

Role of Dissolution Rate and Solubility in Biodegradation of Aromatic Compounds

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Strains of *Moraxella* sp., *Pseudomonas* sp., and *Flavobacterium* sp. able to grow on biphenyl were isolated from sewage. The bacteria produced 2.3 to 4.5 g of protein per mol of biphenyl carbon, and similar protein yields were obtained when the isolates were grown on succinate. Mineralization of biphenyl was exponential during the phase of exponential growth of *Moraxella* sp. and *Pseudomonas* sp. In biphenyl-supplemented media, *Flavobacterium* sp. had one exponential phase of growth apparently at the expense of contaminating dissolved carbon in the solution and a second exponential phase during which it mineralized the hydrocarbon. Phase-contrast microscopy did not show significant numbers of cells of these three species on the surface of the solid substrate as it underwent decomposition. *Pseudomonas* sp. did not form products that affected the solubility of biphenyl, although its excretions did increase the dissolution rate. It was calculated that *Pseudomonas* sp. consumed 29 nmol of biphenyl per ml in the 1 h after the end of the exponential phase of growth, but 32 nmol of substrate per ml went into solution in that period when the growth rate had declined. In a medium with anthracene as the sole added carbon source, *Flavobacterium* sp. converted 90% of the substrate to water-soluble products, and a slow mineralization was detected when the cell numbers were not increasing. *Flavobacterium* sp. and *Beijerinckia* sp. initially grew exponentially and then arithmetically in media with phenanthrene as the sole carbon source. Calculations based on the growth rates of these bacteria and the rates of dissolution of phenanthrene suggest that the dissolution rate of the hydrocarbon may limit the rate of its biodegradation.

In the routine testing for biodegradation, organic chemicals are added to aqueous solutions at concentrations of 2 to 100 µg/ml. Because these concentrations exceed the water solubilities of many organic compounds, the validity of such tests has been questioned (2, 6). The metabolism of several organic substrates with water solubilities below 10 µg/ml at 25°C is well characterized (7, 10).

To mineralize or grow on substrates having low solubilities in water, microorganisms may require some physiological adaptation. Particular attention has been given to the growth and utilization of aliphatic hydrocarbons by bacteria (3, 14). Several mechanisms to facilitate the uptake of aliphatic hydrocarbons are known, for example, the formation of emulsifiers (9, 16) or the modification of the cell surface to increase its affinity for hydrophobic substrates and thus facilitate their absorption (11, 12). Aromatic compounds are of special interest because many are significant environmental pollutants, and millions of tons of such chemicals are used each year. Concern with the possible ecological effects of some of the aromatic compounds that are poorly soluble in water has resulted in a request by the U.S. Environmental Protection Agency for information on their biodegradation, as in the cases of anthraquinone, cumene, and biphenyl (20). The bacterial utilization of several aromatic hydrocarbons with low water solubilities has been investigated (14, 21). It has also been reported recently that the rate of mineralization at the end of the active phase of biodegradation by a mixed culture of microorganisms was less than the rate of spontaneous dissolution of palmitic acid but greater than the rate of spontaneous dissolution of octadecane (18).

The present study was designed to relate the kinetics of bacterial growth on several poorly soluble aromatic compounds to their rates of dissolution and solubilities in water.

MATERIALS AND METHODS

Medium. The inorganic salts solution contained (per liter of deionized water) 775 mg of K₂HPO₄, 350 mg of KH₂PO₄, 200 mg of (NH₄)₂SO₄, and 100 mg of MgSO₄ · 7H₂O. One milliliter of a trace element solution (17) was added after the salts solution was sterilized. The final pH of the medium was 7.2. Glucose and succinate were autoclaved separately and added aseptically to the autoclaved medium. Stock solutions of aromatic hydrocarbons prepared in dichloromethane (5 and 50 g per liter) were added to empty sterile 250-ml incubation bottles by means of positive displacement pipettes (Scientific Manufacturing Industries, Emeryville, Calif.). The inorganic salts solution was added to these bottles after complete evaporation of the solvent. All glassware was cleaned in Nochromix (Godax Laboratories, Inc., New York, N.Y.).

Isolation of microorganisms. Sewage samples from the settling tanks of the Ithaca, N.Y., and Marathon, N.Y., sewage treatment plants were passed through Whatman no. 1 filter papers to remove particulate matter. Portions (1.0 ml) of the filtrate were added to screw-capped 30-ml test tubes containing 1 to 5 mg of an insoluble carbon source and 10 ml of the inorganic salts solution. The test tubes were incubated for 7 days at 29°C on a rotary shaker operating at 120 rpm, and 100-µl portions of the enrichment cultures were transferred to new medium and incubated for another 7 days. Portions (0.1 ml) of 10-fold dilutions of the second enrichment cultures were plated on a medium containing 13 g of Bacto-Agar (Difco Laboratories, Detroit, Mich.) and 3.5 g of Trypticase soy broth (BBL Microbiology Systems, Cocksville, Md.) per liter of deionized water. After incubation of

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the plates at 29°C for 3 to 10 days, individual colonies were picked and inoculated into fresh media. Each bacterial culture was successively transferred three times from solid nonselective medium to liquid medium containing the test compound.

Beijerinckia sp. (wild type) was provided by D. T. Gibson, University of Texas, Austin.

Quantification of growth. Bacterial growth yields on limiting carbon sources were determined with 10 ml of medium contained in 30-ml screw-capped test tubes. The media contained no added carbon source or 0.50, 1.0, 2.5, 5.0, or 7.5 mM succinate, 0.065, 0.13, 0.26, 0.325, or 0.49 mM biphenyl, or 0.070, 0.14, 0.21, or 0.28 mM phenanthrene. Duplicate tubes of media at each concentration were inoculated with 0.03 ml of a culture that had been grown on the test chemical, and the cultures were incubated for 10 days at 29°C on a rotary shaker operating at 60 rpm. Growth was then quantified by duplicate determinations of cell protein (13), and the yield was determined from the slope of a plot of protein against substrate concentration.

The kinetics of growth were determined with duplicate 50-ml cultures contained in 250-ml bottles that were sealed with gas-tight stoppers (Pierce Chemical Co., Rockford, Ill.) and shaken at 90 rpm at 29°C. The inocula were obtained by diluting cultures in the exponential phase of growth. Samples were taken at intervals with sterile 1-ml syringes to determine $^{14}\text{CO}_2$ and viable counts by plating on Trypticase soy agar.

Quantification of mineralization. Mineralization was determined on duplicate samples by trapping the $^{14}\text{CO}_2$ that was formed during bacterial growth on ^{14}C -labeled compounds. The apparatus to collect $^{14}\text{CO}_2$ contained four sealed 20-ml glass vials; the caps of each were fitted with 20-gauge needles to allow incoming air to pass through the liquid in the vial and to permit the air to leave via the headspace. The needle attached to one vial was connected to the needle fit into the next vial with polyethylene tubing. The first vial contained 1.0 ml of 1 M H_2SO_4 to drive off the $^{14}\text{CO}_2$. The second vial contained 8.0 ml of Liquiscint (National Diagnostics Inc., Somerville, N.J.) to trap volatile organic compounds. The last two vials contained 5.0 ml of 1 M NaOH to trap $^{14}\text{CO}_2$. Air was slowly bubbled through the system. The trapping of $^{14}\text{CO}_2$ was initiated by adding a 1.0-ml portion from the culture to the first vial and flushing air through the system for 10 min. In tests involving the addition of labeled bicarbonate, which was obtained from New England Nuclear Corp. (Boston, Mass.), more than 90% of the radioactivity added (18.3 kBq) was recovered in the third vial, and usually less than 0.5% of the counts was recovered in the fourth vial. Duplicate 2.0-ml portions each from vials 3 and 4 were added to plastic vials containing 9 ml of Liquiscint. The radioactivity was counted with a liquid scintillation counter (model LS 7500; Beckman Instruments, Inc., Irvine, Calif.).

Mass transfer rates and solubility. The rates of mass transfer from solid to liquid state (or dissolution) were determined by using the identical type of bottle and the same conditions used for the tests of mineralization, except that no organisms were added. The rates for the labeled compounds were determined by counting the radioactivity, whereas the rates for unlabeled compounds were determined by gas chromatography. The test compound (50 μg) was dissolved in dichloromethane, the solution was added to 250-ml bottles, and the solvent was evaporated. The inorganic salts solution then was added, and the bottle was placed immediately in a water bath set at 29°C and operating

at 90 rpm. Duplicate samples that contained no visible particles were taken from the soluble phase with a syringe after 1, 2.5, 5, 10, 15, and 20 min. Samples (1 ml) were added to plastic vials, and 9 ml of Liquiscint was added to count the radioactivity. For gas chromatographic analyses, 4.0-ml samples were vigorously shaken with 0.5 ml of hexane, and 2- μl portions of the organic phase were injected into a gas chromatograph (model 3920B; Perkin Elmer Corp., Norwalk, Conn.) equipped with a flame ionization detector and fitted with a glass column packed with 3% OV17 on 100/120 mesh Gas Chrom Q (Supelco Inc., Bellefonte, Pa.). The injector and detector temperatures were maintained at 220 and 250°C, respectively. The column temperatures were 150, 190, and 200°C for biphenyl, phenanthrene, and anthracene, respectively, and the retention times of these compounds were 2.3, 3.8, and 3.2 min, respectively. The nitrogen flow was maintained at about 35 ml/min. The dissolution rates were measured twice with duplicate determinations each time.

The solubility of the aromatic compounds was determined in duplicate by adding 50 μg of chemical, which had first been dissolved in dichloromethane, to a 250-ml bottle. After the solvent had evaporated, 50 ml of the salts solution was added, and the bottle was incubated at 29°C for 24 h on a rotary shaker operating at 90 rpm. Samples (2.0 ml) containing no visible particles were then withdrawn for analysis of radioactivity. Because of the small variation between duplicate analyses, it is likely that particles were not present in the aqueous phase of the samples.

Chemicals. [^{14}C]biphenyl (588 MBq/mmol) and [^{14}C]phenanthrene (796 MBq/mmol) were purchased from Pathfinder Laboratories, St. Louis, Mo. The radiopurity of the compounds was stated by the manufacturers to exceed 98%. [^{14}C]anthracene (559 MBq/mmol; 86% radiopurity) was obtained from Amersham Corp., Arlington Heights, Ill.

Unlabeled biphenyl and phenyl ether were purchased from Aldrich Chemical Co., Milwaukee, Wis., acenaphthene and anthracene were from Eastman Kodak Co., Rochester, N.Y., and 1,2-dihydroxyanthraquinone and anthraquinone were from Fisher Scientific Co., Fair Lawn, N.J. All other chemicals were of analytical grade and were purchased from either Mallinckrodt Inc., Paris, Ky., or Sigma Chemical Co., St. Louis, Mo.

RESULTS

Enrichment and isolation of pure cultures. Enrichments were performed with acenaphthene, anthraquinone, anthracene, biphenyl, 1,2- and 1,8-dihydroxyanthraquinone, phenanthrene, and phenyl ether as sole carbon sources. Growth was observed in all enrichments except for the anthraquinones, but, except for the isolates acting on biphenyl, the growth appeared slowly. Three cultures able to grow on biphenyl were isolated. Strain 43 also grew on phenanthrene. In a medium containing labeled anthracene, strain 43 caused the disappearance of the insoluble substrate, but it formed only trace amounts of $^{14}\text{CO}_2$; however, nearly all the ^{14}C was recovered as water-soluble products.

Bacterium 11 was identified as a strain of *Moraxella* sp. It was a gram-negative, short, plump rod (1.1 by 1.2 μm), it was oxidase and catalase positive, and it used no carbohydrates as growth substrates but grew on succinate and biphenyl in the absence of growth factors. Bacterium 31 was identified as *Pseudomonas* sp. Electron micrographs showed it to be a rod-shaped bacterium (1.2 by 0.6 μm) with one or two polar flagella. It accumulated polyphosphate when

TABLE 1. Yields of bacterial protein and bacterial growth rates (μ) on several substrates

Substrate	Bacterium	Protein yield (g/mol of C) ^a	μ (doublings per h)
Biphenyl	<i>Flavobacterium</i> sp.	2.3	0.04
	<i>Moraxella</i> sp.	4.4	0.14
	<i>Pseudomonas</i> sp.	4.5	0.27
Phenanthrene	<i>Flavobacterium</i> sp.	3.3	0.19
	<i>Beijerinckia</i> sp.	3.5	0.23
Succinate	<i>Flavobacterium</i> sp.	3.0	ND ^b
	<i>Moraxella</i> sp.	4.0	ND
	<i>Pseudomonas</i> sp.	4.2	ND
	<i>Beijerinckia</i> sp.	3.5	ND

^a Error of mean, $\pm 5\%$.

^b ND, Not determined.

grown in a succinate-inorganic salts medium. It was gram negative, oxidase positive, and it liquified gelatin. Both bacteria produced visible turbidity in 2 days. Strain 43 was identified as *Flavobacterium* sp. It produced a yellow, water-insoluble pigment on solid media. It was a facultatively aerobic, gram-negative, and oxidase- and catalase-positive rod (1.9 by 0.7 μm) with multitrichous flagella. When grown on solid media in a closed jar in which the air phase contained biphenyl, *Flavobacterium* sp. released a yellow, water-soluble compound into the medium.

Growth on aromatic hydrocarbons. The growth rates and yields of the three isolates and of *Beijerinckia* sp. on biphenyl, phenanthrene, or both were determined. *Moraxella* sp. and *Pseudomonas* sp. did not grow in media with phenanthrene as the sole carbon source. The cell yield on succinate was also measured; this compound is a presumed intermediate in biphenyl degradation (7). The three isolates produced similar amounts of cell protein in media containing biphenyl or succinate, although the yield of protein on both substrates was lower for *Flavobacterium* sp. than for the other two isolates (Table 1). The three organisms differed markedly in their rates of growth on biphenyl. Gas chromatographic analysis, which had a sensitivity for biphenyl of 0.1 $\mu\text{g/ml}$, failed to show the presence of the compound after 8 days of incubation, indicating complete metabolism of the chemical. *Flavobacterium* sp. and *Beijerinckia* sp. also used phenanthrene as their sole carbon and energy sources, and their yields and growth rates in such media were similar.

The kinetics of growth of the three isolates and their formation of $^{14}\text{CO}_2$ in media containing 162 μM labeled biphenyl were measured. *Flavobacterium* sp. had two exponential growth phases in such media (Fig. 1). Because the rate and extent of growth in the first phase were about the same in solution without biphenyl (data not shown), it is likely that the bacterium grew first on contaminating dissolved organic carbon in the medium before it degraded biphenyl. No such biphasic growth was evident during the development of *Moraxella* sp. or *Pseudomonas* sp. The formation of $^{14}\text{CO}_2$ paralleled the increase in cell number and was logarithmic during the single exponential phase of the latter two isolates and during the second exponential phase of *Flavobacterium* sp. The extents of mineralization were 37, 63, and 64% for *Flavobacterium* sp., *Moraxella* sp., and *Pseudomonas* sp., respectively.

Flavobacterium sp. grew exponentially on 84 or 318 μM phenanthrene (Fig. 2); 40 and 30% of the substrate were mineralized at those concentrations. *Beijerinckia* sp. grew at

a slightly faster rate than *Flavobacterium* sp., and it mineralized 40 and 33% of the hydrocarbon at the lower and higher concentrations, respectively. Gas chromatographic analysis, which was sensitive to 25 ng/ml, revealed no residual substrate at the end of the experiment with 84 μM phenanthrene and less than 0.6 $\mu\text{g/ml}$ for both bacteria in media with 318 μM phenanthrene. The exponential phase of growth of *Flavobacterium* sp. slowed down at densities of about 7×10^6 and 3×10^7 cells per ml at the lower and higher substrate concentrations, respectively, but growth and mineralization still continued thereafter. The accumulation of products inhibiting growth did not seem to be the cause of the termination of the exponential phase because the rate of increase in cell number of *Flavobacterium* sp. was the same in sterile spent medium [prepared by growing the culture in 280 μM phenanthrene for 100 h, removing the cells by centrifugation, filter sterilizing the solution, and supplementing it with 200 mg of $(\text{NH}_4)_2\text{SO}_4$ per liter and 84 μM phenanthrene as in the original culture].

Flavobacterium sp. and *Beijerinckia* sp. grew in liquid media containing 5.6 μM labeled anthracene, and the population increased from 10^3 to 10^6 cells per ml within 24 h (data not shown), probably at the expense of contaminating dissolved organic carbon. The substrate particles initially present disappeared in 14 days or less. After 14 days, 90% of the ^{14}C initially added was found in solution; in contrast, less than 10% of the ^{14}C was in solution after 24 h.

To study the mineralization of anthracene, *Flavobacterium* sp. was grown in the salts solution containing 2 mM succinate. Duplicate flasks containing 5.6 μM labeled anthracene and 2 mM succinate were then inoculated with 2.5×10^8 cells per ml, and flasks with anthracene alone received 5×10^4 cells per ml. Succinate (7 mM) and 100 mg of $(\text{NH}_4)_2\text{SO}_4$ per liter were added at 24 days to each of the former flasks. The bacterium grew in the medium with only the hydrocarbon, but it brought about detectable mineralization only when the cells were in the stationary phase (Fig. 3). The decline in CO_2 probably resulted from a decline in the rate of $^{14}\text{CO}_2$ production and dilution of labeled CO_2 in solution with unlabeled CO_2 in the headspace in the bottle. In the succinate-supplemented medium, which also had higher cell densities, the mineralization rate was the same, but the period of mineralization lasted considerably longer; 18% of the substrate was mineralized in 40 days. Succinate also increased the cell numbers, although the initial period of mineralization occurred as the cell numbers were falling.

Influence of solubility and dissolution rate. Most of the hydrocarbon added to the media in the previous experiments was initially insoluble. Phase-contrast microscopy did not reveal the presence of cells of *Flavobacterium* sp. *Moraxella* sp., or *Pseudomonas* sp. on the surface of the solid biphenyl that had been added to the inoculated salts solution; therefore, extensive colonization of the solid did not occur.

The solubilities of the hydrocarbons at 29°C in the inorganic salts solutions, which were determined by measuring the amount of ^{14}C in the aqueous phase, were 42 and 9.5 μM for biphenyl and phenanthrene, respectively (Table 2). These values are similar to those cited by Pearlman et al. (15) at 25°C, namely, 46 ± 5 and 7.2 ± 1.9 μM . These authors also reported the solubility of phenanthrene to be 0.37 ± 0.1 μM . Because the rates of dissolution declined somewhat after the first point, the values presented, which are based on the analyses of the first points, may be somewhat greater than those shown. The two methods of measuring dissolution rates gave similar results, namely, about 9 and 3 nM/s for biphenyl and phenanthrene, respectively (Table 2). Be-

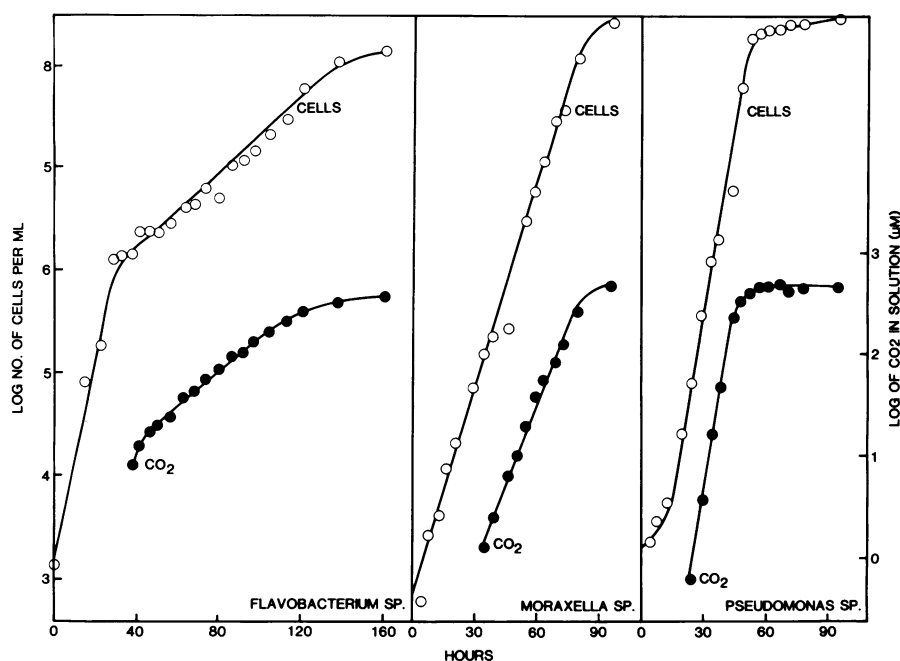


FIG. 1. Mineralization of 162 μM biphenyl by growing cells of *Flavobacterium* sp., *Moraxella* sp., and *Pseudomonas* sp.

cause the radiochemical contaminants may be responsible for the higher apparent dissolution rates determined by the procedure involving ^{14}C , subsequent calculations used only the rates estimated by the chromatographic procedure. The rates determined by means of ^{14}C were always higher than those obtained by gas chromatography.

Because 3.5×10^8 cells of *Pseudomonas* sp., the fastest-growing biphenyl user, were formed per ml in a medium with 162 μM biphenyl, each cell consumed an average of 0.47 fmol of substrate. At the end of the exponential phase in this medium, during which the growth rate was 0.27 doubling per h, the bacterial density was 2.0×10^8 cells per ml. Based on these values, the population at the end of the exponential phase would be expected to produce 6.2×10^7 cells per ml in the next 1 h, a time when some factor is causing a decline in the growth rate. Taking the value of 0.47 fmol of biphenyl consumed per cell, these new cells would consume 29 nmol of substrate per ml, i.e., 29 μM . From the measured dissolution rate of 8.8 nM/s, it can be calculated that 32 μM biphenyl went into solution in this 1-h period. Thus, the dissolution rate did not appear to have limited the biodegradation rate in the preceding phase of exponential growth, although the subsequent multiplication may have been governed by the dissolution rate.

To determine whether solubilizing agents were produced by *Pseudomonas* sp. during its growth in media with 162 μM biphenyl or 10 mM succinate, a culture taken in the stationary phase was centrifuged at $10,000 \times g$, the supernatant fluid was sterilized by passage through filters with a pore size of 0.2 μm , and 55 μM biphenyl was suspended in the liquid. The mixture was incubated at 29°C for 24 h, and the amount of biphenyl in the aqueous phase was determined in duplicate. The solubility in this spent medium was $41 \pm 4 \mu\text{M}$. Because this value is essentially the same as that previously determined for the uninoculated medium, the bacteria apparently did not produce solubilizing agents. On the other hand, the duplicate determinations of the initial dissolution rates of biphenyl in spent medium of succinate-grown and biphenyl-

grown cells gave values of 18.1 ± 2.2 and 29.1 ± 3.2 nM/s, respectively, which are higher than the value of 9.2 nM/s found in the original uninoculated inorganic salts solution.

Flavobacterium sp. failed to grow in media containing 280 μM anthracene, whether the inoculum was derived from cultures grown on 2 nM succinate or 84 μM phenanthrene.

DISCUSSION

Although the mineralization by pure cultures of aromatic compounds with low water solubilities has not been investigated previously, bacteria have been isolated that grew on biphenyl and phenanthrene as the sole carbon source (8, 19, 23), and anthracene has been shown to be oxidized to water-soluble intermediates (4; H. N. Fernley, E. Griffiths, and W. C. Evans, *Biochem. J.* **91**:15P-16P, 1964). Growth of the bacteria on biphenyl in the present study was exponential and was paralleled by the production of CO_2 . The extent of mineralization and the cell yield were lowest for *Flavobacterium* sp.

Growth of *Flavobacterium* sp. and *Beijerinckia* sp. on phenanthrene was exponential only initially. The growth of *Flavobacterium* sp. in media with anthracene as the sole added carbon source was poor and probably resulted from growth on contaminating dissolved organic carbon; although anthracene was readily converted to water-soluble products, the yield of CO_2 was low. Similar observations were made in studies of the transformation of anthracene in sediments, where 89% of the substrate disappeared but only 11% was converted to CO_2 (1).

Most of the hydrocarbon was initially present in the medium as a solid, but microorganisms growing on the surface of the solid substrate were not detected by phase-contrast microscopy. Thus, it may be assumed that the isolates grew at the expense of the dissolved substrate, as suggested by Wodzinski and Coyle (22). In contrast, growth of *Pseudomonas pseudoflava* on palmitic acid was associated with extensive colonization of the surface of the sub-

strate (J. M. Thomas and M. Alexander, unpublished data). Low water solubility does not necessarily mean slow biodegradation, and *Flavobacterium* sp. grew faster on the less soluble phenanthrene than on biphenyl. On the other hand, bacteria may facilitate the uptake of poorly soluble compounds by producing emulsifiers (9) or by the possession of a hydrophobic cell surface (14). It is also possible that the organisms grow only at the expense of the compound in solution and that the rate of dissolution of such chemicals might govern the rate of their biodegradation. Based on the dissolution rate, it can be calculated that dissolved biphenyl would only become limiting when the *Pseudomonas* sp. densities reached 2.5×10^8 cells per ml. Tests were not conducted with higher biphenyl concentrations because the O_2 supply might become limiting at the higher population levels.

Flavobacterium sp. and *Beijerinckia* sp. grew exponentially on phenanthrene until densities of about 10^7 cells per ml were attained. Afterward, the rate of multiplication declined. The cell density reached by exponentially growing cells was the result of phenanthrene consumption, since the bacteria grew only to densities of 5×10^5 cells per ml when no carbon source was added. The increase in cell number in the period between 40 and 80 h for *Flavobacterium* sp. and *Beijerinckia* sp. growing on $318 \mu\text{M}$ phenanthrene was better

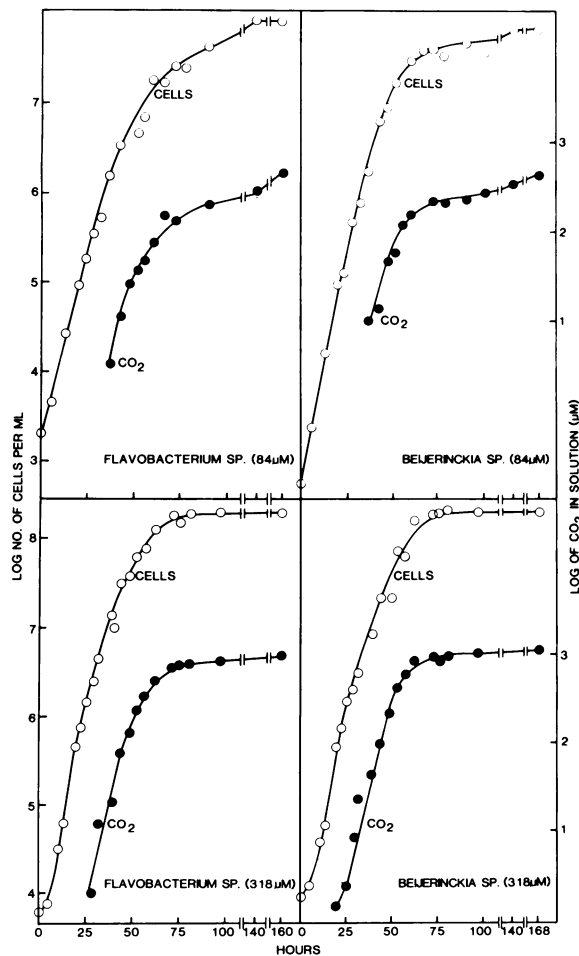


FIG. 2. Mineralization of 84 or $318 \mu\text{M}$ phenanthrene by growing cells of *Flavobacterium* sp. or *Beijerinckia* sp.

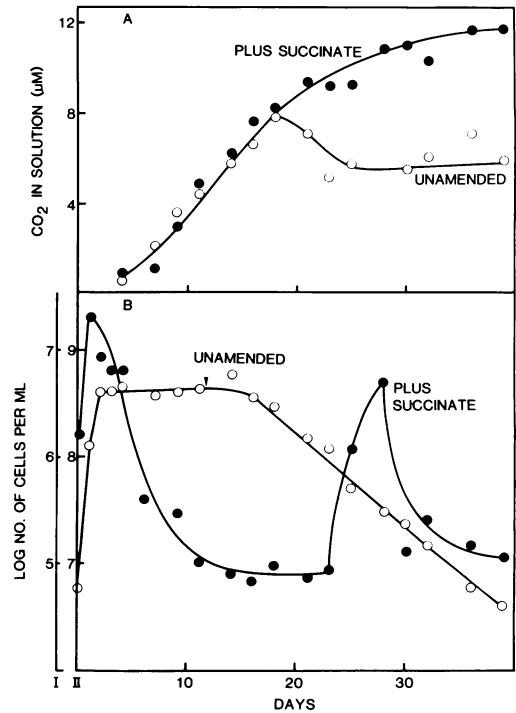


FIG. 3. Mineralization of $5.6 \mu\text{M}$ anthracene and growth of *Flavobacterium* sp. in media with and without succinate. The values for cell numbers on the y axis are for media without (I) and with (II) succinate.

fit by linear plots (correlation coefficient of 0.974 and 0.945 for *Flavobacterium* sp. and *Beijerinckia* sp., respectively) than by exponential plots (correlation coefficients of 0.955 and 0.916, respectively), indicating linear growth on aliphatic hydrocarbons has been reported (5).

From the data that 2.0×10^8 *Flavobacterium* sp. and *Beijerinckia* sp. cells per ml were formed in media initially containing $318 \mu\text{M}$ phenanthrene, it can be calculated that each cell consumed 1.6 fmol of the hydrocarbon. Based on the growth rates of *Flavobacterium* sp. (0.19 doublings per h) and *Beijerinckia* sp. (0.23 doublings per h), the numbers of cells produced in the hour following the end of the exponential phase are calculated to be 4.2×10^6 and 5.2×10^6 cells per ml, respectively. The preceding estimates are based on a population density of 2.0×10^7 cells per ml. Taking the value of 1.6 fmol of phenanthrene per cell, such newly formed cells appearing after the end of the exponential phase would consume $6.7 \mu\text{M}$ substrate for *Flavobacterium* sp. and $9.1 \mu\text{M}$ for *Beijerinckia* sp. Based on the dissolution rate of 2.2 nM/s, $8.3 \mu\text{M}$ phenanthrene should have appeared in solution in this 1-h period, a value which should have supported 5.0×10^6 cells per ml per h. This calculated value is quite similar to the values of 4.5×10^6 *Flavobacterium* sp. cells

TABLE 2. Solubility and initial rates of dissolution of biphenyl and phenanthrene

Chemical	Solubility (μM)	Initial dissolution rate (nM/s)	
		Determined by chromatography	Determined with ^{14}C
Biphenyl	42 ± 4	8.8 ± 0.9	9.2 ± 0.1
Phenanthrene	9.5 ± 1.5	2.2 ± 0.2	4.2 ± 0.3

and 5.6×10^6 *Beijerinckia* sp. cells per ml that were formed during the linear phase of growth of these bacteria in the period between 40 and 80 h. Such estimates support the view that growth is limited by the rate of dissolution of the hydrocarbon.

The exponential growth rate of both bacteria in media containing 84 μ M phenanthrene also declined at densities of about 4×10^6 cells per ml. The dissolution rate should have allowed exponential growth to a fivefold higher cell density. It is not clear why exponential growth ended so soon.

It is not certain whether the rate of dissolution governs the rate of biodegradation of sparingly soluble organic chemicals in natural ecosystems. In nature, microbial growth rates are often low, and the cell densities of species acting on such substrates are probably small. Nevertheless, because many synthetic chemicals have low water solubilities, studies should be conducted to assess the rate of dissolution in governing microbial metabolism in natural conditions.

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