerevisiae* was stored in the tube culture at 4 °C. It was inoculated into the liquid culture medium after activation. All chemicals of analytical reagent grade were purchased from Beijing Chemical Factory (Beijing, China). Chitosan was bought from Beijing Biological Technology Factory (Beijing, China).

Preparation of bio-flocculants: Chitosan flocculant (CTS) was prepared by adding chitosan to 1 % acetic acid solution, forming chitosan solution (10 mg/mL), as a backup solution of chitosan flocculant. Ferric chloride polymerization (PFC) solution was produced by diluting $FeCl_3$ solution (20 mL 0.5 mol/L) to 400 mL,and then dropping NaOH solution(100 mL 0.25 mol/L) to it under the condition of rapid agitation, forming ferric chloride polymerization (PFC) solution as a backup solution of PFC flocculant, the iron concentration of which was 1.1 mg/mL. Chitosan and ferric chloride polymer flocculant compound (PFC-CTS) was synthesized by taking a certain amount of the CTS solution and then mixing with the PFC solution evenly according to a predetermined CTS to PFC ratio, finally aging 1 h for the reserve. Iron- chitosan flocculant (Fe-CTS) was made by adding a certain amount of $FeCl_3$ to the chitosan solution and then dropping dilute NaOH solution in case of slow agitation after the solid completely dissolving, forming the solution with a certain pH value.

Simulated wastewater treatment: The simulated wastewater was 3 % kaolin simulation water sample. The adequate amount of bioflocculant was mixed with the simulation water sample at the rotate speed of 120 r/min and rotate time of 60s. Then the rotate speed was changed to 30 r/min and the rotate time was 15 min. Finally the solution was settled for 10 min and the optical density of the supernatant fluid was measured in the spectrophotometer. The flocculation performance of flocculants can be indicated by the sedimentation rate.

The sedimentation rate (SR) can be calculated by the following equation:

$$SR = \frac{V - V_0}{V_0} \times 100\ \% \quad (1)$$

where V is the optical density value of the supernatant fluid and V_0 is the optical density value of initial wastewater (mg/L).

Ethanol wastewater treatment: The corn residue (30 g) was weighted and mixed with 125 mL water (tap water or treated wastewater). Then the mixture was heat-treated (70 °C, 0.5 h), boiled (125 °C, 1 h) and saccharified (60 °C, 60 min). Prior to fermentation, the pH was adjusted to 4.5 with dilute sulfuric acid. The fermented condition was: culture temperature 30 °C, rotation speed 100 rpm, inoculation volume 12 % and fermentation time 90 h.

During the distillation process after the good fermentation, distillery wastewater was generated at the bottom of distillation tower. The vinasses were passed through a filter screen (50 meshes) to remove coarse solids and then treated by NaOH to control the pH of the solution (pH = 8). Then the flocculant (100 ppm) was added to the solution The wastewater supplemented with different flocculants was settling for 10 min then filtered. The COD, carbohydrates, aldehydes, inorganic ions (such as iron, magnesium and calcium), water-soluble proteins, as well as the suspended substance (including insoluble proteins) of the treated wastewater were measured.

Detoxification of acid hydrolyzate: The hydrolyzate used in the experiments was produced from waste corn straw. First of all, corn straw was cut into 1 cm × 2 cm size and then treated by pulverizer to get cotton-like form. Afterward, the raw materials were mixed with 78.5 % sulfuric acid at the solid-liquid ratio of 1:2, temperature of 45-50 °C, the reaction time of 15 min. And then the mixture was diluted to form 4.7 % sulfuric acid solution. This reaction lasted for 2 h at boiling condition. Finally, the precipitate was removed and the acid hydrolyzates were formed. The filtrate temperature was 65 °C.The acid hydrolyzate was boiled for 15 min to remove the volatile compounds and then the lost water was complemented.

A certain amount of flocculant was added into the conical flask containing with the acid hydrolyzates which was treated by NaOH to get a suitable pH and then the flask was placed in the water bath (the stirring speed of 100 rpm and the stirring time of 10-30 min). After the reaction, the solution was standing for 25 min. Finally the solution was filtered by filter paper or filter cloth (pore size = 0.12 mm). The concentration of acid-soluble lignin in the solution was measured and the detoxification rate (DR) was calculated as

$$DR\ \% = \frac{C_1 - C_2}{C_1} \times 100\ \% \tag{2}$$

in which DR % was the detoxification rate, C_1 was the concentration of acid-soluble lignin in the acid hydrolyzates before detoxification and C_2 was the concentration of acid-soluble lignin after detoxification.

During the detoxification process, a small amount of reducing sugar was absorbed. The loss rate of reducing sugar was calculated as

$$LR\% = \frac{S_1 - S_2}{S_1} \times 100\ \% \tag{3}$$

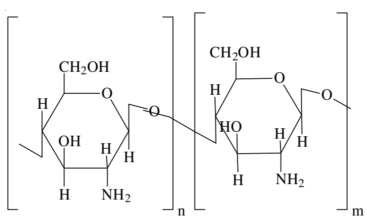
in which LR % was the loss rate, S_1 was the concentration of reducing sugar in the acid hydrolyzates before detoxification and S_2 was the concentration of reducing sugar after detoxification.

Analysis method: The reducing sugars were analyzed by the DNS method[13]. Water-soluble protein content in different batches of distillery wastewater was measured by Biuret method[14]. A calibration curve was made with pure protein at different concentrations and the absorbance of the samples was read at 540 nm. The concentration of Ca^{2+}, Mg^{2+} and Fe^{3+} was measured by the Hana C200 Water Analyzer (Italy) throughout the studies. Total aldehyde (as acetaldehyde) was determined chemically using iodimetry[15]. The total acid content was measured by titration method with sodium hydroxide solution. Phenol red was used as indicator or automatic potentiometric titration was used and the end point of pH was 8. The pH was measured in a pH meter (PHS-3B, Shanghai Precision & Scientific Instrument Co. Ltd, China). Measurements of COD and SS (suspended solids) were performed according to the standard methods[16] (APHA, 1998). The acid-soluble lignin was measured by ultraviolet absorption[17].

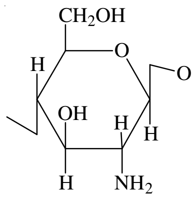
RESULTS AND DISCUSSION

Preparation of new bio-flocculant Fe-CTS: Amino and hydroxy groups are on the sugar residue of chitosan. These are the equatorial bonds, so certain ionic radius of metal ion can be combined on the chitosan molucle to form chelated structure. Fe^{3+} ions in the formation of chitosan-iron compound, did not change the original chitosan molecular structure, with five or six group, to form the reticular structure. The reaction between Fe^{3+} ions and chitosan was complex. The specific reactions were analyzed.

The molecular structure of chitosan is:



And M was the structure of sugar residue of chitosan:



The probable reactions were as follows:

A reaction: $M + FeCl_3 + H_2O \rightarrow [Fe(H_2O)M]Cl_3.H_2O$

M provided a amino group and a oxygen-containing group and the water molecules provided an oxygen-containing group, finally the remainder was constituted by chloride ion.

B reaction: $M + FeCl_3 + 4H_2O \rightarrow [Fe(H_2O)_3MCl]Cl_2.H_2O$

M provided a amino and a oxygen group and the other three oxygen groups were provided by water molecules, finally the remainder was constituted by chloride ion.

C reaction: $2M + FeCl_3 + 2H_2O \rightarrow [Fe(H_2O)(M)_2Cl]Cl_2.H_2O$

There was only one oxygen-containing group provided by water molecule and other two was provided by the two sugar residues involved in the reaction and then the substance was combined with a chloride ion.

D reaction: $3M + FeCl_3 + 2H_2O \rightarrow [Fe(H_2O)(M)_3]Cl_3.H_2O$

Four oxygen-containing groups were combined, wherein the water molecules provided one, so that the other three grounds and two amino groups were provided by sugar residues.

E reaction: $3M + FeCl_3 + H_2O \rightarrow [Fe(M)_3]Cl_3.H_2O$ (5)

Three sugar residues were combined, while providing amino-containing groups and oxygen-containing groups.

The molecular structure of these five kind reactions was show in Fig. 1.

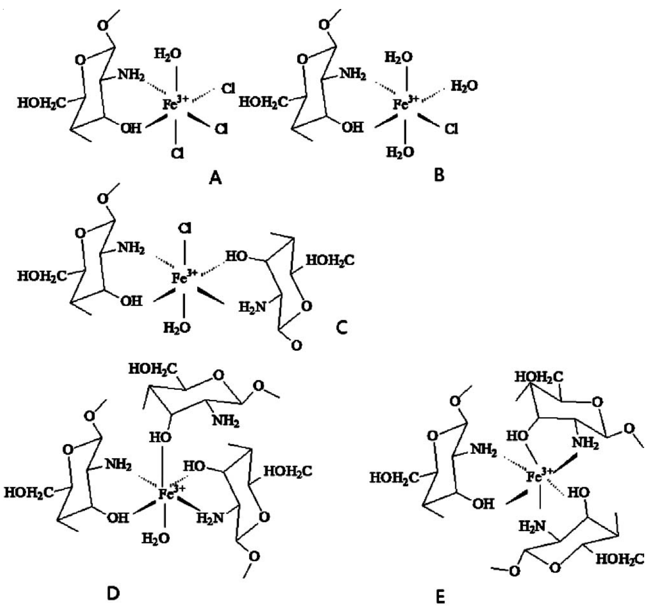


Fig. 1. Molecular structure of these five kind reactions

The effect of Fe and CTS mass ratio on the property of the Fe-CTS polymer as flocculant was measured. Five kinds of different mass ratio of Fe-CTS flocculants were added to the simulated wastewater and the sedimentation rate was shown in Fig 2. The dosage of CTS was 5 ppm in water samples.

As shown in Fig. 2, as the proportion of Fe to CTS was different and the dosage of CTS was in the same dosage, water treatment effects were changed. With the decrease of contents of Fe, the sedimentation rate decreased significantly. When the iron ratio of CTS: Fe was 2:1, the best effect was obtained. Without adding alkali to adjust the pH of water samples, good effect can be achieved.

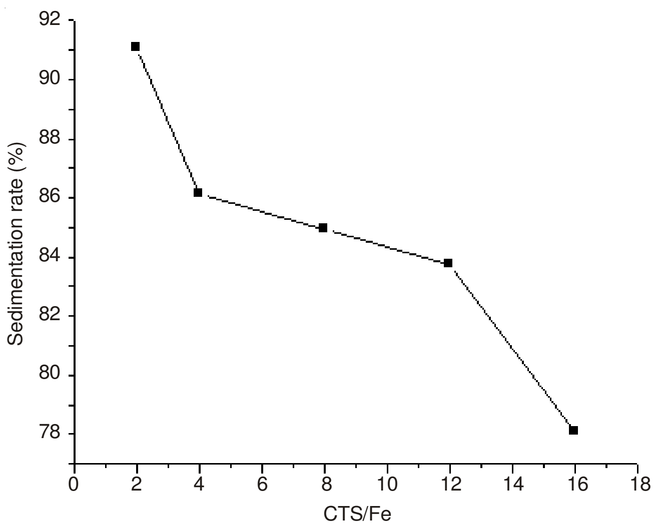


Fig. 2. Effect of Fe and CTS mass ratio on the property of the Fe-CTS polymer

At the best Fe and CTS mass ratio, the Fe-CTS structure had good flocculation settling effect and good enmeshment function on pollutants, which formed a huge reticular structure. So the molecular structure was most likely for D and E shown in Fig. 1.

Simulated wastewater treatment by bioflocculant: Chitosan is always with small charge density and low molecular weight. These shortcomings restrict its widespread use. In order to improve the chitosan adaptation scope, through the reaction with metal ions, the performance of composite flocculant was modified, without changing its linear macromolecular structure, as a result of the metal ion intervention, thereby enhancing contamination enmeshment ability of flocculating agent; at the same time to improve electric neutralization and increase flocculant molecular weight (easy to precipitate).

In order to determine the flocculation performance after chitosan molecule modification, three kinds of bioflocculant CTS, Fe-CTS and PFC-CTS were added to the simulated wastewater. Through several experiments, the best conditions for different flocculants were determined firstly. The optimal dosage of CTS was 30 ppm under the pH value of 9. As the increase of the alkali in the wastewater, the treatment effect was better. The electronegativity of the suspended particles was increased at a relatively high pH. So the electrostatic force between CTS and suspended particles was strengthened to increase the flocculation effect. But if the pH value was too large, the solubility of the CTS in the water can be reduced. The optimal dosage of PFC-CTS was 30 ppm under the pH value of 9. The optimal ratio of CTS to PFC was 2:3. This complex-flocculant effected better than CTS. As shown in Fig. 3, in order to get the more intuitive characterization of the best three flocculant applicable conditions, Fe-CTS, the PFC-CTS and Fe-CTS in the best pH value and the best proportion dealt with the water samples. Fe-CTS showed the best flocculant effect.

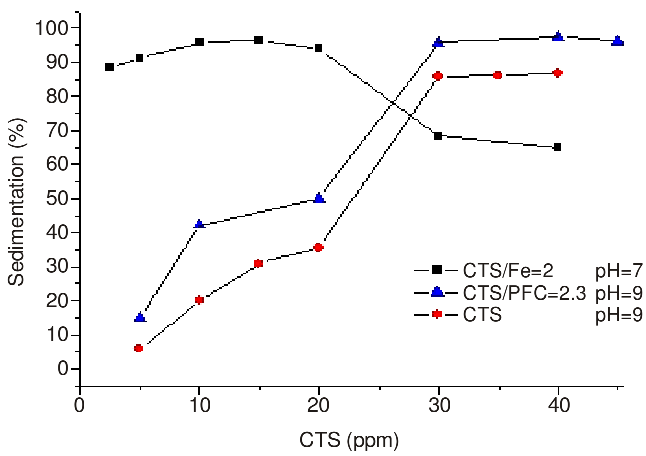


Fig. 3. Sedimentation rate of wastewater with the treatment of different flocculants

The best dosage of Fe-CTS flocculant was determined through experiments. The consumption of the flocculant was minimal. That is to say the modification is useful and appropriate. The optimal ratio of CTS to Fe was 2:1 under the neutral condition. The best treatment effect can be achieved under these conditions. Chitosan modified *via* the inorganic embed organic mode can enhance the characteristics of the cationic flocculant capture and bridging capability, thereby enhancing the ability of the flocculation flocculant.

Ethanol wastewater treatment: Production of bio-ethanol will help to cope with the over consumption of fossil fuels and further work for the reduction of carbon dioxide emissions. However, ethanol manufacture from different raw materials generates large volumes of high strength wastewater that is of serious environmental concern. Several methods have been proposed for the treatment of distillery wastewater such as anaerobic fermentation[18,19] membrane filtration process[20], DDGS[21], *etc*. In our research published in 2009[22], a new clean technology was applied in the ethanol production industry to treat the distillery wastewater and through introducing a bioflocculation process, the distillery waste could be recycled to the fermentation step. The average ethanol production yield was similar to that in the conventional process using tap water. In this research, the new prepared flocculant was used for the treatment of ethanol wastewater and the result was compared to the former research. The value of COD, carbohydrates, aldehydes, inorganic ions (such as iron, magnesium and calcium), water-soluble proteins, as well as the suspended substance (including insoluble proteins) in the wastewater before treatment and after treatment was measured and the result was shown in Table-1.

As shown in Table-1, the concentration of different substances in the wastewater after treatment was changed. Hazardous Materials influencing distillery wastewater recycles on fermentation were also considered in the research published in 2009. It was found that the contents of solid suspension,

TABLE-1
VARIATION OF COD, CARBOHYDRATES, ALDEHYDES, REDUCING SUGARS, INORGANIC IONS (SUCH AS IRON, MAGNESIUM AND CALCIUM), WATER-SOLUBLE PROTEINS, AND SS IN THE WATER

Water	Concentration							
	SS (mg/L)	Total aldehyde (as acetaldehyde (mg/L)	Reducing sugar (mg/L)	Fe^{2-} (mg/L)	Ca^{2-} (mg/L)	Mg^{2-} (mg/L)	Water soluble protein (g/L)	COD (g/L)
Wastewater	3875 ± 82 (4)	20.0 ± 1.0 (4)	1754 ± 21 (4)	0.53 ± 0.01 (4)	128.3 ± 10.2 (4)	48.6 ± 3.5 (4)	1.8 ± 0.1 (4)	30.8 ± 1.7 (4)
PAC	41.1 ± 2.8 (4)	20.0 ± 2.1 (4)	838 ± 20 (4)	0.50 ± 0.02 (4)	24.1 ± 2.5 (4)	29.4 ± 2.8 (4)	0.99 ± 0.02 (4)	20.0 ± 1.4 (4)
CTS	36.2 ± 2.4 (4)	20.1 ± 1.4 (4)	504.7 ± 18.4 (4)	0.3 ± 0.02 (4)	132.9 ± 25.7 (4)	27.5 ± 1.9 (4)	1.54 ± 0.07 (4)	24.0 ± 1.8 (4)
Fe-CTS	17.0 ± 1.2 (4)	20.2 ± 1.5 (4)	543.4 ± 17.6 (4)	0.51 ± 0.05 (4)	113.0 ± 22.4 (4)	15.8 ± 1.6 (4)	1.16 ± 0.04 (4)	20.1 ± 1.5 (4)
PFC-CTS	28.5 ± 2.5 (4)	20.4 ± 2.5 (4)	521.2 ± 26.2 (4)	0.55 ± 0.05 (4)	173.1 ± 35.4 (4)	28.2 ± 1.7 (4)	1.18 ± 0.05 (4)	22.0 ± 2.1 (4)

acid and Fe ions inhibited fermentation and resulted in a decreased ethanol yield[22]. The solid suspension gave a severe negative effect on the respiratory metabolism of yeast cell and ethanol fermentation. The solid suspension was greatly reduced by the flocculation and filtration processes. The solid suspension value was lowest under the wastewater treatment by Fe-CTS. In this research, the pH of the wastewater was controlled to 8 before the flocculation treatment to reduce the effect of acid on fermentation. And the concentration of Fe ion was not increased by Fe-CTS flocculation treatment. Because the stable structure of Fe-CTS molecule was formed and the precipitate after treatment was removed by filtration.

Through the contrast with the research in 2009, this new bio-flocculat Fe-CTS was shown to be an effective way to diminish the content of inhibitory compounds and could be used for the ethanol wastewater treatment.

Detoxification of acid hydrolyzate: A number of by-products are formed during the acid hydrolysis of lignocelluloses, such as furfural, acetate and aromatic substances, which inhibit the fermentation process[23,24]. The aromatic substances generated from lignin have a serious impact on the subsequent fermentation process and difficult to remove[25,26].

In this research, four kinds of flocculants were chosen for the detoxification process including PASP, PAC, CTS and Fe-CTS to remove the aromatic substances. All these flocculants belong to bio-flocculants except PAC. The detoxification process was carried out under the same condition (pH = 2.5; Temperature = 25 °C; Reaction time = 10 min) for contrast. As shown in Table-2, the species and dosage of flocculants had great effect on the detoxification rate and loss rate of sugars. With the increasing of flocculant concentration, the detoxification effect was strengthened for the opportunity of adsorption in bridge and electrical neutralization was improved. At the same time, the adsorption of reducing sugar was increased accordingly. Among the four kinds of selective flocculants, CTS showed the best detoxification rate and the smallest adsorption of reducing sugar (the minimum dosage). In general, the greater the flocculant dosages lead to the stronger absorption effect of reducing sugar. The common flocculant PAC did not show ideal effect. The dosage of PASP and PAC was large (4 g/L-5 g/L) to get an optimal detoxification rate and when the dosage was small, there was no significant adsorption effect, but the small dosage of CTS and Fe-CTS (0.25 g/L) was already resulting in better adsorption. CTS and Fe-CTS had stronger adsorption effect on the acid-soluble lignin. The possible reason was that there were a larger number of active groups for the adsorption in the CTS molecules, so the adsorption of acid-soluble lignin was better than PAC and PASP.

Conclusion

Bioflocculants are harmless to the environment and humans, indicating their potential to replace the existing chemical flocculants. The new bioflocculant Fe-CTS was prepared in order to enhance the solubility, stability and flocculation performance of CTS. After the new modified chitosan flocculant was prepared, the structure model of the modified chitosan flocculant was discussed. At the best CTS and Fe mass ratio of 2 , the Fe-CTS structure had good flocculation settling effect and good enmeshment function on pollutants, which formed a huge reticular structure. So the molecule structure was most likely for $[Fe(H_2O)(M)_3]Cl_3.H_2O$ and $[Fe(M)_3]Cl_3.H_2O$.

In order to determine the flocculation performance after chitosan molecule modification, three kinds of bioflocculant CTS, Fe-CTS and PFC-CTS were added to the simulated wastewater. Fe-CTS showed the best performance. With the

TABLE-2
DETOXIFICATION EFFECT WITH DIFFERENT FLOCCULANTS

PASP (g/L)	DR (%)	LR (%)	PAC (g/L)	DR (%)	LR (%)	CTS (g/L)	DR (%)	LR (%)	Fe-CTS (g/L)	DR (%)	LR (%)
0.99	10	0	1.08	27.9	0	0.02	48.56	0	0.02	44.06	0
20.2	13.38	0	2.07	28.5	0	0.05	49.41	0	0.05	46.7	0
3	19.6	4.3	3.03	35.79	3.05	0.12	50.53	0.8	0.12	47	0
4.05	41.8	13.2	4.08	50.65	4.87	0.20	64.52	1.4	0.20	51.7	1.7
5.1	49.37	16.52	4.99	38.7	7.69	0.25	63.87	3.4	0.25	56.9	3.2
6.2	40.7	25.92	6.1	35.56	10.87	0.30	63.76	7.3	0.30	55.4	7.2

dosage of 10 ppm, the sedimentation rate reached 95 %. Without changing the linear macromolecular structure, as a result of the metal ion intervention, contamination enmeshment ability of flocculating agent was enhanced and at the same time electric neutralization and flocculant molecular weight (easy to precipitate) was increased.

The prepared flocculants were used for the ethanol wastewater treatment and this new bio-flocculant Fe-CTS was shown to be an effective way to diminish the content of inhibitory compounds and could be used for the ethanol wastewater treatment. In order to expand the biological flocculation agent application scope, the new modified chitosan flocculant was used in the detoxification of the acid hydrolyzate of lignocelluloses materials for ethanol production. The new bio-flocculant was proved to have stronger adsorption effect on the acid-soluble lignin.

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