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Production of a bioflocculant from methanol wastewater and its application in arsenite removal

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HIGHLIGHTS

- The methanol wastewater was first time used as a medium for EPS production.
- The bioflocculant-producing bacteria were isolated from methanol wastewater sludge.
- The application of MBF83 in arsenite removal was investigated.
- MBF83 was found to be safe in Zebrafish in toxicity studies.

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ABSTRACT

A novel bioflocculant (MBF83) prepared using methanol wastewater as nutrient resource was systematically investigated in the study. The optimal conditions for bioflocculant production were determined to be an inoculum size of 8.6%, initial pH of 7.5, and a methanol concentration of 100.8 mg L^{-1}. An MBF83 of 4.61 g L^{-1} was achieved as the maximum yield. MBF83 primarily comprised polysaccharide (74.1%) and protein (24.2%). The biopolymer, which was found to be safe in zebrafish in toxicity studies, was characterized using Fourier-transform infrared spectroscopy and elemental analysis. Additionally, conditions for the removal of arsenite by MBF83 were found to be MBF83 at 500 mg L^{-1}, an initial pH of 7.0, and a contact time of 90 min. Under the optimal conditions, the removal efficiency of arsenite was 86.1%. Overall, these findings indicate bioflocculation offers an effective alternative method of decreasing arsenite during wastewater treatment.

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1. Introduction

Arsenic-contaminated wastewater represents a great threat to the environment and human health. Arsenic contamination caused by both natural processes and anthropogenic activities is a worldwide problem. In aquatic systems, the predominant forms of As are the inorganic species arsenate and arsenite, with the latter being more labile and 25–60 times more noxious than the former (Dax et al., 2014). Reducing conditions at low redox potential result in conversion of arsenate into arsenite. Conversely, under oxidizing conditions such as those found in surface water, arsenate is the major arsenic species (Amrose et al., 2013). Owing to the high virulence of arsenic, the World Health Organization (WHO) has lowered the permissible limit of arsenic in drinking water from 50 to 10 μg L^{-1} (Xu et al., 2013); accordingly, it is necessary to develop feasible, efficient methods to diminish both arsenic species concentration from water, particularly arsenite. To date, a substantial number of methods have been developed to remove arsenite (Balasubramanian et al., 2009; Altun et al., 2014), among which flocculation is recognized as one of the best available options because of its low cost and high efficiency (Bolto and Gregory, 2007; Mishra et al., 2014).

Some flocculants, like diatom silica shells and *Arthrobacter* sp. biomass, were used to remove arsenite from the wastewater (Prasad et al., 2013; Zhang et al., 2015). However, most of these flocculants preferentially flocculate arsenate instead of arsenite, and consequently a preliminary oxidation step for the transformation of arsenite to arsenate to achieve efficient arsenic removal is required (Luo et al., 2010). Undoubtedly, this will increase the operation cost and complexity.

Accordingly, "green" bioflocculation has attracted increasingly scientific and technological attention in the wastewater treatment

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field because it is not dangerous to humans, uses easily biodegraded materials, and is free of secondary pollution by degradation intermediates. Moreover, bioflocculants have been widely employed in a variety of processes including wastewater clarification, purification of carbohydrates from plant biomass, paper production, chemical operations, dredging, dewatering and thickening in mineral operations (Aljuboori et al., 2013; Zhao et al., 2013). Nevertheless, there has been no published research regarding the removal of arsenite using microbially produced bioflocculants.

High-costs are the major bottleneck in bioflocculants development for commercial use (Fujita et al., 2000; Zhang et al., 2013). Hence, industrial-scale production and application of bioflocculants as potential alternatives to synthetic ones has yet to be achieved. Although several investigations using inexpensive substrates for bioflocculant production have been conducted (Zhang et al., 2007; Gong et al., 2008), there have been no studies of the production of bioflocculants from methanol wastewater.

Methanol, which is colorless and has a characteristic distinctive irritating odor, is especially useful for HPLC, UV/VIS spectroscopy, and LCMS due to its low UV cutoff. It is also an alternative fuel for internal combustion and other engines, either in combination with gasoline. Although methanol wastewater is very harmful to ecological systems and human health, it is a potentially inexpensive medium and a rich source of carbon and other nutrients that have the potential for use as bioflocculants. Hence, microorganisms that use wastes as substrates for the production of interesting materials not only contribute to the production of these value added compounds, but also focus on the minimization of waste disposal.

Therefore, the present study was conducted to: (1) isolate and identify bioflocculant-producing strains from methanol wastewater sludge; (2) produce bioflocculant using strains isolated from methanol wastewater; (3) evaluate the performance of this bioflocculant and its application to arsenite removal.

2. Materials and methods

2.1. Isolation and identification of bioflocculant-producing microorganisms

Bioflocculant-producing strains were isolated from activated sludge samples (pH 7.6–7.8) taken from a methanol wastewater treatment plant located in Jiangsu, China. The procedure was the same as previously described (Bala Subramanian et al., 2010). Each isolated strain was cultivated in screening medium (50 mL) containing 2% methanol, 0.05% $(NH_4)_2SO_4$, 0.5% K_2HPO_4, 0.2% KH_2PO_4, 0.05% $MgSO_4$, and 0.01% NaCl at 30 °C for 3 d. Next, 1 mL of fermentation broth was added into 100 mL kaolin suspension (4 g L^{-1}) in a 250-mL beaker and the flocculating activities of the suspensions were measured. Culture broths propitious to flocculating rate were further explored. Five strains were found to produce flocculants, among which ZCY83 exhibited the excellent flocculating activity in kaolin suspension. Therefore, ZCY83 was inoculated onto an isolation slant culture-medium and cultivated at 30 °C for 8 d, after which it was preserved at 4 °C for further study. PCR amplification of the 16S rDNA was conducted by Takara Biotechnology Co., Ltd. (Bala Subramanian et al., 2010).

2.2. Bioflocculant production and flocculating activity tests

Methanol wastewater (COD_{Cr} 1,060 mg L^{-1}, pH 7.7, methanol 350 mg L^{-1}) was collected from the primary sedimentation tank of the JiHua Chemical Plant in Jiangsu, China. The concentrations of methanol, formaldehyde and methanoic acid were determined by GC-FID techniques. The culture medium consisted of 1 L diluted methanol wastewater containing 0.05% $(NH_4)_2SO_4$ and 0.5 g yeast extract. Prior to cultivation, the methanol wastewater was diluted to the desired methanol concentration, after which the initial pH of methanol wastewater medium was adjusted to the determined value. Batch anaerobic fermentations were conducted in a 5-L stirred tank reactor (14 cm ID × 45 cm height) at 30 °C for 8 d with agitation at 120 rpm. Samples were drawn and monitored for flocculation properties. After 144 h of fermentation, the pH of the culture broth was adjusted to 12 and the culture broth was stirred for 30 min to extract the bioflocculant from the cells. After the alkali extraction, viscous culture broth was centrifuged at 8000*g* for 30 min. Two volumes of cold ethanol were added to the supernatant and left overnight at 4 °C. The precipitate was collected by centrifugation at 14,000*g* for 10 min and dissolved in ultrapure water. Cetyltrimethyl ammonium bromide (2%) was added to the solution with stirring. The mixture was set aside for 6 h at room temperature. Then two volumes of ethanol were added to recover the precipitate, which was lyophilized for further experiments (Tang et al., 2014).

2.3. Statistical analyses

Statistical analyses were conducted using Design Expert Version 8.0. In this design, the central composite design and response surface methodology were applied to optimize the three most important operating variables: methanol concentration, inoculum size and initial pH. Experiments were initiated as a preliminary study for determining a narrower range of methanol concentration, inoculum size and initial pH prior to designing the experimental runs. Accordingly, methanol concentration from 25 mg L^{-1} were tried and the increments continued until appreciable reductions were observed in the process responses. Likewise, the wide pH range of 4–10 and inoculum size range of 4.5–20% were examined to search for a narrower and more effective range. As a result the study ranges were chosen as methanol concentration 50–150 mg L^{-1}, inoculum size 6.5–10.5% and pH 6–8 for bioflocculant production (Table 1). Each of these three significant variables was assessed at five different levels (−1.682, −1, 0, +1, +1.682). The average yield which obtained in these experiments was used as the response variable (Y) and all the experiments were conducted in triplicate.

The second-order model for the three quantitative factors can be described as follows:

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

where Y is the predicted response, β_0 is the offset term, β_i is the linear effect, β_{ii} is the quadratic effect and β_{ij} is the interaction effect, X_i and X_j are input variables which influence the response variable Y.

2.4. Characteristics of the bioflocculant

The purified bioflocculant was analyzed using a Fourier transform infrared (FTIR) spectrophotometer (Made in Germany Model EQUINOX55). The spectrum of the sample was recorded on the spectrophotometer over a wave length range of 400–4000 cm^{-1} under ambient conditions. The polysaccharide concentration of the purified biopolymer was determined by the phenol–sulfuric method (Aljuboori et al., 2015). The protein concentration of the purified bioflocculant was determined by the Coomassie brilliant blue G-250 dye binding method using bovine serum albumin as the protein standard (Ugbenyen et al., 2014). Neutral sugar, amino sugar, and uronic acid content were determined using the standard methods (Ghosh et al., 2009). The monosaccharide composition of the purified biopolymer was analyzed after hydrolysis with 3 M TFA at 100 °C for 4 h using

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