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Production and application of a novel bioflocculant by multiple-microorganism consortia using brewery wastewater as carbon source

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Abstract

The flocculating activity of a novel bioflocculant MMF1 produced by multiple-microorganism consortia MM1 was investigated. MM1 was composed of strain BAFRT4 identified as *Staphylococcus* sp. and strain CYGS1 identified as *Pseudomonas* sp. The flocculating activity of MMF1 isolated from the screening medium was 82.9%, which is remarkably higher than that of the bioflocculant produced by either of the strains under the same condition. Brewery wastewater was also used as the carbon source for MM1, and the cost-effective production medium for MM1 mainly comprised 1.0 L brewery water (chemical oxygen demand (COD) 5000 mg/L), 0.5 g/L urea, 0.5 g/L yeast extract, and 0.2 g/L $(NH_4)_2SO_4$. The optimal conditions for the production of MMF1 was inoculum size 2%, initial pH 6.0, cultivating temperature 30°C, and shaking speed 160 r/min, under which the flocculating activity of the MMF1 reached 96.8%. Fifteen grams of purified bioflocculant could be recovered from 1.0 L of fermentation broth. MMF1 was identified as a macromolecular substance containing both protein and polysaccharide. It showed good flocculating performance in treating indigotin printing and dyeing wastewater, and the maximal removal efficiencies of COD and chroma were 79.2% and 86.5%, respectively.

Key words: bioflocculant; multiple-microorganism consortia; brewery wastewater; carbon source; indigotin printing and dyeing wastewater

Introduction

Flocculating agents have been widely used in industrial processes, including wastewater treatment, downstream processing, food, and fermentation process (Seo, 1993; Zhang *et al*., 1999; Salehizadeh and Shojaosadati, 2001). In general, flocculants are classified into three groups: inorganic flocculants, such as aluminum sulfate and polyaluminum chloride; organic synthetic flocculants, such as polyacrylamide derivatives and polyethylene imine; and naturally occurring flocculants, such as chitosan, sodium alginate, and microbial flocculants. Despite the effective flocculating performance and low cost of the synthetic chemical flocculants, their use has resulted in some health and environmental problems. Aluminum has been found to induce Alzheimer's disease (Master *et al*., 1985; Kowall *et al*., 1989). It is evident that the acrylamide monomer is not only neurotoxic and carcinogenic but also nonbiodegradable in nature (Vanhorick and Moens, 1983; Dearfield *et al*., 1988). Bioflocculants produced by microorganisms during their growth are safe and biodegradable polymers (Deng *et al*., 2003). Because of the above alarming concerns, it is believed that the use of bioflocculants will increase (Kurane *et al*., 1986a).

Over the past decades, emphasis with regard to the bioflocculant field was mainly placed on sole microorganisms (Takagi and Kadowaki, 1985; Kwon *et al*., 1996; Toeda and Kurane, 1991; Huang *et al*., 2005). Kurane and Matsuyama (1994) reported that R-3 mixed strains, using starch and glucose (1:1) as carbon source, could produce bioflocculants. Zhu *et al*. (2004) found that the combination of strains F2 and F6 possessed better floccu1ating activity and was higher than that of any pure strains. Flocculating activity and cultivation cost are the major limiting factors with regard to their application (Kurane and Nohata, 1994; Li *et al*., 2003; Jang *et al*., 2001). It is of interest to develop a cost-effective bioflocculant. Some wastes, like soybean juice and fishmeal wastewater, have been used as substrate for flocculant-producing microorganisms (Huang *et al*., 2001; Zhou *et al*., 2003). When yeast extract in the medium of microorganism producing NOC-1 was replaced by bean cake, aquafarm wastewater, or cattle blood, two-thirds of the cultivation cost could be saved (Kurane *et al*., 1994). Because of the presence of nutrient substances, brewery wastewater can possibly be used as a good substrate for certain microorganisms (Chen *et al*., 2003). While there are few reports about

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bioflocculant produced by microorganism using brewery wastewater.

In this article, multiple-microorganism consortia producing bioflocculant of high flocculating activity were constructed, and brewery wastewater was used as its carbon source. The bioflocculant produced by the consortia was applied to the treatment of indigotin printing and dyeing wastewater.

1 Materials and methods

1.1 Microorganisms

Strain BAFRT4, HXCS2, HXTD2, CYGS1, and CYGS4 are all flocculant-producing microorganisms screened by the Laboratory of Nano-Material and Microbiology of Nanchang University, China. These strains were used to construct the multiple-microorganism consortia producing bioflocculant of high flocculating activity. Their stock cultures were all maintained at 4°C on slant media and were subcultured on a monthly basis.

1.2 Media and cultivation conditions

The medium for slant consisted of (per liter): 3 g beef extract, 10 g tryptone, 5 g NaCl, and 20 g agar. The screening medium was composed of (per liter): 20 g glucose, 0.5 g urea, 0.5 g yeast extract, 0.2 g $(NH_4)_2SO_4$, 5 g K_2HPO_4, 2 g KH_2PO_4, 0.1 g NaCl, and 0.2 g $MgSO_4 \cdot 7H_2O$. The production medium contained the same components as the screening medium except glucose, which is replaced by 1 L of brewery wastewater. The chemical oxygen demand (COD) and pH of the wastewater collected from Asia Brewery Co., Ltd. (Nanchang, China) were 30287.6 mg/L and 4.2, respectively. Using NaOH (0.1 mol/L) and HCl (0.1 mol/L), the initial pH of all the media were adjusted to 7.0 except the production medium in which the initial pH was adjusted to 6.0. All media were prepared with distilled water. After fermentation, the pH of the cultivation and the production media were about 5.

1.3 Construction of multiple-microorganism consortia

The stock culture from a slant was inoculated into a 150-ml flask containing 50 ml screening medium and incubated for 72 h in a rotatory shaker at 30°C, 160 r/min. This preculture procedure was then used as the standard inoculum preparation for all experiments. To construct the multiple-microorganism consortia producing bioflocculant of high flocculating activity, two or three strains of the five strains were combined to incubate: 0.5 ml preculture of each strain in the same consortia was inoculated into a 150-ml flask containing 50 ml screening medium. Cultivations were conducted in flasks with a shaking speed of 160 r/min at 30°C for 72 h. The fermentation broth that was obtained was centrifuged (6000×*g*, 30 min) to separate the cells. The multiple-microorganism consortia of cell-free supernatant that showed high flocculating activity were selected for further studies. All experiments were performed in triplicates for the mean calculation.

1.4 Production of bioflocculant

A 150-ml flask containing 50 ml production medium was inoculated with 0.5 ml preculture of each strain in the same multiple-microorganism consortia and incubated at 30°C in a rotatory shaker at 160 r/min for 72 h. The fermentation broth obtained was centrifuged (6000×*g*, 30 min) to separate the cells. The cell-free culture supernatant was the liquid bioflocculant, which was used for the analysis of flocculating activity.

1.5 Assay of flocculating activity

The flocculating activity was measured using a previous method with a slight modification, in which Kaolin clay was chosen as the suspended solid (Kurane *et al*., 1986b; Yokoi *et al*., 1995). 3.0 ml of 1% $CaCl_2$ and 2.0 ml bioflocculant were added to 100 ml Kaolin suspended solution (4.0 g/L) in 100-ml test tube in turn. The mixture was vigorously stirred and was allowed to stand for 5 min. The optical density (OD) of the clarifying solution was measured with a spectrophotometer at 550 nm. A control experiment was prepared using the same method, but the bioflocculant was replaced by the fresh culture medium (*B*). The flocculating activity was calculated according to the equation.

$$\text{Flocculating activity} = \frac{B - A}{B} \times 100\% \quad (1)$$

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

1.6 Identification of strains

The strains were identified according to Bergey's Manual of Systematic Bacteriology (Buchanan and Gibbens, 1984).

1.7 Purification and identification of the bioflocculant

To purify the bioflocculant, viscous fermentation broth was diluted with four volumes of distilled water and centrifuged to remove cells by centrifugal separation (6000×*g*, 30 min), and thereafter the diluted fermentation broth was concentrated to half volume with a rotary evaporator. Three volumes of cold ethanol were then added to the broth. The precipitate obtained was redissolved in distilled water followed by the addition of 2% cetylpyridinium chloride solution (CPC) with stirring. After 2 h, the precipitate collected by centrifugal separation of the bioflocculant and the CPC complex was dissolved in NaCl (0.5 mol/L). Three volumes of cold ethanol were then added to obtain the precipitate, and then it was washed several times with ethanol and the precipitate was vacuum-dried.

The purified native bioflocculant was analyzed for α-amino acids by the ninhydrin reaction, following the procedures of Plummer (1978). Polysaccharide was determined by the anthrone reaction, using the procedures of Chaplin and Kennedy (Chaplin and Kennedy, 1986).

1.8 Indigotin printing and dyeing wastewater treatment using the bioflocculant

Indigotin printing and dyeing wastewater was collected from the overflow of the settling pond at Kaili Group (Nanchang, China). The COD and the pH of the wastewater

were 367.6 mg/L and 9.1, respectively. The adsorption peak of the blue-black wastewater appeared at 660 nm, and the optical density (OD_{660}) was 1.100. The bioflocculant produced by multiple-microorganism consortia grown in the production medium was applied to treat this wastewater. Orthogonal experiments were carried out to optimize the pH, the bioflocculant, and the $CaCl_2$ dosages, which are the major factors affecting the COD and the chroma removal efficiencies. The results obtained were the mean of the results of three independent experiments.

2 Results and discussion

2.1 Multiple-microorganism consortia

Although the screened strains are all flocculant-producing microorganisms, their ability of producing bioflocculant is weaker. By combined cultivation of these strains, the authors of this study attempted to find some multiple-microorganism consortia, which could produce bioflocculant with higher efficiency.

All combined cultivations of two or three strains from the five strains were carried out, and the flocculating activities of their cell-free supernatant were investigated. It was found that the flocculating activities of the bioflocculants produced by multiple-microorganism consortia MM1 (BAFRT4+CYGS1) and MM2 (CYGS4+HXCS2+HXTD2) in the screening medium were 82.9% and 83.3%, respectively. Whereas the flocculating activities of the bioflocculants produced by sole microorganisms BAFRT4, CYGS1, CYGS4, HXCS2, and HXTD2 were 26.2%, 42.4%, 42.9%, 23.3%, and 14.3%, respectively. Obviously, both MM1 and MM2 could produce bioflocculants with higher flocculating activity. The bioflocculants produced by MM1 and MM2 were named MMF1 and MMF2, respectively. When microorganisms holding different niches coexist in the same environment and form the relationship of protocooperation, they will benefit each other and promote the production of high-efficiency bioflocculant (Kurane *et al*., 1994; Zhou and Gao, 2002). However, it is more difficult to screen strains producing bioflocculant with higher efficiency than to screen strains producing bioflocculant with lower efficiency (Kurane and Matsuyama, 1994; Zhu *et al*., 2004). Therefore, the construction of multiple-microorganism consortia indicates a novel method for the study of bioflocculant.

2.2 Factors affecting the bioflocculant production

The bioflocculant production is affected by many factors, such as the constituents of the culture medium and the cultivation conditions (Zhou and Gao, 2002; He *et al*., 2004; Nakata and Kurane, 1999). The effects of the key factors, like the concentration of brewery wastewater, initial pH of the production medium, temperature of cultivation, shaking speed, and inoculum size, on the flocculating activity of the bioflocculant by multiple-microorganism consortia were investigated.

2.2.1 Effect of the concentration of brewery wastewater on bioflocculant production

As brewery wastewater was used as the carbon source in the production medium, its concentration would affect bioflocculant production. As shown in Fig.1, the flocculating activity of the bioflocculant by either consortia considerably varied with the concentration, and the average flocculating activity of the MMF1 was apparently higher than that of the MMF2. Compared with MM2, MM1 was more adaptable to secrete flocculant in the production medium. When COD of the brewery wastewater in the production medium was 5000 mg/L, OD_{550} of the Kaolin clarifying solution lowered from 0.640 to 0.225, and the flocculating activity of MMF1 reached a peak. The production medium with lower concentration of brewery wastewater cannot meet nutrient need of MM1, whereas the production medium with higher strength of brewery wastewater may contain higher concentration of inhibitors for MM1 (Chen *et al*., 2003). Therefore, MM1 was chosen as the object for studying multiple-microorganism consortia in the production medium, and 5000 mg/L brewery wastewater was used in the following studies.

2.2.2 Effect of initial pH on the bioflocculant production

The effect of initial pH of the production medium on the flocculating activity of MMF1 was investigated (Fig.2). When the initial pH of the production medium was 6.0, OD_{550} of the Kaolin clarifying solution declined to 0.084, and the flocculating activity of MMF1 reached a peak. The initial pH of the production medium determines the electric charge of the cells and the oxidation-reduction potential, which can affect absorption of nutrients and enzymatic reaction of MM1 (Salehizadeh and Shojaosadati, 2001; Nakata and Kurane, 1999). pH 6.0 was chosen as the initial pH in the following studies.

2.2.3 Effect of cultivating temperature on the bioflocculant production

Different cultivating temperature was set to study its effect on the flocculating activity of MMF1 (Fig.3). When

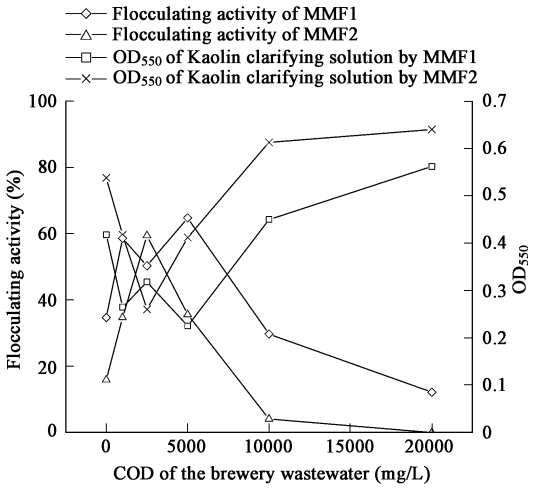

Fig. 1 Effect of strength of brewery wastewater on the flocculating activity of bioflocculant.

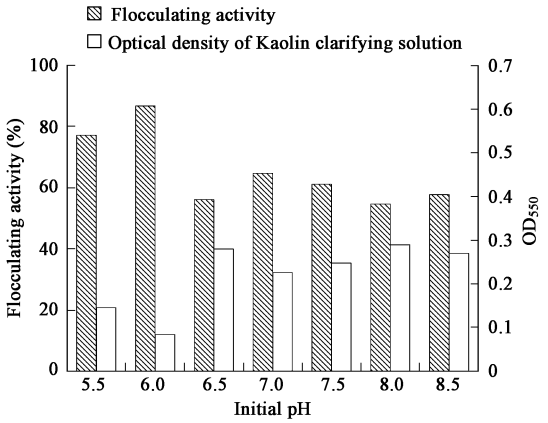

Fig. 2 Effect of initial pH of the production medium on the flocculating activity of MMF1.

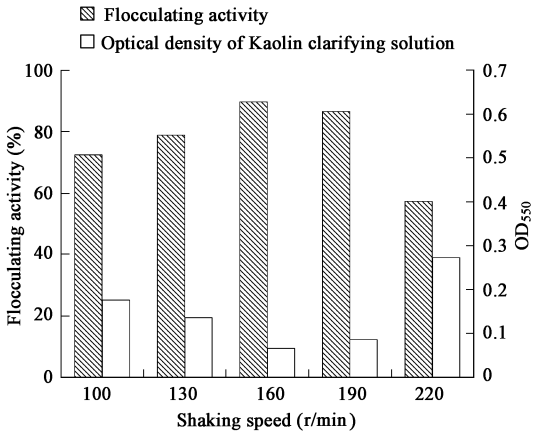

Fig. 4 Effect of shaking speed on the flocculating activity of MMF1.

the cultivating temperature was 30°C, OD_{550} of the Kaolin clarifying solution was 0.066, and the flocculating activity of MMF1 was up to 89.7%. The metabolism of microorganisms has direct relationship with cultivating temperature. Maximum enzymatic activation can be obtained at optimal temperature (Nakata and Kurane, 1999). The optimal temperature for MMF1 production was 30°C, which was used in the following studies.

2.2.4 Effect of shaking speed on the bioflocculant production

The effect of shaking speed on MMF1 production is shown in Fig.4. It can be seen that the shaking speed of 160 r/min was the most preferred. Either higher or lower shaking speed than 160 r/min caused a decrease in the flocculating activity, because the shaking speed determines the concentration of the dissolved oxygen, which can also affect the absorption of nutrients and enzymatic reaction of MM1 (Salehizadeh and Shojaosadati, 2001). The optimal shaking speed of 160 r/min was used in the following studies.

2.2.5 Effect of inoculum size on the bioflocculant production

The strains BAFRT4 and strain CYGS1 were designed such that they have similar inoculum sizes, and the effect of inoculum size on bioflocculant production is shown in Fig.5. When the inoculum size of each strain was 0.5 ml preculture per 50 ml production medium, that is to say, when the inoculum size of each strain was 1% (v/v), the flocculating activity of MMF1 was up to 75.6%. A small inoculum will prolong the stagnant time, whereas a large inoculum will make niche of MM1 overlap excessively and restrain the bioflocculant production (Salehizadeh and Shojaosadati, 2001).

2.2.6 Orthogonal experiments for the bioflocculant production

On the basis of experiments of single factors, the orthogonal experiments ($L_9(3^3)$) for MMF1 production were carried out (Table 1). The optimal cultivation condition for MMF1 production induced by the experiments was listed as follows. In terms of the inoculum size of 2% (v/v), the precultures of the two strains were inoculated into the production medium (pH 6.0). The inoculated medium was cultivated in a rotatory shaker at 30°C, 160 r/min for 72 h. Under this condition, OD_{550} of the Kaolin clarifying solution dropped to 0.020, and the flocculating activity of the MMF1 reached up to 96.8%.

The flocculating activities of MMF1 isolated from the screening medium and from the production medium were 82.9% and 96.8%, respectively. This indicates that it is

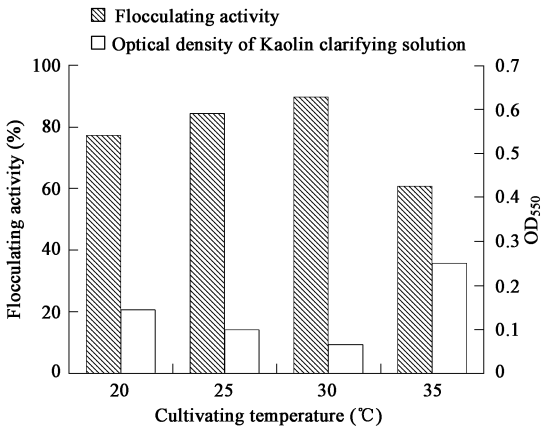

Fig. 3 Effect of cultivating temperature on the flocculating activity of MMF1.

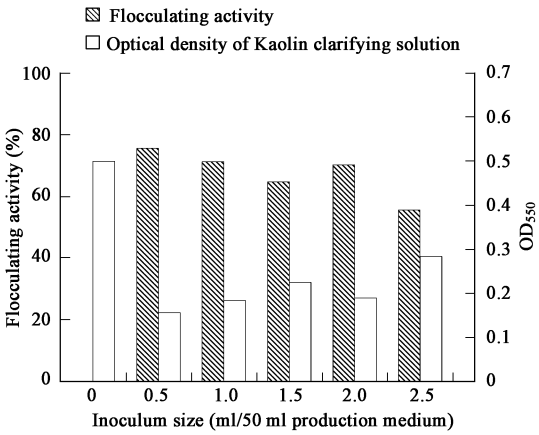

Fig. 5 Effect of inoculum size on the flocculating activity of MMF1.

Table 1 Orthogonal experiments of cultivation conditions

	COD of brewery wastewater (mg/L)	Inoculum size (%)	Initial pH	Flocculating activity (%)
1	1000	1	6.0	67.2
2	1000	2	6.5	50.3
3	1000	3	7.0	56.6
4	2500	1	6.5	44.1
5	2500	2	7.0	56.9
6	2500	3	6.0	68.1
7	5000	1	7.0	67.8
8	5000	2	6.0	96.8
9	5000	3	6.5	64.1
$\overline{k}_1$	58.0	59.7	75.0	
$\overline{k}_2$	56.4	65.6	52.8	
$\overline{k}_3$	73.8	62.9	60.4	
R	17.4	5.9	22.2	

$\overline{k}_i$ (i=1, 2, 3) refers to the average of the flocculating activity of the same level in the same column; R indicates the varying range of $\overline{k}_i$ in the same column, equal to the maximal value of $\overline{k}_i$ minus the minimal value of $\overline{k}_i$.

feasible to replace glucose with brewery wastewater as carbon source of MM1, which can realize resource reuse of the wastewater. Accordingly, brewery wastewater acts as a novel carbon source for flocculant-producing microorganisms.

2.3 Identification of strains

Scanning electron microscopic (SEM) images of strain BAFRT4, strain CYGS1, and multiple-microorganism consortia MM1 are shown in Fig.6. Strain BAFRT4 was ball-shaped with a flagellum, and had a diameter of approximately 1.2 μm and was found to be a facultative aerobe. Strain CYGS1 was rod-shaped with a flagellum and had a size of approximately (0.9–2.4) μm × (0.4–0.6) μm and was found to be a facultative aerobe. Some of the biochemical and physiological characteristics of the bacteria are shown in Table 2.

For comparison, the known strain *Staphylococcus* sp. and strain *Pseudomonas* sp. were also cultivated under the same culture condition, and the characteristics of their colonies were similar to those of the strain BAFRT4 and the strain CYGS1, respectively. According to the results, strain BAFRT4 and strain CYGS1 were identified as *Staphylococcus* sp. and *Pseudomonas* sp., respectively.

As shown in Fig.6c, after combined cultivation, the biomass of the multiple-microorganism consortia MM1 increased obviously and strain CYGS1 exhibited the advantage of high productivity. It may be explained that strain BAFRT4, during the combined cultivation, could use the nutrients in the culture first, and continuously provide more nutrients that favored the growth of strain CYGS1; on the contrary, strain CYGS1 could provide new nutrients for Strain BAFRT4. Therefore, both of them grew well under the new nutritional condition and produced bioflocculant MMF1 with high efficiency.

2.4 Purification and identification of the bioflocculant

Fifteen grams of purified MMF1 was recovered from 1 L of fermentation broth. The ninhydrin reaction produced blue-purple compounds, which indicated that the bioflocculant contained protein. The anthrone reaction produced green compounds, which indicated that the bioflocculant contained polysaccharide. So MMF1 is a macromolecular substance containing both protein and polysaccharide. Several types of bioflocculants have been reported including polysaccharides, proteins, lipids, glycolipids, and glycoproteins (Kurane and Matsuyama, 1994; Zhang *et al*., 2002). Therefore, the protein and the polysaccharide found in MMF1 may explain its high flocculating activity.

The productivity of the bioflocculant is one of the factors affecting its industrial application (Master *et al*., 1985). Many articles have reported the yield of bioflocculants by sole microorganisms. The final concentration of bioflocculant WF-1 produced by Enterobacter aerogenes W-23 was 1.3 g/L (Lu *et al*., 2005). About 4 g/L of purified biopolymer EPS450 by *Bacillus* sp. I-450 was obtained (Kumar *et al*., 2004). In this study, the yield of MMF1 by

Table 2 Biochemical and physiological characteristics of strain BAFRT4 and strain CYGS1

No.	Subject	Strain	
		BAFRT4	CYGS1
1	Gram stain procedure	–	–
2	Capsule stain procedure	+	–
3	Spore stain procedure	–	–
4	Catalase production	+	+
5	Glucose fermentation	Acid, gas	Acid, gas
6	Methyl red reaction	+	–
7	Starch hydrolysis	–	–
8	Levan production	–	+
9	Gelatin liquefaction	–	–

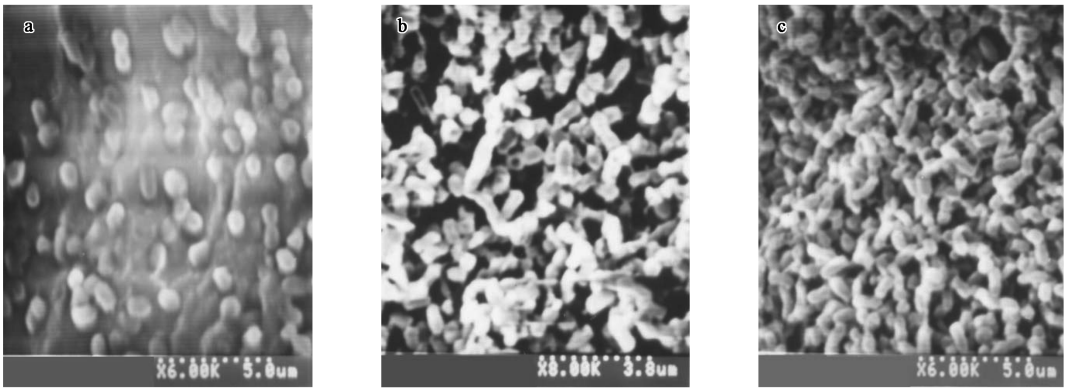

Fig. 6 SEM images of (a) strain BAFRT4 (×6000), and (b) strain CYGS1 (×8000), and (c) MM1 (×6000).

multiple-microorganism consortia was up to 15 g/L, which was markedly higher than those reported in the literature. The high yield of MMF1 can meet the need for wide application of bioflocculant.

2.5 Application of the bioflocculant

MMF1 obtained under the optimal condition was applied to treat indigotin printing and dyeing wastewater (Table 3). The optimal processes of the bioflocculant treatment of the wastewater were deduced from the table. The optimal process for removing the COD and the chroma was as follows: 3.5 ml 1% $CaCl_2$ and 1.7 ml MMF1 were added to 100 ml indigotin printing and dyeing wastewater in 100-ml test tube, and pH of the system was adjusted to 9.0. The mixture was then vigorously stirred and allowed to settle for 10 min. An aliquot of the clarifying solution was withdrawn from the test tube, and the values of its COD and OD_{660} were 76.5 mg/L and 0.149, respectively. The effluent reached the second-grade discharge standard for municipal wastewater treatment plant.

Table 3 Orthogonal experiments of the bioflocculant treating indigotin printing and dyeing wastewater

	Bioflocculant dosage (ml)	1%$CaCl_2$ dosage (ml)	pH of the system	Removal efficiency (%) COD	Chroma
1	0.9	2.5	8.0	41.8	32.3
2	0.9	3.0	8.5	64.8	82.9
3	0.9	3.5	9.0	74.4	73.8
4	1.3	2.5	8.5	63.3	76.5
5	1.3	3.0	9.0	65.0	77.2
6	1.3	3.5	8.0	64.6	75.2
7	1.7	2.5	9.0	63.3	76.9
8	1.7	3.0	8.0	69.1	86.7
9	1.7	3.5	8.5	64.4	83.1
$\overline{k}_1$	60.3/63.0	56.1/61.9	58.5/64.7		
$\overline{k}_2$	64.3/76.3	66.3/82.3	64.2/80.8		
$\overline{k}_3$	65.6/82.2	67.8/78.0	67.6/76.0		
R	5.3/19.2	11.7/20.4	9.1/16.1		

As for $\overline{k}_i$ (i=1, 2, 3) and R, the values front "/" refer to removal efficiency of COD; back refer to removal efficiency of chroma.

Because of the presence of complex compounds, the biodegradation and discoloration of indigotin printing and dyeing wastewater is difficult (Crini, 2006). Utilization of MMF1 in the wastewater treatment could further testify its flocculating performance. The results showed that MMF1 produced from brewery wastewater medium was an efficient agent for the treatment of indigotin printing and dyeing wastewater. The maximum removal efficiencies of the COD and the chroma were up to 79.2% and 86.5%, respectively. As the bioflocculant contains protein and polysaccharide, which are macromolecules with a long molecular chain and many active radicles, it can be assisted by Ca^{2+}, and neutralize the negative charge to absorb colloids. Then, bridging mechanisms occur, leading to the formation of three-dimensional flocs, which can settle fast. Thus, MMF1 exhibits good flocculating capability.

3 Conclusions

In the present study, multiple-microorganism consortia MM1 were constructed to produce bioflocculant MMF1 with high efficiency; brewery wastewater was successfully used as its carbon source. The yield of MMF1 was up to 15 g/L, markedly higher than those reported in other literature. Protein and polysaccharide were simultaneously detected in the bioflocculant. The application of MMF1 to the treatment of indigotin printing and dyeing wastewater was also studied. The results showed, when 3.5 ml of 1% $CaCl_2$ and 1.7 ml of MMF1 were added to 100 ml indigotin printing and dyeing wastewater in 100-ml test tube, at pH of 9.0, the maximum removal efficiencies of COD and chroma were up to 79.2% and 86.5%, respectively. From this study, it can be found that brewery wastewater can not only provide an effective carbon source for the flocculant-producing microorganisms but also realize its resource reuse.

Further studies on the mechanism of multiple-microorganism consortia and scaling up of the cultivation process are being conducted to enhance the bioflocculant production and realize its industrial utilization.

References

Buchanan R E, Gibbens N E, 1984. Bergey's manual of systematic bacteriology[M]. 8th ed. Beijing: Science Press.

Chaplin M F, Kennedy J F, 1986. Carbohydrate analysis: a practical approach[M]. Washington, DC: IRL Press.

Chen Y P, Fu Y S, Li X M, 2003. Characteristics and treatment of brewery wastewater[J]. Pollution Control Technology, 16(4): 146–149.

Crini G, 2006. Non-conventional low-cost adsorbents for dye removal: A review[J]. Bioresource Technol, 97: 1061–1085.

Dearfield K L, Abermathy C O, Ottley M S *et al*., 1988. Acrylamide: its metabolism, developmental and reproductive effects, genotoxicity, and carcinogenicity[J]. Mutation Res, 195: 45–77.

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

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

Huang M S, Shen R H, Xia J, 2001. Microbial flocculates: production and application on wastewater treatment[J]. Journal of Shanghai University (Natural Science), 7(3): 244–248.

Huang X W, Cheng W, Hu Y Y, 2005. Screening of flocculant-producing strains by NTG mutagenesis[J]. J Environ Sci, 17(3): 494–498.

Jang J H, Michihiko I, Shin M K, 2001. Production of a novel bioflocculant by fed-batch culture of *Citrobacter* sp.[J]. Biotechnol Lett, 23: 593–597.

Kowall N W, Pendleury W W, Kessler J B *et al*., 1989. Aluminium-induced neurofibrillary degeneration affects a subset of neurons in rabbit cerebral cortex, basal forebrain and upper brain stem[J]. Neuroscience, 29: 329–337.

Kumar C G, Joo H S, Choi J W *et al*., 2004. Purification and characterization of an extracellular polysaccharide from haloalkalophilic *Bacillus* sp. I-450[J]. Enzyme Microb Tech, 34: 673–681.

Kurane R, Takeda K, Suzuki T, 1986a. Screening and characteristics of microbial flocculants[J]. Agric Biol Chem, 50: 2301–2307.

Kurane R, Toeda K, Takeda K *et al*., 1986b. Culture condition for production of microbial flocculant by *Rhodococcus erythropolis*[J]. Agric Biol Chem, 50: 2309–2313.

Kurane R, Hatamochi K, Kakuno T *et al*., 1994. Production of a bioflocculant by *Rhodococcuse erythropolis* S-1 grown on alcohols[J]. Biosci Biotechnol Biochem, 58(2): 428–429.

Kurane R, Matsuyama H, 1994. Production of a bioflocculant by mixed culture[J]. Biosci Biotechnol Biochem, 58(9): 1589–1594.

Kurane R, Nohata Y, 1994. A new water-absorbing polysaccharide from *Alcaligenes latus*[J]. Biosci Biotechnol Biochem, 58(2): 235–238.

Kwon G S, Moon S H, Hong S D *et al*., 1996. A novel flocculant biopolymer produced by *Pestalotiopsis* sp. KCTC-8637[J]. Biotechnol Lett, 18: 1459–1464.

Li Y, He N, Guan H *et al*., 2003. A novel polygalacturonic acid bioflocculant REA-11 produced by *Corynebacterium glutamicum*: a proposed biosynthetic pathway and experimental confirmation[J]. Appl Microbiol Biot, 63: 200–206.

Lu W Y, Zhang T, Zhang D Y *et al*., 2005. A novel bioflocculant produced by *Enterobacter aerogenes* and its use in defecating the trona suspension[J]. Biochem Eng J, 27: 1–7.

Master C L, Multhaup G, Simms G *et al*., 1985. Neuronal origin of a cerebral amyloid: neurofibrallary tangles of Alzheimer's disease contain the same protein as the amyloid of plaque cores and blood vessels[J]. EMBO Journal, 4: 2757–2763.

Nakata K, Kurane R, 1999. Production of an extracellular polysaccharide bioflocculant by *Klebsiella pneumoniae*[J]. Biosci Biotechnol Biochem, 63(12): 2064–2068.

Plummer D T, 1978. An introduction to practical biochemistry, 2nd edn[M]. London: McGraw-Hill.

Salehizadeh H, Shojaosadati S A, 2001. Extracellular biopolymeric flocculants: Recent trends and biotechnological importance[J]. Biotechnol Adv, 19: 371–385.

Seo H, 1993. Bioflocculant production from *Bacillus* sp. A56[J]. Sanop Misaengmul Hakhoechi, 21: 486–493.

Takagi H, Kadowaki K, 1985. Poly galactosamine produced by a microorganism[J]. Chitin Nat Technol, 3: 121–128.

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

Vanhorick M, Moens W, 1983. Carcinogen of acrylamide[J]. Carcinogenesis, 4:1452–1463.

Yokoi H, Natsuda O, Hirose J *et al*., 1995. Characteristics of biopolymer flocculant produced by *Bacillus* sp. PY-90[J]. J Ferment Bioeng, 79: 378–380.

Zhang T, Lin Z, Zhu H L, 1999. Microbial flocculant and its application in environmental protection[J]. J Environ Sci, 11: 1–12.

Zhang J, Liu Z, Wang S *et al*., 2002. Characterization of a bioflocculant produced by the marine myxobacterium *Nannocystis* sp. NU-2[J]. Appl Microbiol Biot, 59: 517–522.

Zhou Q X, Gao T Y, 2002. Microbiology of environmental engineering[M]. 2nd ed. Beijing: Higher Education Press.

Zhou X, Wang J, Zhou J T, 2003. Studies on properties of a bioflocculant produced by *Pseudomonas* sp. GX4-1 in fish meal wastewater[J]. Research of Environmental Sciences, 16(3): 31–34.

Zhu Y B, Feng M, Yang J X *et al*., 2004. Screening of complex bioflocculant producing bacterium and their flocculating mechanism[J]. Journal of Harbin Institute of Technology, 36(6): 759–762.