Bioresource Technology 177 (2015) 393–397

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Short Communication

Characterization and flocculation mechanism of a bioflocculant from hydrolyzate of rice stover

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HIGHLIGHTS

- Hydrolyzate from acid treated rice stover were used to produce bioflocculant.
- Characteristics of the bioflocculant were examined.
- Flocculating mechanisms were detected by using kaolin suspension in present of Ca^{2+}.
- The application of the bioflocculant in real wastewater treatment was investigated.

ARTICLE INFO

Article history:
Received 2 October 2014
Received in revised form 11 November 2014
Accepted 15 November 2014
Available online 20 November 2014

Keywords:
Bioflocculant
Rice stover
Sludge dewatering
Swine wastewater pretreatment

ABSTRACT

This study investigated the characterization and flocculation mechanism of a bioflocculant from hydrolyzate of rice stover. Production of the bioflocculant was positively associated with cell growth and a highest value of 2.4 g L^{-1} was obtained. During the kaolin suspension flocculation, charge neutralization and inter-particle bridging were proposed as the reasons for enhanced performance. Apart from this, the bioflocculant showed good performances in sludge dewatering and swine wastewater pretreatment. After conditioning by the bioflocculant, dry solids (DS) and specific resistance to filtration (SRF) of the sludge reached 18.4% and 4.8 $\times$ 10^{12} m kg^{-1}, respectively, which were much better than that by conventional chemical flocculants. In the swine wastewater pretreatment, the removal efficiencies of COD, ammonium, and turbidity reached 48.3%, 43.6% and 75.8% at pH 8.0 when the bioflocculant dose was adjusted to 20 mg L^{-1}.

1. Introduction

Microbial bioflocculant (MBF), secreted by microorganisms during their growth and cell-lysis, was a kind of environmentally safe material with the character of harmless and biodegradable. Along with the increasing requirement to environmental quality, bioflocculants have been regarded efficiently in removing pollutants (like suspended solids, dye pigments, and heavy metal ions) from wastewaters on the laboratory scale (Li et al., 2013; Yang et al., 2009). To date, studies on bioflocculants commonly focused on isolating functional strains, adopting these strains to produce bioflocculants in synthetic media, and utilizing these bioflocculants for wastewater treatment. As a result, active ingredients of the bioflocculants and the mechanisms during flocculation process were not entirely clear. However, determination of the active ingredients of bioflocculants is the most important to elucidate their flocculation mechanisms, which would be beneficial for optimizing the flocculating parameters thus improving efficiencies in practical application (Li et al., 2014). Besides, high costs associated with relatively expensive substrates gradually became impediments for the production and application of the bioflocculants (Zhao et al., 2012). Recently, attempts have been made to get new efficient mutant and seeking for low-cost substrates to reduce the production cost. For example, activated sludge was used as raw material to produce bioflocculant (More et al., 2012). Agriculture wastes were rich in lignocelluloses, whose hydrolyzate can be used by some strains to produce biological products (Zheng et al., 2005). Thus, strains that can effectively utilize the substrates in hydrolyzate of agriculture wastes to produce bioflocculants are of academic and practical interests.

In this study, experiments were first determined the active ingredient and characterization of a bioflocculant, which was harvested from hydrolyzate of rice stover. Subsequently, the actual application of this bioflocculant in swine wastewater treatment and sludge dewatering were investigated under a variety of

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http://dx.doi.org/10.1016/j.biortech.2014.11.066
0960-8524/

conditions. Furthermore, based on the performance in the flocculation of kaolin clay suspension (4.0 g L^{-1}), the flocculating mechanisms of this bioflocculant were discussed.

2. Methods

2.1. Reagents

Ensure the fresh diluent of kaolin clay (Tianjin Hengxing Chemical Preparation Co., Ltd., China) was used in each experiment which was prepared by diluting the 4.0 g L^{-1} stock solution.

2.2. The hydrolyzate, strain and its bioflocculant

The dried rice stover, collected from Santai, Sichuan province, China, was hydrolyzed by diluted sulfuric acid (1.7%, w/w) with solid–liquid ratio 1:8 at 121 °C for 2 h. After hydrolysis, the supernatant was collected by centrifugation (6000 rpm, 30 min), and was mixed with sufficient $Ca(OH)_2$ to neutralize pH. The yield liquid by discarding the precipitates by centrifugation (6000 rpm, 30 min), was the hydrolyzate, and was used as raw material to produce bioflocculant.

Bioflocculant-production strain, *Rhodococcus erythropolis*, was obtained from China Center for Type Culture Collection (No. ACCC.10543). The strain was first inoculated in 150 mL seed medium consisted of glucose 20 g, yeast 5 g, beef extracts 2 g, $MgSO_4$ 2 g, and NaCl 10 g dissolved in 1 L distilled water with the pH adjusted to 7.0, and was incubated on a reciprocal shaker at 150 rpm and 35 °C for 24 h. After the cultivation, 2.0% v/v of the above inoculum was used to inoculate the cultivation medium of composition (per liter): 200 mL hydrolyzate, 5 g K_2HPO_4, 2 g KH_2PO_4, 0.2 g $MgSO_4$, 0.1 g NaCl, 0.5 g urea and 0.5 g yeast extract with pH value of 7.0. The inoculated sample was incubated in the same procedure to produce bioflocculant. The bioflocculant was extracted from the whole fermentation liquor and was purified using the methods proposed (Guo et al., 2013).

2.3. Physical and chemical analysis of the bioflocculant

Total sugar and protein content were measured by the methods described in our previous study (Guo et al., 2014). The molecular weight was determined by gel permeation chromatography (GPC) using a Hitachi L-6200 system controller. Functional groups of the bioflocculant were determined using a Fourier transform infrared (FTIR) spectrophotometer (EQUINOX 55, Bruker Company, Germany).

2.4. Assay of flocculating activity

The flocculating activity of the bioflocculant was conducted in jar testers by measuring turbidities of 4.0 g L^{-1} kaolin suspensions (Guo et al., 2014). After adjusting the pH value to 7.5 using 1.0 mol L^{-1} NaOH or HCl, 50 mg of $CaCl_2$ and 2.0 mg of bioflocculant were added into the 100 mL of kaolin suspension in a 300 mL beaker. The mixture was vigorously stirred (180 rpm) for 1.0 min and slowly stirred (80 rpm) for 4.0 min, and then allowed to stand 10 min. The optical density (OD) of the clarifying solution was measured with a spectrophotometer (Unic-7230, Shanghai Lianhua Company, China) at 550 nm. A control experiment was conducted in the same manner without adding bioflocculant. All the bioflocculant measurements were carried out in triplicates and the average values were presented (with standard error less than 5% of the mean). The flocculating activity was calculated by the following equation:

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

where FR is the flocculating activity; A and B are the OD values of the sample and control.

2.5. Zeta potential analysis during the flocculation process

The variation of Zeta potential during the process of flocculation was measured by Zetasizer 2000 (Malvern Instruments Ltd., Company, England). And the procedure of sampling was carried out at three different time-points: kaolin suspension without additives, after adding the bioflocculant, and after adding Ca^{2+}.

2.6. Purification of real wastewater

Swine wastewater taken from Jiancha pig farm, Sichuan province, China, was chosen as a representative suspended sample. The concentrations of COD, ammonium, and turbidity of this solution were 1064, 828 mg L^{-1}, and 157 NTU, respectively. Experiments were performed to obtain the optimal bioflocculant dose and pH value for COD, ammonium and turbidity removal from swine wastewater. A sample of 1.0 L wastewater was poured in a beaker and the pH value was adjusted using 1.0 mol L^{-1} NaOH or HCl if necessary. The bioflocculant was then added, and the mixture was stirred at the design agitation speed for 10 min, and then allowed to stand 30 min. The supernatant was collected and the residual COD, ammonium, and turbidity were determined according to the APHA Standard Methods (APHA, 2005). Sludge for dewatering tests was obtained from the secondary settling tank at Tuanjie Wastewater Treatment Co., Ltd., Sichuan province, China. The dewaterability of the sludge was expressed in terms of dry solids (DS) and specific resistance to filtration (SRF). Flocculants, including the biopolymer, $Al_2(SO_4)_3$, PAC, and PAM, were separately added into a 200 mL mixing chamber with 100 mL sludge, and the mixtures were stirred at the design agitation speed for 10 min. After agitation, all the samples were allowed to stand for 30 min, and then were poured into the funnel fitted with a filter paper separately. After 2 min of gravitational drainage, a vacuum of 0.04 MPa was applied. The volume of the filtrate collected every 15 s was recorded.

The DS of dewatered sludge was determined according to Eq. (2):

$$\mathrm{DS} = \frac{W_2}{W_1} \times 100\% \quad (2)$$

where W_1 is the weight of wet filter cake and W_2 is the weight of filter cake after drying at 105 °C for 8 h.

The SRF was calculated by the following equation:

$$\frac{dt}{dV} = \frac{\mu}{A(\Delta p)}\left(\frac{\alpha c V}{A} + R_{\mathrm{m}}\right) \quad (3)$$

where t is the time, V is the filtrate volume, μ is the filtrate viscosity, A is the filter area, Δp is the pressure drop across filter, c is the slurry concentration, α is the SRF and R_{m} is the resistance of filter medium (neglected).

3. Results and discussions

3.1. Time course assay of flocculating rate and cell quantity

As seen from the growth curve of the strain in hydrolyzate-containing cultivation medium in Fig. S1, the cells were in logarithm growth phase during 6–54 h, with a rapid growth period occurring during 12–18 h, and entered stationary phase since 60 h with a maximum cell number of 19.6×10^7 (mL^{-1}). The average