Bioresource Technology 152 (2014) 490–498

Contents lists available at ScienceDirect

Bioresource Technology

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

# Preparation and characteristics of bacterial polymer using pre-treated sludge from swine wastewater treatment plant

Junyuan Guo [a,*], Chunping Yang [b,c], Lanyan Peng [c]

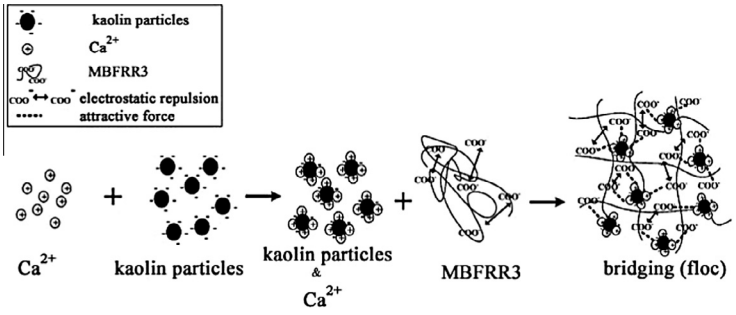
[a] College of Resources and Environment, Chengdu University of Information Technology, Chengdu, Sichuan 610225, China
[b] Zhejiang Provincial Key Laboratory of Waste Treatment and Recycling, College of Environmental Science and Engineering, Zhejiang Gongshang University, Hangzhou, Zhejiang 310018, China
[c] College of Environmental Science and Engineering, Hunan University, Changsha, Hunan 410082, China

## HIGHLIGHTS

- Swine wastewater sludge was first time used as medium for bioflocculant production.
- Effect of sludge pretreatments on MBFRR3 production and flocculation was determined.
- Different forms (slime, capsular, and broth) exhibited distinct flocculation behavior.
- The main component of the purified sludge bioflocculant is a protein.
- MBFRR3 combined with $Ca^{2+}$ acted as conditioning agent.

## GRAPHICAL ABSTRACT

Schematic diagram of flocculation mechanism of bioflocculant MBFRR3. The flocculation of kaolin particles was completed by charge neutralization and bridging mechanism by addition $Ca^{2+}$ in two steps, coagulation and flocculation. First step was the coagulation, in which $Ca^{2+}$ draw closer to the negatively charged kaolin particles through columbic attraction and $Ca^{2+}$–kaolin complexes were formed. $Ca^{2+}$ reduced the thickness of the diffuse double layer of adjacent kaolin particles and hence, reducing the inter particle distance between particles. This phenomenon was evidenced by the decrease of *Zeta* potentials of kaolin suspensions after $Ca^{2+}$ addition. Second step was the flocculation, in which bioflocculants act like a bridging agent of two or more $Ca^{2+}$–kaolin complexes and reduces inter-particle distances through the ionic bonds mechanism, and bridging occurred after the $Ca^{2+}$–kaolin complexes adsorbed onto the bioflocculants chains.




## ARTICLE INFO

*Article history:*
Received 14 September 2013
Received in revised form 14 November 2013
Accepted 16 November 2013
Available online 23 November 2013

*Keywords:*
Swine wastewater sludge
Pre-treatment
Fermentation
Polymeric substances
Flocculating activity

## ABSTRACT

Sterilization, alkaline-thermal, and acid-thermal treatments were applied to different suspended sludge solids (SSS) concentrations and the pre-treated sludge was used as raw material for bioflocculant-producing bacteria R3 to produce bioflocculant. After 60 h of fermentation, three forms of bioflocculant (broth, capsular, and slime) were extracted, and maximum broth bioflocculant of 2.9 and 4.1 g $L^{-1}$ were produced in sterilized and alkaline-thermal treated sludge as compared to that of 1.8 g $L^{-1}$ in acid-thermal treated sludge. Higher bioflocculant quantity was produced in SS of 15, 25, and 35 g $L^{-1}$ compared to that produced in SS of 45, 55, and 65 g $L^{-1}$. Bioflocculant combined with 0.5 g $Ca^{2+}$ in 1.0 L kaolin suspension acted as conditioning agent, and maximum flocculating activity of 94.5% and 92.8% was achieved using broth and slime bioflocculant, respectively. The results demonstrated that wastewater sludge could be used as sources to prepare bioflocculants.



* Corresponding author. Tel.: +86 28 85966913; fax: +86 28 85966089.
*E-mail address:* gjy@cuit.edu.cn (J. Guo).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.11.037

## 1. Introduction

Inorganic flocculants and organic synthetic polymers widely used for coagulation–flocculation processes in wastewater treatment have been reported to be toxic and non-readily degradable (Shih et al., 2001; Gao et al., 2009). Despite the effective flocculating performance of these flocculants, their degraded monomers always carry serious health and environmental concerns including Alzheimer's disease caused by aluminum salts (Arezoo, 2002), as well as the formation of neurotoxic and carcinogenic acrylamide monomers that are harmful to environment during polyacrylamide derivatives degradation (Rudén, 2004). On the contrary, bioflocculant, secreted by microorganisms during their active secretion and cell lysis, is a kind of environment friendly material with the character of harmless and biodegradable, which has been considered as a potential solution to the toxicity to aquatic life and environment pollution in recent years (Li et al., 2009; Liu et al., 2010; Sun et al., 2012). Broadly, due to the special properties (adsorption capability and degradability), bioflocculants have attracted wide attention in wastewater treatment, drinking water purification, and downstream processes in biotechnology (You et al., 2008).

Over the past decades, most of research focused on screening new strains producing bioflocculants with high flocculating activity and optimization of their culture conditions for a higher yield and lower cost (Wang et al., 2007; Yang et al., 2009; Bo et al., 2012). And the production of bioflocculant reported till date mostly is in synthetic media (Gong et al., 2008; More et al., 2012). Thus, high production costs associated with relatively expensive substrates is still the major impediments to its application (Deng et al., 2005; Liu et al., 2010; 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 (Gong et al., 2008). Wastewater sludge, from swine wastewater treatment plant through biological process, is potentially economical media as it is a rich source of carbon, nitrogen, phosphorus, and other nutrients, such as polysaccharide, protein, cellulose, and so on, which have been supposed to be a source of bioflocculant (More et al., 2010), and hence, the use of sludge will be advantageous for growth of the microorganisms isolated from the wastewater sludge which are already well adapted to it, and the strains that can effectively utilize the substrates in wastewater sludge to produce bioflocculants are of academic and practical interests. In general, sludge treatments disintegrated the organic fractions and released soluble carbon and nitrogen into the sludge medium, thus, pre-treatment of the sludge is essential for solubilising complex carbon sources to simpler ones which helps in accelerating growth and product formation by bacteria (More et al., 2012). Moreover, concentration of suspended solids (SS) of the wastewater sludge always affects the cell growth rate and product formation by the bacteria cultured due to the fact that nutrients required for cell growth are mostly embedded into it (Drouin et al., 2008).

In this study, a bioflocculant-producing bacteria (named R3), screened from wastewater sludge, was identified as *Rhodococcus* by 16S rDNA sequence and its biochemical and physiological characteristics. And a series of experiments were conducted to investigate the bioflocculant production and flocculation performance. Bioflocculant-producing R3 was cultured in sludge to determine the optimum SS concentration for the maximum bioflocculant yield. Three types of sludge pre-treatments at different SS concentration, sterilization (ST), alkaline-thermal (ALT), and acid-thermal (ACT) for their impact on bioflocculant production were also investigated. Kaolin flocculation activities of different forms (broth, capsular, and slime) were studied in presence of coagulant aid ($Ca^{2+}$). In addition, the characteristic of purified bioflocculant was examined. Moreover, based on the experimental results, the performance of this bioflocculant and its flocculating mechanisms were further proposed. By the above methods, not only wastewater sludge could be recycled but also the source of bioflocculant was be broadened.

## 2. Methods

### 2.1. Bacteria strains

Bioflocculant-production strain R3, identified as *Rhodococcus* by 16S rDNA sequence and its biochemical and physiological characteristics, was isolated from activated sludge (Guo et al., 2013). The activated sludge was collected from a dewatering workshop at Jinxia Wastewater Treatment Co., Ltd., Hunan province, China, which treated municipal domestic sewage mainly with a treatment capacity of $0.8 \times 10^4$ $m^3$ $h^{-1}$. The bacteria R3 was stored at 4 °C and sub-cultured fortnightly, and the medium for slant and subculture consisted of (per liter): peptone 10 g, yeast extracts 5.0 g, NaCl 10 g, agar 20 g, with initial pH of 7.0.

A total of 1.0 mL of activated sludge sample (prepared with swine wastewater at the sludge concentration of 100 g $L^{-1}$) was serially diluted with distilled water ($10^1$–$10^{10}$folds), and subsequently, 1.0 mL of each dilution was spread onto agar plates. The composition of the agar plates was as follows (per liter): urea 5.0 g, yeast extracts 0.5 g, sucrose 20 g, $K_2HPO_4$ 5.0 g, $KH_2PO_4$ 2.0 g, $MgSO_4$ 2.0 g, NaCl 10 g, and agar 10 g. Then, the plates were sealed and inverted and incubated at 35 °C in an incubator. Visible colonies appeared after 48 h of cultivation. After 4–6 cycles of replanting onto the agar plates, a total of 34 morphologically different isolates were obtained, in which the 9 large and viscous colonies were chosen and individually inoculated on a reciprocal shaker (SHA-A, Shanghai Lianhua Industrial Co., Ltd., China) at 150 rpm and 35 °C for 24 h in 100 mL culture medium. The culture medium consisted of urea 5.0 g, yeast extract 0.5 g, sucrose 20 g, $K_2HPO_4$ 5.0 g, $KH_2PO_4$ 2.0 g, $MgSO_4$ 2.0 g, and NaCl 10 g dissolved in 1.0 L distilled water with the pH value adjusted to 7.0. The flocculating activities of the 9 strains were measured by 4.0 g $L^{-1}$ kaolin clay suspensions method (as Section 2.5) and the strains which showed high flocculating activity were selected as bioflocculant-producing bacteria for further studies. All the 9 strains yielded flocculating activities above 70%, and the bioflocculant-producing bacteria with the highest flocculating activity of 92.3%, named R3, was selected for further tests. The strain R3 was stored at 4 °C and sub-cultured fortnightly, and the medium for slant and subculture consisted of (per liter): peptone 10 g, yeast extracts 5.0 g, NaCl 10 g, agar 20 g, with initial pH value of 7.0. The 16S rRNA gene fragment of the strain R3 was amplified using individual bacterial colony PCR. PCR amplification of 16S rDNA was carried out using forward primer (50-GAG AGT TTG ATC CTG GCT CAG-30) and reverse primer (50-CTA CGG CTA CCT TGT TAC GA-30). The PCR amplification 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 end at 4 °C. The PCR product was sequenced and analyzed by Guangdong Institute of Microbiology (Guangzhou, China). Results showed that the similarity of the 16S rDNA sequences of the strain R3 and the *Rhodococcus erythropolis* CCTCC 10543 reached 99%. According to the 16S rDNA gene sequence, R3 could be identified as *R. erythropolis*.

### 2.2. Sludge sample

The sludge (without addition of chemical polymers) for bioflocculant production was collected from biofiltration unit at a