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Production of a bioflocculant from chromotropic acid waste water and its application in steroid estrogen removal

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ABSTRACT

A novel strain (designated as SW-2) which could convert chromotropic acid into bioflocculants was isolated from chromotropic acid wastewater. Conditions for bioflocculants production were optimized by response surface methodology (RSM) and determined to be inoculum size 7.74%, initial pH 6.9, and COD_{Cr} of the chromotropic acid wastewater 425 mg/L. The yielded bioflocculant was primarily consisting of polysaccharide and protein. It could maintain its flocculating activity to 0.4% (w/w) kaolin suspensions over pH 3–9 and 20–80 °C. In addition, conditions for the removal of estrogens with the bioflocculant were investigated and determined to be bioflocculant dosage 50 mg/L, initial pH 3, reaction time 60 min, and temperature 45 °C. Under these optimal conditions, the removal efficiencies of E1, E2, EE2, and E3 were 87%, 92%, 88% and 96%, respectively. The bioflocculant was shown to offer a promising alternative method of removing estrogens from water in pretreatment applications.

1. Introduction

The occurrence of endocrine disrupting compounds (EDCs) such as estrogen has become more and more noted, sparking discussions among scientists, politicians, industrial and environmental organizations on the significance of the effect of EDCs on wildlife and humans. EDCs interfere with the endocrine system and may alter diverse physiological functions including reproduction and development in different species, including humans [1]. Among a variety of EDCs, estrone (E1), 17β-estradiol (E2), estriol (E3), and 17α-ethinylestradiol (EE2) are reported to be highly disruptive to the endocrine system [2]. The estrogens are released into the aquatic environment mainly via the effluents from wastewater treatment plants (WTPs). So far, the occurrence and removal of estrogens in WTPs have been reported in many studies around the world [3]. As conventional processes in municipal wastewater treatment plants are not able to eliminate estrogens to an ineffective level [4], advanced treatment processes is needed to minimize the discharge of those estrogens. Therefore, the application of flocculation technology is investigated here, aiming to evaluate the potential impact on increased estrogens removal.

Bioflocculation method has several advantages over other methods, such as its low level of toxicity, high biodegradable nature, high selectivity and specificity when at extreme conditions like pH, temperature and salinity [5]. What is more, bioflocculation has been proved to be highly effective and widely applied, but there is no published research on bioflocculation removal of estrogens.

The high-cost and low yield, however, are the major limitation of bioflocculants development for commercial use in wastewater treatment. Although several studies using synthetic and low-cost substrates for bioflocculant production have been reported [5,6], there has been no report on the production of bioflocculants using chromotropic acid (1,8-dihydroxy-3,6-naphthalene disulfonic acid) wastewater as a substrate.

Chromotropic acid is an important dye intermediate for the synthesis of direct, reactive and azo dyes. During chromotropic acid manufacturing, wastewater is released from the acidulation precipitation process and exhibits very high COD (30,000–50,000 mg/L), acidity (pH 1.5–3.5) and chroma. Chromotropic acid wastewater may cause ecological problems and pose potential impact on the quality of drinking water if not treated with proper and efficient methods before discharge. However, there is no report on biodegradation of chromotropic acid. Furthermore, none of the studies considered the use of chromotropic acid wastewater for biological

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products. Additionally, wastewater from the manufacturing processes is rich in various substituted derivatives of chromotropic acid, such as H acid (amino-8-naphthol-3,6-disulfonic acid) and T acid (1-aminonaphthalene-3,6,8-trisulfonic acid), which can inhibit microbial activities. Strains that can effectively utilize chromotropic acid wastewater to produce bioflocculants are of academic and practical interests. The aim of this study was to isolate bioflocculant-producing bacterium using chromotropic acid as a carbon source, optimize the culture conditions for the strain, produce a new bioflocculant and apply it in estrogens removal.

2. Experimental

2.1. Wastewater sample collection and isolation of bioflocculant producing strains

Wastewater samples were collected from industrial effluent of JiHua Chemical Plant in Jiangsu Province, China. With serial dilution techniques, different strains of bacteria were isolated onto agar plates containing the following sterilized medium at pH 7.5 (per liter): 0.2 g chromotropic acid, 5 g K_2HPO_4, 2 g KH_2PO_4, 0.2 g $MgSO_4$, 0.1 g NaCl, 0.5 g urea, 0.5 g yeast extract and 20 g agar. Plates were incubated at 30 °C for 48–72 h. Kaolin suspensions were then used to evaluate the flocculating capability of these microorganisms at the concentration of 4 g/L, and those with high flocculating efficiency would be selected as the bioflocculant-producing strains for further investigation.

Morphological, physiological and biochemical characteristics of the bacteria were identified according to Bergey's manual of systematic bacteriology. PCR amplification of 16S rDNA was identified by Takara Biotechnology Co., Ltd. [7].

2.2. Bioflocculant production and flocculating activity tests

Chromotropic acid wastewater (COD_{Cr} = 24798 mg/L, Chromotropic acid = 3.54 g/L, H acid = 1.40 g/L, T acid = 1.77 g/L, pH 2.5, BOD_5 = 69 mg/L, Color (multiple) = 1100, Na_2SO_4 = 245.85 g/L) was blending black liquor from the primary sedimentation tank of JiHua Chemical Plant in Jiangsu, China. The culture medium consisted of diluted chromotropic acid wastewater (COD_{Cr} = 500 mg/L) 1 L, 5 g K_2HPO_4, 2 g KH_2PO_4, 0.2 g $MgSO_4$, 0.5 g urea and 0.5 g yeast extract. Chromotropic acid wastewater was diluted to the desired COD_{Cr} before cultivation. Initial pH of the chromotropic acid wastewater medium was adjusted to the determined value. Batch fermentations were performed in a 5-L stirred tank fermenter (Model KF5L, KoBio-Tech Co., Inchon, Korea) with 3-L working volume at 37 °C and agitation of 200 rpm for 96 h. Samples were drawn at appropriate time intervals and monitored for pH, biomass, COD, BOD_5 and flocculation properties. The fermented medium was harvested (10,000 rpm; 4 °C, 20 min) and the cell-free supernatant was used as the source of bioflocculant.

The flocculating activity (FA) was measured using jar testers [8] with minor modifications. Briefly, 5.0 mL of a 1% (w/v) $CaCl_2$ solution and 0.2 mL of a centrifuged fermentation culture supernatant were added in turn to 95 mL of kaolin suspension (4.0 g/L, pH 7.5). The solution was stirred at 160 rpm for 160 s and then 40 rpm for 20 min. Then the suspension was settled for 20 min with liquid samples collected at 5 cm beneath water surface for optical density using a spectrophotometer (Shimadzu, UV–vis 2410PC, Japan). A control was prepared in the same way except that 0.2 mL of cell-free culture medium replaced the culture sample. The flocculating activity was calculated according to the following equation:

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

where A and B were $OD_{550\,nm}$ (optical density) of the control and of the sample supernatant, respectively. The FA was expressed as the mean value from duplicate determinations.

2.3. Optimization of bioflocculant production medium by RSM

Plackett–Burman (PB) design was used in the present report, 6 independent factors were investigated using PB design to identify the components that significantly affected bioflocculant production. The factors included COD_{Cr} of chromotropic acid wastewater, agitation speed, inoculum size, temperature, metal ions and initial pH. The design was developed by the Design-Expert software package (version 8.0.4.1, State-Ease, Inc., Minneapolis, USA). All the variables were evaluated in twelve experimental trails and the average bioflocculant yield for each trial was used as the response variable. Regression analysis determined the variables that had a significant (95% level) effect on bioflocculant yield, and these variables were evaluated in further optimization experiments. After the factors were identified by PB design, RSM was employed to optimize the factors to improve bioflocculant production. Three variables (COD_{Cr} of chromotropic acid wastewater, inoculum size and initial pH) were selected from PB design that significantly affected bioflocculant production and were further optimized by RSM. A central composite design (CCD) obtained by using the software Design-Expert was applied to elucidate the interactions of these variables on the bioflocculant production. Experiments were initiated as a preliminary study to determine a narrower range of the COD_{Cr} of the chromotropic acid wastewater, inoculum size and initial pH before designing the experimental runs. Accordingly, COD_{Cr} of the chromotropic acid wastewater from 100 mg/L was tried and the increments continued until appreciable reductions were observed during cell growth. Likewise, inoculum size range of 1–15% and initial pH range of 2–11 were examined to search for a narrower and more effective range. As a result, it was chosen as follows: COD_{Cr} of chromotropic acid wastewater 300–500 mg/L, inoculum size 4–12% and initial pH 6–8 for bioflocculant production. These three independent factors with five different levels (−1.682, −1, 0, +1, +1.682) of the bioflocculant production were investigated and the experimental designs are shown in Table 1.

The response variable (Y) that represented flocculating rate was fitted by a second-order model in the form of quadratic polynomiale equation:

$$Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_i^2 + \Sigma\beta_{ij} X_i X_j, \quad i, j = 1, 2, 3, \ldots, k \quad (2)$$

where Y is the predicted response, X_i and X_j are independent factors, β_0 is the intercept, β_i is the linear coefficient, β_{ii} is the quadratic coefficient and β_{ij} is the interaction coefficient.

2.4. Purification of the bioflocculant

To purify the bioflocculant, the viscous culture broth was centrifuged to remove cell pellets at 12,000 × g for 15 min. The supernatant was poured into two volumes of cold ethanol to precipitate the biopolymer. The resultant precipitate was collected by filtration (Whatman GF Filter) and dialyzed extensively against deionized water. Crude bioflocculant was reprecipitated by addition of a 10% solution of cetylpyridinium chloride. The precipitated polymer complex was collected by centrifugation at 10,000 × g for 20 min at 4 °C and re-dissolved in 10% NaCl solution.

Three volumes of ethanol were added to recover the purified bioflocculant, which was vacuum-dried for 24 h, and then bioflocculant was obtained.