Bioresource Technology 127 (2013) 489–493

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

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

Production and characterization of a bioflocculant produced by *Aspergillus flavus*

Ahmad H. Rajab Aljuboori [a,*], Azni Idris [a], Norhafizah Abdullah [a], Rosfarizan Mohamad [b]

[a] Department of Chemical and Environmental Engineering, University Putra Malaysia, Sri Serdang 43400, Selangor, Malaysia
[b] Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, University Putra Malaysia, Sri Serdang 43400, Selangor, Malaysia

HIGHLIGHTS

- Cation-independent bioflocculant produced by *Aspergillus flavus*.
- Sucrose and peptone are the most favorable sources for bioflocculant production.
- The bioflocculant mainly consists of polysaccharide and protein with a molecular weight of 2.57×10^4 Da.
- The bioflocculant is stable at wide ranges of pH and temperature.

ARTICLE INFO

Article history:
Received 13 December 2011
Received in revised form 28 July 2012
Accepted 7 September 2012
Available online 14 September 2012

Keywords:
Aspergillus flavus
Bioflocculant
Microbial flocculant
Bioflocculant stability
Extracellular biopolymeric substance

ABSTRACT

The production and characterization of a bioflocculant, IH-7, by *Aspergillus flavus* was investigated. About 0.4 g of purified bioflocculant with an average molecular weight of 2.574×10^4 Da could be obtained from 1 L of fermentation medium. The bioflocculant mainly consisted of protein (28.5%) and sugar (69.7%), including 40% of neutral sugar, 2.48% of uronic acid and 1.8% amino sugar. The neutral sugar components are sucrose, lactose, glucose, xylose, galactose, mannose and fructose at a molar ratio of 2.4:4.4:4.1:5.8:9.9:0.8:3.1. Fourier-transform infrared spectroscopy analysis revealed that purified IH-7 contained hydroxyl, amide, carboxyl and methoxyl groups. The elemental analysis of purified IH-7 showed that the weight fractions of the elements C, H, O, N and S were 29.9%, 4.8%, 34.7%, 3.3%, and 2.0%, respectively. IH-7 had good flocculating rate in kaolin suspension without cation addition and stable over wide range of pH and temperature.

1. Introduction

Flocculants are useful agents in the aggregation of colloids, cells and suspended solids and are commonly used for drinking water production, waste water treatment, fermentation processes, and food production (Shih et al., 2001). These flocculants are usually classified into three groups: (1) inorganic flocculants such as polyaluminum chloride, (2) organic synthetic flocculants such as polyacrylaminde derivatives and (3) naturally occurring flocculants such as chitosan. Inorganic and organic synthetic flocculants are widely used in industrial fields because of their cost effectiveness and efficiency, although their use may incur some environmental and health problems (Liu et al., 2009). In contrast, bioflocculants, extracellular biopolymeric substances secreted by bacteria, fungi, algae and yeast (Salehizadeh and Shojaosadati, 2001) are biodegradable and nontoxic flocculants (Dermlim et al., 1999).

Bioflocculants are mainly composed of macromolecular substances, such as polysaccharide and protein (Lu et al., 2005; Zheng et al., 2008). The composition and properties of bioflocculants depend on type of bioflocculant-producing microorganisms (BPMs), composition of media and environmental conditions. The differences in the composition and properties of polysaccharides and proteins lead to differences in the charge of bioflocculant (Bala Subramanian et al., 2010). The flocculation ability of bioflocculants produced by *Vagococcus* sp. (Gao et al., 2006), *Halomonas* sp. (He et al., 2010), *Bacillus circulans* (Li et al., 2009a), *Pseudoalteromonas* sp. (Li et al., 2008), *Serratia ficaria* (Gong et al., 2008), *Bacillus licheniformis* (Li et al., 2009b), and *Klebsiella mobilis* (Wang et al., 2007) depends on the presence of cations. Ca^{2+} is known to link negatively charge groups on flocculants and particles (He et al., 2010). Addition of cations to the flocculation process can produce secondary pollution and increase costs. Thus, finding cation-independent bioflocculants is desirable.

In the present study, the production of a bioflocculant, IH-7, produced by *Aspergillus flavus*, was investigated to determine optimal culture medium composition and environmental conditions. Various factors influencing the production of IH-7, like carbon source, nitrogen source, C/N ratio, initial pH of culture medium,

* Corresponding author. Address: Chemical and Environmental Engineering Department, Faculty of Engineering, University Putra Malaysia. Tel.: +60 12 357 5668; fax: +60 38 656 7120.
E-mail address: ahmaddjla2001@yahoo.com (Ahmad H. Rajab Aljuboori).

0960-8524/$ - see front matter © 2012 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.biortech.2012.09.016

culture temperature, metal ions, inoculum size and time course were investigated. The composition and properties of the IH-7 were investigated as well.

2. Materials and methods

2.1. Microorganism and culture conditions

A. flavus S44-1, isolated by the Department of Biotechnology and preserved at the Microbial Culture Collection Unit (UNiCC), Laboratory of Industrial Biotechnology, Institute of Bioscience, University Putra Malaysia (Mohamad and Ariff, 2007) (Selangor, Malaysia), was maintained on slant media at 4 °C and sub-cultured every 30–40 days. The medium for slant and subculture consisted of (g/l): potato extract, 4; glucose, 20; agar, 15; and the initial pH was adjusted to 5.6 ± 0.2. The production medium consisted of (g/l): sucrose, 30; peptone, 3.0; $MgSO_4{\cdot}7H_2O$, 0.5; KCl, 0.5; $FeSO_4$, 0.01; K_2HPO_4, 1.0; and the initial pH was adjusted to 6.0. The fungus was cultured in 100-mL Erlenmeyer flasks containing 50 ml of medium and incubated in a shaker at 180 rpm for 3 days at 30 °C. Samples were taken at different time intervals to determine flocculation rate and fungal biomass weight. The biomass samples were filtered and dried at 80 °C in an oven for 4 h (Deng et al., 2005). Distilled water was used to prepare all medium solutions and media were sterilized at 121 °C for 20 min.

2.2. Optimization of culture conditions of A. flavus for IH-7 production

Nine factors including carbon source, nitrogen source, C/N ratio, initial pH, culture temperature, inoculum size, metal ions and culture time were investigated. To determine the effect of carbon and nitrogen sources on bioflocculant production, sucrose was replaced with glucose, fructose, lactose, starch, and glycerol (30 g/l for each type of carbon source) and peptone was replaced with $(NH_4)_2SO_4$, NH_4NO_3, $NaNO_3$, yeast extract, urea and glutamic acid (3 g/l for each type of nitrogen source). To study the effect of metal ions on bioflocculant production, KCl was replaced with NaCl, $CaCl_2$, $MgCl_2$, $MnCl_2$ and $FeCl_3$ at same concentration. For the C/N ratio different concentrations of sucrose were used in order to get the different C/N ratio of 0/1–50/1 while peptone kept constant. In initial pH of production media were adjusted at 2–9. Temperature of production media were adjusted at 15–45 °C. Inoculum size used 0.2–10% v/v. The effect of time course of IH-7 production was investigated between 0 and 96 h. All the experiments were conducted in triplicate.

2.3. Bioflocculant purification

Two volumes of cold ethanol (at 4 °C) were added to 1 L culture broth (supernatant).

The precipitate was dissolved in 100 ml deionized water and 50 mL of 2% cetylpyridinium chloride solution (CPC) was added to the solution and thoroughly mixed. After three hours, the precipitate was collected and dissolved in 100 mL of 0.5 M NaCl. Two volumes of cold ethanol were added and the precipitate was washed with ethanol, Dissolved in 5 mL of deionized water and vacuum-dried (Deng et al., 2005).

2.4. Physical and chemical analysis of IH-7 bioflocculant

The total sugar content of IH-7 bioflocculant was determined according to the phenolsulfuric acid method using glucose as standard (Chaplin and Kennedy, 1994). The total protein content was determined by the Bradford method with bovine serum albumin as standard (Bradford, 1976). The uronic acid was determined using the carbazole–sulfuric acid method (Chaplin and Kennedy, 1994). Amino sugars were determined using the Elson–Morgan method with glucose amine as standard (Chaplin and Kennedy, 1994). In order to determine of its sugar composition, IH-7 was hydrolyzed with trifluoroacetic acid at 121 °C for 1 h. The resulting sugars were analyzed by high-performance liquid chromatography (HPLC) with a Rezex RCMMonosaccharide Ca^{2+} column (300 × 7.8 mm) using deionized distilled water as eluent at a flow rate of 0.4 ml/min, a column temperature of 85 °C and back pressure of 331 psi. Elemental analysis was carried out with a Truspec CHN/CHNS elemental analyzer (LECO, USA). The functional groups of IH-7 were determined with a Spectrum 100 FTIR spectrometer (PerkinElmer, USA). The molecular weight of IH-7 bioflocculant was measured by a Waters-Alliance 2695 GPC system (Waters, USA), equipped with a Waters 2410 Reactive Index Detector (RID) and a Waters Ultrahydrogel, linear column (7.8 × 300 mm). The mobile phase was 0.1 M sodium nitrate at a flow rate of 0.6 ml/min and the column was operated at room temperature. The injection volume was 20 μL. A calibration curve was constructed using dextran T standards. The average molecular weight was calculated using Empower soft ware (System Software, Empower option GPC, Waters Co.)

2.5. Determination of flocculating rate

A kaolin suspension was used to determine the flocculating rate of the bioflocculant in culture broth. Two gram of Kaolin clay (Merck, Germany) was suspended in 1 L of deionized water. One ml of culture broth was added to 99 ml of kaolin suspension in a 400-ml beaker and the pH value was adjusted to 7.0 using 1 M NaOH or HCl. The mixture was stirred at 200 rpm for 1 min, slowly stirred at 80 rpm for 5 min, and allowed to stand for 5 min using jar tester (JLT6, VELP SCIENTIFICA, Italy). The optical density (OD) of the supernatant was measured with a spectrophotometer (GENESYS 10 UV, Thermo Scientific, USA) at 550 nm. In the control experiment, 1 ml of culture broth was replaced with 1 ml of fresh culture medium. The flocculating rate was calculated according to the following equation:

$$\text{Flocculating rate } (\%) = (A550 - B550)/A550 \times 100$$

where A550 and B550 were the OD550 (optical density at 550 nm) of control and sample supernatant, respectively.

2.6. pH stability of purified IH-7

Pure IH-7 was dissolved in a suitable volume of deionized water to achieve an initial flocculating rate of over 90% and divided into eight aliquots. The pH of the aliquots was adjusted to 3, 4, 5, 6, 7, 8, 9 and 10 with 1 M of NaOH or HCl. The aliquots were kept at 4 °C for 24 h (He et al., 2004), and the flocculating rates were measured at room temperature, kaolin suspension 2 g/l adjusted at pH 7, the mixture stirred at 200 rpm for 1 min, slowly stirred at 80 rpm for 5 min, and allowed to stand for 5 min.

2.7. Thermo-stability of purified IH-7

Pure IH-7 was dissolved in a suitable volume of deionized water to achieve an initial flocculating rate of over 90%, and divided into six groups with a pH of 3, 4, 5, 6, 7 and 8. Six aliquots of each pH were treated at temperatures of 10, 20, 40, 60, 80 and 100 °C for 1 h in a water bath (He et al., 2004), and the flocculating rates were measured at room temperature, kaolin suspension 2 g/l adjusted at pH 7, the mixture stirred at 200 rpm for 1 min, slowly stirred at 80 rpm for 5 min, and allowed to stand for 5 min.