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Optimized production of a novel bioflocculant M-C11 by *Klebsiella* sp. and its application in sludge dewatering

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ABSTRACT

The optimized production of a novel bioflocculant M-C11 produced by *Klebsiella* sp. and its application in sludge dewatering were investigated. The optimal medium carbon source, nitrogen source, metal ion, initial pH and culture temperature for the bioflocculant production were glucose, $NaNO_3$, $MgSO_4$, and pH 7.0 and 25°C, respectively. A compositional analysis indicated that the purified M-C11 consisted of 91.2% sugar, 4.6% protein and 3.9% nucleic acids (*m/m*). A Fourier transform infrared spectrum confirmed the presence of carboxyl, hydroxyl, methoxyl and amino groups. The microbial flocculant exhibited excellent pH and thermal stability in a kaolin suspension over a pH range of 4.0 to 8.0 and a temperature range of 20 to 60°C. The optimum bioflocculating activity was observed as 92.37% for 2.56 mL M-C11 and 0.37 g/L $CaCl_2$ dosages using response surface methodology. The sludge resistance in filtration (SRF) decreased from 11.6×10^{12} to 4.7×10^{12} m/kg, which indicated that the sludge dewaterability was remarkably enhanced by the bioflocculant conditioning. The sludge dewatering performance conditioned by M-C11 was more efficient than that of inorganic flocculating reagents, such as aluminum sulfate and polymeric aluminum chloride. The bioflocculant has advantages over traditional sludge conditioners due to its lower cost, benign biodegradability and negligible secondary pollution. In addition, the bioflocculant was favorably adapted to the specific sludge pH and salinity.

Introduction

Sludge dewatering is of great significance in sludge processing because it reduces the sludge volume and, as a result, decreases the cost of the transportation and disposal (Yuan et al., 2011b). Chemical flocculation (Lo et al., 2001), thermal treatments (Guan et al., 2012), ultrasound conditioning (Feng et al., 2009), electrolysis (Yuan et al., 2010) and Fenton oxidation (Tony et al., 2008, 2009) have been investigated as means of enhancing sludge dewaterability. Inorganic and organic synthetic flocculants are extensively employed in sludge dewatering because of their high coagulating effectiveness, despite the fact that they posed consequent environmental threats and risks. As chemical flocculating substances, polyacrylamides (PAM) were confirmed to be harmful and carcinogenic (Dearfield et al., 1988), resulting in human health problems, including Alzheimer's disease, which is correlated with the application of aluminum salts (Bondy, 2010).

Bioflocculants are organic macromolecular substances produced during microorganism growth that can efficiently coagulate and aggregate suspended colloids. Attention has been focused on the production and component analysis of novel bioflocculants with high flocculation efficiencies (Li et al., 2009; Liu et al., 2010; Zheng et al., 2008). Various strains have been reported to be bioflocculant-producing microorganisms (BPMs),

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of which *Bacillus* sp. has been the most widely reported (Lian et al., 2008; Yuan et al., 2011c; Zheng et al., 2008). Previously, bioflocculants were reported to remove metal ions (Salehizadeh and Shojaosadati, 2003), defecate trona suspension (Lu et al., 2005), and treat dye solutions (Deng et al., 2003) and low-temperature drinking water (Li et al., 2009). Nevertheless, relevant research studies on improved sludge dewaterability by bioflocculant conditioning have rarely been reported. Compared with conventional flocculating agents, bioflocculants have attracted considerable scientific and biotechnological attention because of their lower costs, benign biodegradability, and negligible secondary pollution (Liu et al., 2010). Salinity has been correlated with lipid content and cell growth, which was believed to have a significant effect on the sludge dewaterability (Bartley et al., 2013; Chu et al., 1997; Lo et al., 2001). In particular, the salinity levels of the wastewater and the activated sludge were relatively high. The reported bioflocculants for wastewater treatment were primarily screened and isolated from soils, wastewater, rivers and foods (Gao et al., 2009). Bioflocculants from other environmental media may not efficiently adapt to the sludge salinity, which may inhibit the sludge conditioning effect.

The cation dosage was proven to neutralize the negative charges in the sludge particles, and to decrease the absolute value of the zeta potential during the flocculating process (Zhang et al., 2010b). The bioflocculant concentration also exhibited a remarkable influence on the flocculating activity (Zheng et al., 2008). To obtain the optimum flocculating activity under the combined effects of the pH value, the bioflocculant dosage and the cation dosage, response surface methodology (RSM) was used to estimate the interaction between the multiple factors. Compared with conventional and single-factor methods, which are time-consuming because they require a large number of experiments to determine the optimal content of each factor one at a time, the response surface methodology can distinguish the interaction between individual variables (He et al., 2009). In this study, a novel bioflocculant named M-C11, produced by *Klebsiella* sp., was isolated from the activated sludge. The optimal cultivation and flocculation conditions were determined using response surface methodology. In addition, the bioflocculant was finally employed in sludge dewatering such that it was adjusted in the specific pH, salinity, and biological conditions.

1. Materials and methods

1.1. Isolation and culture composition

Bioflocculant-producing bacteria were isolated from the activated sludge of a secondary sedimentation tank in a wastewater treatment plant (WWTP) located in Jiangsu Province, China. To obtain bioflocculant-producing microorganisms (BPMs), repeated screening and purification experiments were conducted with a culture-medium composition as follows (per liter): 0.5 g KCl; 2.0 g $NaNO_3$; 0.01 g $FeSO_4$; 1.0 g K_2HPO_4; 0.5 g $MgSO_4$; and 30.0 g sucrose. All medium solutions were prepared with deionized water and were sterilized at 120°C for 30 min. The isolated strains were then inoculated with 50-mL screening broth and incubated in a rotary shaker for 48 hr. The fermentation broth obtained was centrifuged (4000 r/min, 30 min) to separate the cells. The cell-free culture supernatant was the liquid bioflocculant and preserved at 4°C in a refrigerator to analyze flocculating activity and sludge dewatering effect. The screened bioflocculant-producing strain was named C11 and further identified by the 16S rDNA gene sequence and its morphological characteristics.

1.2. Optimization of bioflocculant production

The effects of the carbon source, nitrogen source, metal ions, initial pH and culture temperature on the M-C11 production were investigated to identify the optimal cultivation conditions. Sucrose (30.0 g/L) was compared with glucose, starch, compound carbon (glucose: starch = 1:1, *m*/*m*) and beef extract to determine the effect of the carbon source on the bioflocculant production; $NaNO_3$ (2.0 g/L) was compared with NH_4Cl, yeast and urea to determine the effect of the nitrogen source on the bioflocculant production; $MgSO_4$ (0.5 g/L) was compared with NaCl, $CaCl_2$, $FeSO_4$, $CuSO_4$ and $Al_2(SO_4)_3$ to determine the effect of the metal ions on the bioflocculant production; the initial pH was adjusted to 4.0–10.0 by NaOH (0.1 mol/L) and HCl (0.1 mol/L), and the culture temperature was adjusted to 20 to 40°C to determine the effect of the initial pH and the culture temperature, respectively, on the bioflocculant production. All experiments were conducted in triplicate for a mean calculation, and the standard deviations were included in the figure plotting. SigmaPlot software (v10.0) was used to create the figures.

1.3. Measurement of flocculating activity

Kaolin clay was chosen as the suspended solid to calculate the flocculating activity. First, 93 mL kaolin suspension (4.0 g/L), 5.0 mL $CaCl_2$ (1%, *m*/*V*) and 2.0 mL liquid bioflocculant were mixed and stirred at 400 r/min for 1 min and then stirred at 150 r/min for 5 min. After settling for 5 min in the cylinder, the supernatant absorbance was measured by a spectrophotometer at 550 nm (Shimadzu UV-2550, Japan). The fermentation supernatant was replaced with a culture medium at the same concentration in the control experiment. The flocculating activity (FA, %) was calculated according to Eq. (1):

$$\mathrm{FA} = \frac{A_0 - A}{A_0} \times 100\% \quad (1)$$

where, A_0 and A were the absorbance variables at 550 nm of the control and the sample supernatant, respectively.

1.4. Bioflocculant purification

According to Salehizadeh and Shojaosadati (2003), to obtain the purified bioflocculant, the fermentation broth was centrifuged to remove the cells by centrifugal separation (5000 r/min, 30 min). Two volumes of cold ethanol were then added to the supernatant and left overnight at 4°C. The precipitate was re-dissolved in distilled water, followed by the addition of 2% cetylpyridinium chloride solution (CPC) with stirring. After 2 hr, the resulting precipitate was collected by centrifugation at 5000 r/min for 30 min and re-dissolved in NaCl (0.5 mol/L). Three volumes of cold ethanol was added to obtain the precipitate, which was then washed two times with ethanol, and then the purified bioflocculant was vacuum-dried.

1.5. Physical and chemical analysis of M-C11

The total sugar content of the M-C11 bioflocculant was measured according to the phenol–sulfuric acid method using glucose as the standard (Chaplin and Kennedy, 1994). The total protein content was measured by the Bradford method with bovine