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Flocculation behavior and mechanism of bioflocculant produced by *Aspergillus flavus*

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ABSTRACT

In this study, the flocculation behavior and mechanism of a cation-independent bioflocculant IH-7 produced by *Aspergillus flavus* were investigated. Results showed 91.6% was the lowest flocculating rate recorded by IH-7 (0.5 mg L^{-1}) at pH range 4–8. Moreover, IH-7 showed better flocculation performance than *polyaluminum chloride* (*PAC*) at a wide range of flocculant concentration (0.06–25 mg L^{-1}), temperature (5–45 °C) and salinity (10–60% w/w). The current study found that cation addition did not significantly enhance the flocculating rate and IH-7 is a positively charged bioflocculant. These findings suggest that charge neutralization is the main flocculation mechanism of IH-7 bioflocculant. IH-7 was significantly used to flocculate different types of suspended solids such as activated carbons, kaolin clays, soil solids and yeast cells.

1. Introduction

Flocculating agents are widely used in industrial processes such as, drinking water purification, food processing and wastewater treatment. Flocculants are classified into inorganic flocculants such as polyaluminum chloride (PAC), synthetic organic flocculants such as polyacrylamide derivatives and natural occurring flocculants such as bioflocculants and chitosan (Bezawada et al., 2013; Prazeres et al., 2013; Riaño and García-González, 2014). Chemical flocculants are commonly used in water and wastewater treatment industries because of their cost effectiveness and efficient flocculation activities (More et al., 2014). However, the use of these chemical flocculants can cause health and environmental problems. For example, a residual aluminum from PAC and acrylamide monomers from polyacrylamide is known to be neurotoxic and carcinogenic toward humans (Hierrezuelo et al., 2010). Currently most of bioflocculants have attracted research and industry interests, as alternative flocculant, due to their high flocculation performance, ecofriendly, biodegradability and some of them could be produced from biological or agro-industrial wastes (Aljuboori et al., 2013, 2014; Bezawada et al., 2013).

To date, over 100 species of bioflocculant-producing microorganisms have been reported and their bioflocculant characterized. Most studies have shown that bioflocculants are primarily formed by polysaccharides and proteins. The chemical composition and physical properties of bioflocculants are usually determined their flocculating capability and mechanism. For instance, the main bioflocculation mechanisms are achieved through polymer bridging and charge neutralization. Polymer bridging proposes that cation-mediated bridges between the kaolin particles and bioflocculant chains primarily form flocs (Sobeck and Higgins, 2002). The role of cations is to increase the initial adsorption of bioflocculants onto kaolin particles, by decreasing the negative charges on bioflocculant chain and kaolin particles (Yim et al., 2007). As for charge neutralization, the negative charges on impurities (either colloids or particles) are neutralized by a positively charged bioflocculant (Lian et al., 2008). This promotes electrostatic interaction between the positively charged bioflocculant and colloids. As a result, it produces attraction and charge neutralization of the colloids surface, leading to the flocs formation and decreasing electrical repulsion between them (Feng et al., 2013).

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Based on the main bioflocculation mechanisms, bioflocculants can be classified into cation-dependent bioflocculants (bioflocculant that require cation addition) such as bioflocculant produced by *Serratia ficaria* (Gong et al., 2008), *Corynebacterium glutamicum* (He et al., 2004) and *Halomonas* sp. (He et al., 2010) and cation-independent bioflocculants (bioflocculant that function without cation) such as bioflocculant produced by *Bacillus mucilaginosus* (Deng et al., 2003) and *Klebsiella pneumoniae* (Zhao et al., 2013). The flocculation performance of bioflocculant can be affected by many factors including bioflocculant concentration, pH, temperature, metal ions, salinity, mixing speed and types of colloids. Therefore, understanding the flocculation behavior and identifying the flocculation mechanism of bioflocculant would highly improve the bioflocculation process.

In this study, the flocculation behavior, factors affecting the flocculting rate *and flocculation mechanism of* cation-independent bioflocculant (IH-7) produced by *Aspergillus flavus* were investigated. In addition, the flocculation performance of IH-7 and PAC were investigated at wide range of chemical and environmental conditions. To our knowledge, *this is the first report on flocculation behavior and mechanism of* cation-independent *bioflocculant*.

2. Materials and methods

2.1. Microorganism, culture conditions and preparation of IH-7

A bioflocculant-producing microorganism, *A. flavus* link S44-1, isolated by the Department of Biotechnology and preserved at the **Microbial Culture Collection Unit (UNiCC),** University Putra Malaysia was maintained on Potato Dextrose Agar (PDA) (Merck, Germany) slant at 4 °C. In order to carryout the experiment, the *A. flavus* link S44-1 was sub-cultured every 30–40 days. The optimized production medium consisted of (g L^{-1}): sucrose (11.87) (C source), peptone (7.52) (N source), $MgSO_4 \cdot 7H_2O$ (0.5), KCl (0.5), $FeSO_4$ (0.01), K_2HPO_4 (1.0). The initial pH of the production medium was adjusted to 7.0. The fungus was cultured in 1000 mL Erlenmeyer flasks containing 500 mL medium and incubated in a shaker at 200 rpm for 60 h at 40 °C (Aljuboori et al., 2013). Subsequently, the IH-7 was prepared and purified according to the method previously described by Aljuboori et al. (2013). A purified IH-7 consisted of 28.5% protein and 69.7% sugar, including 40% neutral sugar, 2.48% uronic acid and 1.8% amino sugar. The average molecular weight of IH-7 is 2.574×10^4 Da. The IR spectrum showed purified IH-7 contained hydroxyl, amide, carboxyl and methoxyl groups (Aljuboori et al., 2013).

2.2. Determination of flocculating rate

The flocculating rate of the IH-7 and PAC was measured using a Kaolin suspension. Briefly, 2 g of Kaolin clay (Merck, Germany) was suspended in a 1 L of deionized water. Different concentrations of flocculants were added to 200 mL aliquots of kaolin suspension in 400 mL beaker, and the pH value was adjusted to 7.0 using 1 M NaOH or HCl. The mixture was then vigorously stirred at 150 rpm for 1 min, slowly stirred at 80 rpm for 5 min, and then allowed to stand for 5 min using a 6-breaker jar tester (JLT6, VELP Scientifica, Italy). The optical density (OD) of the clarified solution was measured with a spectrophotometer (Genesys 10 UV, Thermo Scientific, USA) at 550 nm. Finally, the flocculating rate was calculated according to the following equation:

$$\text{Flocculating rate \%} = (A - B)/A \times 100 \quad (1)$$

where *A* and *B* are the OD_{550} (optical density at 550 nm) of the control and sample supernatant, respectively.

2.3. Factors affecting the flocculating rate

The effects of IH-7 and PAC concentrations of 0.06–25 mg L^{-1}, pH values of 4, 5, 6, 7, 8, 9 and 10 and temperatures of 5, 10, 15, 20, 25, 30, 35, 40 and 45 °C on the flocculating rate were investigated. In addition, the effects of metal ions (KCl, NaCl, $MgCl_2$, $CaCl_2$, $FeSO_4$ and $FeCl_3$) and salinity at 0, 10, 20, 40, 60, 80–100% (w/w) on flocculating rate were measured. The influence of different initial concentrations of kaolin (0.5, 1, 2, 4, 6, 8–10 g L^{-1}) and mixing speeds on the flocculating rate were also studied. Finally, the effects of different suspended solids, yeast, activated carbon, and soil solids were examined. All the experiments were conducted in triplicate.

2.4. Flocculation mechanism

2.4.1. Surface structure of flocs and zeta potential

The surface structure of kaolin particles, IH-7 bioflocculant and floc were examined using a Hitachi SU-70 *Scanning Electron Microscope* (*SEM*; *Hitachi*, *USA*). *The zeta potential of the kaolin suspension* (2 g L^{-1}), bioflocculant IH-7, treated water (purified water) and kaolin clay flocculated by IH-7 (flocs) were measured using a Zetasizer Nano ZS90 (Malvern, United Kingdom).

3. Results and discussion

3.1. Flocculation performance of bioflocculant IH-7

3.1.1. Effects of IH-7 concentration

As shown in Fig. 1, the flocculating rate increased dramatically as the IH-7 concentration increased from 0.06 to 0.25 mg L^{-1}. The highest flocculating rate of 97.4% was recorded with 1 mg L^{-1} of IH-7, above which, the flocculating rate gradually decreased. These results showed that excessive doses of bioflocculant caused restabilization of the colloids, while low doses of IH-7 were insufficient to destabilize and aggregate most of the colloids in the kaolin suspension, both excessive and low doses resulted in low flocculating rates (Bratskaya et al., 2005; Wu and Ye, 2007). Overall, IH-7 showed over 80% of flocculating rate at a wide range of concentrations (0.12–25 mg L^{-1}) which was similar to those bioflocculants produced by *Pseudoalteromonas* sp. (Li et al., 2008), *Chryseobacterium daeguense* (Liu et al., 2010) and *Halomonas* sp. (He et al., 2010).

In comparison to common chemical flocculant PAC, the flocculating rate of IH-7 was higher at wide range of flocculant concentrations (0.06–25 mg L^{-1}). However, the flocculating rate of PAC was rapidly increased from 0.06 to 0.75 mg L^{-1}, above which, the

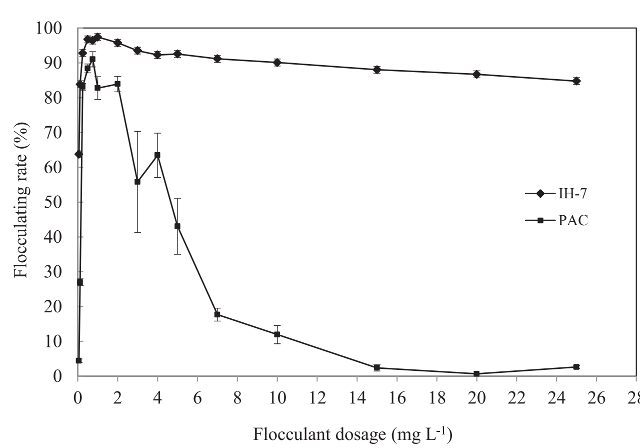

Fig. 1. Effects of flocculant concentration on flocculation performance.