International Journal of Mineral Processing 137 (2015) 15–25

Contents lists available at ScienceDirect

International Journal of Mineral Processing

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

Production and characterization of bioflocculants for mineral processing applications

K. Karthiga devi [a], K.A. Natarajan [b,*]

[a] Department of Materials Engineering, Indian Institute of Science, Bangalore, 560 012, India
[b] Department of Materials Engineering, Indian Institute of Science, Bangalore 560 012, India

ARTICLE INFO

Article history:
Received 6 June 2014
Received in revised form 19 November 2014
Accepted 14 February 2015
Available online 17 February 2015

Keywords:
Bacillus licheniformis
Bacillus firmus
Bioflocculants
Clay flocculation
Bioremediation

ABSTRACT

A comparative study of two bacterial strains namely, *Bacillus licheniformis* and *Bacillus firmus* in the production of bioflocculants was made. The highest bioflocculant yield of 16.55 g/L was obtained from *B. licheniformis* (L) and 10 g/L from *B. firmus* (F). The bioflocculants obtained from the bacterial species were water soluble and insoluble in organic solvents. FTIR spectral analysis revealed the presence of hydroxyl, carboxyl and sugar derivatives in the bioflocculants. Thermal characterization by differential scanning calorimetry (DSC) showed the crystalline transition and the melting point (T_m) at 90-100 °C. Effects of bioflocculant dosage and pH on the flocculation of clay fines were evaluated. Highest bioflocculation efficiency on kaolin clay suspensions was observed at an optimum bioflocculant dosage of 5 g/L. The optimum pH range for the maximum bioflocculation was at pH 7–9. Bioflocculants exhibited high efficiency in dye decolorization. The maximum Cr (VI) removal was found to be 85 % for L (bioflocculant dosage at 2 g/L). This study demonstrates that microbial bioflocculants find potential applications in mineral processing such as selective flocculation of mineral fines, decolorization of dye solutions and in the remediation of toxic metal solutions.

1. Introduction

Microbially-produced flocculants or bioflocculants can be defined as biopolymers which induce particle to particle flocculation through formation of bridges resulting in the agglomeration and settling of suspended fine particles. Bioflocculants may be economically produced on a large scale and easily harvested from fermentation broths. They find wide industrial applications in the manufacture and processing of textiles and detergents, in clay and turbidity removal, oil recovery, metal recovery and waste water treatment (Kumar et al., 2004).

Major components of bioflocculants include extracellular polymeric substances such as polysaccharides, proteins, glycoproteins or nucleic acids (Lazarova and Manem, 1995; Gao et al., 2006). Use of bioflocculants in place of toxic chemical reagents is advantageous since they are biodegradable, nontoxic and free of secondary pollution risk. Bioflocculants could replace currently used organic synthetic flocculants which possess inherent drawbacks of being a source of carcinogenic monomers, and often nonbiodegradable. Bioflocculants have been widely used in wastewater treatment, and in food-processing and fermentation industries (Wang et al., 2007). The problem of removal of kaolinite turbidity from industrial effluents before discharging to the environment has been widely studied. Kaolinite suspensions have often been chosen as a model for control of natural turbidity of surface waters. Alum in combination with lime have been conventionally used for the removal of clay particles in the effluents.

Increased release of organics and toxic heavy metals from mining operations enters natural water systems causing water pollution and ecological damages. Heavy metals cannot be degraded to harmless products and hence persist in the environment. Several abiotic methods have been used for the removal of heavy metals in contaminated sites, such as chemical precipitation, ion exchange, activated carbon adsorption and reverse osmosis. Use of biosorbents derived from microbial biomass is an emerging trend since it is cost effective and ecofriendly. Several kinds of microorganisms, which secrete flocculating biopolymers, have been screened and isolated from activated sludge, soil and industrial effluents. Environments containing high levels of dissolved metals include active and abandoned mine sites, where the production of acid mine drainage (AMD) is catalyzed by the action of microorganisms (Ledin and Pederson, 1996). The generation of mine wastes causes a huge environmental problem where drainage from tailings and waste rock releases high levels of acid and toxic heavy metals.

Production and characterization of bioflocculants using two types of bacterial strains namely, *Bacillus licheniformis* and *Bacillus firmus* are illustrated in this paper. The utility of extracted bioflocculants in some mineral processing applications is demonstrated with respect to clarification of turbid aqueous solutions, removal of organics and

* Corresponding author.
E-mail addresses: k.karthigabio@gmail.com (K. Karthiga devi), kan@materials.iisc.ernet.in (K.A. Natarajan).

http://dx.doi.org/10.1016/j.minpro.2015.02.007
0301-7516/

metal remediation. Clarification of kaolin clay suspensions, decolorisation of dye solutions and removal of Cr (VI) are taken as typical examples representative of mining and mineral processing operations.

2. Materials and methods

2.1. Minerals

Handpicked pure mineral samples of kaolinite were procured from Alminrock Indscer Fabriks and Indian Bureau of Mines, Bangalore, India. The sample was subjected to dry grinding in a porcelain ball mill, sieved and fractioned to obtain different size fractions followed by sedimentation to separate fine particles of size less than 10 μm. Particle size was determined by Malvern Zetasizer and an average particle size of 8-10 μm was used for flocculation studies. The purity of the mineral was determined by X-ray diffraction and petrographic analysis as 98.6% kaolinite.

2.2. Bacterial culture

Pure strains of *B. licheniformis* (NCIM 2472) and *B. firmus* (NCIM 2264) used in this study were procured from National Collection of Industrial Microorganisms, National Chemical Laboratory, Pune, India.

Potassium nitrate was used to maintain the ionic strength, while sulphuric acid and sodium hydroxide were used as pH modifiers. All the reagents used in the present studies were of analytical reagent grade. Deionized double distilled water was used in all the tests.

B. licheniformis and *B. firmus* were subcultured by inoculating 10 ml of pure strain to 90 ml of LB medium (tryptone — —10 g/L, yeast extract — —5 g/L, NaCl —10 g/L) prepared in a 250 ml Erlenmeyer flask. This was incubated at 30 °C on an Orbitek rotary shaker at 200 rpm.

To study the production of the bioflocculant, pure isolates of *B. licheniformis* and *B. firmus* (10% inoculum) were inoculated in the minimal medium (Table 1) (Sambrook et al., 1989). The initial pH was 7.0–-7.2, autoclaved at 115 °C for 30 min and incubated at 30 °C on a Orbitek rotary shaker at 200 rpm for 2–3 days. Samples were taken at regular time intervals to determine the pH and $OD_{600\ nm}$ (optical density at 600 nm) using aUV–VIS spectrophotometer (Systronics UV–VIS spectrophotometer-117). Changes in pH of the culture medium during growth were monitored at regular time intervals using a Systronics-μ pH SYSTEM 361 digital pH meter.

2.3. Extraction and purification of bioflocculants

The grown bacterial culture broth was taken and centrifuged at 10,000 rpm for 15 min. The collected supernatant was filtered through 0.2 μm sterile filter membrane to obtain cell free extract. The cell free extract was poured into two volumes of ice cold solvents such as ethanol, methanol and acetone (1:2 v/v) to selectively precipitate the bioflocculant. It was allowed to stand overnight at 4 °C in a refrigerator. The resulting precipitate was separated by centrifugation at 10,000 rpm for 15 min and washed by redissolving in double distilled water followed by reprecipitation which was repeated twice. The precipitated bioflocculant was dehydrated for 2 h to remove all water and solvent in vaccum by a rotary evaporator and the crude extract was subjected to dialysis against distilled water and kept overnight at 4 °C (Gao et al., 2009).

Table 1
Composition of minimal media.

Media components (analytical grade)	Concentration
$Na_2HPO_4.\cdot 7H_2O$	12.8 g/L
KH_2PO_4	3 g/L
NaCl	0.5 g/L
NH_4Cl	1 g/L
$MgSO_4$	2 mM
$CaCl_2$	0.1 mM
Glucose	50 g/L

2.4. Characterization of bioflocculants

Total protein content of the bioflocculants was determined using the Bradford method with Bovine serum albumin as a standard (Bradford, 1976). Total sugar content was determined using the phenol-sulphuric acid method with glucose as the standard (Chaplin and Kennedy, 1994).

Thin layer chromatographic (TLC) analysis was performed on silica G-50 plates with butanol, acetic acid and water (3:1:1) as the developing phase, and the spot was detected by spraying agent with 20% H_2SO_4 in methanol followed by drying at 120 °C for 5–10 min. Authentic monosaccharide standards (glucose, fructose, galactose) were run along with the test samples (Patil et al., 2009; Boual et al., 2012). Amino acids were detected by spraying with ninhydrin reagent followed by drying at 120 °C for 5–10 min. Amino acid standards (L-cysteine, glutamic acid) were run along with the test samples. R_f values of different spots were determined by measuring the movement of solute divided by the movement of solvent.

Pure bioflocculant samples were characterized using a Fourier-transform infrared spectrophotometer (FT-IR, Thermo-Nicolet 6700). The dried sample was blended with KBr powder and pressed into pellets for FT-IR spectra measurement in the frequency range of 4000–400 cm^{-1}. The UV-–Visible spectra of the bioflocculants were also analyzed (Spectro UV-VIS dual beam, Labomed, Inc.) (Deng et al., 2003; Abdel-Aziz et al., 2011; Ugbenyen and Okoh, 2013).

Thermal analysis by differential scanning calorimetry (DSC) of bioflocculants was also performed on METTLER DSC 822, using a nitrogen atmosphere with a flow rate of 80 ml/min and heating rate of 10 °C/min (Prasertsan et al., 2006; Kumar et al., 2004).

Additional analysis of the bioflocculants included the solubility test in distilled water and several solvents such as acetone, ethanol, methanol, butanol, propanol and isoamylalcohol (Prasertsan et al., 2006).

2.5. Flocculation efficiency of purified bioflocculants

Experiments were carried out to determine the optimum dosage of the bioflocculants for the kaolin clay suspension (10 g/L) at neutral pH. Different dosages of the purified bioflocculants ranging from 1 mL to 7 mL (5 g/L) were used. The mixture containing 100 mL kaolin clay suspension (10 g/L, pH-7-7.2), 1-7 mL (5 g/L) of bioflocculant sample and 3 mL of 10 g/L of $CaCl_2$ was stirred vigorously and allowed to stand for 2–5 min. The addition of calcium ions neutralizes the negative repulsive changes facilitating enhanced flocculation. The optical density (OD) of the clarified solution was measured at 550 nm. A control experiment was also carried out replacing the bioflocculant with deionized water. The flocculation efficiency was calculated according to the following equation (Gao et al., 2009).

$$\text{Percent flocculation efficiency} = \frac{F_2 - F_1}{F_2} \times 100 \quad (1)$$

where F_1 and F_2 are the optical densities of the sample and control at 550 nm respectively. Effect of pH on flocculation efficiency was also similarly studied.

2.6. Decolorization experiments

Dye concentration of 10 mg/L was used in all experiments. 4 mL of bioflocculant solution (2 g L^{-1}) was added to 10 mL orange G, methylene blue, crystal violet and malachite green dye solution (10 mg /L) and well mixed in a shaker for 30 min. The absorbance of the sample was measured under the maximum wavelength of the dye (500, 660,580 and 620 nm for orange G, methylene blue, crystal violet and