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Removal of arsenite by a microbial bioflocculant produced from swine wastewater

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HIGHLIGHTS

- A microbial bioflocculant was produced from swine wastewater.
- Application of the bioflocculant in arsenite removal was investigated.
- Mechanisms of arsenite removal by the bioflocculant were detected.

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ABSTRACT

This paper focused on the production and characteristics of a bioflocculant by using swine wastewater and its application in removing arsenite from aqueous solution. A series of experimental parameters including bioflocculant dose, calcium ions concentration, and solution pH value on arsenite uptake were evaluated. Results have demonstrated that a bioflocculant of 3.11 g L^{-1} was achieved as the maximum yield after 60 h fermentation, with a main backbone of polysaccharides. Maximum arsenite removal efficiency of 99.2% can be reached by adding bioflocculant in two stages: 3×10^{-3}% (w/w) in the 1.0 min's rapid mixing (180 rpm) and 2×10^{-3}% (w/w) after 2.0 min's slow mixing (80 rpm) with pH value fixed at 7. Negative Gibbs free energy change (ΔG^{o}) indicated the spontaneous nature of arsenite removal. Arsenite was removed by the bioflocculant through bridging mechanisms.

1. Introduction

Microbial bioflocculant (MBF) are extracellular polymeric substances which mainly contained glycoproteins, polysaccharides, and proteins that produced by microorganisms during their active secretion and celllysis (Aljuboori et al., 2014). It was considered as an environment-friendly material and was advantageous over traditional flocculants (such as $FeCl_3$, $Al_2(SO_4)_3$, *polyaluminum chloride* (PAC), and *polyacrylamide* (PAM) due to its harmless, biodegradable, and free of secondary pollution (Okaiyeto et al., 2015). Therefore, MBF has been extensively employed in removing pollutants (such as suspended solids, organic pollutants, and heavy metal ions) from wastewaters (Guo et al., 2013; Guo and Yu, 2014) and sludge dewatering (Guo et al., 2015a) on a laboratory scale. However, the high production cost compared with traditional flocculants was still the major limitation in bioflocculants' commercial applications (Zaki et al., 2013; Zhang et al., 2013).

Extensive swine breeding has already been highlighted as an effective way to increase meat production efficiency in China (Song et al., 2011; Wang et al., 2006), and thus, a large quantity of swine wastes were generated, which contained high ammonia as well as high levels of organics (Ryu and Lee, 2010). After anaerobic treatment of swine wastes to recover biogas energy, there still remained high-levels of organic matters and ammonia in the effluent, named digested swine wastewater, which can be used to cultivate bioflocculant-producing strains. The strains' metabolites could be a source to extract bioflocculant (Guo et al., 2013). Thus, strains that can effectively utilize the substrates in digested swine wastewater to produce bioflocculant are of academic and practical interests.

Although most of the bioflocculants can be used to flocculate particles/colloids and remove pollutants from aqueous solution (Nie et al., 2011; Patil et al., 2011; Peng et al., 2014), there has been few published research regarding arsenite removal from wastewater by using microbial bioflocculants. In fact, arsenite-contaminated

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wastewater has been a great threat to human health and environment due to the carcinogenicity and high virulence of arsenite, whose ingestion may deleteriously affect the gastrointestinal tract, cardiac, vascular system and central nervous system, and thus, in most cases excepting the presence of unacceptable level of arsenic, the groundwater is otherwise quite fit for drinking purpose (Altun et al., 2014; Balasubramanian et al., 2009). Accordingly, it is necessary to develop feasible, efficient methods to diminish the presence of arsenite in water. Currently, some methods have been developed to get rid of arsenite, including coagulation or flocculation with ferric salts, aluminum salts, and other flocculants; adsorption onto activated alumina/carbon; ion-exchange, reverse osmosis, and electrodialysis (Balasubramanian et al., 2009; Mohan and Pittman, 2007), among which coagulation or flocculation by using flocculants has been demonstrated to be very efficient and inexpensive (Wang et al., 2007). The adsorption on activated alumina/carbon was not efficient for arsenite removal (Balasubramanian et al., 2009). While, though the chemical precipitation with ferric and salts was quite effective, it yields large quantities of solid sludge that requires further treatment. The other treatment methods such as ion exchange and reverse osmosis were expensive and always presented limitations (Balasubramanian et al., 2009; Mohan and Pittman, 2007).

The objectives of this study were: 1) Isolation of a bioflocculant-producing strain from biological sludge and production of a microbial bioflocculant from swine wastewater; 2) Analysis of chemical compositions, thermal stabilities, physico-chemical and ultra-structure characteristics to determine the active ingredient and characteristics of the bioflocculant; 3) Investigation of bioflocculant dose, $CaCl_2$ dose, and solution pH value on arsenite removal efficiencies to determine the application potential of the bioflocculant in arsenite-contaminated wastewaters; and 4) Analysis of arsenite removal mechanisms by the bioflocculant, where surface structure and texture of the arsenite-loaded bioflocculant were determined and the variation of zeta potential during arsenite removal process by the bioflocculant was monitored. It is an economic and resourceful way to produce an environmental friendly flocculant by using swine wastewater as fermentation medium, and the application of this bioflocculant in arsenite removal is a feasible and efficient way for removing arsenite from wastewaters.

2. Materials and methods

2.1. Isolation and identification of bioflocculant-producing strains

Bioflocculant-producing strain was isolated from biological sludge (pH value 6.5–6.8) taken from a swine wastewater treatment plant of a pig farm located in Sichuan Province, China. The isolation process was given as follows: a total of 1 mL of sludge sample was diluted with distilled water (10^1–10^6 folds), subsequently, 1 mL of each dilution was spread onto agar plates, and was incubated at 35 °C in an incubator till substantial microbial growth. Composition of the agar plates were (per liter): urea 5 g, sucrose 20 g, K_2HPO_4 5 g, KH_2PO_4 2.5 g, $MgSO_4$ 1 g, NaCl 5 g, and agar 10 g. Large and viscous colonies were then inoculated in the same plates and was incubated at the same procedure, after 10 cycles of replanting onto the agar plates, a total of 7 morphologically different isolates were isolated. Each of these 7 large and viscous strains were individually inoculated on a rotary shaker at 150 $r \cdot min^{-1}$ and 35 °C for 24 h in screening medium consisted of swine wastewater 200 mL, K_2HPO_4 1 g, and KH_2PO_4 0.5 g, $MgSO_4$ 0.2 g, NaCl 1 g. After 60 h of cultivation, fermentation broths were obtained, whose flocculating activities toward to kaolin suspension (4 g L^{-1}) were measured by the flocculating method (section 2.3) and the ones with the highest flocculating activity of 90.2%, named G04, was selected to produce bioflocculant.

Cell forms and colony characteristics of the strain G04 on nutrient agar was observed with bio-microscope (CX31, Olympus, Japan), and it's physiological and biochemical characteristics were identified according to Bergey's Manual of systematic bacteriology. The 16S rRNA gene fragment was then amplified using individual bacterial colony PCR. PCR amplification was carried out using forward primer 27A (5′-GAG AGT TTG ATC CTG GCT CAG-3′) and reverse primer 1495R (5′-CTA CGG CTA CCT TGT TAC GA-3′), and was run on a MyCycler thermal cycle (Bio-Rad, USA) using cycling conditions as follows: 94 °C for 4 min; followed by 30 cycles of 94 °C for 90 s, 55 °C for 60 s, 72 °C for 90 s; followed by 72 °C for 7 min, and ended at 4 °C.

2.2. Bioflocculant production and purification

Swine wastewater for culturing strains to produce bioflocculant was taken from Jiancha pig farm, Sichuan province, China. Concentrations of COD, ammonia, and total phosphorus (TP) of this wastewater were 1065, 828, and 26 mg L^{-1}, respectively. PH value of this wastewater was 6.5. To produce bioflocculant, the strain G04 was inoculated with 200 mL of swine wastewater (as “fermentation medium”) in 500 mL flask directly, and the flask was then shaken on a rotary shaker for 60 h (30 °C, 150 $r \cdot min^{-1}$). After the incubation, the culture solution (as “fermentation broth”) was centrifuged at 3000 $r \cdot min^{-1}$ for 30 min to remove bacterial cells. The remained supernatant was combined with one volume of distilled water, and then concentrated about three times using a reverse osmosis process. The concentrated solution was centrifuged at 3000 $r \cdot min^{-1}$ for 30 min and the obtained supernatant was filtered using a Durapore membrane (0.45 μm diameter of pore). The filtrated solution was combined with 2 vol of cold anhydrous ethanol, and the precipitate was produced by incubating the mixture at 4 °C for 24 h. The precipitate was collected by centrifuged at 3000 $r \cdot min^{-1}$ for 15 min, and then re-suspended in a minimal volume of distilled water, washed with 75% ethanol twice, dialyzed against distilled water at 4 °C overnight, dried by vacuum evaporation, and the resulting dry matter was denoted as the bioflocculant.

2.3. Assay of the flocculating activity

Referred to Magdalena et al. (2016)'s method, flocculating activity of the bioflocculant was measured using kaolin clay suspension with $CaCl_2$. Fresh diluent of kaolin clay suspension (Tianjin Kemiou Chemical Preparation Co., Ltd., China) was used. With slightly modified from Magdalena et al. (2016)'s method, 4 g of kaolin and 50 mg of $CaCl_2$ were suspended in 1.0 L of distilled water. 0.1 mL of the fermentation broth was added to 9 mL of this solution, stirred during 30 s using Vortex, and left to stand for 5 min. The absorbance of the upper phase and blank control without the fermentation broth was measured at 550 nm (as OD_{sample} and OD_{blank}, respectively) using a spectrophotometer. Flocculating activity (FA, %) was calculated as follows:

$$FA = \frac{OD_{blank} - OD_{sample}}{OD_{sample}} \times 100 \quad (1)$$

2.4. Physico-chemical analysis and ultra-structure characteristics of the bioflocculant

Total sugar content of the purified bioflocculant was measured by the phenol-sulphuric acid method, using glucose as a standard (Kurane and Matsuyama, 1994). Protein content of the purified bioflocculant was measured by the Bradford method, using bovine