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ORIGINAL PAPER

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Characteristics of a bioflocculant produced by *Bacillus mucilaginosus* and its use in starch wastewater treatment

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Abstract A bioflocculant, MBFA9, was produced from a strain of bioflocculant-producing bacteria isolated from a soil sample and identified as *Bacillus mucilaginosus*. MBFA9 had a good flocculating capability and could achieve a flocculating rate of 99.6% for kaolin suspension at a dosage of only 0.1 ml/l. The major component of MBFA9 was found to be polysaccharide composed mainly of uronic acid (19.1%), neutral sugar (47.4%) and amino sugar (2.7%). Infrared spectrum analysis showed the presence of carboxyl and hydroxyl groups in the bioflocculant. MBFA9 is nontoxic and can be used in food industries for suspended solids (SS) recovery. When applied to starch wastewater treatment, MBFA9 greatly accelerated the formation of flocs and the settling of organic particles in the presence of Ca^{2+} salt. After 5 min of settling, the removal rate of SS and chemical oxygen demand were up to 85.5% and 68.5%, respectively, which is better than traditional chemical flocculants.

Introduction

Bioflocculants (microbial flocculants) are polymers produced by microorganisms during their growth. In recent years, the study of bioflocculants has attracted wide attention. Many microorganisms, such as *Rhodococcus erythropolis* (Takeda et al. 1991; Kurane et al. 1994), *Paecilomyces* (Hiroaki and Kiyoshi 1985), *Klebsiella pneumoniae* (Nakata and Kurane 1999), *Citrobacter* (Ike et al. 2000), have been found to produce bioflocculants. However, low flocculating capability and large dosage requirement have been a major problem in bioflocculant development for actual wastewater treatment.

In wastewater treatment, flocculation is an easy and effective method of removing suspended solids (SS). Many chemical flocculants, including aluminum sulfate, ferric chloride, and polyacrylamide (PAM), have been widely used, although there are concerns about the toxicity of these chemicals for recovering organics, especially in the food and fermentation industries. Since bioflocculants can be nontoxic, harmless and without secondary pollution, they have a great potential for use in those industries. Oh Hee-Mock reported that a bioflocculant successfully harvested *Chlorella vulgaris* from culture broth (Oh et al. 2001).

Starch wastewater is one of the most common wastewaters in the food industry. During the production of starch, starch wastewater is produced in the process of bran-starch separation of corn, with lots of fine suspended bran particles and almost all corn proteins retained in the wastewater. Recovering SS not only decreases the amount of pollutants for discharge, but also increases the income for the factories as the recovered solids can be used as feed additives for animals. In most starch factories, settling separation is commonly used to recover SS, but the settling time is often very long and the separation efficiency is normally low. Therefore, flocculants are usually used to accelerate or improve the settling of SS in starch wastewater. Because most conventional flocculants have some adverse effects on animals and the environment, their application in this field is not desirable. In this paper, we report the development of the bioflocculant MBFA9 and its application in starch wastewater treatment.

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Materials and methods

Screening and culturing of bioflocculant-producing bacteria

Many types of bacteria were screened from wastewater, activated sludge, and soil samples. The composition of the medium for screening was as follows: 10 g glucose, 2 g KH_2PO_4, 5 g K_2HPO_4,

0.2 g $MgSO_4 \cdot 7H_2O$, 0.1 g NaCl, 0.5 g carbamide, and 0.5 g yeast extract dissolved in 1 l deionized water with the initial pH adjusted to 7.0. After sterilization and inoculation of the medium, the bacterium was cultured on a rotary shaker at 150 rpm and 30°C for 3 days. Kaolin suspensions at a concentration of 5,000 mg/l were then used to evaluate the flocculating capability of a series of the culture broths. From these results, a bacterium with good flocculating capability was selected and cultivated in 100 ml medium in a 250 ml flask on a shaker at 150 rpm and 30°C for 84 h. Medium samples were taken at appropriate time intervals to determine the pH, OD_{660} (optical density at 660 nm), viscosity and flocculation properties. The OD_{660} was determined with a spectrophotometer, and the apparent viscosity was measured with a CS-Rheometer (RS100; Haake, Karlsruhe, Germany) with a DG40 sensor at a shear rate of 10 s^{-1}.

Determination of the flocculating activity

Flocculating rate was used as a measurement of the flocculating activity of the bioflocculants. A 0.5 g amount of kaolin clay (average diameter 4 μm) was suspended in 100 ml deionized water, and 0.01 ml of the bioflocculant (culture broth) was added to the kaolin suspension. The mixture was stirred at 60 rpm for 30 s with a vortex mixer and then kept still for 5 min. The absorbance of the supernatant and the blank control without bioflocculant was measured at 550 nm (as OD_{550} and OD_{blank}, respectively) with a spectrophotometer. The flocculating rate was defined and calculated as follows:

$$\text{Flocculating rate } (\%) = (OD_{blank} - OD_{550})/OD_{blank} \times 100 \qquad (1)$$

Bioflocculant purification

In order to purify the bioflocculant, the viscous culture broth was diluted with nine volumes of distilled water and then centrifuged to remove cells at 20,000 *g* for 10 min to concentrate the cultures. Two volumes of cold ethanol were added to the cold culture broth. The precipitate obtained was redissolved in deionized water and 2% cetylpyridinium chloride solution was added with stirring. After several hours, the precipitate was collected and dissolved in a 0.5 N NaCl solution. Two volumes of cold ethanol were then added to obtain the precipitate, which was then washed with ethanol. The crude bioflocculant was dialyzed at 4°C overnight against deionized water and the precipitate was vacuum-dried. About 700 mg pure bioflocculant was obtained from 1 l culture broth.

Bioflocculant composition analysis

The total sugar content of the bioflocculant was determined by the phenol-sulfuric acid method using glucose as standard solution (Chaplin and Kennedy 1994). The protein content was measured by the Lowry-Folin method. The contents of neutral sugar, uronic acid, and amino sugar in the bioflocculant were determined after hydrolysis with sulfuric acid. The methods of phenol-sulfuric and carbazol-sulfuric acid were used to determine the contents of uronic acid and neutral sugar, respectively, and amino sugar was measured by the Elson-Morgan method (Chaplin and Kennedy 1994). The average molecular weight of the bioflocculant was determined by a capillary viscosity meter (0.5 mm) at 30°C.

Bioflocculant toxicity test

Sixty white mice (20±2 g) were obtained at 1 month of age from China Medical University. The mice were divided into two groups randomly without consideration of male and female. The mice in one group were fed with the bioflocculant in food, and the mice in the other group were fed with food only. The animals were kept in a room with a temperature maintained at 22°C, and a relative humidity of 55%. The bioflocculant was dissolved in water and mixed with the food, and a dosage of 1 g bioflocculant (kg animal)$^{-1}$ day^{-1} was used. The mice were raised for 15 days and their posture, bite and sup, movement, and weight were monitored.

Starch wastewater flocculation test

The starch wastewater was taken from the overflow of the settling pond at Nanta Starch Company (Shenyang, China). There were some fine organic solids in the wastewater (mainly starch and protein) that could be used as high-grade feed additive for animals. The SS and chemical oxygen demand (COD) of the starch wastewater were 2,145 mg/l and 6,222 mg/l, respectively. A sample of 475 ml starch wastewater and 25 ml of 1% $CaCl_2$ solution were mixed in a 1,000 ml beaker, and the pH was adjusted with HCl or NaOH as necessary. The flocculant was then added to the wastewater, and the contents of the beaker were mixed using a blender at 200 rpm for 1 min, and then at 60 rpm for another 5 min. The wastewater was allowed to settle for 5 min and the supernatant was taken for analysis.

Results

Identification of MBFA9-producing bacterium

About 50 types of bioflocculant-producing bacteria were screened from soil samples on the basis of a flocculating rate for kaolin suspension of over 50%. The most effective bacterium, named A-9, had a flocculating rate exceeding 90%. The bacterium was rod-shaped with a flagellum, had a size of around 4.16–4.83×0.5–0.83 μm, and was determined to be a Gram-positive, aerobic, thick-capsule-producing strain, with an optimum growth temperature in the range of 25–30°C. The bacterium A-9 could grow on potassium silicate and Ashby's N-free medium, and could hydrolyze starch, but not casein. When A-9 was incubated on potato dextrose agar medium, some oval spores were produced. Colonies of A-9 were colorless, transparent, viscous, smooth and elastic when cultivated on solid substrate containing potassium silicate. When A-9 was incubated on Ashby's N-free medium, the colonies were smooth and high. For comparison, the known strain *Bacillus mucilaginosus* was also cultivated on the above media, and the characteristics of the colonies of the two strains were similar. Based on the references Malinovskaya et al. (1990) and Podgorskii et al. (1991), strain A-9 was identified as *B. mucilaginosus* and the bioflocculant produced was named MBFA9.

Bioflocculant-producing properties

The effects of carbon and nitrogen sources and initial pH on bioflocculant-producing properties of strain A-9 were investigated, and the optimum composition of the medium for bioflocculant production was found to be the following: 1.5 g soluble starch, 0.6 g K_2HPO_4, 0.3 g yeast extract, 0.02 g $MgSO_4 \cdot 7H_2O$, 0.01g NaCl in 1 l deionized water. The initial pH value of the medium was adjusted to

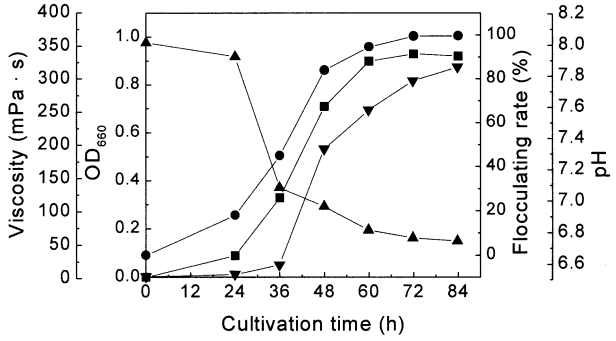

Fig. 1 Time course of the growth (OD_{660}, *squares*), flocculating rate (*circles*), pH (*triangles up*) and viscosity (*triangles down*) of culture broth of strain A-9 on a rotary shaker at 150 rpm, 30°C for 84 h

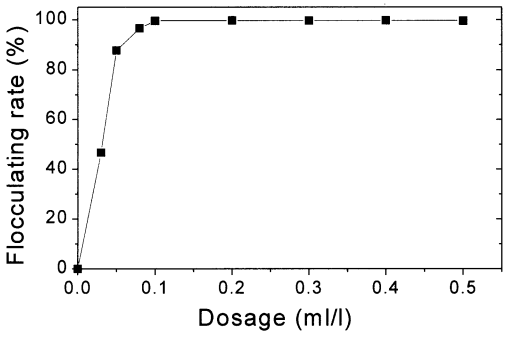

Fig. 2 Relationship between dosage of the bioflocculant MBFA9 and flocculating rate for 5 g/l kaolin suspension

8.0, and the bacterium was cultured at 30°C and shaken at 140 rpm in a rotary shaker for 72 h (Deng et al. 1999). The flocculating rate of the culture broth was 99.6% without the synergistic effects of Ca^{2+}, Al^{3+} etc., evaluated by flocculation of kaolin suspension (5,000 mg/l). Strain A-9 was found to be an effective flocculant-producing bacterium.

The growth curve of the strain, the flocculating rate, viscosity and pH variation of the culture broth are shown in Fig. 1. The flocculating rate curve is parallel to the growth curve and flocculating rates increase with increasing cultivation time, indicating that bioflocculant is produced by strain A-9 during its growth. This is supported by the fact that the flocculating rates increased rapidly during the logarithmic growth period (from 24 h to 60 h), reaching 94.7% at 60 h. The viscosity of the culture broth increased with increasing cultivation time, from 0.68 mPa s at the beginning to 316 mPa s at 84 h. The increase in viscosity of the culture broth was caused by high molecular weight polymer produced by strain A-9 during the growth period. The results in Fig. 1 also show that the pH of the culture broth decreased from 8.0 to 6.73 with the increase in cultivation time from 0 to 84 h, which suggests that some organic acids were produced and released into the medium by strain A-9 during growth.

Characteristics of bioflocculant MBFA9

5 ml of culture broth of the strain A-9 was heated in a test tube at 100°C in air for 30 min and the flocculating rate was investigated. The results showed that the flocculating rate was still almost 99%, suggesting that MBFA9 is a heat-stable bioflocculant.

MBFA9 was also found to be an effective flocculant with low dosage requirements. For kaolin suspension, the relationship between flocculating rate and the dosage of MBFA9 is shown in Fig. 2. It is apparent that the flocculating rate can reach 99.6% with a dosage as low as only 0.1 ml/l. In the literature (listed in Table 1), the dosage of culture broths used as bioflocculants for kaolin suspensions is usually in the range of about 1.0 to 150 ml/l, and often Ca^{2+}, Fe^{3+} or Al^{3+} are used as coagulants to achieve high flocculating rates.

Bioflocculant MBFA9 toxicity test

The results of toxicity tests showed bioflocculant MBFA9 to be nontoxic for white mice. When the white mice were fed at a dosage of 1 g pure MBFA9 (kg animal)$^{-1}$ day^{-1} for 15 days, they were seen to be normal in posture, bite and sup, and other activities, and no obvious weight differences between the mice in the two groups were found, which indicates that bioflocculant MBFA9 has no acute toxicity, at least for white mice.

Table 1 Dosage of different bioflocculants for flocculating kaolin suspension

Bioflocculant-producing bacterium	Optimum concentration (ml culture broth/l)	Flocculating rate (%)	Ions added	Reference
Alcaligenes sp.	20	90	Ca^{2+}	Wang et al. 1994
Rhodococcus erythropolis	5	5.6[a]	Ca^{2+}	Kurane et al. 1986
Alcaligenes latus	1	8.8[a]	Ca^{2+}	Kurane et al. 1991
Bacillus coagulants As101	40	90	Ca^{2+}, Fe^{3+}, Al^{3+}	Salehizadeh et al. 2000
Bacillus licheniformis	150	8.5[a]	Ca^{2+}, Fe^{3+}, Al^{3+}	Shih et al. 2001
Citrobacter sp.	100	98.4	No ions	Fujita et al. 2000
Klebsiella sp.	10	1.38[a]	Ca^{2+}	Dermlim et al. 1999
Streptomyces griseus	40	78	Ca^{2+}	Shimofuruya et al. 1996
Bacillus mucilaginosus	0.1	99.6	No ions	Present article

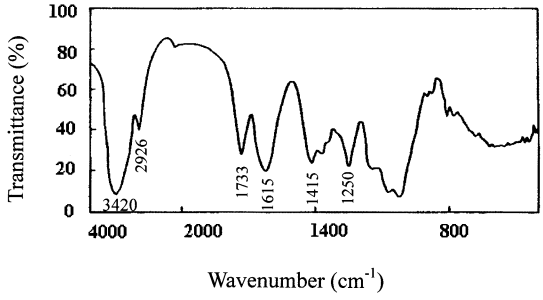

Fig. 3 Infrared spectrum of the purified flocculant MBFA9

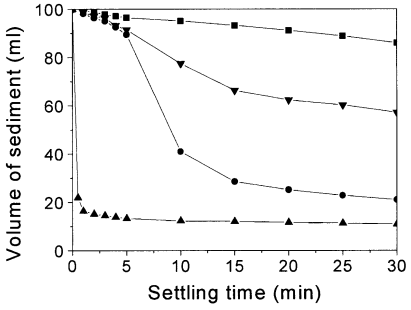

Fig. 4 Settling curves of starch wastewater. *Circles* 0.5 g/l $CaCl_2$, *triangles up* 0.5 g/l $CaCl_2$ and 0.2 ml/l MBFA9, *triangles down* 0.2 ml/l MBFA9, *squares* no chemicals

Composition of bioflocculant MBFA9

From the study of the components of bioflocculant MBFA9, total sugar content was found to be 93% (w/w) and no protein was detected, indicating that the bioflocculant was mainly polysaccharide. Since polysaccharides can consist of many saccharides including neutral sugar, uronic acid and amino sugar, pure bioflocculant MBFA9 was hydrolyzed with sulfuric acid to determine the content of different sugars. The analyses showed that the contents of neutral sugar, uronic acid and amino sugar were 47.4%, 19.1% and 2.7%, respectively. The average molecular weight of the bioflocculant was estimated to be 2.6×10^6 by the method of viscosity.

The pure bioflocculant was analyzed by infrared spectrophotometry, and the results are shown in Fig. 3. The spectrum displays clear absorption peaks at 3,420, 2,926, 1,733, 1,615, 1,415 and 1,250 cm^{-1} wavenumbers. The broad O-H stretching absorption band can be observed at 3,420 cm^{-1} and a weak C-H stretching vibration band at 2,926 cm^{-1}. The peak at 1,733 cm^{-1} is characteristic of the C=O stretching vibration in COOH, and the bands at 1,615 cm^{-1} and 1,415 cm^{-1} may be assigned to the C=O antisymmetrical and symmetrical stretchings in the carboxylate, respectively (Dermlim et al. 1999), indicating the presence of carboxyl group in bioflocculant MBFA9.The sorption peak at 1,250 cm^{-1} is an indication of the C-O stretching in ether or alcohol.

Starch wastewater treatment using bioflocculant MBFA9

MBFA9 could be used in drinking water processing and downstream processing in the food and fermentation industries because of its lack of toxicity. In this study, starch wastewater was used to evaluate the flocculating activity of the bioflocculant MBFA9.

The effects of $CaCl_2$ and bioflocculant MBFA9 on the settling properties of starch wastewater are shown in Fig. 4. The very slow rate of particle removal from starch wastewater without addition of any chemicals was attributed to the small size and light weight of the particles. After 30 min settling, the volume of solids remained in 86 ml of the total 100 ml wastewater. When $CaCl_2$ was added to the wastewater, many small flocs were produced and the settling velocities of the particles were increased. After 10 min settling, the volume of the sediment was only 41 ml. The volume was further reduced to 21 ml in the following 20 min. When $CaCl_2$ and MBFA9 were both added to the water, the flocs became bigger and denser and the settling velocity was much greater. The sediment volume reached 22 ml with 0.5 min settling, and became 11 ml after 30 min settling. However, the settling velocities of the particles were slow when MBFA9 was added alone, and the sediment volume was 57 ml after 30 min settling. Apparently, bioflocculant MBFA9 significantly improved the separation of SS from starch wastewater in the presence of $CaCl_2$.

Table 2 Experimental results of starch wastewater treated with different flocculants after 5 min settling. *SS* Suspended solids, *COD* chemical oxygen demand, *PAC* poly aluminium chloride, *PAM* nonionic polyacrylamide, *HPAM* anionic polyacrylamide

Flocculant	Dosage (mg/l)	pH	SS (mg/l)	COD (mg/l)
Blank	–	4.3	2,145	6,222
PAC	100	7.0	2,090	6,196
PAM	10	9.0	2,100	6,270
HPAM	10	9.0	520	2,330
MBFA9	0.2 ml/l[a]	9.0	310	1,962

[a] 0.2 ml culture broth (MBFA9)/l starch wastewater

Table 2 presents a comparison of experimental results obtained using bioflocculant MBFA9 and other conventional chemical flocculants, such as PAC (poly aluminium chloride), HPAM (anionic polyacrylamide, MW $=3.2\times10^6$) and PAM (nonionic polyacrylamide, MW $=5.8\times10^6$) for the treatment of starch wastewater in the presence of 0.5 g/l $CaCl_2$. The results show that PAC and PAM had little effect on separation, HPAM performed much better than PAC and PAM, but MBFA9 was the best. After settling, SS and COD of the wastewater treated with MBFA9 were 310 mg/l (removal rate 85.5%) and 1,962 mg/l (removal rate 68.5%), respectively. About 2 kg dry sediments were recovered from 1 tonne wastewater when bioflocculant MBFA9 was used.

Discussion

Most bioflocculants are produced by microorganisms during their growth periods (Kwon et al. 1996; Nakata and Kurane 1999; Shih et al. 2001). Bacteria can utilize the nutrients in the culture medium to synthesize high molecular weight polymers internally within the cell under the action of specific enzymes, and these polymers can be excreted and exist in the medium or on the surface of the bacteria as capsule. Therefore, the action of bacteria converts the simple substances in their environment into complex polymers that can be used as flocculant. From Fig. 1, it can be seen that flocculating rate, viscosity of the culture broth and growth (OD_{660}) of *B. mucilaginosus* increase with increasing cultivation time, indicating that the flocculant is produced by the bacterium during its growth. Secretion of bioflocculant MBFA9, which contains a COOH group (Fig. 3), into the culture medium was one of the reasons for the decrease in pH of the medium during cultivation. In our research, *B. mucilaginosus* made use of common starch and other nutrients and produced a high molecular weight (2.6×10^6) polysaccharide bioflocculant. Although starch can be modified into a flocculant through chemical reaction (Khalil and Aly 2001), the grafting degree is low and the flocculating capability of the product is usually unsatisfactory. Through the action of bacteria, starch can be easily changed into an effective polysaccharide bioflocculant. Therefore, bioflocculants may represent a new solution for the development of polysaccharide flocculants.

Different bacteria may produce different bioflocculants. For example, *R. erythropolis* S-1 (Takeda et al. 1991), *Bacillus licheniformis* (Shih et al. 2001), *Pacilomyces* sp. (Hiroaki and Kiyoshi 1985), and *Nocardia amarae* YK1 (Takeda et al. 1992) produce protein bioflocculants. *Alcaligenes latus* KT201 (Toeda and Kurane 1991), and *Bacillus subtilis* IFO3335 (Yokoi et al. 1996) produce polysaccharide bioflocculant, while *Arcuadendron* sp. TS-4 (Lee et al. 1995) and *Arathrobacter* sp. (Wang et al. 1995) produce glycoprotein bioflocculant. In our research, the major component of bioflocculant MBFA9 is polysaccharide, and it is heat-stable. In comparison, protein bioflocculants are usually not heat-stable as protein can be destroyed upon heating; for example, the flocculating capability of the protein bioflocculant NOC-1 produced by *R. erythropolis* decreased by 50% after 30 min heating at 100°C (Takeda et al. 1991). If the major component of a bioflocculant is a glycoprotein, its stability will depend on the relative contents of protein and polysaccharide.

As the dosage of bioflocculant MBFA9 for flocculating kaolin suspension, the molecular weight, and the concentration of MBFA9 in culture broth are 0.1 ml/l, 2.6×10^6 and 700 mg/l, respectively, the number of molecules used in 1 l kaolin suspension should be 2.7×10^{-11}. While the molecular weight and the concentration in the culture broth of most bioflocculants are less than 2×10^6 and 1 g/l, respectively (Hiroaki and Kiyoshi 1985; Dermlim et al.1999; Fujita et al. 2000; Shih et al. 2001), and the dosages of other bioflocculants for kaolin suspensions are much higher than that of MBFA9 (shown in Table 1), it can be calculated that the number of molecules of other bioflocculants in 1 l kaolin suspension is much higher than that of MBFA9. That is to say, the flocculating activity per molecule of MBFA9 is higher.

MBFA9 was found to be an effective bioflocculant to flocculate kaolin suspension and suspended organic solids in starch wastewater. The molecular weight and functional groups in the molecular chains are the important factors in the flocculating activity of bioflocculants. For protein bioflocculants, the amino and carboxyl groups are the effective groups for flocculation, but their molecular weights are usually low (Kurane et al. 1994). In contrast, polysaccharide bioflocculants have high molecular weights and many functional groups (Kurane et al. 1991). In fact, the components and structures of bioflocculants are complex, and different bioflocculants produced by different bacteria can have different properties. The flocculation of yeast cells with a bioflocculant produced by *Aspergillus sojae* has been suggested to occur via bridging between cells and bioflocculant (Nakamura et al. 1976). For the bioflocculant MBFA9 in our research, the high molecular weight (2.6×10^6) and appropriate content of uronic acid (19.1%) are beneficial for flocculation. The carboxyl groups present on the molecular chain make the chain stretched-out because of electrostatic repulsion and the stretched molecular chains provide more effective sites for particle attachment. In addition, the high molecular weight makes bridging between bioflocculant and discrete particles effective.

A major condition for flocculation is that the molecules of flocculant can adsorb onto the surface of particles. When MBFA9 is approaching particles in solution, an attractive force must overcome the electrostatic repulsion force. At first, the van der Waals force may be the attractive force, then OH, COOH, COO^- groups of the bioflocculant and H^+, OH^- groups on the surface of particles may form hydrogen bonds as the bioflocculant chains approach the surface of particles. As carboxyl groups are present in bioflocculant MBFA9, and Ca^{2+}, Al^{3+} are on the surface of the particles, chemical bonds may also be formed. During the flocculation of kaolin suspension, the flocculating rate was high without adding Ca^{2+}, but Ca^{2+} was needed when MBFA9 was used to flocculate organic particles from starch wastewater. This may be due to the fact that more negative electrical charges are present on the surface of the organic particles than on kaolin particles.

Bridging mechanisms occur after the particles have adsorbed onto the chains of bioflocculant. Many particles could adsorb to a long molecular chain, and the particles adsorbed on the chain could be adsorbed simultaneously by other flocculant chains, leading to the formation of three-dimensional flocs that are capable of settling fast; thus, the bioflocculant MBFA9 has a good flocculating capability.

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References

Chaplin MF, Kennedy JF (1994) Carbohydrate analysis, 2nd edn. Oxford University Press, New York

Deng SB, Hu XM, Luo Q (1999) Culture conditions and flocculation characteristics of an efficient bioflocculant (in Chinese). Dongbei Daxue Xuebao/J North-Eastern University 20:525–528

Dermlim W, Prasertsan P, Doelle H (1999) Screening and characterization of bioflocculant produced by isolated *Klebsiella* sp. Appl Microbiol Biotechnol 52:698–703

Fujita M, Ike M, Tachibana S, Kitada G, Kim SM, Inoue Z (2000) Characterization of a bioflocculant produced by *Citrobacter* sp. TKF04 from acetic and propionic acids. J Biosci Bioeng 89:40–46

Hiroaki T, Kiyoshi K (1985) Purification and chemical properties of a flocculant produced by *Paecilomyces*. Agric Biol Chem 49:3159–3164

Ike M, Tachibana S, Kitada G, Kim SM, Inoue Z (2000) Characterization of a bioflocculant produced by *Citrobacter* sp. TKF04 from acetic and propionic acids. J Biosci Bioeng 89:40–46

Khalil MI, Aly AA (2001) Preparation and evaluation of some cationic starch derivatives as flocculants. Starch/Staerke 53:84–89

Kurane R, Toeda K, Takeda K, Suzuki T (1986) Culture conditions for production of microbial flocculant by *Rhodococcus erythropolis*. Agric Biol Chem 50:2309–2313

Kurane R, Hatamochi K, Kakuno T, Kiyohara M, Kawaguchi K, Mizuno Y, Hirano M, Taniguchi Y (1991) Microbial flocculation of waste liquid and oil emulsion by a bioflocculant from *Alcaligenes latus*. Agric Biol Chem 55:1127–1129

Kurane R, Hatamochi K, Kakuno T, Kiyohara M, Kawaguchi K, Mizuno Y, Hirano M, Taniguchi Y (1994) Purification and characterization of liquid bioflocculant produced by *Rhodococcus erythropolis*. Biosci Biotechnol Biochem 58:1977–1982

Kwon GS, Moon SH, Hong SD, Lee HM, Kim HS, Oh HM, Yoon BD (1996) A novel flocculant biopolymer produced by *Pestalotiopsis* sp KCTC 8637P. Biotechnol Lett 18:1459–1464

Lee SH, Lee SO, Jang KL, Lee TH (1995) Microbial flocculant from *Arcuadendron* sp. TS-49, Biotechnol Lett 17:95–100

Malinovskaya IM, Kosenko LV, Votselko SK, Podgorskii VS (1990) Role of *Bacillus mucilaginosus* polysaccharide in degradation of silicate minerals. Microbiology 59:49–55

Nakamura J, Miyashiro S, Hirose Y (1976) Modes of flocculation of yeast cells with flocculant produced by *Aspergillus sojae* AJ7002. Agric Biol Chem 40:1565–1571

Nakata K, Kurane R (1999) Production of an extracellular polysaccharide bioflocculant by *Klebsiella pneumoniae*. Biosci Biotechnol Biochem 63:2064–2068

Oh HM, Lee SJ, Park MH, Kim HS, Kim HC, Yoon JH, Kwon GS, Yoon BD (2001) Harvesting of *Chlorella vulgaris* using a bioflocculant from *Paenibacillus* sp. AM 49. Biotechnol Lett 23:1229–1234

Podgorskii VS, Gromozova EN, Boldareva AI, Stepanyuk VV, Lysenko AM, Kanyuk NI, Berezkin OA (1991) Biological features of a strain of *Bacillus mucilaginosus*, isolated from soddy-podzolic soil in the Ukrainian-SSR. Microbiology 60:492–495

Salehizadeh H, Vossoughi M, Alemzadeh I (2000) Some investigations on bioflocculant producing bacteria. Biochem Eng J 5:39–44

Shih IL, Van YT, Yeh LC, Lin HG, Chang YN (2001) Production of a biopolymer flocculant from *Bacillus licheniformis* and its flocculation properties. Bioresour Technol 78:267–272

Shimofuruya H, Koide A, Shirota K, Tsuji T, Nakamura M, Suzuki J (1996) The production of flocculating substance(s) by *Streptomyces griseus*. Biosci Biotechnol Biochem 60:498–500

Takeda M, Kurane R, Koizumi J, Nakamura I (1991) A protein bioflocculant produced by *Rhodococcus erythropolis*. Agric Biol Chem 55:2663–2664

Takeda M, Koizumi J, Matsuoka H, Hikuma I (1992) Factors affecting the activity of a protein bioflocculant produced by *Nocardia amarae*. J Ferment Bioeng 74:408–409

Toeda K, Kurane R (1991) Microbial flocculant from *Alcaligenes cupidus* KT201. Agric Biol Chem 55:2793–2799

Wang Z, Wang KX, Xie YM (1994) Screening of flocculant-producing microorganisms and some characteristics of flocculants. Biotechnol Tech 8:831–836

Wang Z, Wang K, Xie Y (1995) Bioflocculant-producing microorganisms. Acta Microbiol Sin 35:121–129

Yokoi H, Arima T, Hayashi S, Takasaki Y (1996) Flocculation properties of poly(gamma-glutamic acid) produced by *Bacillus subtilis*. J Ferment Bioeng 82:84–87