ABSTRACT

A lipopeptide biosurfactant is produced by *Bacillus licheniformis* JF-2. The biosurfactant has found application in *in-situ* microbial enhanced crude petroleum recovery whereby the bacteria is able to grow facultatively and anaerobically under extreme conditions of temperature, pH and salinity (Javaheri *et al.*, 1985). An attempt was made in this thesis to formulate a cultivation medium which would enhance the growth of the microorganism in order to achieve high yields of biomass. Process optimization was achieved in Nutrient broth whereby the initial pH was recorded at 7.0, temperature of 40°C and at an agitation speed of 250 rpm in shake flask cultures. The maximum absorbance achieved was 1.14 at the end of the log phase (8 hours) which was similar to the maximum absorbance achieved by Jenneman *et al.* (1983) between 1.0 to 1.2 obtained after 20 hours of growth in Medium E. The log phase lasted 5 hours in our study, approximately 14 hours was reported in Medium E conducted by Jenneman *et al.* (1983) to achieve maximum absorbance.

Media optimization in shake flask studies resulted in the formulation of the Optimized Medium which consisted of 0.3% (w/v) yeast extract, 3% (w/v) sodium chloride and 5% (w/v) glucose added with nutrient broth. *Bacillus licheniformis* JF-2 grown in the Optimized Medium at an initial pH of 7.0, temperature of 40°C and shaken at 250 rpm achieved a maximum biomass yield averaging 1.61 g/L and crude lipopeptide yield of 1.82 g/L whereas the maximum absorbance read at 480 nm achieved a maximum of 1.58 at the 8th hour of fermentation.

Studies conducted in a 1.5L capacity Biolab fermenter at an optimum agitation speed of 400 rpm and at an air flow rate of 1000 cm^3/min resulted in a yield of 3.15 g/L for biomass and 2.96 g/L of crude lipopeptide, respectively whereas the maximum absorbance achieved was 2.90 at the end of the 24th hour fermentation period. Biomass in terms of dry cell weight obtained by Lin *et al.* (1994b) were measured between 1.2 to 1.3 g/L under 30% saturation of dissolved oxygen when *B. licheniformis* JF-2 was grown in a Mineral Salts Medium supplemented with 1% glucose after approximately 12 hours of fermentation . There was a marked increase in the biomass when *B. licheniformis* JF-2 was grown on the Optimized Medium as shown by our laboratory studies.

Due to the intended application of *B. licheniformis* JF-2 in treating wastewater which requires the seed cultures to be produced in large volumes, the use of the Optimized Medium would be costly and impractical whereby making it unprofitable on a long term basis. Therefore, locally available wastewater, namely, dairy wastewater from the processing of sweetened condensed milk, rubber effluent and palm oil mill effluent were used in shake flask studies to cultivate *B. licheniformis* JF-2. Undiluted dairy wastewater with an initial pH of 7.0 was found to be suitable for the growth of *B. licheniformis* JF-2.

The maximum biomass obtained averaged 0.71 g/L at the 8th hour of fermentation. The surface tension of the inoculated dairy wastewater was reduced from an initial value of 48.4 mN/m to 38.8 mN/m, a reduction of 19.8 % after 8 hours of fermentation, indicating the presence of the lipopeptide biosurfactant in the dairy wastewater.

Bacillus licheniformis JF-2 was found to successfully treat edible palm oil processing wastewater during a 15 day treatment period. The Chemical Oxygen Demand (COD) of the palm oil processing wastewater was reduced by 65% in an activated sludge process and the surface tension of the treated wastewater was reduced by 49%. *Bacillus licheniformis* JF-2 has found a new application in the treatment of palm oil processing wastewater. The reduction of the surface tension by 49% indicates the presence of the lipopeptide biosurfactant in the treated wastewater.