Available online at www.sciencedirect.com
ScienceDirect
Bioresource Technology 99 (2008) 4825–4831
BIORESOURCE TECHNOLOGY

Microbial flocculation by *Bacillus mucilaginosus*: Applications and mechanisms

Bin Lian [a,*], Ye Chen [a,b], Jin Zhao [b], H. Henry Teng [d], Lijun Zhu [b], Sheng Yuan [c]

[a] *State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, Guiyang 550002, People's Republic of China*
[b] *Key Laboratory of Karst Environment and Geological Hazard Prevention (Guizhou University), Ministry of Education, Guiyang 550003, People's Republic of China*
[c] *Key Laboratory of Microbial Engineering, College of Life Sciences, Nanjing Normal University, Nanjing 210097, People's Republic of China*
[d] *Department of Chemistry, The George Washington University, Washington, DC 20052, USA*

Received 31 January 2007; received in revised form 16 September 2007; accepted 17 September 2007
Available online 29 October 2007

Abstract

Microbiological flocculation in wastewater by a strain of *Bacillus mucilaginosus* was examined and the flocculation mechanism was investigated using Coomassie brilliant blue reaction, Anthrone reaction, thin-layer chromatography (TLC), scanning electronic microscope, and infrared spectroscopy. The GY03 strain, isolated from farmland soil, was identified as *B. mucilaginosus* by its morphology and 16S rDNA sequence. Cultivated in a nitrogen-free medium, the flocculation material (bacterium–mineral complex) was used to treat domestic, brewage, and pharmaceutical wastewater. On the basis of one-way experimental results, orthogonal experiments were carried out and the optimum processing conditions of the microbial flocculants were obtained. In the optimal conditions, the maximal removal ratio of COD in domestic, brewage, and pharmaceutical wastewater reached 74.6%, 70.5%, and 66.2%, respectively; the maximal removal ratio of BOD was 42.3%, 77.4%, and 41.7%, respectively; and the maximal removal of SS amounted 93.3%, 93.6%, and 88.4%, respectively. The treatment to kaolin suspended liquid by the GY03 strain may act as a model of studying microbial flocculation mechanisms in which bridging and charge neutralization hypothesis were proposed as the critical reasons based upon the experimental observations.

Keywords: Microbial flocculant; *Bacillus mucilaginosus*; Flocculation; Flocculation mechanism; Wastewater treatment

1. Introduction

Microbial flocculants (MBFs) are polymers produced by microorganisms during growth. In recent years, the study of MBFs has increasingly attracted wide attention. MBFs are mainly composed of macromolecular substances, such as glycoprotein, polysaccharide, protein, cellulose, and nucleic acid (Hejzlar and Chudoba, 1986; AI-Shahwani and AI-Rawi, 1989; Labille et al., 2005). Many microorganisms, such as *Rhodococcus erythropolis* (Kurane et al., 1994), *Paecilomyces* sp. (Hiroaki and Kiyoshi, 1985), *Klebsiella pneumonia*e (Nakata and Kurane, 1999), *Citrobacter* sp. (Fujita 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. Since bioflocculants are largely nontoxic and hence produce no secondary pollution, they have a great potential to industrial applications (Kurane et al., 1994; Lee et al., 1995; Yokoi et al., 1996; Kumar et al., 2004; Chen and Lian, 2005; Sheng et al., 2006; Raul and Benoit, 2006; Prasertsan et al., 2006; Joung et al., 2007).

* Corresponding author. Tel./fax: +86 0851 5895148.
E-mail address: bin2368@vip.163.com (B. Lian).

0960-8524/$ - see front matter
doi:10.1016/j.biortech.2007.09.045

Studying the flocculation mechanism could help us to understand the role of MBFs in wastewater treatment and to improve the actual treating effects. Numerous hypotheses, such as Butterfield's viscousness proposal (Butterfield, 1935), the cellulose behavior outside the cell (Friedman et al., 1969), "lectin-like" hypothesis propose (Taylor and Orton, 1978), the three-dimensional model between discrete cellular and stretched bridge (Nakaura et al., 1976), the alginate theory (Bruus et al., 1992), Derjaguin, Landau, Verwey, and Overbeek (DLVO) theory (Adamson, 1990), and divalent cation bridging (DCB) theory(McKinney, 1952; Tezuka, 1969), are available in the literatures to address the flocculation mechanisms of MBFs. Although these models have helped to understand the flocculation process and MBF has been used in actual wastewater treatment (Suh et al., 1997; Shih et al., 2001; He et al., 2004; Zouboulis et al., 2004; Deng et al., 2005; Lu et al., 2005; Li et al., 2006), it has been difficult to apply this technique directly because of the high cost, low consistency in quality of the treated wastewater, the lack of clear understanding in the flocculation mechanism, and the frequent degeneration of the microbial species. This study investigated the MBF produced by the strain of *Bacillus mucilaginosus* and its applications to the treatment of different types of wastewater. Based on the experimental results, we explored the flocculation mechanism of MBFs.

2. Methods

2.1. Bacterium acquisition and identification

A strain of bacterium, GY03, was isolated from soil samples collected from the maize-farming fields in the nearby suburbs of Guiyang City, Guizhou Province, China (Lian et al., 2004). Isolation and purification of the bacterium were conducted using a nitrogen free medium (sucrose 5.0 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $CaCO_3$ 0.1 g, Na_2HPO_4 2.0 g, $FeCl_3$ 0.005 g, illite powder 1.0 g, distilled water 1000 mL, pH 7.0–7.2), autoclaved for 20 min at 121 °C.

This bacterium was Gram negative and of bacilliform, measuring 0.8–1.2 μm × 3–9 μm in size, with thick capsules in the cell surroundings. Individual cells were seen to be linked by the capsule to form zoogloea. The capsule was generally as 2–5 times large in size as the bacterial bodies. The shape of spores was round or elliptic. No strict nutrient conditions were required for the growth of this bacterium since the organism can make full use of a variety of sugars. In addition, this species fixes nitrogen biologically, produces little acids, and is capable of releasing potassium and phosphorus from soil minerals (Malinovskaya et al., 1990; Lian, 1998).

The bacterial genomic DNA of GY03 was extracted using a commercially available extraction kit (Genomic DNA Purification Kit 713, Shenergy Biocolor Biotech Ltd., Shanghai). The PCR (polymerase chain reaction) amplification of the 16S rDNA was carried out using a forward (5′-AGAGTTTGATCCTGGTCAGAAC-3′) and a reverse primer (5′-TACGGCTACCTTGTTACGACTT-3′) by a thermal cycler (MJ Research PTC-100). The PCR conditions are: pre-denaturation, 94 °C for 5 min; denaturation, 95 °C for 1 min; annealing, 55 °C for 1 min; extension: 72 °C for 2 min except the last cycle that had 5 min. A total of 30 cycles were completed for the PCR amplification. The PCR products were purified and sequenced commercially. The resultant sequence was submitted (Accession Number: EU048557) to the GenBank database (http://www.ncbi.nlm.nih.gov) and analyzed following the method given by Altschul et al. (1997). The results showed a 99% similarity between GY03 and *B. mucilaginosus*. Combining the colony features, growth characteristics, cell morphology, as well as the 16SrDNA sequence, a positive identification in *B. mucilaginosus* was given to this strain.

2.2. Wastewater source and treatment procedure

2.2.1. Wastewater source

Three wastewater samples (domestic, brewage, and pharmaceutical wastewater) were collected from a university campus, a Brewery, and a Pharmaceutical Factory. The pH, COD_{cr} (chemical oxygen demand), BOD_5 (biochemical oxygen demand for five day), and SS (solid suspension) were determined using the methods given by the National Standard of People's Republic of China. The pH of the three types of wastewater was 7.2 mg L^{-1}, 7.8 mg L^{-1}, and 8.2 mg L^{-1}, respectively; the COD_{cr} was 193, 1479, and 1586 mg L^{-1}, the SS was 164, 281, and 327 mg L^{-1}; and the BOD_5 was 78, 761, and 278 mg L^{-1}.

2.2.2. Procedure of wastewater treatment experiments

It was determined from our previous studies (Lian et al., 2003, 2004; Chen and Lian, 2005) that the major influence factors on flocculation effect are pH of the wastewater, treating time, and the dosage ratio between wastewater and flocculent. Orthogonal experiment for the optimal treating process condition was thus designed by using a $L_{16}(4^5)$ orthogonal table based upon the results from these one-way experiments (Mason et al., 2003).

2.2.3. Extraction of flocculant material

The activated strain (approximately 2 mL) was inoculated into a 250-mL triangular flask containing 100 mL culture medium (described earlier) and was statically cultured for 6 days at 28 °C. The stable floccules were concentrated in the lower portion of the liquid culture (Lian et al., 2003) at the end of culture. The supernatant (approximately 85% of the total volume) was decanted and the remaining 15 mL concentrated floccus substance was used as the flocculation material (FM).

After collecting the crude FM, further purification was conducted in order to determine the physical and chemical characteristics of the bioflocculent. In the purification step, the FM was first centrifuged for 20 min at 5000 rpm, followed by adding three times as much absolute ethyl alco-

hol. The product was allowed to settle for overnight in refrigerator. The deposit was then collected and washed twice by absolute ethyl alcohol and dehydrated at 40 °C to yield the raw bioflocculants.

2.2.4. Determination of flocculation ratio in wastewater by GY03's MBFs

Flocculation ratio, F_{ratio} was used to represent flocculability quantitatively in the experiments. The ratio was computed by equation (Nakamura et al., 1976)

$$F_{ratio} = \frac{OD^1_{550} - OD^2_{550}}{OD^1_{550}} \times 100\%$$

where OD^1_{550} is the optical density of the blank control, and OD^2_{550} is the value after adding of FM. For kaolin experiments, 0.5 g kaolin of mineral (4 μm average particle size) was added in a 100 mL beaker, followed by the addition of 95 mL water at a steady stirring condition. Five milliliter of FM was then introduced. After 5 min of reaction, the supernatant portion was collected to determine its OD_{550} by a 751 spectrophotometer. The control was prepared by the similar procedure except that 5 mL of distilled water instead FM was used.

2.3. Abstraction and compositions analysis of exocellular polymers

Five thousand milliliters of culture liquid was concentrated to 1000 mL at 50 °C by revolving vacuum evaporator, and then was centrifugalized for 20 min at 5000 rpm. The supernatant liquid was subsequently separated and concentrated to 300 mL. The final product was mixed with 900 mL alcohol (95%) and stored in refrigerator for 12 h at 5 °C, followed by centrifuging at 5000 rpm for 20 min to obtain the precipitate. The raw material, about 8.52 g, was harvested, washed for by the Sevag Reagent (chloroform(4):pentanol(1)), and dried at 40 °C to yield 8.31 g exocellular polymer. Monosaccharide compositions analysis was performed using the method of thin-layer chromatography (TLC) (Kirchner, 1978).

2.4. Physical and chemical analysis of exocellular polymers

The total sugar was determined by the Anthrone reaction (Graham, 1963), proteins were measured by the Coomassie brilliant blue reaction (Blakesley and Boezi, 1977), proteins and nucleic acid were determined qualitatively by UV spectrophotometry (Harrington and Raper, 1968), and the constituent sugar was analyzed by infrared spectrophotometry.

The thermal stability of the FM was tested at room (23 °C) and elevated temperature in water-bath (30, 40, 50, 60, 70, 80, and 90 °C) for 30 min, respectively. Ten milliliters of FM heated at different temperatures was mixed with100 mL of kaolin (3‰) suspension. Flocculation ratios were determined at each temperature. The grain morphology in kaolin suspension was observed before-and-after the flocculation by transmission electron microscope (JEM-2000FXII, high-resolution, with the EM-ASID20 scanning accessories).

3. Results and discussion

3.1. Wastewater treatment

3.1.1. Domestic wastewater

Orthogonal experiments were carried out to determine the optimal conditions. The experimental results for domestic wastewater were shown in Table 1. The results of range analysis suggest that the flocculation ratio was influenced by the following factors in the descending order: system pH > the amount of FM used > treatment time. Thus, the optimal factor combination for flocculation ratio of wastewater from the result above is $A_1B_4C_1$: 6 mL of FM; system pH of 9, and a treatment time of 6 h.

Results of the treatment at the optimal condition of $A_1B_4C_1$ show a significant improvement in the water quality (Fig. 1). While the pH remained unchanged, the SS was decreased from 164 to 11 mg L^{-1}, and COD decreased from 193 to 49 mg L^{-1}, and BOD_5 decreased from 78 to 45 mg L^{-1}.

3.1.2. Brewage wastewater

The range analysis in the orthogonal experiments (Table 2) indicates that the flocculation ratio is influenced by the following factors in a descending order: pH > treatment time > volume ratio between wastewater and flocculent.

Table 1
The orthogonal experiment L_{16} (4^5) of domestic wastewater treatment

	A (mL)	B	C (h)	D	E	Flocculation ratio
1	1(6)	1(6)	1(6)	1	1	0.507
2	1	2(7)	2(8)	2	2	0.821
3	1	3(8)	3(10)	3	3	0.921
4	1	4(9)	4(12)	4	4	0.941
5	2(8)	1	1	3	4	0.460
6	2	2	2	4	3	0.853
7	2	3	3	1	2	0.909
8	2	4	4	2	1	0.936
9	3(10)	1	1	4	2	0.292
10	3	2	2	3	1	0.683
11	3	3	3	2	4	0.915
12	3	4	4	1	3	0.939
13	4(12)	1	1	2	3	0.314
14	4	2	2	1	4	0.727
15	4	3	3	4	1	0.932
16	4	4	4	3	2	0.919
I	0.798	0.393	0.798	0.771	0.764	
II	0.789	0.771	0.788	0.747	0.735	
III	0.707	0.913	0.719	0.746	0.757	
IV	0.723	0.934	0.712	0.754	0.761	
R	0.090	0.541	0.087	0.025	0.029	

Note: A, FM adding amount; B, pH; C, treatment time; D, blank control; E, blank control.

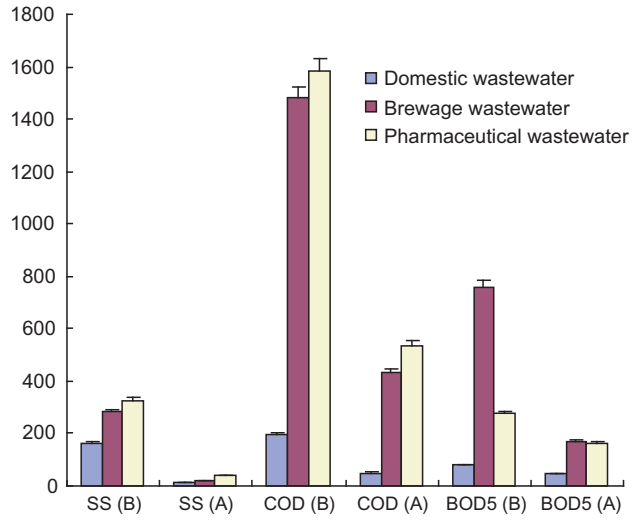

Fig. 1. The treatment results of domestic, brewage, and pharmaceutical wastewater (A): after flocculation (B): before flocculation.

Thus, the optimal factor combination for flocculation ratio of wastewater from the result above is $A_4B_4C_3$, pH of 8.5, a standing time of 8 h, and a volume ratio between wastewater and flocculant of 8:1.

Based on the orthogonal experiment result, the brewery waste was treated directly by the optimal factor combination $A_4B_4C_3$ and the results were shown in Fig. 1. The water quality after the treatment improved significantly. The SS decreased from 281 to 18 mg L^{-1}, the COD was decreased from 1479 to 436 mg L^{-1}, and BOD_5 decreased from 761 to 172 mg L^{-1}.

Table 2
The orthogonal experiment L_{16} (4^5) of brewery wastewater treatment

	A	B (h)	C	D	E	Flocculation ratio
1	1(7)	1(2)	1(4:1)	1	1	0.117
2	1	2(3)	2(6:1)	2	2	0.283
3	1	3(6)	3(8:1)	3	3	0.408
4	1	4(8)	4(10:1)	4	4	0.475
5	2(7.5)	1	1	3	4	0.233
6	2	2	2	4	3	0.125
7	2	3	3	1	2	0.050
8	2	4	4	2	1	0.575
9	3(8)	1	1	4	2	0.833
10	3	2	2	3	1	0.733
11	3	3	3	2	4	0.833
12	3	4	4	1	3	0.942
13	4(8.5)	1	1	2	3	0.867
14	4	2	2	1	4	0.892
15	4	3	3	4	1	0.942
16	4	4	4	3	2	0.975
I	0.321	0.513	0.513	0.500	0.592	
II	0.246	0.508	0.600	0.640	0.535	
III	0.836	0.558	0.677	0.587	0.586	
IV	0.919	0.742	0.531	0.594	0.608	
R	0.673	0.541	0.164	0.140	0.073	

Note: A, pH; B, treatment time; C, volume of wastewater/volume of flocculant; D, blank control; E, blank control.

3.1.3. Pharmaceutical wastewater

The range analysis (Table 3) from the orthogonal experiment indicates that the flocculation ratio is influenced by the following factors in a descending order: pH > volume ratio between wastewater and flocculant > treatment time > amount of zeolite added. Thus, the optimal factor combination for the best flocculation ratio of wastewater from the result above is $A_4B_3C_4D_2$, that is, pH 8.5, a standing time of 6 h, a volume ratio between wastewater and flocculant of 10:1, and a ratio between zeolite added and wastewater 1:1000.

Based on the orthogonal experiment result, the pharmaceutical waste was treated directly by the optimal factor combination $A_4B_3C_4D_2$ and the results Fig. 1) show SS decreased from 327 to 38 mg L^{-1}, COD from 1586 to 536 mg L^{-1}, and BOD_5 from 278 to 162 mg L^{-1}. The results demonstrated a significant improvement in SS and COD but not in BOD_5. This is probably because pharmaceutical waste tends to contain certain organic matter that may restrain the growth of the strain GY03 to retard biodegradation.

3.2. Mechanistic study of flocculation

3.2.1. Physical and chemical characteristics of flocculation materials

The extracted and dried raw flocculation material appeared as white powder. The extraction rate of the exocellular polymer ranged from 1.58 to 2.19 g per 1000 mL culture liquid due to differences between culture and

Table 3
The orthogonal experiment L_{16} (4^5) of pharmaceutical wastewater treatment

	A	B (h)	C	D	E	Flocculation ratio
1	1(7)	1(2)	1(4:1)	1(1:500)	1	0.748
2	1	2(3)	2(6:1)	2(1:1000)	2	0.809
3	1	3(6)	3(8:1)	3(1:1500)	3	0.814
4	1	4(8)	4(10:1)	4(1:2000)	4	0.877
5	2(7.5)	1	1	3	4	0.485
6	2	2	2	4	3	0.389
7	2	3	3	1	2	0.672
8	2	4	4	2	1	0.524
9	3(8)	1	1	4	2	0.832
10	3	2	2	3	1	0.804
11	3	3	3	2	4	0.843
12	3	4	4	1	3	0.737
13	4(8.5)	1	1	2	3	0.944
14	4	2	2	1	4	0.958
15	4	3	3	4	1	0.972
16	4	4	4	3	2	0.947
I	0.812	0.752	0.732	0.779	0.762	
II	0.518	0.740	0.751	0.780	0.815	
III	0.804	0.825	0.782	0.762	0.721	
IV	0.955	0.771	0.824	0.768	0.791	
R	0.438	0.085	0.092	0.018	0.094	

Note: A, pH; B, treatment time; C, volume of wastewater/volume of flocculant; D, The ratio of zeolite adding amount to the wastewater volume; E, blank control.

abstraction condition. These rates are higher than the reported one of 0.75 g per 1000 mL culture liquid by Feng (2002). The results of color reaction showed that the Anthrone reaction was positive while Coomassie brilliant blue reaction was negative. Thus, we concluded that the effective components of this flocculation material were mainly polysaccharides, not proteins. The results of UV spectrophotometry showed that there were no characteristic absorption peaks at 260, 280 nm, confirming that the flocculant consists of primarily polysaccharide with little proteins and nucleic acids.

Compared to the partial absorption peaks of amylose infrared spectrum reported by Feng (2002), the infrared spectrophotometry results of GY03 showed certain distinct features. At 3430.36 cm^{-1}, the absorption peak is caused by the stretching vibration of associating –OH in the saccharide; the peak at 2917.41 cm^{-1} is the result of the C–H bond stretching vibration of saccharide. Both the peaks at 1352.10 and 1633.36 cm^{-1} are the consequences of COO^- bond asymmetric oscillation. The peak concurrence at the two positions indicates the existence of carboxylic acid ions. There is no characteristic absorption peaks to denote the C=O bond stretching vibration in COOH in the range of 1720 to 1680 cm^{-1}. It is therefore indicating that the MBF of GY03 contains COO^- bond instead of –COOH.

3.2.2. Analysis on the thermal stability of FM

The thermal stability of *FM* was tested at different temperature for 30 min each. It can be seen from the results that the MBF is fairly heat-resistant. The flocculation ratio varies from approximately 85% to 89% in the temperature range of 23 to 70 °C, and only begins to decrease noticeably with further temperature increase from 70 to 90 °C. The slight increase in the flocculation ratio from 23 to 40 °C may be explained by the higher random motion of the flocculant molecules, and hence, higher collision frequency with kaolin particles. Thus, it appears that temperature has little impact on the physical and chemical properties of the flocculant molecules in the temperature range of 23–70 °C. However, when temperature goes above 70 °C, the physiochemical characteristics of flocculant molecules may have changed, given the noticeable reduction in the flocculation ratio that falls to about 70%. The heat resistance of the MBF is consistent with the general understanding that flocculants rich in polysaccharides have a better thermal resistance than those consisted of mainly protein and nucleic acid (Kurane et al., 1986; Nam et al., 1996).

3.2.3. Monosaccharide composition analysis

The effects on wastewater treatment were related to extracellular polysaccharides surrounding the GY03 cells. Therefore, studying extracellular polysaccharides and their components was critical in explaining flocculation mechanism. The extraction test showed that 8.31 g of the 8.52 g exocellular substances was polysaccharide, and the remaining 0.21 g was likely protein based upon a de-protein test with the Sevag Reagent. Analysis on monosaccharide compositions demonstrated that the polysaccharides of *B. mucilaginosus* were composed of rhamnose, xylose, sorbose, glucose, galactose, and two unknown types of sugars. The tests indicated that large quantities of the polysaccharides were contained in the capsular matrix of *B. mucilaginosus*. The active substance from *B. mucilaginosus* that produced FM was mainly composed of polysaccharides, carbohydrate and other bio-macromolecule organic matter.

3.3. Proposed flocculation mechanisms

Given the abundance of acidic polysaccharides in the MBF, we speculate that the flocculation process may involve chemical interactions between the MBF molecules and the kaolin particles. The net effect of such interactions may be that the MBF serves as bridging agents to coagulate kaolin particles. We can see that individual mineral grains are clearly visible with distinct morphology and inter-grain spaces before flocculation from the TEM observations of kaolin particles. The particles are interlaced with flocculi after flocculation to show soft edges, and there are no more 'individual' mineral grains left.

We propose the flocculation takes place mainly by linking and charge neutralization mechanism. First of all, individual flocculant molecules and kaolin particles draw closer to each other possibly through ionic attractions to form mineral–MBF complexes (oppositely charged polyelectrolytes reduce the particle surface charge density such that particles may approach each other sufficiently close, so that the attractive forces become effective). If the extension of MBF from the particle surface is greater than the distance over which the interparticle repulsion acts, the MBF can adsorb onto another particle thereby bridging the two, then the aggregates entangle to form larger particles, and finally settle down and precipitate. Therefore, it is likely that the microbial flocculants promote the formation of flocculi through bridging and charge neutralization to increase flocculation efficiency.

4. Concluding remarks

It is shown in this study that *B. muscilaginosus* bacterium GY03 is a flocculant producing strain. The bacterium can be cultured easily with simple nutritional requirement and is capable of secreting exocellular polysaccharides in nitrogen-free medium. The MBF has a desired effective flocculation ability with a significant removal ratio of COD, BOD, and SS for waste waters.

We suggest that the flocculation mechanism by biopolymer from *B. mucilaginosus* is similar to that by synthetic polyelectrolytes (Friedman et al., 1968). We postulate that polyvalent ions can complex with functional groups on two different elementary fibrils and thereby cause an effective bridge and alteration of the polymer charge. We propose

that the biopolymer bridges between bacterial cells resemble that in the polystyrene latex balls reported by Ries and Meyers (1968).

Acknowledgements

This research was financially supported by national natural science foundation of China (NSFC40463001) and Guizhou Province for science and teaching talents program (2005-356).

References

AI-Shahwani, M.F., AI-Rawi, E.H., 1989. Bacterial extracellular material from brewery wastewater for raw water treatment. Biological Wastes 28 (4), 271–276.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Research 25 (17), 3389–3402.

Adamson, A.W., 1990. Physical Chemistry of Surfaces, fifth ed. Wiley, NY, pp. 20–25.

Blakesley, R.W., Boezi, J.A., 1977. A new staining technique for proteins in polyacrylamide gels using Coomassie brilliant blue G250. Analytical Biochemistry 82, 580–582.

Bruus, J.H., Nielsen, P.H., Keiding, K., 1992. On the stability of activated sludge flocs with implications to dewatering. Water Research 26, 1597–1604.

Butterfield, C.T., 1935. Studies of sewage purification. II. A zoogloea-forming bacterium isolated from activated sludge. Public Health Reports 50, 671–681.

Chen, Y., Lian, B., 2005. Ability of *Bacillus mucilaginosus* GY03 Strain to adsorb chromium ions. Pedosphere 15 (2), 225–231.

Deng, S.B., Yu, G., Peng, Y.T., 2005. Production of a bioflocculant by *Aspergillus parasiticus* and its application in dye removal. Colloids and Surfaces B: Biointerfaces 44 (4), 179–186.

Feng, D.L., 2002. The production of microbial flocculant and the research of its flocculating mechanism. Ph.D. Dissertation, Wuhan Science and Engineering University, pp. 43–68.

Friedman, B.A., Dugan, P.R., Pfister, R.M., Remsen, C.C., 1968. Fine structure and composition of the zoogloeal matrix surrounding Zoogloea ramigera. Journal of Bacteriology 96, 2144–2153.

Friedman, B.A., Dugan, P.R., Pfister, R.M., Remsen, C.C., 1969. Structure of exocellular polymers and their relationship to bacterial flocculation. Journal of Bacteriology 98 (3), 1328–1334.

Fujita, M., Ike, M., Tachibana, S., Kitada, G., Kim, S.M., Inoue, Z., 2000. Characterization of a bioflocculant produced by *Citrobacter* sp. Journal of Bioscience and Bioengineering 89 (1), 40–46.

Graham, H.D., 1963. Reaction of sugar alcohols with the anthrone reagent. Journal of Food Science 28 (4), 440–445.

Harrington, B.J., Raper, K.B., 1968. Use of a fluorescent brightener to demonstrate cellulose in the cellular slime molds. Applied Microbiology 16, 106–113.

He, N., Li, Y., Chen, J., 2004. Production of a novel polygalacturonic acid bioflocculant REA-11 by *Corynebacterium glutamicum*. Bioresource Technology 94 (1), 99–105.

Hejzlar, J., Chudoba, J., 1986. Microbial polymers in the aquatic environment: II. Isolation from biologically nonpurified and purified municipal waste water and analysis. Water Research 20 (10), 1217–1221.

Hiroaki, T., Kiyoshi, K., 1985. Purification and chemical properties of a flocculant produced by *Paecilomyces*. Agricultural and Biological Chemistry 49 (11), 3159–3164.

Joung, H.Y., Sung, J.K., Se, H.A., Hong, K.L., 2007. Characterization of a novel bioflocculant, p-KG03, from a marine dinoflagellate, *Gyrodinium impudicum* KG03. Bioresource Technology 98 (2), 361–367.

Kirchner, J.G., 1978. Thin-Layer Chromatography. Wiley-Interscience, New York, pp. 150–180.

Kumar, C.G., Joo, H.S., Choi, J.W., Koo, Y.M., Chang, C.S., 2004. Purification and characterization of an extracellular polysaccharide from haloalkalophilic *Bacillus* sp. I-450. Enzyme and Microbial Technology 34 (7), 673–681.

Kurane, R., Hatamochi, K., Kakuno, T., Kiyohara, M., Hirano, M., Taniguchi, Y., 1994. Production of a bioflocculant by *Rhodococcus erythropolis* S-l grown on alcohols. Bioscience, Biotechnology and Biochemistry 58 (2), 428–429.

Kurane, R., Takeda, K., Suzuki, T., 1986. Screening for and characteristics of microbial flocculants. Agricultural and Biological Chemistry 50 (9), 2301–2307.

Labille, J., Thomas, F., Milas, M., Vanhaverbeke, C., 2005. Flocculation of colloidal clay by bacterial polysaccharides: effect of macromolecule charge and structure. Journal of Colloid and Interface Science 284 (1), 149–156.

Lee, S.H., Lee, S.O., Jang, I.L., Lee, T.H., 1995. Microbial flocculant from *Arcuadendron* sp. TS-49. Biotechnology Letters 17 (1), 95–100.

Li, J., Lian, B., Hao, J.C., Zhao, J., Zhu, L.J., 2006. Non parallelism between the effect of microbial flocculants on sewerage disposal and the flocculation rate. Chinese Journal of Geochemistry 25 (2), 139–142.

Lian, B., 1998. Study on the Potassium Releasing from Minerals by Silicate Bacteria. Guizhou Science and Technology Publishing House, Guiyang, pp. 53–108 (in Chinese).

Lian, B., Chen, Y., Yuan, S., Liu, C.Q., 2003. Study on the flocculability of silicate bacterial GY03 strain. Acta Mineralogica Sinica 23 (4), 303–307 (in Chinese).

Lian, B., Chen, Y., Yuan, S., Zhu, L.J., Liu, C.Q., 2004. Study on the flocculability of metal ions by *Bacills mucilaginosus* GY03 strain. Chinese Journal of Geochemistry 23 (4), 380–386.

Lu, W.Y., Zhang, T., Zhang, D.Y., Li, C.H., Wen, J.P., Du, L.X., 2005. A novel bioflocculant produced by *Enterobacter aerogenes* and its use in defecating the trona suspension. Biochemical Engineering Journal 27 (1), 1–7.

Malinovskaya, I.M., Kosenko, L.V., Votselko, S.K., Podgorskii, V.S., 1990. Role of *Bacillus mucilaginosus* polysaccharide in degradation of silicate minerals. Microbiology 59 (1), 49–55.

Mason, L.R., Gunst, F.R., Hess, L.J., 2003. Statistical Design and Analysis of Experiments, Second ed. John Wiley & Sons, New York, pp. 247–254.

McKinney, R.E., 1952. A fundamental approach to the activated sludge process II. A proposed theory of floc formation. Sewage and Industrial Wastes 24, 280–287.

Nakamura, J., Miyashiro, S., Hirose, Y., 1976. Screening: isolation and some properties of microbial cell flocculants. Agricultural and Biological Chemistry 40 (2), 377–383.

Nakata, K., Kurane, R., 1999. Production of an extracellular polysaccharide bioflocculant by *Klebsiella pneumoniae*. Bioscience, Biotechnology, and Biochemistry 63 (12), 2064–2068.

Nakaura, J., Miygeyshi, S., Hirose, Y., 1976. Modes of flocculation of yeast cells with flocculant produced by *Aspergillus sojae* AJ7002. Agricultural and Biological Chemistry 40 (8), 1565–1571.

Nam, J.S., Kwon, G.S., Lee, S.O., Hwang, J.S., Lee, J.D., Yoon, B.D., Lee, T.H., 1996. Bioflocculant produced by *Aspergillus* sp. JS-42. Bioscience, Biotechnology, and Biochemistry 60 (2), 325–327.

Prasertsan, P., Dermlim, W., Doelle, H., Kennedy, J.F., 2006. Screening, characterization and flocculating property of carbohydrate polymer from newly isolated *Enterobacter cloacae* WD7. Carbohydrate Polymers 66 (3), 289–297.

Raul, M., Benoit, G., 2006. Algal–bacterial processes for the treatment of hazardous contaminants: a review. Water Research 40 (15), 2799–2815.

Ries, H.E., Meyers, B.L., 1968. Flocculation mechanism: charge neutralization and bridging. Science 160, 1449–1450.

Sheng, Y.L., Zhang, Q., Sheng, Y.R., Li, C.B., Wang, H.J., 2006. Screening and flocculating properties of bioflocculant-producing microorganisms. Journal of University of Science and Technology Beijing 13 (4), 289–292 (in Chinese).

Shih, I.L., Van, Y.T., Yeh, L.C., Lin, H.G., Chang, Y.N., 2001. Production of a biopolymer flocculant from *Bacillus licheniformis* and its flocculation properties. Bioresource Technology 78 (3), 267–272.

Suh, H.H., Kwon, G.S., Lee, C.H., Kim, H.S., Oh, H.M., Yoon, B.D., 1997. Characterization of bioflocculant produced by *Bacillus* sp. DP-152. Journal of Fermentation and Bioengineering 84 (2), 108–112.

Taylor, N.W., Orton, W.L., 1978. Aromatic compounds and sugars in flocculation of *Saccharomyces cerevisiae*. Journal of the Institute of Brewing 84, 113–114.

Tezuka, Y., 1969. Cation-dependent flocculation in a *Flavobacterium* species predominant in activated sludge. Applied Microbiology 17, 222–226.

Yokoi, H., Shiraki, M., Hirose, J., Hayashi, S., Takasaki, Y., 1996. Flocculation properties of xanthan produced by *Xanthomonas campestris*. Biotechnology Techniques 10 (10), 789–792.

Zouboulis, A.I., Chai, X.L., Katsoyiannis, I.A., 2004. The application of bioflocculant for the removal of humic acids from stabilized landfill leachates. Journal of Environmental Management 70 (1), 35–41.