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Production and characteristics of a bioflocculant produced by *Bacillus* sp. F19

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Abstract

A bioflocculant-producing bacterium isolated from soil was identified as *Bacillus* sp. and the bioflocculant produced was named MBFF19. Effects of physico-chemical conditions including pH, carbon sources and nitrogen sources on MBFF19 production were studied. Chemical analyses of the purified bioflocculant MBFF19 indicated that it was a sugar-protein derivative, composed of neutral sugar (3.6%, w/w), uronic acid (37.0%, w/w), amino sugars (0.5%, w/w) and protein (16.4%, w/w). The two neutral sugar components were mannose and glucose and the molar ratio was 1.2:1. Infrared spectrophotometry analysis revealed that MBFF19 contained carboxyl, hydroxyl and methoxyl groups in its structural. Flocculating properties of bioflocculant MBFF19 was examined using kaolin, activated carbon and fly coal suspension. Cation supplement had no positive effects on the flocculating activity whereas the presence of Fe^{3+} inhibited flocculation. Influences of pH and bioflocculant dosage on the flocculation were also examined.

Keywords: *Bacillus* sp.; Bioflocculant; Flocculation; Polysaccharide

1. Introduction

At present, flocculants are prevalent in the variety of industrial processes such as wastewater treatment, drinking water purification and downstream processes in fermentation processes (Shih et al., 2001). Although chemically synthetic flocculants are playing dominant roles associated with their effectiveness and low cost, they are hard degraded. Some of these synthetic flocculating substances have threat to public heath and increase environmental risks. For example, polyacrylamide, one of the most popular flocculants, includes acrylamide monomers which are verified as both neurotoxin and strong carcinogens to human beings (Dearfield and Abermathy, 1988). Recently, bioflocculant has attracted considerable attention as a promising substitute of the chemical flocculants (Jang et al., 2001) because of their biodegradability and safety for ecosystems (He et al., 2004).

Bioflocculants, secreted by algae, bacteria, fungi and yeast, are kinds of extracellular biopolymer including proteins, glycoproteins, polysaccharides, lipids and glycolipids (Salehizadeh and Shojaosadati, 2003). Many particular microorganisms and their bioflocculants had been announced lately. Joung Han and his cooperators discovered the *Gyrodinim impudicum* KG03, a bioflocculant produced by a marine dinoflagellate, which was characterized as an acidic heteropolysaccharide, with galactose and uronic acid as main and minor components, respectively (Yim et al., 2007). The bioflocculant secreted by *Nannocystis* sp. Nu-2 was found to be a glycoprotein which was effective in bleaching acid red and direct emerald blue (Zhang et al., 2002). In addition, *Rhodococcus erythropolis* was able to generate a protein bioflocculant which lost the flocculating capability by enzymatic digestion (Takeda et al., 1991). Although these bioflocculants showed strong flocculating activities, it was noted that their flocculation abilities were depended strongly on cations. The bioflocculant generated

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by *Klebsiella* sp. S11 was unable to flocculate without addition of $CaCl_2$ solution (Dermlim et al., 1999) and the bioflocculant produced by *Enterobacter aerogenes* required Zn^{2+} for flocculating activity (Lu et al., 2005). The Ca^{2+} was suggested to be the common ions to seduce flocculating ability of bioflocculants (Salehizadeh and Shojaosadati, 2001). Since addition of cations to flocculation causes cost increase and second pollution, screening of new microorganisms which could produce cation-independent bioflocculant is preferable.

The objective of present research was basically developed by three sections. The first is to report a bioflocculant-producing bacterium with cation-independent flocculating capability. In the second part, optimization of bioflocculant production was performed and the third part involved the characteristics and flocculation properties of the corresponding bioflocculant for further application.

2. Methods

2.1. Isolation and culture of bioflocculant bacteria

Bioflocculant-producing strains were isolated from soil collected from Shanghai Jiao Tong University in Shanghai, China. The composition of the selective medium was as follows: glucose, 10 $g\,l^{-1}$; yeast extract, 0.5 $g\,l^{-1}$; KH_2PO_4, 2 $g\,l^{-1}$; K_2HPO_4, 5 $g\,l^{-1}$; $(NH_4)_2SO_4$, 0.2 $g\,l^{-1}$; urea, 0.5 $g\,l^{-1}$ and NaCl, 0.1 $g\,l^{-1}$ with the initial pH 7. Each isolated strain was inoculated in 250 ml Erlenmeyer flasks containing 30 ml medium and incubated in a shaker at 200 rpm for 48 h at 30 °C. The culture broth was determined for flocculating activity. Strains with high flocculating ability were selected for further studies.

2.2. Identification of the bioflocculant-producing microorganism

Cell forms and colony characteristics of the strain on nutrient agar were observed after 2 days' incubation. Phylogenetic analysis and 16S rDNA sequence determination were conducted to identify the microorganism. The chromosomal DNA was isolated in conformity to the method described previously (Ausubel et al., 2002). PCR amplification of 16S rDNA was carried out (Wu et al., 2007) using universal primers, 27F (5′AGAGTTTGATCCPATGGCTCAG3′) and 1492R (5′ GGTTACCTTGTTACGACTT3′). The PCR product was sequenced by Invitrogen Company (Shanghai) and the results obtained were contrasted to the 16S rDNA sequences available in the GenBank from the National Center for Biotechnology Information Database.

2.3. Bioflocculant collection and purification

Production of the bioflocculant was performed in several 500 ml flasks containing 100 ml culture medium with 200 rpm agitation at 30 °C. The composition of the culture medium was as follows: sucrose, 20 $g\,l^{-1}$; yeast extract, 2.5 $g\,l^{-1}$; KH_2PO_4, 2 $g\,l^{-1}$; K_2HPO_4, 5 $g\,l^{-1}$ and NaCl, 0.1 $g\,l^{-1}$. The initial pH was 7. After incubation for 48 h, the culture was centrifuged at 10,000 rpm for 15 min. Bioflocculant was initially remained in liquid form, therefore, the supernatant was collected. Cold ethanol was added into the supernatant with stirring at the ratio of 2:1 (V/V). The precipitate was obtained by centrifugation at 10,000 rpm for 3 min and purified with distilled water. After three such ethanol precipitations, the bioflocculant was dialyzed against de-ionized water overnight and then lyophilized to obtain purified bioflocculant.

2.4. Physical and chemical analysis of the bioflocculant

Total sugar content of the bioflocculant was measured by phenol–sulfuric acid method (Chaplin and Kennedy, 1994) using glucose as the standard solution. Protein content was determined by the Lowery method (Zhang, 2003) with bovine serum albumin as a standard. After hydrolysis of the bioflocculant with trifluoroacetic acid at 121 °C for 2 h, the amino sugar compositions were examined according to the Elson-Morgan method (Chaplin and Kennedy, 1994) with glucose amine as the standard solution. The uronic acid was assayed using carbazole–sulfuric acid method (Chaplin and Kennedy, 1994) with glucuronic acid as the standard solution. The method of carbazole–sulfuric was used to determine the content of neutral sugar (Zhang, 2003). Amino acid was estimated by ninhydrin method (Zhang, 2003). The sugar units of the bioflocculant were analyzed with GC on column DB-5 (Agilent, 0.25 mm × 30 m) according to the protocol described previously (Zhang, 2003).

Elemental analysis was achieved with an elemental analyzer (PE 2400 II, Perkin Elmer Company, USA). Infrared spectra of the dried polysaccharide sample was recorded in the frequency range of 4000–400 cm^{-1} by a fourier transform infrared-raman Spectrophotometer (EQUINOX 55, Bruker Company, Germany) with KBr disks.

2.5. Flocculating activity assessment

Flocculating activity of the purified biopolymer was calculated by flocculating rate. The bioflocculant was added into the diverse suspensions of 5 $g\,l^{-1}$. The mixture was stirred at 50 rpm for 2 min using a magnetic mixer. After 5 min settlement, samples were withdrawn at the half height of the suspension for optical density determination at the wave length of 550 nm. The flocculating rate was defined by following formula:

$$\text{Flocculating rate} = (A - B)/A \times 100\%$$

where A and B were OD_{550} (optical density at 550 nm) of the control and sample supernatant, respectively. Effects of pH, flocculant concentration and various cations on flocculation were investigated.

3. Results

3.1. Isolation of the bioflocculant-producing microorganisms and phylogenetic analysis

Totally 24 mucoid colonies were isolated from the soil. Morphological test presented that these isolates were all convex and round edge. Most of the colonies were creamy colour except for two yellow colonies. The assessment of flocculation activities was carried out after 2 days cultivation, which found out that three isolates, F19, P2 and P4 had the flocculating capabilities but only F19 still had when without existence of $CaCl_2$ solution.

The streak plate technique was applied to purify the strain F19. According to the morphological and physiological characteristics, the strain was rod-shaped, Gram positive and aerobic bacteria. The 16S rDNA were partially sequenced following PCR amplification and compared with sequences deposited in databases. Totally 912 bp of the 16S rDNA of strain F19 was determined. The phylogenetic tree (not shown) showed that strain F19 formed evolutionary lineage within the radiation of a cluster comprising the *Bacillus* sp. the highest level of 16S rDNA sequence similarity being to *Bacillus megaterium* (97%). Strain F19 and its bioflocculant were named *Bacillus* sp. F19 and MBFF19, respectively.

3.2. Bioflocculant MBFF19 production

Effects of pH variation between the ranges of 5–12 on MBFF19 production were investigated after 48 h cultivation. From Table 1, it was obvious that changes of the pH values (pH 5–12) had no significant effect on *Bacillus* sp. F19 growth. However, production of MBFF19 in acidic conditions was distinctly much lower than in either of the neutral and alkaline. MBFF19 was hardly harvested at initial pH 5 and 6 due to its too low production while significant production of MBFF19 (0.56 $g l^{-1}$) was detected at initial pH 7. Moreover, the yield of MBFF19 maintained higher than 0.48 $g l^{-1}$ when the pH ranged at 7–12. The generation of MBFF19 was affected by the initial pH value of the culture medium. The Alkaline culture medium was favored while the MBFF19 production was inhibited in acidic culture medium. Comparison between final pH and initial pH values clearly presented that the cultural medium had the buffer capability especially in alkaline conditions. This buffer ability may come from the organic acid contained in bioflocculant, by-production like acetic acid, or the unspent K_2HPO_4 and KH_2PO_4.

Table 1
Effects of pH on cell growth and MBFF19 production

Initial pH	Final pH	Yield of MBFF19 ($g l^{-1}$)	Cell dry weight ($g l^{-1}$)
5.06	5.65	–	3.62
6.08	5.72	0.08	3.08
7.09	6.46	0.56	3.15
7.92	7.09	0.66	3.95
8.95	7.37	0.75	3.11
10.03	7.31	0.71	3.91
10.98	7.21	0.61	2.95
12.01	7.38	0.48	2.89

Samples of cultural broth were withdrawn after 48 h incubation.

The effects of various carbon sources on the production of MBFF19 were studied (Fig. 1). Among the carbon source studied, sucrose was the most favorable carbon source for both production of bioflocculant and cell growth. The generation of MBFF19 was also enhanced when glucose, lactose and fructose were added to the medium. The cell grew well in the medium of maltose and ethanol, but the yield of MBFF19 was relatively low. Nevertheless, the cells growth was poor on sodium acetate leading to none yield of bioflocculant. Since the sucrose medium induced the highest generation of MBFF19 and also was a cheap substrate, it could serve as an appropriate carbon source for MBFF19 production. In addition, the effect of nitrogen source on MBFF19 production was examined (Fig. 2) with sucrose as carbon source. Among

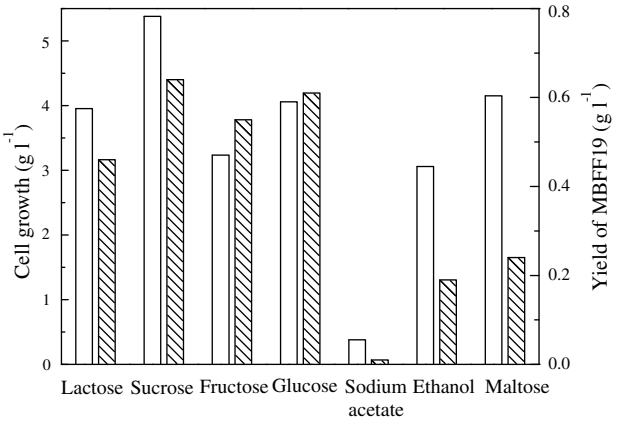

Fig. 1. Effect of carbon sources on MBFF19 production (grid columns) and cell growth (open columns) of *Bacillus* sp. The carbon concentration was 8 $g l^{-1}$.

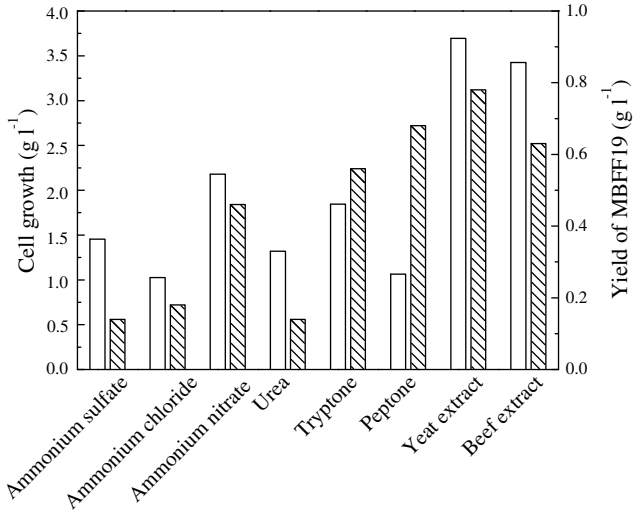

Fig. 2. Effect of nitrogen sources on MBFF19 production (grid columns) and cell growth (open columns) of *Bacillus* sp. The nitrogen concentration was 0.25 $g l^{-1}$.

the inorganic nitrogen compounds investigated, ammonium nitrate was the only one which could lead to a yield of higher than 0.3 gl^{-1}. Other inorganic nitrogen led to poor MBFF19 production. Organic nitrogen was obviously better source for MBFF19 production than inorganic nitrogen. Yeast extract was therefore selected as the nitrogen source in following experiments as it caused highest MBFF19 secretion of 0.78 gl^{-1}.

Production of bioflocculant MBFF19 corresponding to the growth curve of the isolated *Bacillus* sp. F19, variation of the flocculating rate and pH values were illustrated in Fig. 3. The flocculating rate of cultural medium was measured by adding 1 ml cultural medium to 100 ml kaolin suspension. A delay of cell growth was observed in the first 12 h. However a rapid increase of the flocculating rate was detected which might indicated a forming of MBFF19 in the first period. Slight amount of biopolymer generated in the lag phase resulted in the remarkable improvement of flocculating rate. The flocculating rate was 85.8% at 26 h. However, significant product of MBFF19 was not determined due to the limitation of the collection method applied at low MBFF19 concentration. The flocculating activity remained stable from 36 h to 86 h while the output of bioflocculant increased sharply to 1.47 gl^{-1}. Profile of bioflocculant yield showed a good corresponding to the cell growth curve, indicating that the bioflocculant was formed during cell growth but not cell-lysis.

3.3. Characterization of the purified MBFF19

3.3.1. Composition analysis of MBFF19

The total sugar and total protein content of bioflocculant were 66.4% and 16.4% (w/w), respectively, indicating that MBFF19 was mainly a polysaccharides bioflocculant. Since polysaccharides could include different saccharides like neutral sugar, uronic acid and amino sugar, it was necessary to distinguish specific sugar components. The results from the analysis of the hydrolyzed bioflocculant which was treated by trifluoroacetic acid revealed that the contents of neutral sugar, uronic acid and amino sugars were 3.6%, 37.0% and 0.5%, respectively. Besides that, ninhydrin-positive reaction illuminated that the bioflocculant contains amino acids. Analysis by GC designated that the molar ratio of mannose to glucose was approximate 1.2:1. The elemental analysis of the bioflocculant revealed the mass proportion of C, H, O, N and S is 36.9:5.9:51.7:4.4:1.1 (w/w) correspondently.

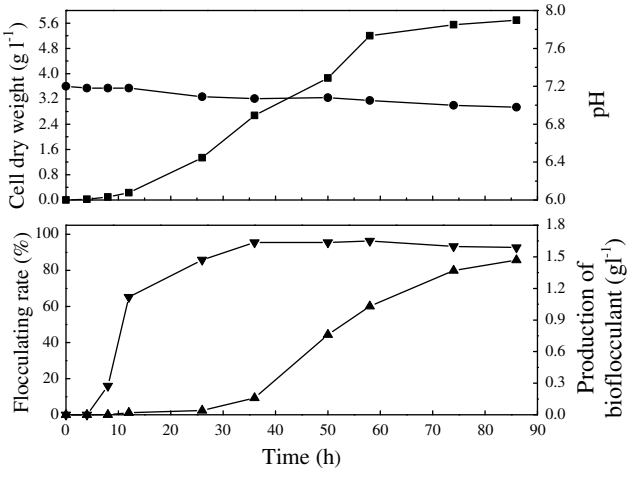

Fig. 3. Time course of a batch culture of *Bacillus* sp. F19. The flocculating rate was measured by the mixture of 5 gl^{-1} kaolin clay. ■, Cell dry weight (gl^{-1}); ●, pH; ▲, production of bioflocculant (gl^{-1}); ▼, flocculating rate (%).

3.3.2. Analysis of functional group in bioflocculant MBFF19

The infrared spectrophotometry was used to analyze the functional group in bioflocculant MBFF19. The spectrum displayed a broad stretching intense peak at around 3420 cm^{-1} characteristic for hydroxyl. A weak C–H stretching vibration band was observed at 2933 cm^{-1}. Furthermore, an asymmetrical stretching peak was noticed at 1645 cm^{-1} and a week symmetrical stretching peak at 1409 cm^{-1}, indicating the presence of carboxyl group in bioflocculant MBFF19. Methoxyl group was detected due to the bands at 1056 cm^{-1} and 1137 cm^{-1}. The sorption peak at 1236 cm^{-1} suggested the C–O stretching in ether or alcohol. The absorption peaks around 1000–1100 cm^{-1} are known to be characteristic for all sugar derivatives. The spectrum showed the presence of carboxyl, hydroxyl and methoxyl groups, and these groups were the preferred groups for flocculation process. The OH, COOH, COO^- groups in the bioflocculant and H^+, OH^- group on the surface of the particles may form hydrogen bonds when the bioflocculant chains approach the surface of particles (Deng et al., 2003).

3.4. Flocculating properties of bioflocculant MBFF19

3.4.1. Effect of various cations on flocculating activity

Flocculating properties of bioflocculant MBFF19 was examined using three different diverse suspensions (kaolin, activated carbon and fly coal). The effects of various cations on flocculating activity were evaluated using NaCl, $CaCl_2 \cdot 2H_2O$, $MgCl_2 \cdot 6H_2O$, $ZnSO_4$, $MnSO_4$, $FeSO_4$, $Fe_2(SO_4)_3$ and $AlCl_3 \cdot 6H_2O$ and the initial MBFF19 concentrations were 1 mgl^{-1} for kaolin and activated carbon and 50 mgl^{-1} for fly coal, respectively. The results indicated that the addition of any above cations could not evidently enhance flocculating rate of the three suspensions. Besides that, when 0.5 $mmoll^{-1}$ of the Fe^{3+} was added into the suspensions, the flocculating rates of kaolin, activated carbon and fly coal reduced from 86.0%, 80.3% and 82.6% to 0, 41.7% and 22.4%, respectively. The presence of Fe^{3+} completely inhibited the flocculation of kaolin and markedly reduced the flocculating activities on the rest two suspensions. Cations were important to the flocculation process. The flocculating activity of bioflocculant from a haloalkalophilic *Bacillus* was significantly enhanced by the addition of divalent cations like Ca^{2+}, Cu^{2+}, Zn^{2+},

Mn^{2+}, Co^{2+} and Fe^{2+}, and dropped by the addition of Al^{3+}, Fe^{3+}, Ni^{2+} and Na^{+} (Kumar et al., 2004). The bioflocculant MS-102 was a cation-dependent, whose flocculating capability was strongly increased by Ca^{2+} and Mg^{2+} (Salehizadeh and Shojaosadati, 2002). Cations improved the flocculating ability by neutralizing the residual negative charge of functional groups (Salehizadeh and Shojaosadati, 2001). Nevertheless, the supplement of cations has no positive effect on the flocculating performance of bioflocculant MBFF19. This may be because the flocculation of MBFF19 is primarily depending on bridging function instead of the mechanism of charge neutralization. Similar observation (Deng et al., 2003) also reported that the polysaccharide bioflocculant was able to flocculate kaolin suspension without adding of ions.

3.4.2. Effect of the bioflocculant dosage

As shown in Fig. 4, the typical flocculation curve of the biopolymer showed the relationship between the concentration of the bioflocculant and its flocculating activity. The flocculating activities of kaolin suspension was above 90% when the MBFF19 concentrations were adjusted to the range of 1.0–20.0 mgl^{-1} with pH at 3 and the corresponding maximum flocculating rates were achieved at bioflocculant dosage of 2.0 mgl^{-1}. Flocculation mainly ceased once the MBFF19 concentration exceeded 80 mgl^{-1}. The flocculating rate of activated carbon stayed higher than 90% when the MBFF19 concentration was between 1.0 and 20.0 mgl^{-1} at pH 2. The same flocculating rate was obtained on the suspension of fly coal, when the MBFF19 concentration ranged from 22.5 to 135.0 mgl^{-1} with pH at 4. The depression of flocculating activity at high concentration of MBFF19 is largely due to incomplete dispersion of excess bioflocculant (Suh et al., 1997). Only particles around flocculants participated in the flocculating reaction while others were excluded. Table 2 summarizes the bioflocculant producing microorganisms and their optimum dosage for flocculation on kaolin suspension reported in the literature which indicated that the bioflocculant produced by *Bacillus* sp. F19 was an effective flocculating agent with low dosage requirement.

3.4.3. Effect of pH

The experiments concerning the effect of pH on the flocculation were carried out with the range of pH 2–9. The initial MBFF19 concentrations were 1 mgl^{-1} for kaolin and activated carbon and 50 mgl^{-1} for fly coal, respectively. As can be seen from Fig. 5, the flocculating capabilities were observed at pH ranging from 2 to 9, 2 to 6, 4 to 6 for kaolin, activated carbon and fly coal suspension, respectively. The flocculating activities of all the three suspensions dropped with increasing pH. The maximum flocculating rates of kaolin and activated carbon suspen-

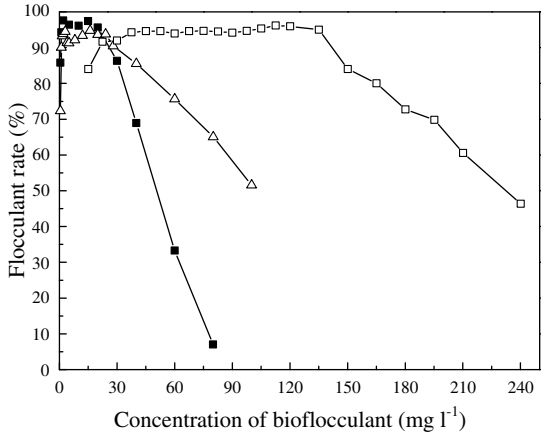

Fig. 4. Effect of MBFF19 dosage on the flocculating rate (■, kaolin; △ activated carbon; □, fly coal). The pH: kaolin, 3; activated carbon, 2; fly coal, 4.

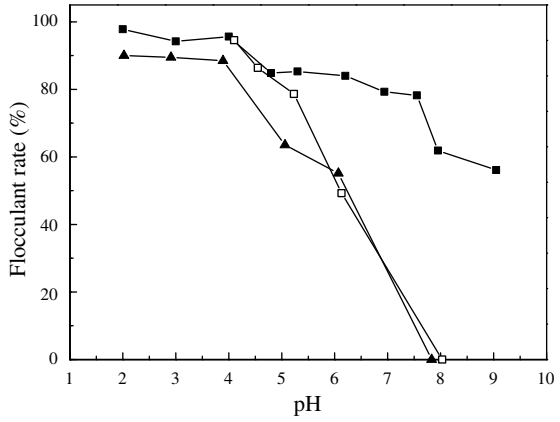

Fig. 5. Effect of the pH on the flocculating rate (■, kaolin; ▲, activated carbon; □, fly coal). The initial MBFF19 concentrations: kaolin and activated carbon, 1 mgl^{-1}; fly coal, 50 mgl^{-1}.

Table 2
Comparison of the flocculating rates at optimum dosage of different bioflocculants

Microorganism	Optimum concentration (mgl^{-1})	Flocculating rate	Cations	Reference
Enterobacter sp.BY-29	40	23[a]	Fe^{3+}	(Yokoi et al., 1997)
Gyrodinium impudicum KG30	1	92%	No cation	(Yim et al., 2007)
Enterobacter aerogenes	90	Data not shown	Zn^{2+}	(Lu et al., 2005)
Bacillus sp. DP-152	1	43[a]	Ca^{2+}	(Suh et al., 1997)
Bacillus coagulants As-101	30	92%	Al^{3+}, Fe^{3+},Ca^{2+}	(Salehizadeh et al., 2000)
Klebsiella pneumoniae H12	1	Data not shown	Ca^{2+}	(Nakata and Kurane, 1999)
Citrobacter sp. TKF04	1–10	Above 90%	No cation	(Fujita et al., 2000)
Bacillus sp. F19	2	97%	No cation	Present article

[a] Flocculating activity, $1/OD_{550}$.

sion were observed at pH 2. The optimized pH for flocculation of fly coal was 4. At pH lower than 4, the fly coal suspension was partially soluble. These results were slight different from a previous study reporting that the flocculating activity of the bioflocculant produced by *S. griseus* was observed within the pH 2–6 and the maximum value was obtained at pH 4 (Shimofuruya et al., 1996). The maximum activity of the bioflocculant produced by *Gyrodinium impudicum* KG03 was observed at pH 4 with pH range of approximately 3–6 (Yim et al., 2007).

4. Conclusions

A bioflocculant-producing bacterium *Bacillus* sp. F19 was isolated from soil and production of the bioflocculant (named MBFF19) was optimized. The bioflocculant was a biopolymer consists of neutral sugar (3.6%, w/w), uronic acid (37.0%, w/w), amino sugars (0.5%, w/w) and protein (16.4%, w/w). MBFF19 was cation-independent and effective for flocculation of kaolin, activated carbon and fly coal suspension. Its practical application in industry would also be developed in further progress.

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References

Ausubel, F.M., Kingston, R.E., Seidman, J.G., 2002. Short Protocols in Molecular Biology. Wiley, NewYork.

Chaplin, M.F., Kennedy, J.F., 1994. Carbohydrate Analysis, second ed. Oxford University Press, New York.

Dearfield, K.L., Abermathy, C.O., 1988. Acrylamide: its metabolism developmental and reproductive effects, genotoxicity, and carcinogenicity. Mutat. Res. 195, 45–77.

Deng, S.B., Bai, R.B., Hu, X.M., Luo, Q., 2003. Characteristics of a bioflocculant produced by *Bacillus mucilaginosus* and its use in starch wastewater treatment. Appl. Microbiol. Biot. 60, 588–593.

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

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

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

Jang, J.H., Ike, M., Kim, S.M., Fujita, M., 2001. Production of a novel bioflocculant by fed-batch culture of *Citrobacter* sp. Biotechnol. Lett. 23, 593–597.

Kumar, C.G., Joo, H.S., Kavali, R., Choi, J.W., Chang, C.S., 2004. Characterization of an extracellular biopolymer flocculant from a haloalkalophilic *Bacillus* isolate. World J. Microb. Biot. 20, 837–843.

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. Biochem. Eng. J. 27, 1–7.

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

Salehizadeh, H., Shojaosadati, S.A., 2001. Extracellular biopolymeric flocculants – recent trends and biotechnological importance. Biotechnol. Adv. 19, 371–385.

Salehizadeh, H., Shojaosadati, S.A., 2002. Isolation and characterisation of a bioflocculant produced by *Bacillus firmus*. Biotechnol. Lett. 24, 35–40.

Salehizadeh, H., Shojaosadati, S.A., 2003. Removal of metal ions from aqueous solution by polysaccharide produced from *Bacillus firmus*. Water Res. 37, 4231–4235.

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

Shih, I.L., Van, Y.T., Yeh, L.C., Lin, H.G., Chang, Y.N., 2001. Production of a biopolymer flocclant from *Bacillus licheniformis* and its flocculation properties. Bioresource 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. Biotech. Biochem. 60, 498–500.

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. J. Ferment. Bioeng. 84, 108–112.

Takeda, M., Kurane, R., Koizumi, J.I., Nakamuea, I., 1991. A protein bioflocculant produced by *Rhodocccus erythropolis*. Agric. Biol. Chem. 5, 2663–2664.

Wu, X., Xi, W.Y., Ye, W., Yang, H., 2007. Bacterial community composition of a shallow hypertrophic freshwater lake in China, revealed by 16S rRNA gene sequences. Fems Microbiol. Ecol. 61, 85–96.

Yim, J.H., Kim, S.J., Ahn, S.H., Lee, H.K., 2007. Characterization of a novel bioflocculant, p-KG03, from a marine dinoflagellate, *Gyrodinium impudicum* KG03. Bioresource Technol. 98, 361–367.

Yokoi, H., Yoshida, T., Mori, S., Hirose, J., Hayashi, S., Takasaki, Y., 1997. Biopolymer flocculant produced by an *Enterobacter* sp. Biotechnol. Lett. 19, 569–573.

Zhang, J., Liu, Z., Wang, S., Jiang, P., 2002. Characterization of a bioflocculant produced by the marine mycobacterium *Nannocystis* sp. NU-2. Appl. Microbiol. Biot. 59, 517–522.

Zhang, W., 2003. Biological–chemical analysis of Glycoconjugates, second ed. Zhejiang University Press, Zhejiang.