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Modulation of Affinity of a Marine Pseudomonad for Toluene and Benzene by Hydrocarbon Exposure

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Trace (microgram liter^{-1}) quantities of either toluene or benzene injected into an amino-acid-limited continuous culture of *Pseudomonas* sp. strain T2 were utilized immediately with affinities of 2.6 and 6.8 liters g of cells^{-1} h^{-1}, respectively, and yielded large amounts of organic products, carbon dioxide, and cells. The immediate utilization of hydrocarbons by hydrocarbon-deprived organisms helps to establish the nutritional value of nonpolar substrates in the environment. The observation of small Michaelis constants for toluene transport led to tests of metabolic competition between hydrocarbons; however, competitive inhibition of toluene metabolism was not found for benzene, naphthalene, xylene, dodecane, or amino acids. Benzene and terpenes were inhibitory at milligram liter^{-1} concentrations. Toluene was metabolized by a strongly inducible system when compared with benzene. The capacity of toluene to effect larger affinity values increased with exposure time and concentration. The kinetics of induction suggested saturation phenomena, resulting in an induction constant, K_{ind}, of 96 μg of toluene liter^{-1}. Maximal induction of amino-acid-grown cells required about 80 h, with the affinity reaching 317 liters g of cells^{-1} h^{-1}.

The metabolism kinetics of toluene are characterized by very small Michaelis constants for toluene uptake in raw seawater (8) and in cultures of the isolate *Pseudomonas* sp. strain T2 (6). These constants are in the 0.5- to 44-μg-liter^{-1} range, similar to those reported for cell-free systems (unpublished data) and to those reported by Pfaender and Bartholomew (26) for *m*-cresol and chlorobenzene. Such small Michaelis constants raise questions about the effects of structurally similar hydrocarbons on hydrocarbon uptake kinetics, because if, as Gibson (17) suggests, metabolic (or transport) routes are shared, then competition between substrates for common active sites could reduce rates at environmentally relevant concentrations. Also, as the Michaelis constant for transport (K_t) becomes small, the specific affinity (4) must increase if equivalent nutrient flux is to be sustained at high substrate concentration as a result of saturation phenomena. Thus, unusually large specific affinities must be induced and maintained for growth on substrates with small K_t.

The metabolism of one carbon source is often restricted through regulation due to the presence of another in high-substrate-concentration systems (23). In nutrient-limited systems, various additional substrates can enhance the ability of cells to utilize glucose at low concentrations (21). Even in the absence of added hydrocarbon, the ability of the marine isolate *Corynebacterium* sp. strain 198 to metabolize dodecane at concentrations of >1 μg liter^{-1} was maintained for extended periods during arginine-limited continuous culture (3). Hydrocarbons added at equally low concentrations to several aquatic systems (5, 8; D. K. Button and B. R. Robertson, Limnol. Oceanogr., in press) were attacked within minutes to hours. Therefore, hydrocarbon metabolism may be an important component of aquatic microbiological processes, as further indicated by the substantial activity of indigenous marine microflora toward terpenes (5).

In contrast to the somewhat constitutive nature of hydrocarbon metabolism implied above, it is well known that hydrocarbon metabolism often proceeds by inducible systems (15, 24). However, information on the induction kinetics of substrates is very limited (25). Spain et al. (28) found that *p*-nitrophenol concentrations of 10 μg liter^{-1} were required for accelerated decomposition in a mixed community. Such amphipathic (29) substrates may be accumulated differently than those which are more lipophilic (6), leading to different conditions required for induction. Therefore, the dependency of hydrocarbon metabolism capacity on culture history is unclear.

Accurate evaluation of nutrient transport ability is difficult. Early experiments with phosphate limitation demonstrated that harvested organisms could lose significant capacity for nutrient transport. Dependable capacity measurements are necessary for understanding potential contributions from substrates at given concentrations (6). Rapid transfer procedures (27) were developed to avoid this artifact, with success confirmed by analysis of isotope relaxation profiles (7). Other attempts to avoid imprecisions in the transport kinetic data derived from disturbed cultures include a perturbation technique (10, 18), in which the limiting nutrient is raised to analytically detectable levels and the rate of decrease is measured. To study the kinetics and metabolic interactions of hydrocarbons as they may occur in the environment, where other substrates can provide the main carbon source, a technique is needed that will yield accurate analyses of trace substrates without disturbing the system. The present technique involves establishing carbon-(amino acid) limited growth in continuous culture and evaluating the distribution kinetics of traces of radioactive hydrocarbon injected into the reactor. Organisms with a well defined culture history can then be evaluated for their ability to metabolize combinations of hydrocarbons while simulating the nutrient-limited, quasi-steady-state conditions of the environment.

This trace-injection technique failed to show competitive inhibition between toluene and benzene. Instead, the specific affinity for toluene was increased by the addition of small amounts of benzene. This led to an examination of the

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TABLE 1. Nomenclature

Symbol	Definition	Units
A	Substrate, toluene; $A_{out(0)}$ concentration of A injected into reactor at time zero; A_{out}, substrate surrounding cells	g liter^{-1}
B	Substrate, benzene (see A)	g liter^{-1}
C	Constant of integration	Dimensionless
a_A	Affinity of organisms for substrate as defined by equation 3; superscript gives the substrate concentration in micrograms liter^{-1} at which the value is observed; $a^{max}{}_A$, maximal value of the affinity for substrate A; a_{QA}, partial affinity based on product Q produced from substrate A	Liters g of cells^{-1} h^{-1}
D	Dilution rate, F/V	h^{-1}
F	Flow of medium into and from the reactor	Liters h^{-1}
K	Saturation constant; K_t, Michaelis or substrate concentration at half-maximal transport rate; K_{ind}, induction constant (concentration at 0.5 $a^{max}{}_A$)	g liter^{-1}
P	Organic products, in units of substrate mass on a carbon basis	g liter^{-1}
Q	Second product of substrate, CO_2 (See P)	g liter^{-1}
t	Time	h
V	Volume of reactor	Liters
v	Rate of substrate uptake; v_A, rate of uptake of A; v_{PA}, rate of formation of P from A; v_{K_t}, rate of uptake at concentration K_t	g of A liter^{-1} h^{-1}
X	Biomass (wet weight)	g liter^{-1}
Y	Yield; Y_{XA}, yield of cells produced from substrate A; Y_{QA}, yield of Q produced from A	g of product g of substrate consumed^{-1}

concentration dependency of induction rates. Levels of induction resulting from exposure to low hydrocarbon concentrations are reported in terms of specific affinity, a value which gives an absolute measure of the concentration-dependent ability of organisms to metabolize the substrate.

MATERIALS AND METHODS

Organism and medium. The marine isolate *Pseudomonas* sp. strain T2 was isolated by growth from toluene vapor (8) and maintained on agar slants with periodic transfer and growth in shake flasks. The medium was made up of an amino-acid-amended artificial seawater solution (19) without further additions of hydrocarbon. Growth was at 10°C. For continuous culture, equal quantities of the 20 proteogenic amino acids were added to achieve a total of 1.0 mg of carbon liter^{-1}. Noninduced organisms were obtained by withholding hydrocarbon from the medium during batch growth on amino acids (100 mg of casein hydrolysate liter^{-1}; Difco Laboratories, Detroit, Mich.) for at least 1 week. Induced cells were produced by suspending an open glass bulb containing the appropriate hydrocarbon above the amino-acid-amended culture medium (11). For toluene, this resulted in a concentration of about 35 mg liter^{-1}. Medium sterilization was by passage through filters (pore size, 0.2 μm; Nuclepore Corp., Pleasanton, Calif.).

Continuous culture. The previously described (21) two-phase (medium and air) system was initially supplied with 20 liters of medium; carboys of fresh medium were connected as required. The 2-liter reactor was operated with 1,200 ml of culture fluid and either autoclaved unlabeled or aseptic radioactive (9) hydrocarbons were injected with a syringe through a rubber-stopper port.

Isotopes. [U-^{14}C]benzene (30 mCi mmol^{-1}) and [ring-1,2,3,4,5,6-^{14}C]toluene (50 mCi mmol^{-1}) were obtained from New England Nuclear Corp., Boston, Mass. Purification was by cold-finger transfer to minimize carry-over of nonhydrocarbon constituents (9).

Cell preparations. Cells were harvested (2,000 × *g*) from a batch growth in exponential phase, washed twice with basal medium, and suspended with hydrocarbons at the concentrations and times indicated.

Analytical. Biomass was measured by monitoring electrical resistance (Coulter Counter model Z_{B1}; Coulter Electronics, Inc., Hialeah, Fla.). [^{14}C]toluene and [^{14}C]benzene were determined by the amount of radioactivity lost upon sparging with nitrogen at pH 7.8 or by A_{260}. $^{14}CO_2$ was determined by liberating with acid, drying the resulting gas stream, purifying the vapor with hydrophobic resin, collecting it in scintillation fluid, and measuring the amount by scintillation spectrometry (9). Organic products were estimated from the soluble nonvolatile radioactivity produced from toluene or benzene, by comparison with cell-free controls. Hydrocarbons were removed from 100 ml of culture at a rate of 0.4 min^{-1} by a 200-ml min^{-1} stream of nitrogen for 10 min.

Formulations. The general carbon distribution equation for the microbial utilization of organic substrate A (Table 1) is

$$\begin{array}{ccccccc} \text{Substrate} & \rightarrow & \text{Organic products} & + & CO_2 & + & \text{Cells} \\ A & \rightarrow & P & + & Q & + & X \end{array} \quad (1)$$

The rate of substrate depletion, v_A, is given by the sum of the product formation rates:

$$v_A = v_{PA} + v_{QA} + v_{XA} \quad (2)$$

The rate of accumulation of substrate A from solution by microorganisms is (4)

$$v_A = a_A X A_{out} \quad (3)$$

Since the uptake rate v_A is equivalent to the sum of the product formation rates, the portion of uptake devoted to each product is specified by the same portion of the affinity designated as the respective partial affinity: e.g., a_{QA} is the part of the affinity devoted to carbon dioxide formation. The kinetics of substrate accumulation are then computed from the rate of formation of CO_2 from $v_A/v_{QA} = a_A/a_{QA}$ according to yield (see Table 3).

The kinetics of accumulation of a substrate such as A (toluene) injected in a small amount into a continuous culture at steady state are calculated as follows. From a material balance the rate of change of A_{out} in the reactor is given by change = input − loss through the outflow − uptake by the

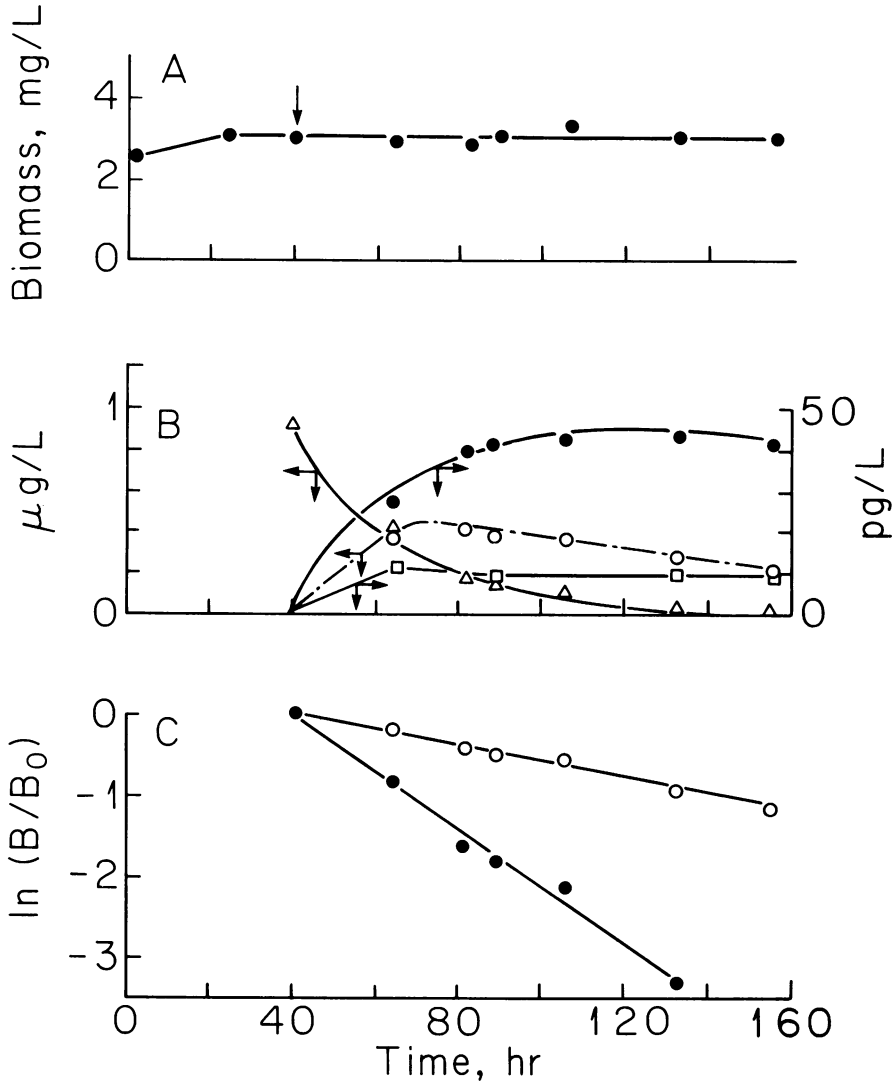

FIG. 1. Kinetics of metabolism of benzene injected (1 μg liter^{-1}) at a dilution rate D (equal to the specific growth rate) of 0.011 h^{-1}. (A) Biomass versus time after benzene injection. Arrow, time of [^{14}C]benzene injection. (B) Conversion kinetics of benzene (△) to carbon dioxide (●), organic products (○), and cells (□) (notice scale change [marked by arrows] and that ^{14}C-labeled products exceed $^{14}CO_2$). (C) Kinetics of change in total radioactivity (○) ($B/B_{out(0)}$, where B is the sum of all benzene-derived components present) and change in radioactivity due to benzene outside the cells (●) ($B_{out}/B_{out(0)}$), both according to equation 4.

culture: $V(dA_{out}/dt) = 0 - FA_{out} - a_A XVA_{out}$. Since $F/V = D$ by definition, $dA_{out}/A_{out} = -(D + a_A X)dt$. If X is constant, integration gives $\ln A_{out} = -(D + a_A X)t + C$. At time zero ($t = 0$), $A_{out} = A_{out(0)}$ and $C = \ln A_{out(0)}$. At time t,

$$\ln (A_{out}/A_{out(0)}) = -(D + a_A X)t \quad (4)$$

The relationship between specific affinity, growth rate, and the transport constant can be evaluated as follows. Substitution of the Michaelian relationship for transport into equation 2 gives the specific affinity at the Michaelis concentration for transport $a_A^{K_t}$ as $a_A/2$, which reflects the effects of saturation. Then the specific uptake rate at the Michaelis concentration v_{K_t}/X is

$$v_{K_t}/X = a_A/2^{K_t} = a_A^{K_t} K_t \quad (5)$$

From the relationship between uptake rate and specific growth rate, i.e., $v_A/X = \mu/Y_{XA}$, the maximal growth rate is set by an uptake rate that is two times the rate effected by the Michaelis concentration K_t:

$$\mu_{max} = 2\, v_{K_t}\, Y_{XA}/X \quad (6)$$

Combining equations 5 and 6,

$$\mu_{max} = a_A\, K_t\, Y_{XA} \quad (7)$$

The growth rate at the Michaelis concentrations can then be reduced to the following:

$$\mu_{K_t} = (a_A/2)K_t\, Y_{XA} = a_A^{K_t}\, K_t\, Y_{XA} \quad (8)$$

RESULTS

Affinity in noninduced continuous culture. The kinetics of benzene metabolism in an amino-acid-limited (1 mg of carbon liter^{-1}) continuous culture following an injection of benzene at a concentration $B_{out(0)} = 1$ μg liter^{-1} are shown in Fig. 1. Since biomass (Fig. 1A) and growth rate remained

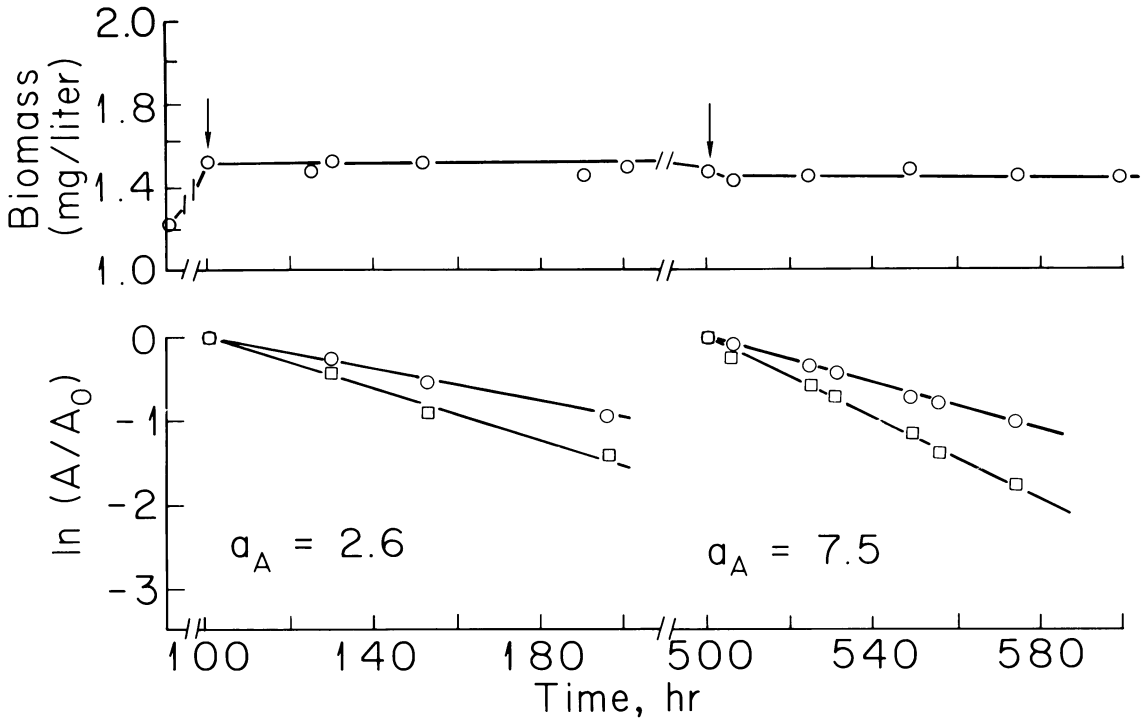

FIG. 2. Kinetics of metabolism of toluene injected into a continuous culture both alone (0.96 μg liter $^{-1}$; first arrow) and when administered (1.24 μg liter $^{-1}$) along with a larger quantity of benzene (50 μg liter $^{-1}$; second arrow). Other conditions are as in Fig. 1. Top panel: ○, biomass versus time. Bottom panel: ○, ^{14}C (total); □, [^{14}C]toluene.

constant, the rate of utilization could be observed in an undisturbed culture and calculated from an exact solution (equation 4) of the second-order rate equation (equation 3). Utilized benzene appeared as organic products (see below), carbon dioxide, and cell material in a ratio of 89:9:2 (Fig. 1B). The slope of total radioactivity with respect to time, $-0.0095\ h^{-1}$ (Fig. 1C), was in reasonable agreement with the expected loss through dilution by fresh medium at the rate $-0.011\ h^{-1}$. This agreement also demonstrated that the reactor medium was in rapid equilibrium with the captured air contained above it and that the presence of the air phase did not disturb the kinetics. Since the oxygen required was dissolved in the fresh medium, the only purpose of this air phase was to prevent the organisms of the continuous culture from swimming back up the feed line which supplied fresh medium. Radioactivity from the benzene in solution disappeared at a rate greater than the dilution rate because of metabolic consumption. Losses of hydrocarbon into the lipid of the small population present at a partition coefficient of 300 (22) were estimated to be 1 part in 9×10^6 and were neglected. Computation of the affinity from the rate of benzene loss (determined by the decrease in volatile radioactivity at pH 7.8) in three experiments of the type shown in Fig. 1C gave $a_B^1 = 6.8 \pm 0.05$ liters g of cells^{-1} h^{-1}.

A similar experiment was performed for toluene addition (Fig. 2). Soluble organic compounds were again the main product of toluene metabolism. During batch culture, organic compounds were consistently produced along with carbon dioxide and cell material in a ratio of about 55:35:10. The organic products formed from toluene appear to be made up of toluene dihydrodiol, 3-methylcatechol, a yellow C_7 keto acid (as often reported [1, 11, 20, 31]), acetate, and a trace of formate (B. R. Robertson and D. K. Button, manuscript in preparation).

Inhibition. Having observed the response of the continuous culture to both benzene and toluene, we examined the ability of benzene, as a structural analog, to inhibit the accumulation of toluene. A steady state was reestablished after the first toluene addition (1 μg liter^{-1}), then toluene was combined with a large amount (50 μg liter^{-1}) of benzene in a second injection. Benzene at 50 μg liter^{-1} did not appear to compete with toluene for metabolism because the lower affinity expected for toluene (according to the kinetics of competitive inhibition [12]) was not observed. K_I is 44 μg of toluene liter^{-1} and is induction independent (B. R. Robertson, D. K. Button, R. A. T. Law, and K. S. Craig, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, N74, p. 235). Thus, if the metabolic pathways of toluene and benzene share a common step which is rate limiting, the amount of benzene present should have been sufficient to lower the rate of toluene accumulation and result in a smaller affinity for toluene. Instead, the affinity increased from $a_A^1 = 2.6$ to 7.5 liters g of cells^{-1} h^{-1} as shown. Inhibition of toluene metabolism by benzene did not occur until a concentration of 200 μg of benzene liter^{-1} was reached (Fig. 3). Other hydrocarbons were equally impotent inhibitors and gave no indication of competition with toluene or benzene (Table 2). Only benzene at 2 mg liter^{-1} and the terpene mixture at saturation level caused observable inhibition: concentrations which could have been sufficient for physical modification of the cell membranes. *Pseudomonas* sp. strain T2 will grow in terpene media; gas chromatography indicates that it can utilize many of these hydrocarbons from a mixture (5). Although *Pseudomonas* sp. strain T2 will also grow on limonene alone, growth on single terpene substrates has been difficult to achieve, perhaps because of the combination of low K_I values and toxicity reported above.

Induction. Sequential additions of benzene at 1 μg liter^{-1}

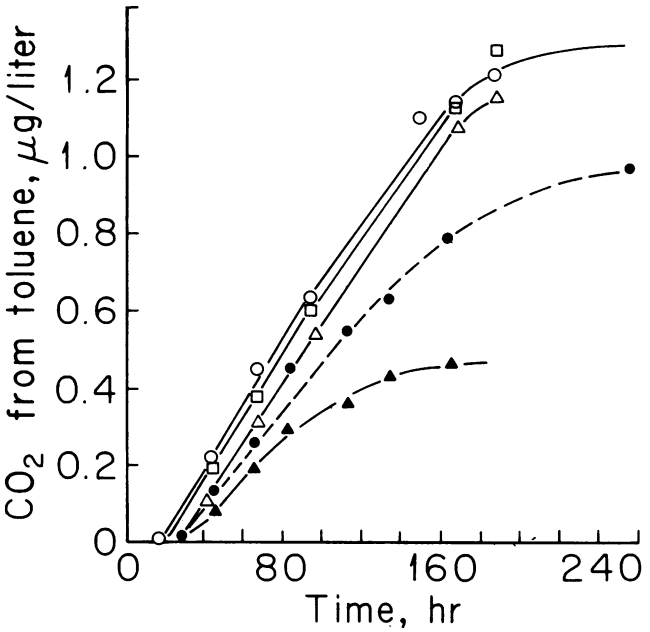

FIG. 3. Effect of benzene on the metabolism rate of toluene. Amino-acid-grown toluene-induced cells were washed and suspended at 1 mg liter^{-1} with [^{14}C]toluene (2.1 μg liter^{-1}) along with benzene at the following concentrations (micrograms liter^{-1}): ○, 0; □, 10; △, 50; ●, 200; ▲, 1,000; then the time course of $^{14}CO_2$ formation was followed.

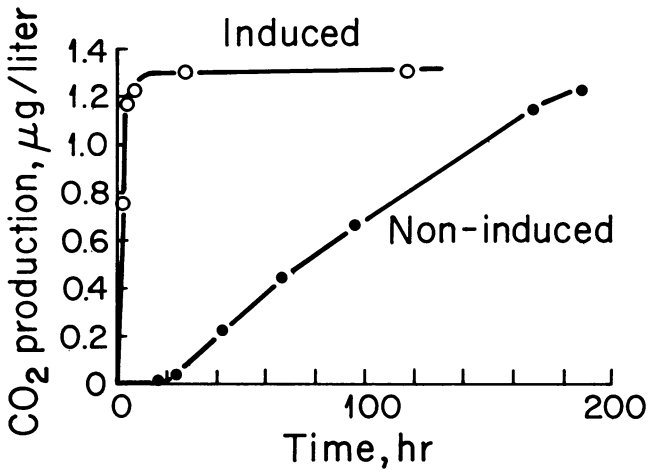

FIG. 4. Effect of induction on the time course of $^{14}CO_2$ production from toluene. Cells were grown from toluene (induced) or amino acids (noninduced), washed twice, and suspended in a [^{14}C]toluene solution at a biomass of approximately 1 mg liter^{-1}, and the $^{14}CO_2$ was collected at the times shown.

appeared to have no effect on the ability of the culture to metabolize the benzene. The enhanced toluene metabolism after addition of the toluene-benzene mixture (Fig. 2) was reexamined by comparing the time course of benzene metabolism by organisms grown from amino acids (casein hydrolysate) with metabolism by those which had also been exposed to benzene. Benzene induction under these growth conditions required at least 6 days and resulted in an increase in the yield of $^{14}CO_2$ from benzene (15 to 20% noninduced versus 30 to 45% induced) and also caused an increase in affinity to 21.6 liters g of cells^{-1} h^{-1} (data not shown).

When the time course of toluene metabolism by harvested toluene-induced organisms was compared with that for noninduced organisms, the response was more apparent. No lag time was observed before the initiation of toluene metabolism as compared with a 25-h lag time for benzene, and there was a larger increase in the partial affinity relative to benzene (Fig. 4). Affinities for toluene and benzene under various conditions are compared in Table 3. To examine the effects of both inducing concentration and time of exposure on the rate of toluene metabolism, we examined the ability of amino-acid-grown, washed cells to produce $^{14}CO_2$ from radioactive toluene after exposure to [^{12}C]toluene at various concentrations for various times. The extent of induction, as was reflected by the value of the partial affinity, increased with both inducing concentration and time (Fig. 5). By subtracting the value of the partial affinity in the control (which gives a base value) from the values obtained following induction, converting rates from carbon dioxide production to toluene consumption, and fitting the results to the rectangular hyperbola of saturation kinetics, constants analogous to those of Michaelian kinetics were obtained. The maximal value of the specific affinity (a^{max}_{QA}) was 123 ± 45 liters g of cells^{-1} h^{-1}, with an induction constant or toluene concentration at half a^{max}_{QA} (K_{ind}) of 96 ± 45 μg liter^{-1}.

TABLE 2. Inhibition of toluene metabolism[a]

Inhibitor	Concn (μg liter^{-1})	% Inhibition
Toluene	200	0
Benzene	200	30
Benzene[b]	200	0
Benzene[b]	2,000	83
Naphthalene	100	0
p-Xylene	100	0
Toluene, naphthalene, and *p*-xylene	100 each	0
Dodecane	Saturated	0
Casein hydrolysate	100	0
Casein hydrolysate	1,000	18[c]
Monoterpene mixture	Saturated	98

[a] Experiments were conducted with toluene metabolism induced as described in the legend to Fig. 3.
[b] Cells were grown in the presence of benzene rather than toluene. At 2,000 μg of benzene liter^{-1} there was, in addition to the inhibition shown, a lag time of 4 h before benzene metabolism began.
[c] Percent stimulation, not percent inhibition.

TABLE 3. Affinity of *Pseudomonas* sp. strain T2 for benzene and toluene under various conditions

Substrate	Expt	Affinity (liters g of cells^{-1} h^{-1}
Benzene	Amino acid limited, continuous (Fig. 1)	6.83
Toluene	Amino acid limited, continuous (Fig. 2)	2.6–7.5
Benzene[a]	Amino acid grown, noninduced	2.2
Benzene[a]	Amino acid grown, benzene induced	21.6
Toluene[b]	Amino acid grown, partially induced	2.6
Toluene[b]	Amino acid grown, induced (Fig. 4)	317
Toluene	Amino acid grown (19 months)[c]	>0.03
Toluene	Toluene grown[c]	500

[a] Affinity calculated from a_{QB} by using v_{QB}/v_B = 0.5.
[b] Calculated from a_{QA} by using v_{QA}/v_A = 0.35.
[c] B. R. Robertson and D. K. Button, manuscript in preparation.

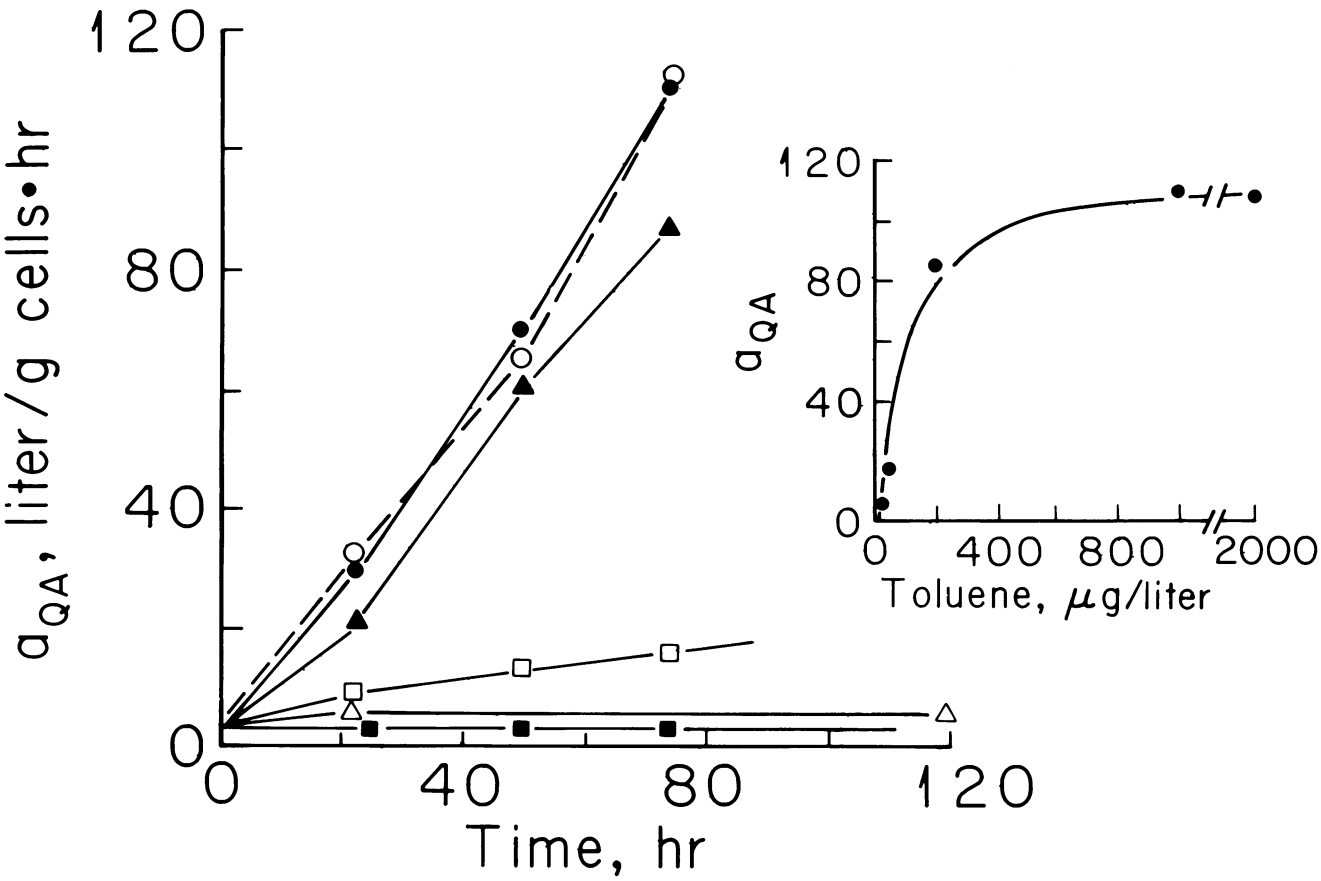

FIG. 5. Induction: the effect of exposure to toluene, at concentrations (1 [■], 10 [△], 50 [□], 200 [▲], 1,000 [○], and 2,000 [●] μg liter $^{-1}$) and at the times shown, on the affinity a_{QA}. Values were calculated from the partial affinity a_{QA} as observed from CO_2 production according to the relationship $a_A = a_{QA}/0.35$. At the indicated times, 50-ml subsamples were withdrawn, the toluene was removed with nitrogen, 1 μg of [^{14}C]toluene liter $^{-1}$ was added, and the rate of $^{14}CO_2$ production was monitored over 5 h. Inset shows the specific affinities after induction for 68 h at the concentrations shown.

DISCUSSION

Experiments showed that *Pseudomonas* sp. strain T2 maintained the ability to metabolize benzene during carbon-limited growth on amino acids for a considerable number of generations so that the addition of a trace quantity was metabolized immediately. The isotope injection procedure appeared to be a good way to establish the absolute value of the ongoing capacity of a culture to utilize a substrate and at the same time avoid the trauma of culture manipulation. Compared with the perturbation technique (10, 18), disturbance to the system was minimal, and attendant formulations had an exact solution. Compared with the relaxation procedure (7, 27), methods were operationally and computationally simple. According to equation 8, the affinity required for an organism to grow at a half-maximal rate of 0.05 h^{-1} from a substrate metabolized with a yield of unity in a culture limited by A as transported by a perfectly Michaelian system is 0.05 $h^{-1}/(4.4 \times 10^{-5}$ g liter$^{-1} \times 1) =$ 1,100 liters g of cells h^{-1}. Although such large affinities are seldom indicated in the literature (5), they are needed for growth from single substrates with K_t values in the microgram liter^{-1} range, as the toluene-*Pseudomonas* system appears to have. These kinetics help to explain why growth from single hydrocarbons is difficult for many bacteria. The highest affinity observed here is $a_{QA}/Y_{QA} = 120/0.35 = 343$ liters g of cells h^{-1}, which is a rather typical value for this pseudomonad after several days of growth in amino acid-toluene media: conditions which are optimal for induction. According to equation 7, the maximal growth rate on toluene alone is then only approximately 2×10^{-3} h^{-1}, which indicates limitation by the rate of toluene transport or metabolism. However, in agreement with studies involving the use of amino acids with sugars (20) and alkanes (2), trace aromatic hydrocarbon concentrations can help to support growth along with other more commonly investigated substrates.

Competitive interactions with other substrates were not observed; the addition of relatively large concentrations of hydrocarbon with respect to K_t, i.e., 100 to 300 μg liter^{-1}, did not impair the ability of *Pseudomonas* sp. strain T2 to accumulate the substrate. Although the apparent lack of competitive inhibition indicated by the continuous culture data could have been explained by induction of increased affinity which outweighed the reduction in rate caused by both inhibition and saturation, the batch culture inhibition observations at higher concentrations of hydrocarbon rule out this possibility. Therefore, benzene and several other hydrocarbons appear to be incorporated by pathways which have independent rate-limiting steps, as corroborated by the finding of numerous metabolic pathways for hydrocarbons (13, 17, 20). The products liberated furnish evidence that

toluene utilization begins in this organism with hydroxylation of ring carbons, followed by metabolism through the *meta* pathway, which is thought to be chromosomally encoded (30). Such action is thought to suppress *ortho* pathway activity, which is reportedly chromosomally mediated and highly specific (14, 24). Although low specificity has been reported for the enzymes of the *meta* pathway (16) and toluene metabolism is thought to be possible through benzene pathway enzymes (16, 17) (in which case toluene and benzene could interact directly), we observed mutually independent uptakes. Observations of the dynamics of hydrocarbons in water samples amended with radiolabeled toluene or benzene for study are therefore probably not influenced by the presence of additional low levels of hydrocarbon contaminants. *Pseudomonas* sp. strain T2 will grow in toluene vapors (about 35 mg of toluene liter^{-1} in solution), and so our observation of benzene inhibition at 200 μg liter^{-1} is not completely understood. Perhaps benzene is more toxic; we could not grow the organism in benzene vapors. In addition, these hydrocarbons may be metabolized by more than one pathway, which becomes apparent only at substantially different concentrations, as suggested by kinetic curves for metabolism that indicate a degree of first-order kinetics well above K_t (data not shown).

The ability of this organism to metabolize a very large number of substrates, including hydrocarbons, terpenes, sugars, and amino acids, shows that a surprisingly large number of catabolic pathways can be present within an organism having space, according to calculations, for relatively few proteins. Space limitation, which is also suggested by analysis of electrophoresis gels of the type produced by Benson et al. (2), helps to explain the unusually slow growth of the organism normally observed when it is provided with just one of the members of one of these substrate groups as the sole source of carbon and energy.

Sequential additions of benzene at a concentration of 1 μg liter^{-1} to a continuous culture gave constant and moderate values for the affinity, whereas larger additions to harvested cells produced a somewhat larger value. However, metabolism rates after toluene additions suggested that the metabolism of toluene was more strongly inducible. Induction showed saturation at a concentration K_{ind} of 96 μg of toluene liter^{-1}, which was similar to K_t (44 μg liter^{-1}). The concept that effective inducing concentrations are related to K_t is further supported by K_t values for the indigenous microflora of seawater. These Michaelis concentrations for uptake were measured at 0.5 to 3.0 μg liter^{-1}, whereas the induction constant was measured in raw seawater at 1.87 ± 0.06 μg of toluene liter^{-1} (D. K. Button and B. R. Robertson, Mar. Ecol. Prog. Ser., in press). The approximate equivalence between K_{ind} and K_t appears reasonable, considering that the amount of induction may rest on the internal substrate concentration, which depends on the rate of accumulation, a rate influenced by K_t. It is not suggested that the shape of the induction curve is theoretically exactly hyperbolic. Neither induction nor the loss of an induced system appears to be an all-or-none phenomenon. Our experiences with this organism suggest that affinity, following induction, is gradually but reversibly lost over a period of years. It is clear, however, that the affinity of the organisms for toluene increases gradually over a 3-day period at a rate that increases with the concentration of toluene present when toluene is in the 100-μg liter^{-1} range.

The absolute values of the affinities as well as their relative change values are noteworthy. Apparently this organism modulates its ability to utilize toluene over a wide range of affinities which depend on inducer concentration. When toluene was in the 1- to 10-μg liter^{-1} range, it could supply between 0.01 and 0.1% of the metabolic needs of the organism (equation 8); at concentrations exceeding 50 μg liter^{-1}, it could supply much, sometimes all, of the carbon required (equation 7). The demonstrated ability of organisms to utilize small quantities of individual compounds is also noteworthy, because aquatic systems contain very many compounds which, in combination, support a major portion of marine biomass. These data provide the first quantitative measure of the ability of a culture to respond, with new transport capacity, to very small concentrations of nutrient.

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