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Environmental Factors Influencing the Rate of Hydrocarbon Oxidation in Temperate Lakes

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Rates of hydrocarbon biodegradation were estimated by following oxygen uptake during mineral oil oxidation or oxidation of [1-^{14}C]hexadecane to $^{14}CO_2$, when these substrates were added to natural water samples from Wisconsin lakes. A lag phase preceded hydrocarbon oxidation, the length of which depended on population density or on factors influencing growth rate and on the presence of nonhydrocarbon organic compounds. Hydrocarbon oxidation was coincident with growth and presumably represented the development of indigenous hydrocarbon-degrading microorganisms in response to hydrocarbon additions. In detailed studies in Lake Mendota, it was found that, despite the continued presence of hydrocarbon-degrading microorganisms in water samples, seasonal variations in the rates of mineral oil and hexadecane oxidation occurred which correlated with seasonal changes in temperature and dissolved inorganic nitrogen and phosphorus. The temperature optimum for oil biodegradation remained at 20 to 25 C throughout the year, so that temperature was the main limiting factor during winter, spring, and fall. During summer, when temperatures were optimal, nutrient deficiencies limited oil biodegradation, and higher rates could be obtained by addition of nitrogen and phosphorus. The rates of hydrocarbon biodegradation were thus high only for about 1 month of the ice-free period, when temperature and nutrient supply were optimal. Nutrient limitation of oil biodegradation was also demonstrated in 25 nutrient-poor lakes of northern Wisconsin, although in almost every case oil-degrading bacteria were detected. Knowledge of temperature and nutrient limitations thus will help in predicting the fate of hydrocarbon pollutants in freshwater.

Massive oil pollution of the sea has resulted in extensive research on hydrocarbon biodegradation, which has recently been reviewed (4, 10, 27). Recent studies (1, 2, 11, 12) have demonstrated that, despite the abundance of hydrocarbon-degrading microorganisms, oil biodegradation depends on various environmental parameters which limit the process in seawater (i.e., temperature, low nutrient concentration).

Perhaps the magnitude and catastrophic nature of oil spillage in marine environments has overshadowed the occurrence of freshwater oil pollution. Refineries, pipelines, automobiles, industrial effluents, and watercraft have been suggested as sources of freshwater oil pollutants (18, 26). Numerous examples of actual pollution incidents are documented. For example, more than 50 spillages of at least 100 gallons (ca. 380 liters) of various hydrocarbons occurred in navigable waters of the State of Wisconsin between 1969 and 1973 (records of the Department of Natural Resources, State of Wisconsin). Included among these was a recent spillage of approximately 273,000 gallons of crude oil resulting from a pipeline failure near Cambridge, Wisconsin, on 17 March 1973. A wrecked barge spilled 1,600 gallons of lubricating oil into Lake Michigan near Milwaukee, Wisconsin, in February 1975 (personal communication, Captain of the Port Office, U.S. Coast Guard Base, Milwaukee).

Since early reviews of the literature (25) the widespread occurrence of hydrocarbon-degrading microorganisms in nature, including many freshwater environments, has been generally accepted. Yet, few studies have addressed themselves to oil biodegradation in freshwaters or to environmental limitations of the process. Vorosilova and Dionova (see reference 13) demonstrated the selective enrichment of hydrocarbon-degrading bacteria in relation to other heterotrophic bacteria associated with oil pollutants downstream from oil refineries on the rivers Moskwa and Volga. Ludzack et al. (17) attributed buildup of hydrocarbons in sediments of the Ottawa River, Ohio, downstream

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from a refinery to the inability of sediment microorganisms to cause a significant reduction in hydrocarbons during a 125-day anaerobic laboratory incubation. In other studies (18) enrichment cultures from various soils that supposedly contributed inoculum to nearby freshwater would degrade motor oil at 25 C but not at 4 C. Brown and Tischer (8) noted that enrichment cultures from freshwater inocula decomposed oil better if nitrogen and phosphorus were added. Hunter et al. (15) related the oxidation rate of octadecane and motor oil to substrate concentration and turbulence in a continuous-flow sediment system seeded with sewage. Caparello and LaRock (9) measured the metabolism of [^{14}C]hexadecane by natural samples of lake water and demonstrated a correlation between number of hydrocarbon-oxidizing bacteria present and the lag before hexadecane metabolism.

In view of the occurrence of freshwater oil pollution and because the role of microorganisms in freshwater oil biodegradation has not been clearly established, we measured variations in hydrocarbon oxidation seasonally in the epilimnion of Lake Mendota, and in a number of lakes of varying water quality in northern Wisconsin, to identify critical environmental parameters affecting hydrocarbon biodegradation in freshwater.

MATERIALS AND METHODS

Study areas. Lake Mendota, a temperate eutrophic lake located in south central Wisconsin, was sampled during seasonal studies. On each sampling date, three stations were sampled, and data are presented as the average of data obtained for all stations. The stations were on a transect from the University of Wisconsin Limnology Lake Laboratory to Picnic Point and were located at approximately 100 m, 500 m, and 1 km from the Lake Laboratory toward Picnic Point. Previous studies at more locations showed that oil biodegradation rates did not vary with respect to location in the surface of the lake on a given date (D. M. Ward, M.S. thesis, Univ. of Wisconsin, Madison, 1973).

Nutrient limitation studies were performed on 25 oligotrophic lakes of Vilas County, Wisconsin. Stations were established at least 100 to 200 m away from shore by canoe, except at Pauto, Little Rock, Sparkling, and Trout lakes, where samples were collected a few meters off shore near public boat landings. Sampling was done over a 4-day period, and assays were initiated immediately after sample collection. Samples were incubated at ambient temperature (usually 20 to 30 C) until being placed in a 25 C incubator upon return to the field station no longer than 12 h after sampling.

Field observations and sampling protocol. Water temperature and dissolved oxygen were measured in situ using a model 51-A oxygen meter and thermistor (Yellow Springs Instrument Co., Yellow Springs, Ohio).

Surface water samples were collected by immersion of sterile sample bottles over the side of the sampling craft during the ice-free period or through a hole in the ice during winter. Sample bottles and glassware used in studies on Vilas County lakes were acid cleaned in 3 N HCl and rinsed in glass redistilled water before sterilization to prevent nutrient contamination. In some cases, bottles and vials used in rate assays were filled while sampling either by immersion of BOD bottles or by dispensing 40 ml of surface water to serum vials using a sterile 60-ml plastic syringe. Otherwise, samples were collected in 1-liter Nalgene bottles and distributed aseptically in the laboratory immediately after sampling. Samples collected for nutrient analysis were kept frozen until chemical assays were performed. Samples collected for temperature optima studies during colder months were returned to the laboratory in an ice chest before distribution to various incubation temperatures.

Oxygen uptake. The rate of oxygen uptake during mineral oil oxidation was studied in lake water samples contained in standard 300-ml BOD bottles. Each assay consisted of two control bottles to which a sterile 47-mm membrane filter (0.45-μm pore size, Millipore Corp.) was added and two oil-supplemented bottles to which mineral oil (Fisher Scientific Co.; white, heavy, laboratory grade) sterilized by filtration through a 0.45-μm membrane filter (Millipore Corp.) was added adsorbed onto an identical filter, providing a concentration of about 0.6 g of oil per liter. Samples were incubated in the dark. Dissolved oxygen concentrations were assayed periodically using an oxygen electrode (YSI 5420 Self Stirring BOD bottle electrode, Yellow Springs Instrument Co.). Oxygen uptake was taken as the difference in oxygen content of averaged control and oil-emended bottles. The rate of oxygen uptake was estimated from the linear region of oxygen uptake during mineral oil oxidation. In a separate experiment, nonbiological oxygen demand in formaldehyde controls (4.2% formaldehyde final concentration) was found to be insignificant. Other controls showed that membrane filters (Millipore Corp.) did not increase the BOD of samples.

Hexadecane oxidation. The rate of hexadecane oxidation in lake water samples was measured by incubating 40-ml samples in sterile 60-ml rubber-stoppered serum vials, which contained 0.1 μCi of [1-^{14}C]hexadecane (Amersham/Searle Corp.) diluted with nonradioactive hexadecane (Fisher Scientific Co.) to a specific activity of 0.13 μCi/μmol, to provide a final hexadecane concentration of about 0.02 mM. Periodically, duplicate vials plus a formaldehyde control (as above) were acidified to less than pH 1 by addition of 0.5 ml of 10 N H_2SO_4, and $^{14}CO_2$ was transferred via an air stream from the serum vial to a liquid scintillation cocktail containing phenethylamine as a CO_2 binding agent (see reference 21). Radioactivity was counted with a Packard Tri-Carb liquid scintillation counter, model 3375, using a 0-600 window setting with 6% gain, counts per minute were corrected for background, and non-

biological oxidation and duplicates were averaged. Counting efficiency was 88%. Nonbiological oxidation was always less than 0.25% of the total radioactivity added. The rate of hexadecane oxidation was estimated from the linear region of the $^{14}CO_2$ production time course. At the completion of [1-^{14}C]hexadecane oxidation, approximately 50% of the radioactivity added was recovered as $^{14}CO_2$. The remainder was recovered as material retained by a glass-fiber filter (Gelman type A), not extracted by rinses of the serum vial and filter with two pulses of 5 ml of heptane.

Chemical assays. Nutrient assays were performed on samples clarified by filtration through prewashed 0.45-μm membrane filters (Millipore Corp.), using glassware cleaned in 3 N HCl and using only glass redistilled water. Phosphate, nitrate, and ammonia were assayed according to standard analytical procedures (see reference 22, methods II.2.I, II.6, and II.9, for phosphate, nitrate, and ammonia, respectively).

Protein was determined by the method of Lowry et al. (16), using lysozyme standards, after concentrating protein from lake water samples contained in BOD bottles on glass-fiber filters (Gelman type A).

Glucose was assayed by the Nelson-Somogyi reducing sugar assay (14). Acetate was measured enzymatically (20).

Enumeration of microorganisms. Heterotrophic microorganisms were enumerated by counting the number of colonies that developed after incubation at 25 C on a medium designed to promote growth of aquatic heterotrophic bacteria (see modified Allen and Brock medium, reference 7).

The most-probable-number of mineral oil- and hexadecane-oxidizing microorganisms was estimated using a liquid mineral salts medium containing 0.4 g of NaCl, 0.5 g of NH_4Cl, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.05 g of $NaH_2PO_4 \cdot H_2O$, and 0.05 g of KH_2PO_4 per liter of distilled water. A concentrated phosphate salts solution (pH 7) was autoclaved separately and added to an autoclaved solution (pH 7) containing the other salts. Tenfold dilutions from lake water were made using the salts medium as diluent. One drop of sterile mineral oil or hexadecane was added per 10 ml of salts medium as sole carbon and energy source. Growth after incubation at 25 C was detected by pellicle formation at the oil droplet surface.

Nutrient additions. Oxygen uptake and hexadecane oxidation studies were done after the addition of nitrogen (as KNO_3) and/or phosphorus (as KH_2PO_4) from sterile, concentrated stock solutions (pH 7) to samples prior to incubation in the laboratory. Hydrocarbon-emended samples were compared with controls, which also contained nitrogen and phosphorus but no hydrocarbons.

RESULTS

Hydrocarbon oxidation in natural samples. In all hydrocarbon oxidation experiments on lake water samples the results were similar to that shown in Fig. 1. After an initial lag phase, the oxygen uptake due to mineral oil oxidation and $^{14}CO_2$ release due to hexadecane oxidation proceed linearly. Incubation of samples on a rotary shaker to promote mixing did not influence the kinetics of hydrocarbon oxidation.

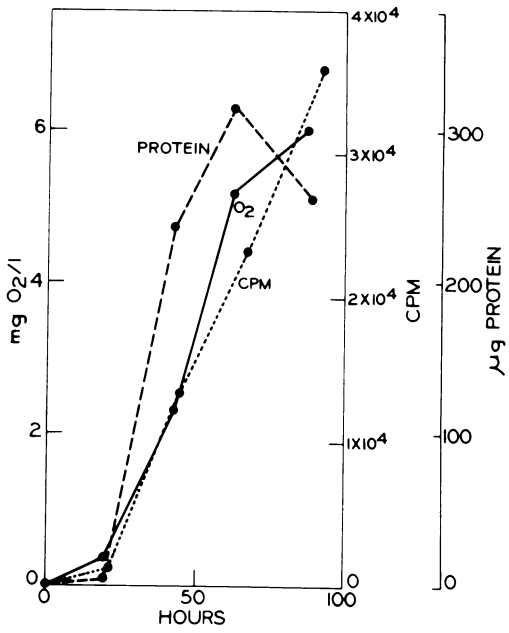

FIG. 1. *Oxygen uptake during mineral oil oxidation (——) and protein increase during growth on mineral oil (- - -) for Lake Mendota surface water collected 1 May 1974; and counts per minute of $^{14}CO_2$ produced during hexadecane oxidation (·····) by Lake Mendota surface water samples collected 27 April 1973. Values on the ordinate represent protein amount per 300-ml lake water sample supplemented with oil, and the protein increase in unsupplemented samples has been deducted. Incubation was at 25 C.*

Several observations suggest that development of a population of hydrocarbon-degrading microorganisms occurs during the incubation period. (i) Protein concentration increased coincident with oxygen uptake (Fig. 1), indicating that growth had occurred. (ii) The lag phase was prolonged by factors that retard growth, such as low temperature (see below). (iii) When mineral oil and hexadecane oxidation rates were studied on a series of samples serially diluted twofold with filter-sterilized lake water (i.e., 1/2, 1/4, 1/8, and 1/16 of the initial lake water sample), the lag phase before the onset of hydrocarbon breakdown was increased in proportion to the decrease in initial population density. However, the rate of hydrocarbon oxidation was independent of the initial population density over the range of dilutions tested. Thus, oxygen uptake and hexadecane oxidation rates, estimated from similar time courses,

may be considered as indicating the potential growth rate of hydrocarbon-oxidizing microorganisms inhabiting natural samples, which develop in response to hydrocarbon additions.

The results of other experiments support the possibility that hydrocarbon-oxidizing enzymes were repressed and had to be induced when hydrocarbons were added to natural samples. (i) During an experiment in which lake water was aged to remove indigenous oxidizable organic compounds (BOD) by incubation at 25 C for 24 h before addition of hydrocarbons, the lag before hydrocarbon oxidation was shortened from about 40 h to about 20 h. (ii) It was possible to extend the lag phase when glucose was added in addition to [1-^{14}C]hexadecane. Figure 2 demonstrates that $^{14}CO_2$ was not produced from [1-^{14}C]hexadecane until after glucose had been completely consumed, whereas glucose was consumed without a lag. In the presence of both glucose and hexadecane the lag phase was lengthened by approximately the length of time required for glucose metabolism. Dissolved oxygen levels were always near saturation. It was necessary to increase nitrogen and phosphorus levels in this experiment, since indigenous levels of these nutrients were too low to balance the carbon added as glucose. Addition of acetate in a separate experiment did not increase the lag phase before [^{14}C]hexadecane oxidation, although acetate was rapidly consumed.

Since the measurement of the rate of hydrocarbon biodegradation depended on the enrichment of an active population of hydrocarbon-degrading microorganisms, such measurements must be considered potential rates.

Seasonal variation in hydrocarbon oxidation rate in Lake Mendota. The rates of hydrocarbon oxidation by natural surface water microbial communities of Lake Mendota were followed over a complete season from 14 March 1973, the day after ice break-up, until 20 October 1973. Figure 3A shows the seasonal variation of mineral oil and hexadecane oxidation rates in samples incubated at temperatures that corresponded to indigenous water temperatures. The rates were low initially and increased during the spring, attaining maximum levels in June before decreasing and remaining low throughout the remainder of the study period. During the study period the numbers of heterotrophic and oil- and hexadecane-oxidiz-

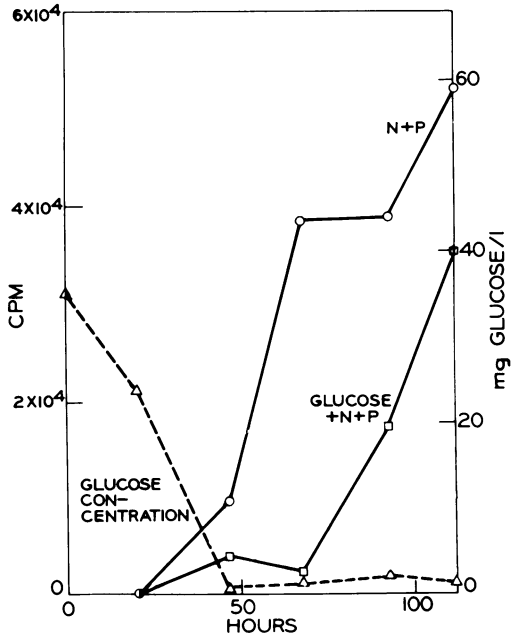

FIG. 2. *Production of $^{14}CO_2$ during the oxidation of [1-^{14}C]hexadecane in Lake Mendota, Wisconsin, surface water samples collected 15 January 1975, with (squares) and without (circles) addition of 35 mg of glucose per liter and with addition of 85 mg of KNO_3 and 10 mg of KH_2PO_4 per liter. Dashed lines represent glucose concentrations in glucose plus N + P emended samples.*

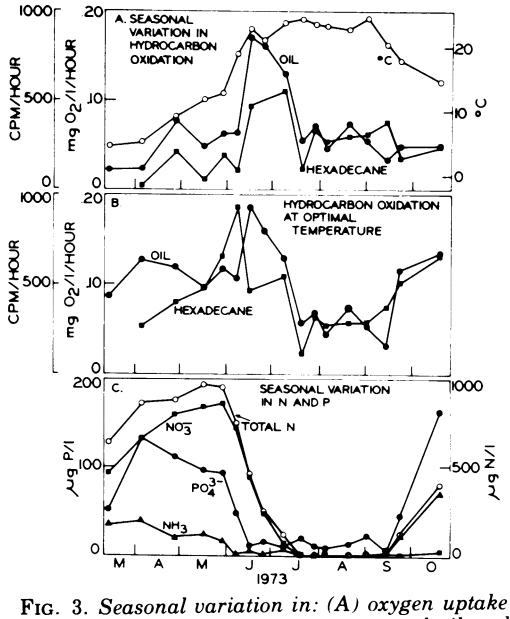

FIG. 3. *Seasonal variation in: (A) oxygen uptake and $^{14}CO_2$ production rates during mineral oil and hexadecane oxidation by natural samples at in situ temperatures and Lake Mendota surface water temperature; (B) oxygen uptake and $^{14}CO_2$ production rates during mineral oil and hexadecane oxidation by natural samples incubated at 25 C; (C) dissolved inorganic phosphate, nitrate, ammonia, and total N (nitrate plus ammonia) in Lake Mendota surface water samples.*

ing bacteria varied over a range of about 1 order of magnitude (Fig. 4). The proportion of hydrocarbon-utilizing to heterotrophic bacteria was always small, and minor variations in population levels did not affect hydrocarbon oxidation rates after the end of the lag, as mentioned above.

A gradual increase in rates during spring correlated with a gradual increase in water temperature, suggesting temperature limitation (see Fig. 3A). Temperature limitation of oil biodegradation was studied in greater detail by incubating identical lake water samples at various temperatures and measuring rates of mineral oil and hexadecane oxidation (Fig. 5). In addition to affecting the rate of hydrocarbon oxidation, low water temperatures increased the lag phase before the onset of hydrocarbon metabolism. The optimum temperature for hydrocarbon oxidation was 25 C or greater, regardless of the indigenous water temperature, when temperature optimum experiments were run in summer (25 C), fall (12 C), or winter (0 C) (Fig. 6). Below 20 C low temperature limited hydrocarbon oxidation rate.

During midsummer, factors other than temperature must have limited hydrocarbon oxidation rates. Excellent correlation of non-temperature-limited rates of hydrocarbon oxidation (Fig. 3B) and nitrogen and phosphorus levels in the Lake Mendota epilimnion (Fig. 3C) suggested nutrient limitation. A similar correlation was found in Lake Mendota during 1972 (Ward, M.S. thesis, Univ. of Wisconsin, Madison, 1973).

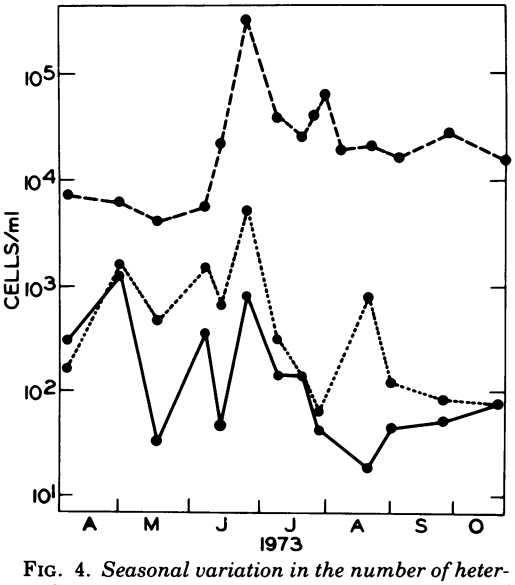

FIG. 4. *Seasonal variation in the number of heterotrophic (- - -), mineral oil-oxidizing (——), and hexadecane-oxidizing (·····) bacteria present in Lake Mendota surface water samples. Incubation was at 25 C.*

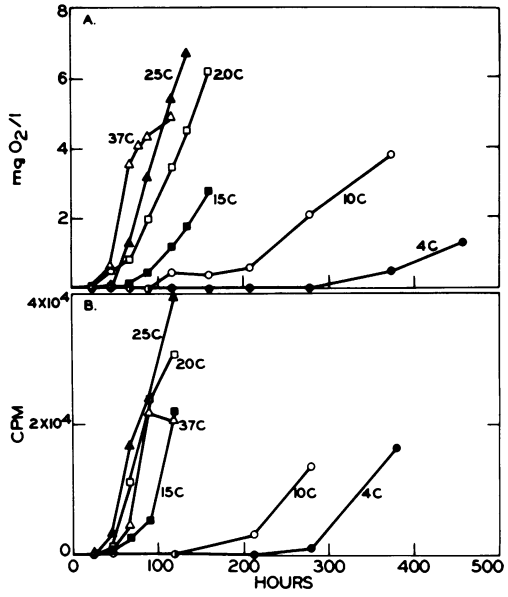

FIG. 5. *Effect of temperature on (A) oxygen uptake during mineral oil oxidation and (B) $^{14}CO_2$ production during hexadecane oxidation by Lake Mendota surface water samples collected 18 January 1975 when the indigenous water temperature was 0 C. Symbols correspond to different incubation temperatures: 4 C (●); 10 C (○); 15 C (■), 20 C (□); 25 C (▲); and 37 C (△).*

Nutrient limitation of oil biodegradation was demonstrated in nutrient addition experiments on samples collected when indigenous nutrient levels were low. When either nitrogen (provided as KNO_3) or phosphorus (as KH_2PO_4) was added alone, mineral oil or hexadecane oxidation rates were not stimulated to maximum levels. However, when either nitrogen or phosphorus was provided in excess, the rates depended on the amount of the other nutrient (Fig. 7A and B).

Nutrient limitation in oligotrophic lakes of Vilas County. Table 1 presents data on rates of mineral oil and hexadecane oxidation for 25 lakes studied in Vilas County during July 1974. Chemical analyses in Table 1 demonstrate that all of these lakes are nutrient deficient. Enumeration of microbial populations showed that low levels of heterotrophic bacteria inhabited these systems, a small proportion of which could grow on mineral oil or hexadecane as sole carbon and energy source. For Pauto, Little Rock, and Sparkling lakes, the most probable number of oil-oxidizing bacteria was high relative to the number of heterotrophic bacteria

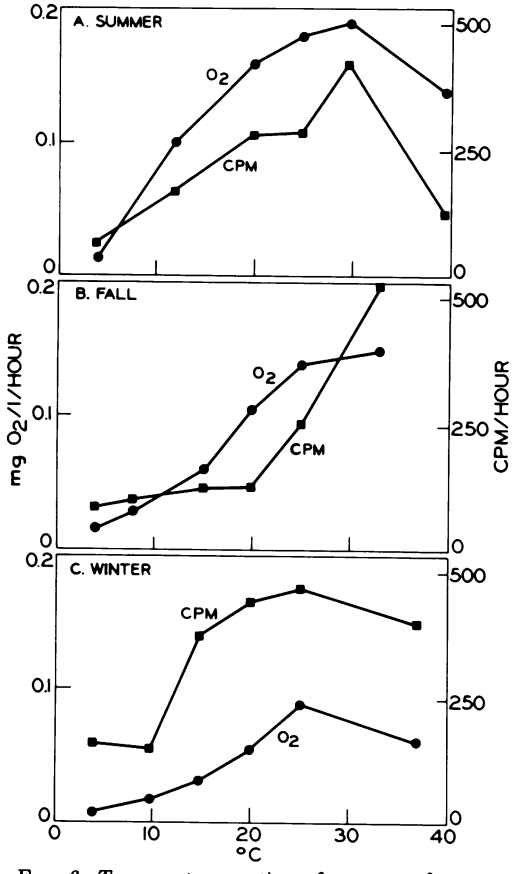

FIG. 6. *Temperature optima for rates of oxygen uptake during mineral oil oxidation (●) and $^{14}CO_2$ production during hexadecane oxidation (■) of Lake Mendota surface water samples collected (A) 14 June 1973, (B) 31 October 1974, and (C) 18 January 1975, when indigenous water temperatures were 23, 12, and 0 C, respectively.*

that formed colonies on the test medium. In fact, the greater number of oil-oxidizing bacteria than heterotrophic bacteria in Pauto and Little Rock lakes probably reflects differences in efficiency between the most probable number and plating techniques. Samples for these lakes were collected a few meters off shore near public boat landings, where a continuous supply of hydrocarbons may have resulted in enrichment of oil-degrading microorganisms. Oil-oxidizing bacteria were not detected in Black Oak Lake and Fence Lake. Since low numbers of heterotrophic bacteria also inhabited these lakes, it is presumed that the number of oil-oxidizing bacteria was too low to be detected by the lowest dilution tested in most-probable-number determinations. The observance of hydrocarbon oxidation attests to the fact that such microorganisms did inhabit these lakes.

Mineral oil and hexadecane oxidation rates were studied directly on lake water samples and also on lake water samples to which 300 μg of N per liter as KNO_3 and 100 μg of P per liter as KH_2PO_4 were added. All assays were performed at 25 C. In all cases, indigenous rates of hydrocarbon oxidation were lower than in samples enriched with nitrogen and phosphorus. Enrichment of samples with nitrogen and phosphorus resulted in an increase in the rate of mineral oil and hexadecane oxidation of 2.7 to 27.7 times the indigenous rates.

When the data of Vilas County and Lake Mendota studies are combined, the correlation of hydrocarbon oxidation rates with indigenous nutrient levels describes the dependence of rate on concentrations from near detection limits to maximum environmental levels. Figure 8 shows the correlation between mineral oil oxidation rate and dissolved inorganic phosphate levels (Vilas County lakes containing less than 2.5 μg of P per liter are excluded as being below detection limits for the phosphate assay). The data resemble a saturation relationship of the

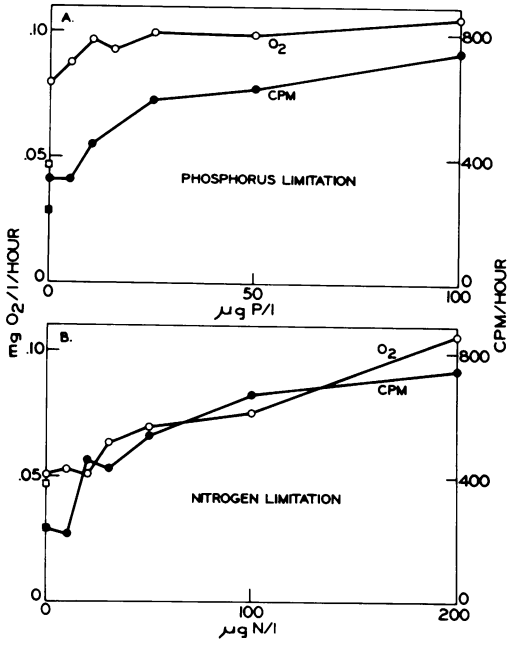

FIG. 7. *Rates of oxygen uptake during mineral oil oxidation (○) and $^{14}CO_2$ production during hexadecane oxidation (●) by Lake Mendota surface water samples collected 7 September 1974 at various concentrations of added (A) phosphate with nitrogen provided at 300 μg/liter as nitrate and (B) nitrate with phosphorus provided at 100 μg/liter as phosphate. The rate of mineral oil oxidation (□) and hexadecane oxidation (■) in samples without added nutrients is included in each figure.*

TABLE 1. *Hydrocarbon oxidation rates, cell counts, and chemical data for Vilas County lakes*

Lake	Nutrient analyses			Cells/ml		Hydrocarbon oxidation rate[a]			
						Mineral oil (mg of O_2/liter per h)		Hexadecane (counts/min per h)	
	μg of PO_4^{-3}-P/liter	μg of NO_3^--N/liter	μg of NH_3-N/liter	Heterotrophic	MPN oil[b]	Indigenous	N + P[c]	Indigenous	N + P
Big Crooked	0[d]	0	22.1	365	23	0.007	0.083	23.9	311.2
Palmer	9.8	0	27.7	1,295	43	0.027	0.083	111.7	399
Black Oak	0	0	15.1	815	0	0.000	0.075	13.3	266
Lac Vieux Desert	4.2	2.2	5.3	1,295	9	0.018	0.063	98.4	399
Big Sand	1.2	0	0	3,100	3	0.016	0.058	26.6	332.5
North Twin	2.6	0	11.6	6,700	4	0.010	0.075	45.2	234.1
Pickerel	4.6	0	4.0	5,850	4	0.010	0.054	66.5	665
Muskellunge	3.8	0	23.5	12,500	15	0.029	0.083	117.0	532
Catfish	5.4	0	14.7	6,800	15	0.025	0.067	111.7	353.8
Little St. Germaine	2.4	0.9	5.1	3,700	93	0.005	0.044	29.3	311.2
Otto Mielke	0.4	0	0	540	4	0.003	0.047	45.2	465.5
Manitowish	1.2	0	2.6	1,070	20	0.009	0.081	39.2	266
Pokegama	0	0	0	1,065	4	0.015	0.090	87.8	239.4
Lac du Flambeau	0.2	0	0.2	495	4	0.011	0.075	29.3	199.5
Fence	0.4	0	3.0	315	0	0.014	0.069	26.6	311.2
Johnson	1.8	0	0	640	9	0.003	0.083	29.3	300.6
Snake	3.2	0	42.6	5,150	240	0.025	0.071	77.1	444.2
Arrowhead	1.8	0	6.7	1,125	15	0.005	0.108	31.9	465.5
Erickson	1.6	0	12.1	380	23	0.006	0.100	18.6	266
Big Muskellunge	3.4	0	16.5	235	4	0.015	0.075	29.3	220.8
Crystal	0	0	9.1	150	15	0.006	0.081	26.6	433.6
Pauto	0	0	28.6	2,235	15,000	0.009	0.081	47.9	292.6
Little Rock	0	0	3.5	4,100	9,300	0.011	0.088	37.2	284.6
Sparkling	0	0.78	0.5	5,206	2,300	0.008	0.083	26.6	266
Trout	5.6	3.0	5.1	690	43	0.006	0.094	47.9	353.8

[a] Milligrams of O_2 per liter per hour during mineral oil oxidation and counts per minute of $^{14}CO_2$ per hour.

[b] Most probable number (mpn) of bacteria per milliliter of lake water able to develop in a mineral salts medium containing mineral oil as a sole carbon and energy source.

[c] 100 μg of PO_4^{-3}-P per liter and 300 μg of NO_3^--N per liter added.

[d] Zero indicates a lower absorbance than the blank. Values below 2.5 μg of PO_4^{-3}-P, 2 μg of NO_3^--N, and 5 μg of NH_3-N per liter we considered to be below the sensitivity of our assays and are probably not absolute.

Michaelis-Menten type, and the line drawn in Fig. 8 is a statistical fit of the linear regression of a double-reciprocal plot of the same data. The three anomalous points in Fig. 8 may be due to high N/P ratios in Lake Mendota in early summer when phosphate levels are very low, possibly causing changes in the affinity of the microbial community for phosphorus. Plots of nitrogen content versus hydrocarbon oxidation rate also resembled a saturation curve, although correlation was not as good.

DISCUSSION

A small percentage of the heterotrophic bacterial population inhabiting all lake water samples could metabolize hydrocarbons. After addition of hydrocarbons to lake water, a lag phase of at least 20 h was always encountered before measurable hydrocarbon oxidation began, coincident with the development of bacteria on added hydrocarbons. Factors causing the lag phase presumably include (i) contact between cell and insoluble substrate, (ii) population growth to a level sufficient to produce measurable oxygen uptake or $^{14}CO_2$ evolution, and (iii) enzyme induction. Since shaking did not influence the kinetics of hydrocarbon oxidation, contact or solubility problems do not appear to explain the lag phase. The dependence of the duration of the lag phase on factors that either retard the growth rate (temperature) or cause a decrease in the initial population density (dilution of the water) suggests that the lag, at least in part, represents the time needed for the growth of the initially low hydrocarbon-degrading population to higher levels. Caparello and LaRock (9) related the lag before [^{14}C]hexadecane oxidation in pure cultures of hydrocarbon-oxidizing bacteria to the initial population density and suggest that their results can be applied to estimate the initial population density of hydrocarbon-oxidizing bacteria in natural samples. Using their reasoning, the

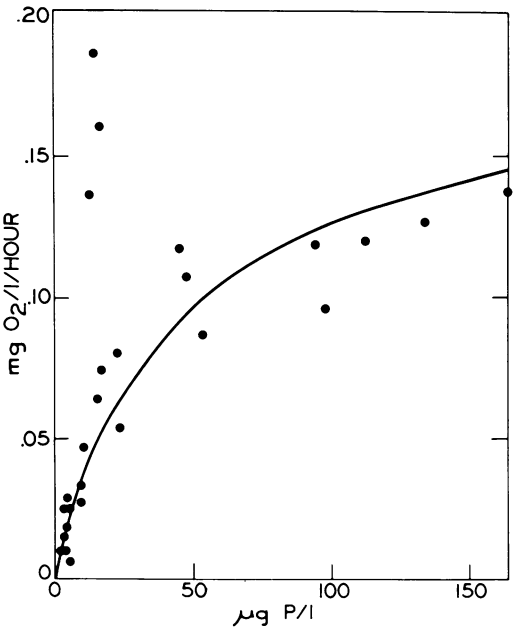

FIG. 8. *Correlation between the indigenous rates of oxygen uptake during mineral oil oxidation and the indigenous dissolved inorganic phosphate concentrations for Lake Mendota and Vilas County samples (exclusive of Vilas County lakes containing less than 2.5 µg of P per liter). The line was obtained by plotting the reciprocal of the linear regression line fitting a double-reciprocal plot of the data.*

length of the lag phase (about 20 h) and the initial density of hydrocarbon-degrading bacteria (about 10^2/ml) present in our samples roughly correspond.

On the other hand, some observations support the necessity of enzyme induction and suggest that hydrocarbon oxidation was not ongoing in the lake water samples we collected. The lag coincides with the period when oxidizable organic compounds (BOD) indigenous to lake water are metabolized. Repression of hydrocarbon-oxidizing enzymes during growth on preferred indigenous substrates might explain part of the lag. In Lake Mendota, hydrocarbon-oxidizing bacteria are nutritionally versatile (24) and may grow as normal heterotrophic community members until they are selectively enriched when hydrocarbons are added. In water samples aged to remove BOD before the addition of hydrocarbons, the lag was shortened, although it was not eliminated. It was possible to increase the lag by addition of low levels of glucose (about 0.2 mM) in addition to hexadecane. Hexadecane metabolism began about 20 h after glucose was completely consumed. Induction and repression of hydrocarbon-degrading enzyme systems have been previously demonstrated in pure cultures (6, 19, 23). In contrast to observations of an increase in the ratio of hydrocarbon-degrading to total heterotrophic bacteria in areas where hydrocarbon metabolism is ongoing (3, 13), the ratio was always small in Lake Mendota.

It is, thus, impossible to conclude that hydrocarbon metabolism was not ongoing when lake water was collected. However, this possibility cannot be ignored. Since nonhydrocarbon organic compounds are probably continuously supplied to lake water microorganisms, and because oil pollution may occasionally occur in areas where high levels of organic compounds are found, regulatory interference of hydrocarbon metabolism in nature might be significant and should be further investigated.

Although the lag phase remains unresolved, the methods used enabled a comparison of the potential rate of hydrocarbon breakdown by natural populations that develop in response to hydrocarbon addition, such as might occur after an oil spill. Comparable results were obtained when O_2 uptake was measured during the oxidation of a mixed hydrocarbon substrate (mineral oil) and when $^{14}CO_2$ was measured during oxidation of [1-^{14}C]hexadecane.

Despite the presence of hydrocarbon-oxidizing microorganisms, the potential rate of hydrocarbon oxidation varies with the physical limitations of the environment. In temperate lakes, temperature is a major variable affecting hydrocarbon breakdown. Populations of psychrophilic hydrocarbon-oxidizing microorganisms did not adapt to seasonally cold water temperatures in Lake Mendota, as shown by the constant temperature optimum at 25 C or greater in different seasons. Thus, seasonally low water temperatures limit the rate at which hydrocarbon degradation might occur, except during summer when temperature is optimal.

Our experiments on relationships between nitrogen and phosphorus concentration and hydrocarbon oxidation rates demonstrated nutrient limitation typical of the saturation relationship often used to show dependence of growth rate on limiting nutrient concentration. Half-saturation concentrations for growth rates of natural communities on mineral oil and hexadecane were approximately 20 µg of P and 50 µg of N per liter. This suggests that, although quite high amounts of nitrogen and phosphorus may be necessary to balance the carbon used when substantial hydrocarbon degradation occurs, the rate of oxidation by natural communities only becomes limited at quite low nutrient levels. For example, substantial decreases in hydrocarbon oxidation rates did not occur until the indigenous nutrient levels decreased to lev-

els near the limits of detection (Fig. 3B and C). The fact that such low levels are common to the epilimnion of eutrophic lakes (presumably due to efficient competition for nitrogen and phosphorus by bloom-forming algae) and in oligotrophic lakes, which receive little nutrient input, indicates that nutrient limitation of oil biodegradation is of widespread occurrence in natural freshwater systems. It will be of interest to consider the ability of pollutant-degrading microorganisms to compete for nutrients that limit the growth of all the members of natural communities. For oil biodegradation the provision of forms of nitrogen and phosphorus soluble in oil but insoluble in water may selectively increase the competitiveness of oil-degrading microorganisms (5).

In temperate lakes the potential rate at which hydrocarbon pollutants can be degraded is usually limited due to seasonal and regional variations in temperature and nutrient content. Despite the constant presence of oil-degrading bacteria, such lakes do not provide optimal conditions for hydrocarbon metabolism.

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