

REFERENCES

- Atlas, R.M. (1981) Microbial Degradation of petroleum hydrocarbons: an environmental perspective. Microbiological Reviews, 45(1), 180-209.
- Banat, I.M., Makkar, R.S., and Cameotra, S.S. (2000) Potential commercial applications of microbial surfactants. Applied Microbiology and Biotechnology, 53, 495-508.
- Blanch, H.W. and Clark, D.S. (1997) Biochemical Engineering. New York: Marcel Dekker, Inc.
- Chung, N. and Alexander, M. (1999) Effect of concentration of sequestration and bioavailability of two polycyclic aromatic hydrocarbons. Environmental Science and Technology, 33, 3605-3608.
- Diaz, M.P., Boyd, K.G., Grigson, S.J.W., and Burgess, J.G. (2002) Biodegradation of crude oil across a wide range of salinities by an extremely halotolerant bacterial consortium MPD-M, immobilized onto polypropylene fibers. Biotechnology and Bioengineering, 79(2), 145-153.
- Dutta, T.K. and Harayama, S. (2000) Fate of crude oil by the contamination of photooxidation and biodegradation. Environmental Science and Technology, 34, 1500-1505.
- Ekpo, B.O., Ibok, U.J., and Umoh, N.D. (2000) Geochemical evaluation of suitability of sites for hazardous waste disposal: a case study of recent and old waste-disposal sites in Calabar Municipality, SE Nigeria. Environmental Geology, 39(11), 1286-1294.
- Garcia, J.M., Wick, L.Y., and Harma, H. (2001) Influence of the nonionic surfactant Brij 35 on the bioavailability of solid and sorbed dibenzofuran. Environmental Science and Technology, 35, 2033-2039.
- Gogolev, A. and Wilke, B.M. (1997) Combination effects of heavy metals and fluoranthene on soil bacteria. Biology and Fertility of Soils, 25, 274-278.
- Huesemann, M.H. and Truex, M.J. (1996) The role of oxygen diffusion in passive bioremediation of petroleum contaminated soils. Journal of Hazardous Materials, 51, 93-113.

- J, B., A, R., and A, S., (1994) Optimization technique or sewage-sludge conditioning with polymer and skeleton builders. Water Research, 28(10), 2067-2073.
- Janiyani, K.L., Wate, S.R., and Joshi, S.R. (1993) Solubilization of hydrocarbons from oil sludge by synthetic surfactants. Journal of Chemical Biotechnology, 56, 305-308.
- JD, V.H. and OP, W. (1999) Influence of chemical surfactants on the biodegradation of crude oil by a mixed bacterial culture. Canadian Journal of Microbiology, 45(2), 130-137.
- JD, V.H. and OP, W. (2001) Physical and metabolic interactions of *Pseudomonas* sp strain JA5-B45 and *Rhodococcus* sp strain F9-D79 during growth n crude oil and effect of a chemical surfactant on them. Applied and Environmental Microbiology, 67(10), 4874-4879.
- McKetta, J.J. (Ed.). (1992) Petroleum Processing Handbook. New York:Marcel Dekker, Inc.
- Mackey, D.M., Roberts, P.V., and Cherry, J.A. (1985) Transport of organic contaminants in groundwater. Environmental Science and Technology, 19, 384-392.
- Margesin, R. and Schinner, F. (2001) Biodegradation and bioremediation of hydrocarbons in extreme environments. Applied Microbiology and Biotechnology, 56, 650-663.
- Oh, Y.S., Maeng, J., and Kim, S.J. (2000) Use of microorganism-immobilized polyurethane foams to absorb and degrade oil on water surface. Applied Microbiology and Biotechnology, 54, 418-423.
- P, B., H, B., and K, E., (1999) Effects of surfactant mixtures, including Corexit 9527, on bacterial oxidation of acetate and alkanes in crude oil. Applied and Environmental Microbiology, 65(4), 1658-1661.
- Rhykerd, R.L., Crews, B., McInnes, K.J., and Weaver, R.W. (1999) Impact of bulking agents, forces aeration, and tillage on remediation of oil-contaminated soil. Bioresource Technology, 67, 279-285.
- Riser-Roberts, E. (Ed.). (1992) Bioremediation of Petroleum Contaminated Sites. The united states of america: C.K. Smoley.

- Samanta, S.K., Bhushan, B., and Jain, R.K. (2001) Efficiency of naphthalene and salicylate degradation by a recombinant *Pseudomonas putida* mutant strain defective in glucose metabolism. Applied Microbiology and Biotechnology, 55, 627-631.
- Samiullah, Y. (Ed.). (1990) Prediction of The Environmental Fate of Chemical. London: Elsevier.
- SE, P. and AK, Z., (1996) Stabilization of a mixed waste sludge for land disposal. Hazardous Waste and Hazardous Materials, 13(4), 515-524.
- SR, J., YF, L., CS, H., and DY, L., (1999) Evaluation of effective conditioners for enhancing sludge dewatering and subsequent detachment from filter cloth. Journal of Environmental Science and Health Part A-Toxic/Hazardous Substances and Environmental Engineering, 34(7), 1517-1531.
- Sugiura, K., Ishihara, M., Shimauchi, T., and Harayama, S. (1997) Physicochemical properties and biodegradability of crude oil. Environmental Science and Technology, 31, 45-51.
- Vasudevan, N. and Rajaram, P. (2001) Bioremediation of oil sludge-contaminated soil. Environment International, 26, 409-411.
- YF, L., SR, J., and DY, L., (2001) Enhancing dewaterability and amenability of sludge for subsequent stabilization processes by using organic waste solids as conditioners. Journal of Environmental Science and Health part a-Toxic/Hazardous Substances and Environmental Engineering, 36(2), 191-202.

APPENDICES

Appendix A Experimental data of enhanced solubilization of hydrocarbons in oil sludge by nonionic surfactants.

1. Experiment Data of Effect of Contact Time on Solubilization of Oil Sludge by Nonionic Surfactants

Table A-1 Effect of contact time on solubilization of oil sludge by Brij 30

Time (h.)	Total Organic Carbon (S+O) (ppm)			C from surfactant (ppm)	C from oil sludge (ppm)			average C from oil sludge (ppm)
	sample1	sample2	sample3		sample1	sample2	sample3	
control	40.0	40.2	40.0	0.0	40.0	40.2	40	40.1
0	13,599	13,598	13,602	13,449.0	150.0	149	153	150.7
2	14,000	14,011	13,950	13,500.0	500.0	511	450	487.0
6	14,362	14,420	14,356	13,500.0	862.0	920	856	879.3
12	14,953	14,957	14,900	13,489.0	1,463.6	1,468	1,411	1,447.5
20	16,944	17,002	16,894	13,488.0	3,456.0	3,514	3,406	3,458.7
28	17,930	17,942	17,900	13,500.0	4,430.0	4,442	4,400	4,424.0
48	19,000	19,031	19,035	13,487.0	5,513.0	5,544	5,548	5,535.0
70	19,312	19,400	19,287	13,501.0	5,811.0	5,899	5,786	5,832.0
93	19,322	19,311	19,320	13,510.0	5,812.0	5,801	5,810	5,807.7
120	19,294	19,295	19,300	13,495.0	5,799.0	5,800	5,805	5,801.3
150	19,297	19,310	19,289	13,497.0	5,800.0	5,813	5,792	5,801.7
154	19,290	19,320	19,290	13,495.0	5,795.0	5,825	5,795	5,805.0

C = Carbon

S = Nonionic surfactant

O = Oil sludge

Table A-2 Effect of contact time on solubilization of oil sludge by Tween 80

Time (h.)	Total Organic Carbon (S+O) (ppm)			C from surfactant (ppm)	C from oil sludge (ppm)			average C from oil sludge (ppm)
	sample1	sample2	sample3		sample1	sample2	sample3	
control	40.0	40.2	40.0	0.0	40.0	40.2	40.0	40.1
0	9,786	9,780	9,779	9650	136.0	130	129	131.7
2	9,995	9,901	10,014	9658	336.8	243	356	311.7
6	10,055	10,103	10,070	9652.1	402.9	450	418	423.6
12	10,842	10,895	10,902	9655	1,187.2	1,240	1,247	1,224.9
20	11,665	11,600	11,599	9663.4	2,001.6	2,135	2,041	2,059.2
28	13,766	13,700	13,799	9661.5	4,104.8	4,039	4,137	4,093.5
48	14,376	14,390	14,376	9645.5	4,730.8	4,744	4,730	4,735.0
70	14,374	14,397	14,400	9647.9	4,725.6	4,749	4,752	4,742.1
93	14,381	14,302	14,400	9652.3	4,728.4	4,650	4,748	4,708.6
120	14,394	14,420	14,385	9660.7	4,733.0	4,760	4,724	4,738.8
150	14,385	14,365	14,396	9654.8	4,730.1	4,710	4,741	4,727.1
154	14,387	14,365	14,400	9657.1	4,729.6	4,708	4,743	4,726.9

Table A-3 Effect of contact time on solubilization of oil sludge by Brij 30

Time (h.)	Total Organic Carbon (S+O) (ppm)			C from surfactant (ppm)	C from oil sludge (ppm)			average C from oil sludge (ppm)
	sample1	sample2	sample3		sample1	sample2	sample3	
control	40.0	40.2	40.0	0.0	40.0	40.2	40	40.1
0	658	660	665	561.3	97.0	99	104	99.8
2	679	675	684	560	118.8	115	124	119.2
6	846	859	840	565.2	280.8	294	275	283.3
12	895	900	909	570	325.2	330	339	331.4
20	960	978	973	566.1	394.3	412	407	404.4
28	970	970	962	570.5	399.0	399	392	396.6
48	962	959	962	562.4	399.1	396	399	398.2
70	964	965	957	566.3	398.0	399	390	395.7
93	966	956	970	568.7	397.6	387	401	395.2
120	961	970.4	962.3	565.9	395.4	405	396	398.8
150	961	970	971	564.7	396.7	405	407	402.8
154	965	967	971	570	395.0	397	401	397.4

2. Experimental Data of Enhanced Solubilization of Hydrocarbons in Oil Sludge by Nonionic Surfactants

2.1 COD Method

Table A-4 Enhanced solubilization of hydrocarbons in oil sludge by Brij 30 at various concentrations

Brij 30 concentration		COD exerted by Brij 30 (ppm)			Soluble COD exerted by Brij 30 + Oil sludge (ppm)			COD exerted by hydrocarbons in aqueous phase (ppm)			average of COD exerted by hydrocarbons in aqueous phase (ppm)
%w/v	n x cmc	sample1	sample2	sample3	sample1	sample2	sample3	sample1	sample2	sample3	
control	control	0.00	0.00	0.00	115.00	114.00	116.00	115.00	114.00	116.00	115.00
0.01	3	194.19	194.19	196.14	956.60	976.70	970.97	762.41	782.51	774.83	773.25
0.05	15	971.00	972.85	979.30	2,003.10	2,106.50	2,112.80	1,032.10	1,133.65	1,133.50	1,099.75
0.1	30	2,039.03	2,075.90	2,112.90	4,112.50	4,208.70	4,231.00	2,073.47	2,132.80	2,118.10	2,108.12
0.5	145	11,023.00	11,000.00	11,011.00	14,522.30	14,264.00	15,022.00	3,499.30	3,264.00	4,011.00	3,591.43
1	299	22,065.00	21,356.00	21,855.00	26,023.00	25,988.00	25,947.00	3,958.00	5,864.99	4,092.00	4,638.33
1.5	449	33,102.00	33,098.50	33,096.50	38,102.00	38,114.00	38,021.00	5,000.00	5,015.50	4,924.50	4,980.00
2	599	40,258.00	39,887.00	39,065.70	47,276.10	47,112.00	45,899.00	7,018.10	7,225.00	6,833.30	7,025.47
2.5	748.5	54,986.10	55,123.00	55,420.00	60,562.50	60,744.80	60,200.00	5,576.40	5,621.80	4,780.00	5,326.07
3	898	65,988.00	66,085.80	66,000.00	70,952.00	71,223.00	70,855.00	4,964.00	5,137.20	4,855.00	4,985.40
4	1197	88,215.20	88,358.09	86,998.74	92,556.00	93,200.00	91,574.00	4,340.80	4,841.91	4,575.26	4,585.99

Table A-5 Enhanced solubilization of hydrocarbons in oil sludge by Tween 80 at various concentrations

Tween-80 concentration		COD exerted by Tween-80 (ppm)			Soluble COD exerted by Tween 80 + Oil sludge (ppm)			COD exerted by hydrocarbons in aqueous phase (ppm)			average of COD exerted by hydrocarbons in aqueous phase (ppm)
% w/v	n x cmc	sample1	sample2	sample3	sample1	sample2	sample3	sample1	sample2	sample3	
control	control	0.00	0.00	0.00	115.00	114.00	116.00	115.00	114.00	116.00	115.00
0.03	19	322.37	314.50	397.13	1,162.95	1,170.89	1,198.20	840.58	856.39	801.07	832.68
0.05	32	639.63	685.91	679.20	1,553.55	1,456.45	1,490.30	913.92	770.54	811.10	831.85
0.10	64	1,650.65	1,747.30	1,941.94	4,500.23	4,552.90	4,480.60	2,849.58	2,805.60	2,538.66	2,731.28
0.20	127	3,500.00	3,580.50	3,796.70	5,631.61	5,782.10	5,623.00	2,131.61	2,201.60	1,826.30	2,053.17
0.30	191	5,253.23	5,621.00	5,738.71	7,122.00	7,398.65	7,236.10	1,868.77	1,777.65	1,497.39	1,714.60
0.40	255	7,906.30	7,825.31	7,885.80	9,582.40	9,729.60	9,688.20	1,676.10	1,904.29	1,802.40	1,794.26
0.50	319	9,838.00	9,525.00	9,698.41	11,531.30	10,624.85	11,087.00	1,693.30	1,099.85	1,388.59	1,393.91
1.00	637	17,989.00	18,956.00	17,529.40	19,025.60	20,084.00	19,051.30	1,036.60	1,128.00	1,521.90	1,228.83
2.00	1,274	32,652.10	32,569.10	33,051.20	33,755.20	33,620.10	34,562.30	1,103.10	1,051.00	1,511.10	1,221.73
3.00	1,911	58,622.10	59,632.10	59,632.10	59,984.70	61,122.80	61,023.50	1,362.60	1,490.70	1,391.40	1,414.90
4.00	2,548	75,347.12	72,240.02	70,686.47	76,602.30	73,298.50	71,682.30	1,255.18	1,058.48	995.83	1,103.16

Table A-6 Enhanced solubilization of hydrocarbons in oil sludge by Triton X-100 at various concentrations

Triton X-100 concentration		COD exerted by Triton X-100 ppm			Soluble COD exerted by Triton X-100 + Oil sludge ppm			COD exerted by hydrocarbons in aqueous phase ppm			average of COD exerted by hydrocarbons in aqueous phase (ppm)
% w/v	n x cmc	sample1	sample2	sample3	sample1	sample2	sample3	sample1	sample2	sample3	
control	control	0.00	0.00	0.00	115.00	114.00	116.00	115.00	114.00	116.00	115.00
0.03	3	527.24	524.32	524.32	1,068.06	1,165.16	970.97	540.83	640.84	446.65	542.77
0.05	5	878.30	879.60	873.87	1,553.55	1,456.45	1,553.55	675.25	576.85	679.68	643.93
0.10	10	2,039.03	2,000.00	2,085.10	3,058.55	3,254.10	3,349.84	1,019.52	1,254.10	1,264.74	1,179.45
0.50	50	10,589.00	10,502.60	10,885.00	12,336.00	12,300.00	12,450.30	1,747.00	1,797.40	1,565.30	1,703.23
1.00	100	21,945.50	20,985.60	20,887.40	24,102.50	23,065.00	23,012.00	2,157.00	2,079.40	2,124.60	2,120.33
1.50	150	32,926.10	32,856.10	33,000.00	36,205.40	36,552.80	36,987.00	3,279.30	3,696.70	3,987.00	3,654.33
2.00	200	43,110.98	43,693.56	44,858.72	44,982.50	45,099.10	46,323.00	1,871.52	1,405.54	1,464.28	1,580.44
2.50	250	51,267.11	53,815.00	53,625.00	52,988.00	55,422.10	54,882.30	1,720.89	1,607.10	1,257.30	1,528.43
3.00	300	65,238.60	68,598.60	65,621.30	66,544.00	69,901.50	66,845.20	1,305.40	1,302.90	1,223.90	1,277.40
4.00	400	83,114.87	83,114.87	79,230.99	84,552.00	84,332.60	80,631.70	1,437.13	1,217.73	1,400.71	1,351.86

2.2 TOC Analyzer

Table A-7 Enhanced solubilization of hydrocarbons in oil sludge by Brij 30 at various concentrations

Brij30 concentration		Total Organic Carbon (S+O) ppm			C from surfactant (S) ppm			C from oil sludge ppm			average C from oil sludge ppm
% w/v	n x cmc	sample1	sample2	sample3	sample1	sample2	sample3	sample1	sample2	sample3	
control	control	36.5	38	38.7	0	0	0	36.5	38	38.7	37.7
0.01	3	277	278	277	61	63	63	216.06	215.5	214.6	215.4
0.05	15	523	526	525	304	310	313	219.1	216	212	215.7
0.1	30	895	840	837	623	625	630	272.4	214.3	207.3	231.3
0.5	145	3,610	3,532	3,521	3,289	3,290	3,300	320.5	241.9	220.5	261.0
1	299	7,005	6,600	6,590	6,525	6,198	6,144	480	402	446	442.7
1.5	449	10,323	10,223	10,257	9,790	9,782	9,758	532.5	441	499	490.8
2	599	13,500	13,600	13,587	12,850	12,836	12,845	650	764	742	718.7
2.5	748.5	16,220	16,200	16,328	15,880	15,870	15,842	340	330	486	385.3
3	898	19,224	19,219	19,244	18,860	18,866	18,842	363.5	353	402	372.8
4	1197	24,960	25,096	25,003	24,590	24,750	24,700	370	346	303	339.7

Table A-8 Enhanced solubilization of hydrocarbons in oil sludge by Tween 80 at various concentrations

Tween-80 concentration		Total Organic Carbon (S+O) ppm			C from surfactant (S) ppm			C from oil sludge ppm			average C from oil sludge ppm
% w/v	n x cmc	sample1	sample2	sample3	sample1	sample2	sample3	sample1	sample2	sample3	
control	control	36.5	38	38.7	0	0	0	36.5	38	38.7	37.73
0.03	19	271.8	272.9	272.4	157.7	160.5	161.8	114.1	112.4	110.6	112.37
0.05	32	587.3	586.1	586.7	271.9	285.3	274.8	315.4	300.8	311.9	309.37
0.1	64	941.3	967	930.5	541.3	562.1	571.3	400	404.9	359.2	388.03
0.2	127	1183	1201	1192	1022	1082.2	1015.3	161	118.8	176.7	152.17
0.3	191	1795	1823	1809	1641	1689.3	1701.5	154	133.7	107.5	131.73
0.4	255	2325	2344	2335	2179	2150.3	2169.1	146	193.7	165.9	168.53
0.5	319	2904	2951	2928	2740	2803.1	2796.7	164	147.9	131.3	147.73
1	637	5470	5515	5490	5350	5415	5360	120	100	130	116.67
2	1,274	10620	10710	10665	10510	10593	10542.3	109.8	117.4	122.7	116.63
3	1,911	15680	15840	15760	15520	15721	15614.7	160	119	145.3	141.43
4	2,548	20400	20280	20190	20290	20125	20090.3	110	154.9	99.7	121.53

Table A-9 Enhanced solubilization of hydrocarbons in oil sludge by Triton X-100 at various concentrations

Triton X-100 concentration		Total Organic Carbon (S+O) ppm			C from surfactant (S) ppm			C from oil sludge ppm			average C from oil sludge ppm
% w/v	n x cmc	sample1	sample2	sample3	sample1	sample2	sample3	sample1	sample2	sample3	
control	control	36.5	38.0	38.7	0.0	0.0	0.0	36.5	38.0	38.7	37.7
0.03	3	292.0	293.7	290.2	193.9	192.6	191.8	98.1	101.1	98.4	99.2
0.05	5	417.2	413.1	421.2	308.8	305.8	315.2	108.4	107.3	106.0	107.2
0.10	10	890.1	884.8	895.4	642.3	640.9	645.1	247.8	243.9	250.3	247.3
0.50	50	3,485.0	3,521.0	3,584.0	3,165.0	3,158.9	3,201.5	320.0	362.1	382.5	354.9
1.00	100	6,723.0	6,725.0	6,698.2	6,335.0	6,341.1	6,304.8	388.0	383.9	393.4	388.4
1.50	150	9,674.0	9,711.0	9,737.5	9,245.0	9,235.0	9,250.0	429.0	476.0	487.5	464.2
2.00	200	12,489.0	12,645.0	12,700.0	12,280.0	12,350.1	12,415.0	209.0	294.9	285.0	263.0
2.50	250	15,512.0	16,012.0	15,864.0	15,320.0	15,825.0	15,658.0	192.0	187.0	206.0	195.0
3.00	300	17,998.0	18,720.0	18,359.0	17,860.0	18,562.0	18,221.0	138.0	158.0	138.0	144.7
4.00	400	23,550.0	24,098.0	24,175.0	23,430.0	23,895.0	24,051.0	120.0	203.0	124.0	149.0

Appendix B Experimental data of enhanced biodegradation of hydrocarbons in oil sludge by nonionic surfactants.

1. Surfactant Effects on Growth and TPH Degradation of Indigenous Bacteria

Table B-1 The effect of oil sludge on growth and biodegradation of indigenous bacteria

day	wt before (g)		wt after (g)		TPH extract (mg)		dry wt cell (mg)		TPH degradation (mg)		ave TPH degradation (mg)	ave dry wt cell (mg)
	sample1	sample2	sample 1	sample2	sample1	sample2	sample1	sample2	sample 1	sample2		
control	0.09051	0.09044	0.09098	0.09085	984.33	983.2	1.175	1.025	0	0	0	1.1
d1	0.09575	0.09021	0.10072	0.09506	974.24	972.68	12.445	12.125	10.09	10.52	10.305	12.285
d2	0.09251	0.09011	0.10773	0.10509	966.52	968.74	38.05	37.45	17.81	14.46	16.135	37.75
d3	0.09533	0.09013	0.11216	0.10672	964.011	962.21	42.075	41.475	20.319	20.99	20.6545	41.775
d4	0.09419	0.09004	0.11355	0.10949	950.21	948.95	48.4	48.625	34.12	34.25	34.185	48.5125
d5	0.09515	0.09011	0.11573	0.11098	897.33	901.65	51.45	52.175	87	81.55	84.275	51.8125
d7	0.09035	0.0921	0.111	0.1129	899.01	899.58	51.625	52	85.32	83.62	84.47	51.8125

TPH = Total Petroleum hydrocarbons

Ave = average

Table B-2 The effect of Brij 30 at 600 times of its cmc on growth and biodegradation of indigenous bacteria

day	wt before (g)		wt after (g)		TPH extract (mg)		dry wt cell (mg)		TPH degradation (mg)		ave TPH degradation (mg)	ave dry wt cell (mg)
	sample 1	sample2	sample 1	sample2	sample 1	sample2	sample 1	sample2	sample 1	sample2		
control	0.08897	0.09114	0.08912	0.09164	988.74	990.45	0.375	0.5	0	0	0	0.4375
d1	0.09024	0.09233	0.09763	0.09946	930.47	932.5	18.475	17.825	58.27	57.95	58.11	18.15
d2	0.0893	0.09014	0.10914	0.10913	882.96	885.61	49.6	47.475	105.78	104.84	105.31	48.5375
d3	0.09091	0.09053	0.1117	0.11153	876.94	879.43	51.975	52.5	111.8	111.02	111.41	52.2375
d4	0.09014	0.09017	0.1125	0.11248	797.08	795.49	55.9	55.775	191.66	194.96	193.31	55.8375
d5	0.09013	0.09112	0.1146	0.11563	696.63	692.96	61.175	61.275	292.11	297.49	294.8	61.225
d7	0.09	0.09014	0.11458	0.11477	607.84	607.15	61.45	61.575	380.9	383.3	382.1	61.5125

Table B-3 The effect of Tween 80 at 64 times of its cmc on growth and biodegradation of indigenous bacteria

day	wt before (g)		wt after (g)		TPH extract (mg)		dry wt cell (mg)		TPH degradation (mg)		ave TPH degradation (mg)	ave dry wt cell (mg)
	sample 1	sample2	sample 1	sample2	sample 1	sample2	sample 1	sample2	sample 1	sample2		
control	0.09044	0.08998	0.09055	0.09017	985.98	989.84	0.275	0.475	0	0	0	0.375
d1	0.08956	0.09004	0.0959	0.09616	971.05	972.05	15.85	15.3	14.93	17.79	16.36	15.575
d2	0.0893	0.0901	0.10732	0.10866	835.96	840.865	45.05	46.4	150.02	148.975	149.4975	45.725
d3	0.09091	0.09011	0.10988	0.10963	822.01	819.47	47.425	48.8	163.97	170.37	167.17	48.1125
d4	0.09022	0.09016	0.1122	0.11221	785.64	788.11	54.95	55.125	200.34	201.73	201.035	55.0375
d5	0.09013	0.08997	0.11321	0.11294	742.84	744.46	57.7	57.425	243.14	245.38	244.26	57.5625
d7	0.08994	0.09009	0.11295	0.11319	739.28	741.12	57.525	57.75	246.7	248.72	247.71	57.6375

Table B-4 The effect of Triton X-100 at 150 times of its cmc on growth and biodegradation of indigenous bacteria

day	wt before (g)		wt after (g)		TPH extract (mg)		dry wt cell (mg)		TPH degradation (mg)		ave TPH degradation (mg)	ave dry wt cell (mg)
	sample 1	sample2	sample 1	sample2	sample 1	sample2	sample 1	sample2	sample 1	sample2		
control	0.0902	0.0902	0.09035	0.0903	986.08	985.43	0.375	0.25	0	0	0	0.3125
d1	0.09015	0.09015	0.09548	0.09541	971.87	971.84	13.325	13.15	14.21	13.59	13.9	13.2375
d2	0.0893	0.0902	0.10533	0.10631	936.55	933.42	40.075	40.275	49.53	52.01	50.77	40.175
d3	0.09017	0.09018	0.10752	0.10746	927.01	925.58	43.375	43.2	59.07	59.85	59.46	43.2875
d4	0.08979	0.09016	0.1092	0.10952	947.81	945.51	48.525	48.4	38.27	39.92	39.095	48.4625
d5	0.09013	0.08994	0.10848	0.10821	944.33	944.05	45.875	45.675	41.75	41.38	41.565	45.775
d7	0.09001	0.09014	0.10842	0.1085	943.09	942.01	46.025	45.9	42.99	43.42	43.205	45.9625

2. Surfactant Effects on Growth and TPH Degradation of *Pseudomonas aeruginosa*

Table B-5 The effect of nonionic surfactants on growth of *Pseudomonas aeruginosa* by using spectrophotometer

day	Type of Nonionic Surfactants		
	OD ₆₀₀ Brij 30 at 600cmc	OD ₆₀₀ Tween 80 at 64 cmc	OD ₆₀₀ Triton X-100 at 150cmc
d0	0.008	0.0085	0.0095
d1	0.009	0.0165	0.366
d2	0.0105	0.0195	0.465
d3	0.0125	0.024	0.502
d4	0.0105	0.0215	0.5895
d5	0.011	0.0215	0.5845
d6	0.012	0.0205	0.5855
d7	0.012	0.021	0.586

Table B-6 The effect of oil sludge on growth and biodegradation of *Pseudomonas aeruginosa*

day	wt before (g)		wt after (g)		dry wt cell (mg)		TPH extract (mg)		TPH degradation (mg)		ave TPH degradation (mg)	ave dry wt cell (mg)
	sample 1	sample2	sample 1	sample2	sample 1	sample2	sample 1	sample2	sample 1	sample2		
control	0.09051	0.09044	0.09098	0.09085	1.175	1.025	984.33	983.2	0	0	0	1.1
d1	0.09422	0.09311	0.10288	0.10104	21.65	19.825	966.35	963.22	17.98	19.98	18.98	20.7375
d2	0.09251	0.09256	0.11107	0.10509	46.4	45.575	965.33	960.54	19	22.66	20.83	45.9875
d3	0.09534	0.09247	0.11569	0.10672	50.875	51.1	952.66	950.33	31.67	32.87	32.27	50.9875
d4	0.09317	0.09311	0.11602	0.10949	57.125	57.525	889.56	890.54	94.77	92.66	93.715	57.325
d5	0.0941	0.09347	0.11711	0.11098	57.525	59.125	890.33	886.97	94	96.23	95.115	58.325
d7	0.09215	0.0921	0.11569	0.1129	58.85	57.32	890.23	887.65	94.1	95.55	94.825	58.085

Table B-7 The effect of Brij 30 at 600 times of its cmc on growth and biodegradation of *Pseudomonas aeruginosa*

day	wt before (g)		wt after (g)		dry wt cell (mg)		TPH extract (mg)		TPH degradation (mg)		ave TPH degradation (mg)	ave dry wt cell (mg)
	sample 1	sample2	sample 1	sample2	sample 1	sample2	sample 1	sample2	sample 1	sample2		
control	0.08897	0.09114	0.08912	0.09134	0.375	0.5	988.74	990.45	0	0	0	0.4375
d1	0.09135	0.09233	0.10058	0.10186	23.075	23.825	928.55	925.61	60.19	64.84	62.515	23.45
d2	0.09138	0.0913	0.11143	0.11116	50.125	49.65	881.56	880.32	107.18	110.13	108.655	49.8875
d3	0.09265	0.09124	0.11632	0.11422	59.175	57.45	850.33	849.63	138.41	140.82	139.615	58.3125
d4	0.09331	0.09235	0.1172	0.1163	59.725	59.875	753.21	769.88	235.53	220.57	228.05	59.8
d5	0.09187	0.09306	0.1169	0.11804	62.575	62.45	694.33	690.87	294.41	299.58	296.995	62.5125
d7	0.09226	0.09014	0.11704	0.11499	61.95	62.125	600.32	610.45	388.42	380	384.21	62.0375

Table B-8 The effect of Tween 80 at 64 times of its cmc on growth and biodegradation of *Pseudomonas aeruginosa*

day	wt before (g)		wt after (g)		dry wt cell (mg)		TPH extract (mg)		TPH degradation (mg)		ave TPH degradation (mg)	ave dry wt cell (mg)
	sample 1	sample2	sample 1	sample2	sample 1	sample2	sample 1	sample2	sample 1	sample2		
control	0.09044	0.08998	0.09055	0.09017	0.275	0.475	985.98	989.84	0	0	0	0.375
d1	0.08997	0.09051	0.0989	0.09962	22.325	22.775	970.05	969.56	15.93	20.28	18.105	22.55
d2	0.09127	0.09132	0.11061	0.11117	48.35	49.625	833.22	835.62	152.76	154.22	153.49	48.9875
d3	0.09235	0.09225	0.1137	0.11392	53.375	54.175	795.68	787.63	190.3	202.21	196.255	53.775
d4	0.0922	0.09124	0.11487	0.11367	56.675	56.075	783.65	779.86	202.33	209.98	206.155	56.375
d5	0.09067	0.0923	0.11422	0.11538	58.875	57.7	712.65	708.27	273.33	281.57	277.45	58.2875
d7	0.09147	0.0922	0.11508	0.11571	59.025	58.775	699.32	700.89	274.18	277.32	275.75	58.9

Table B-9 The effect of Triton X-100 at 150 times of its cmc on growth and biodegradation of *Pseudomonas aeruginosa*

day	wt before (g)		wt after (g)		dry wt cell (mg)		TPH extract (mg)		TPH degradation (mg)		ave TPH degradation (mg)	ave dry wt cell (mg)
	sample 1	sample2	sample 1	sample2	sample 1	sample2	sample 1	sample2	sample 1	sample2		
control	0.0902	0.0902	0.09035	0.0903	0.375	0.25	986.08	985.43	0	0	0	0.3125
d1	0.09113	0.09233	0.09976	0.10017	21.575	19.6	967.83	965.48	18.25	19.95	19.1	20.5875
d2	0.09065	0.09187	0.1086	0.10945	44.875	43.95	965.14	961.23	20.94	24.2	22.57	44.4125
d3	0.09204	0.09214	0.11172	0.11211	49.2	49.925	958.33	957.18	27.75	28.25	28	49.5625
d4	0.09235	0.09207	0.1128	0.112	51.125	49.825	895.76	896.74	90.32	88.69	89.505	50.475
d5	0.09168	0.09067	0.11435	0.11254	56.675	54.675	893.66	894.37	92.42	91.06	91.74	55.675
d7	0.09077	0.09184	0.11375	0.11329	57.45	53.625	894.05	893.99	92.03	91.44	91.735	55.5375

3. GC-MS

Table B-10 TPH degradation of hydrocarbons by Indigenous bacteria (reported as type of hydrocarbons)

Type of Hydrocarbons	No surfactant	Brij 30 at 600xcmc	Tween 80 at 64xcmc	Triton X-100 at 150xcmc
2,4- Dimethylheptane	0.25	11.27	10.48	2.50
Phenanthrene	13.57	91.21	50.16	14.98
2-Methylphenanthrene	8.86	76.15	95.91	5.50
9,10-Dimethlyanthracene	3.26	79.40	53.70	3.67
1-Methylanthracene	2.62	63.14	43.28	1.57
4-Methylpyrene	3.39	25.70	38.23	2.74
1-Methylpyrene	7.57	83.04	31.58	6.58
5-Methylchrysene	0.54	47.58	20.50	0.48

Table B-11 TPH degradation of hydrocarbons by *Pseudomonas aeruginosa* (reported as type of hydrocarbons)

Type of Hydrocarbons	No surfactant	Brij 30 at 600xcmc	Tween 80 at 64xcmc	Triton X-100 at 150xcmc
2,4- Dimethylheptane	0.70	12.88	12.81	0.07
Phenanthrene	17.29	94.53	89.07	1.37
2-Methylphenanthrene	9.14	78.98	97.22	1.14
9,10-Dimethlyanthracene	12.96	90.51	91.62	0.20
1-Methylanthracene	5.83	92.98	85.12	0.38
4-Methylpyrene	13.45	28.69	62.05	1.08
1-Methylpyrene	16.64	88.02	62.60	1.39
5-Methylchrysene	1.78	48.57	37.95	0.65

4. Rate and Yield of Biodegradation

Table B-12 Rate of TPH degradation of Indigenous bacteria

Type of nonionic surfactants	Rate of TPH degradation (mg/day)	Rate of TPH degradation (mg/day*dry wt.cell)
no surfactant	12.07	0.23
Brij600cmc	54.59	0.89
Tween64cmc	35.39	0.61
Triton150cmc	6.17	0.13

Table B-13 Rate of TPH degradation of *Pseudomonas aeruginosa*

Type of nonionic surfactants	Rate of TPH degradation (mg/day)	Rate of TPH degradation (mg/day*dry wt.cell)
no surfactant	13.55	0.23
Brij600cmc	54.89	0.88
Tween64cmc	41.12	0.70
Triton150cmc	13.11	0.24

Table B-14 Yield of Indigenous bacteria and *Pseudomonas aeruginosa*

Type of nonionic surfactant	yield(mg dry weight cell/mg TPH degrading)	
	Indigenous bacteria	<i>P. aeruginosa</i>
no surfactant	0.61	0.61
Brij 30	0.16	0.16
Tween80	0.23	0.21
Triton X-100	1.06	0.61

Appendix C Analytical method.

1. Enhanced Solubilization

$$= (\text{Solubilization}_{\text{oil+surf}} - \text{Solubilization}_{\text{surf}}) - \text{Solubilization}_{\text{control}}$$

where Surf = Surfactant

2. % Enhanced Solubilization

$$= (\text{Enhanced solubilization} \times 100) / \text{Solubilization}_{\text{control}}$$

3. TPH Degradation

$$= \text{TPH}_{\text{control}} - \text{TPH}_d$$

where TPH = Total Petroleum Hydrocarbon

d = d1 to d7

4. % TPH Degradation

$$= (\text{TPH degradation} \times 100) / \text{TPH}_{\text{control}}$$

5. Yield of Bacteria

$$= \text{Dry weight cell} / \text{TPH degradation}$$

6. Rate of TPH Degradation

$$= \text{TPH degradation} / 7 \text{ days}$$

$$= \text{TPH degradation} / (7 \text{ days} \times \text{dry weight cell})$$

CURRICULUM VITAE

Name: Ms. Anusa Ruttanapol

Date of Birth: March 6, 1980

Nationality: Thai

University Education:

1998-2000 Bachelor Degree of Science in Chemical Engineering, Faculty of Science, Chulalongkorn University, Bangkok, Thailand.