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APPENDICES

Appendix A Experimental Data of Biosurfactant-Producing Bacteria

Table A-1 Data for screening biosurfactant-producing bacteria from various sources

strain	clear zone (cm)	oil displacement (cm)
CS1	0.6	0.9
CS11	0.3	0.6
CS14	0.7	0.9
RY7	0.5	0.8
BC1	0.8	1.9
BC4	1.0	2.0
BC9	1.3	2.3
PT2	1.1	2.5
PT4	1.2	2.1
PT6	0.9	2.2

Table A-2 Surface properties of bacteria from various sources

strain	surface tension (mN/m)	%reduction of surface tension
CS1	38.2	18.72
CS11	45.0	4.26
CS14	32.0	31.91
RY7	43.3	7.87
BC1	33.0	29.79
BC4	32.5	30.85
BC9	30.0	36.17
PT2	26.5	43.62
PT4	29.4	37.45
PT6	34.7	26.17

Table A-3 Effect of palm oil on surface activity of biosurfactant from PT2

% palm oil	Media	surface tension (mN/m)	% Reduction of surface tension	Oil displacement test (cm ²)
2	Nutrient broth	26.5	43.62	19.64
4	Nutrient broth	26.5	43.62	19.65
6	Nutrient broth	26.5	43.62	19.65
8	Nutrient broth	26.5	43.62	19.62
10	Nutrient broth	26.5	43.62	19.64

Table A-4 Effect of %inoculum on surface activity of biosurfactant from PT2

% Inoculum	Media	surface tension	% Reduction of surface tension	Oil displacement test (cm ²)
2	Nutrient broth	26.5	43.62	19.64
4	Nutrient broth	26.5	43.62	19.65
6	Nutrient broth	26.6	43.40	19.64
8	Nutrient broth	26.7	43.19	19.65

Table A-5 Effect of %inoculum on surface activity of biosurfactant from PT2

strain	surface tension	% Reduction of surface tension	Oil displacement test (cm ²)	Oil displacement test (cm)
CS14	32.0	31.91	2.55	0.9
BC4	32.5	30.85	12.57	2.0
BC9	30.0	36.17	16.63	2.3
PT2	26.5	43.62	19.64	2.5
PT4	29.4	37.45	13.86	2.1

Table A-6 Growth curve of PT2 with palm oil

time (hr)	SFT. (mN/m)	oil dis. (cm)	oil dis. (cm ²)	Cell weight (g/l)
0	47.0	0.0	0.00	0.0000
3	46.5	0.0	0.00	0.0000
6	44.3	0.0	0.00	0.3467
9	41.2	0.0	0.00	1.1267
12	39.3	0.3	0.28	1.6798
15	38.7	0.7	1.54	2.1356
18	37.6	0.8	2.01	2.5743
21	34.5	0.8	2.01	2.5764
24	32.4	0.9	2.55	2.8954
27	31.7	0.9	2.55	3.2679
30	27.5	1.0	3.14	3.4527
33	27.2	1.0	3.14	3.9854
36	27.0	1.4	6.16	4.2876
39	26.8	1.7	9.08	4.6754
42	26.7	1.6	8.05	4.9432
45	26.6	1.9	11.35	5.0340
48	26.5	2.2	15.21	5.2356
51	26.4	2.5	19.64	5.3264
54	26.4	2.5	19.64	5.2157
57	26.4	2.3	16.63	5.0230
60	26.4	2.6	21.25	4.9532
63	26.4	2.7	22.91	4.2675
66	26.4	2.1	13.86	3.9652
69	26.4	1.9	11.35	4.1467
72	26.4	2.2	15.21	4.6872
75	26.4	2.3	16.63	3.4996
78	26.4	2.4	18.10	3.9853
81	26.3	2.1	13.86	4.0235
84	26.3	2.5	19.64	4.5823
87	26.2	2.4	18.10	4.3876
90	26.2	2.1	13.86	3.7623
93	26.2	2.7	22.91	3.8955
96	26.2	2.6	21.25	4.7230

Table A-7 Growth curve of PT2 with extracted oil

time (hr)	SFT. (mN/m)	oil dis. (cm)	oil dis. (cm ²)	Cell weight (g/l)
0	47.0	0.0	0.00	0.0000
3	46.9	0.0	0.00	0.0000
6	43.6	0.0	0.00	0.3354
9	41.6	0.2	0.13	0.6798
12	37.9	0.4	0.50	1.1235
15	35.7	1.2	4.53	1.6345
18	32.3	1.5	7.07	2.2453
21	31.6	1.7	9.08	2.3413
24	28.9	2.1	13.86	2.9321
27	27.6	2.1	13.86	3.2456
30	27.2	2.2	15.21	3.6431
33	26.8	2.4	18.10	3.8631
36	26.4	2.5	19.64	4.2167
39	26.4	2.5	19.64	4.3256
42	26.4	2.6	21.25	4.2467
45	26.4	2.4	18.10	4.1643
48	26.4	1.7	9.08	4.2790
51	26.4	1.8	10.18	4.3853
54	26.4	1.9	11.35	4.4257
57	26.4	2.0	12.57	4.3679
60	26.4	2.1	13.86	4.1246
63	26.3	2.1	13.86	4.0335
66	26.3	2.2	15.21	4.3866
69	26.3	2.4	18.10	4.4723
72	26.3	2.3	16.63	4.3985
75	26.3	2.7	22.91	4.2345
78	26.2	2.5	19.64	3.4256
81	26.2	2.5	19.64	3.1225
84	26.2	2.6	21.25	2.5325
87	26.2	2.4	18.10	2.6643
90	26.2	2.5	19.64	2.8654
93	26.2	2.6	21.25	1.9743
96	26.2	2.2	15.21	2.4567

Table A-8 Growth curve of SP4 with extracted oil

time (hr)	SFT. (mN/m)	oil dis. (cm)	oil dis. (cm ²)	Cell weight (g/l)
0	47.0	0.0	0.00	0.0000
3	46.9	0.0	0.00	0.0000
6	43.6	0.0	0.00	0.2134
9	41.6	0.3	0.28	0.4278
12	37.9	0.5	0.79	0.8923
15	35.7	0.5	0.79	1.3678
18	32.3	0.8	2.01	1.4679
21	31.6	1.3	5.31	2.2456
24	28.9	1.5	7.07	2.5690
27	27.6	1.6	8.05	2.6370
30	27.2	1.7	9.08	3.3467
33	26.8	1.8	10.18	3.7893
36	26.4	1.9	11.35	3.8642
39	26.4	2.2	15.21	3.9435
42	26.4	2.4	18.10	4.4235
45	27.0	2.5	19.64	4.6892
48	27.0	2.5	19.64	4.8932
51	27.0	2.3	16.63	4.9853
54	27.0	2.4	18.10	4.3258
57	27.0	2.5	19.64	4.8763
60	27.0	2.1	13.86	4.3996
63	27.0	2.2	15.21	4.1987
66	27.1	2.3	16.63	4.2368
69	27.0	2.4	18.10	4.3886
72	27.0	2.1	13.86	3.8720
75	27.0	1.9	11.35	4.9852
78	27.2	1.8	10.18	4.3587
81	26.9	2.1	13.86	4.2479
84	27.0	2.6	21.25	3.9776
87	27.1	2.2	15.21	3.8723
90	26.9	2.1	13.86	3.7842
93	27.0	2.2	15.21	3.7513
96	27.3	2.3	16.63	3.9876

Table A-8 Microbial growth determination (600 nm)

time (hr.)	absorbance
0	
3	0.50
6	0.64
9	0.70
12	0.68
15	0.70
18	0.71
21	0.73
24	0.74
27	0.84
30	1.91
33	1.86
36	1.73
39	1.49
42	1.38
46	1.35
48	1.23

Table A-9 Critical micelle concentration of biosurfactant from PT2

Broth Concentration	Surface tension
0	72.3
1	69.2
2	65.1
3	54.3
4	44.3
5	40.2
6	37.5
7	32.6
8	26.4
9	26.4
10	26.4
15	26.4
20	26.4

Appendix B Experimental Data of Oil Recovery from Ottawa Sand

Table B-1 The percentage of carbon content concentration (mg/L) of motor oil recovered from column using biosurfactants from PT2 in free-cell broth

Time (min)	Total carbon (mg/L)	TC-hexane (mg/L)	pH
11	67.34	28.12	7.65
22	98.32	59.10	7.64
33	149.24	110.02	7.68
44	224.96	185.74	7.43
55	385.34	346.12	7.23
66	483.22	444.00	7.34
77	450.93	411.71	7.12
88	683.42	644.20	7.23
99	783.94	744.72	7.43
110	734.97	695.75	6.98
121	1094.56	1055.34	6.70
132	1298.34	1259.12	7.10
143	1432.78	1393.56	7.20
154	1509.46	1470.24	7.29
165	1423.05	1383.83	7.24
176	1367.93	1328.71	6.87
187	1287.45	1248.23	6.53
198	1265.68	1226.46	7.21
209	1198.56	1159.34	7.11
220	1196.78	1157.56	6.89
231	1167.34	1128.12	6.87
242	1287.23	1248.01	6.56
253	1203.54	1164.32	7.26
264	1098.65	1059.43	7.11
275	1022.34	983.12	6.34
286	1092.45	1053.23	6.54
297	940.56	901.34	7.65
308	845.23	806.01	7.78
319	765.23	726.01	6.54
330	659.83	620.61	6.33
360	450.25	411.03	6.87
390	302.56	263.34	6.34
420	276.34	237.12	6.38
450	178.83	139.61	6.87
480	102.49	63.27	6.59
510	90.72	51.50	6.52
540	60.34	21.12	6.43
570	40.23	1.01	6.97
600	39.44	0.22	6.23
630	39.30	0.08	6.43
660	39.33	0.11	6.33
		27230.48	

Table B-2 The percentage of carbon content concentration (mg/L) of palm oil recovered from column using biosurfactants from PT2 in free-cell broth

Time (min)	Total carbon (mg/L)	TC-hexane (mg/L)	pH
11	46.78	7.56	7.83
22	79.45	40.23	7.63
33	112.54	73.32	7.68
44	178.54	139.32	7.33
55	250.56	211.34	7.22
66	339.45	300.23	7.12
77	340.54	301.32	7.34
88	534.67	495.45	6.53
99	643.23	604.01	7.21
110	650.98	611.76	7.11
121	689.43	650.21	6.70
132	722.45	683.23	7.10
143	643.89	604.67	7.20
154	689.23	650.01	7.29
165	712.34	673.12	7.24
176	849.61	810.39	6.87
187	873.49	834.27	6.33
198	750.65	711.43	6.87
209	829.34	790.12	6.34
220	934.82	895.60	6.89
231	953.49	914.27	7.23
242	987.45	948.23	6.87
253	1000.34	961.12	6.59
264	1038.95	999.73	6.52
275	1023.56	984.34	6.43
286	944.23	905.01	6.97
297	832.97	793.75	6.23
308	765.32	726.10	7.78
319	694.67	655.45	6.54
330	583.56	544.34	6.89
360	439.45	400.23	6.78
390	284.21	244.99	7.23
420	142.97	103.75	6.38
450	94.56	55.34	7.11
480	90.34	51.12	7.23
510	60.23	21.01	6.45
540	55.32	16.10	7.43
570	50.46	11.24	6.98
600	48.54	9.32	7.11
630	44.35	5.13	6.43
660	41.23	2.01	6.33
690	40.22	1.00	6.34
720	39.96	0.74	6.54
750	39.45	0.23	7.65
770	39.41	0.19	6.84
		19442.33	

Table B-3 The percentage of carbon content concentration (mg/L) of palm oil recovered from column using biosurfactants from SP4 in free-cell broth

Time (min)	Total carbon (mg/L)	TC-hexane (mg/L)	pH
11	47.64	8.42	7.83
22	55.95	16.73	7.63
33	90.78	51.56	7.68
44	180.26	141.04	7.33
55	243.98	204.76	7.22
66	320.56	281.34	7.12
77	409.23	370.01	7.34
88	450.67	411.45	6.53
99	410.00	370.78	7.21
110	650.98	611.76	7.11
121	660.45	621.23	6.70
132	745.03	705.81	7.10
143	630.45	591.23	7.20
154	711.28	672.06	7.29
165	703.59	664.37	7.24
176	820.54	781.32	6.87
187	840.56	801.34	6.33
198	770.23	731.01	6.87
209	750.00	710.78	6.34
220	820.00	780.78	6.89
231	910.58	871.36	7.23
242	950.83	911.61	6.87
253	1183.07	1143.85	6.59
264	1231.45	1192.23	6.52
275	1023.56	984.34	6.43
286	944.23	905.01	6.97
297	832.97	793.75	6.23
308	765.32	726.10	7.78
319	694.67	655.45	6.54
330	583.56	544.34	6.89
360	439.45	400.23	6.78
390	284.21	244.99	7.23
420	142.97	103.75	6.38
450	94.56	55.34	7.11
480	90.34	51.12	7.23
510	60.23	21.01	6.45
540	55.32	16.10	7.43
570	50.46	11.24	6.98
600	48.54	9.32	7.11
630	44.35	5.13	6.43
660	41.23	2.01	6.33
690	40.22	1.00	6.34
720	39.96	0.74	6.54
750	39.45	0.23	7.65
770	39.41	0.19	6.84
		19178.22	

Table B-4 The percentage of carbon content concentration (mg/L) of motor oil recovered from column using Tween 80.

Time (min)	Total carbon (mg/L)	TC-hexane (mg/L)	pH
11	43.23	4.01	7.65
22	54.87	15.65	7.64
33	82.39	43.17	7.68
44	123.54	84.32	7.43
55	156.98	117.76	7.23
66	213.95	174.73	7.34
77	295.38	256.16	7.12
88	347.85	308.63	7.23
99	432.67	393.45	7.43
110	564.85	525.63	6.98
121	673.73	634.51	6.70
132	739.50	700.28	7.10
143	685.93	646.71	7.20
154	745.69	706.47	7.29
165	984.38	945.16	7.24
176	990.34	951.12	6.87
187	883.46	844.24	6.53
198	904.68	865.46	7.21
209	1098.00	1058.78	7.11
220	1196.78	1157.56	6.89
231	1098.19	1058.97	6.87
242	893.45	854.23	6.56
253	908.93	869.71	7.26
264	803.24	764.02	7.11
275	823.57	784.35	6.34
286	870.28	831.06	6.54
297	995.17	955.95	7.65
308	1093.67	1054.45	7.78
319	1134.96	1095.74	6.54
330	1234.67	1195.45	6.33
360	1416.83	1274.63	6.37
390	904.58	865.36	6.34
420	634.73	595.51	6.38
450	467.23	428.01	6.87
480	237.45	198.23	6.59
510	94.67	55.45	6.52
540	69.54	30.32	6.43
570	42.78	3.56	6.97
600	53.78	14.56	6.23
630	40.65	1.43	6.43
660	39.44	0.22	6.33
			23308.01

Table B-5 The percentage of carbon content concentration (mg/L) of motor oil recovered from column using SDBS.

Time (min)	Total carbon (mg/L)	TC-hexane (mg/L)	pH
11	42.67	3.45	6.79
22	45.39	6.17	7.11
33	78.93	39.71	6.75
44	110.92	71.70	6.89
55	128.46	89.24	7.43
66	168.93	129.71	6.98
77	222.53	183.31	6.70
88	313.48	274.26	6.97
99	411.23	372.01	7.34
110	561.75	522.53	6.9
121	674.23	635.01	6.54
132	699.34	660.12	7.65
143	646.38	607.16	7.78
154	690.54	651.32	6.54
165	834.52	795.30	6.33
176	950.68	911.46	6.56
187	814.64	775.42	6.78
198	935.68	896.46	7.34
209	911.49	872.27	7.12
220	850.96	811.74	7.23
231	934.06	894.84	7.43
242	1065.39	1026.17	6.98
253	956.36	917.14	6.70
264	856.38	817.16	7.65
275	934.06	894.84	7.64
286	855.39	816.17	7.68
297	823.95	784.73	7.43
308	952.89	913.67	7.57
319	1052.98	1013.76	7.23
330	840.32	801.10	6.87
360	980.73	941.51	6.34
390	905.78	866.56	7.10
420	1107.36	1068.14	7.20
450	811.04	771.82	7.29
480	539.50	500.28	7.24
510	249.50	210.28	6.87
540	124.95	85.73	7.11
570	56.98	17.76	6.89
600	45.96	6.74	6.87
630	40.59	1.37	6.56
660	39.65	0.43	7.26
		22658.55	

Table B-5 The percentage of carbon content concentration (mg/L) of motor oil recovered from column using Alfoterra

Time (min)	Total carbon (mg/L)	TC-hexane (mg/L)	pH
11	45.67	6.45	7.10
22	55.34	16.12	7.20
33	81.97	42.75	7.29
44	112.45	73.23	7.24
55	157.65	118.43	6.87
66	249.46	210.24	6.33
77	304.57	265.35	6.87
88	349.84	310.62	6.34
99	547.96	508.74	7.11
110	686.45	647.23	6.33
121	659.37	620.15	6.87
132	756.34	717.12	6.34
143	687.98	648.76	6.89
154	889.56	850.34	7.23
165	987.87	948.65	7.11
176	997.35	958.13	7.04
187	885.39	846.17	7.65
198	910.34	871.12	7.64
209	1109.56	1070.34	7.68
220	1145.68	1106.46	7.43
231	1003.47	964.25	6.74
242	1098.34	1059.12	6.91
253	1002.35	963.13	7.12
264	989.46	950.24	6.87
275	958.36	919.14	6.56
286	1004.79	965.57	7.26
297	989.26	950.04	7.83
308	1045.34	1006.12	7.63
319	1196.37	1157.15	7.68
330	1267.45	1228.23	7.33
360	1299.45	1260.23	7.22
390	1098.34	1059.12	7.12
420	723.06	683.84	7.34
450	475.73	436.51	6.53
480	240.96	201.74	7.21
510	88.38	49.16	7.11
540	69.54	30.32	6.70
570	44.32	5.10	7.10
600	55.79	16.57	7.20
630	44.58	5.36	7.11
660	39.46	0.24	6.79
		24747.58	

Table B-1 PZC point of Ottawa Sand

pH initial	pH Final
1	1.42
2	2.93
3	3.20
4	5.10
5	5.61
6	5.80
7	6.36
8	6.45
9	7.80
10	7.77
11	8.09
12	8.95
13	9.24
14	10.53

Appendix C Calculation of oil recovery

- In case of biosurfactant PT2 with motor

Initial diesel motor oil used = 45 ml

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

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

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

Therefore, Mass of diesel motor oil = (0.7292 g/ml)(24 ml)
= 17.5008 g

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

After analyzed by TOC, total carbon content (excluding hexane)
= 27230.48 mg/L

Therefore, solution 1000 ml has carbon content = 27230.48 mg

solution biosurfactant use 396 ml has carbon content
= [(27230.48 mg)(396 ml) / (1000 ml)]

$$= 10783.46 \text{ mg} = 10.7834 \text{ g}$$

Therefore, the oil recovery [(10.7834 g) / (17.5008 g)] × 100 % = 63.56 %

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