1.0. INTRODUCTION

Biosurfactants of microbial origin have become important products of biotechnology for potential industrial applications. As high value microbial products, they are specific in action, nontoxic and easily produced (Kosaric, 1992). Preliminary toxicity tests carried out on *Daphnia magna* of the Crustacean family using the non-purified biosurfactant from *Corynebacterium xerosis* was found to be non-toxic at concentrations of less than 0.2% (w/v) whereas the non-purified biosurfactant from *Corynebacterium* sp. was found to be totally non-toxic (Margaritis *et al.*, 1979). They are produced generally by certain microorganisms grown on hydrocarbons, although it is also possible to produce them from other substrates such as carbohydrates. These microbial cultures are able to produce high yields of surface-active substances that emulsify or wet the hydrocarbon phase thus making it available for cellular absorption (Margaritis *et al.*, 1979).

Microbial surfactants offer two important advantages relative to synthetic ones. Firstly, the structural diversity of biosurfactants offers a wide range of surface active compounds which may be better suited for specific applications. Secondly, as environmental considerations are becoming increasingly important for the selection of industrial chemicals, biosurfactants which are biodegradable appear promising for applications in bioremediation and the dispersion of oil spills (Harvey *et.al.*, 1990; Oberbremer *et al.*, 1990). In 1987, Emulsan was the only commercially marketed industrial biosurfactant for use in cleaning oil contaminated vessels, oil spills, MEOR and to facilitate pipeline transportation of heavy crude oil because of its ability to reduce viscosity which contributes to reduced transportation costs (Fiechter, 1992).

Commercially available chemical surfactants are generally toxic and are non-biodegradable. They are produced from hydrocarbons which are non-replenishable and their manufacture involves a series of steps where several chemical reactions are followed by a number of costly purifications (Margaritis *et al.*, 1979). The unique properties of biosurfactants allow their use and possible replacement of chemical surfactants in a large number of industrial applications, operations, such as in the production of cosmetics, pharmaceuticals and as functional food additives (Kosaric, 1992). Table 1 shows the various potential applications of microbial surfactants in various industries. The replacement of these with biologically active compounds could be possible, if they are produced more cheaply (Cooper, 1986).

Biosurfactants have the potential to become important industrial products and process optimization results in high yields with reduced cost (Kosaric *et al.*, 1987). Also their capacity to substitute existing synthetic surfactants needs further evaluation (Jenny *et al.*, 1991).

The production of biosurfactants with inexpensive substrates could reduce the cost to Ringgit Malaysia 1.85 to 5.55/kg (equivalent to Canadian $1.00/kg to $3.00/kg), whereas commercial surfactants consisting of non-ionic alcohol ethoxylate and alkylphenolethoxylate which are used in enhanced oil recovery costs to Ringgit Malaysia 2.59 to 2.96/kg (equivalent to Canadian $1.4-1.6/kg) (Kosaric, 1992). Cooper & Paddock (1984) estimated the microbial production of sophorose lipids to be at Ringgit Malaysia 5.09/kg (equivalent to Canadian $2.75/kg) compared to Ringgit Malaysia 6.01/kg (equivalent to Canadian $3.25/kg) for the chemically synthesized Span 60. Since sophorose lipids are produced in high yields (70 g/L) on inexpensive

Table 1: Biosurfactant functions most likely to be needed and potential industrial users (Kosaric *et al.*, 1987)

Function	Industrial users										
	Agriculture	Building and construction	Elastomer and plastic	Foods and beverages	Industrial cleaning	Leather	Metals	Paper	Pain and protective coating	Petroleum and petrochemical products	Textiles
Emulsification	X		X	X		X	X		X	X	X
Deemulsification										X	
Wetting, spreading, penetration	X	X	X	X	X	X	X	X	X	X	X
Solubilization, solids disposal	X		X	X						X	X
Air entrainment, foaming		X	X	X			X			X	
Defoaming				X				X			
Detergency				X	X	X		X		X	X
Antistatic			X						X		X
Corrosion inhibition					X					X	

substrates such as vegetable oil, it is likely to be of commercial value. Several commercial surfactants such as perfluorinated anionics resist biodegradation, accumulate in the environment and cause ecological problems. However, microbial surfactants are susceptible to degradation in water and soil (Zajic *et al.*, 1977).

In 1987, the only commercial industrial biosurfactant in the market was Emulsan, marketed by Petroleum Fermentations (Petroferm) for use in cleaning oil-contaminated vessels, oil spills and MEOR. It was also used to facilitate pipeline transportation of heavy crude which resulted in the reduction of the viscosity and reduced the transportation cost (Fiechter, 1992). The sophorose lipids produced by *Torulopsis bombicola* has been successfully used recently for making detergents in Japan by Kao Industries (Sasidharan, 1995).

The oil spill from Exxon Valdez at Alaska's Prince William Sound resulted in the destruction of the aquatic habitat and the environment (Harvey *et al.*, 1990). Chemical surfactants are generally used widely for the clean up of oil spills which is the cause of major pollution problems in shallow coastal waters. However, biosurfactants are ideally suited for environmental application because of their ability to disperse or solubilize insoluble pollutants. This is particularly useful in bioremediation whereby emulsification facilitates the assimilation of organic matter by microorganisms. The application of oleophilic fertilizers containing nitrogen and phosphorus provides nutrients to indigenous microoganisms to speed up the biodegradation process *in situ* and it appears to have helped in removing some of the spilled oil from the Alaskan soil (Harvey *et al.*, 1990). Biosurfactants also have little impact on the environment and there is the possibility of *in situ* application (Harvey *et al.*, 1990; Oberbremer *et al.*, 1990).

The potential application of biosurfactants to de-water fuel grade peat has unique advantages over conventional agents (Cooper *et al.*, 1986). These are added to the process before mechanical pressing to improve significant loss of water. Wastewater streams with dissolved organics, are a potential source of pollution. However, these generally support the growth of microorganisms, notably *Bacillus subtilis* (Mulligan & Cooper, 1985).

The use of biosurfactants in the industry depends solely on the cost of production. There must be sufficient biological and engineering inputs in order to produce biosurfactants economically. Stricter environmental and health regulations will strongly influence the application of chemical surfactants in future (Cooper, 1986).

The use of palm oil mill effluent (POME), rubber effluent (RE) and dairy wastewater from the processing of sweetened condensed milk as substrates is aimed at utilizing locally available agro-industrial by-products for the cultivation of *B. licheniformis* JF-2. This is viewed to reduce the cost of production of biomass and perhaps increase the yield of the product. Thus, in our study, the biomass was used for wastewater treatment and the qualitative emulsification of crude petroleum.

The study focused on process optimization for the production of high yields of biomass for *in-situ* application of the biosurfactant with the following objectives:

1. Growth studies and media optimization in shake flasks for maximum production of biomass and crude biosurfactant yield.

2. Scale-up to 1.5L capacity Biolab fermenter to increase biomass and crude biosurfactant yield. This also includes a media comparison study with Mineral Salts Medium I and II.

3. Separation of the major components of the lipopeptide by thin layer chromatography (TLC).

4. Utilization of locally available agro-industrial by-products for the cultivation of *B. licheniformis* JF-2 for reducing the cost of seed culture production for use in wastewater treatment.

5. *In-situ* application of *B. licheniformis* JF-2 in treating edible palm oil processing wastewater.