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APPENDICES

APPENDIX A

1. Calculation of Oil Concentration

$$Sg = \frac{\rho l}{\rho h}$$

$$\rho l = \frac{m}{V}$$

where:

Sg = Specific gravity

ρl = density of liquid (g/cm^3)

ρh = density of water (= $1g/cm^3$)

m = mass (cm^3)

V = volume (cm^3)

Specific gravity of lubricant oil (Sg) = 0.994

ρl = Sg x ρh

ρl = 0.994 x 1 = 0.994 g/cm3

Adding lubricant 1 cm^3 in water 1 L, thus

Calculating mass of lubricant m = ρl x V

m = 0.994 x 1=0.994 g

but 1 g = 1000 mg, thus

m = 0.994 x 1000 = 994 mg

Therefore, stock concentration = 994 mg/L

Required concentration = 200 mg/L at 1 L for the experiment

Take stock solution = $\frac{200 \text{ x1/994 mg/Lx L}}{\text{mg/L}}$ = 200 mL

and diluted with water to 1 L

2. Standard curve of lubricating oil

The calibration curve was plotted between ratio of area (lubricant oil/stearyl alcohol) and ratio of concentration (lubricant oil/stearyl alcohol).

Amount of lubricating oil = Ratio of concentration x Amount of stearyl alcohol

Ratio of concentration (x) = Ratio of area (y)/1.612

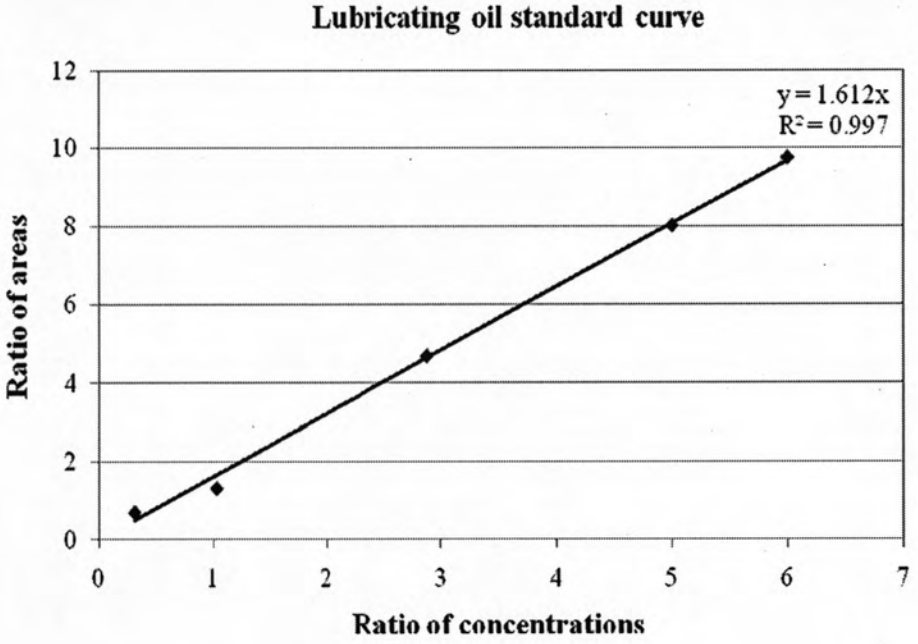

Figure A-1 Standard curve of lubricating oil from TLC-FID. Each data point was averaged from triple spots on chromatorods.

APPENDIX B

1. Oil and water sorption by chitosan

The maximum water and oil sorped by powder chitosan, flake shrimp shell chitosan, and flake squid pen chitosan were shown in Table B-1, B-2, and B-3 respectively.

Table B-1 Water and oil sorption capacity of powder chitosan

Triplicates	Dry chitosan weight (So)	Damp chitosan weight (Sw)	Water adsorbency (Sw-So)/So
1	0.19	1.98	9.49
2	0.18	2.05	10.43
3	0.20	2.20	10.13
ave	0.19	2.08	10.13

Table B-2 Water and oil sorption capacity of flake shrimp shell chitosan

Triplicates	Dry chitosan weight (So)	Damp chitosan weight (Sw)	Water adsorbency (Sw-So)/So
1	1.32	3.03	1.30
2	1.32	4.67	2.54
3	1.32	3.91	1.96
ave	1.32	3.87	1.96

Table B-3 Water and oil sorption capacity of flake squid pen chitosan

Triplicates	Dry chitosan weight (So)	Damp chitosan weight (Sw)	Water adsorbency (Sw-So)/So
1	0.35	1.19	2.40
2	0.35	1.57	3.49
3	0.35	1.11	2.17
ave	0.35	1.29	2.17

2. Optimum oil-in-water emulsion treatment condition of chitosan

2.1 Effect of chitosan dosage

Table B-4a Peak area of saturate, aromatic, and total oil remained in water after treated by different chitosan dose. Each data point was averaged from triple spots on chromatorods.

Chitosan dose (g/L)	Triplicates	Peak area			
		Saturate	Aromatic	Total oil	Stearyl
0	1	28240	20736	48976	38491
	2	20232	14825	35057	28658
	3	20899	14825	35724	28658
Average		23124	16795	39919	31936
0.1	1	4049	2291	6340	17000
	2	3931	1857	5788	14286
	3	4856	3155	8011	16037
Average		4279	2435	6713	15774
0.5	1	2591	1501	4092	15161
	2	2781	1715	4496	17171
	3	3272	2570	5842	16795
Average		2881	1929	4810	16376
1	1	2580	1702	4282	15491
	2	2332	1531	3863	16304
	3	2137	1107	3244	15272
Average		2350	1447	3796	15689
3	1	2073	1229	3303	14429
	2	2392	1751	4144	19600
	3	3400	2028	5428	22011
Average		2622	1669	4291	18680
5	1	2543	1350	3894	18635
	2	3089	1685	4775	17383
	3	1737	996	2733	17763
Average		2456	1344	3800	17927

Table B-4b Amount of total oil remained in water after treated by different chitosan dose and percent oil removal efficiency.

Chitosan dose (g/L)	Triplicates	Ratio of area (y)	Ratio of concentration (x)	Amount of lubricants (mg)	% Removal
0	1	1.2724	0.7892	9.8653	0
	2	1.2233	0.7588	9.4846	0
	3	1.2466	0.7732	9.6650	0
Average		1.2474	0.7737	9.6716	0
0.1	1	0.3730	0.2313	2.8917	71
	2	0.4052	0.2513	3.1413	67
	3	0.4995	0.3099	3.8731	60
Average		0.4259	0.2642	3.3020	66
0.5	1	0.2699	0.1674	2.0925	79
	2	0.2618	0.1624	2.0301	79
	3	0.3479	0.2158	2.6972	72
Average		0.2932	0.1819	2.2732	76
1	1	0.2764	0.1715	2.1432	78
	2	0.2370	0.1470	1.8372	81
	3	0.2124	0.1318	1.6469	83
Average		0.2419	0.1501	1.8758	81
3	1	0.2289	0.1420	1.7747	82
	2	0.2114	0.1311	1.6392	83
	3	0.2466	0.1530	1.9120	80
Average		0.2290	0.1420	1.7753	82
5	1	0.2089	0.1296	1.6200	84
	2	0.2747	0.1704	2.1296	78
	3	0.1538	0.0954	1.1928	88
Average		0.2125	0.1318	1.6475	83

Table B-5 The data of turbidity removal by different chitosan dose compared with no chitosan. Each data point was averaged from triple measurement.

Chitosan dose (g/L)	Triplicates	Adsorbance (500 nm)			% Removal
		1	2	3	
0	1	0.108	0.109	0.108	0
	2	0.106	0.107	0.109	0
	3	0.105	0.108	0.108	0
Average		0.106	0.108	0.108	0
0.1	1	0.060	0.060	0.057	47
	2	0.061	0.060	0.061	44
	3	0.063	0.062	0.062	43
Average		0.061	0.061	0.060	45
0.5	1	0.031	0.032	0.032	70
	2	0.031	0.033	0.031	72
	3	0.032	0.034	0.031	71
Average		0.031	0.033	0.031	71
1	1	0.023	0.024	0.025	77
	2	0.022	0.022	0.023	79
	3	0.024	0.023	0.024	78
Average		0.023	0.023	0.024	78
3	1	0.021	0.022	0.021	80
	2	0.020	0.022	0.022	80
	3	0.023	0.022	0.023	79
Average		0.021	0.022	0.022	80
5	1	0.020	0.019	0.019	82
	2	0.020	0.021	0.021	81
	3	0.021	0.020	0.019	82
Average		0.020	0.020	0.020	82

2.2 Effect of mixing time

Table B-6a Peak area of saturate, aromatic, and total oil remained in water after treated by different mixing time. Each data point was averaged from triple spots on chromatorods.

Mixing time (min)	Triplicates	Peak area			
		Saturate	Aromatic	Total oil	Stearyl
0	1	20261	11405	31667	28760
	2	15281	10555	25836	24328
	3	20973	10509	31482	29860
Average		18838	10823	29662	27649
10	1	12403	5958	18361	22185
	2	13252	6510	19762	23737
	3	13503	7993	21496	26109
Average		13053	6820	19873	24011
20	1	10128	6259	16288	22436
	2	11393	6182	17575	24421
	3	11899	7677	19576	25541
Average		11140	6706	17813	24133
40	1	8222	5562	13783	24232
	2	8736	5960	14697	24702
	3	9094	4995	14090	25053
Average		8684	5506	14190	24662
60	1	8660	4832	13492	33702
	2	5687	3207	8894	26603
	3	5770	3324	9094	24872
Average		6706	3788	10494	28393
80	1	5943	3593	9536	27231
	2	4447	3405	7853	25591
	3	5939	3157	9096	26613
Average		5443	3385	8828	26478

Table B-6b Amount of total oil remained in water after treated by different mixing time and percent oil removal efficiency compared with no chitosan.

Mixing time (min)	Triplicates	Ratio of area (y)	Ratio of concentration (x)	Amount of lubricants (mg)	% Removal
0	1	1.1011	0.6830	8.5371	22
	2	1.0620	0.6587	8.2339	31
	3	1.0543	0.6540	8.1745	32
Average		1.0725	0.6652	8.3152	29
10	1	0.8276	0.5133	6.4167	45
	2	0.8325	0.5164	6.4550	46
	3	0.8233	0.5107	6.3833	47
Average		0.8278	0.5135	6.4184	46
20	1	0.7260	0.4503	5.6288	53
	2	0.7197	0.4464	5.5799	54
	3	0.7664	0.4754	5.9425	50
Average		0.7374	0.4574	5.7170	52
40	1	0.5688	0.3528	4.4102	63
	2	0.5950	0.3690	4.6129	62
	3	0.5624	0.3488	4.3605	64
Average		0.5754	0.3569	4.4612	63
60	1	0.4003	0.2483	3.1040	74
	2	0.3343	0.2074	2.5921	78
	3	0.3656	0.2268	2.8350	76
Average		0.3668	0.2275	2.8437	76
80	1	0.3502	0.2172	2.7152	77
	2	0.3068	0.1903	2.3791	80
	3	0.3418	0.2120	2.6500	78
Average		0.3329	0.2065	2.5815	78

Table B-7 The data of turbidity removal by different mixing time compared with no chitosan. Each data point was averaged from triple measurement.

Mixing time (min)	Triplicates	Adsorbance (500 nm)			% Removal
		1	2	3	
0	1	0.176	0.165	0.170	4
	2	0.175	0.175	0.174	2
	3	0.164	0.163	0.162	9
Average		0.172	0.168	0.169	5
10	1	0.120	0.118	0.127	29
	2	0.127	0.119	0.117	34
	3	0.124	0.125	0.122	31
Average		0.124	0.121	0.122	31
20	1	0.098	0.094	0.097	46
	2	0.102	0.101	0.102	43
	3	0.100	0.097	0.103	42
Average		0.100	0.097	0.101	44
40	1	0.049	0.053	0.053	71
	2	0.058	0.064	0.068	62
	3	0.063	0.067	0.067	65
Average		0.057	0.061	0.063	66
60	1	0.032	0.034	0.033	81
	2	0.043	0.044	0.045	75
	3	0.031	0.034	0.033	81
Average		0.035	0.037	0.037	79
80	1	0.039	0.036	0.038	80
	2	0.036	0.037	0.034	81
	3	0.032	0.035	0.040	78
Average		0.036	0.036	0.037	80

3. Lubricating oil and oil-in-water emulsion degradation by isolated bacteria

Table B-8a Peak areas of each fraction remained in water after degrade lubricating oil 25,000 mg/L by isolated bacteria. Each data point was averaged from triple spots on chromatorods.

Bacteria	Triplicates	Peak area			
		Saturate	Aromatic	Total oil	Stearyl
Ch1	1	9558	2811	12369	4701
	2	8802	2947	11749	4508
	3	9431	3253	12684	4662
Average		9264	3004	12267	4623
Ch2	1	9042	2427	11470	5209
	2	8393	3251	11644	5721
	3	7912	2741	10653	5416
Average		8449	2806	11255	5449
Ch3	1	8712	3817	12529	4894
	2	9280	2927	12206	4476
	3	8220	3241	11461	4965
Average		8737	3328	12066	4779
Ch4	1	10947	4017	14965	7310
	2	10234	3593	13827	7140
	3	12164	4469	16633	7314
Average		11115	4026	15141	7255
Ch5	1	8540	2508	11048	4220
	2	9148	2773	11921	4743
	3	8971	2304	11275	4569
Average		8886	2528	11415	4511
None	1	10303	5457	15761	4850
	2	8883	3943	12826	4554
	3	9182	3119	12301	4254
Average		9456	4173	13629	4553

Table B-8b Amounts of total oil remained in water 30 mL after degrade lubricating oil 25,000 mg/L by isolated bacteria.

Bacteria	Triplicates	Ratio of peak area (total/stearyl)	Ratio of concentration	Amount of total oil (mg)
Ch1	1	2.6313	1.6321	204.02
	2	2.6064	1.6167	202.09
	3	2.7210	1.6877	210.97
Average		2.6529	1.6455	205.69
Ch2	1	2.2020	1.3659	170.73
	2	2.0351	1.2623	157.79
	3	1.9670	1.2201	152.51
Average		2.0681	1.2828	160.34
Ch3	1	2.5601	1.5880	198.50
	2	2.7269	1.6914	211.42
	3	2.3083	1.4318	178.97
Average		2.5318	1.5704	196.29
Ch4	1	2.0471	1.2697	158.72
	2	1.9366	1.2012	150.16
	3	2.2740	1.4105	176.31
Average		2.0859	1.2938	161.72
Ch5	1	2.6178	1.6237	202.97
	2	2.5135	1.5590	194.88
	3	2.4676	1.5306	191.32
Average		2.5330	1.5711	196.38
None	1	3.2498	2.0158	251.97
	2	2.8165	1.7470	218.37
	3	2.8917	1.7936	224.21
Average		2.9860	1.8521	231.51

Table B-8c Amounts of saturate and aromatic fraction remained in water 30 mL after degrade lubricating oil 25,000 mg/L by isolated bacteria.

Bacteria	Triplicates	Ratio of peak area (saturate/total)	Amount of saturate (mg)	Ratio of peak area (aromatic/total)	Amount of aromatic (mg)
Ch1	1	0.7727	157.65	0.2273	46.37
	2	0.7491	151.39	0.2509	50.70
	3	0.7435	156.86	0.2565	54.10
Average		0.7551	155.30	0.2449	50.39
Ch2	1	0.7884	134.60	0.2116	36.13
	2	0.7208	113.74	0.2792	44.05
	3	0.7427	113.27	0.2573	39.24
Average		0.7506	120.54	0.2494	39.81
Ch3	1	0.6953	138.02	0.3047	60.48
	2	0.7602	160.73	0.2398	50.69
	3	0.7172	128.36	0.2828	50.61
Average		0.7243	142.37	0.2757	53.93
Ch4	1	0.7315	116.11	0.2685	42.61
	2	0.7401	111.14	0.2599	39.02
	3	0.7313	128.94	0.2687	47.37
Average		0.7343	118.73	0.2657	43.00
Ch5	1	0.7730	156.89	0.2270	46.08
	2	0.7674	149.55	0.2326	45.33
	3	0.7957	152.23	0.2043	39.09
Average		0.7787	152.89	0.2213	43.50
None	1	0.6537	164.72	0.3463	87.25
	2	0.6926	151.24	0.3074	67.13
	3	0.7465	167.36	0.2535	56.85
Average		0.6976	161.11	0.3024	70.41

Table B-9a Peak areas of each fraction remained in water after degrade oil-in-water emulsion 200 mg/L by Bacteria Ch2. Each data point was averaged from triple spots on chromatorods.

Incubation time (h)	Triplicates	Peak area			
		Saturate	Aromatic	Total oil	Stearyl
0	1	4751	2969	7721	7775
	2	3125	2524	5649	7942
	3	3867	2216	6083	7330
Average		3914	2570	6484	7682
1	1	1770	722	2492	7708
	2	3099	1308	4407	7729
	3	1320	1115	2435	8092
Average		2063	1048	3111	7843
4	1	1020	628	1648	4784
	2	1534	1066	2601	8047
	3	1563	937	2501	7443
Average		1373	877	2250	6758
12	1	1177	674	1851	7241
	2	2290	1328	3618	7296
	3	1614	807	2421	7064
Average		1694	936	2630	7201
24	1	787	460	1247	7642
	2	825	340	1165	7464
	3	899	408	1306	7834
Average		837	403	1240	7647

Table B-9b Amounts of total oil remained in water 50 mL after degrade oil-in-water emulsion 200 mg/L by bacteria Ch2.

Incubation time (h)	Triplicates	Ratio of peak area (total/stearyl)	Ratio of concentration	Amount of total oil (mg)
0	1	0.9930	0.6159	7.70
	2	0.7113	0.4412	5.51
	3	0.8298	0.5147	6.43
Average		0.8447	0.5239	6.55
1	1	0.3233	0.2006	2.51
	2	0.5701	0.3536	4.42
	3	0.3009	0.1866	2.33
Average		0.3981	0.2469	3.09
4	1	0.3445	0.2137	2.67
	2	0.3232	0.2005	2.51
	3	0.3360	0.2084	2.60
Average		0.3345	0.2075	2.59
12	1	0.2557	0.1586	1.98
	2	0.4958	0.3076	3.84
	3	0.3428	0.2126	2.66
Average		0.3648	0.2262	2.83
24	1	0.1632	0.1012	1.27
	2	0.1561	0.0968	1.21
	3	0.1668	0.1034	1.29
Average		0.1620	0.1005	1.26

Table B-9c Amounts of saturate and aromatic fraction remained in water 50 mL after degrade oil-in-water emulsion 200 mg/L by bacteria Ch2.

Incubation time (h)	Triplicates	Ratio of peak area (saturate/total)	Amount of saturate (mg)	Ratio of peak area (aromatic/total)	Amount of aromatic (mg)
0	1	0.6154	4.74	0.3846	2.96
	2	0.5532	3.05	0.4468	2.46
	3	0.6357	4.09	0.3643	2.34
Average		0.6014	3.96	0.3986	2.59
1	1	0.7102	1.78	0.2898	0.73
	2	0.7032	3.11	0.2968	1.31
	3	0.5422	1.27	0.4578	1.07
Average		0.6519	2.05	0.3481	1.04
4	1	0.6189	1.65	0.3811	1.02
	2	0.5900	1.48	0.4100	1.03
	3	0.6252	1.63	0.3748	0.98
Average		0.6114	1.59	0.3886	1.01
12	1	0.6359	1.26	0.3641	0.72
	2	0.6329	2.43	0.3671	1.41
	3	0.6667	1.77	0.3333	0.89
Average		0.6452	1.82	0.3548	1.01
24	1	0.6308	0.80	0.3692	0.47
	2	0.7082	0.86	0.2918	0.35
	3	0.6879	0.89	0.3121	0.40
Average		0.6757	0.85	0.3243	0.41

Table B-10a Peak areas of each fraction remained in water after degrade oil-in-water emulsion 200 mg/L by Bacteria Ch4. Each data point was averaged from triple spots on chromatorods.

Incubation time (h)	Triplicates	Peak area			
		Saturate	Aromatic	Total oil	Stearyl
0	1	3122	2012	5134	8138
	2	4298	2813	7111	7959
	3	3856	2620	6476	8038
Average		3759	2482	6240	8045
1	1	2839	1645	4483	8444
	2	2027	1104	3131	7821
	3	2557	1442	3999	7285
Average		2474	1397	3871	7850
4	1	1314	716	2030	5974
	2	1331	690	2020	7416
	3	1783	865	2648	9751
Average		1476	757	2233	7714
12	1	2038	1093	3131	7621
	2	2299	1590	3889	8646
	3	2349	1494	3843	9572
Average		2228	1393	3621	8613
24	1	1634	1298	2932	7774
	2	1487	1051	2538	6869
	3	1442	1056	2498	7834
Average		1521	1135	2656	7409

Table B-10b Amounts of total oil remained in water 50 mL after degrade oil-in-water emulsion 200 mg/L by bacteria Ch4.

Incubation time (h)	Triplicates	Ratio of peak area (total/stearyl)	Ratio of concentration	Amount of total oil (mg)
0	1	0.6309	0.3913	4.89
	2	0.8934	0.5542	6.93
	3	0.8056	0.4997	6.25
Average		0.7766	0.4817	6.02
1	1	0.5309	0.3293	4.12
	2	0.4004	0.2483	3.10
	3	0.5490	0.3405	4.26
Average		0.4934	0.3061	3.83
4	1	0.3398	0.2108	2.63
	2	0.2724	0.1690	2.11
	3	0.2716	0.1684	2.11
Average		0.2946	0.1827	2.28
12	1	0.4108	0.2548	3.19
	2	0.4498	0.2790	3.49
	3	0.4015	0.2490	3.11
Average		0.4207	0.2609	3.26
24	1	0.3772	0.2339	2.92
	2	0.3695	0.2292	2.86
	3	0.3189	0.1978	2.47
Average		0.3552	0.2203	2.75

Table B-10c Amounts of saturate and aromatic fraction remained in water 50 mL after degrade oil-in-water emulsion 200 mg/L by bacteria Ch4.

Incubation time (h)	Triplicates	Ratio of peak area (saturate/total)	Amount of saturate (mg)	Ratio of peak area (aromatic/total)	Amount of aromatic (mg)
0	1	0.6081	2.97	0.3919	1.92
	2	0.6044	4.19	0.3956	2.74
	3	0.5955	3.72	0.4045	2.53
Average		0.6027	3.63	0.3973	2.39
1	1	0.6332	2.61	0.3668	1.51
	2	0.6473	2.01	0.3527	1.09
	3	0.6394	2.72	0.3606	1.54
Average		0.6399	2.45	0.3601	1.38
4	1	0.6473	1.71	0.3527	0.93
	2	0.6586	1.39	0.3414	0.72
	3	0.6733	1.42	0.3267	0.69
Average		0.6598	1.50	0.3402	0.78
12	1	0.6509	2.07	0.3491	1.11
	2	0.5911	2.06	0.4089	1.43
	3	0.6112	1.90	0.3888	1.21
Average		0.6177	2.01	0.3823	1.25
24	1	0.5573	1.63	0.4427	1.29
	2	0.5860	1.68	0.4140	1.19
	3	0.5772	1.43	0.4228	1.05
Average		0.5735	1.58	0.4265	1.18

Table B-11 Number of bacteria during the experiment of oil-in-water emulsion 200 mg/L degradation by bacteria Ch2 and Ch4. Each data point was averaged from triple examinations.

Bacteria	time	Triplicates			AVE	SD
		1	2	3		
Ch2	0	5.00E+06	6.00E+07	2.90E+07	3.13E+07	2.76E+07
	3	1.30E+07	1.00E+07	1.50E+07	1.27E+07	2.52E+06
	4	6.00E+06	6.00E+06	5.00E+06	5.67E+06	5.77E+05
	24	1.60E+07	1.50E+07	1.20E+07	1.43E+07	2.08E+06
Ch4	0	7.00E+07	4.00E+07	4.00E+07	5.00E+07	1.73E+07
	3	2.20E+07	2.30E+07	2.20E+07	2.23E+07	5.77E+05
	4	2.80E+07	1.80E+07	3.50E+07	2.70E+07	8.54E+06
	24	3.00E+07	2.50E+07	2.80E+07	2.77E+07	2.52E+06

4. Oil-in-water emulsion treatment by sorption by chitosan, degradation by isolated bacteria, and sorption and degradation by chitosan-immobilized cells technique.

The initial oil-in-water emulsion concentration was 200 mg/L. Then, lubricating oil at 200 mg/L was added to the oil-in-water emulsion everyday until finish the study. Control treatment was the oil-in-water emulsion that did not have any treatment.

Table B-12a Peak areas of each fraction remained in water after treated by powder chitosan-immobilized cells. Each data point was averaged from triple spots on chromatorods.

Day	Triplicates	Peak area			
		Saturate	Aromatic	Total oil	Stearyl
0	1	1761	1061	2822	5891
	2	1871	969	2840	6352
	3	1649	979	2628	5858
Average		1760	1003	2763	6034
1	1	1335	643	1978	3231
	2	1947	898	2845	5011
	3	2855	1355	4210	6216
Average		2046	965	3011	4819
2	1	4544	2173	6717	5311
	2	2755	1423	4178	5386
	3	3677	1397	5074	5621
Average		3659	1664	5323	5439
3	1	2953	1524	4477	5645
	2	6047	2257	8304	5910
	3	8423	2426	10849	6429
Average		5808	2069	7877	5995
4	1	8991	5577	14567	5766
	2	11761	7053	18815	6206
	3	8423	5426	13849	6429
Average		9725	6019	15744	6134

Table B-12b Amounts of total oil remained in water 50 mL after treat oil-in-water emulsion by powder chitosan-immobilized cells.

Day	Triplicates	Ratio of peak area (total/stearyl)	Ratio of concentration	Amount of total oil (mg)
0	1	0.4790	0.2971	3.71
	2	0.4470	0.2773	3.47
	3	0.4486	0.2783	3.48
Average		0.4582	0.2842	3.55
1	1	0.6122	0.3797	4.75
	2	0.5679	0.3522	4.40
	3	0.6773	0.4201	5.25
Average		0.6191	0.3840	4.80
2	1	1.2648	0.7845	9.81
	2	0.7756	0.4811	6.01
	3	0.9027	0.5599	7.00
Average		0.9810	0.6085	7.61
3	1	0.7932	0.4920	6.15
	2	1.4049	0.8714	10.89
	3	1.6875	1.0467	13.08
Average		1.2952	0.8034	10.04
4	1	2.5263	1.5670	19.59
	2	3.0317	1.8805	23.51
	3	2.1541	1.3362	16.70
Average		2.5707	1.5945	19.93

Table B-12c Amounts of saturate and aromatic fraction remained in water 50 mL after treated oil-in-water emulsion by powder chitosan-immobilized cells.

Day	Triplicates	Ratio of peak area (saturate/total)	Amount of saturate (mg)	Ratio of peak area (aromatic/total)	Amount of aromatic (mg)
0	1	0.6239	2.32	0.3761	1.40
	2	0.6589	2.28	0.3411	1.18
	3	0.6275	2.18	0.3725	1.30
Average		0.6368	2.26	0.3632	1.29
1	1	0.6750	3.20	0.3250	1.54
	2	0.6844	3.01	0.3156	1.39
	3	0.6781	3.56	0.3219	1.69
Average		0.6792	3.26	0.3208	1.54
2	1	0.6765	6.63	0.3235	3.17
	2	0.6594	3.97	0.3406	2.05
	3	0.7246	5.07	0.2754	1.93
Average		0.6868	5.22	0.3132	2.38
3	1	0.6595	4.06	0.3405	2.09
	2	0.7282	7.93	0.2718	2.96
	3	0.7764	10.16	0.2236	2.93
Average		0.7214	7.38	0.2786	2.66
4	1	0.6172	12.09	0.3828	7.50
	2	0.6251	14.69	0.3749	8.81
	3	0.6082	10.16	0.3918	6.54
Average		0.6168	12.31	0.3832	7.62

Table B-13a Peak areas of each fraction remained in water after treated oil-in-water emulsion by flake shrimp shell chitosan-immobilized cells. Each data point was averaged from triple spots on chromatorods.

Day	Triplicates	Peak area			
		Saturate	Aromatic	Total oil	Stearyl
0	1	1698	900	2598	9356
	2	1574	696	2270	8603
	3	1579	733	2312	8542
Average		1617	776	2393	8834
1	1	1533	637	2170	7333
	2	1486	544	2030	8673
	3	1633	663	2295	8321
Average		1551	615	2165	8109
2	1	1653	714	2367	9283
	2	1803	826	2629	8588
	3	1851	951	2801	9189
Average		1769	830	2599	9020
3	1	2782	1384	4166	8515
	2	2196	1276	3472	10121
	3	2934	1345	4279	8807
Average		2638	1335	3972	9148
4	1	3806	2251	6057	10155
	2	4176	1641	5817	8893
	3	3738	1698	5437	8757
Average		3907	1863	5770	9268

Table B-13b Amounts of total oil remained in water 50 mL after treated oil-in-water emulsion by flake shrimp shell chitosan-immobilized cells.

Day	Triplicates	Ratio of peak area (total/stearyl)	Ratio of concentration	Amount of total oil (mg)
0	1	0.2776	0.1722	2.15
	2	0.2639	0.1637	2.05
	3	0.2707	0.1679	2.10
Average		0.2707	0.1679	2.10
1	1	0.2959	0.1835	2.29
	2	0.2341	0.1452	1.81
	3	0.2758	0.1711	2.14
Average		0.2686	0.1666	2.08
2	1	0.2550	0.1582	1.98
	2	0.3062	0.1899	2.37
	3	0.3049	0.1891	2.36
Average		0.2887	0.1791	2.24
3	1	0.4892	0.3034	3.79
	2	0.3430	0.2128	2.66
	3	0.4859	0.3014	3.77
Average		0.4394	0.2725	3.41
4	1	0.5965	0.3700	4.62
	2	0.6541	0.4057	5.07
	3	0.6208	0.3851	4.81
Average		0.6238	0.3869	4.84

Table B-13c Amounts of saturate and aromatic fraction remained in water 50 mL after treated oil-in-water emulsion by flake shrimp shell chitosan-immobilized cells.

Day	Triplicates	Ratio of peak area (saturate/total)	Amount of saturate (mg)	Ratio of peak area (aromatic/total)	Amount of aromatic (mg)
0	1	0.6535	1.41	0.3465	0.75
	2	0.6932	1.42	0.3068	0.63
	3	0.6830	1.43	0.3170	0.67
Average		0.6766	1.42	0.3234	0.68
1	1	0.7066	1.62	0.2934	0.67
	2	0.7319	1.33	0.2681	0.49
	3	0.7113	1.52	0.2887	0.62
Average		0.7166	1.49	0.2834	0.59
2	1	0.6983	1.38	0.3017	0.60
	2	0.6857	1.63	0.3143	0.75
	3	0.6606	1.56	0.3394	0.80
Average		0.6815	1.52	0.3185	0.71
3	1	0.6678	2.53	0.3322	1.26
	2	0.6326	1.68	0.3674	0.98
	3	0.6857	2.58	0.3143	1.18
Average		0.6620	2.27	0.3380	1.14
4	1	0.6283	2.91	0.3717	1.72
	2	0.7179	3.64	0.2821	1.43
	3	0.6876	3.31	0.3124	1.50
Average		0.6780	3.29	0.3220	1.55

Table B-14a Peak areas of each fraction remained in water 50 mL after treated oil-in-water emulsion by flake squid pen chitosan-immobilized cells. Each data point was averaged from triple spots on chromatorods.

Day	Triplicates	Peak area			
		Saturate	Aromatic	Total oil	Stearyl
0	1	1545	639	2184	8657
	2	1192	733	1925	8655
	3	1303	740	2043	7114
Average		1346	704	2051	8142
1	1	996	685	1681	8538
	2	1292	722	2013	7872
	3	1466	800	2266	7930
Average		1251	736	1987	8113
2	1	1573	724	2297	8853
	2	1703	760	2463	8990
	3	1830	752	2582	8752
Average		1702	745	2447	8865
3	1	3648	2152	5800	10703
	2	4094	2190	6284	9230
	3	2593	1837	4429	8794
Average		3445	2060	5504	9576
4	1	3957	2587	6544	9369
	2	2648	1431	4079	8289
	3	4006	1938	5944	9095
Average		3537	1985	5522	8918

Table B-14b Amounts of total oil remained in water after treated oil-in-water emulsion by flake squid pen chitosan-immobilized cells.

Day	Triplicates	Ratio of peak area (total/stearyl)	Ratio of concentration	Amount of total oil (mg)
0	1	0.2523	0.1565	1.96
	2	0.2224	0.1380	1.72
	3	0.2871	0.1781	2.23
Average		0.2539	0.1575	1.97
1	1	0.1969	0.1221	1.53
	2	0.2558	0.1586	1.98
	3	0.2858	0.1773	2.22
Average		0.2461	0.1527	1.91
2	1	0.2595	0.1609	2.01
	2	0.2739	0.1699	2.12
	3	0.2950	0.1830	2.29
Average		0.2761	0.1713	2.14
3	1	0.5419	0.3361	4.20
	2	0.6808	0.4223	5.28
	3	0.5037	0.3124	3.91
Average		0.5755	0.3569	4.46
4	1	0.6985	0.4332	5.42
	2	0.4921	0.3052	3.82
	3	0.6536	0.4054	5.07
Average		0.6147	0.3813	4.77

Table B-14c Amounts of saturate and aromatic fraction remained in water after treated oil-in-water emulsion by flake squid pen chitosan-immobilized cells.

Day	Triplicates	Ratio of peak area (saturate/total)	Amount of saturate (mg)	Ratio of peak area (aromatic/total)	Amount of aromatic (mg)
0	1	0.7073	1.38	0.2927	0.57
	2	0.6191	1.07	0.3809	0.66
	3	0.6377	1.42	0.3623	0.81
Average		0.6547	1.29	0.3453	0.68
1	1	0.5923	0.90	0.4077	0.62
	2	0.6416	1.27	0.3584	0.71
	3	0.6469	1.43	0.3531	0.78
Average		0.6269	1.20	0.3731	0.71
2	1	0.6848	1.38	0.3152	0.63
	2	0.6915	1.47	0.3085	0.66
	3	0.7087	1.62	0.2913	0.67
Average		0.6950	1.49	0.3050	0.65
3	1	0.6290	2.64	0.3710	1.56
	2	0.6515	3.44	0.3485	1.84
	3	0.5853	2.29	0.4147	1.62
Average		0.6219	2.79	0.3781	1.67
4	1	0.6047	3.27	0.3953	2.14
	2	0.6492	2.48	0.3508	1.34
	3	0.6739	3.41	0.3261	1.65
Average		0.6426	3.06	0.3574	1.71

Table B-15a Peak areas of each fraction remained in water after treated oil-in-water emulsion by chitosan alone. Each data point was averaged from triple spots on chromatorods.

Day	Triplicates	Peak area			
		Saturate	Aromatic	Total oil	Stearyl
0	1	4794	2727	7521	27123
	2	3309	1625	4934	27560
	3	3952	2054	6006	24824
Average		4018	2135	6154	26503
1	1	10724	5566	16290	27603
	2	8187	3568	11755	23008
	3	8042	4273	12315	26918
Average		8984	4469	13453	25843
2	1	7691	3659	11351	5350
	2	11005	3207	14212	6587
	3	7790	4254	12044	6327
Average		8829	3707	12536	6088
3	1	12703	8311	21013	6425
	2	12869	6780	19649	5713
	3	11345	7190	18535	6422
Average		12306	7427	19732	6187
4	1	14000	9261	23261	5698
	2	17991	10616	28607	5759
	3	11884	10107	21991	4870
Average		14625	9995	24620	5442

Table B-15b Amounts of total oil remained in water 50 mL after treated oil-in-water emulsion by chitosan alone.

Day	Triplicates	Ratio of peak area (total/stearyl)	Ratio of concentration	Amount of total oil (mg)
0	1	0.2773	0.1720	2.15
	2	0.1790	0.1111	1.39
	3	0.2419	0.1501	1.88
Average		0.2328	0.1444	1.80
1	1	0.5902	0.3661	4.58
	2	0.5109	0.3169	3.96
	3	0.4575	0.2838	3.55
Average		0.5195	0.3222	4.03
2	1	2.1216	1.3160	16.45
	2	2.1575	1.3382	16.73
	3	1.9034	1.1806	14.76
Average		2.0609	1.2783	15.98
3	1	3.2704	2.0285	25.36
	2	3.4395	2.1334	26.67
	3	2.8862	1.7902	22.38
Average		3.1987	1.9841	24.80
4	1	4.0823	2.5321	31.65
	2	4.9676	3.0813	38.52
	3	4.5159	2.8011	35.01
Average		4.5220	2.8048	35.06

Table B-15c Amounts of saturate and aromatic fraction remained in water after treated oil-in-water emulsion by chitosan alone.

Day	Triplicates	Ratio of peak area (saturate/total)	Amount of saturate (mg)	Ratio of peak area (aromatic/total)	Amount of aromatic (mg)
0	1	0.6374	1.37	0.3626	0.78
	2	0.6707	0.93	0.3293	0.46
	3	0.6580	1.23	0.3420	0.64
Average		0.6553	1.18	0.3447	0.63
1	1	0.6583	3.01	0.3417	1.56
	2	0.6965	2.76	0.3035	1.20
	3	0.6530	2.32	0.3470	1.23
Average		0.6693	2.70	0.3307	1.33
2	1	0.6776	11.15	0.3224	5.30
	2	0.7743	12.95	0.2257	3.78
	3	0.6468	9.55	0.3532	5.21
Average		0.6996	11.21	0.3004	4.76
3	1	0.6045	15.33	0.3955	10.03
	2	0.6549	17.47	0.3451	9.20
	3	0.6121	13.70	0.3879	8.68
Average		0.6238	15.50	0.3762	9.30
4	1	0.6019	19.05	0.3981	12.60
	2	0.6289	24.22	0.3711	14.29
	3	0.5404	18.92	0.4596	16.09
Average		0.5904	20.73	0.4096	14.33

Table B-16a Peak areas of each fraction remained in water after treated oil-in-water emulsion by bacteria alone. Each data point was averaged from triple spots on chromatorods.

Day	Triplicates	Peak area			
		Saturate	Aromatic	Total oil	Stearyl
0	1	787	460	1247	7642
	2	825	340	1165	7464
	3	899	408	1306	7834
Average		837	403	1240	7647
1	1	1770	722	2492	7708
	2	3099	1308	4407	7729
	3	1320	1115	2435	8092
Average		2063	1048	3111	7843
2	1	4544	2640	7184	5311
	2	3755	2423	6178	5386
	3	5010	2931	7941	5621
Average		4436	2664	7101	5439
3	1	7020	3858	10877	5645
	2	6713	3257	9970	5910
	3	7757	3426	11182	6429
Average		7163	3513	10677	5995
4	1	12324	6910	19234	5766
	2	14428	8720	23148	6206
	3	12757	7759	20516	6429
Average		13170	7796	20966	6134

Table B-16b Amounts of total oil remained in water 50 mL after treated oil-in-water emulsion by bacteria alone.

Day	Triplicates	Ratio of peak area (total/stearyl)	Ratio of concentration	Amount of total oil (mg)
0	1	0.1632	0.1012	1.27
	2	0.1561	0.0968	1.21
	3	0.1668	0.1034	1.29
Average		0.1620	0.1005	1.26
1	1	0.3233	0.2006	2.51
	2	0.5701	0.3536	4.42
	3	0.3009	0.1866	2.33
Average		0.3981	0.2469	3.09
2	1	1.3527	0.8390	10.49
	2	1.1469	0.7114	8.89
	3	1.4127	0.8762	10.95
Average		1.3041	0.8089	10.11
3	1	1.9270	1.1953	14.94
	2	1.6869	1.0464	13.08
	3	1.7394	1.0789	13.49
Average		1.7844	1.1068	13.84
4	1	3.3356	2.0690	25.86
	2	3.7299	2.3136	28.92
	3	3.1911	1.9794	24.74
Average		3.4189	2.1206	26.51

Table B-16c Amounts of saturate and aromatic fraction remained in water after treated oil-in-water emulsion by bacteria alone.

Day	Triplicates	Ratio of peak area (saturate/total)	Amount of saturate (mg)	Ratio of peak area (aromatic/total)	Amount of aromatic (mg)
0	1	0.6308	0.80	0.3692	0.47
	2	0.7082	0.86	0.2918	0.35
	3	0.6879	0.89	0.3121	0.40
Average		0.6757	0.85	0.3243	0.41
1	1	0.7102	1.78	0.2898	0.73
	2	0.7032	3.11	0.2968	1.31
	3	0.5422	1.27	0.4578	1.07
Average		0.6519	2.05	0.3481	1.04
2	1	0.6325	6.63	0.3675	3.85
	2	0.6078	5.40	0.3922	3.49
	3	0.6309	6.91	0.3691	4.04
Average		0.6238	6.32	0.3762	3.79
3	1	0.6453	9.64	0.3547	5.30
	2	0.6733	8.81	0.3267	4.27
	3	0.6937	9.35	0.3063	4.13
Average		0.6708	9.27	0.3292	4.57
4	1	0.6407	16.57	0.3593	9.29
	2	0.6233	18.03	0.3767	10.89
	3	0.6218	15.38	0.3782	9.36
Average		0.6286	16.66	0.3714	9.85

Table B-17a Peak areas of each fraction remained in control. Each data point was averaged from triple spots on chromatorods.

Day	Triplicates	Peak area			
		Saturate	Aromatic	Total oil	Stearyl
0	1	1051	690	1741	1786
	2	1145	504	1648	1646
	3	1280	909	2189	2191
Average		1158	701	1859	1874
1	1	2110	1781	3891	1774
	2	1935	1251	3186	1684
	3	2610	1311	3921	2000
Average		2218	1448	3666	1819
2	1	4636	2967	7603	1987
	2	4887	2151	7038	2202
	3	4300	2175	6476	2384
Average		4608	2431	7039	2191
3	1	6709	4352	11061	2260
	2	6731	3725	10456	1984
	3	6883	5223	12106	2316
Average		6775	4433	11208	2187
4	1	9306	5887	15194	2465
	2	9445	7191	16636	2339
	3	9041	6762	15803	2285
Average		9264	6614	15877	2363

Table B-17b Amounts of total oil remained in control.

Day	Triplicates	Ratio of peak area (total/stearyl)	Ratio of concentration	Amount of total oil (mg)
0	1	0.9748	0.6046	7.56
	2	1.0016	0.6213	7.77
	3	0.9989	0.6196	7.75
Average		0.9918	0.6152	7.69
1	1	2.1938	1.3607	17.01
	2	1.8925	1.1739	14.67
	3	1.9602	1.2158	15.20
Average		2.0155	1.2501	15.63
2	1	3.8264	2.3734	29.67
	2	3.1956	1.9821	24.78
	3	2.7167	1.6851	21.06
Average		3.2462	2.0135	25.17
3	1	4.8937	3.0354	37.94
	2	5.2702	3.2689	40.86
	3	5.2262	3.2417	40.52
Average		5.1300	3.1820	39.77
4	1	6.1629	3.8227	47.78
	2	7.1135	4.4123	55.15
	3	6.9168	4.2903	53.63
Average		6.7311	4.1751	52.19

Table B-17c Amounts of saturate and aromatic fraction remained in control.

Day	Triplicates	Ratio of peak area (saturate/total)	Amount of saturate (mg)	Ratio of peak area (aromatic/total)	Amount of aromatic (mg)
0	1	0.6035	4.56	0.3965	3.00
	2	0.6944	5.39	0.3056	2.37
	3	0.5847	4.53	0.4153	3.22
Average		0.6276	4.83	0.3724	2.86
1	1	0.5423	9.22	0.4577	7.79
	2	0.6074	8.91	0.3926	5.76
	3	0.6656	10.12	0.3344	5.08
Average		0.6051	9.42	0.3949	6.21
2	1	0.6098	18.09	0.3902	11.58
	2	0.6944	17.20	0.3056	7.57
	3	0.6641	13.99	0.3359	7.08
Average		0.6561	16.43	0.3439	8.74
3	1	0.6066	23.01	0.3934	14.93
	2	0.6438	26.31	0.3562	14.56
	3	0.5686	23.04	0.4314	17.48
Average		0.6063	24.12	0.3937	15.66
4	1	0.6125	29.27	0.3875	18.52
	2	0.5677	31.31	0.4323	23.84
	3	0.5721	30.68	0.4279	22.95
Average		0.5841	30.42	0.4159	21.77

Table B-18a Peak areas of each fraction remained in powder chitosan. Each data point was averaged from triple spots on chromatorods.

Day	Triplicates	Peak area			
		Saturate	Aromatic	Total oil	Stearyl
0	1	7923	5536	13459	16510
	2	13918	5999	19917	17164
	3	15750	7243	22993	26235
Average		12530	6259	18790	19970
1	1	3657	2855	6512	3777
	2	4856	3316	8172	5033
	3	4634	2677	7311	4356
Average		4382	2949	7332	4388
2	1	9219	6427	15647	6596
	2	8628	5929	14557	6977
	3	8552	4996	13549	6530
Average		8800	5784	14584	6701
3	1	4116	2777	6893	2905
	2	4926	3145	8071	3023
	3	5206	2526	7733	3294
Average		4750	2816	7566	3074
4	1	10640	7189	17829	6876
	2	11062	7634	18696	6864
	3	11300	7576	18876	7021
Average		11000	7466	18467	6920

Table B-18b Amounts of total oil remained in powder chitosan.

Day	Triplicates	Ratio of peak area (total/stearyl)	Ratio of concentration	Amount of total oil (mg)
0	1	0.8152	0.5056	6.321
	2	1.1604	0.7197	8.997
	3	0.8764	0.5436	6.795
Average		0.9507	0.5897	7.371
1	1	1.7240	1.0694	13.37
	2	1.6239	1.0072	12.59
	3	1.6786	1.0412	13.01
Average		1.6755	1.0393	12.99
2	1	2.3721	1.4714	18.39
	2	2.0865	1.2942	16.18
	3	2.0748	1.2870	16.09
Average		2.1778	1.3508	16.89
3	1	2.3728	1.4718	18.40
	2	2.6697	1.6559	20.70
	3	2.3475	1.4561	18.20
Average		2.4633	1.5279	19.10
4	1	2.5931	1.6084	20.10
	2	2.7237	1.6894	21.12
	3	2.6885	1.6676	20.85
Average		2.6684	1.6551	20.69

Table B-19a Peak areas of each fraction remained in flake chitosan-immobilized cell. Each data point was averaged from triple spots on chromatorods.

Day	Triplicates	Peak area			
		Saturate	Aromatic	Total oil	Stearyl
0	1	763	302	1065	18413
	2	830	0	830	28780
	3	444	131	575	25701
Average		679	144	823	24298
1	1	437	336	773	21803
	2	706	0	706	13469
	3	303	188	491	30875
Average		482	175	657	22049
2	1	441	150	591	28210
	2	360	171	531	24539
	3	383	208	591	27133
Average		395	176	571	26627
3	1	667	265	932	21539
	2	684	197	881	15978
	3	382	254	636	21043
Average		578	239	816	19520
4	1	318	237	555	8188
	2	405	0	405	8446
	3	1151	498	1649	19425
Average		624	245	870	12019

Table B-19b Amounts of total oil remained in flake chitosan-immobilized cell.

Day	Triplicates	Ratio of peak area (total/stearyl)	Ratio of concentration	Amount of total oil (mg)
0	1	0.0579	0.0359	0.45
	2	0.0288	0.0179	0.22
	3	0.0224	0.0139	0.17
Average		0.0364	0.0226	0.28
1	1	0.0355	0.0220	0.28
	2	0.0524	0.0325	0.41
	3	0.0159	0.0099	0.12
Average		0.0346	0.0215	0.27
2	1	0.0209	0.0130	0.16
	2	0.0216	0.0134	0.17
	3	0.0218	0.0135	0.17
Average		0.0214	0.0133	0.17
3	1	0.0433	0.0268	0.34
	2	0.0551	0.0342	0.43
	3	0.0302	0.0187	0.23
Average		0.0429	0.0266	0.33
4	1	0.0678	0.0420	0.53
	2	0.0479	0.0297	0.37
	3	0.0849	0.0527	0.66
Average		0.0669	0.0415	0.52

5. Number of bacteria survival in chitosan and emulsion

Table B-20 Number of bacteria in media from the oil-in-water emulsion treatment by three chitosan types.

Day	Number of bacteria in media			AVE	S.D.
	1	2	3		
Powder chitosan					
0	2.00E+09	3.00E+09	2.00E+09	2.33E+09	5.77E+08
1	1.70E+09	1.90E+09	1.60E+09	1.73E+09	1.53E+08
3	5.00E+08	4.00E+08	4.00E+08	4.33E+08	5.77E+07
5	9.00E+07	8.00E+07	9.00E+07	8.67E+07	5.77E+06
Flake shrimp shell chitosan					
0	3.00E+08	2.00E+08	2.00E+08	2.33E+08	5.77E+07
1	6.00E+08	5.00E+08	3.00E+08	4.67E+08	1.53E+08
3	5.00E+08	6.00E+08	7.00E+08	6.00E+08	1.00E+08
5	8.00E+08	6.00E+08	6.00E+08	6.67E+08	1.15E+08
Flake squid pen chitosan					
0	2.00E+08	1.00E+08	2.00E+08	1.67E+08	5.77E+07
1	2.00E+08	5.00E+08	3.00E+08	3.33E+08	1.53E+08
3	6.00E+08	5.00E+08	8.00E+08	6.33E+08	1.53E+08
5	1.80E+09	1.40E+09	1.80E+09	1.67E+09	2.31E+08

Table B-21 Number of bacteria in emulsion from the oil-in-water emulsion treatment by three chitosan types.

Day	Number of bacteria in emulsion			AVE	S.D.
	1	2	3		
Powder chitosan					
0	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1	4.00E+05	3.00E+05	2.00E+05	3.00E+05	1.00E+05
3	4.00E+05	2.00E+05	2.00E+05	2.67E+05	1.15E+05
5	4.00E+05	3.00E+05	4.00E+05	3.67E+05	5.77E+04
Flake shrimp shell chitosan					
0	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1	5.00E+05	6.00E+05	8.00E+05	6.33E+05	1.53E+05
3	1.00E+06	1.30E+06	1.00E+06	1.10E+06	1.73E+05
5	7.00E+06	6.00E+06	6.00E+06	6.33E+06	5.77E+05

Table B-19 (cont.) Number of bacteria in emulsion from the oil-in-water emulsion treatment by three chitosan types.

Day	Number of bacteria in emulsion			AVE	S.D.
	1	2	3		
Flake squid pen chitosan					
0	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
1	1.00E+05	4.00E+05	1.00E+05	2.00E+05	1.73E+05
3	1.00E+07	9.00E+06	8.00E+06	9.00E+06	1.00E+06
5	1.20E+07	1.50E+07	1.30E+07	1.33E+07	1.53E+06

APPENCIX C

Full paper in the 7th National Environmental Conference (March, 12-14th 2008) on the topic of Treatment of Oily Wastewater by Chitosan Immobilized Bacteria. This full paper was presented as oral presentation by Miss Nichakorn Khondee.

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การบำบัดน้ำเสียปนเปื้อนน้ำมันโดยใช้แบคทีเรียตรึงบนไคโตซาน

Treatment of Oily Wastewater by Chitosan Immobilized Bacteria

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บทคัดย่อ

ในการศึกษานี้ไคโตซานตรึงแบคทีเรียได้ถูกพัฒนาขึ้นเพื่อทดสอบประสิทธิภาพในการบำบัดน้ำเสียที่ปนเปื้อนน้ำมัน ไคโตซานเป็นสารโพลิเมอร์ชีวภาพที่มีประจุบวก (cationic biopolymer) ซึ่งสามารถผลิตได้จากการตรึงหมู่อะซิทิล (acetyl group) ของไคตินออกไป สำหรับน้ำเสียปนเปื้อนน้ำมันที่ใช้ในการศึกษานี้เป็นน้ำเสียที่สังเคราะห์จากการผสมน้ำมันหล่อลื่นกับน้ำกลั่นและสารลดแรงตึงผิวชนิดไม่มีประจุ โดยในการศึกษาเบื้องต้นพบว่าไคโตซานสามารถดูดซับน้ำมันและน้ำในสารละลายบัฟเฟอร์พีเอช 7 ได้ถึง 0.5 กรัมน้ำมัน/กรัมไคโตซาน และ 10 กรัมน้ำ/กรัมไคโตซานตามลำดับ จากผลการทดลองนี้แสดงให้เห็นว่าไคโตซานมีความสามารถในการดูดซับน้ำมันเมื่อนำไคโตซานไปประยุกต์ใช้ในการบำบัดน้ำเสียปนเปื้อนน้ำมัน นอกจากนี้ในการทดลองหาสภาวะที่เหมาะสมในการบำบัดน้ำเสียปนเปื้อนน้ำมันโดยไคโตซาน พบว่าปริมาณไคโตซาน 1.0 กรัม/ลิตร และระยะเวลาผสม 60 นาทีเป็นสภาวะที่ไคโตซานสามารถกำจัดน้ำมันออกจากน้ำได้มากที่สุด โดยสามารถลดความขุ่นของน้ำเสียสังเคราะห์ได้ถึง 80% ของปริมาณความขุ่นเริ่มต้นและมีน้ำมันในน้ำเหลืออยู่เพียง 20% ของปริมาณน้ำมันเริ่มต้น จากการคัดแยกแบคทีเรียจาก 5 ดินตัวอย่างเพื่อศึกษาประสิทธิภาพในการย่อยสลายน้ำมันโดยจุลินทรีย์พบว่ามีแบคทีเรีย 2 ชนิดที่สามารถย่อยสลายน้ำมันได้มากที่สุด โดยแบคทีเรียทั้ง 2 ชนิดมีศักยภาพในการย่อยสลายสารกลุ่มอะโรมาติกได้มากกว่าสารกลุ่มอื่น หลังจากนั้นจึงทำการนำแบคทีเรียที่เลือกไว้ไปตรึงบนไคโตซานเพื่อใช้ในการบำบัดน้ำเสียปนเปื้อนน้ำมันและพบว่าแบคทีเรียตรึงบนไคโตซานมีศักยภาพในการบำบัดน้ำเสียปนเปื้อนน้ำมัน โดยน้ำมันที่ถูกดูดซับโดยไคโตซานจะถูกย่อยสลายโดยแบคทีเรียที่ตรึงบนไคโตซาน

คำสำคัญ : ไคโตซาน; การตรึง; น้ำเสียปนเปื้อนน้ำมัน; อิมัลชัน; การย่อยสลายทางชีวภาพ

Abstract

Removal of oily wastewater by a natural sorbent integrated with biodegradation technique was explored in this study. Chitosan is a cationic biopolymer produced by the extensive deacetylation of chitin obtained from shrimp shell wastes. To simulate wastewater from gas station, oil emulsion was prepared by mixing lubricating oil with distilled water and a nonionic emulsifier. The final concentration of oil was equivalent to 200 mg/L. From preliminary study, the maximum oil and water sorption capacity of chitosan in a pH 7.0 buffer solution were 0.5 g oil/g chitosan and 10 g water/g chitosan, respectively. These results suggested that chitosan has the possibility to sorp most oil while applying to the wastewater. The amount of chitosan and mixing time were varied to find the conditions for maximum oil removal efficiency. At the optimum treatment conditions (dosage: 1.0 g/l, contact and mixing time: 60 min), the turbidity of wastewater was reduced by 80% and the amount of remaining oil was only 20% of the initial. In the biodegradation experiment, oil-degrading bacteria were isolated from five soil samples. Two bacterial isolates provided the highest lubricating oil degradation efficiency and specifically degraded the aromatic fraction of lubricant oil. A selected strain was later immobilized on chitosan to treat the oily wastewater. Results showed that chitosan immobilized bacteria had the potential to remove oil in water. The inoculants formulated with chitosan as carrier materials improved the survival and the activity of the oil-degrading strains. In addition, the oil was sorped and concentrated on chitosan, thus facilitated its degradation by the immobilized bacteria.

Keywords : Chitosan; Immobilization; Oily wastewater; Emulsion; Biodegradation

Introduction

Over the past ten years, Thailand has an increase of petroleum industries and gas stations. This is especially from 1994 to 1997 when the numbers of gas stations increased from 9,000 to 14,000 stations and also produced a large volume of wastewater containing various pollutants [1]. These gas stations generate an average of 20 m^3 per day of wastewater [1]. US-EPA has raise awareness of the following parameters from vehicle and equipment wash waters: TSS, pH, salts, particulate matter, oil, grease, organics, COD, chlorinated solvents, detergents, lubricants, additives, heavy metals, antifreeze, and acid/alkaline wastes [2]. Thus, this enormous volume of wastewater and its harmful properties has become an extreme environmental concern in Thailand. Generally, wastewater from gas station is generated from various activities such as car washing, floor cleaning, toilet and cafeteria usage, lubricant changing, etc. Wastewater from car washing operations contains suspended solid, emulsifier and oily wastewater. Oily wastewater not only present in free oil form, but also in oil emulsion form caused by admixture of automotive oil such as lubricant oil with emulsifier and wash water [3].

The conventional treatment method such as American Petroleum Institute (API) gravitational oil water separation, Corrugated Plate Interceptor (CPI), or grease trap can remove only free oil and settled solid [1]. These techniques can not satisfactorily handle the other types of oily wastewater such as oil emulsion. Therefore, the car wash wastewater in oil emulsion form has become a crucial problem to the public sewer system. Emulsion oil can be removed by several methods such as heating, configuration, biological treatment, filtration, adsorption, and chemical treatment [3]. In recent year, biodegradation has been a focus of interest for the treatment of petroleum contaminated area. However, in the natural environment microbial degradation is slow essentially because of the lack of hydrocarbon-degrading microorganisms, of the toxicity of some

components, such as aromatic compounds, of the limited oil-water interface, the insufficiency of dissolved oxygen, the non-optimal temperature, and the lack of nutrients [4]. The use of oil sorbent materials, for example chitin, chitosan, bentonite, and activated carbon, can enhance oil contaminated water biodegradation [6].

Chitosan is a natural sorbent, which have been used in many applications, ranging from food and separation technology to wastewater treatment [5]. Many reports demonstrate that chitosan has excellent properties, such as biodegradability, biocompatibility, adsorption property, and flocculating ability, consequently it can be applied to various processes such as sorption, coagulation, and biodegradation [6]. This research used chitosan instead of conventional sorbents or coagulants to treat oily wastewater containing lubricating oil and an emulsifier. The application of chitosan for oil removal was previously studied but with residue palm oil mill effluent and sea water contaminated with crude oil [6, 7]. To improve the oil removal efficiency, chitosan was later used as matrix for bacterial immobilization. Immobilized cells can degrade toxic chemicals faster than conventional wastewater treatment systems since high densities of specialized microorganisms are used [8]. The use of bacterial immobilized chitosan to clean-up oily wastewater has never been done. Therefore, this research immobilized oil-degrading bacteria on chitosan and then investigated the potential and effectiveness of the immobilized bacteria on oily wastewater treatment.

Materials and Methods

Synthetic oil/water emulsion preparation

SKG Blender with 350 W, 13,000 rpm motor and maximum capacity of 1.5 L was used for preparing stock oil/water emulsion. One mL of Oil (PTT V-120) was injected into the blender and mixed with 800 mL distilled water and 100 mL 0.1% emulsifier (tween 80) for 1 min. The admixture was later stabilized by stirring for 10 min and diluted to 1 L with distilled water [1, 9]. This concentration of stock oil/water emulsion was equivalent to 994 mg/L with respect to the specific gravity of lubricant oil. This mixture was diluted to the required oil concentration (i.e. 100-500 mg/L) by adding distilled water. The oil/water emulsion should remain stable during the experimental run.

Oil sorption by chitosan

1) Effect of chitosan dosage

The synthetic oil/water emulsion was prepared and diluted to 200 mg/mL. 50 mL of 200 mg/mL emulsion was transferred into 125 mL flasks and the different dose of chitosan between 0.1 to 10 g/L was added to each flask. The sample was mixed for 30 min at 200 rpm, and sedimentation time of 60 min. Then, the residual oil in water was extracted and analyzed for the quantities of oil. Turbidity of oil/water emulsion was also analyzed.

2) Effect of mixing time

The effect of mixing time between 5 to 80 min on the adsorption of chitosan was analyzed using the optimized chitosan dosage from the previous study, mixing rate of 200 rpm, and sedimentation time of 60 min. Then, the residual oil in water was extracted and analyzed for the quantities of oil. Turbidity of oil/water emulsion was also analyzed

Oil degradation by isolated bacteria

1) Oil-degrading bacteria isolation

10 g of soil samples is mixed with 100 mL CFMM media containing 5 mg/mL lubricating oil and shaken at RT, 200 rpm for 5 days. Cultures were enriched by transferring into new CFMM media when the change in lubricating oil is observed. The process was repeated several times. Isolates were purified by spreading on CFMM agar that has lubricating oil spread on surface [10].

2) Inoculums preparation

Isolates were cultured in CFMM agar for 5 days. Then, colonies were brought to 300 mL CFMM broth containing 5 mg/mL lubricating oil for 5 days. Cells were centrifuged at 8,000 rpm, 4°C for 10 minute and washed using 0.85% sodium chloride solution. To remove the remaining oil, the cells was resuspended in sodium chloride solution and incubated for overnight at room temperature, 200 rpm. Cells were washed and adjusted the optical density (OD) to 1.0 at 600 nm.

3) Biodegradation test

30 mL cell suspensions from 2.5.1 were added to 125 mL flask containing lubricating oil with the final concentration of 10 mg/mL. The flask with only saline solution and oil was used as control and all flasks were done in triplicates. All flasks were shaken at 200 rpm, room temperature for 20 days. Then, the sample was analyzed for the amount of residual oil.

The strain that provide highest activity was selected to determine the oil degradability over time. In addition, number of bacteria was determined by total plate count to determine the relationship between oil removal and bacterial growth. Then, oil-degrading ability of the selected strain was examined with oil-in-water emulsion.

Oil-in-water emulsion treatment by chitosan immobilized bacteria

1) Production of immobilized cells

To immobilize the selected oil-degrading bacteria on chitosan, the bacteria was cultured together with chitosan in CFMM with lubricating oil as the sole source of carbon and energy. Oil-degrading bacteria cells suspension was prepared as in 2.5.1. Then, 1.25 g sterilized chitosan was incubated in 500 mL cell suspensions with 0.25% (v/v) of oil [11]. The suspension was shaken at 150 rpm and room temperature for 5 days. Subsequently, the immobilized cultures was washed with mineral salt (MS) medium and filtrated through sterilized filter paper for removing unattached cells. A filtrated culture material was air dried in sterile hood for 24 h.

2) Oil-in-water emulsion biodegradation test by immobilized cells

The dried immobilized cells were transferred to 125 mL sterile flask containing 50 mL 200 mg/L oil emulsion. The initial concentrations of immobilized cells was 1.0 g/L. The samples of chitosan immobilized with bacteria were taken from the flasks after finish treatment to analyze for the number of attached bacteria per dry weight chitosan. The amount of residual oil in water phase was analyzed.

At the end of experiment, three types of oil-in-water emulsion treatment were examined with different emulsion concentrations: (I) oil-in-water emulsion treatment by chitosan, (II) biodegradation, and (III) oil-in-water emulsion treatment by immobilized cells. Then, residue oil in water removal efficiency of each treatment was determined.

Analytical methods

Amount of residual in water was quantified by Thin Layer Chromatography with flame ionized detector (TLC-FID). NaCl was added to the water phase after treatment at the final concentration of 0.25 M [12] and mixed with chloroform at the ratio equal to 1:5. The chromarod development conditions are *n*-hexane for 10 cm (25 min), dichloromethane (DCM) for 6.5 cm (2 min) and 4 cm (5 min), and DCM/methanol (95/5, v/v) for 1 cm (1 min). Finally, the amounts of oil on the Chromarods were quantified using the FID of the Iatroscan with scan speed equal to 30s/scan (normal scan). Flow rate of hydrogen for the FID were 160 mL/min. Retention time of stearyl alcohol, saturates, aromatics, resin, and asphaltenes were approximately 0.35, 0.13, 0.24, 0.42, and 0.47 min, respectively.

Results and Discussion

Oil sorption by chitosan

1) Effect of chitosan dosage

In this study, the amount of chitosan was varied to find the lowest amount of chitosan that provide maximum oil removal efficiency. From preliminary test, the maximum oil and water sorptions of chitosan in pH 7.0 buffer solutions were 0.5 g oil/g chitosan and 10 g water/g chitosan, respectively. These results indicated that chitosan has the potential to sorp oil from water phase. About the effect of chitosan dosage, percent of oil removal was intensely increased from 66% to 80% when the concentration of chitosan was increased from 0.1 to 1.0 g/L (Figure 1). However, when the concentrations of chitosan were increased from 1.0 to 5.0 g/L, the percentage of oil removal was relatively stable. Therefore, the optimum concentration of chitosan is 1.0 g/L because it is the lowest concentration of chitosan that provides oil removal efficiency around 80%. During the study, turbidity of wastewater was reduced by 80%. Chitosan chain structure has positively charged amine (NH_2) functional groups which are very attracted to anionic ions [13]. Therefore, it could easily bind and bridge with negatively charged material into a floc. The overall charge of chitosan is positive; whereas, the majority charge of oil/water emulsion is negatively charged [1]. Thus, attractions between the charges enhance the agglomeration process and this mechanism is called as charge neutralization [6]. Consequently, chitosan not only acts as an adsorbent but at the same time as a coagulant.

2) Effect of mixing time

The effect of mixing time for oil/water emulsion removal by chitosan was analyzed using 1 g/L of chitosan. The results were demonstrated in Figure 1b and it was found that chitosan required 60 min to adsorb maximum amount of oil in aqueous phase. It was noticed that as the time was prolong from 10 to 80 min, the removal was increasing. This is because the breakage of the oil droplets is enhanced by emulsification process. Thus, the reduction of oil droplets into emulsion form causes more interfacial area for adsorption to happen [14].

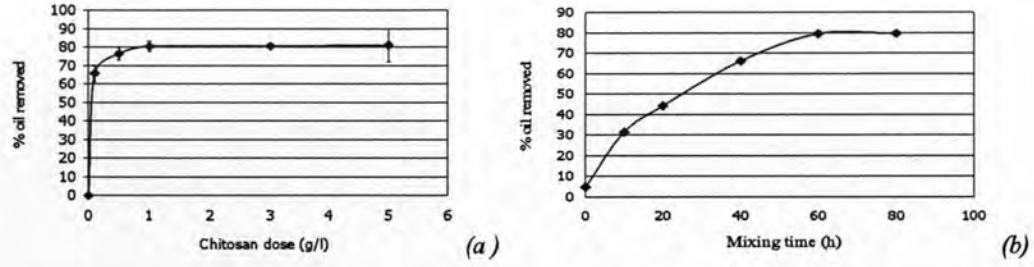

Figure 1 Percentage of oil removed from water vs. dosage of chitosan (a) and mixing time of chitosan (b)

Oil degradation by the isolated bacteria

Bacteria used in this study were isolated from soil sample collected from Chanthaburi province. Then, five strains of bacteria, which have different colony morphology, were selected to determine the oil degradation efficiency. Lubricants was added to CFMM media at 5 mg/ml and incubated for 20 days. Figure 2a show that bacteria strain Ch2 and Ch4 achieve the highest oil degradation efficiency. For Ch2, percent degradation of total oil, saturates, and aromatics were 33%, 22%, and 54%, respectively. For Ch4, percent degradation of total oil, saturates, and aromatics were 34%, 25%, and 51%, respectively. These results demonstrated that Ch2 and Ch4 could degrade aromatics fraction more efficiently than saturates fraction in the lubricants.

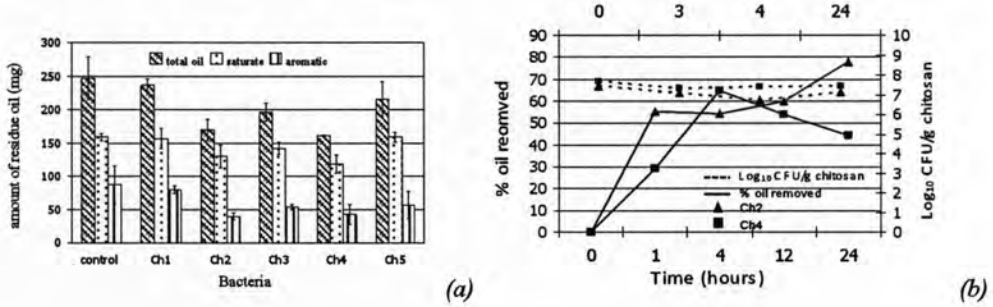

Figure 2 Amount of oil after 20 days degradation by five isolate bacteria (a) and percentage of oil removed from the oil/water emulsion by Ch2 and Ch4 bacteria (b)

Bacteria Ch2 and Ch4 were later examined with oil/water emulsion to determine the effects of emulsifier on oil degradation. In 24 hours, bacteria Ch2 could degrade 80% of initial oil concentration; whereas, bacteria Ch4 could degrade only 50% of initial oil/water emulsion concentration (Figure 2b). The amounts of both bacteria were constant throughout the study. Thus, the present of emulsifier did not affect the bacteria as well as their oil-degrading activity. Bacteria Ch2 was selected for further experiment.

Oil-in-water emulsion treatment by chitosan immobilized bacteria

Chitosan immobilized bacteria at 1.0 g/L were used in the treatment of five concentrations of oil/water emulsion i.e. 100, 200, 300, 400, and 500 ppm. It was found that percent of oil/water emulsion removal by chitosan immobilized bacteria was decreased when the concentration of oil/water emulsion was increased (Figure 3). At the beginning, the amounts of bacteria in chitosan were 1.9×10^{9} CFU/g chitosan, however the number was decreased to 4.0×10^{8} CFU/g chitosan. It is well known that the surface-attached population bacteria were protected from environmental stress [6] thus the number of bacteria in chitosan was not extremely decreased after used to treat oil/water emulsion. Meanwhile, the application of chitosan immobilized bacteria should be optimized to increase its activity and survival when apply to wastewater with high concentrations of oil. For example, longer incubation time and higher amounts of chitosan immobilized bacteria are suggested.

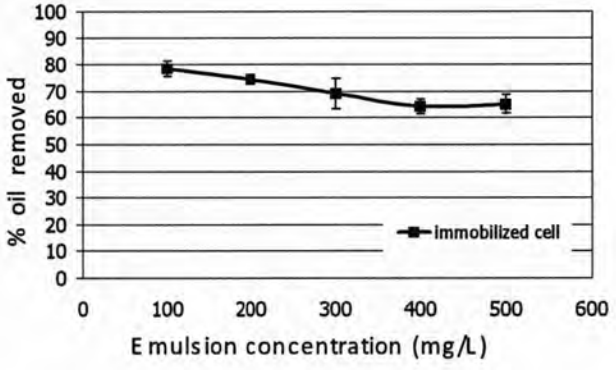

Figure 3 Percentage of oil in water emulsion removed from water by chitosan immobilized bacteria

Conclusions

In this research, chitosan was used to remove oil from oil/water emulsion. The optimum conditions were 1.0 g/L chitosan and 60 min mixing time. However, the use of chitosan to sorp oil/water emulsion will become a crucial problem because the oil sorped chitosan is considered as hazardous waste. Biodegradation of oily wastewater was therefore added to the sorption process. Here, the chitosan immobilized bacteria was used to sorp and simultaneously degrade oil from oil/water emulsion wastewater. The success of oil removal may also due to the detached bacteria from chitosan, which might degrade oil in the aqueous phase. The developed technique may be applied to treat wastewater from gas station.

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ทำเนียบวิทยากร

ชื่อบทความ	การบำบัดน้ำเสียปนเปื้อนน้ำมันโดยใช้แบคทีเรียตรึงบนไคโตซาน Treatment of Oily Wastewater by Chitosan Immobilized Bacteria
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