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Full Length Research Paper

Production of extra-cellular polymer in *Azotobacter* and biosorption of metal by exopolymer

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Two *Azotobacter* strains were isolated from alkaline and acid soils. The production of alginate and exopolymer from these two strains showed that, strain AC2 produced high polymer in 2% beet molasses or 1% sucrose broth and addition of nitrogen sources (yeast extract) reduced production of this polymer. The optimum condition for production of maximum polymer production (7.5 mg/ml) was at 200 rpm shaking, pH 7 without addition of nitrogen sources. The production of polymer was reduced at pH 4. The polymer adsorbed Cu, Zn, and Fe, at 15.5, 20 and 25 mg, respectively.

Key words: *Azotobacter*, exopolymer, of extracellular polysaccharide, biosorption of metal.

INTRODUCTION

Production of extracellular polysaccharide had been studied in *Pseudomonas_aeruginosa* (Castaneda et al., 2000) *Erwinia*, (Deretie et al., 1987) *Ralstonia* (Dolph et al., 1988; Kao et al., 1992), and *Azotobacter vinelandii* (Saile et al., 1997). Alginate is used for encapsulation of microorganisms and animal cells as well as metals. Extracellular polysaccharide is required for wild-type virulence of *Pseudomonas solanacearum* and other microorganisms (Willis et al., 2001). However in *Azotobacter vinelandii*, alginate protects nitrogenase from oxygen and increases nitrogen fixation (Sabra et al., 2000). Here extracellular polysaccharide from *Azotobacter* is used to biosorbe metals.

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MATERIAL AND METHODS

Isolation and identification

Azotobacter isolation media is composed of 0.25 g/L KH_2PO_4, 0.125 g/L $MgSO_4.7H_2O$, 0.125 g/L NaCl, 0.005 g/L $FeSO_4.7H_2O$, 0.005 g/L $Na_2MnO_4.2H_2O$, 0.005 g/L $MnSO_4.4H_2O$, 0.1 g/L $CaCO_3$ and 10 g/L glucose at pH 7.2.

Exo-polysaccharide extraction

Several different media were used to produce exo-polysaccharides. The media for maximum polysaccharide production in *Azotobacter* contains the following 20 g/L sucrose, 3.2 g/L K_2HPO_4, 0.8 g/L KH_2PO_4, 0.4 g/L $MgSO_4.7H_2O$, 0.2 g/L NaCl, 0.020 g/L $FeSO_4.6H2O$, 0.03 g/L Na_2MoO_4, 0.05 g/L CaCO3 at pH 7.2. The bacteria was grown on the optimal media and incubated at 20°C at 200 rpm. The cells were centrifuged at 9000 rpm in 1 mM EDTA. The supernatant was removed and equal volume of cold acetone was added. The precipitated was collected by centrifugation at 20,000 rpm for 30 min.

Biomass as metal biosorption

The harvested biomass was washed with deionized water and then dried at 60°C for 24h in an oven. The growth rate was obtained by optical density of 600 nm.

Bioadsorption experiments

Bioadsorption experiments were conducted using separate solutions containing 10PPM Cu, Fe and Zn^{2+} in distilled water. Known amount of polysaccharide or bacteria cells mixed with each metal solution. The reaction mixture was agitated at 125 rpm on rotary shaker. After 1 h of contact time, the pellet was obtained by centrifugation of mixture at 10000 rpm. Metals concentration was measured using a varian AA-10 atomic absorption spectrophotometer. Bioadsorption experiments were carried out in duplicate and average values were used in the analysis. Bioadsorption capacity, i.e. amount of metal ion (mg) bioadsorbed per g (dry mass or polysaccharide) was calculated using the following equation (3).

$$Q = (\frac{Ci\text{-}Cf}{M}).V$$

Where:
Q = mg of metal ion bioadsorbed per g of biosensor
Ci = initial metal ion concentration (mg/l)
Cf = final metal ion concentration (mg/l)
M = mass of biosensor in the reaction mixture (g)
V = volume of the reaction (L)

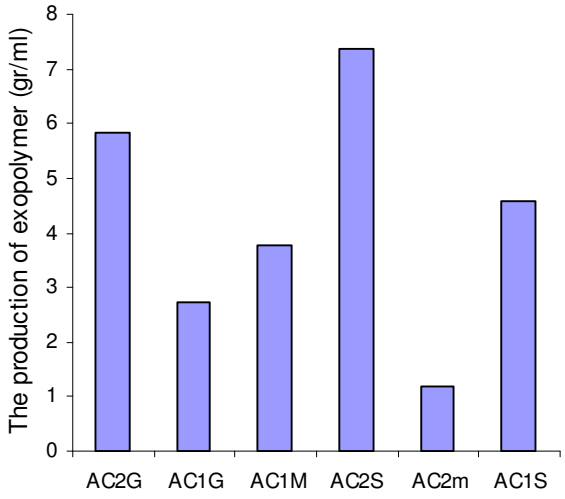

Figure 1. The effect of culture media on exopolymer production by from *Azotobacter* strains AC2 and AC1 (G=Glucose, M=Manitol, S=sucrose).

RESULTS AND DISCUSSION

Two *Azotobacter* strains, AC1 and AC2, were isolated from dry and wet soils. These isolates were grown in different media. In all media AC2 grew better than AC1 (Figure 1). Both strains had good growth in sucrose (1%). However AC2 produced maximum (7.5 mg/ml) exopolymer in media with sucrose as the only carbon

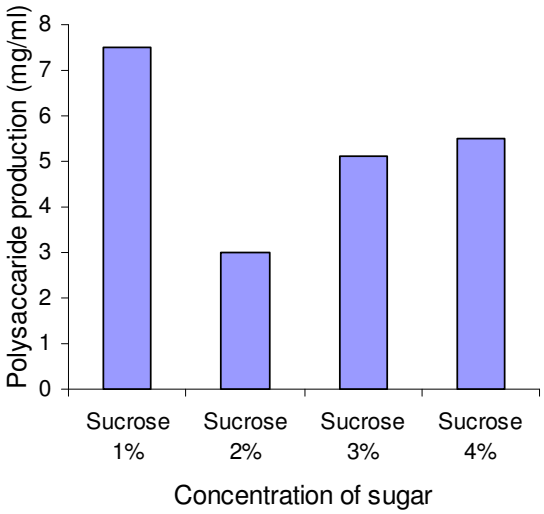

Figure 2. Production of exopolysaccharide frome *Azotobacter* strain C2.

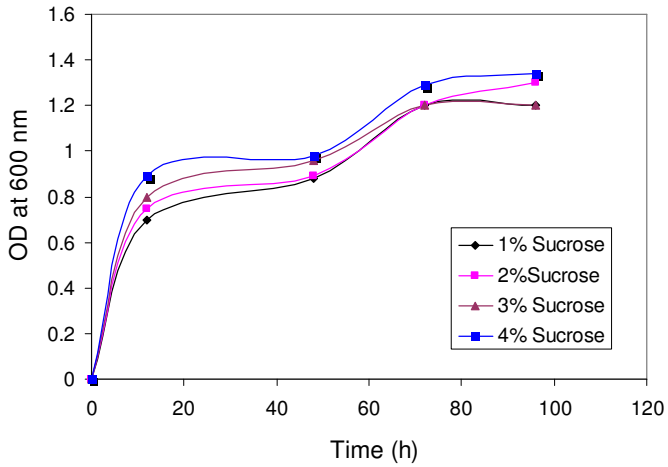

Figure 3. The growth rate of *Azotobacter* (AC1).

source (Figure 2). AC2 had maximum growth at 4% sucrose (Figure 3). However this strain had maximum exopolymer in 1% sucrose (Figure 2). Different concentration of beet molasses were used for production of polymer. The maximum growth and exopolymer production of AC2 was on 2% beet molasses (Figures 4 and 5).

Addition of vitamin, different nitrogen sources (Ammonium salts, yeast extract and peptone) did not effect exopolymer production in *Azotobacter*. However, shaking had significant effect on exopolymer production by *Azotobacter* AC2 (Table 1). Production of maximum polymer was at 30ºC, 200 rpm shaking during 4 days in 1% sucrose. The production of this polymor at pH 4 and pH 8 was reduced significantly. This polymer biosorbe metals more than cells. The biosorption of Cu, Zn, Fe

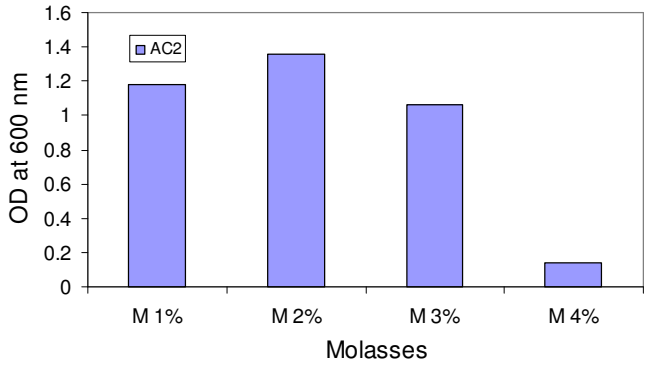

Figure 6. The growth rate of *Azotobacter* in beet molasses (M).

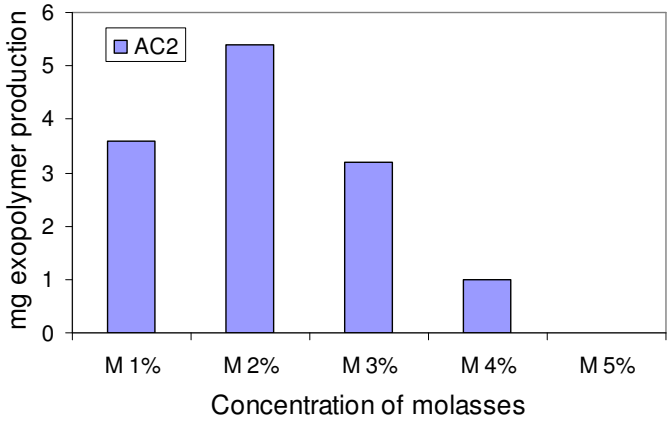

Figure 6. Production of exopolymer at *Azotobacter* (Strain AC2) in beet molasses media (M).

Table 1. The effect of O_2 and nitrogen sources on exopolymer production in *Azotobacter* AC2 grown on Sucrose (1%).

Treatment	Exopolymer production (mg/ml)
Without shaking (Without nitrogen sources)	2.5
Shaking at 200 (rpm) without any nitrogen sources	7.5
Shake at (100 rpm) without yeast extract as nitrogen sources	5
Shaking at (100 rpm) with vitamin	4

Table 2. Biosorption of metal by exopolymer from *Azotobacter* (AC2).

Metals	Metal biosrption per polysaccharide (mg/g)	Metal biosorption per biomass (mg/g)
Cu^{++}	15.5	12.5
Zn^{++}	20	6.5
Fe^{++}	25	18

were 15.5, 20, and 25 mg/g polysaccharide, respectively. However, the whole cell only biosorp these metal by 12.5, 6.5 and 18 mg/g dry cells, respectively (Table 2).

The removal of toxic metals from waste waters has directed attention to biosorption based on the metal binding capacities of algae, bacteria, fungi and yeast as potential metal sorbents (Veglio and Boelchini, 1998; Say et al., 2001). Also various biological materials like live and dead cells of mucor (Yan. and Viraraghavan, 2000), DNA (Sponer et al., 1998; Jaroslav et al., 1997) outer membrane of *Escherichia coli* (Hoyle and Beveridge, 1983; Ferris and Beveridge, 1986) and microbial envelope can be used for metal removal (Weppen and Homburg, 1995). In this work it was shown that exopolysaccharide from *Azotobacter* had high capacity to bisorbe metals and sucrose is the best substrate to produce this polymer.

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