APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1985, p. 822–827
0099-2240/85/040822-06$02.00/0

Vol. 49, No. 4

Effects of Dissolved Organic Carbon and Second Substrates on the Biodegradation of Organic Compounds at Low Concentrations

STEVEN K. SCHMIDT AND MARTIN ALEXANDER*

Laboratory of Soil Microbiology, Department of Agronomy, Cornell University, Ithaca, New York 14853

Received 9 October 1984/Accepted 22 January 1985

Pseudomonas acidovorans **and *Pseudomonas* sp. strain ANL but not *Salmonella typhimurium* grew in an inorganic salts solution. The growth of *P. acidovorans* in this solution was not enhanced by the addition of 2.0 μg of phenol per liter, but the phenol was mineralized. Mineralization of 2.0 μg of phenol per liter by *P. acidovorans* was delayed 16 h by 70 μg of acetate per liter, and the delay was lengthened by increasing acetate concentrations, whereas phenol and acetate were utilized simultaneously at concentrations of 2.0 and 13 μg/liter, respectively. Growth of *Pseudomonas* sp. in the inorganic salts solution was not affected by the addition of 3.0 μg each of glucose and aniline per liter, nor was mineralization of the two compounds detected during the initial period of growth. However, mineralization of both substrates by this organism occurred simultaneously during the latter phases of growth and after growth had ended at the expense of the uncharacterized dissolved organic compounds in the salts solution. In contrast, when *Pseudomonas* sp. was grown in the salts solution supplemented with 300 μg each of glucose and aniline, the sugar was mineralized first, and aniline was mineralized only after much of the glucose carbon was converted to CO_2. *S. typhimurium* failed to multiply in the salts solution with 1.0 μg of glucose per liter. It grew slightly but mineralized little of the sugar at 5.0 μg/liter, but its population density rose at 10 μg of glucose per liter or higher. The hexose could be mineralized at 0.5 μg/liter, however, if the solution contained 5.0 mg of arabinose per liter. In solutions with this arabinose concentration and glucose levels too low to support growth, the percentage of glucose carbon incorporated into *S. typhimurium* cells was the same as when the bacterium was grown in solutions with high concentrations of glucose alone. When glucose was the only carbon source for *S. typhimurium*, the percentage of the glucose carbon assimilated and mineralized progressively declined as the sugar concentration was reduced to levels approaching the threshold for growth. These results indicate that second substrates and uncharacterized dissolved organic carbon may play an important role in controlling the rate and extent of biodegradation of organic compounds at low concentrations.**

Microorganisms can degrade a variety of synthetic organic compounds present in samples from natural environments at concentrations below 10 μg/liter (1, 16). However, it is not certain whether microorganisms can grow at such low concentrations. It has been postulated that substrate uptake will just meet maintenance energy requirements and growth is not possible at sufficiently low substrate concentrations (12). Of the few bacteria whose ability to grow at very low substrate concentrations has been studied, threshold concentrations for growth have been found to be ca. 2 μg/liter (17) or much higher (8, 14, 17).

An explanation for the biodegradation of organic compounds at concentrations below the threshold level is that the organisms are simultaneously using higher concentrations of other compounds for maintenance energy and growth. Simultaneous use of two substrates has been shown for a number of bacteria (4, 7), and levels of available but largely uncharacterized dissolved organic carbon (DOC) are high enough in many environments to support considerable bacterial growth (13, 19). However, demonstrating the existence of threshold concentrations for growth is difficult because many bacteria can readily grow on the DOC in distilled water (2, 6) or on the volatile organic compounds from air in contact with culture media (3, 10).

The aim of the present study was to determine the effects of DOC in laboratory media prepared with distilled water on the abilities of bacteria to degrade low concentrations of added substrate. For this purpose, species able and unable to use the uncharacterized DOC were used. In addition, to understand the effect of DOC on the metabolism of synthetic organic substrates, studies were also conducted on the effects of second substrates on the biodegradation of low concentrations of organic compounds.

MATERIALS AND METHODS

Radioisotopes. [*U*-^{14}C]aniline hydrogen sulfate (99 mCi/mmol) and [*U*-^{14}C]phenol (87 mCi/mmol) were obtained from Amersham Corp., Arlington Heights, Ill. [*U*-^{14}C] glucose (348 mCi/mmol) was purchased from New England Nuclear Corp., Boston, Mass.

Bacterial isolates. *Salmonella typhimurium* LT_2 was obtained from J. M. Calvo, Department of Biochemistry, Cornell University. *Pseudomonas acidovorans* was obtained from N. C. Dondero, Department of Microbiology, Cornell University. *Pseudomonas* sp. strain ANL was isolated from the primary settling tank of the Ithaca, N.Y., sewage treatment plant. Sewage (1.0 ml) was added to 100 ml of an inorganic salts solution containing 30 mg of aniline per liter. After 10 days, 1.0 ml of the enrichment culture was transferred to 200 ml of fresh medium, and the culture was incubated for 7 days at 21°C. Serial dilutions were then plated on a medium containing inorganic salts and 100 mg of aniline and 15 g of Difco Bacto-Agar per liter. Isolated colonies appearing after 4 days of incubation at 21°C were restreaked on fresh agar four times before transferring back to liquid media.

Media. The inorganic salts solution contained 13 mg of KH_2PO_4, 60 mg of Na_2HPO_4, 40 mg of NH_4Cl, 10 mg of

* Corresponding author.

$MgSO_4 \cdot 7H_2O$, 10 mg of $CaCl_2 \cdot 2H_2O$, 0.5 mg of $FeCl_3 \cdot 6H_2O$, 0.5 mg of $MnSO_4 \cdot H_2O$, 0.01 mg of $ZnSO_4 \cdot 7H_2O$, 5 μg of $CuSO_4 \cdot 5H_2O$, and 5 μg of $CoCl_2 \cdot 6H_2O$ per liter. Individual salt solutions were sterilized separately before being added to test solutions. The final pH of the mixture was 6.9.

The water was distilled and then treated with a Milli-Q reagent grade water system (Millipore Corp., Bedford, Mass). Where indicated, water was further purified by using a wet oxidation procedure. Pretreated water was distilled twice in glass containers from 1.0-liter volumes containing 1.0 ml of H_3PO_4 and 10 g of $K_2S_2O_8$ (9). All glassware was soaked overnight in concentrated sulfuric acid containing 15 g of Nochromix (Godax Laboratories, New York, N.Y.) per liter, followed by three rinses in doubly distilled water.

Inocula preparation. The bacteria were initially grown in the inorganic salts solution containing 100 mg of substrate per liter. The substrates were phenol and aniline for *P. acidovorans* and *Pseudomonas* sp. strain ANL, respectively, and glucose or arabinose for *S. typhimurium*. To adapt the cells to low concentrations of substrate, the inoculum was grown from a low cell density to the stationary phase in media containing 100 μg of substrate per liter. Unwashed cells which had been in the stationary phase for 24 to 48 h were used as the inoculum. To minimize the carry-over of residual carbon, the inoculum was diluted at least 200-fold to obtain the initial cell densities that were used. For experiments involving the simultaneous utilization of glucose and arabinose by *S. typhimurium*, the inoculum was grown to mid-logarithmic phase in the inorganic salts solution containing 10 mg of arabinose per liter. The cells were centrifuged and resuspended in the salts solution before being diluted 1,000-fold to obtain the cell suspensions that were used.

Determination of growth. Erlenmeyer flasks (250 ml) containing 150 ml of the inorganic salts solution and the test compounds were incubated in the dark at 29°C on a rotary shaker operating at 125 rpm. At regular intervals, the number of cells was determined by using the spread-plate technique. For this purpose, duplicate 0.1-ml portions of 10-fold dilutions were plated on a medium containing 15 g of Difco Bacto-Agar and 3 g of Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) per liter of deionized water, and colony counts were made after 48 h of incubation at 29°C. The data represent means of duplicate plate counts from individual flasks. The generation time of *S. typhimurium* in media with different glucose concentrations was calculated by using data from the exponential phase of growth.

Analytical techniques. All flasks received similar amounts of ^{14}C-labeled compounds (1,000 to 2,000 dpm/ml), but the final substrate concentration was varied by adding different amounts of unlabeled compounds. At regular intervals, 3.5-ml samples were removed from the flasks and acidified with 4 drops of concentrated H_2SO_4. To drive off CO_2, the samples were bubbled vigorously for 5 min with compressed air. Subsamples (3.0 ml) then were added to plastic vials containing 9 ml of Liquiscint (National Diagnostics, Inc., Somerville, N.J.), and the radioactivity was counted with a liquid scintillation counter (model LS7500; Beckman Instruments, Inc., Irvine, Calif.).

All ^{14}C-depletion curves were obtained from the flasks that were used to obtain cell counts. Unless indicated otherwise, the curves showing ^{14}C disappearance show ^{14}C remaining in the medium containing the cells. The ^{14}C remaining in the medium is assumed to be assimilated into the cells, present as the added substrate, or present as excreted products.

To determine the amount of ^{14}C assimilated by the cells, samples were passed through a 0.2-μm polycarbonate filter (Nuclepore Corp., Pleasanton, Calif.), and the radioactivity in the filtrate was counted as described above. The amount of ^{14}C in the filtrate was subtracted from the amount in the original suspension to obtain the amount of ^{14}C assimilated. Controls with heat- or acid-killed cells indicated that negligible amounts of [^{14}C]glucose or [^{14}C]phenol were retained on the polycarbonate filters during filtration. [^{14}C]aniline, on the other hand, was readily sorbed by the polycarbonate filters in the presence of killed cells, and therefore aniline assimilation could not be measured reliably by this technique.

To study the simultaneous utilization of glucose and aniline, solutions were prepared in duplicate with equal amounts of unlabeled glucose and aniline. To one flask of each pair was added an appropriate amount of either [^{14}C]aniline or [^{14}C]glucose. The concentrations were made equal by adding either unlabeled glucose or aniline. These experiments were repeated twice with similar results.

RESULTS

Characterization of the aniline-degrading bacterium. The isolate is a gram-negative, oxidase-positive, catalase-positive, polarly flagellated, aerobic rod that does not require growth factors. These characteristics suggest that it is a *Pseudomonas* strain (11). It was designated strain ANL.

DOC-using bacteria. To test the ability of the bacteria to grow in the absence of added organic substrate, each organism was inoculated into glass-stoppered 250-ml Erlenmeyer flasks containing 150 ml of the inorganic salts solution. *P. acidovorans* grew to final cell densities of from 1×10^5 to 5×10^5 cells per ml in unamended inorganic salts solution, whereas *Pseudomonas* sp. strain ANL reached a density of 1.8×10^6 cells per ml. Subsequent studies of *P. acidovorans* indicated that the unamended salts solution contained enough available carbon to support a population equivalent to that expected in a medium containing ca. 90 μg of phenol per liter. Only at phenol concentrations of 100 μg/liter or more was the maximum cell number greater than that obtained in the inorganic salts solution (Fig. 1). The salts solution prepared from doubly glass-distilled water which had been oxidized with $K_2S_2O_8$ still supported the growth of 6×10^4 and 2×10^4 *P. acidovorans* cells per ml in two separate experiments. These values were 50 and 15% of the counts in untreated distilled water, indicating that treatment with $K_2S_2O_8$ removed from 50 to 85% of the DOC that supported growth of *P. acidovorans*.

The effects of the uncharacterized DOC on the utilization of low concentrations of added substrate were determined by concurrently measuring growth of the bacteria and mineralization of the ^{14}C-labeled compounds. *P. acidovorans* was able simultaneously to grow on the uncharacterized DOC and mineralize phenol supplied at a concentration of 2.0 μg/liter (Fig. 2). The data indicate that the rate of growth and the number of cells were essentially the same with and without the added substrate. Moreover, based on the maximum cell counts obtained in media with higher phenol concentrations, 2.0 μg of phenol per liter should have supported the growth of only 3×10^3 cells per ml. The fact that 100 times that many cells were produced indicates that the growth observed was almost entirely a result of the use of constituents of DOC other than the added phenol.

The uncharacterized DOC had a different effect on the ability of *Pseudomonas* sp. strain ANL to utilize low concentrations of added substrates. The bacterium was inoculated into the inorganic salts solution containing either no added carbon source or a mixture of 3.0 μg each of glucose and aniline per liter. In the latter medium, mineralization of each of the two test compounds was also determined. The growth curves were essentially identical in the presence or absence of the two added substrates (Fig. 3). Moreover, the final yield of cells was far greater than could be explained by the small amounts of glucose and aniline added, indicating that much of the cell carbon came from unknown components of the DOC. Under these conditions, mineralization of glucose and aniline was initially undetectable or was extremely slight, as the cells were growing at the expense of the uncharacterized DOC. Once a substantial amount of growth on the DOC had occurred, however, the glucose and aniline were simultaneously utilized. The pattern of metabolism was quite different when *Pseudomonas* sp. strain ANL was inoculated into a medium containing 300 μg each of glucose and aniline per liter. Under these conditions, glucose was mineralized first (Fig. 4). Only when the rate of disappearance of ^{14}C from labeled glucose began to decline was there appreciable mineralization of aniline.

In addition to the loss of ^{14}C as the bacteria mineralized labeled glucose as shown in Fig. 3 and 4, 21% of the added ^{14}C was incorporated into biomass after 70 h, leaving ca. 7% of the ^{14}C in the bacteria-free solution. This soluble ^{14}C may represent excreted products.

P. acidovorans is unable to grow on glucose (11), so acetate was used as a second carbon source. The addition of 70, 180, and 480 μg of acetate per liter to the inorganic salts solution containing 2.0 μg of phenol per liter caused the onset of phenol mineralization to be delayed for 16, 19, and 22 h, respectively. This lag in phenol mineralization coincided with the growth of *P. acidovorans*, which was inoculated at a density of 10^3 cells per ml, on the added acetate. In a subsequent experiment, *P. acidovorans* was able to min-

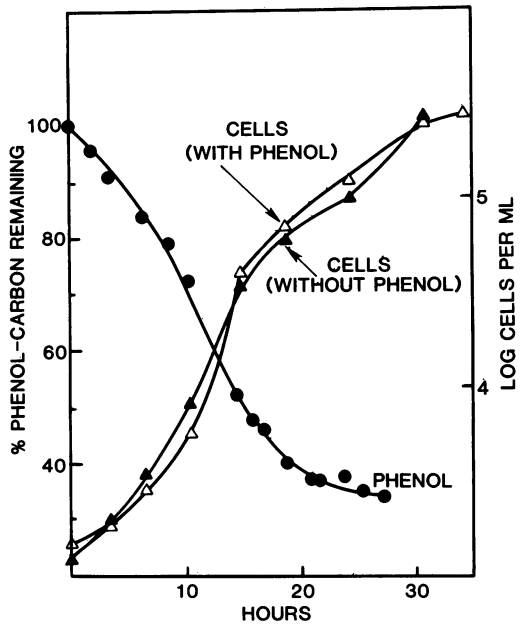

FIG. 2. Growth of *P. acidovorans* in inorganic salts solution with and without phenol (2.0 μg/liter) and phenol mineralization in the former medium.

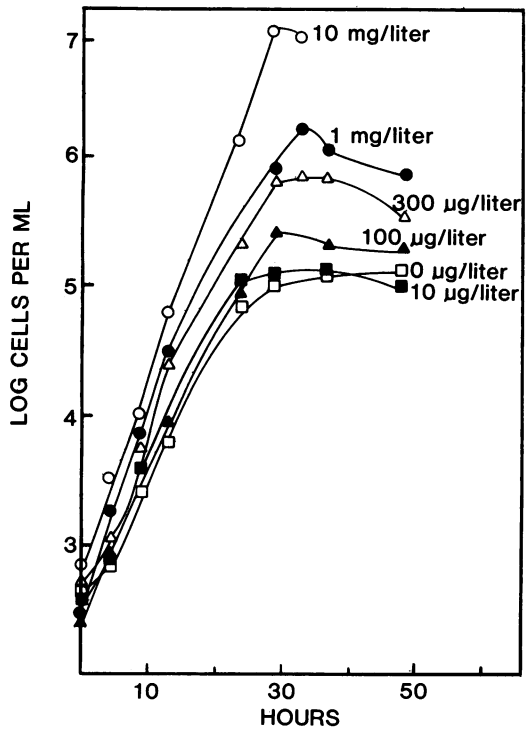

FIG. 1. Growth of *P. acidovorans* in an inorganic salts solution containing different concentrations of phenol.

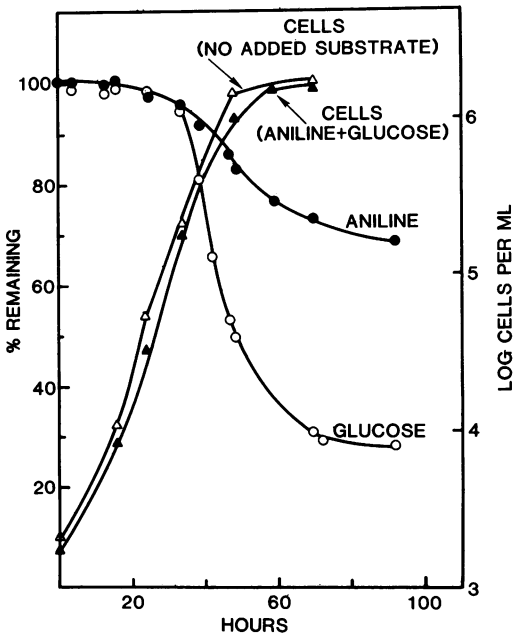

FIG. 3. Growth of *Pseudomonas* sp. strain ANL in salts solution containing 3.0 μg each of glucose and aniline per liter or in unamended salts solution and mineralization of the two compounds in the former medium. The mineralization curves show the percentage of ^{14}C remaining in the bacterial suspensions.

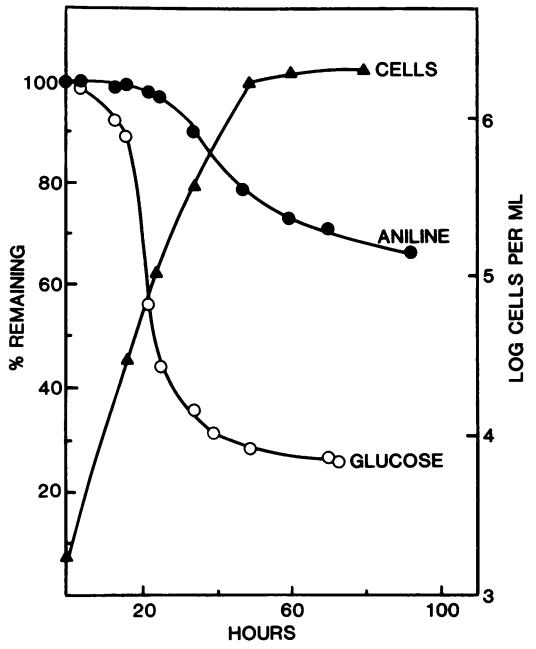

FIG. 4. Mineralization of glucose and aniline and the growth of *Pseudomonas* sp. strain ANL in a medium containing 300 μg each of glucose and aniline per liter. The mineralization curves are for the percentage of ^{14}C remaining in the bacterial suspensions.

eralize phenol and acetate simultaneously when they were present at concentrations of 2.0 and 13 μg/liter, respectively.

Growth of *S. typhimurium*. To determine how bacteria unable to use the uncharacterized DOC respond to low concentrations of added substrate, *S. typhimurium* was chosen as a model organism. The growth and [^{14}C]glucose utilization of *S. typhimurium* were concurrently measured over a range of glucose concentrations. *S. typhimurium* was unable to grow in the unamended inorganic salts solution, and unlike the other bacteria, which used the uncharacterized DOC, its growth rate and maximum cell density decreased as the initial glucose concentration was lowered (Fig. 5). By using the data in Fig. 5, mean generation times of 79, 42, 23, 14, and 7.3 h were calculated for initial glucose concentrations of 5.0, 10, 20 (data not shown) 40, and 80 μg/liter, respectively. A linear regression on the generation times versus the inverse of concentration (17) yields a K_s value for growth of 95 μg of glucose per liter ($r = 0.99$). The organism did not grow at a glucose concentration of 1.0 μg/liter, the inoculum size being reduced in this instance to allow for detection of a possible population increase. Growth at an initial glucose concentration of 5 μg/liter ceased before a substantial amount of the glucose carbon was mineralized (Fig. 5b). These data indicate that the threshold concentration for the growth of *S. typhimurium* is between 1.0 μg and 5.0 μg of glucose per liter. The variability between replicate growth curves was greater at glucose concentrations less than 40 μg/liter than at higher levels, and so both growth curves are presented in Fig. 5 for these lower concentrations. The area between the curves of radioactivity remaining in solution and ^{14}C left in the filtrate represents the percentage of the ^{14}C assimilated by the cells.

A study was conducted to determine whether a bacterium which could not use the uncharacterized DOC could metabolize a characterized substrate (glucose) at levels too low to support growth when that organism is provided with a second substrate (arabinose) at high enough concentrations to support growth. For this purpose, *S. typhimurium* was inoculated into the salts solution containing 0.5 μg of labeled glucose per liter as the sole carbon source or into the same medium containing 5.0 mg of arabinose per liter as well as the glucose. The cell numbers and mineralization of glucose were determined at regular intervals. The population size did not increase in the medium with only the trace amount of glucose, but its numbers rose if arabinose was present (Fig. 6). As *S. typhimurium* grew on arabinose, it metabolized the glucose, despite the fact that the hexose was at a concentration well below the level of glucose that would sustain growth.

A study was then conducted to determine the percentage of [^{14}C]glucose converted to CO_2 and cells in a medium containing 5.0 mg of arabinose per liter and different concentrations of glucose. The percentage of [^{14}C]glucose incorporated into the cells in the presence of arabinose was the same at glucose concentrations of 5, 0.5, and 0.25 μg/liter and was comparable to the amounts incorporated by *S. typhimurium* grown in a medium with high concentrations of glucose as the sole carbon and energy source (Table 1). In media with glucose as the sole carbon source, reducing the sugar concentration to levels of 40 μg/liter or lower progressively reduced the extent of glucose incorporation, and lowering the concentration to 10 or 5 μg/liter reduced the extent of mineralization.

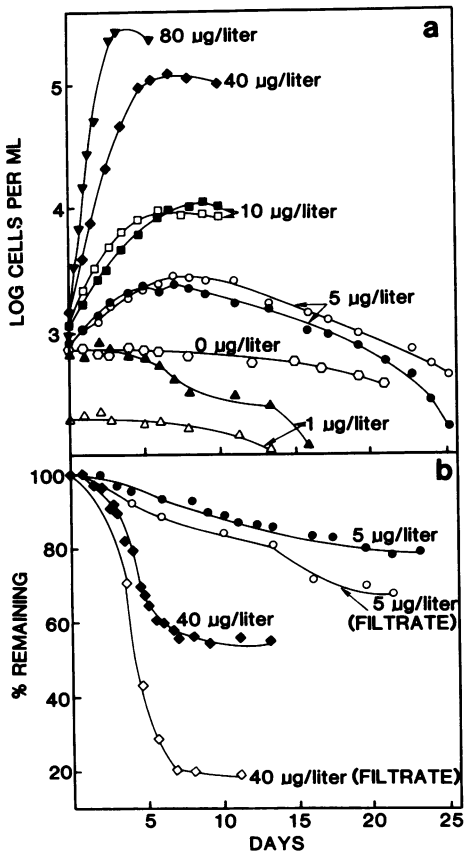

FIG. 5. (a) Growth of *S. typhimurium* at different concentrations of glucose added to minimal salts media. (b) The percentage of ^{14}C remaining in the suspensions (closed symbols) and filtrates (open symbols) at initial glucose concentrations of 5 and 40 μg/liter.

DISCUSSION

The ability of *P. acidovorans* and *Pseudomonas* sp. strain ANL to multiply in an unamended inorganic salts solution is attributed to their utilization of uncharacterized DOC. Similar oligocarbophilic growth has been observed for a number of bacteria (6, 18) and is usually attributed to the dissolving in the media of volatile organic substances from the air (3, 5, 10). Although our experiments were conducted with glass-stoppered flasks, air exchange with the atmosphere did occur when samples were taken, so that volatile organic substances could be responsible for some of the growth; nevertheless, because 50 to 85% of the DOC available to *P. acidovorans* could be removed by pretreatment of the water with $K_2S_2O_8$, volatile organic substances were not responsible for all the growth. In different experiments, *P. acidovorans* reached different final counts in the unamended inorganic salts solution, indicating dissimilar DOC levels in these solutions. Regardless of its source, the uncharacterized DOC had a significant effect on the biodegradation of test substrates present at very low concentrations. Because the concentration of available DOC was usually at least 100 times greater than that of the added substrates, the growth rates and cell yields of organisms capable of utilizing the uncharacterized DOC were determined by those unknown compounds. After most of the DOC in the media had been exhausted, *Pseudomonas* sp. strain ANL was able to utilize simultaneously low concentrations of aniline and glucose.

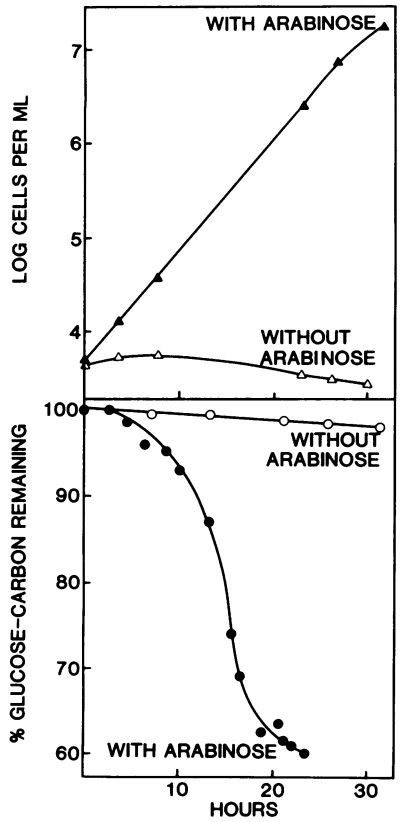

FIG. 6. Numbers of *S. typhimurium* cells and mineralization of labeled glucose in a medium with an initial glucose concentration of 0.5 μg/liter with and without 5 mg of unlabeled arabinose per liter. Only glucose mineralization was determined.

TABLE 1. Conversion of [^{14}C]glucose to CO_2 and cells by *S. typhimurium* in medium with 5.0 mg of unlabeled arabinose per liter and different glucose concentrations

Glucose concn (μg/liter)	% [^{14}C]glucose converted to CO_2 and cells in medium:			
	Without arabinose		With arabinose	
	CO_2	Cells	CO_2	Cells
0.25	ND[a]	ND	42	46
0.50	ND	ND	42	48
5.0	9	3	42	46
10	27	11	ND	ND
20	42	31	ND	ND
40	44	36	ND	ND
500	41	47	ND	ND
10,000	42	46	ND	ND

[a] ND, not determined.

The ability of heterogeneous bacterial communities to metabolize mixtures of carbon compounds simultaneously has long been known (15), and the simultaneous use of several substrates by pure cultures of bacteria has been reviewed by Harder and Dijkhuizen (4). Recently, it was shown that a synthetic compound (methylene chloride) can be degraded simultaneously with acetate by a pure culture of bacteria (7). The present study is the first demonstration that aromatic pollutants can be utilized simultaneously with other compounds by pure cultures of bacteria.

The simultaneous use of two substrates is concentration dependent. Thus, *Pseudomonas* sp. strain ANL mineralized glucose and aniline simultaneously when present at 3.0 μg/liter but metabolized them diauxically at 300 μg/liter. In addition the uncharacterized DOC repressed the ability of this organism to utilize low concentrations of glucose or aniline. Although this DOC did not inhibit phenol mineralization by *P. acidovorans*, repression could be demonstrated with as little as 70 μg of acetate per liter. The metabolism of aromatic compounds by members of the genus *Pseudomonas* is usually repressed by the presence of more easily metabolized compounds, especially intermediates of the tricarboxylic acid cycle (20).

The fact that *S. typhimurium* is unable to grow on DOC in distilled water makes it a good organism for studying growth and substrate utilization at very low concentrations of a single substrate. This inability to grow in the inorganic salts solution is not a result of the failure of the organism to grow at low substrate concentrations, because its numbers increased in solutions with a glucose concentration of 5 μg/liter, which is 200 times lower than the level required to qualify it as a facultative oligotroph (6). *S. typhimurium*, however, is not an oligocarbophile (5, 10) because it is unable to grow in unamended inorganic salts solutions.

S. typhimurium was unable to grow at a glucose concentration of 1 μg/liter, a concentration similar to the threshold value of 2.5 μg/liter for *Aeromonas hydrophila* (17). The presence of arabinose at levels adequate for growth allowed *S. typhimurium* to mineralize glucose at concentrations well below the threshold for replication. This ability to metabolize low levels of glucose is not the result of an increased affinity for glucose in the presence of the pentose, since the rate of mineralization of 5 μg of glucose per liter is ca. 1 fg/h per cell whether arabinose is present or not. The ability to mineralize glucose rapidly at concentrations below the threshold appears to be related to (i) the concentration of arabinose being high enough to meet maintenance and growth requirements and thus the number of cells capable of

utilizing glucose increased and (ii) arabinose not interfering with utilization of glucose.

The results with the three bacteria suggest that the rate and extent of biodegradation of low concentrations of synthetic organic compounds in nature may be controlled by the presence of higher concentrations of other organic molecules. The data also suggest that the stimulation or inhibition by second substrates of the biodegradation of synthetic compounds depends on the organisms in the environment, the concentration of the second substrate, and the particular synthetic chemical that is being degraded.

ACKNOWLEDGMENTS

This investigation was supported by funds provided by the U.S. Army Research Office, the U.S. Environmental Protection Agency under assistance agreement CR809735-02-0, and the Cooperative State Water Resources Research Program.

LITERATURE CITED

1. **Boethling, R. S., and M. Alexander.** 1979. Effect of concentration of organic chemicals on their biodegradation by natural microbial communities. Appl. Environ. Microbiol. **37:** 1211–1216.
2. **Favero, M. S., L. A. Carson, W. W. Bond, and N. J. Petersen.** 1971. *Pseudomonas aeruginosa*: growth in distilled water from hospitals. Science **173:**836–838.
3. **Geller, A.** 1983. Growth of bacteria in inorganic medium at different levels of airborne organic substances. Appl. Environ. Microbiol. **46:**1258–1262.
4. **Harder, W., and L. Dijkhuizen.** 1982. Strategies of mixed substrate utilization in microorganisms. Philos. Trans. R. Soc. Lond. B Biol. Sci. **297:**459–479.
5. **Hirsch, P.** 1964. Oligocarbophilie (Wachstum auf Kosten von Luftverunreinigungen) bei Mycobakterien und einigen ihnen nahestehenden Actinomyceten. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Ref. **194:**70–82.
6. **Ishida, Y., I. Imai, T. Miyagaki, and H. Kadota.** 1982. Growth and uptake kinetics of a facultatively oligotrophic bacterium at low nutrient concentrations. Microb. Ecol. **8:**23–32.
7. **LaPat-Polasko, L. T., P. L. McCarty, and A. J. B. Zehnder.** 1984. Secondary substrate utilization of methylene chloride by an isolated strain of *Pseudomonas* sp. Appl. Environ. Microbiol. **47:**825–830.
8. **Law, A. T., and D. K. Button.** 1977. Multiple-carbon-source-limited growth kinetics of a marine coryneform bacterium. J. Bacteriol. **129:**115–123.
9. **Menzel, D. W., and R. F. Vaccaro.** 1964. The measurement of dissolved organic and particulate carbon in seawater. Limnol. Oceanogr. **9:**138–142.
10. **Moaledj, K., and J. Overbeck.** 1980. Studies on uptake kinetics of oligocarbophilic bacteria. Arch. Hydrobiol. **89:**303–312.
11. **Palleroni, N. J.** 1983. *Pseudomonas* Migula 1894, p. 141–142. *In* J. G. Holt and N. R. Krieg (ed.), Bergey's manual of determinative bacteriology, 9th ed., vol. 1. The Williams & Wilkins Co., Baltimore.
12. **Powell, E. O.** 1967. The growth rate of microorganisms as a function of substrate concentration, p. 34–56. *In* E. O. Powell, C. G. T. Evans, R. E. Strange, and D. W. Tempest (ed.), Microbial physiology and continuous culture. Her Majesty's Stationery Office, London.
13. **Romanenko, V. I., and E. P. Nikiforova.** 1974. Growth of bacteria on dissolved organic matter of freshwater reservoirs. Microbiology (Engl. Transl. Mikrobiologiya) **43:**112–115.
14. **Shehata, T. E., and A. G. Marr.** 1971. Effect of nutrient concentration on the growth of *Escherichia coli*. J. Bacteriol. **107:**210–216.
15. **Stumm-Zollinger, E.** 1966. Effects of inhibition and repression on the utilization of substrates by heterogeneous bacterial communities. Appl. Microbiol. **14:**654–664.
16. **Subba-Rao, R. V., H. E. Rubin, and M. Alexander.** 1982. Kinetics and extent of mineralization of organic chemicals at trace levels in freshwater and sewage. Appl. Environ. Microbiol. **43:**1139-1150.
17. **van der Kooij, D., A. Visser, and W. A. M. Hijnen.** 1980. Growth of *Aeromonas hydrophila* at low concentrations of substrates added to tap water. Appl. Environ. Microbiol. **39:**1198–1204.
18. **van der Kooij, D., A. Visser, and J. P. Oranje.** 1982. Multiplication of fluorescent pseudomonads at low substrate concentrations in tap water. Antonie van Leeuwenhoek J. Microbiol. Serol. **48:**229–243.
19. **van Es, F. B., and L. A. Meyer-Reil.** 1982. Biomass and metabolic activity of heterotrophic marine bacteria. Adv. Microb. Ecol. **6:**111–170.
20. **Weitzman, P. D. J., and D. Jones.** 1968. Regulation of citrate synthase and microbial taxonomy. Nature (London) **219:** 270–272.