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APPENDICES

Appendix A Experimental Data of Characterization of Mineral medium and Palm Oil

Table A1 Characterization of mineral medium and palm oil

Parameters	Mineral medium (MM)	palm oil
COD (mg/l)	0	1,250,821.88
TOC (mg/l)	0	42,131.57
Total nitrogen (mg/l)	290	1,500
Total phosphorus (mg/l)	291.33	90
C/N ratio	16/1	28.09
C/P ratio	14/1	468.13
Total suspended solids (mg/l)	0	0
Surfactant concentration (xCMC)	0	0
pH	8.6	4.6
Surface tension (mN/m)	67.09	-

Table A2 Density of palm oil

No.	Density (g/ml)
1	0.97918
2	0.97687
3	0.94572
4	0.91481
5	0.91752
6	0.90076
7	0.96076
8	0.98900
Avg.	0.9481

Table A3 Chemical oxygen demand (COD) of palm oil

No.	Palm Oil (g)	Palm Oil (ml)	DI water (ml)	Diluted COD (mg/l)	COD (mg/l)	Avg. COD (mg/l)
1	0.00051	0.000537931	2.499462069	284	1,319,872.60	1,250,821.88
2	0.00051	0.000537931	2.499462069	280.33	1,302,816.50	
3	0.0005	0.000527383	2.499472617	238.33	1,129,776.55	

Table A4 Total organic carbon (TOC) of palm oil

Substance	Sample No.	wt. of SFT (g)	RO water (ml)	[SFT] (%w/v)	TOC (mg/l)			
					NO.1	NO.2	Avg.	Total Avg.
SFT solution	1	0.150038	150	0.10003	158.6	157.3	157.95	174.22
	2	0.150038	150	0.10003	158.7	153.7	156.2	
	3	0.150038	150	0.10003	204.6	209.4	208.5	

Substance	Sample No.	wt. of SFT (g)	[SFT] (%w/v)	SFT solution (ml)	Palm oil (ml)	[Palm oil] (%v/v)	TOC (mg/l)					
							Sample dilution			Palm oil (mg/l)	100% v/v Palm oil	Avg.
							NO.1	NO.2	Avg.			
Palm oil + SFT solution	4	0.150038	0.10003	10	0.04	0.4	352.2	353.4	352.8	178.58	44,645.83	42,131.57
	5	0.150038	0.10003	10	0.05	0.5	381.4	389.8	385.6	211.38	42,276.67	
	6	0.150038	0.10003	10	0.06	0.6	412.5	409.6	411.05	236.83	39,472.22	

; Surfactant (SFT): Triton X-100

Table A5 Total nitrogen (TP) and total nitrogen (TN) of mineral medium and palm oil

Substances	No.	Volume of test (ml)		Diluted TP (mg/l)	Actual TP (mg/l)	Avg. TP (mg/l)	Diluted TN (mg/l)	Actual TN (mg/l)	Avg. TN (mg/l)
		Sample	DI water						
Palm oil	1	1	99	0.9	90	90	19	1,900	1,500
	2	1	99	0.8	80		13	1,300	
	3	1	99	1	100		13	1,300	
K ₂ HPO ₄ 0.1005 g/100 ml DI water	1	0.5	4.5	16.9	169	168	-	-	-
	2	0.5	4.5	16.7	167		-	-	
	3	0.5	4.5	16.8	168		-	-	
KH ₂ PO ₄ 0.0503 g/ 100 ml DI water	1	0.5	4.5	12.3	123	123.333	-	-	-
	2	0.5	4.5	12.3	123		-	-	
	3	0.5	4.5	12.4	124		-	-	
NaNO ₃ 0.2003 g/ 100 ml DI water	1	0.1	0.4	-	-	-	57	285	290
	2	0.1	0.4	-	-		57	285	
	3	0.1	0.4	-	-		60	300	

Table A6 Surface tension of the mineral medium in the influent at an oil loading rate of $2 \text{ kg/m}^3\text{d}$ with C:N of 16:1 and C:P of 14:1

Cycle Time (d/cycle)	Surface tension (mN/m)										
	No.1	No.2	No.3	No.4	No.5	No.6	No.7	No.8	No.9	No.10	Avg.
1	69.17	71.60	70.29	70.60	70.71	70.87	71.19	71.26	71.67	71.56	70.89
2	69.74	70.91	70.95	70.92	70.80	71.03	71.21	71.40	71.25	71.37	70.96
3	68.45	70.78	70.71	70.78	70.80	70.84	71.73	71.93	71.78	71.86	70.97

Table A7 Surface tension of the mineral medium in the influent at an oil loading rate of $2 \text{ kg/m}^3\text{d}$ at 2 d/cycle

C:N ratio	Surface tension (mN/m)										
	No.1	No.2	No.3	No.4	No.5	No.6	No.7	No.8	No.9	No.10	Avg.
16:0.57	70.76	70.98	71.91	71.12	71.51	71.21	71.68	71.77	71.76	71.57	71.43
16:1	69.74	70.91	70.95	70.92	70.80	71.03	71.21	71.40	71.25	71.37	70.96
16:3	68.49	70.65	69.96	70.12	70.21	70.7	70.46	70.66	70.7	70.53	70.25

Table A8 COD and TOC of oil and mineral medium in the influent at an oil loading rate of 2 kg/m³ d

Cycle time (d/cycle)	[Oil] (g/ml)	[Oil] (%w/v)	[Oil] (%v/v)	[Media] (%v/v)	Vol. of Media (ml)	Vol. of Oil (ml)	COD of oil (mg/l)	COD of media (mg/l)	Total COD (mg/l)	TOC of oil (mg/l)
1	0.006	0.6	0.63	99.37	496.836	3.164	7,915.76	0	7,915.76	266.63
2	0.006	0.6	0.63	99.37	493.672	6.328	15,831.52	0	15,831.52	533.25
3	0.006	0.6	0.63	99.37	490.507	9.493	23,747.28	0	23,747.28	799.88
			100	-	0	500	1,250,821.88			42,131.57
				100	500	0				

Table A9 Composition of mineral medium with C/N of 16/1 and C/P of 14/1 at an oil loading rate of 2 kg/m³d

Cycle time (d/cycle)	[Oil] (%w/v)	TN of oil (mg/l)	TN of media (mg/l)	Total TN (mg/l)	Wanted TN (mg/l)	Wanted TN in media (mg/l)	Fill NaNO ₃ in media (g/l)	C:N ratio
1	0.6	9.492	288.165	297.657	16.664	7.172	0.1151	16:1
2	0.6	18.984	286.330	305.314	33.328	14.344	0.2302	16:1
3	0.6	28.476	284.495	312.971	49.993	21.517	0.3453	16:1
		1500	290					

Cycle time (d/cycle)	[Oil] (%w/v)	TP of oil (mg/l)	TP of media (mg/l)	Total TP (mg/l)	Wanted TP (mg/l)	Wanted TP in media (mg/l)	Fill KH ₂ PO ₄ in media (g/l)	Fill K ₂ HPO ₄ in media (g/l)	C:P ratio
1	0.6	0.570	289.486	290.056	19.045	18.475	0.0270	0.0691	14:1
2	0.6	1.139	287.643	288.782	38.089	36.950	0.0540	0.1383	14:1
3	0.6	1.709	285.799	287.508	57.134	55.426	0.0811	0.2074	14:1
		90	291.33						

Table A10 Composition of mineral medium with C/N of 16/3 and 16/0.57, C/P of 14/1 at an oil loading rate of 2 kg/m³d

Cycle time (d/cycle)	[Oil] (g/ml)	[Oil] (%w/v)	[Oil] (%v/v)	[Media] (%v/v)	Vol. of Media (ml)	Vol. of Oil (ml)	COD of oil (mg/l)	COD of media (mg/l)	Total COD (mg/l)	TOC of oil (mg/l)
2	0.006	0.6	0.63	99.37	493.672	6.328	15,831.52	0	15,831.52	533.25

Cycle time (d/cycle)	[Oil] (%w/v)	TN of oil (mg/l)	TN of media (mg/l)	Total TN (mg/l)	Wanted TN (mg/l)	Wanted TN in media (mg/l)	Fill NaNO ₃ in media (g/l)	C:N ratio
2	0.6	18.984	286.330	305.314	99.984	81	0.5595	16:3
2	0.6	18.984	-	18.984	18.984	-	-	16:0.57

Cycle time (d/cycle)	[Oil] (%w/v)	TP of oil (mg/l)	TP of media (mg/l)	Total TP (mg/l)	Wanted TP (mg/l)	Wanted TP in media (mg/l)	Fill KH ₂ PO ₄ in media (g/l)	Fill K ₂ HPO ₄ in media (g/l)	C:P ratio
2	0.6	1.139	287.643	288.782	38.089	36.950	0.0540	0.1383	14:1

Appendix B Experimental Data of Biosurfactant Production at an Oil Loading Rate of 2 kg/m³d with Different of Cycle Times at C/N 16/1 and C/P 14/1

1. Effect of Cycle Time on Biosurfactant Production.

1.1 Chemical Oxygen Demand (COD)

Table B1 Influent, effluent COD and COD removal on days 1-10 of operation in the SBR at 1 d/cycle

Day	Volume of test (ml)		Centrifuge				Avg. COD (mg/l)	Actual effluent COD (mg/l)	Influent COD (mg/l)	% COD removal
			Diluted effluent COD (mg/l)							
	Sample	RO. Water	No.1	No.2	No.3	avg.				
0	-	-	-	-	-	-	-	-	-	
1	0.1	2.4	122	123	124	123	123	3,075	7,915.76	61.15
			123	122	124	123				
			122	123	124	123				
2	0.05	2.45	76	67	69	70.67	71.22	3,561.11	7,915.76	55.01
			78	67	70	71.67				
			76	69	69	71.33				
3	0.05	2.45	77	77	77	77	73.00	3,650.00	7,915.76	53.89
			70	70	70	70				
			72	72	72	72				
4	0.05	2.45	25	25	25	25	44	2,217	7,915.76	71.99
			54	54	54	54				
			54	54	54	54				
5	0.075	2.425	67	67	67	67	68	2,266.67	7,915.76	71.36
			68	68	68	68				
			69	69	69	69				
6	0.025	2.475	18	18	18	18	20	2,000	7,915.76	74.73
			22	22	22	22				
			21	20	19	20				
7	0.025	2.475	19	18	18	18.33	21.56	2,155.56	7,915.76	72.77
			26	26	26	26				
			21	20	20	20.33				
8	0.025	2.475	25	25	25	25	23	2,333	7,915.76	70.52
			23	23	23	23				
			22	22	22	22				
9	0.025	2.475	26	26	26	26	25	2,467	7,915.76	68.84
			20	20	20	20				
			28	28	28	28				
10	0.025	2.475	21	21	21	21	21.00	2,100.00	7,915.76	73.47
			20	20	20	20				
			22	22	22	22				

Table B2 Influent and effluent COD and COD removal on days 1-20 of operation in the SBR at 2 d/cycle

Day	Volume of test (ml)		Centrifuge				Avg. COD (mg/l)	Actual COD (mg/l)	Influent COD (mg/l)	% COD removal
	Sample	RO. Water	Diluted effluent COD (mg/l)							
			No.1	No.2	No.3	avg.				
0	-	-	-	-	-	-	-	-	-	
2	0.025	2.475	125	126	127	126.00	103.56	10,355.56	15,831.52	34.59
			127	127	131	128.33				
			56	56	57	56.33				
4	0.025	2.475	22	22	23	22.33	30.33	3,033.33	15,831.52	80.84
			38	39	39	38.67				
			29	30	31	30.00				
6	0.025	2.475	24	25	26	25.00	25.78	2,577.78	15,831.52	83.72
			24	25	25	24.67				
			27	28	28	27.67				
8	0.025	2.475	29	29	31	29.67	24.44	2,444.44	15,831.52	84.56
			21	21	22	21.33				
			22	22	23	22.33				
10	0.025	2.475	16	17	17	16.67	13.78	1,377.78	15,831.52	91.30
			11	11	12	11.33				
			13	13	14	13.33				
12	0.025	2.475	18	18	18	18.00	17.44	1,744.44	15,831.52	88.98
			18	18	18	18.00				
			16	16	17	16.33				
14	0.025	2.475	10	10	10	10.00	17.22	1,722.22	15,831.52	89.12
			23	24	24	23.67				
			18	18	18	18.00				
16	0.025	2.475	29	30	31	30.00	27.56	2,755.56	15,831.52	82.59
			26	27	27	26.67				
			26	26	26	26.00				
18	0.025	2.475	25	25	27	25.67	19.78	1,977.78	15,831.52	87.51
			13	13	13	13.00				
			20	20	22	20.67				
20	0.025	2.475	13	15	11	13.00	12.89	1,288.89	15,831.52	91.86
			13	13	13	13.00				
			13	13	12	12.67				

Table B3 Influent and effluent COD and COD removal on days 1-30 of operation in the SBR at 3 d/cycle

Day	Volume of test (ml)		Centrifuge				Avg. COD (mg/l)	Actual COD (mg/l)	Influent COD (mg/l)	% COD removal
	Sample	RO. Water	Diluted effluent COD (mg/l)							
			No.1	No.2	No.3	avg.				
0	-	-	-	-	-	-	-	-	-	
3	0.025	2.475	139	139	139	139	141	14,100	23,747.28	40.62
			143	143	143	143				
			141	141	141	141				
6	0.025	2.475	165	165	165	165	162.00	16,200	23,747.28	31.78
			155	155	155	155				
			166	166	166	166				
9	0.025	2.475	141	141	141	141	141.67	14,166.67	23,747.28	40.34
			144	144	144	144				
			140	140	140	140				
12	0.025	2.475	140	140	140	140	138	13,789	23,747.28	41.93
			144	144	144	144				
			127	127	135	130				
15	0.025	2.475	144	144	144	144	144	14,400	23,747.28	39.36
			145	145	145	145				
			143	143	143	143				
18	0.025	2.475	139	139	139	139	145	14,467	23,747.28	39.08
			131	131	131	131				
			164	164	164	164				
21	0.025	2.475	165	165	165	165	147.67	14,766.67	23,747.28	37.82
			164	164	164	164				
			127	107	108	114				
24	0.025	2.475	127	127	127	127	140	14,033	23,747.28	40.91
			145	145	145	145				
			149	149	149	149				
27	0.025	2.475	142	143	143	143	141	14,111	23,747.28	40.58
			140	140	140	140				
			142	140	140	141				
30	0.02	2.48	107	107	107	107	107.00	13,375	23,747.28	43.68
			104	104	104	104				
			110	110	110	110				

1.2 Suspended Solid (SS)

Table B4 Effluent SS on days 1-10 of operation in the SBR at 1 d/cycle

Day	Vol. sample (ml)	wt. filter (g)	wt. filter+ TSS (g)	Effluent SS (g)	Effluent SS (mg)	Effluent SS (mg/l)	Avg. Effluent SS (mg/l)
0	-	-	-	-	-	-	-
1	4	0.0724	0.0837	0.0113	11.3	2,825	2,850
	4	0.0724	0.0839	0.0115	11.5	2,875	
2	4	0.0728	0.0781	0.0053	5.3	1,325	1,350
	4	0.0728	0.0783	0.0055	5.5	1,375	
3	4	0.0739	0.0788	0.0049	4.9	1,225	1,225
	4	0.074	0.0789	0.0049	4.9	1,225	
4	4	0.0722	0.0765	0.0043	4.3	1,075	1,075
	4	0.0723	0.0766	0.0043	4.3	1,075	
5	5	0.0729	0.0754	0.0025	2.5	500	500
	5	0.0732	0.0757	0.0025	2.5	500	
6	5	0.0728	0.0766	0.0038	3.8	760	760
	5	0.0728	0.0766	0.0038	3.8	760	
7	5	0.0743	0.077	0.0027	2.7	540	630
	5	0.0744	0.078	0.0036	3.6	720	
8	4	0.0721	0.0774	0.0053	5.3	1,325	1,325
	4	0.0723	0.0776	0.0053	5.3	1,325	
9	5	0.0715	0.0807	0.0092	9.2	1,840	1,810
	5	0.0718	0.0807	0.0089	8.9	1,780	
10	5	0.0721	0.082	0.0099	9.9	1,980	1,910
	5	0.0728	0.082	0.0092	9.2	1,840	

Table B5 Effluent SS on days 2-20 of operation in the SBR at 2 d/cycle

Day	Vol. sample (ml)	wt. filter (g)	wt. filter+ SS (g)	Effluent SS (g)	Effluent SS (mg)	Effluent SS (mg/l)	Avg. Effluent SS (mg/l)
0	-	-	-	-	-	-	-
2	5	0.071	0.0782	0.0072	7.2	1,440	1,180
	5	0.0732	0.0778	0.0046	4.6	920	
4	5	0.0724	0.0787	0.0063	6.3	1,260	1,420
	5	0.0712	0.0791	0.0079	7.9	1,580	
6	5	0.0722	0.0744	0.0022	2.2	440	490
	5	0.0732	0.0759	0.0027	2.7	540	
8	5	0.0723	0.0751	0.0028	2.8	560	520
	5	0.0725	0.0749	0.0024	2.4	480	
10	5	0.0712	0.0736	0.0024	2.4	480	520
	5	0.0701	0.0729	0.0028	2.8	560	
12	5	0.0709	0.0728	0.0019	1.9	380	420
	5	0.0713	0.0736	0.0023	2.3	460	
14	5	0.0726	0.0742	0.0016	1.6	320	270
	5	0.0716	0.0727	0.0011	1.1	220	
16	5	0.0719	0.0749	0.003	3	600	570
	5	0.0717	0.0744	0.0027	2.7	540	
18	5	0.0712	0.075	0.0038	3.8	760	500
	5	0.0708	0.072	0.0012	1.2	240	
20	5	0.0716	0.073	0.0014	1.4	280	260
	5	0.0705	0.0717	0.0012	1.2	240	

Table B6 Effluent SS on days 3-30 of operation in the SBR at 3 d/cycle

Day	Vol.sample (ml)	wt.filter (g)	wt.filter+ SS (g)	Effluent SS (g)	Effluent SS (mg)	Effluent SS (mg/l)	Avg. Effluent SS (mg/l)
0	-	-	-	-	-	-	-
3	4	0.073	0.0944	0.0214	21.4	5,350	5,350
	4	0.073	0.0944	0.0214	21.4	5,350	
6	3	0.0728	0.0862	0.0134	13.4	4,467	4,467
	3	0.0728	0.0862	0.0134	13.4	4,467	
9	4	0.0724	0.094	0.0216	21.6	5,400	5,388
	4	0.0724	0.0939	0.0215	21.5	5,375	
12	4	0.0729	0.0959	0.023	23	5,750	5,788
	4	0.0729	0.0962	0.0233	23.3	5,825	
15	4	0.0721	0.0908	0.0187	18.7	4,675	4,663
	4	0.0722	0.0908	0.0186	18.6	4,650	
18	5	0.0731	0.1145	0.0414	41.4	8,280	8,280
	5	0.0731	0.1145	0.0414	41.4	8,280	
21	5	0.0733	0.1214	0.0481	48.1	9,620	9,620
	5	0.0733	0.1214	0.0481	48.1	9,620	
24	5	0.072	0.1174	0.0454	45.4	9,080	9,080
	5	0.072	0.1174	0.0454	45.4	9,080	
27	5	0.072	0.1163	0.0443	44.3	8,860	9,290
	5	0.0727	0.1213	0.0486	48.6	9,720	
30	5	0.0721	0.1195	0.0474	47.4	9,480	9,270
	5	0.0725	0.1178	0.0453	45.3	9,060	

1.3 Mixed Liquor Suspended Solid (MLSS)

Table B7 Effluent MLSS on days 1-10 of operation in the SBR at 1d/cycle

Day	Vol. sample (ml)	wt. filter (g)	wt. filter+ MLSS (g)	Effluent MLSS (g)	Effluent MLSS (mg)	Effluent MLSS (mg/l)	Avg. effluent MLSS (mg/l)
0	-	-	-	-	-	-	-
1	5	0.0714	0.087	0.0156	15.6	3,120	3,210
	5	0.0721	0.0886	0.0165	16.5	3,300	
2	5	0.0687	0.0805	0.0118	11.8	2,360	2,480
	5	0.0682	0.0812	0.013	13	2,600	
3	5	0.0697	0.0802	0.0105	10.5	2,100	2,080
	5	0.0692	0.0795	0.0103	10.3	2,060	
4	5	0.0717	0.0865	0.0148	14.8	2,960	2,690
	5	0.0724	0.0845	0.0121	12.1	2,420	
5	5	0.0715	0.0821	0.0106	10.6	2,120	2,050
	5	0.0707	0.0806	0.0099	9.9	1,980	
6	5	0.0698	0.0818	0.012	12	2,400	2,370
	5	0.0698	0.0815	0.0117	11.7	2,340	
7	5	0.0698	0.0868	0.017	17	3,400	3,370
	5	0.0699	0.0866	0.0167	16.7	3,340	
8	5	0.0705	0.0894	0.0189	18.9	3,780	3,870
	5	0.0706	0.0904	0.0198	19.8	3,960	
9	5	0.0715	0.082	0.0105	10.5	2,100	1,940
	5	0.0718	0.0807	0.0089	8.9	1,780	
10	5	0.0721	0.0825	0.0104	10.4	2,080	2,060
	5	0.0728	0.083	0.0102	10.2	2,040	

Table B8 Effluent MLSS on days 2-20 of operation in the SBR at 2 d/cycle

Day	Vol. sample (ml)	wt. filter (g)	wt. filter+ MLSS (g)	Effluent MLSS (g)	Effluent MLSS (mg)	Effluent MLSS (mg/l)	Avg effluent MLSS (mg/l)
0	-	-	-	-	-	-	-
2	5	0.0713	0.1303	0.059	59	11,800	7,090
	5	0.071	0.0829	0.0119	11.9	2,380	
4	5	0.0728	0.0961	0.0233	23.3	4,660	4,820
	5	0.0711	0.096	0.0249	24.9	4,980	
6	5	0.0721	0.0856	0.0135	13.5	2,700	2,820
	5	0.0715	0.0862	0.0147	14.7	2,940	
8	5	0.071	0.084	0.013	13	2,600	2,640
	5	0.0708	0.0842	0.0134	13.4	2,680	
10	5	0.0714	0.0854	0.014	14	2,800	3,560
	5	0.0714	0.093	0.0216	21.6	4,320	
12	5	0.0719	0.087	0.0151	15.1	3,020	3,210
	5	0.0725	0.0895	0.017	17	3,400	
14	5	0.0732	0.0936	0.0204	20.4	4,080	3,630
	5	0.0722	0.0881	0.0159	15.9	3,180	
16	5	0.0712	0.0763	0.0051	5.1	1,020	1,050
	5	0.0724	0.0778	0.0054	5.4	1,080	
18	5	0.0721	0.0762	0.0041	4.1	820	1,030
	5	0.0713	0.0775	0.0062	6.2	1,240	
20	5	0.0708	0.0782	0.0074	7.4	1,480	1,140
	5	0.0712	0.0752	0.004	4	800	

Table B9 Effluent MLSS on days 3-30 of operation in the SBR at 3 d/cycle

Day	Vol. sample (ml)	wt. filter (g)	wt. filter+ MLSS (g)	Effluent MLSS (g)	Effluent MLSS (mg)	Effluent MLSS (mg/l)	Avg. Effluent MLSS (mg/l)
0	-	-	-	-	-	-	-
3	5	0.0725	0.1505	0.078	78	15,600	15,800
	5	0.0718	0.1518	0.08	80	16,000	
6	5	0.0723	0.1495	0.0772	77.2	15,440	11,310
	5	0.0717	0.1076	0.0359	35.9	7,180	
9	5	0.0714	0.1275	0.0561	56.1	11,220	11,590
	5	0.0717	0.1315	0.0598	59.8	11,960	
12	5	0.0722	0.1252	0.053	53	10,600	11,240
	5	0.0716	0.131	0.0594	59.4	11,880	
15	5	0.0721	0.131	0.0589	58.9	11,780	11,910
	5	0.0722	0.1324	0.0602	60.2	12,040	
18	5	0.0731	0.1245	0.0514	51.4	10,280	10,330
	5	0.0731	0.125	0.0519	51.9	10,380	
21	5	0.0733	0.1231	0.0498	49.8	9,960	10,020
	5	0.0733	0.1237	0.0504	50.4	10,080	
24	5	0.072	0.1245	0.0525	52.5	10,500	10,520
	5	0.072	0.1247	0.0527	52.7	10,540	
27	5	0.072	0.1312	0.0592	59.2	11,840	10,780
	5	0.0727	0.1213	0.0486	48.6	9,720	
30	5	0.0721	0.1232	0.0511	51.1	10,220	10,010
	5	0.0725	0.1215	0.049	49	9,800	

1.4 pH

Table B10 Influent and effluent pH in the SBRs with 1, 2 and 3 d/cycle.

Influent pH

Palm oil	4.6
Mineral medium	8.6

Effluent pH

1 d/cycle		2 d/cycle		3 d/cycle	
days	Effluent pH	days	Effluent pH	days	Effluent pH
1	5.33	2	6	3	5.3
2	5.27	4	6.27	6	5.59
3	5.3	6	6.06	9	5.45
4	5.51	8	6.03	12	5.58
5	5.37	10	6.24	15	5.51
6	5.37	12	6.08	18	5.51
7	5.47	14	5.81	21	5.63
8	5.64	16	5.8	24	5.68
9	5.7	18	6.05	27	5.67
10	5.63	20	6.05	30	5.65
11	5.68			33	6.02
12	5.91				
13	5.9				
14	6.29				
15	6.6				

1.5 Palm Oil Content

Table B11 Effluent palm oil and oil removal of 1 d/cycle

Day	Volume of test (ml)			Wt.flask (g)	Wt.flask + oil (g)	Wt. oil (g)	Effluent [oil] (g/500ml)	Effluent [oil] (g/l)	Avg. effluent [oil] (g/l)	Oil removal (%)
	sample	1:1 HCl	DCM							
0	-	-	-	-	-	-	-	-	-	-
1	24	0.18	90	51.3823	51.402	0.0197	0.4104	0.8208	0.825	86.25
	24	0.18	90	161.3255	161.3454	0.0199	0.4146	0.8292		
2	23	0.18	90	161.201	161.2257	0.0247	0.5370	1.0739	1.052	82.46
	23	0.18	90	113.1286	113.1523	0.0237	0.5152	1.0304		
3	24.4	0.18	90	113.2945	113.3047	0.0102	0.2090	0.4180	0.415	93.08
	25	0.18	90	101.2579	101.2682	0.0103	0.2060	0.4120		
4	26.2	0.18	90	110.9911	110.9997	0.0086	0.1641	0.3282	0.328	94.53
	25	0.18	90	109.1258	109.134	0.0082	0.1640	0.3280		
5	28.2	0.18	90	109.0928	109.1077	0.0149	0.2642	0.5284	0.526	91.23
	25	0.18	90	101.0928	101.1059	0.0131	0.2620	0.5240		
6	25	0.18	90	109.0995	109.1094	0.0099	0.1980	0.3960	0.398	93.37
	25	0.18	90	113.0852	113.0952	0.01	0.2000	0.4000		
7	25	0.18	90	108.5147	108.5412	0.0265	0.5300	1.0600	0.970	83.83
	25	0.18	90	161.1682	161.1902	0.022	0.4400	0.8800		
8	25	0.18	90	108.3316	108.3731	0.0415	0.8300	1.6600	1.652	72.46
	25	0.18	90	110.0849	110.126	0.0411	0.8220	1.6440		
9	25	0.18	90	110.8707	110.9113	0.0406	0.8120	1.6240	1.642	72.63
	25	0.18	90	109.0903	109.1318	0.0415	0.8300	1.6600		
10	25	0.18	90	101.1061	101.1303	0.0242	0.4840	0.9680	1.056	82.40
	25	0.18	90	109.0918	109.1204	0.0286	0.5720	1.1440		

Table B12 Effluent palm oil and oil removal of 2 d/cycle

Day	Volume of test (ml)			Wt. flask (g)	Wt. flask + oil (g)	Wt. oil (g)	Effluent [oil] (g/500ml)	Effluent [oil] (g/l)	Avg. effluent [oil] (g/l)	Oil removal (%)
	sample	1:1 HCl	DCM							
0	-	-	-	-	-	-	-	-	-	-
2	25	0.18	90	108.5093	108.5362	0.0269	0.5380	1.0760	1.448	87.93
	25	0.18	90	110.8330	110.8785	0.0455	0.9100	1.8200		
4	25	0.18	90	100.3536	100.4007	0.0471	0.9420	1.8840	1.962	83.65
	25	0.18	90	111.4922	111.5432	0.0510	1.0200	2.0400		
6	25	0.18	90	101.1100	101.1300	0.0200	0.4000	0.8000	2.308	80.77
	25	0.18	90	111.4936	111.5890	0.0954	1.9080	3.8160		
8	25	0.18	90	108.5151	108.5440	0.0289	0.5780	1.1560	0.952	92.07
	25	0.18	90	113.2877	113.3064	0.0187	0.3740	0.7480		
10	25	0.18	90	113.2896	113.2924	0.0028	0.0560	0.1120	0.11	99.08
	25	0.18	90	111.4968	111.4995	0.0027	0.0540	0.1080		
12	25	0.18	90	108.5169	108.5187	0.0018	0.0360	0.0720	0.096	99.20
	25	0.18	90	101.1123	101.1153	0.0030	0.0600	0.1200		
14	25	0.18	90	110.3500	110.3565	0.0065	0.1300	0.2600	0.996	91.70
	25	0.18	90	111.4728	111.5161	0.0433	0.8660	1.7320		
16	25	0.18	90	101.0915	101.1028	0.0113	0.2260	0.4520	0.326	97.28
	25	0.18	90	108.4955	108.5005	0.0050	0.1000	0.2000		
18	25	0.18	90	110.3719	110.3746	0.0027	0.0540	0.1080	0.094	99.22
	25	0.18	90	111.4980	111.5000	0.0020	0.0400	0.0800		
20	25	0.18	90	113.2874	113.2939	0.0065	0.1300	0.2600	0.246	97.95
	25	0.18	90	108.5191	108.5249	0.0058	0.1160	0.2320		

Table B13 Effluent palm oil and oil removal of 3 d/cycle

Day	Volume of test (ml)			Wt. flask (g)	Wt. flask +oil (g)	Wt. oil (g)	Effluent [oil] (g/500ml)	Effluent [oil] (g/l)	Avg. effluent [oil] (g/l)	Oil removal (%)
	sample	1:1 HCl	DCM							
0	-	-	-	-	-	-	-	-	-	-
3	24.2	0.18	90	108.6068	108.6951	0.0883	1.8244	3.6488	3.716	79.35
	25	0.18	90	109.5612	109.6558	0.0946	1.8920	3.7840		
6	24	0.18	90	109.0997	109.1527	0.0530	1.1042	2.2083	2.286	87.30
	25	0.18	90	113.1256	113.1847	0.0591	1.1820	2.3640		
9	25	0.18	90	161.2344	161.3373	0.1029	2.0580	4.1160	4.130	77.05
	25	0.18	90	113.2025	113.3061	0.1036	2.0720	4.1440		
12	25	0.18	90	51.3807	51.5152	0.1345	2.6900	5.3800	5.360	70.22
	25	0.18	90	109.3207	109.4542	0.1335	2.6700	5.3400		
15	26.4	0.18	90	109.0989	109.2518	0.1529	2.8958	5.7917	5.792	67.82
	25	0.18	90	113.0257	113.1705	0.1448	2.8960	5.7920		
18	25	0.18	90	112.9468	113.0994	0.1526	3.0520	6.1040	6.104	66.09
	25	0.18	90	109.5568	109.7094	0.1526	3.0520	6.1040		
21	28.8	0.18	90	113.2862	113.5210	0.2348	4.0764	8.1528	8.232	54.26
	25	0.18	90	113.3122	113.5200	0.2078	4.1560	8.3120		
24	25	0.18	90	113.2992	113.5134	0.2142	4.2840	8.5680	8.608	52.17
	25	0.18	90	113.2852	113.5014	0.2162	4.3240	8.6480		
27	25	0.18	90	113.2703	113.4256	0.1553	3.1060	6.2120	6.69	62.83
	25	0.18	90	109.0692	109.2484	0.1792	3.5840	7.1680		
30	25	0.18	90	101.1046	101.2285	0.1239	2.4780	4.9560	4.772	73.49
	25	0.18	90	110.7921	110.9068	0.1147	2.2940	4.5880		

1.6 Surface Tension

Table B14 Surface tension and surface tension of 1 d/cycle

Day	Surface tension (mN/m)						Surface tension reduction (%)
	No.1	No.2	No.3	No.4	No.5	Avg.	
0	70.89	70.89	70.89	70.89	70.89	70.89	0
1	28.96	29.42	29.81	29.06	29.29	29.308	58.66
2	28.92	29.07	28.43	28.94	28.53	28.778	59.40
3	29.85	29.18	29.76	29.45	29.3	29.508	58.37
4	28.51	29.02	29.74	29.16	29.74	29.234	58.76
5	29.52	29.73	29.36	29.57	29.74	29.584	58.27
6	29.86	29.74	29.79	29.79	29.2	29.676	58.14
7	31.6	32.21	31.99	31.85	31.66	31.862	55.05
8	31.76	31.61	31.98	31.88	31.92	31.83	55.10
9	31.04	31.56	31.44	31.12	31.16	31.264	55.90
10	30.59	30.52	30.89	30.94	30.76	30.74	56.64

Table B15 Surface tension and surface tension of 2 d/cycle

Day	Surface tension (mN/m)						Surface tension reduction (%)
	No.1	No.2	No.3	No.4	No.5	avg.	
0	70.96	70.96	70.96	70.96	70.96	70.96	0
2	29.23	29.28	29.10	29.08	29.17	29.17	58.89
4	30.89	30.39	30.67	30.44	30.49	30.58	56.91
6	32.56	32.45	32.00	32.30	33.23	32.51	54.19
8	29.55	29.62	29.49	29.45	29.39	29.50	58.43
10	29.02	29.31	29.28	29.36	29.22	29.24	58.80
12	28.47	28.56	26.89	26.68	27.26	27.57	61.14
14	29.87	29.74	29.46	29.70	29.46	29.65	58.22
16	29.36	29.18	29.30	29.38	28.98	29.24	58.79
18	30.46	30.49	30.42	30.39	30.47	30.45	57.09
20	29.59	29.58	29.28	29.41	29.44	29.46	58.48

Table B16 Surface tension and surface tension of 3 d/cycle

Day	Surface tension (mN/m)						Surface tension reduction (%)
	No.1	No.2	No.3	No.4	No.5	avg.	
0	70.97	70.97	70.97	70.97	70.97	70.97	0
3	28.36	28.44	28.18	28.25	28.48	28.342	60.06
6	28.81	29.94	29.29	29.33	29.49	29.372	58.61
9	29.34	29.6	29.79	29.63	29.53	29.578	58.32
12	29.45	29.41	29.77	29.85	29.79	29.654	58.22
15	33.62	33.52	33.42	33.61	33.67	33.568	52.70
18	33.38	33.7	33.08	33.63	33.35	33.428	52.90
21	30.75	29.99	29.86	29.54	29.74	29.976	57.76
24	30.21	30.23	29.63	29.5	29.95	29.904	57.86
27	31.72	31.34	31.58	31.71	31.95	31.66	55.39
30	31.91	31.74	31.72	31.64	31.62	31.726	55.30

Table B17 Surface tension and surface tension reduction at steady state operation

Parameter	Cycle time		
	1 d/cycle	2 d/cycle	3 d/cycle
SFT	30.37	28.82	30.93
% Reducion	57.15	59.39	56.41

Table B18 Percent reduction of COD, oil and surface tension at steady state operation

Parameters	Cycle time		
	1 d/cycle	2 d/cycle	3 d/cycle
COD removal (%)	72.95	89.8	40.54
Oil removal (%)	89.48	96.66	71.7
Surface tension (%)	57.15	59.39	56.41

2. Measurement of Surface Tension and Determining Critical Micelle Dilution (CMD)

Table B19 Surface tension of supernatant from *Pseudomonas aeruginosa* SP4 with aeration step during steady state cycle at 2 d/cycle

Time (hr)	Surface Tension (mN/m)										
	No.1	No.2	No.3	No.4	No.5	No.6	No.7	No.8	No.9	No.10	Avg.
0	65.92	65.77	65.84	65.92	65.66	65.76	65.37	67.2	67.01	65.15	65.96
2	64.95	64.66	64.97	64.67	64.59	64.05	64.47	63.66	63.29	63.63	64.29
4	63.07	63.29	63.33	62.76	62.74	62.61	62.72	62.55	62.74	62.21	62.80
6	33.93	34.88	34.31	34.31	33.46	33.39	33.38	33.15	33.09	33	33.69
8	35.27	35.26	35.44	35.2	35.37	35.28	35.38	37.73	37.51	37.54	36.00
10	33.29	33.76	33.66	33.43	33.32	33.25	33.56	31.07	30.98	30.93	32.73
12	32.96	32.96	32.88	32.78	32.95	30.85	30.75	30.9	30.88	30.87	31.88
14	31.2	31.07	31.14	31.16	31.17	31.33	31.44	32.21	32.28	32.11	31.51
16	29.49	29.55	29.41	29.38	29.4	29.3	29.24	28.94	28.84	28.94	29.25
18	28.88	28.94	28.96	28.96	28.95	28.95	28.95	29.01	29.02	29.04	28.97
20	28.61	28.28	28.31	28.63	28.32	28.44	28.53	28.42	28.3	28.42	28.43
22	28.3	28.3	28.33	28.56	28.63	28.52	28.52	29.34	29.23	29.23	28.72
24	28.48	28.05	28.39	28.05	28.39	28.19	28.25	28.55	28.48	28.36	28.32
26	28.21	28.04	28.25	28.33	28.59	28.49	28.48	28.27	28.39	28.25	28.33
28	29.34	29.66	29.08	29.54	29.32	29.12	29.45	31.91	31.85	29.28	29.86
30	29.65	29.53	29.23	29.67	29.58	29.34	29.04	33.77	33.53	33.58	30.69
32	29.72	29.73	29.68	29.69	29.64	29.59	29.61	29.57	29.28	28.15	29.47
34	29.16	29.16	29.07	28.54	28.84	28.81	28.83	28.56	28.51	28.43	28.79
36	29.64	29.61	29.73	29.93	29.51	29.47	29.39	29.32	29.26	28.23	29.41
38	29.65	29.77	29.57	29.6	29.58	29.75	29.58	28.79	29.57	29.29	29.52
40	29.21	29.23	29.3	29.28	29.21	29.02	29.84	29	29.07	29.14	29.23
42	29.49	29.52	29.54	29.53	29.41	29.17	29.14	29.22	29.24	29.21	29.35
44	30.85	30.86	30.94	30.36	30.31	30.3	30.4	30.73	30.85	30.92	30.65
46	30.09	30.14	30.41	30.18	30.26	30.26	30.24	30.25	30.22	30.17	30.22
48	31.04	29.86	29.39	31.23	33.72	32.97	30.29	29.74	33.86	32.08	31.42

Table B20 The surface tension of serially dilutions of 2 d/cycle with 10, 20, 30 and 40 h of aeration time.

Aeration time (h)	Surface tension (mN/m)						
	1:0	99:1	95:5	90:10	75:25	50:50	25:75
10	32.73	53.30	60.41	62.21	59.35	68.59	69.85
20	28.43	47.55	58.37	62	62.51	65.61	66.51
30	30.69	39.95	53.98	54.36	58.55	64.96	66.45
40	29.23	29.31	31.14	57.9	56.37	62.8	64.97

Appendix C Experimental Data of Biosurfactant Production at an Oil Loading Rate of 2 kg/m³d with Different C/N ratio of 16/0.57 and 16/3 at 2 d/cycle

1. Mixed Liquor Suspended Solid (MLSS)

Table C1 Effluent MLSS of 2 d/cycle in the C/N ratio of 16/0.57 at oil loading rate of 2 kg/m³d

Day	Vol. sample (ml)	wt. filter (g)	wt. filter+ MLSS (g)	Effluent MLSS (g)	Effluent MLSS (mg)	Effluent MLSS (mg/l)	Avg. effluent MLSS (mg/l)
0	-	-	-	-	-	-	-
2	5	0.0691	0.0704	0.0013	1.3	260	250
	5	0.0689	0.0701	0.0012	1.2	240	
4	5	0.0692	0.0707	0.0015	1.5	300	290
	5	0.0692	0.0706	0.0014	1.4	280	
6	5	0.0695	0.07	0.0005	0.5	100	140
	5	0.0695	0.0704	0.0009	0.9	180	
8	5	0.0693	0.0701	0.0008	0.8	160	160
	5	0.0692	0.07	0.0008	0.8	160	

Table C2 Effluent MLSS of 2 d/cycle in the C/N ratio of 16/3 at oil loading rate of 2 kg/m³d

Day	Vol.sample (ml)	wt.filter (g)	wt.filter+ MLSS (g)	Effluent MLSS (g)	Effluent MLSS (mg)	Effluent MLSS (mg/l)	Avg. effluent MLSS (mg/l)
0	-	-	-	-	-	-	-
2	5	0.0695	0.0704	0.0009	0.9	180	190
	5	0.0692	0.0702	0.001	1	200	
4	5	0.0685	0.0705	0.002	2	400	430
	5	0.0685	0.0708	0.0023	2.3	460	
6	5	0.0685	0.0708	0.0023	2.3	460	470
	5	0.0684	0.0708	0.0024	2.4	480	
8	5	0.0685	0.0708	0.0023	2.3	460	460
	5	0.0685	0.0708	0.0023	2.3	460	

2. Suspended Solid (SS)

Table C3 Effluent SS of 2 d/cycle in the C/N ratio of 16/0.57 at oil loading rate of 2 kg/m³d

Day	Vol. sample (ml)	wt. filter (g)	wt. filter+ SS (g)	Effluent SS (g)	Effluent SS (mg)	Effluent SS (mg/l)	Avg. effluent SS (mg/l)
0	-	-	-	-	-	-	-
2	5	0.0691	0.0704	0.0013	1.3	260	230
	5	0.0691	0.0701	0.001	1	200	
4	5	0.0696	0.0707	0.0011	1.1	220	200
	5	0.0697	0.0706	0.0009	0.9	180	
6	5	0.0696	0.07	0.0004	0.4	80	120
	5	0.0696	0.0704	0.0008	0.8	160	
8	5	0.0695	0.0701	0.0006	0.6	120	100
	5	0.0696	0.07	0.0004	0.4	80	

Table C4 Effluent SS of 2 d/cycle in the C/N ratio of 16/3 at oil loading rate of 2 kg/m³d

Day	Vol. sample (ml)	wt. filter (g)	wt. filter+ SS (g)	Effluent SS (g)	Effluent SS (mg)	Effluent SS (mg/l)	Avg. effluent SS (mg/l)
0	-	-	-	-	-	-	-
2	5	0.069	0.0699	0.0009	0.9	180	180
	5	0.069	0.0699	0.0009	0.9	180	
4	5	0.0687	0.0705	0.0018	1.8	360	360
	5	0.0681	0.0699	0.0018	1.8	360	
6	5	0.0684	0.0705	0.0021	2.1	420	440
	5	0.0686	0.0709	0.0023	2.3	460	
8	5	0.0685	0.0703	0.0018	1.8	360	420
	5	0.0685	0.0709	0.0024	2.4	480	

Table C5 Average effluent SS and MLSS (days 4-8) of 2 d/cycle in the difference of C/N ratio of 16/0.57 and 16/3 at oil loading rate of 2 kg/m³d

C/N ratio	MLSS	SS
	(mg/l)	(mg/l)
16/0.57	197	140
16/1	3,467	403
16/3	453	407

3. Surface Tension and Surface Tension Reduction

Table C6 Surface tension and surface tension reduction of 2 d/cycle in the C/N ratio of 16/0.57 at oil loading rate of 2 kg/m³d

Day	Surface tension (mN/m)						Surface tension reduction (%)
	No.1	No.2	No.3	No.4	No.5	avg.	
0	70.96	70.96	70.96	70.96	70.96	70.96	-
2	48.94	48.45	47.66	46.35	46.57	47.59	32.93
4	47.93	47.70	47.71	44.83	46.40	46.91	33.89
6	45.45	45.35	45.47	45.15	45.14	45.31	36.14
8	42.36	42.17	42.11	42.01	41.95	42.12	40.64

Table C7 Surface tension and surface tension reduction of 2 d/cycle in the C/N ratio of 16/3 at oil loading rate of 2 kg/m³d

Day	Surface tension (mN/m)						Surface tension reduction (%)
	No.1	No.2	No.3	No.4	No.5	avg.	
0	70.96	70.96	70.96	70.96	70.96	70.96	-
2	42.98	42.60	42.00	41.43	40.86	41.97	40.85
4	41.76	41.67	40.12	40.10	40.03	40.74	42.59
6	42.13	42.25	42.56	42.15	42.23	42.26	40.44
8	43.88	43.68	43.74	44.28	44.84	44.08	37.87

Table C8 The average surface tension and surface tension reduction of 2 d/cycle (days 4-8) in the different C/N ratio of 16/0.57 and 16/3 at oil loading rate of 2 kg/m³d

C/N ratio	Surface tension (mN/m)	% Reduciton
16/0.57	44.78	36.89
16/1	28.82	59.39
16/3	42.36	40.3

4. pH

Table C9 Effluent pH of 2 d/cycle in the C/N ratio of 16/0.57 and 16/3.

Day	Effluent pH	
	16/0.57	16/3
0	-	-
2	5.7	5.69
4	5.73	5.71
6	5.24	6.06
8	5.27	5.69

Table C10 Average effluent pH (days 4-8) in the C/N ratio of 16/0.57 and 16/3 at oil loading rate of 2 kg/m³d

C/N ratio	Avg. pH
16/0.57	5.41
16/1	6.04
16/3	5.82

CURRICULUM VITAE

Name: Ms. Onsiri Huayyai

Date of Birth: August 16, 1983

Nationality: Thai

University Education:

2001-2005 Bachelor Degree of Engineering, Faculty of Engineering and Industriail Technology, Silpakorn University, Nakhon Prathom, Thailand

Working Experience:

2005 Position: Internship Student
Company name: APEX Petrochemical Co., Ltd.

Proceedings:

1. Huayyai, O., Chavadej, S., Rujiravanit, R., and Abe, M. (2008, April 23) Biosurfactant Production from Palm Oil Using Sequencing Batch Reactors. Proceedings of 14th PPC Symposium on Petroleum, Petrochems. and Polymers. Bangkok, Thailand.
2. Huayyai, O., Chavadej, S., Rujiravanit, R., and Abe, M. (2008, April 22-25) Biosurfactant Production from Palm Oil using Sequencing Batch Reactors: Effect of Cycle Time. Proceedings of Smartmat-'08 & IWOFFM-2, Chiangmai, Thailand.

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1. Huayyai, O., Chavadej, S., Rujiravanit, R., and Abe, M. (2008, April 23) Biosurfactant Production from Palm Oil Using Sequencing Batch Reactors. Poster presented at the 14th PPC Symposium on Petroleum, Petrochems. and Polymers. Bangkok, Thailand.
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