

1.0. APPENDIX I

Optimized Medium (OM)

*In case of change in
use of units
in Appendices*

Medium composition:

g/L

(a) Nutrient broth (Difco, USA)

(i) Bacto Beef

3.0

(ii) Bacto Peptone

5.0

(b) NaCl

30.0

(c) Yeast extract (Difco, USA)

3.0

(d) Glucose (BDH)

50.0

APPENDIX II

Mineral Salts Medium (MSM I) (Lin *et al.*, 1993)

Medium composition

	<u>% (w/v)</u>
(a) Glucose	1.0
(b) $(\text{NH}_4)_2\text{SO}_4$	0.1
(c) MgSO_4	0.025
(d) NaCl	0.5
(e) Trace Metal Solution	0.5

Composition of trace metal solution

(i) EDTA	0.1
(ii) MnSO_4	0.3
(iii) FeSO_4	0.01
(iv) CaCl_2	0.01
(v) CoCl_2	0.01
(vi) ZnSO_4	0.01
(vii) CuSO_4	0.01
(viii) $\text{AlK}(\text{SO}_4)_2$	0.001
(ix) H_3BO_4	0.001
(x) Na_2MoO_4	0.001

APPENDIX III

Mineral Salts Medium (MSM II) (Juwarkar, 1994)

Medium composition

	<u>g/L</u>
(a) NaNO_3	3.04
(b) K_2HPO_4	1.0
(c) KH_2PO_4	0.5
(d) MgSO_4	0.5
(e) KCl	0.1
(f) FeSO_4	0.01
(g) CaCl_2	0.01
(h) Glucose	20.0
pH	7.0

APPENDIX IV

Medium E (Jenneman *et al.*, 1983)

Medium composition:

	<u>%</u>	
(a) NaCl	5.0	in 100mM
(b) $(\text{NH}_4)_2\text{SO}_4$	1.0	phosphate
(c) MgSO_4	0.025	buffer
(d) Sucrose	1.0	(pH 7.0)
(e) Trace Metal Solution	1.0	

Constituents of the Trace Metal Solution:

	<u>g/L</u>
(f) EDTA	1.0
(g) FeSO_4	3.0
(h) CaCl_2	0.1
(i) CoCl_2	0.1
(j) ZnSO_4	0.1
(k) CuSO_4	0.01
(l) $\text{AlK}(\text{SO}_4)_2$	0.01
(m) H_3BO_3	0.01
(n) NaMoO_4	0.01
(o) MnSO_4	3.0

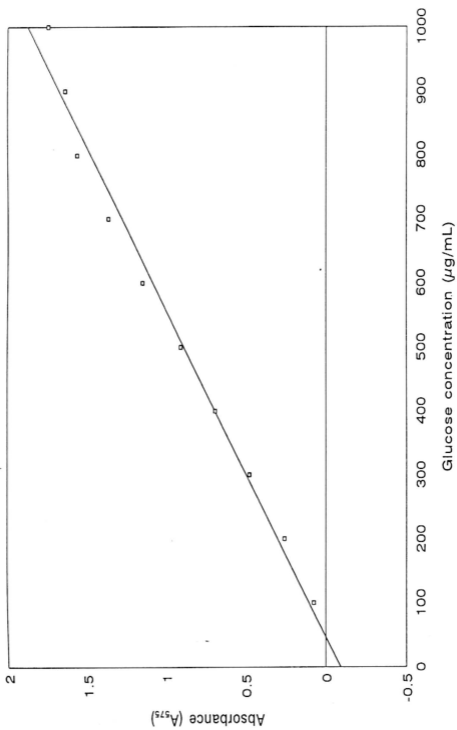
APPENDIX V

Microorganisms present in JAD 969P bioaugmentation culture complex:

- (a) *Bacillus subtilis*
- (b) *Bacillus licheniformis*
- (c) *Pseudomonas aeruginosa*
- (d) *Pseudomonas stutzeri*
- (e) *Pseudomonas fluorescens*
- (f) *Escherichia hermannii*

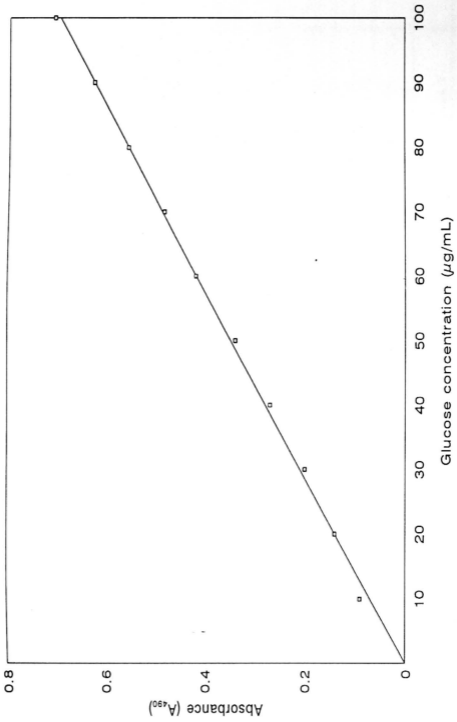
Appendix VI

Standard Reducing Sugar Curve (DNS method)



Appendix VII

Standard curve to determine Total Sugar Content



APPENDIX VIII

Parameter under study	Nutrient Broth (NB)	Optimized Medium : NB + 0.3% Yeast Extract + 3.0 % NaCl + 5.0 % Glucose			Wastewater		
		8	8	24	Rubber effluent (Dilution 1:100)	Dairy effluent (Dilution 1:0)	P.O.M.E. (Dilution 1:10)
Incubation time (h)	8	8	8	24	8	8	8
Initial pH / Final pH	7.0/8.0	7.0/5.0	7.0/6.1	7.0/5.1	7.1/7.1	6.7/7.8	6.9/7.0
Temp. (°C) at high biosurfactant yield	40	40	40	40	40	40	40
Shake flask (s.f.) / Fermenter capacity	s.f	s.f	1.5	1.5	s.f	s.f	s.f
Agitation speed (rpm)	250	250	400	400	250	250	250
Air flowrate (L/min)	nd	nd	1.0	1.0	nd	nd	nd
Maximum biomass (g/L)	1.14	1.61	2.20	3.15	0.07	0.70	nd
Crude lipopeptide yield (g/L)	0.22	1.82	nd	2.96	nd	nd	nd
Maximum absorbance (A ₄₈₀)	1.14	1.58	2.0	2.90	nd	nd	nd
Initial surface tension (mN/m) (with inoculum)	60.8	48.6	55.8	55.8	71.8	48.4	44.6
Final surface tension (mN/m)	37.3	36.2	61.8	62.4	70.4	39.4	37.6
% Reduction in surface tension	23.5	25.5	-10.8	-11.8	1.9	18.6	15.7

nd = not determined

P.O.M.E. = Palm oil mill effluent

= = increase (due to biosurfactant removal from the cultivation medium through the foam fractionation method)
= = negative value denotes an increase