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APPENDIX

Experimental Data of Biosurfactant-Producing Bacteria

Table 1 Oil displacement test & the appearance of clear zone of 16 bacterial colonies

Strain	Radius of Oil Displacement Test (cm)	Oil Displacement Test (cm²)	Clear zone (cm)	Diameter of a bacterial colony (cm)	Clear zone- Diameter of a bacterial colony (cm)
Sp. 3	0.300	0.283	2.1	1	1.1
Sp. 4	2.500	19.643	1	0.3	0.7
Sp. 7	2.000	12.571	2.3	0.9	1.4
Sp.10	0.500	0.786	2.4	1.3	1.1
Sp.11	0.000	0.000	1.8	1	0.8
Sp.12	2.100	13.860	1.5	0.5	1
Sp.15	1.300	5.311	1.9	0.8	1.1
Sp.16	2.000	12.571	2.6	1.2	1.4
Sp.20	2.000	12.571	2.2	0.8	1.4
Sp.27	2.000	12.571	2.3	1	1.3
Sp.32	2.100	13.860	2.2	1	1.2
Sp.36	0.500	0.786	2	0.7	1.3
Sp.40	2.200	15.211	1.4	0.4	1
Sp.49	0.500	0.786	2.2	1	1.2
Sp.61	0.300	0.283	2.6	1.3	1.3
Sp.65	0.400	0.503	1.7	1	0.7

Table 2 Surface properties of bacteria SP3

Clear zone (cm)	Diameter of a bacterial colony (cm)	Clear zone - Diameter of a bacterial colony (cm)	Oil Displacement Test (cm)	Hours	Surface tension (mN/m)	Area for ODT (cm²)
2.1	1	1.1	0.3	24	45	0.283
3.4	2.1	1.3	0.4	48	41	0.503
4.8	3	1.8	0.5	72	39	0.786
5.8	4	1.8	0	96	39	0.000

Table 3 Surface properties of bacteria SP4

Clear zone (cm)	Diameter of a bacterial colony (cm)	Clear zone - Diameter of a bacterial colony (cm)	Oil Displacement Test (cm)	Hours	Surface tension (mN/m)	Area for ODT (cm^2)
1	0.3	0.7	2.5	24	28.7	19.643
3.8	0.9	2.9	6	48	27	113.143˙
5.5	1.4	4.1	6.3	72	27	124.740
7.2	1.7	5.5	6.7	96	27	141.083

Table 4 Surface properties of bacteria SP7

Clear zone (cm)	Diameter of a bacterial colony (cm)	Clear zone - Diameter of a bacterial colony (cm)	Oil Displacement Test (cm)	Hours	Surface tension (mN/m)	Area for ODT (cm^2)
2.3	0.9	1.4	2	24	38	12.571
3.4	1.2	2.2	2.1	48	36	13.860
4.2	2	2.2	2.3	72	34	16.626
5	3	2	5	96	34	78.571

Table 5 Surface properties of bacteria SP10

Clear zone (cm)	Diameter of a bacterial colony (cm)	Clear zone - Diameter of a bacterial colony (cm)	Oil Displacement Test (cm)	Hours	Surface tension (mN/m)	Area for ODT (cm^2)
2.4	1.3	1.1	0.5	24	32	0.786
3.7	2.4	1.3	0.1	48	30.5	0.031
4.5	2.8	1.7	5	72	30	78.571
5	3	2	5	96	30	78.571

Table 6 Surface properties of bacteria SP11

Clear zone (cm)	Diameter of a bacterial colony (cm)	Clear zone - Diameter of a bacterial colony (cm)	Oil Displacement Test (cm)	Hours	Surface tension (mN/m)	Area for ODT (cm^2)
1.8	1	0.8	0	24	44.5	0.000
3.7	2	1.7	0.3	48	40	0.283
4.5	3	1.5	2.5	72	40	19.643
5.3	4	1.3	0	96	40	0.000

Table 7 Surface properties of bacteria SP12

Clear zone (cm)	Diameter of a bacterial colony (cm)	Clear zone - Diameter of a bacterial colony (cm)	Oil Displacement Test (cm)	Hours	Surface tension (mN/m)	Area for ODT (cm^2)
1.5	0.5	1	2.1	24	32.5	13.860
3.9	1.5	2.4	3.1	48	30	30.203
6	2.4	3.6	4.8	72	30	72.411
7.3	2.2	5.1	6.3	96	30	124.740

Table 8 Surface properties of bacteria SP15

Clear zone (cm)	Diameter of a bacterial colony (cm)	Clear zone - Diameter of a bacterial colony (cm)	Oil Displacement Test (cm)	Hours	Surface tension (mN/m)	Area for ODT (cm^2)
1.9	0.8	1.1	1.3	24	33	5.311
3	1.2	1.8	1.3	48	32.5	5.311
3.8	1.6	2.2	1.5	72	32.5	7.071
4.5	1.9	2.6	3.5	96	32	38.500

Table 9 Surface properties of bacteria SP16

Clear zone (cm)	Diameter of a bacterial colony (cm)	Clear zone - Diameter of a bacterial colony (cm)	Oil Displacement Test (cm)	Hours	Surface tension (mN/m)	Area for ODT (cm^2)
2.6	1.2	1.4	2	24	39	12.571
3.8	2	1.8	2	48	36.5	12.571
5	3.2	1.8	2	72	36.5	12.571
6	4	2	3.8	96	36.5	45.383

Table 10 Surface properties of bacteria SP20

Clear zone (cm)	Diameter of a bacterial colony (cm)	Clear zone - Diameter of a bacterial colony (cm)	Oil Displacement Test (cm)	Hours	Surface tension (mN/m)	Area for ODT (cm^2)
2.2	0.8	1.4	2	24	38	12.571
3.1	1.3	1.8	2	48	34	12.571
4	2.4	1.6	2	72	32.5	12.571
4.8	3.7	1.1	4.5	96	32.5	63.643

Table 11 Surface properties of bacteria SP27

Clear zone (cm)	Diameter of a bacterial colony (cm)	Clear zone - Diameter of a bacterial colony (cm)	Oil Displacement Test (cm)	Hours	Surface tension (mN/m)	Area for ODT (cm^2)
2.3	1	1.3	2	24	36.5	12.571
3.7	1.5	2.2	2	48	31	12.571
4.8	3.5	1.3	5	72	31	78.571
6	3.5	2.5	5	96	31	78.571

Table 12 Surface properties of bacteria SP32

Clear zone (cm)	Diameter of a bacterial colony (cm)	Clear zone - Diameter of a bacterial colony (cm)	Oil Displacement Test (cm)	Hours	Surface tension (mN/m)	Area for ODT (cm^2)
2.2	1	1.2	2.1	24	36	13.860
3.2	1.5	1.7	2.8	48	32	24.640
4.5	2	2.5	3.7	72	32	43.026
5.5	2.6	2.9	5.5	96	32	95.071

Table 13 Surface properties of bacteria SP36

Clear zone (cm)	Diameter of a bacterial colony (cm)	Clear zone - Diameter of a bacterial colony (cm)	Oil Displacement Test (cm)	Hours	Surface tension (mN/m)	Area for ODT (cm^2)
2	0.7	1.3	0.5	24	34.5	0.786
3.4	1.4	2	3.2	48	34.5	32.183
4.3	1.5	2.8	6	72	32	113.143
4.8	1.7	3.1	5	96	32	78.571

Table 14 Surface properties of bacteria SP40

Clear zone (cm)	Diameter of a bacterial colony (cm)	Clear zone - Diameter of a bacterial colony (cm)	Oil Displacement Test (cm)	Hours	Surface tension (mN/m)	Area for ODT (cm^2)
1.4	0.4	1	2.2	24	28	15.211
3.8	0.8	3	6.2	48	28	120.811
6.5	1.4	5.1	6.4	72	27.5	128.731
8	1.5	6.5	6.8	96	27.5	145.326

Table 15 Surface properties of bacteria SP49

Clear zone (cm)	Diameter of a bacterial colony (cm)	Clear zone - Diameter of a bacterial colony (cm)	Oil Displacement Test (cm)	Hours	Surface tension (mN/m)	Area for ODT (cm^2)
2.2	1	1.2	0.5	24	39	0.786
3	2	1	0.9	48	37	2.546
4.1	2.5	1.6	1.7	72	35	9.083
5	3.2	1.8	2	96	35	12.571

Table 16 Surface properties of bacteria SP61

Clear zone (cm)	Diameter of a bacterial colony (cm)	Clear zone - Diameter of a bacterial colony (cm)	Oil Displacement Test (cm)	Hours	Surface tension (mN/m)	Area for ODT (cm^2)
2.6	1.3	1.3	0.3	24	42	0.283
3.3	2	1.3	0.5	48	40	0.786
4.3	2.9	1.4	0.8	72	39	2.011
5	3.5	1.5	1.2	96	39	4.526

Table 17 Surface properties of bacteria SP65

Clear zone (cm)	Diameter of a bacterial colony (cm)	Clear zone - Diameter of a bacterial colony (cm)	Oil Displacement Test (cm)	Hours	Surface tension (mN/m)	Area for ODT (cm^2)
1.7	1	0.7	0.4	24	41.5	0.503
3.2	1.7	1.5	0.8	48	40.5	2.011
4.4	2.2	2.2	1.5	72	40	7.071
5.2	3	2.2	2.1	96	40	13.860

Table 18 Comparison of 3 culture mediums for *Pseudomonas aeruginosa* SP4 between 0 and 72 hours

Before cultivate			After cultivate														
Medium	pH	SFT	pH			SFT (mN/m)			Dried weight (g/50ml)			% Reduction of SFT			ODT (cm^2)		
0	0	0	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
NB+2%	6.80	47.00	7.8200	7.6000	6.5200	29.402	28.573	26.712	0.4806	0.6173	0.3400	37.443	39.206	43.166	9.083	124.74	132.79
BM+2%	6.52	44.00	6.0200	5.6600	5.7500	27.958	26.615	26.014	0.4500	0.5000	0.4900	36.459	39.511	40.877	95.071	50.286	19.643
DM+2%	5.35	45.00	5.9600	5.7000	5.6500	44.019	31.879	35.402	0.1000	0.2300	0.3400	2.180	29.158	21.329	0.031	3.143	3.143
NB+4%	6.80	47.00	7.8700	7.5700	6.6700	30.668	29.455	27.846	0.5047	1.0462	0.5000	34.749	37.330	40.754	11.346	103.91	95.071
BM+4%	6.52	44.00	6.0400	5.6200	5.6900	28.416	26.122	26.015	0.9500	0.6200	0.3900	35.419	40.632	40.875	34.226	69.426	69.426
DM+4%	5.35	45.00	5.9000	5.7000	5.7700	44.965	31.519	35.636	0.3900	0.5500	1.0100	0.078	29.958	20.809	0.031	3.143	4.526
NB+6%	6.80	47.00	7.7500	7.3400	6.3400	31.021	29.081	26.395	0.5859	0.7349	0.2800	33.998	38.126	43.840	15.211	95.071	75.460
BM+6%	6.52	44.00	6.1100	5.8900	5.2400	31.223	26.712	26.671	1.1200	1.0700	0.9500	29.039	39.291	39.384	55.440	38.500	40.731
DM+6%	5.35	45.00	5.8900	5.7300	5.7500	43.801	34.774	35.754	0.9800	1.2300	1.6600	2.664	22.724	20.547	0.031	7.071	1.540
NB+8%	6.80	47.00	7.7000	7.1700	6.4700	30.090	28.669	27.431	0.6114	0.9353	0.4200	35.979	39.002	41.637	18.103	95.071	95.071
BM+8%	6.52	44.00	6.2300	6.0400	5.3200	30.254	26.853	27.368	1.1000	2.3400	1.7600	31.241	38.970	37.800	78.571	32.183	19.643
DM+8%	5.35	45.00	5.7200	5.7100	5.6800	41.708	33.350	34.139	0.6000	1.6700	0.9600	7.316	25.889	24.136	0.031	0.786	3.143
NB+10%	6.80	47.00	7.6000	7.4800	6.4100	34.856	32.287	28.107	0.4418	1.5179	0.8300	25.838	31.304	40.199	10.183	88.283	113.14
BM+10%	6.52	44.00	6.2400	6.0600	5.3800	29.209	27.458	27.323	1.8300	2.0100	1.2000	33.617	37.595	37.902	19.643	38.500	12.571
DM+10%	5.35	45.00	5.7800	5.7200	5.7800	36.599	34.356	32.597	0.8200	1.3500	1.1100	18.669	23.653	27.562	0.126	7.071	4.526

SFT = Surface tension (mN/m)

ODT = Oil displacement test (cm^2)

Table 19 Microbial growth determination at 600 nanometers

Time (hr.)	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48
NB*	0.571	0.629	0.690	0.705	0.745	0.762	1.242	1.952	1.909	1.862	1.700	1.650	1.593	1.532	1.507	1.353
	0.543	0.568	0.673	0.625	0.691	0.687	1.135	1.901	1.877	1.850	1.696	1.683	1.618	1.560	1.525	1.393
	0.542	0.551	0.675	0.682	0.701	0.673	1.124	1.873	1.885	1.847	1.717	1.655	1.625	1.590	1.537	1.381
Average	0.552	0.583	0.679	0.671	0.712	0.707	1.167	1.909	1.890	1.853	1.704	1.662	1.612	1.561	1.523	1.376
NB (+G)**	0.484	0.592	0.571	0.613	0.616	0.632	1.159	1.892	1.816	1.793	1.670	1.610	1.542	1.487	1.277	1.108
	0.567	0.608	0.641	0.679	0.685	0.720	0.822	1.958	1.869	1.843	1.727	1.671	1.552	1.521	1.344	1.181
	0.414	0.639	0.689	0.716	0.733	0.752	0.738	1.949	1.864	1.852	1.718	1.642	1.571	1.511	1.358	1.286
Average	0.488	0.613	0.634	0.669	0.678	0.701	0.906	1.933	1.850	1.829	1.705	1.641	1.555	1.506	1.326	1.192

* Nutrient broth

** Nutrient broth + 0.02%glucose

Table 20 Growth curve of biosurfactant-producing bacteria SP4

Time (hour)	Surface Tension (mN/m)	pH	Oil displacement test (cm^2)	Cell dried weight (g/l)
0	47.459	7.17	0.000	0.000
3	46.127	7.21	0.011	1.040
6	43.746	7.20	0.031	2.176
9	39.722	7.29	0.126	4.240
12	35.605	7.30	0.786	5.212
15	35.225	7.48	0.786	5.320
18	35.109	7.29	1.540	5.368
21	32.858	7.32.	3.803	6.940
24	30.162	7.16	19.643	7.354
30	29.071	7.40	38.500	9.872
36	28.146	7.27	78.571	11.518
42	28.008	7.28	78.571	11.710
48	26.906	7.52	120.811	11.446
54	26.815	7.55	124.740	9.622
60	26.518	7.43	120.811	8.512
66	26.639	7.45	116.946	8.434
72	26.783	7.40	120.811	8.120
78	26.044	7.15	141.083	7.448
84	26.213	7.21	132.786	6.764
90	26.295	7.27	120.811	5.756
96	26.362	7.17	116.946	3.896
102	26.226	7.16	124.740	2.846
108	25.985	6.87	145.326	2.540
114	25.968	6.77	154.000	2.300
120	26.112	6.47	145.326	2.040

Table 21 Comparison of the percentage inoculums after culturing in nutrient broth containing 2% palm oil and incubating at 37°C in a shaking incubator at 200 rpm

Hours	Percent inoculum (v/v)	γ (mN/m)				Oil Displacement Test (cm^2)	Dried cell weight (g/l)
		1	2	3	Average		
24	2.1	29.199	29.207	29.046	29.151	28.286	1.8740
	4.1	29.494	29.301	29.380	29.392	28.286	2.3760
	6.1	29.563	29.479	29.345	29.462	28.286	3.7240
	8.1	31.786	31.849	31.721	31.785	19.643	4.9120
	2.2	29.410	29.435	29.239	29.361	30.203	2.3540
	4.2	29.304	29.790	28.992	29.362	28.286	2.1200
	6.2	29.232	29.270	29.000	29.167	38.500	7.4160
	8.2	28.935	28.834	28.806	28.858	19.643	4.0380
48	2.3	27.033	27.069	27.087	27.063	119.143	5.7460
	4.3	27.090	27.023	27.116	27.076	102.111	2.0080
	6.3	28.177	28.141	27.964	28.094	84.983	7.6640
	8.3	27.822	27.853	27.980	27.885	113.143	12.6580
	2.4	27.224	27.342	27.257	27.274	105.726	8.6960
	4.4	28.021	27.954	27.856	27.944	98.560	7.0560
	6.4	27.874	27.958	27.885	27.906	88.283	7.9160
	8.4	28.011	28.124	28.105	28.080	105.726	10.7760

Table 22 The percentage of carbon content concentration (mg/L) of oil recovered from column using biosurfactants in free-cell broth

Time (min)	Total Carbon (mg/L)	TC - Hexane (mg/L)	Time (min)	Total Carbon (mg/L)	TC - Hexane (mg/L)	Time (min)	Total Carbon (mg/L)	TC - Hexane (mg/L)
11	75.82	36.6	231	1197.32	1158.1	451	413.92	374.7
22	114.57	75.35	242	1369.14	1329.92	462	254.87	215.65
33	237.98	198.76	253	1247.74	1208.52	473	287.97	248.75
44	457.76	418.54	264	1317.33	1278.11	484	216.43	177.21
55	374.63	335.41	275	1179.24	1140.02	495	321.87	282.65
66	549.18	509.96	286	1158.48	1119.26	506	114.21	74.99
77	678.53	639.31	297	1297.11	1257.89	517	95.79	56.57
88	571.67	532.45	308	1147.53	1108.31	528	81.27	42.05
99	791.24	752.02	319	957.41	918.19	539	69.42	30.2
110	627.92	588.7	330	1094.14	1054.92	550	75.77	36.55
121	924.51	885.29	341	975.32	936.1	561	43.35	4.13
132	845.89	806.67	352	752.54	713.32	572	39.97	0.75
143	1024.81	985.59	363	892.87	853.65	583	45.78	6.56
154	957.37	918.15	374	754.36	715.14	594	40.02	0.8
165	984.35	945.13	385	658.91	619.69	605	42.58	3.36
176	1207.84	1168.62	396	772.54	733.32	616	39.47	0.25
187	897.98	858.76	407	624.52	585.3	627	39.25	0.03
198	1127.62	1088.4	418	481.76	442.54	638	41.07	1.85
209	1247.21	1207.99	429	571.58	532.36	649	39.54	0.32
220	1327.86	1288.64	440	287.97	248.75	660	39.27	0.05

* Hexane has carbon content = 39.22 mg/L

Calculation the percentage of oil recovery in a column.

Initial diesel motor oil used $\qquad$ = 45 ml

Recovery diesel motor oil by a water flooding technique $\qquad$ = 21 ml

Remaining diesel motor oil in the pores of Ottawa sand $\qquad$ = 45-21 = 24 ml

Properties of diesel motor oil; $\qquad$ Density = 0.7292 g/ml

Therefore, $\qquad$ Mass of diesel motor oil = (0.7292 g/ml)(24 ml)

$\qquad$ = 17.5008 g

Total biosurfactant used (each collection tube is 6.6 ml) $\qquad$ = 396 ml

After analyzed by TOC, total carbon content (excluding hexane)

$\qquad$ = 33751.17 mg/L

Therefore, solution   1000 ml has carbon content   =33751.17 mg

biosurfactant   396   ml has carbon content

$$= [(33751.17 \text{ mg})(396 \text{ ml}) / (1000 \text{ ml})]$$

$$= 13365.46 \text{ mg} = 13.3654 \text{ g}$$

Therefore, the oil recovery $[(13.3654 \text{ g}) / (17.5008 \text{ g})] \times 100 \% = 76.37\%$
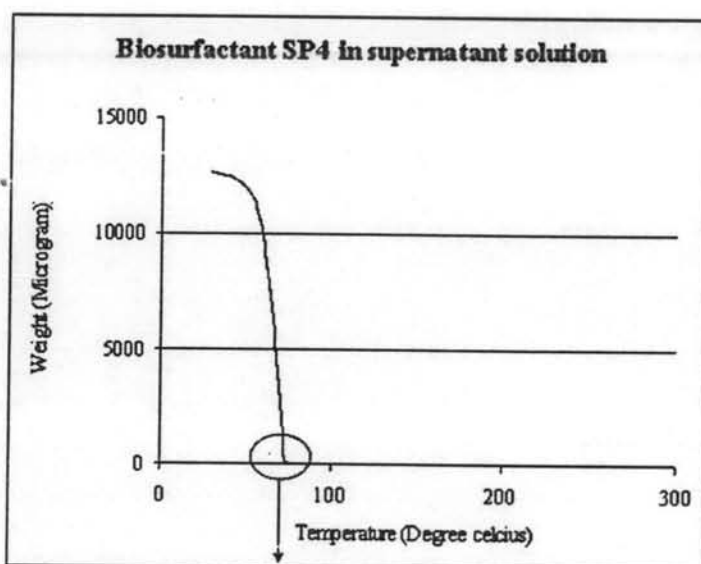

Figure 1   Thermal Gravimetric Analysis of biosurfactant SP4 in supernatant solution.
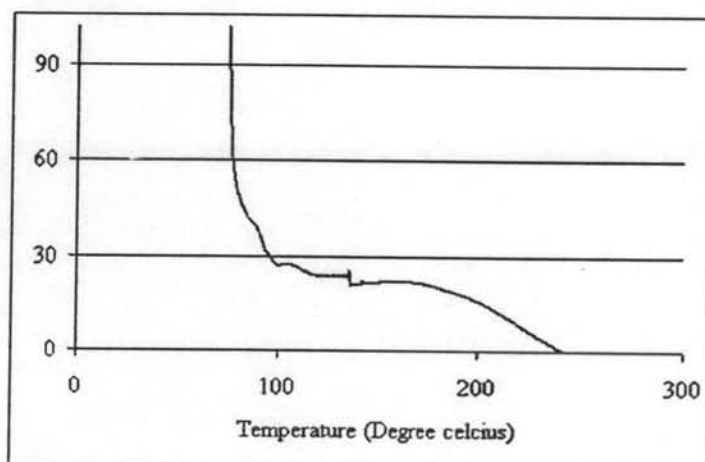

Figure 2 Expansion from figureA1to point out solid content at 100°C.

Calculation of CMC in term of mg/L

From TGA;

At 0°C,   Sample weight = 12.725 mg, Sample volume = 0.0159 ml

At 100°C, Solid content (biosurfactant) remained 27.05078 μg. (0.02705078 mg)

From   Density = Mass/Volume

Therefore,   Density = (0.02705078 mg) / (0.0159 ml)

                                    = 1.701306918 mg/ml

At CMC 3.6 %v/v, $C_1V_1 = C_2V_2$

        (1.701306918 mg/ml)(3.6 ml)= C_2(100 ml)

                            C_2 = [(1.701306918 mg/ml)(3.6 ml)] / (100 ml)

Thus, C_2 = 0.06125 mg/ml

                                = 0.06125 mg/ml × 1000 ml/L

                                = 61.25 mg/L

Therefore,   CMC 3.6 %v/v = CMC 61.25 mg/L

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