Chapter 2

Culture Condition Effect on Bioflocculant Production and Actual Wastewater Treatment Application by Different Types of Bioflocculants

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Abstract

The effect of culture condition on different types of bioflocculant production and its application on actual wastewater treatment were studied in this chapter. The advantages of mixed strain HXJ-1 were as follows: directly using acidic wine wastewater, adapting to wastewater at high concentrations and the presence of less nitrogen. HXJ-1 achieved good flocculating rate when the chemical oxygen demand (COD) was 12,000 mg/L, C/N 20:1. Three kinds of bioflocculants had some good treatment results on starch wastewater, printing and dyeing wastewater and landfill leachate. The treatment effect of XJBF-1 (produced by mixed strain HXJ-1) on the starch wastewater was better than that of traditional polyacrylamide and other bioflocculants produced by a single bacterial (X15BF-1) and yeast strain (J1BF-1). XJBF-1 had better treatment results on three types of wastewater. It also had good removal rate of chromaticity, especially on the starch wastewater , the printing and dyeing wastewater; the removal rate was up to 88%, and the starch wastewater COD removal rate was up to 86%.

Keywords: Alcoholic wastewater, bioflocculant, mixed strains, culture condition, actual wastewater treatment

1. Introduction

Microbial flocculent, the secondary metabolites with flocculating activity and produced by microorganisms [1], is a new water treatment agent with efficient, safe, natural degradation characteristic to flocculate and sediment the solid suspended particles and colloidal particles which are not easily degradable in water [2, 3]. Most bioflocculants are still in the developmental stage and under research because of the high cost of the culture medium and the lack of high production of flocculating engineering fungus. Therefore, the synthesis of microbial flocculants from inexpensive carbon source showed its potential benefit since it could address the critical problems of substrate costs [4]. The use of industrial wastewater, such as soy sauce wastewater, and biological hydrogen production waste as an alternative medium to reduce the cost of production of microbial flocculants has been reported in recent years [5, 6]. China is one of the largest alcoholic beverage-producing and -consuming countries; nutrients in the alcoholic wastewater might be used as free resources for microorganism growth and synthesis of bioflocculants to reduce the cost of culture medium, and the pollutant in the wastewater might also be reduced after it is utilized by microorganisms. Therefore, the idea 'use waste to treat waste' may realize material recycling and will benefit the environment and society. In addition, *Bacillus cereu*s, as feed additives, is widely used in feed production, and a symbiosis relationship was reported between *Bacillus cereu*s and yeast [7]. *Bacillus cereu*s has a strong resistance to adverse environments and is fast growing, and yeasts are widely used in food and wine industry; so it should have good adaptability to wine, but its growth rate is usually slower compared with bacteria. The synergy between different microorganisms has the potential benefit to adapt to a wider range and promote the production of flocculants.

Only a few references on actual wastewater treatment by bioflocculant could be found at present [8]. In order to put bioflocculant into use on actual wastewater treatment, based on previous study [9, 10], different culture conditions on single strains and mixed strains were studied in this chapter. Three kinds of bioflocculants produced by different strains were used in actual wastewater treatment. These results will provide reference for complex bioflocculant research and future applications.

2. Materials and Methods

2.1. Materials

2.1.1. Strains

The flocculant-producing strains used in the experiments were isolated and selected from activated sludge from Chengdu wastewater treatment plant. They were primarily identified to be *Bacillus cereus* and *Pichia membranifaciens* according to morphological and genetic sequence identification, and then kept in the Lab.

2.1.2. Wastewater quality parameters

Wastewater came from Chengdu alcoholic production plant. The water quality parameters are reducing sugar 20.80 g/L, chemical oxygen demand (COD) concentration of 90,000 mg/L and PH3.6.

2.1.3. Wastewater fermentation medium

Appropriate dilution of alcoholic wastewater and pH adjustment according to the testing requirements, adding nitrogen (urea as the nitrogen source) according to a certain C/N ratio, and sterilization at 121℃ after 30 min were performed.

2.1.4. Actual wastewater for treatment

The landfill leachate: from Chengdu landfill plant. The water quality parameters: COD concentration: 1944 mg/L, turbidity: 1440 degrees, chromaticity: 512 times, SS: 11.04 g/L and pH: 6.5.

The starch wastewater: from Chengdu medicine production plant. The water quality parameters: COD concentration: 9660 mg/L, turbidity: 2098 degrees, chromaticity: 320 times, SS: 1.094g/L and pH: 2.3.

The printing and dyeing wastewater: from Chengdu textile plant. The water quality parameters: COD concentration: 760 mg/L, turbidity: 165 degrees, chromaticity: 1200 times, SS: 0.348g/L and pH: 8.7.

2.2. Methods

2.2.1. Measurement of flocculating activity

The flocculating activity was measured using the previous method [11] with a slight modification [12]. Ninety-three mL Kaolin suspension (1g/L), 5 mL of 1% $CaCl_2$ solution and 2 mL of bioflocculant were taken in a 200 mL beaker, and the pH was adjusted to 7.0. Then the beaker was placed in an electric mixer for 250 r/min of fast stirring for 1 min, 60 r/min of slow stirring for 2 min and then kept unstirred for 15 min at room temperature. The supernatant was carefully transferred to a utensil, and the optical density of the supernatant (OD_{550}) was measured by spectrophotometer, keeping an equal volume of distilled water as a control. The flocculating rate (FR) indicates the flocculating activity, which is calculated as follows:

$$FR = \frac{A-B}{A} \times 100\%$$

where A is the absorbance at 550 nm of control and B is the absorbance at 550 nm of treatment.

2.2.2. The effects of culture conditions on microbial flocculant production and flocculating activity

Ten percent relative inoculum size (V/V cell concentration: 10^8/L) *Bacillus cereus, Pichia membranifaciens* and mixed strains of these 1:1 (V/V) (referred to as HXJ-1) were seeded in different wastewater concentrations (COD concentration), different C/N ratios, initial pH of wastewater fermentation medium, shaking speed of 120 r/min and at a temperature of 30℃. After fermentation for 24 h, 10 mL of fermentation broth by centrifugation was taken, and the flocculating rate to kaolin suspension was measured from the supernatant. The flocculating rate was used to investigate the effect of wastewater concentration, C/N ratio and initial pH value of *Bacillus cereus, Pichia membranifaciens* and HXJ-1-producing flocculants.

2.2.3. Different bioflocculants applications on actual wastewater treatment

After 93 mL starch wastewater, printing and dyeing wastewater and landfill leachate were poured into three 200 mL beakers respectively, 5 mL of 1% $CaCl_2$ solution and 2 mL of four different types of flocculants were added in each of the 200 mL beaker, and the pH was adjusted to 7.0. Then the beaker was placed in an electric mixer for 250 r/min of fast stirring for 1 min and 60 r/min of slow stirring for 2 min and then kept unstirred for 15 min at room temperature. Variations in wastewater COD, turbidity, chromaticity and SS were measured before and after the treatment. The COD speed-measuring device was used to measure COD [13], the spectrophotometric method was used to measure turbidity [14], the dilution factor method was used to measure chromaticity [15] and the constant weight method was used to measure SS [14].

3. Results and discussion

3.1. The effect of wastewater concentration on bioflocculant production

The wastewater concentration of the fermentation liquid with the effect of different microbial flocculant production is shown in Figure 1. *Bacillus cereus* could grow and produce bioflocculants in the wastewater at a low COD concentration, which was corresponding to the characteristics of low nutritional requirements of *Bacillus cereus* [7]. Higher COD concentrations were suitable for the growth of *Pichia membranifaciens* and flocculant production; the growth and bioflocculant production of HXJ-1 needed more carbon source; therefore, HXJ-1 reached a maximum flocculating rate of 90.0% at a higher COD concentration (COD concentration of 12,000 mg/L). However, if COD concentrations are higher than 12,000 mg/L, the lack of oxygen may cause an incomplete substrate oxidation and a large amount of acidic substance accumulation, thereby affecting the physiological activity of microorganisms, as well as reducing the capacity of producing flocculants. Other results also could be seen from Figure 1, which showed that HXJ-1 had the adaptability to higher wastewater concentration and better flocculating activity than a single strain. This result suggested that during flocculant production, yeast fermentation could quickly reduce the COD concentration in the fermentation broth, so that the role of bacteria is enhanced. *Bacillus cereus* and *Pichia membranifaciens* adjusted to niche separation to avoid disorderly competition; hence, it is possible not only to fully use the organic materials in wastewater and improve the synthetic efficiency of the flocculants but also to improve the utilization efficiency of the wastewater to achieve the purpose 'use waste to treat waste' by the utilization of alcoholic wastewater.

3.2. The effect of C/N on bioflocculant production

The effect of C/N in the fermentation broth on bioflocculant production by different strains is shown in Figure 2. It could also be seen that the flocculating rate of HXJ-1 increased rapidly

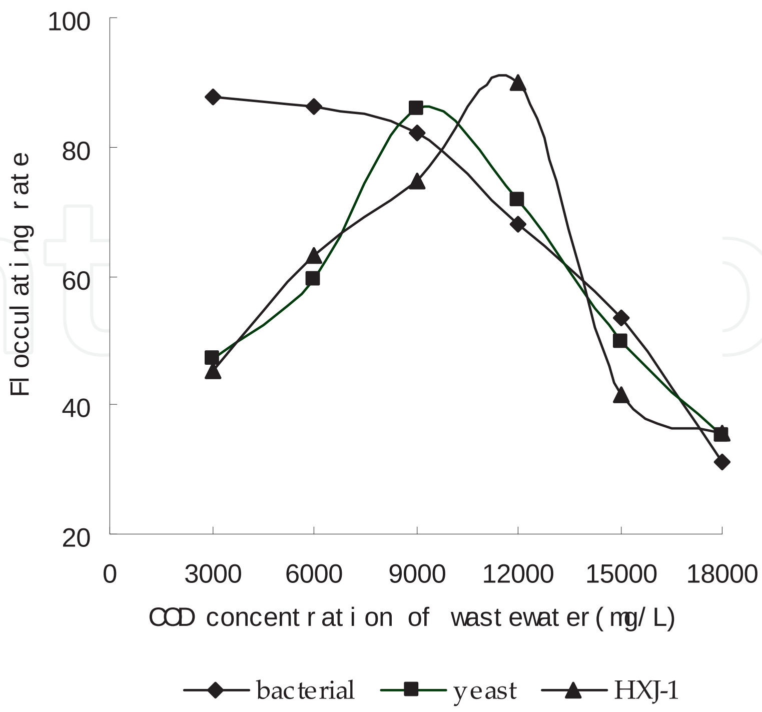

Figure 1. The effect of COD on the flocculating rate of different strains.

with the increasing C/N ratio and achieved the maximum rate 89.3% at C/N 20:1 and then declined slightly. *Pichia membranifaciens* achieved the maximum flocculating rate at C/N 20:1 and then its rapid decline started. C/N had a less effect on the bioflocculant production of *Bacillus cereus*, which achieved the maximum flocculating rate at C/N 15:1 and C/N 10:1–30:1 and could maintain a good rate of flocculation; the results were in line with the strong resistance characteristics of *Bacillus cereus* to adverse environmental conditions [7]. The flocculating rate of *Pichia membranifaciens* decreased rapidly when C/N > 20:1 and indicated that the N source demand of *Pichia membranifaciens* was greater than that of *Bacillus cereus* and HXJ-1. The following results could be observed: HXJ-1 had the same lower demand for N source characteristics as that of *Bacillus cereus*; good flocculating effect was observed when C/N > 20:1; hence, a favourable benefit condition had been set up to reduce the cost of bioflocculant production by mixed strains.

3.3. The effect of initial medium pH on bioflocculant production

The effect of initial pH of alcoholic wastewater fermentation medium on bioflocculant production by different strains is shown in Figure 3. The flocculating rate of *Bacillus cereus* increased with the increase in initial pH and achieved the maximum at pH 7.6; the flocculating rate of *Pichia membranifaciens* was the highest at pH 3.6, and it showed a downward trend with an increase in pH. The flocculating rate of HXJ-1 with the trend of change in pH was more complex than that of a single strain. A good flocculating effect appeared either in acidic or

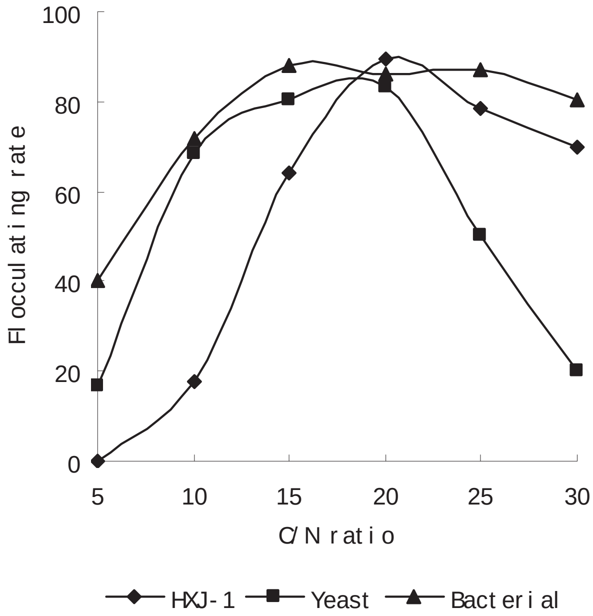

Figure 2. The effect of C/N on the flocculating rate of different strains.

alkaline conditions, but the flocculating rate was low at pH 4.0–6.0. This may be due to conducive acidic conditions for the growth and reproduction of *Pichia membranifaciens* in HXJ-1, in which *Pichia membranifaciens* played a major role, while in the alkaline conditions, *Bacillus cereus* played a major role. However, the flocculant synthesis enzyme of *Bacillus cereus and Pichia membranifaciens* had different activities in the optimum pH range, pH 4.0–6.0, which were not conducive to flocculant production, leading to a reduction in the flocculating rate. The observation of the strain number in the HXJ-1 fermentation broth showed that 82.8% strains were of *Pichia membranifaciens* with an initial pH of 3.6, but 71.6% of the strains were *Bacillus cereus* when the initial pH was 7.6 in the fermentation medium. The observation results further confirmed the correctness of the above inference.

3.4. Treatment effect of different flocculants on the starch wastewater

The starch wastewater normally has a high COD concentration and turbidity. Its main component of starch wastewater is starch, protein and carbohydrate. The results of starch wastewater treatment of four different bioflocculants are shown in Table 1. The COD removal rate was very high, reaching 81% and 86% by X15BF (produced by bacterial strains) and XJBF-1 (produced by mixed strains of bacteria and yeast) respectively, much higher than that of polyacrylamide (PAM) removal rate of COD. At the same time, the removal rate of SS in the starch wastewater and three kinds of microbial flocculants was equal to PAM but was quite superior to turbidity and chromaticity removal in the starch wastewater compared with PAM. The results also showed that the removal rates of all indicators in the starch wastewater by XJBF-1 were the highest, and excellent wastewater treatment results were achieved compared

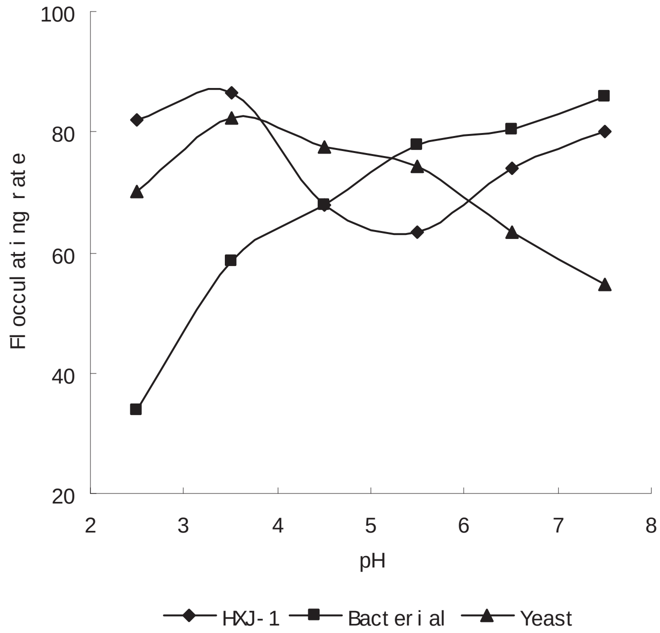

Figure 3. The effect of initial pH on the flocculating rate of different strains.

with the traditional flocculant and other two kinds of bioflocculants in starch wastewater treatment.

Type of flocculants	Water quality before treatment				Water quality after treatment				Removal rate (%)			
	COD mg/L	T NTU	C degree	SS g/L	COD mg/L	T NTU	C degree	SS g/L	COD mg/L	T NTU	C degree	SS g/L
J1BF	9660	2098	320	0.194	4021	966	136	0.128	58	54	57	34
X15BF	9660	2098	320	0.194	1780	856	98	0.124	81	59	69	36
XJBF-1	9660	2098	320	0.194	1350	713	36	0.114	86	66	88	41
PAM	9660	2098	320	0.194	4108	1011	120	0.125	59	55	62	36

J1BF: bioflocculant produced by yeast

X15BF: bioflocculant produced by bacteria

XJBF-1: bioflocculant produced by mixed strains (bacteria and yeast V/V 1:1)

PAM: polyacrylamide

T: turbidity

Table 1. Treatment results of starch wastewater by different kinds of flocculants

3.5. Treatment effect of different flocculants on landfill leachate

The composition of landfill leachate is very complex, with variations in water quality and quantity and a high concentration of organic matter. The landfill leachate treatment results of four kinds of flocculants are shown in Table 2, which indicated that the removal rates of turbidly, chromaticity and SS by three bioflocculants were not as high as PAM, especially the X15BF and J1BF produced by a single bacterial and yeast strain. However, the COD removal rate reached 73% by XJBF-1 (produced by mixed strains), while the PAM COD removal rate was only 58%. Although the removal rates of turbidity, chromaticity and SS of landfill leachate by XJBF-1 were not as good as PAM, the treatment effect had significantly improved compared with the bioflocculants produced by a single strain. The removal rates of chromaticity and SS have been more than 70%, except for the turbidity removal rate which is only 50%. It is difficult to treat landfill leachate because of its complex composition. Therefore, relatively speaking, these types of bioflocculants played a certain role in landfill leachate treatment, especially XJBF-1 produced by mixed bacteria and yeast showed good results on landfill leachate treatment.

Type of flocculants	Water quality before treatment				Water quality after treatment				Removal rate (%)			
	COD mg/L	T NTU	C degree	SS g/L	COD mg/L	T NTU	C degree	SS g/L	COD mg/L	T NTU	C degree	SS g/L
J1BF	1944	1440	512	11.04	1125	793	302	6.15	42	44	41	51
X15BF	1944	1440	512	11.04	1070	747	216	4.48	45	48	58	59
XJBF-1	1944	1440	512	11.04	512	716	154	2.99	73	50	70	74
PAM	1994	1440	512	11.04	439	331	77	1.44	58	55	85	77

J1BF: bioflocculant produced by yeast

X15BF: bioflocculant produced by bacteria

XJBF-1: bioflocculant produced by mixed strains (bacteria and yeast V/V 1:1)

PAM: polyacrylamide

T: turbidity

C: chromaticity

Table 2. Treatment results of landfill leachate by different kinds of flocculants

3.6. Treatment effect of different flocculants on the printing and dyeing wastewater

The printing and dyeing wastewater has a high chromaticity degree and complex composition; the wastewater has greater biological toxicity, which contains dyes, sizing additives, oils, acids, alkalis, fibre impurities and inorganic salts, the structure of dyes, nitro and amino compounds, copper, chromium, zinc, arsenic and other heavy metals. The treatment effects by four kinds of bioflocculants on the printing and dyeing wastewater are shown in Table 3. From the experimental results, COD removal rates on the printing and dyeing wastewater by four flocculants were low, only 45–57%, but there was a high removal rate of suspended particles. The turbidity and chromaticity removal rates by X15BF (produced by a single bacterial strain) and XJBF-1 (produced by mixed bacterial and yeast strains) achieved 70–80%. The results were

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far better than those of J1BF produced by yeast and traditional PAM. Especially in XJBF-1, the chromaticity removal rate was high as 88%, much higher than those of PAM and the other two microbial flocculants produced by bacteria and yeasts respectively. The SS removal efficiency by PAM was slightly better than that of the three types of microbial flocculants.

Type of flocculants	Water quality before treatment				Water quality after treatment				Removal rate (%)			
	COD mg/L	T NTU	C degree	SS g/L	COD mg/L	T NTU	C degree	SS g/L	COD mg/L	T NTU	C degree	SS g/L
J1BF	760	165	1200	0.348	415	83	576	0.146	45	49	46	58
X15BF	760	165	1200	0.348	382	44	352	0.146	49	73	70	58
XJBF-1	760	165	1200	0.348	328	36	124	0.128	57	78	88	63
PAM	760	165	1200	0.348	350	40	108	0.108	54	56	69	69

J1BF: bioflocculant produced by yeast

X15BF: bioflocculant produced by bacteria

XJBF-1: bioflocculant produced by mixed strains (bacteria and yeast V/V 1:1)

PAM: polyacrylamide

T: turbidity

C: chromaticity

Table 3. Treatment results on the printing and dyeing wastewater by different kinds of flocculants

4. Conclusion

The advantages of mixed strain HXJ-1 were as follows: directly using acidic wine wastewater, adapting to wastewater at high concentrations and the presence of less nitrogen. Three kinds of bioflocculants had some good treatment results on the starch wastewater, printing and dyeing wastewater and landfill leachate, respectively. The treatment effect of XJBF-1 (produced by mixed strains) on the starch wastewater was better than that of traditional PAM, and XJBF-1 had better treatment results on the three types of wastewater than those of X15BF-1 and J1BF-1 produced by single bacterial and yeast strain respectively. XJBF-1 had good removal rates for three kinds of wastewater chromaticity, especially for the starch wastewater and dyeing wastewater.

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