Study on optimal culture conditions of bioflocculant produced by brewery wastewater and its application to middle phase wastewater in paper making process

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Abstract—In order to increase the output of bioflocculant, improve efficiency and stability of flocculation and reduce the cultivating cost, Pseudomonas fluorescens C-2 strain was cultured in brewery wastewater culture medium where brewery wastewater replaced glucose as carbon source and energy source to produce bioflocculant, then the bioflocculant obtained was applied to treat middle phase wastewater in paper making process, and the culture conditions were optimized by orthogonal test. The optimum culture conditions of strain C-2 were as follows: the COD Cr in the brewery wastewater was 8000 mg·L⁻¹, the initial pH of brewery culture medium was 7.5, the inoculum size was 2.5 mL/(mL·50mL⁻¹), the cultivating temperature was 30 ℃, the shaking speed was 160 r·min⁻¹. Under the optimum culture conditions, the maximal COD Cr and turbidity removal efficiency were 88.26% and 95.92%, respectively. In a word, the method was feasible, it achieved resources utilization of wastes. Brewery wastewater has opened up a new way for carbon source and energy source supply.

Keywords- brewery wastewater; Pseudomonas fluorescens C-2; bioflocculant; middle phase wastewater in paper making process; optimization of culture conditions

I. INTRODUCTION

Bioflocculant is a dynamic process resulting from the synthesis of extracellular polymers by living cells[1]. As it is biodegradable and nontoxic, and produce no secondary pollution [2,3], so bioflocculant are applied to drinking water purification, sewage and wastewater treatment, food and fermentation processes[4, 5]. The study of bioflocculants has attracted considerable attention over the years. Finding low-cost substrates and optimizing the fermentation process to improve productivity [6] have, therefore, become another research focus in recent years. Brewery wastewater is classed as a strong wastewater characterized by high biological oxygen demand (BOD) and chemical oxygen demand (COD) concentrations, it is composed of protein, as well as yeast and fattiness and non-toxic organic ingredients. In addition to environmental problems that can result from discharge of brewery wastewater, a great deal of valuable substance in the brewery wastewater is loss. The environmental impacts of brewery wastewater may be reduced economically when recovery or re-utilization of proteins and yeast is performed. In this study, Pseudomonas fluorescens C-2 were cultured in brewery wastewater culture medium where brewery wastewater replaced glucose as carbon source and energy source to produce bioflocculant, then the bioflocculant obtained were applied to treat middle phase wastewater in paper making process, and the optimum culture conditions of strain C-2 were obtained by means of the orthogonal test.

II. MATERIALS AND METHODS

A. Microorganism

Bioflocculant-producing strains Pseudomonas fluorescens C-2 were isolated from soil and activated sludge using Kaolin clay solution as test material by our lab[7].

B. Brewery wastewater

Brewery wastewater (COD Cr ≈ 30015.8 mg ·L⁻¹, pH ≈ 5.20) were collected from wash wastewater in Zhenjiang Beer Company. The brewery wastewater was diluted to the desired COD Cr, which was prepared for brewery wastewater medium.

C. Middle phase wastewater in paper making process

Middle phase wastewater in paper making process was collected from Zhenjiang Jinhe paper making industry, water quality: COD Cr(1606.5 mg·L⁻¹), SS(460 mg·L⁻¹), pH5.6, chromaticity 80/times.

D. Bioflocculant-producing medium and culture conditions

The seed culture medium: glucose 20 g, yeast extract 0.5 g, urea 0.5 g, NaCl 0.1 g, (NH4)2SO4 0.2 g, K2HPO4 5 g, and KH2PO4 2 g, distilled water 1 L,and the initial pH value was adjusted to 7.0, autoclaving at 115 ℃ for 30 min. Brewery wastewater medium: brewery wastewater (CODcr 30015.8 mg·L⁻¹) 1 L, KH2PO4 2 g, K2HPO4 5 g, (NH4)2SO4 0.2 g, NaCl 0.1 g, urea 0.5 g, yeast extract, 0.5 g, and the

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initial pH value was adjusted to 7.0, autoclaving at 115 °C for 30 min. The strain C-2 were first inoculated in 250 mL Erlenmeyer flasks containing 50 mL seed culture medium and incubated for 3–4 days on a rotary shaker at 160 r·min⁻¹ and 30 °C, and broth as seed culture liquid. Then the 2.5 mL seed culture liquid was inoculated into 250 mL conical flasks containing 50 mL brewery wastewater medium, after incubating for 72 h on a rotary shaker at 160 r·min⁻¹ and 30°C, fermentation culture broth was centrifuged at 5000 r·min⁻¹ for 20 min to remove cell, the supernatant was used bioflocculant sample.

E. Determination of the flocculating efficiency

In a 200 mL beaker, 93 mL of middle phase wastewater sample in paper making process was added and mixed with 5 mL of 1% CaCl₂ solution, to this mixture, 2 mL of the bioflocculant sample was added, then, the solution pH was adjusted to 7.0 with NaOH and HCl, and vortexed quickly(250 r·min⁻¹) for 1 min with electric blender, vortexed slowly(80 r·min⁻¹) for 2 min, and allowed to stand for 15 min at room temperature. The absorbance of the upper phase at 550 nm (A) was measured with a spectrophotometer. A control experiment in which 2 mL of distilled water instead of the bioflocculant sample addition to the suspension was performed in the same manner, and the absorbance was measured (B). The flocculating efficiency was calculated according to the following equation: CODCr removal efficiency =\((A - B)/A\) × 100%; where A and B are original wastewater sample and after the flocculation treatment CODCr value, respectively. Turbidity removal efficiency =\((A - B)/A\) × 100%; where A and B are the absorbance of the upper phase at 550 nm of the control and after the flocculation treatment, respectively.

The measurements of turbidity and CODCr were measured by Spectrophotometry and potassium dichromate, respectively.

III. RESULTS AND DISCUSSION

A. Effect of the culture conditions on wastewater treatment by single factor test

1) Impact of the strength of brewery wastewater on middle phase wastewater in paper making process treatment

Brewery wastewater replaced glucose of seed culture medium as carbon source and energy source to produce bioflocculant. The impact of strength of brewery wastewater (with various CODCr concentrations) on wastewater treatment was investigated and shown in Figure 1. At the CODCr concentration of 6000 mg·L⁻¹, the maximum CODCr and turbidity removal efficiency were 89.9% and 92.5%, respectively. However, when CODCr concentration was at 12000 mg·L⁻¹, CODCr and turbidity removal efficiency were 47.50% and 65.35%, respectively. It is possible that the lower CODCr could not meet need of C-2 for carbon source and energy source, the higher CODCr concentration could restrain C-2 growth. As a result, the brewery strength of wastewater of 6000 mg·L⁻¹ was used for all subsequent cultures.

Figure 1. Impact of the concentration of brewery wastewater on the wastewater treatment

2) Impact of the initial pH of brewery wastewater on wastewater treatment

The initial pH of brewery wastewater culture medium was adjusted with 1% NaOH and 1% HCl solution, the impact of the initial pH of brewery culture medium on wastewater treatment was examined (Figure 2), when the pH was 7.5, the highest CODCr and turbidity removal efficiency in the wastewater were 84.7% and 89.2%, respectively. The higher pH was more favorable for the secretion and accumulation of the bioflocculant in the culture broth. As initial pH of culture medium could impact electrification state of microorganism cell and redox potential, and affect that the microorganism assimilate nutriment, and affect enzyme reaction.

Figure 2. Impact of the initial pH of brewery culture medium on wastewater treatment

3) Impact of the cultivating temperature on wastewater treatment

The pH of brewery culture medium was adjusted 7.5, seed culture medium was inoculated into brewery culture medium, impact of the cultivating temperature on wastewater treatment was investigated and shown in Figure 3. The maximum CODCr and turbidity removal efficiency occurred at 30°C, and were 88.75% and 92.58%, respectively. As both the microorganism life activity and matter metabolize were connected with the temperature, proper temperature was beneficial to keep fine growth and metabolize rate, lower or higher temperature could affect enzyme activity, make a cell metabolize slowly and affect bioflocculant activity [8].
because variety of shaking speed would alter dissolved oxygen concentration in fermentation broth.

Figure 4. Impact of the inoculum size on wastewater treatment

5) Impact of the shaking speed on wastewater treatment

The pH of brewery culture medium was adjusted 7.5, seed culture solution was inoculated into brewery culture medium, impact of the shaking speed on wastewater treatment was investigated and shown in Figure 5. At the shaking speed of 160 r·min⁻¹, the maximum CODcr and turbidity removal efficiency were 56.80% and 72.50%, respectively.

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<th>Test number</th>
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<th>Turbidity removal efficiency %</th>
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M2 217.68/229.53 230.20/243.05 261.75/274.79 233.31/246.53
M3 253.75/265.95 232.60/244.75 221.77/233.92 244.52/256.57
m1 77.95/82.36 80.83/84.93 73.92/77.95 75.81/79.82
m2 72.56/76.51 76.73/81.02 87.25/91.59 77.77/82.18
M1 217.49/221.16 28.35/26.80 355.36/371.55 50.13/49.20
M2 36.07/36.42 12.28/11.73 39.99/40.92 17.07/17.09
M3 78.36 82.51

<p>| TABLE 3. VARIANCE ANALYSIS OF CODCr REMOVAL EFFICIENCY |
|---------------------------------|------------|-------------|---------|---------|</p>
<table>
<thead>
<tr>
<th>Origin of variance</th>
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<th>$\bar{S}$</th>
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<th>Prominent degree</th>
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<p>| TABLE 4. VARIANCE ANALYSIS OF TURBIDITY REMOVAL EFFICIENCY |
|---------------------------------|------------|-------------|---------|---------|</p>
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Table 2, Table 3, Table 4 showed that the impact of the each factor on wastewater treatment followed by the inoculum size > the concentration of brewery wastewater > the initial pH of brewery culture medium. The impact of the inoculum size was highly prominent, the concentration of brewery wastewater was prominent, then the initial pH was not prominent. $M_1$ value in Table 2 showed that optimal culture conditions of bioflocculant produced by C-2 was $A_3B_1C_2$, that were the concentration of brewery wastewater was $8000 \text{mg}\cdot\text{L}^{-1}(\text{CODCr})$, the initial pH was 7.5, the inoculum size was 2.5 mL/(mL·50mL$^{-1}$). The CODCr and turbidity removal efficiency in the wastewater with bioflocculant produced by C-2 achieved 88.26% and 95.92%, respectively, under the optimal culture conditions.

IV. CONCLUSIONS
- It was feasible that brewery wastewater completely replaced glucose as carbon source and energy source for bioflocculant produced by C-2 and the bioflocculant obtained was applied to treat middle phase wastewater in paper making process.
- The optimal culture conditions of bioflocculant produced by C-2 were the concentration of brewery wastewater was $8000 \text{mg}\cdot\text{L}^{-1}(\text{CODCr})$, the initial pH was 7.5, the inoculum size was 2.5 mL/(mL·50mL$^{-1}$), the cultivating temperature was 30 °C, shaking speed was 160r.min$^{-1}$.
- The CODCr and turbidity removal efficiency in the wastewater with bioflocculant produced by C-2 achieved 88.26% and 95.92%, respectively, under the optimal culture conditions.

REFERENCES