

CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
ABSTRACT	viii
LIST OF TABLES	xi
LIST OF FIGURES	xiii
LIST OF PLATES	xvii
LIST OF APPENDICES	xix
LIST OF ABBREVIATIONS	xx
1.0 INTRODUCTION	1
2.0 LITERATURE REVIEW	7
2.1 Biosurfactants in biotechnology	7
2.2 Biosurfactants	8
2.3 Classification of biosurfactant	11
2.4 <i>Bacillus licheniformis</i> JF-2	17
2.5 The industrial use of <i>Bacillus licheniformis</i>	20
2.6 Biosynthesis of biosurfactants	22
2.7 The physiology of microorganisms affecting the yield of biosurfactants	23
2.7.1 Culture conditions affecting the yield of the lipopeptide biosurfactants	23
2.7.2 Nutritional factors affecting the yield of the biosurfactant	24
2.7.3 Carbon source for biosurfactant production	26
2.8 Surfactants in the petroleum industry	29

3.0 MATERIALS AND METHODS

3.1 Culture	33
3.2 Media	33
3.2.1 Inoculum preparation	33
3.2.2 Fermentation	35
3.3 Physical parameters	35
3.3.1 Biomass	35
3.3.2 pH	35
3.3.3 Surface tension	36
3.3.4 Emulsification Measurement (E_{24})	37
3.3.5 Thin Layer Chromatography	37
3.4 Wastewater analysis	38
3.4.1 Chemical Oxygen Demand (COD)	38
3.4.2 Dissolved phosphate	40
3.4.3 Determination of total sugar	42
3.4.4 Determination of reducing sugar (Miller, 1959)	42
3.4.5 Total Nitrogen	43
3.5 Determination of crude lipopeptide yield (g/l)	44
3.5.1 Acid precipitation method for shake flask cultures	44
3.5.2 Foam fractionation method for isolating the biosurfactant from fermenter runs	44
3.6 Process optimization	48
3.6.1 Initial pH	48
3.6.2 Temperature	48

3.7	Media optimization	49
3.7.1	The effect of various concentrations of sodium chloride on the growth and biosurfactant production by <i>B. licheniformis</i> JF-2 without glucose	49
3.7.2	The effect of various concentrations of sodium chloride on the growth of <i>B. licheniformis</i> JF-2 in Nutrient broth supplemented with glucose	49
3.7.3	The effect of various concentrations of yeast extract on the growth and biosurfactant production by <i>B. licheniformis</i> JF-2.	50
3.7.4	A time course study of <i>B. licheniformis</i> JF-2 in the final optimized growth medium	50
3.8	Fermentation	51
3.8.1	The growth and biosurfactant production by <i>B. licheniformis</i> JF-2 in a 1.5L capacity Biolab fermenter	51
3.8.2	Production of biosurfactant by <i>B. licheniformis</i> JF-2 in selected media	52
3.9	Growth of <i>B. licheniformis</i> JF-2 in agro-industrial wastewater	53
3.10	Treatment of edible palm oil processing wastewater	54
3.10.1	Acclimatisation of seed culture	54
3.10.2	Treatment of edible palm oil processing wastewater by the activated sludge process	54
3.10.3	Treatment of edible palm oil processing wastewater using <i>B. licheniformis</i> JF-2	55

4.0 RESULTS

4.1	Process Optimization	56
4.1.1	Temperature optimization for the growth of <i>B. licheniformis</i> JF-2	56
4.1.2	Determination of optimum pH for growth and production of the lipopeptide by <i>B. licheniformis</i> JF-2	58
4.2	Media Optimization	61
4.2.1	Effect of sodium chloride (NaCl) on the growth and lipopeptide synthesis by <i>B. licheniformis</i> JF-2	61
4.2.2	Effect of 5% and 10% glucose in the cultivation medium containing NaCl on the growth and lipopeptide production by <i>B. licheniformis</i> JF-2	63
4.2.3	Effect of various concentrations of Yeast extract (YE) on the growth and lipopeptide yield of <i>B. licheniformis</i> JF-2	65
4.2.4	Production of biomass and lipopeptide by <i>B. licheniformis</i> JF-2 in shake flask cultures using the Optimized medium (OM)	70
4.2.5	A comparison of Medium E (ME) and our Optimized Medium (OM) in shake flask culture for the production of biomass and lipopeptide from <i>B. licheniformis</i> JF-2	72
4.3	Identification of the crude lipopeptide biosurfactant by thin layer chromatography	74
4.4	Critical Micelles Concentration (CMC) of the crude lipopeptide obtained from the growth of <i>B. licheniformis</i> JF-2 after 8 hours of fermentation in the Optimized Medium in shake flask cultures	77
4.5	Fermentation studies on <i>B. licheniformis</i> JF-2 in a 1.5L Biolab Fermenter	78
4.5.1	The production of biomass and lipopeptide from <i>B. licheniformis</i> JF-2 using the optimized medium (OM) in a 1.5L capacity fermenter (B. Braun Biolab)	78

4.5.2	Production of the biosurfactant by <i>B. licheniformis</i> JF-2 in mineral salts medium compared to the optimized medium in a 1.5 litre fermenter (B. Braun Biolab)	84
4.6	Media formulation with locally available agro-industrial by-products for the cultivation of <i>B. licheniformis</i> JF-2	89
4.6.1	Palm oil mill effluent (POME) as a growth medium for <i>B. licheniformis</i> JF-2	90
4.6.2	Rubber effluent (RE) as a growth medium for <i>B. licheniformis</i> JF-2	94
4.6.3	Dairy wastewater as a cultivation medium for <i>B. licheniformis</i> JF-2	97
4.6.4	Use of sugar cane molasses as an additional carbon source in dairy wastewater for cultivation of <i>B. licheniformis</i> JF-2	101
4.6.5	Use of Fish Meal (FM) as a nitrogen source for the cultivation of <i>B. licheniformis</i> JF-2	105
4.7	Application of the biosurfactant in the removal of crude oil from solid surfaces	108
4.8	<i>In-situ</i> application of <i>B. licheniformis</i> JF-2 compared to a bioaugmentation culture (JAD 969P) for the treatment of edible palm oil processing wastewater	109
5.0 DISCUSSION		
5.1	Process Optimization	114
5.2	Scale-up to a 1.5L capacity Biolab fermenter	117
5.3	Application of agro-industrial by-products in the cultivation media for the growth of <i>B. licheniformis</i> JF-2	121
5.4	Wastewater treatment	122
REFERENCES		
		125

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.