Characteristics of a bioflocculant produced by a consortium of *Cobetia* and *Bacillus* species and its application in the treatment of wastewaters

AM Ugbenyen and AI Okoh*

Applied and Environmental Microbiology Research Group (AEMREG), Department of Biochemistry and Microbiology, University of Fort Hare, Private Bag X1314, Alice 5700, South Africa

ABSTRACT

The characteristics of a bioflocculant produced by a consortium of 2 bacteria belonging to the genera *Cobetia* and *Bacillus* was investigated. The extracellular bioflocculant was composed of 66% uronic acid and 31% protein and showed an optimum flocculation (90% flocculating activity) of kaolin suspension at a dosage of 0.8 mg/mℓ, pH of 8, and with Ca^{2+} as a coagulant aid. The bioflocculant is thermally stable, with a high residual flocculating activity of 86.7%, 89.3% and 87% after heating at 50°C, 80°C and 100°C, respectively, for 25 min. FTIR analysis of the bioflocculant indicated the presence of hydroxyl, amino, carbonyl and carboxyl functional groups. Scanning electron microscopy (SEM) revealed a crystal-linear sponge-like bioflocculant structure and EDX analysis of purified bioflocculant indicated an elemental composition in mass proportions of C:N:O:S:P of 6.67:6.23:37.55:0.38:4.42 (% w/w). The produced bioflocculant was highly efficient in removing turbidity and reducing chemical oxygen demand (COD) in brewery wastewater, dairy wastewater and river water. The bioflocculant could flocculate kaolin clay more efficiently than traditional flocculants; alum and polyethylenimine.

Keywords: Extracellular, bioflocculant, consortium, characteristic, *Cobetia* sp., *Bacillus* sp.

INTRODUCTION

Flocculants are chemicals that stimulate flocculation by aggregation of colloids and other suspended particles, forming a floc (IUPAC, 1997). Flocculants are widely applied in various industrial processes, including wastewater treatment, downstream processing, and food and fermentation processes (Mabinya et al., 2011; Nakata and Kurane, 1999; Salehizadeh and Shojaosadati, 2001). Although chemical flocculants have been widely used due to their effective flocculating activity and low cost, some synthetic flocculants are known to be hazardous to the environment (Master et al., 1985; Kowall et al., 1989; Dearfield and Abermathy, 1988). Some examples of chemical flocculants include: aluminium chlorohydrate, aluminium sulphate, calcium hydroxide, iron sulphide, iron(III)chloride, polyacrylamide, chitosan, guar gum, etc.

In recent years, utilisation of microbial flocculants has been promoted due to their biodegradability and their environmentally inert nature (Li et al., 2009; Liu et al., 2010). Currently, bioflocculants are attracting considerable attention as a promising substitute for chemical flocculants. In nature, microorganisms do not live in isolation; they coexist with many other microorganisms forming relationships that have an effect on the biological adequacy of all interacting species. Nevertheless, over the past decades, emphasis with regard to the bioflocculant field has mainly been placed on pure cultures. However, Zhu et al. (2004) and Zhang et al. (2007) have reported that the combination of strains of microorganisms in consortia produced bioflocculants that possessed better flocculating activity and higher bioflocculant yield than pure strains.

In this study we assessed the characteristics of a bioflocculant produced by a consortium of 2 bacteria, viz. *Cobetia* sp. OAUIFE and *Bacillus* sp. MAYA, which were previously isolated from the sediment of Algoa Bay in the Eastern Cape Province of South Africa, as a result of efforts to discover and explore new bioflocculants offering potential as alternatives to chemical or synthetic flocculants.

EXPERIMENTAL

Bacteria and culture conditions

The bacteria were isolated from sediment samples of Algoa Bay in the Eastern Cape Province of South Africa and maintained in 20% glycerol at −80°C in the culture collection of the Applied and Environmental Microbiology Research Group (AEMREG), University of Fort Hare, Alice, South Africa after affirmation of their bioflocculant production potential.

The culture medium consisted of 20 g glucose, 0.5 g urea, 0.5 g yeast extract, 0.2 g $(NH4)_2SO_4$, 2 g KH_2PO_4, 5 g K_2HPO_4, 0.1 g NaCl and 0.2 g $MgSO_4 \cdot 7H_2O$ in a litre of natural seawater filtered using Whatman filter paper (Zhang et al., 2007). A loopful of each bacterial colony was inoculated separately into 50 mℓ of the medium and incubated for 72 h at 28°C with shaking at 160 r/min, and was used as a pre-culture for subsequent inoculations. For the bulk fermentation 10 mℓ of each preculture was inoculated into 1 ℓ of the culture medium, indicating a 2% (v/v) inoculum size, incubated with shaking at 160 r/min for 72 h at 28°C.

Purification of bioflocculant

Isolation and purification of the bioflocculant was done according to the method described by previous reports (Chang et al., 1998; Chen et al., 2002; Ugbenyen et al., 2012). After 72 h of

* To whom all correspondence should be addressed.
☎ +27 40 602 2365; fax: +27 866 286824;
e-mail: aokoh@ufh.ac.za

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