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Compound Bioflocculant Production and its Application in Low Temperature and Low Turbidity Drinking Water Treatment

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A compound bioflocculant CBF with high flocculating activity, produced by mixed culture of *Rhizobium radiobacter* F2 and *Bacillus sphaeicus* F6 from soil, was investigated with regard to its production and application in low temperature and low turbidity drinking water treatment. The most preferred carbon source, nitrogen source and C/N ratio (w/w) for straina F2 and F6 to produce CBF were found to be glucose, Urea and 20, respectively. The optimal conditions for CBF production were inoculum size 10% (v/v), initial pH 7.5, culture temperature 30°C, and shaking speed 140 r/min for 24 h, under which the flocculating activity of the bioflocculant reached 98.05%. The CBF showed good flocculating performance and industrial potential for treatment of low temperature and low turbidity drinking water. The maximum removal efficiencies of turbidity and Al(III) were 85.54% and 89.32%, respectively, which were better than conventional chemical flocculants.

Key words: Compound bioflocculant; Flocculating activity; Production; Low temperature and low turbidity drinking water.

Flocculants are useful agents in the aggregation of colloids, cells and suspended solids and are commonly used for drinking water production, waste water treatment, fermentation processes and food production[1]. 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. In contrast, bioflocculants, extracellular biopolymeric substances secreted by bacteria, fungi, algae and yeast are biodegradable and nontoxic flocculants[2].

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The study of bioflocculants has attracted considerable scientific and biotechnological attention over the years due to its biodegradability, harmlessness, and lack of secondary pollution[2-4]. Bioflocculants have a great potential to industrial applications[5-7], however, low flocculating capability and large dosage requirement have been a major problem in bioflocculant development for actual wastewater treatment.

For drinking water treatment, flocculation precipitation is the primary method used due to its low cost and established process. Many chemicals flocculants including polyaluminum chloride(PAC) and aluminum sulfate(AS) have been widely used for drinking water treatment because of their high flocculating performance and low cost. However, there is evidence of health problems including Alzheimer's disease and Parkinson's disease[8-10] caused by aluminum salts.

Source Water with low temperature and low turbidity is very difficult to treat in water supply company[11]. The dosage of flocculants significantly increases with the deterioration of coagulation effect at low temperatures, giving rise to a potential safety hazard[12]. In view of these concerns, bioflocculants are attractive alternatives to existing synthetic flocculants such as PAC and AS.

In our previous work, the compound bioflocculant (CBF), which was secreted during the mixed culture of *Rhizobium radiobacter* F2 and *Bacillus sphaeicus* F6, has been found to show an excellent flocculating activity against solid particles and metals aqueous solution[13]. To our knowledge, no previous work describing the compound bioflocculant production and its application in drinking water treatment has been reported to date. In this paper, the compound bioflocculant(CBF) which was produced by mixed culture of bioflocculant-producing microorganisms F2 and F6 was used in the all experiments . A series of experiments was performed to investigate CBF production and its application in low temperature and low turbidity drinking water treatment.

METHODS

Strains

Bioflocculant-producing strains F2 and F6 were originally screened from soil by State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology of China. F2 and F6 were identified as *Rhizobium radiobacter* and *Bacillus sphaeicus* respectively[14].

The stock culture was maintained at 4°C on slant medium which consisted of (per liter) 3 g beef extract, 10 g peptone, 5 g NaCl, and 20 g agar and subcultured every 30 days

Culture media

The composition of production medium was as follows (per liter): 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 . The initial pH was adjusted to 7.2–7.5. All media solutions were prepared with distilled water and sterilized at 115 °C for 30 min.

Bioflocculant production

A set of 250 ml flasks containing 100 ml production medium were used for fermentation. The F2 and F6 strains were separately inoculated from stock cultures into the production mediums and incubated on a shaker (140 r/min) for 24 h at 30°C. In the fermentation experiments, 6.6 ml F2 broth and 3.3 ml F6 broth were inoculated into a flask containing 100 ml production medium together and incubated under the same conditions. The compound bioflocculant used in the study was extracted from fermentation broth of one batch under the same conditions. The fermentation broth obtained was centrifuged (4000g, 30 min) to separate the cells. The cell-free culture supernatant was the liquid bioflocculant, which was used for the analysis of flocculating activity and in experiments of low temperature and low turbidity drinking water treatment. All experiments were performed in triplicates for the mean calculation.

Assay of flocculating activity

Flocculating activity was evaluated by measurement of the turbidity of kaolin suspension based on the method reported by Kurane et al[5]. 1.5 ml of 10% $CaCl_2$ and CBF solution were added into 1.0 L kaolin suspension (5 g/L) in turn, and then pH is adjusted to 7.5 by adding NaOH. The solution was first stirred at 140 r/min for 40 s and then 40 r/min for 160 s. After 20 min settlement, samples were carefully withdrawn from the upper layer for optical density determination at the wave length of 550 nm using a 721 UV spectrometer. A control solution was prepared by replacing CBF solution with distilled water. The flocculating activity was expressed as the following formula:

$$\text{Flocculationg activity (\%)} = (A - B)/A \times 100$$

where A is the optical density of control experiment at550 nm; B is the optical density of at the sample experiment 550 nm.

Each of the experiments was repeated three times and the arithmetic average values were obtained. All the chemicals used in this study were of analytical grade without further purification.

Low temperature and low turbidity drinking water flocculation experiments

Source water with low temperature(0.5-1.0 °C) and low turbidity was collected from the Songhua River. The turbidity and the concentration of Al of the raw water were 10.13 NTU and 0.4434 mg/ L respectively. A sample of 1000 ml raw water

and 10%(w/v) $CaCl_2$ solution were mixed in a 1000 ml beaker. The flocculant was then added to the raw water, and the mixture was vigorously stirred (200 r/min) for 0.5 min and slowly stirred (60 r/min) for 2 min. The mixture was then allowed to stand for 20 min and the supernatant was taken for analysis. The measurements of turbidity and the concentration of Al were determined according to the standard methods issued by the China National Environmental Protection Agency, and the removal efficiency was calculated as follows:

$$\text{Removal efficiency (\%)} = (C_0 - C)/C_0 \times 100$$

where C_0 is the initial value and C is the value after the flocculation treatment.

Orthogonal test of removal of turbidity and Al(III)

Based on the preliminary obtained optimum condition, flocculant dosage, coagulant-aid dosage, pH and sedimentation time were considered as influencing parameterrs. The design of experiment is shown in Table 1

RESULTS AND DISCUSSION

The compound bioflocculant production

The bioflocculant production is affected by many factors, such as the constituents of the culture medium and culture conditions[15-18]. The effects of the key factors, like culture time, carbon source, nitrogen source, C/N ratio, culture temperature, initial pH of the production medium, inoculum size and shaking speed on the flocculating activity of CBF, were investigated with an aim to identify the optimal culture conditions for the CBF production.

Time course of the production of CBF

Fig. 1 shows how the bioflocculant production varies with the growth curve of strains F2 and F6. The flocculating activity reached 93.7% at the early stationary phase (at 24 h) and then flocculating activity slightly increased in stationary phase from 24h to 48h. The flocculating activity reached its maximum flocculating activity(94.7%) at the middle stage of the stationary phase (at 48 h), which is higher than 94.3% of *Serratia ficaria* SF-1 [19], 87.2% of *Aspergillus flavus* IH-7[17]. It indicated that the bioflocculant was produced by biosynthesis during its growth. The pH of the fermentation broth went down smoothly from 7.5 to 6.2 during the logarithm phase and stationary phase, followed by no obvious variation till the end. The reciprocal change of pH and flocculating activity confirmed the production of organic acids in the bioflocculant production[20,21]. To obtain the cost-optimal bioflocculant with high flocculating activity , culture time 24 h was chosen in the following studies.

Effect of carbon source, nitrogen source and C/N ratio on CBF production

The effects of carbon source, nitrogen source and the C/N ratio on CBF production were investigated (Table 2). Table 2 shows the flocculating activity after 24 h cultivation with various carbon sources (sucrose, fructose, lactose, mannitol) replacing glucose at the same concentration. Sucrose, glucose and lactose were favorable carbon sources for CBF production, while the production of CBF was relatively low when fructose and mannitol was added respectively. The highest production of CBF was achieved in glucose medium. Sucrose, glucose and lactose were also reported as the favorable carbon sources in the production of bioflocculant MBFF19[22]. Since glucose was the most preferred and cheap carbon source, it could serve as an appropriate carbon source for CBF production and was used in the following experiments. The effect of nitrogen sources was investigated by cultivating the strains in the same medium, except that the nitrogen source was changed (Table 2). Strains F2 and F6 was able to effectively use organic nitrogen (peptone, yeast extract and urea), inorganic nitrogen (ammonium sulfate and sodium nitrate) as nitrogen sources. Urea was the most cost-effective nitrogen source and used in the following experiments. At a fixed urea concentration of 0.5 g/L, a rapid increase on CBF production was noticed when the C/N ratio was increased up to 20/1 (Table 2), but a further increase in the C/N ratio caused a slight decrease on the production of CBF. That showed lower or higher C/N ratio than 20 would go against CBF production, and then this C/N ratio was chosen for the next experiments.

Effect of the initial pH on CBF production

The effect of the initial pH of the culture medium on CBF production was investigated (Fig. 2). Over the pH range of 6-9, the lowest flocculating activity was 85.36%. The optimal pH for the CBF production was in the range of 7-8, this wide range

of pH should save the amount of acids and alkaline solutions required to adjust pH. When the initial pH was 7.5, the flocculating activity of CBF reached a peak (95.38%). This pH value was chosen as the initial pH in the following studies. Higher pH (above 7) appeared to be more favorable for CBF production. The initial pH of the culture medium determines the electric charge of the cells and the redox potential which can affect nutrient absorption and enzymatic reaction[2]. For different strains, their requirement for the initial pH varied greatly. The alkaline pH, especially pH 9.5, effectively stimulated the flocculant production of *R. erythropolis*[23]. The pH 7.0 was the optimum for TJ-F1 production, which is a neutral pH and can save large numbers of acid and alkali used to adjust pH[18]. The optimal pH for the ZS-7 bioflocculant production was in the range of 6.5-9.0[12].

Effect of temperature, inoculum size and shaking speed on CBF production

The effects of temperature, inoculum size and shaking speed on CBF production was examined (details not shown).When the culture temperature was 30°C, the flocculating activity of CBF reached 96.3% which was maximal flocculating activity in the experiments. When temperature was over 30°C, the flocculating activity of CBF gradually decreased. The optimal temperature for CBF production was 30°C, this temperature was used for the following studies. The metabolism of microorganisms is directly related to culture temperature[18, 24]. Maximum enzymatic activation can only be obtained at an optimum temperature[18].

The flocculating rate of CBF obtained from the cultures media inoculated with 3-15% (v/v) was investigated. The flocculating activity initially increased with inoculum size. At the inoculum size of 10%, the maximum flocculating activity was obtained, which was 97.1%. However, any further increase in inoculum size did not result in any higher flocculating activity. A small inoculum will prolong the stagnant phase, whereas a large inoculum size will make the niche of strains overlap excessively and inhibit the bioflocculant production[2]; As a result, an inoculum size of 10% was used for all subsequent cultures.

The effect of shaking speed on CBF production was also investigated. Shaking speed of 140 r/min was the optimum and flocculating activity was 98.0%. Either higher or lower shaking speed than this speed caused a decrease in the flocculating activity. The shaking speed of 140 r/min was used in the following studies. The shaking speed determines the concentration of dissolved oxygen which can also affect nutrient absorption and enzymatic reaction [2]. The optimum shaking speed for strains F2 and F6 was 140r/min, which was different from the speed listed in various reports[1, 18]. This difference may be because different microorganisms needed the different oxygen demand.

Low temperature and low turbidity drinking water treatment experiments

In this study, low temperature and low turbidity drinking water was used to evaluate the flocculating activity of CBF. Nine sets of

Table 1. Design form of the orthogonal test L9(3^4)

Levels	A Flocculant dosage(ml)	B Coagulant aid dosage(mL)	C pH	D sedimentation time(min)
1	2	0.5	6	10
2	6	1.0	7	20
3	10	1.5	8	30

Table 2. Effects of constituents of the culture medium on CBF production

Carbon sources	glucose	sucrose	fructose	lactose	mannitol
Flocculationg activity (%)	97.0	96.8	92.7	95.4	93.7
nitrogen source	Urea	Yeast extract	$(NH_4)_2$ SO_4	$NaNO_3$	peptone
Flocculationg activity (%)	97.2	96.8	96.3	96.1	95.8
C/N ratio	1/1	5/1	10/1	20/1	30/1
Flocculationg activity (%)	80.1	85.2	89.8	96.9	96.6

experiments were conducted according to the orthogonal design table L9 (3^4), and the experimental results were shown in Table 3.

As shown in Table 3, according to mean value analysis, optimum flocculation condition was the combination of A3B2C3D1, which was flocculant dosage of 10 mL, coagulant aid dosage of 1.0 mL, pH 8.0 and sedimentation time of 10 min. It proved that, under this condition, the removal efficiency of the turbidity was 85.54%. By comparing the range, the influencing factors on the removal efficiency of the turbidity obeyed the following order: C > A > D > B. That is to say, pH value affected mostly, followed by flocculant dosage, sedimentation time and coagulant aid dosage. This is because that the dissociation of flocculant occurs within a certain pH range. Proper pH value could increase the dissociation degree, lead to a higher charge density of flocculant, benefits the spreading of the flocculant molecules, and facilitates the bridging action of the

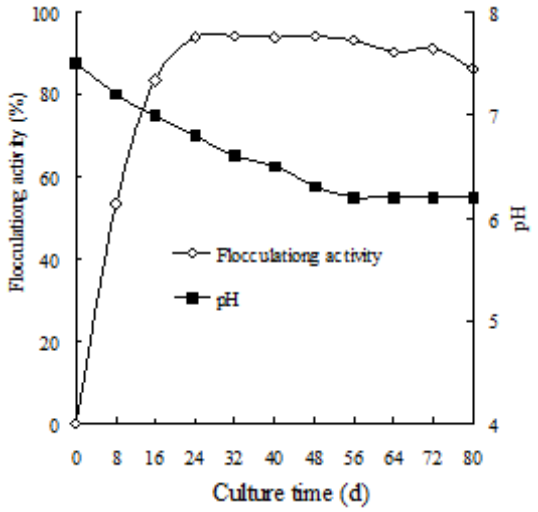

Fig. 1. Growth curve of strains F2 and F6

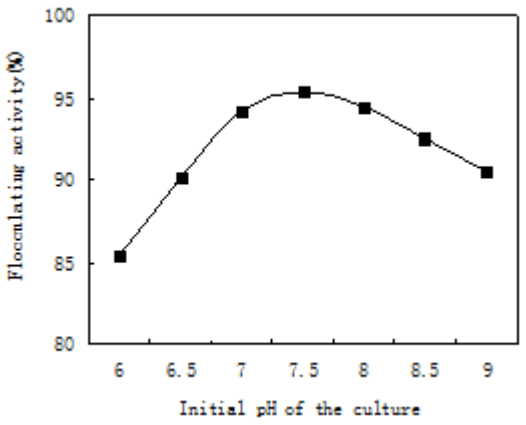

Fig. 2. The effect of initial pH of the culture medium on production of CBF

Table 3. L9(3^4) orthogonal tests and results on removal efficiency of the turbidity and Al (III)

Tests No.		A CBF dosage(ml)	B Coagulant aid dosage(mL)	C pH	D sedimentation time(min)	Removal efficiency of turbidity (%)	Removal efficiency of Al(III) (%)
1		1	1	1	1	2.65	13.21
2		1	2	2	2	63.76	60.34
3		1	3	3	3	65.71	82.45
4		2	1	2	3	63.57	71.47
5		2	2	3	1	83.55	65.70
6		2	3	1	2	5.15	15.57
7		3	1	3	2	70.37	87.52
8		3	2	1	3	9.63	21.60
9		3	3	2	1	80.11	70.69
Removal efficiency of turbidity	Mean Value 1	40.040	45.530	5.810	53.437	Order of the influencing factors:	
	Mean Value 2	50.757	52.313	69.147	46.427	RC > RA > RD > RB	
	Mean Value 3	53.370	50.323	73.210	46.303	Optimum flocculation condition:	
	Range	9.330	6.783	67.400	9.134	A3B2C3D1	
Removal efficiency of Al (III)	Mean Value 1	52.000	57.400	16.793	49.867	Order of the influencing factors:	
	Mean Value 2	50.913	49.213	67.500	54.477	RC > RA > RD > RB	
	Mean Value 3	59.937	56.237	78.557	58.507	Optimum flocculation condition:	
	Range	9.024	8.187	61.764	8.640	A3B1C3D3	

bioflocculant. Thus, pH value plays a critical role[25].

The experimental results of the removal efficiency of CBF on Al(III) were shown in Table 3. The results of range analysis suggest that removal efficiency of Al(III) was influenced by the following factors in the descending order: C >A > D > B . The pH value affected mostly, followed by CBF dosage, sedimentation time and coagulant aid dosage. The results indicated the order of the effect degrees of factors on removal efficiency of Al(III) was same as the order on removal efficiency of the turbidity. That means not only functional groups of CBF, –OH ,–NH, C=O and C–O et al, absorbed Al(III),but the colloidal particles in water absorbed Al(III) also. It was speculated the colloidal particles and Al(III) were removed from water simultaneously and the colloidal particles with Al(III) integrated a whole group under Van der Waals'Force ,charge neutralization and stronger bridging ability, finally precipitating from water by gravity[26]. The optimal factor combination for removal efficiency of Al(III) of low temperature and low turbidity drinking water from the result above was A3B1C3D3, which was CBF dosage of 10 mL, coagulant aid dosage of 1.0 mL, pH 8.0 and sedimentation time of 30 min. Under this condition, the removal efficiency of the turbidity was 89.32%. The results of the treatment at the optimal condition of A3B1C3D3 showed a significant improvement in the water quality. The high removal efficiency of the CBF on Al (III) was because the CBF was characterized to be polysaccharides of high molecular weight which containing abundant chemical groups with chelating ability with metals[13].

CONCLUSIONS

In the present study, strains F2 and F6 producing the compound bioflocculant CBF with high flocculating activity were screened out from soil. The most preferred carbon source,nitrogen source and C/N ratio (w/w) for mixed strains F2 and F6 were glucose, Urea and 20, respectively. The optimal conditions for CBF production were inoculum size 10% (v/v), initial pH 7.5, culture temperature 30°C, and shaking speed 140 r/min for 24 h, under which the flocculating activity of CBF reached 98.0%. The CBF showed good flocculating performance and industrial potential for treatment of low temperature and low turbidity drinking water.The maximal removal efficiencies of turbidity and Al(III) were 85.54% and 89.32% respectively.

These results show a possible use of the compound bioflocculant CBF as an alternative to typically used chemical flocculants for drinking water treatment. Consideration its excellent flocculating activity and harmlessness toward humans and the environment, CBF is expected to be a potential replacement of conventional synthetic flocculants and widely applied in water treatment and downstream processing of food and fermentation industries.

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