A Highly Efficient Biofloculant Produced by a Strain of *Klebsiella* sp.

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Abstract. A biofloculant-producing bacterium isolated from activated sludge, named B3, was identified as *Klebsiella* sp. The biofloculant produced by B3 was named MBFB3. The main component of MBFB3 was glycoprotein analyzed by IR. The water quality of sewage and the rate of sludge dewatering were increased after treating with MBFB3, indicating the biofloculant has a wide application prospect in the sewage biological treatment.

1 Introduction

Biofloculant is a type of biodegradable macromolecular flocculant produced by microorganisms. With the properties of innocuity, high-efficiency, safe and biodegradation, it can become a new kind of product to replace the conventional chemical flocculants [1,2].

The study of biofloculant has attracted considerable attention over the years. Many biofloculant-producing microorganisms including bacteria, fungi and yeast had been screened and isolated from activated sludge, soil and sewage [3-10]. *Rhodococcus erythropolis* was isolated from activated sludge and the biofloculant from it was highly effective in treating the wastewater generated from industrial processes [11]. Microorganisms such as *Bacillus* sp. [12], *Enterobacter* sp. [13], and *Alcaligenes latus* B-16 [14] were all isolated from soil samples. However, Compared to chemical flocculants, the low yields and the high costs of biofloculants are major limitations to their practical application [15].

In the previous research, a flocculant-producing strain of *Klebsiella* sp. B3, with high flocculating activity, was screened from the activated sludge. In this paper, the chemical composition of the biofloculant produced by B3 (MBFB3) was analyzed. And the application of MBFB3 in the treatment of sewage and sludge were discussed.

2 Materials and Methods

2.1 Biofloculant collection and purification

After incubation for 24 hr, Cells were removed from the culture medium, which had been diluted with the two

volumes of distilled water, by centrifugation at 8,000 r/min for 10 min at 4°C. Thereafter, the supernatant was concentrated to 0.1 volumes with a rotary evaporator and precipitated by the addition of 4 volumes of cold anhydrous ethanol. The supernatant was incubated at 4°C for 24 hr, and then centrifuged at 4,000 r/min for 5 min. The precipitate was collected, washed twice using anhydrous ethanol, and then dissolved in distilled water. The supernatant was centrifuged at 5,000 r/min for 10 min at 4°C to remove the precipitate and then the free protein was removed by the method of Sevag. After dialyzed overnight at 4°C in deionized water, the supernatant was precipitated by the addition of 4 volumes of cold anhydrous ethanol again. The resulting precipitate was collected by centrifugation at 10,000 r/min for 15 min, redissolved in distilled water and then lyophilized to obtain purified biofloculant.

2.2 Characterization of the biofloculant

The functional groups of the biofloculant were determined using a Fourier transform infrared spectrometer (Nicolet 8700, Thermo Scientific Instrument Co.U.S.A.) in the frequency range of 4000–400 cm⁻¹ with KBr disks. Elemental analysis was achieved with an elemental analyzer (Vario ELIII, Elementar, Germany).

2.3 Biofloculant toxicity test

Thirty white mice (20±2 g) at the age of 30 days were divided into three groups randomly. Each group included five males and five females. All the animals were kept in a room with a temperature maintained at 22±2°C, a relative humidity of 55±5%, and illumination of 12 hr

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light/dark cycle.

The mice in the first and second group were fed with the fermented liquor as a dosage of 0.2 mL and 1 mL per day, respectively. The mice in the contrast group were fed with physiological saline. All the mice were raised for 14 days and their posture, bite and sup, movement, and weight were monitored.

2.4 Effect of MBFB3 on the quality of sewage

Taking the inlet sewage of Shiwuli river as the research object, the coagulation experiment was carried out at the condition of 250 rpm 40 s and 40 rpm 120 s in the six mixers. The changes of each water quality index were analyzed with the national standard method.

2.5 Effect of MBFB3 on the sedimentation of activated sludge

2.5.1 Effect on the Dewatering Rate of Activated Sludge

100 mL sludge, 98 mL sludge with 2 mL CaCl_2 (10g/L), 96 mL sludge with 2 mL CaCl_2 (10g/L) and 2 mL microbial flocculant were added in three 100 mL colorimetric tubes respectively. The mixture was reversed for 20 times and then allowed to stand for 10 min. The volume and the absorbance of the upper phase at 550 nm using a spectrophotometer were measured after filtering for 5 minutes. The dewatering rate was defined and calculated as follows:

$$\text{dewatering rate} = \text{clear water volume} / \text{total volume}$$

2.5.2 Effect on the Sedimentation Rate of Activated sludge

According to the above methods, the volumes of settling sludge and clean water at different sedimentation time (0, 5, 10, 15, 20, 25, 30min) were recorded in two 100 mL colorimetric tubes, respectively. The sedimentation curves of activated sludge under different conditions were drawn. The volume and the absorbance of the upper phase at 550 nm were also measured.

3 Results and Discussion

3.1 Characterization of the bioflocculant

From the IR spectrum (The IR spectrum of MBFB3 is shown in Figure 1), the characteristic chemical groups of MBFB3 were observed as followed. The absorption peaks at 3369, 2960, 2925, 2853, 1081, 1039 cm^{-1} were the characteristic groups of polysaccharide. The absorption peak at 3369 cm^{-1} was the characteristic of $-\text{OH}$ stretching from the alcoholic hydroxyl group. The peak at 2960 cm^{-1} and 2925 cm^{-1} indicated the antisymmetric stretching vibration of CH_3 and CH_2 , respectively. The peak at 2853 cm^{-1} was an indication of the symmetry stretching vibration of CH_2 . Two weak bands at 1081 cm^{-1} and 1039 cm^{-1} indicated C–OH bands

of saccharide.

The absorption peaks at 1648, 1533, 1239 cm^{-1} were the characteristic groups of protein. The peak at 1648 cm^{-1} was an indication of the stretching vibration of C=O from tertiary amide. The peak at 1533 and 1239 cm^{-1} was attributed to the coupling of N–H bending vibration and C–N stretching vibration.

In summary, the major component of the MBFB3 should be glycoprotein.

The elemental analysis of MBFB3 revealed that the weight fractions of the elements C, H and N were 39.21%, 6.34% and 7.87%, respectively.

Fig.1. IR spectrogram of MBFB3.

3.2 MBFB3 toxicity test

When white mice were fed with the fermented liquor as a dosage for 14 days, no obvious weight differences were observed between treated and untreated mice. They maintained normal posture, bite and movement, indicating that MBFB3 has no acute toxicity, at least for white mice.

3.3 Effect of MBFB3 on the treatment of sewage

The quality of sewage was changed greatly after coagulation and sedimentation with MBFB3 in the six mixers. The results (table1) indicated that MBFB3 could remove the main pollutants in the sewage with the process of coagulation and precipitation.

Table1. Effect of MBFB3 on the sewage quality

Detection Index	Before Treatment	After Treatment	Removal Rate/%
COD/ $\text{mg}\cdot\text{L}^{-1}$	221	96	56.56
Ammonia Nitrogen/ $\text{mg}\cdot\text{L}^{-1}$	30.06	18.76	37.59
SS/ $\text{mg}\cdot\text{L}^{-1}$	220	34	84.55
Chromaticity/times	40	10	75.00

3.4 Effect of MBFB3 on the quality of activated sludge

3.4.1 effect on the dewatering rate of activated sludge

The clarity of upper phase and the sludge dewatering rate of activated sludge were all improved after be treated with MBFB3(Figure 2). The clarity of activated sludge can be reduced from 0.39 to 0.29. And the sludge dewatering rate can be increased from 20% to 35%. The results showed that MBFB3 can flocculate the floats in the sludge and contribute to the dewatering of activated sludge.

Fig.2. Effect of MBFB3 on OD₅₅₀ of the supernatant and the dewatering ratio of sludge.

3.4.2 Kinetic study on Dewatering of activated sludge

The volume of the clean water at different times and the clarity of the supernatant OD₅₅₀ were records after the activated sludge was treated with the microbial flocculant. The dewatering rate was calculated also.

Fig.3. Kinetic curve of sludge dewatering.

Fig.4. Effect of MBFB3 on the OD₅₅₀ of supernatant of activated sludge.

After the activated sludge was treated with MBFB3, the sedimentation rate of the sludge was accelerated and the volume of the sludge decreased from 100 mL to 58 mL, which was lower than that of control group in the initial 0~15 min (Figure 2, 3). The clarity of the supernatant decreased continuously with time and increased 48.15% compared with the blank (Figure 4). The results indicated that MBFB3 was good for the flocculation and the dewatering rate of activated sludge.

4 CONCLUSIONS

A flocculant-producing strain B3 of *Klebsiella* sp. with high flocculating activity was screened from the activated sludge. The chemical composition and structure of the bioflocculant produced by B3 (MBFB3) were analyzed. The pure product of the bioflocculant was attained through the process of ethanol deposition and chloroform-butyl alcohol for getting rid of uncombined protein. The compositions of the flocculants were qualitatively determined as glycoprotein through element analysis and IR.

The quality of sewage, such as COD, ammonia nitrogen, SS and chromaticity, was changed greatly after coagulation and sedimentation with MBFB3. The clarity of the supernatant and the dewatering rate of sludge were all increased after treated by MBFB3, indicating the flocculant can flocculate the suspended solids in the settled sludge. So MBFB3 has a wide application prospect in the sewage biological treatment.

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Conflict of interest

The authors declare that they have no conflict of interest.

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