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Crude Oil Biodegradation In Arctic Tundra Ponds

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ABSTRACT. The degradation of Prudhoe crude oil was studied in arctic tundra ponds. Contained subponds were treated with oil and/or oleophilic phosphate or inorganic phosphate fertilizers in an attempt to enhance the degradation of the oil by the indigenous microflora. Enumeration studies of water and sediment samples indicated that oil treatment alone did not increase numbers of total heterotrophic or oil-degrading bacteria over a short period (28 days). It was also shown that oil spilled years previously on 2 whole ponds at a high (10 $1/m^2$) and a low dose (0.24 $1/m^2$) did not alter the microflora quantitatively, except in a small core spilled with oil. Although oil alone seemed to exhibit neither stimulatory nor toxic effects, oleophilic phosphate, added weekly at a concentration of 0.1 mM, significantly stimulated the microflora in the presence or absence of oil. Since equal concentrations of inorganic phosphate failed to induce this effect, the stimulation was attributed to the hydrocarbon portion of the organic phosphate molecule. ^{14}C-hydrocarbon mineralization studies demonstrated that the microflora would mineralize the saturate fraction of the oil before the polyaromatic fraction. It was concluded that oleophilic fertilizers may provide a useful tool to enhance the biodegradation of crude oil spilled on oligotrohpic waters.

RÉSUMÉ. La dégredation du "brut" de Prudhoe Bay a été étudiée dans des étangs de toundra arctique. On a traité des bassins fermées avec du pétrole et/ou avec du phosphate oléophilique ou avec des engrais de phosphate inorganique, dans l'espoir d'accélérer la dégradation du pétrole par la microflore indigéne. Les techniques énumerées montraient que le phosphate oléophilique (avec un rapport pétrole/engrais de 20 pour 1*), stimulait d'une manière significative la microflore, soit en présence ou en l'absence de pétrole. Ajouté une fois par semaine avec une concentration du 0.1 milligramme, le phosphate inorganique ne stimulait pas les microbes dégradant le pétrole. Les études de minéralisation d'hydrocarbure en C_{14} (glucose) démontraient que la microflore minéraliserait la fraction saturée du pétrole avant la fracture polyaromatique. Les études énumerées démontraient que le pétrole répandu antérieurement sur deux étangs entiers, aux taux de 10 litres/m^2 et á faible dose (0.24 litres/m^2), n'alterait pas la microflore. Cependant, du pétrole répandu dans un petit bac, deux ans auparavent, avait stimulé, d'une manière significative, la microflore indigène pour dégrader le "brut." On en concluait que les engrais oléophiliques pouvaient fournir un outil utile pour accélérer la bio-dégradation du brut répandu sur des eaux oligotrophiques.

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INTRODUCTION

The completion of the Trans Alaskan Pipeline has caused environmental concern about the effects of a possible oil spill on the fragile coastal tundra. Should a spill occur, microbial biodegradation of the hydrocarbon fractions would enhance the removal of oil from the environment, in addition to weathering. A discussion of the microbial response to Prudhoe Bay crude oil on various coastal tundra soils has been published (Sexstone and Atlas, 1977; Sexstone, *et al.*, 1978a, 1978b), but research reports on the effects of crude

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oils on oligotrophic tundra thaw ponds have been few. (Atlas and Busdosh, 1976; Federle *et al.*, 1978; Miller *et al.*, 1978).

Federle, *et al.* (1978) showed that in contained oil spills, primary production was drastically reduced within 5 days. Although recovery reached near normal levels by 15 days, the composition of the phytoplankton had changed due to the toxic oil fractions.

Although studies have been conducted on the effects of Alaskan crude oils on marine microbial populations (Atlas, *et al.*, 1976; Atlas and Schofield, 1975) only one other study has concerned itself with the effects of crude oil on the microbiota of freshwater tundra ponds (Atlas and Busdosh, 1976). These authors showed that oleophilic nitrogen and phosphorus containing fertilizers increased the *in situ* degradation of Prudhoe crude oil about 10% over weathering levels after 21 days. Addition of inorganic nitrogen and phosphorus (as NO_3 and PO_4) did not enhance the degradation of crude oil even though previous work (Barsdate and Prentki, 1972) had shown that phosphorus was limiting in tundra ponds.

This investigation further considers the effects of inorganic and oleophilic phosphate fertilizers on the enhancement of the indigenous microflora to degrade crude oil within 28 days during the ice free season. Degradation of oil fractions was monitored by enumeration of hydrocarbon-degrading bacteria and by ^{14}C-hydrocarbon mineralization techniques. A study of the microflora of two ponds previously spilled with high and low doses of oil would give an idea of whether oil fractions were toxic to the microflora over a period of 2-7 years.

MATERIALS AND METHODS

Pond sites and subpond treatments. All studies were conducted during the summer of 1977 on Ponds C, E, and Omega (Ω). The ponds were located on the International Biological Program (IBP) Site, near the Naval Arctic Research Laboratory (NARL) at Barrow, Alaska. The limnology of these ponds has been reviewed (Hobbie, 1973).

In order to determine the interactions of the sediment microflora with that of the water column, 3 types of samples were taken: 1) pond water from the water column alone (PW); 2) pondwater in contact with the sediment (SW); and 3) sediment samples (SS). The first type, PW, was taken from large (20 l) plastic garbage cans (subponds) which were filled with 18 l of Pond C water (never before treated with oil contaminants), and placed in a similar pond for incubation. The second type of water sample, SW, was taken from unbottomed subponds which were placed through the water column (approximately 18 l) to the permafrost of Pond C. The subponds were acclimated for 2 days before treatment. Water samples were collected in sterile Whirl Paks.

Sediments samples, SS, were taken using a 7 cm I.D. plexiglass core. The top was stoppered until the core was 1-2 cm into the sediment. As the core was twisted into the sediment, air was bled from the top of the core. When the core reached the permafrost, the top was stoppered and the core slowly

removed. Another stopper was placed in the bottom of the core while it was still in the water column. This whole technique allowed for minimal disturbance of the top of the sediment samples, which did not contain appreciable amounts of surface water. The cores were removed to the lab and stored at 10 °C until used, usually within 2-4 hours. The top one centimetre was removed from the sediment column, and divided into four 10 g (wet weight) portions. One portion was placed in a Waring blender with 100 ml of sterile salts medium and agitated for 30 seconds. The clear medium, after setting of the particulates for 30 min, was used for the enumeration and mineralization experiments. The other three portions were dried at 60 °C for 48 hr and used to determine an average dry weight.

The experiment was started on 21 June 1977 about one week after spring thaw and runoff. The experimental design of the subpond treatments is outlined as follows:

Subpond	Treatment
1	Control (no treatment)
2	" + Prudhoe Bay crude oil (1 ml/1)
3	" + Prudhoe Bay crude oil (2 ml/1)
4	" + Oil 0.1mM Na_2HPO_4
5	" + Oleophilic phosphate (0.1 mM)
6	" + Crude oil (1 ml/1) + 0.1 mM Na_2HPO_4
7	" + Crude oil (1 ml/1) + oleophilic phosphate

The fertilizers were added weekly throughout the study period of 28 days. Differences among the subponds were determined by a one-way analysis of variance ($P \leq 0.05$) and Scheff's multiple range test ($P \leq 0.05$; Statistical Package for the Social Sciences, 1970). Dissolved oxygen determinations were made using a YSI Oxygen Meter (Model 51,Yellow Springs Instrument Co., Yellow Springs, OH).

Ponds previously treated with oil. In 1970, Prudhoe Bay crude oil was spilled on Pond E (10 1/m²). In 1975, Prudhoe crude oil was spilled on Pond Ω (0.24 1/m²). Plexiglass cores (7 cm I.D.) were also placed into the water column and sediment of Pond Ω and dosed with 1 ml of crude oil. Water and sediment samples, taken twice during the summer, were designated as follows:

Designation	Sample
Ω W	Pond Ω, water from an unoiled area
Ω O	Pond Ω, water from an oiled area
Ω OC	Pond Ω, water from the oiled core
C W	Pond C, untreated (control) water
E W	Pond E, water from an unoiled area
E O	Pond E, water from an oiled area

The same code was also used to indicate sediment samples.

Enumeration of microorganisms. Water and sediment samples were plated in triplicate on the following media: 1) Salts agar (SA) was prepared with the L-salt of Leadbetter and Foster (1958) plus purified or noble agar (1.5%; Difco); 2) Oil agar (OA) was made with the salts media plus 0.5% Prudhoe crude oil by the method of Atlas and Bartha (1973); 3) Nutrient agar (NA) was half-strength nutrient broth solidified with 1.5% Bacto-Agar (Difco); 4) Hexadecane plates were salts agar with 0.1 ml of hexadecane (99+% pure, Matheson, Coleman, and Bell) added to the inverted lid of the plate. Samples were incubated for 14 days at 10 °C.

Mineralization of labeled substrates. The following method was adapted from Jordan and Hobbie (1978). Duplicate 5 ml samples were added to 20 ml glass scintillation vials, with a sterile water control for each duplicate set of samples. Any label collected from these controls was subtracted from the average of the experimental samples. One μl of n- (1-^{14}C)-hexadecane (s.a. 54.4 mCi/mmol; 100 μCi/ml) or (1-^{14}C)-naphthalene (3.67 mCi/mmol; 100 μCi/ml) were added to each vial. After the vials were stoppered and incubated for 3 days at 10 °C, 0.2 ml of 4N KOH was added. Following a further 2 hour incubation, rubber stoppers containing a plastic cup with flutted filter paper (2 cm^2) were placed on the scintillation vials. One-tenth ml of 4 N KOH was injected into the filter paper. Dissolved $^{14}CO_2$ was liberated with the addition of 0.5 ml of 2 N H_2SO_4 in the aqueous solution. After 2 hours, the papers were placed in 7 ml scintillation vials and dried over night. Scintillation fluid (0.01% POPOP and 0.4% PPO in toluene) was added and samples counted. Correction for quenching was made by the channels ratio method.

Turnover of ^{14}C-glucose. The turnover of ^{14}C-glucose was determined by the method of Wright and Hobbie (1966) as modified by Hobbie and Crawford (1969). The ^{14}C-glucose (164 mCi/mmol; 100 μCi/ml) was added (1, 2, 4 and 8 μl) to duplicate 5 ml water samples. (One μl contained 0.058 μg of glucose). All samples were incubated for 24 hours at 10 °C. Linear regressions between turnover time and substrate concentration of each sample were significant at $P \leq 0.05$ (SPSS, 1970).

Chemicals. The Prudhoe Bay crude oil was provided by the Atlantic Richfield Company. The oleophilic phosphate was "Victawet 12", donated by the Stauffer Chemical Company (Cleveland, OH). It is 2-ethylhexyl-dipolyethylene oxide phosphate. The polyethylene oxide moiety has 3-4 ethylene oxides.

RESULTS

The effects of fertilizers on crude oil biodegradation. The ability of the indigenous microflora of tundra ponds to degrade fractions of Prudhoe Bay crude oil was studied by 2 different methods: 1) enumeration of hydrocarbon-degrading bacteria; and 2) mineralization of selected hydrocarbon substrates. The ability of inorganic phosphate and oleophilic phosphate to stimulate the indigenous microflora to degrade crude oil was also tested. The experimental design allowed for internal comparisons of fertilized and unfertilized subponds. The degradation potential of the water column alone was compared to that of the water column and sediment.

Enumerations of microorganisms were done on half-strength nutrient broth agar (for heterotrophs), a salts agar (for low nutrient bacteria) and oil agar (for oil degrading bacteria). There were no appreciable differences between the numbers of microorganisms/ml between the two water types (PW and SW) grown on each medium (Tables 1 and 2). The NA plates exhibited the highest

TABLE 1. Enumeration of bacteria in the water column from bottomed subponds (PW) on various media, initially and 28 days after oil treatments

	Nutrient Agar (microbes/ml)	Oil Agar (microbes/ml)	Salts Agar (microbes/ml)
Initial Population (all subponds)	1.8×10^4	8.0×10^2	4.0×10^2
Day 28 population Subpond			
1	1.2×10^4a*	1.0×10^2a	5.7×10^3a
2	4.0×10^4a	3.7×10^3a	5.8×10^4a
3	6.6×10^4a	1.1×10^3a	9.2×10^3a
4	4.2×10^5a	8.7×10^2a	5.7×10^4a
5	9.2×10^6c	1.5×10^5b	1.2×10^6b
6	6.0×10^4a	1.4×10^3a	5.1×10^4a
7	4.6×10^6b	1.6×10^5b	7.4×10^6b

*means with identical letters (a, b, or c) are not significantly different at the 0.05 level using Scheffe's Multiple Range Test.

TABLE 2. Enumeration of bacteria in the water column from unbottomed subponds (SW) on various media, initially and 28 days after oil treatments

	Nutrient Agar (microbes/ml)	Oil Agar (microbes/ml)	Salts Agar (microbes/ml)
Initial Population (all subponds)	1.7×10^4	1.0×10^3	6.7×10^2
Day 28 population Subpond			
1	4.4×10^4a*	4.2×10^3a	3.8×10^4a
2	1.3×10^5a	3.5×10^3a	1.5×10^4a
3	3.8×10^4a	1.6×10^3a	1.5×10^5a
4	1.2×10^5a	1.3×10^4a	1.1×10^5a
5	1.5×10^6b	1.4×10^5b	1.3×10^6c
6	6.6×10^4a	2.5×10^3a	2.2×10^4a
7	3.9×10^6c	3.9×10^5c	4.2×10^5b

*means with identical letters (a, b, or c) are not significantly different at the 0.05 level using Scheffe's Multiple Range Test.

TABLE 3. Enumerations of bacteria in the top 1 cm of sediment from unbottomed subponds (SS) on various media, initially and 28 days after oil treatments

	Nutrient Agar (microbes/g dws)	Oil Agar (microbes/g dws)	Salts Agar (microbes/g dws)
Initial Population (all subponds)	2.2×10^8	4.0×10^7	1.7×10^7
Day 28 population Subpond			
1	4.3×10^8a*	8.6×10^6a	1.1×10^8a
2	3.6×10^8a	3.0×10^7a	1.6×10^8a
3	1.4×10^9a	5.2×10^7a	4.4×10^8b
4	1.3×10^8a	4.8×10^6a	1.8×10^7a
5	5.1×10^9b	7.5×10^8b	1.2×10^9c
6	4.9×10^8a	9.3×10^7a	1.3×10^8a
7	7.2×10^9b	1.2×10^9b	5.2×10^8b

*means with identical letters (a, b, or c) are not significantly different at the 0.05 level using Scheffe's Multiple Range Test.

TABLE 4. Ratios of oil degraders to heterotrophs and low nutrient bacteria in the subpond treatments at 28 days after oil treatment

Oil Agar/Nutrient Agar

Subpond	PW	SW	SS
1	.009	.10	.02
2	.09	.03	.08
3	.02	.04	.04
4	.002	.11	.04
5	.02	.09	.15
6	.02	.04	.19
7	.04	.10	.17

Oil Agar/Salts Agar

Subpond	PW	SW	SS
1	.02	.11	.08
2	.11	.23	.19
3	.01	.01	.12
4	.02	.11	.27
5	.13	.11	.63
6	.03	.11	.72
7	.02	.93	2.30

counts, whereas the SA were greater than the OA counts. The sediment enumerations were also consistent with these data (Table 3). The oleophilic fertilizer significantly stimulated the indigenous microflora in the presence or absence of oil (treatments 5 and 7) in the two water types (PW and SW) and the sediment (SS).

Table 4 lists the ratio of oil degraders to heterotrophs and that of oil degraders to low nutrient bacteria. These ratios were highest for subponds receiving oleophilic phosphate, inorganic phosphate and oil, and oleophilic phosphate and oil (treatment 5, 6 and 7) within the sediment samples (SS).

In order to determine the per cent of oil degrading microorganisms, isolates from oil agar plates were inoculated into salts medium plus oil. From over 100 randomly chosen colonies of various colonial morphologies, it was determined by visible turbidity in the tubes of media that 65% of the isolates could grow on the soluble fractions of crude oil at 10 °C within 14 days.

The ability of the indigenous microflora of the subponds to mineralize hexadecane or naphthalene was studied in samples 10 and 24 days after the oil treatments. In Table 5, it is seen that the rates of mineralization of hexadecane are equal to or faster on day 10 than day 24. Regardless of what treatment was applied, neither oil and/or fertilizers had an enhancing affect on the mineralization potential for hexadecane of the indigenous bacteria.

The rate of naphthalene mineralization was highest on day 24. This was especially true for the sediment samples (Table 6). By day 24, the greatest increase in PW subponds was in those treated with oil and inorganic phosphate. In SW subponds, the 2X oil treatment (subpond 3) and the 2 treatments with fertilizers and oil (subponds 6 and 7) had a greatly enhanced ability to degrade naphthalene. All of the sediment treatments showed a greatly enhanced ability to degrade naphthalene by the 24th day.

Glucose metabolism was measured as an indication of microbial heterotrophic activity (Table 7). There were no differences between the PW and SW turnover values of subpond treatments. Although, turnover times of glucose in the sediment appeared much longer than in either of the water types, they cannot be compared since the numbers of organisms was a function of weight of sediment and not volume of water.

Dissolved oxygen ranged from 9.5 to 12.6 mg/l in the subponds. The temperature in oiled subponds, even directly under the oil scum, never differed more than ± 1 °C compared to the control (unoiled) subponds.

Effects on tundra ponds after previous oil perturbation. Water and sediment samples were taken from Pond C (untreated), oiled and unoiled portions of Pond E (contaminated in 1970), Pond Ω (contaminated in 1975), and core tubes placed in Pond Ω which had been dosed with oil. Water and sediment samples for each treatment were enumerated on four types of media (Table 8). There were no significant differences among sample enumerations, except for the cores from Pond Ω. Unfortunately, no unoiled core samples were available to compare with oiled cores.

TABLE 5. Rates of mineralization of ^{14}-C-hexadecane ($^{14}CO_2$ produced) 10 and 24 days after oil treatments on the subponds[2] and ratios of day 10 rates to day 24 rates

	PW			SW			SS		
Subpond	Day 10	Day 24	Ratio (10/24)	Day 10	Day 24	Ratio (10/24)	Day 10	Day 24	Ratio (10/24)
1	156*	95	1.6	192	196	0.98	478	389	1.2
2	256	185	1.4	397	292	1.4	530	350	1.5
3	284	134	2.1	294	176	1.6	515	207	2.5
4	239	129	1.8	201	118	1.7	450	380	1.2
5	102	66	1.5	108	59	1.8	129	77	1.7
6	227	149	1.5	266	84	2.7	600	210	2.9
7	99	40	2.5	188	95	2.0	419	295	1.4

*Data expressed as dpm/day

TABLE 6. Rates of mineralization ($^{14}CO_2$ produced) of ^{14}C-naphthalene, 10 and 24 days after oil treatments on the subponds, and ratios of day 10 rates to day 24 rates

	PW			SW			SS		
Subpond	Day 10	Day 24	Ratio (10/24)	Day 10	Day 24	Ratio (10/24)	Day 10	Day 24	Ratio (10/24)
1	31*	18	1.7	33	9	3.7	42	1444	0.03
2	30	25	1.2	41	27	1.5	32	1110	0.03
3	33	71	0.46	37	359	0.10	33	4362	0.008
4	24	20	1.2	57	13	4.4	33	532	0.06
5	39	116	0.34	35	29	1.2	60	3196	0.02
6	38	1392	0.03	325	7800	0.04	158	9587	0.02
7	34	116	0.29	46	51290	0.0009	38	1088	0.03

*Data expressed as dpm/day

TABLE 7. Kinetic parameters for the metabolism of ^{14}C-glucose by the indigenous microflora of the subponds, 10 and 24 days after oil treatments

	PW		SW		SS	
Subponds (10 days)	Vmax (ug/1.h)	Turnover (h)	Vmax (ug/1.h)	Turnover (h)	Vmax (ug/1.h)	Turnover (h)
1	6.94	44.7	2.06	93.8	0.37	202.4
2	1.34	59.4	2.97	115.3	0.88	222.6
3	1.67	44.7	0.90	69.7	0.50	278.3
4	9.01	40.6	4.25	116.2	0.19	390.9
5	1.22	121.0	3.11	117.5	1.59	120.2
6	3.50	114.6	1.10	119.3	0.61	142.8
7	1.17	99.3	2.33	108.6	0.86	154.4

TABLE 7 (Cont'd)

24 days					
1	3.47	70.4	0.95	62.8	ND
2	3.37	65.9	2.09	118.3	ND
3	3.78	64.0	6.02	76.0	ND
4	9.17	76.6	6.45	67.6	ND
5	3.82	71.2	4.35	58.5	ND
6	2.73	68.0	0.95	50.7	ND
7	2.83	105.5	0.99	50.2	ND

ND = not done

TABLE 8. Recovery of Ponds Omega and E after oil spills in July 1975 and 1970, respectively, compared to Pond C (no previous oil treatment) as indicated by enumerations of bacteria on various media. The data are the averages of two samplings taken on June 25 and July 13, 1977

	Medium Type			
Sample type (water)	Nutrient Agar #/ml	Oil Agar #/ml	Hexadecane vapors #/ml	Salts agar #/ml
Ω W	5.8×10^3a*	5.0×10^2a	2.6×10^3a	2.2×10^3a
Ω O	5.3×10^3a	1.3×10^3a	9.5×10^3a	1.5×10^3a
Ω OC	2.3×10^5b	3.3×10^4b	1.5×10^5b	1.1×10^5b
C W	5.1×10^3a	7.8×10^2a	1.3×10^3a	3.0×10^3a
E W	2.6×10^3a	1.6×10^3a	1.5×10^3a	8.3×10^2a
E O	4.1×10^3a	1.8×10^3a	7.1×10^3a	1.6×10^3a
(Sediment)	#/g (dry wt)	#/g (dry wt)	#/g (dry wt)	#/g (dry wt)
Ω W	5.7×10^7a	3.2×10^7a	1.8×10^8a	1.9×10^8b
Ω O	2.4×10^8a	1.4×10^8a	2.5×10^8a	1.8×10^8b
Ω OC	3.8×10^9b	2.2×10^9b	3.1×10^9b	4.7×10^8c
C W	5.1×10^7a	6.5×10^6a	2.5×10^7a	4.2×10^7a
E W	1.2×10^8a	3.4×10^7a	6.2×10^7a	1.6×10^7a
E O	2.2×10^8a	6.4×10^7a	4.8×10^7a	4.7×10^7a

*means with identical letters (a, b, or c) for each media are not significantly different at the 0.05 level using Scheffe's Multiple Range Test.

DISCUSSION

Previous work (Federle *et al.*, 1978) demonstrated that in contained subponds, Prudhoe Bay crude oil spilled at a dose of 1 ml/l caused serious stress on the biota of tundra pond water. Within 5 days, primary production was drastically reduced and indigenous zooplankton were killed by the toxic effects of the soluble fraction of the oil.

If oil were to contaminate tundra ponds, its biodegradation could be enhanced by stimulation of the indigenous oil degrading bacteria. This study shows that oil perturbation did not significantly alter the water or sediment microflora during the 28 day subpond experiment. Recovery of the microflora to control levels was seen within 2 years after whole pond spills of a similar dose (0.24 l/m^2) and at a higher dose (10 l/m^2) within 7 years. Thus, this indicates that oil itself did not significantly reduce or stimulate total heterotrophic or oil degrading bacterial populations in the short term (28 days) or the long term (2-7 years), except for the 2 year core samples. It can be concluded that a determination of the change in microflora was not an adequate indicator of oil perturbation effects. The bacteria were not sensitive to the toxic fractions of the oil at the doses tested in the subponds. It should be noted that for short term toxicity determinations, primary production, phytoplankton composition or zooplankton mortality would be better indicators of oil effects in tundra ponds (Federle *et al.*, 1978; Miller *et al.*, 1978; O'Brien 1978).

The 2 year old cores provided the only instances of oil stimulation of pond microflora. Although there were no unoiled cores to sample for container effects, the 1-2 log increase in oil and hexadecane degraders indicates stimulation of hydrocarbon utilizers by the crude oil contained in the small cores. It might also be postulated that the increase of cells was due to the fact that the oil was partially protected from abiotic factors, such as wind and mixing, and thus unable to disperse throughout the pond over the 2 year period.

Although oil itself did not normally induce stimulation of the indigenous microflora, and oleophilic phosphate fertilizer did significantly stimulate both heterotrophic and oil degrading bacteria (Tables 1, 2, and 3). Because an equal concentration of inorganic phosphate did not increase the microflora either in the presence or absence of oil, it was thought that the stimulation (with or without oil) by the oleophilic fertilizer was due to enrichment by the soluble hydrocarbon portion of the fertilizer.

Horowitz and Atlas (1977) proposed that a ratio of hydrocarbon utilizers to total heterotrophs would be a useful index of hydrocarbon contamination. Table 4 shows that the SW and SS ratios were generally higher than the PW ratios. This indicates that there was an important sediment-water interaction relating to the stimulation of oil degraders. These ratios were highest in 5SS, 6SS and 7SS compared to the other SS treatments, demonstrating that the sediment was quite active in degrading oil in the presence of oleophilic fertilizer and fertilizers with oil. In the bottomed subponds, (PW), the large source of potential hydrocarbon degraders in the sediment was blocked off, thus explaining why the PW ratios were less than the SW ratios.

The ability of the indigenous microflora to mineralize oil fractions (Tables 5 and 6) indicated that the saturate fraction was mineralized faster just after oil treatment (10 days) compared to later (24 days). Conversely, the polyaromatic fraction required a longer period in which to induce naphthalene degraders. The degradation was higher on day 24 compared to day 10, especially in the

sediments. This finding agrees with previous suggestions from *in situ* losses due to degradation (Atlas, 1975; Atlas and Busdosh, 1976; Bailey *et al.*, 1973; Miller *et al.*, 1978; Westlake *et al.*, 1974).

In the unbottomed subponds, the water and sediment had much higher rates of naphthalene mineralization in the 2X oil treatment (3) and the two treatments containing oil and fertilizer (6 and 7). The fertilizers or increased doses of oil stimulated and indigenous microflora to degrade the polyaromatic fraction of the oil.

The subpond treatments did not appreciably affect the turnover of glucose by the indigenous bacteria in the water column or sediments. It is possible that turnover was not always fastest in subponds with the greatest populations because each treatment caused a different environmental change, such as a stimulation of primary production (Federle *et al.*, 1978). This may have caused a unique selection of bacteria in that subpond. Thus, in some cases, there may have been organisms which favored other substrates besides glucose or there may have been more natural substrate in the pond water.

Excessive oil spillage could cause limited oxygen supply due to the inability of oxygen to exchange with the atmosphere. Temperature enhancement due to absorption of infrared light by the oil, could also result. Neither affect was apparent in these experiments.

It was concluded from this study that oil alone did not have a stimulatory or toxic affect on total numbers of heterotrophic or oil degrading bacteria within 28 days or 2-7 years. However, oil biodegradation was stimulated by the presence of an oleophilic phosphate, due to the hydrocarbon moiety of the fertilizer. In addition to increasing the indigenous microflora, the fertilizer was an excellent detergent and dispersed the oil scum. More work is suggested on the effects of this fertilizer on other portions of the biota of arctic tundra ponds to determine whether it would be a safe means of enhancing recovery from crude oil spills.

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