Kinetics of p-Nitrophenol Mineralization by a *Pseudomonas* sp.: Effects of Second Substrates

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The kinetics of simultaneous mineralization of p-nitrophenol (PNP) and glucose by *Pseudomonas* sp. were evaluated by nonlinear regression analysis. *Pseudomonas* sp. did not mineralize PNP at a concentration of 10 ng/ml but metabolized it at concentrations of 50 ng/ml or higher. The K_s value for PNP mineralization by *Pseudomonas* sp. was 1.1 μg/ml, whereas the K_s values for phenol and glucose mineralization were 0.10 and 0.25 μg/ml, respectively. The addition of glucose to the media did not enable *Pseudomonas* sp. to mineralize 10 ng of PNP per ml but did enhance the degradation of higher concentrations of PNP. This enhanced degradation resulted from the simultaneous use of glucose and PNP and the increased rate of growth of *Pseudomonas* sp. on glucose. The Monod equation and a dual-substrate model fit these data equally well. The dual-substrate model was used to analyze the data because the theoretical assumptions of the Monod equation were not met. Phenol inhibited PNP mineralization and changed the kinetics of PNP mineralization so that the pattern appeared to reflect growth, when in fact growth was not occurring. Thus, the fitting of models to substrate depletion curves may lead to erroneous interpretations of data if the effects of second substrates on population dynamics are not considered.

In natural ecosystems, organic pollutants frequently occur in mixtures with other synthetic as well as natural organic compounds. Therefore, it is important to understand how the biodegradation of a polluting compound is affected by the presence of other compounds. Recent work (4, 12, 13) has shown that the degradation of low concentrations of organic compounds can be stimulated by the addition of readily degraded organic substrates. Such findings may have both practical and ecological significance. In a practical sense, it would be beneficial if these findings could be applied to the operation of waste treatment systems to stimulate the breakdown of synthetic compounds. In this regard, Lapat-Polasko et al. (4) demonstrated that methylene chloride degradation by a *Pseudomonas* sp. can be enhanced if acetate is supplied to the organism as a supplementary substrate. In an ecological context, many natural environments are carbon-limited, and therefore it would be advantageous for an organism to metabolize a variety of organic compounds that are present at low concentrations.

In addition to enhancing the mineralization of synthetic organic compounds, supplementary substrates may affect the kinetics of biodegradation of organic compounds. Schmidt et al. (13) showed that the kinetics of biodegradation of organic compounds are altered by the presence of compounds that the organism can use simultaneously with the test substrate. When the two substrates are metabolized simultaneously, the degradation of the compound that is present at low concentration is enhanced if the growth of the population is increased by the presence of the other substrate (13). This enhancement of biodegradation has been termed secondary substrate utilization (7).

Further work is needed to determine whether the addition of secondary substrates is a feasible way to stimulate biodegradation of synthetic organic molecules. The purpose of the present study was to determine how the kinetics of biodegradation of p-nitrophenol (PNP) by a *Pseudomonas* sp. are affected by its growth on supplementary carbon sources. Nonlinear regression analysis was used to compare the kinetics of PNP degradation when PNP was used as the sole substrate with the kinetics when it was used simultaneously with other substrates.

MATERIALS AND METHODS

Media. The inorganic salts solution contained 125 mg of KH_2PO_4, 60 mg of Na_2HPO_4, 40 mg of NH_4Cl, 10 mg of $MgSO_4 \cdot 7H_2O$, 10 mg of $CaCl_2 \cdot 2H_2O$, 0.5 mg of $FeCl_3 \cdot 6H_2O$, and 0.5 mg of $MnSO_4 \cdot H_2O$ per liter of deionized, distilled water. Individual salt solutions were sterilized separately before they were mixed. The final pH of the mixture was 6.2. The water was distilled and then treated with a reagent-grade water system (Milli-Q; Millipore Corp., Bedford, Mass.). 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.

[U-^{14}C]phenol (87 mCi/mmol) was obtained from Amersham Corp., Arlington Heights, Ill.; and [2,6-^{14}C]PNP (30 mCi/mmol) was purchased from ICN Pharmaceuticals Inc., Irvine, Calif. [U-^{14}C]glucose (348 mCi/mmol) and [1,2-^{14}C]acetic acid, sodium salt (55 mCi/mmol), were purchased from New England Nuclear Corp., Boston, Mass. Reagent-grade phenol was purchased from Mallinckrodt Inc., Paris, Ky. Sodium glutamate was purchased from Sigma Chemical Co., St. Louis, Mo. Glucose was purchased from Fisher Scientific Co., Rochester, N.Y.; and PNP was obtained from J. T. Baker Chemical Co., Phillipsburg, N.J.

Bacterial isolate. *Pseudomonas* sp. strain K was isolated from Mardin silt loam (14). To isolate this bacterium, PNP was added to a 50-g (dry weight) sample of moist soil at a concentration of 10 μg/g, and the soil was incubated at 23°C. After 7 days, a small amount of this soil was transferred to 100 ml of distilled water containing 10 μg of PNP per ml, and the culture was incubated without shaking for 7 days at 23°C.

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Serial dilutions were then plated on a medium containing inorganic salts and 10 mg of PNP and 15 g of Bacto-Agar (Difco Laboratories, Detroit, Mich.) per liter. Isolated colonies were selected and streaked onto PNP plates several times successively. *Pseudomonas* sp. was grown to the early stationary phase in the inorganic salts solution containing 30 μg of PNP per ml. The cultures were then diluted at least 100-fold with inorganic salts solution to obtain the initial cell densities used in the experiments.

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 the spread-plate technique. Duplicate 0.1-ml portions of 10-fold dilutions were plated onto a medium containing (per liter of deionized water) 15 g of Bacto-Agar (Difco) and 3 g of Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.), and colony counts were made after 72 h of incubation at 29°C. The data represent means of duplicate plate counts from individual flasks.

Measurement of mineralization. All flasks received similar concentrations of ^{14}C-labeled compounds (2,000 to 4,000 dpm/ml), but the final substrate concentration was varied by adding different amounts of unlabeled compounds. To measure the simultaneous mineralization of PNP and glucose, for example, the flasks received either ^{14}C-labeled PNP and unlabeled glucose or unlabeled PNP and ^{14}C-labeled glucose. At regular intervals, 3.5-ml samples were removed from each flask and passed through a polycarbonate filter (pore size, 0.2 μm; Nuclepore Corp., Pleasanton, Calif.) into a test tube containing 3 drops of concentrated sulfuric acid. Some samples also were collected without filtering and placed directly into acidified test tubes. Carbon dioxide was driven off by vigorous bubbling for 5 min with compressed air. Subsamples (3.0 ml) then were added to plastic vials containing 9 ml of Liquiscint scintillation cocktail (National Diagnostics, Inc., Somerville, N.J.), and the radioactivity was counted with a liquid scintillation counter (model LS 7500; Beckman Instruments, Inc., Irvine, Calif.). The ^{14}C depletion curves were obtained from the flasks that were used to obtain cell counts. Unless indicated otherwise, the curves of ^{14}C disappearance show ^{14}C remaining in solution after the culture was passed through filters (pore size, 0.2 μm).

To measure the amount of substrate that was assimilated by cells, the amount of ^{14}C in the filtrate was subtracted from the amount remaining in an unfiltered sample that was collected at the same time.

Data analysis. Nonlinear regression analyses of substrate disappearance were performed on all curves. For this purpose, the MARQFIT computer program was used (15). This program fits data by minimizing the least squares of the differences between the data and the model curve by using the Marquardt method (1).

To determine the model that best represents each curve showing substrate disappearance, the residual sums of squares from fits obtained by different models were compared. The model giving the lowest residual sum of squares for a particular data set was deemed the model of best fit if the difference between it and models with fewer parameters was significant at the 95% or higher confidence level when a standard F-test was used (10). For some curves, two unrelated models with the same number of parameters fit the data equally well. In such cases, the model that was used to fit the data was the one that gave parameter estimates closest to

those obtained by independent measurements. In addition, if only one substrate was being used by the bacterium, the Monod family of models (15) was used to analyze the data. For tests in which the organism was using two substrates simultaneously, the models described by Schmidt et al. (13) were used to analyze the data.

RESULTS

Characterization of the isolate. *Pseudomonas* sp. strain K is a short, gram-negative, motile rod that is oxidase-positive and forms catalase but no fluorescent pigments. It has several polar flagella, accumulates poly-β-hydroxybutyrate, does not grow at 41°C, and is capable of *meta* cleavage of protocatechuate. These traits are characteristic of the RNA group III of the genus *Pseudomonas* (8). The bacterium grew on arginine and betaine, however, which is not a trait of group III pseudomonads. Therefore, *Pseudomonas* sp. strain K would be categorized under section V of the genus *Pseudomonas* by the classification of Palleroni (8). The reactions of *Pseudomonas* sp. strain K to various tests compared best with those of *Pseudomonas mesoacidophila* of the organisms listed as section V pseudomonads (8). The results of the tests with our isolate and *P. mesoacidophila* are presented in Table 1.

Growth at low concentrations of PNP. In salts solution with no added carbon source, *Pseudomonas* sp. grew to a final cell density of 4×10^4 cells per ml, whereas in the presence of 50 and 100 ng of PNP per ml, this organism reached final cell densities of 1×10^5 and 2×10^5/ml, respectively (Fig. 1). In a medium with 10 ng of PNP per ml, the final cell density was essentially the same as that obtained in the liquid with no added substrate. Concurrent measurements of the metabolism of ^{14}C-labeled PNP showed that the substrate was mineralized at concentrations of 50 and 100 but not 10 ng/ml (Fig. 1). After 3 months of incubation, the number of *Pseudomonas* sp. only decreased from 4×10^4 to 3×10^4 cells per ml in the presence of 10 ng of PNP per ml, yet the substrate remained unmineralized (data not shown). The mineralization of 50 and 100 ng of PNP per ml stopped before all of the PNP was mineralized. These data, combined with the fact that *Pseudomonas* sp. was unable to mineralize 10 ng of PNP per ml, suggest the existence of a threshold concentration below which PNP is not mineralized by this organism. The exact threshold was not determined. On the other hand, phenol was mineralized by *Pseudomonas* sp. at a concentration of 5.0 ng/ml, which was the lowest concentration of phenol tested (data not shown).

Kinetics of PNP mineralization. To determine the half-saturation constant for growth (K_s), 3×10^5 cells per ml were incubated with various concentrations of PNP. At concentrations of 0.75 and 1.5 μg/ml, the curves of PNP mineralization were sigmoidal in shape, and the model of best fit to each data set was the logistic equation ($P = 0.001$ and 0.05, respectively) (Table 1). Thus, these concentrations were too low for a reliable estimate of K_s to be obtained (15). At a concentration of 6 μg/ml, however, the curve of PNP disappearance was almost completely concave down (Fig. 2), and the model of best fit to the data was the Monod equation. From the fit of the Monod equation to these data, the value of K_s was estimated to be 1.1 ± 0.2 μg/ml (Table 2).

The K_s for phenol mineralization by *Pseudomonas* sp. was determined by incubating 10^5 cells with 0.5 or 1.5 μg of phenol per ml. Under these conditions, the mineralization curves were almost completely concave down (Fig. 2). The Monod equation provided the curve of best fit to the disap-

TABLE 1. Comparison of *Pseudomonas* sp. strain K with *P. mesoacidophila*

Characteristic	P. mesoacidophila[a]	Pseudomonas sp. strain K
Morphology	Rod	Rod
PHB accumulation[b]	+	+
Flagella	Polar (1 to several)	Polar (1 to 5)
Pigment production	−	−
Growth at pH 4.5	+	+
Growth at 41°C	+	−
Oxidase	−	+
Catalase	+	+
Growth on:		
L-Arabinose	+	+
D-Xylose	+	+
Glucose	+	+
D-Mannose	+	+
D-Fructose	+	+
D-Sorbitol	+	+
Mannitol	+	+
Inositol	+	+
Galactose	+	+
Maltose	+	+
Sucrose	+	+
Raffinose	+	+
Glycerol	+	+
2-Ketogluconate	+	ND[c]
Gluconate	ND	+
Citrate	+	+
Acetate	+	+
Succinate	+	+
L-Alanine	+	+
β-Alanine	+	ND
L-Arginine	+	+
Betaine	+	+
Lactose	−	+
Starch	−	−
Benzoate	ND	+
Shikimate	ND	+
Kynurenate	ND	+
p-Nitrobenzoate	ND	+
Anthranilate	ND	+
Salicylate	ND	+

[a] See References 3 and 8.
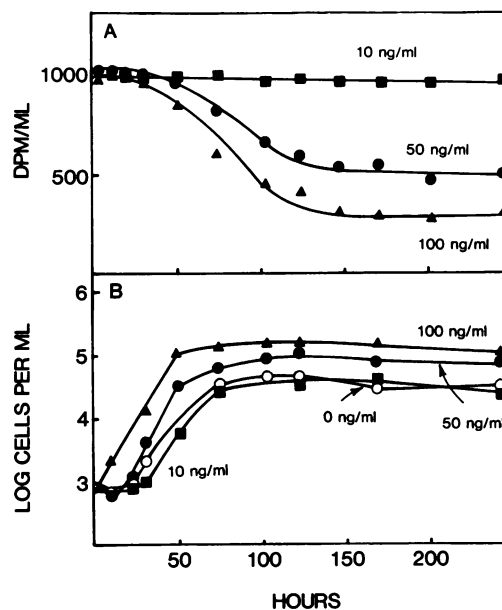[b] PHB, Poly-β-hydroxybutyrate.
[c] ND, Not determined.

FIG. 1. (A) Mineralization of low concentrations of PNP by *Pseudomonas* sp.; (B) growth in media with various levels of PNP or no added carbon source.

neously by *Pseudomonas* sp., 3×10^5 cells were incubated with (i) labeled glucose and unlabeled PNP or (ii) labeled PNP and unlabeled glucose. In both media, the growth of the organism was almost identical, and the two substrates were mineralized simultaneously (Fig. 4). The curve of PNP mineralization in the presence of glucose was best described by model V ($P = 0.01$) of Schmidt et al. (13). This model was formulated for situations such as that depicted in Fig. 4, in which the organism is growing exponentially while simultaneously mineralizing a substrate which is at a concentration

pearance of 1.5 μg of phenol per ml. The estimated value of K_s for phenol mineralization was 0.10 ± 0.08 μg/ml (Table 2), a value that was about 11 times lower than the K_s for *Pseudomonas* sp. growing on PNP. The curve for mineralization of 0.5 μg of phenol per ml was sigmoidal in shape and was fit by the logistic equation.

Experiments were then conducted to determine the effects of additional substrates on the mineralization of PNP. In the presence of 20 μg of glucose, glutamate, acetate, or phenol per ml, *Pseudomonas* sp. did not mineralize 10 ng of PNP per ml (data not shown). Glucose (20 μg/ml) stimulated the mineralization of 1 μg of PNP per ml (Fig. 3A), and the sugar increased the rate of growth of *Pseudomonas* sp. (Fig. 3B). Thus, glucose probably enhances PNP mineralization by stimulating the growth of *Pseudomonas* sp. Of the models of Schmidt et al. (13), model IV was the one that gave the best fit to the curve of PNP mineralization in the presence of glucose. The parameter estimates for the degradation of 1 μg of PNP per ml in the presence and absence of glucose are presented in Table 3.

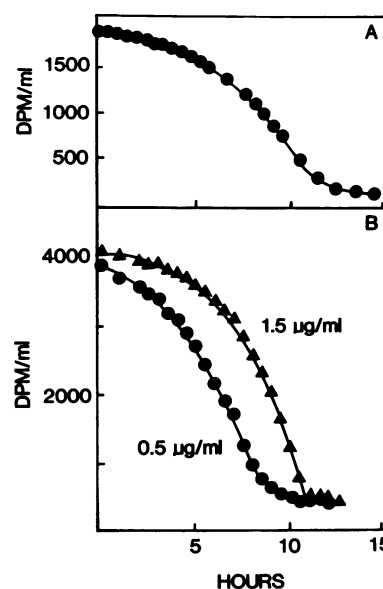To verify that PNP and glucose can be used simulta-

FIG. 2. Mineralization of 6 μg of PNP per ml (A) and 0.5 and 1.5 μg of phenol per ml (B) by *Pseudomonas* sp. The curves were fit by the logistic equation (0.5 μg of phenol per ml) or the Monod equation.

TABLE 2. Parameters and standard deviations of two models of mineralization kinetics fit to data on the mineralization of PNP and phenol by a *Pseudomonas* sp.

Substrate	Substrate concn (μg/ml)	Model	Rate constant	K_s (μg/ml)	$X_0{}^a$	S_0 (dpm/ml)	ζ^b
PNP	0.75	Logistic	$k_4 = 0.0002 \pm 0.00001^c$	NA^d	254 ± 25	$1,773 \pm 12$	0.07 ± 0.003
	1.5	Logistic	$k_4 = 0.0003 \pm 0.00001$	NA	74 ± 13	$1,941 \pm 19$	0.06 ± 0.007
	6.0	Monod	$\mu_{max} = 0.31 \pm 0.002^e$	1.1 ± 0.2	118 ± 13	$1,864 \pm 6$	0.07 ± 0.003
Phenol	0.5	Logistic	$k_4 = 0.0002 \pm 0.00001$	NA	98 ± 16	$3,634 \pm 28$	0.09 ± 0.007
	1.5	Monod	$\mu_{max} = 0.38 \pm 0.02$	0.1 ± 0.002	110 ± 15	$4,063 \pm 15$	0.10 ± 0.002

[a] The initial population size in terms of the amount of substrate (in disintegrations per minute) required to produce it.

[b] Zeta represents the fraction of ^{14}C in unmetabolized substrate and extracellular products.

[c] Constant k_4 has units of milliliters per hour per disintegrations per minute.

[d] NA, Not applicable.

[e] Rate constant for this model is μ_{max}, with units of hours^{-1}.

in the vicinity of the K_s. The parameter estimates from the fit of model V to these data are presented in Table 3. The Monod equation also provided a good fit to these data. The residual sum of squares of the Monod equation fit to the data (127,960) was not statistically different from the sum of squares resulting from the fit by model V (127,430). Model V was used to fit the data because the Monod equation should only be used when one substrate is being mineralized by an organism and that substrate is limiting the rate of growth.

To determine the effect of a second substrate on the mineralization of PNP by nongrowing cells, 3×10^7 cells of *Pseudomonas* sp. per ml were incubated with 4 μg of PNP per ml and 100 μg of either glucose or phenol per ml of inorganic salts solution. In all cases, PNP was mineralized rapidly, and an appreciable increase in population size was not evident (Fig. 5). Glucose had no effect on the mineralization of PNP under these conditions, whereas phenol markedly inhibited the reaction. The curve of PNP mineralization in the absence of glucose or phenol was best de-

FIG. 3. Effect of glucose (20 μg/ml) on mineralization of PNP (A) and growth of *Pseudomonas* in a medium with 1 μg of PNP per ml (B). The curve for PNP mineralization in the presence of glucose was fit by model IV (13), and the curve for PNP alone was fit by the logistic equation.

scribed by Michaelis-Menten kinetics, as predicted by theory (13). The curve of PNP mineralization in the presence of glucose was also best fit by the Michaelis-Menten equation, which gave parameter estimates very similar to those estimated for the same concentration of PNP in the absence of glucose (Table 4). The curve of PNP mineralization in the presence of phenol had a sigmoidal shape and was best fit by the logistic equation. It is evident from the results presented in Fig. 5, however, that an increase in cell number did not occur during the experiment. Therefore, the Michaelis-Menten model was used to fit the data.

DISCUSSION

The inability of *Pseudomonas* sp. to mineralize PNP at 10 ng/ml may be attributable to the fact that the concentration was too low to meet the maintenance requirements of the organism (12) or to induce the enzymes necessary for uptake or degradation of the substrate (5). In the presence of high concentrations of a second substrate, *Pseudomonas* sp. was unable to utilize PNP at concentrations below the threshold, even though these added substrates promoted the growth of the organism. Even glucose, which accelerated the breakdown of high concentrations of PNP, did not enable *Pseudomonas* sp. to mineralize PNP below the threshold concentration. This suggests that the threshold was not a result of the fact that the concentration of PNP was too low to meet the maintenance energy requirements of the organism, but rather supports the hypothesis that the concentration was too low to induce degradative enzymes. The threshold concentration was compound-specific, moreover, because *Pseudomonas* sp. was able to mineralize phenol at a concentration of 5 ng/ml. It is interesting that *Pseudomonas* sp. also had a higher affinity for phenol than for PNP, as evidenced by the 11-fold lower value of K_s for phenol than for PNP mineralization. These findings support the contention of Martin and MacLeod (6) that categorizing bacteria as oligotrophs or eutrophs is arbitrary and that the categories vary, depending on what compound is used as the test substrate.

The mineralization of organic compounds can be accelerated by the growth of the active population on other substrates (13, 16), and the kinetics can be described by the models of Schmidt et al. (13). When second substrates do not significantly influence the growth of the mineralizing population, the Monod family of models adequately describes the kinetics of biodegradation (15). The half-saturation constant (K_s or K_m) is a parameter common to both families of models. K_s is the half-saturation constant for growth on the
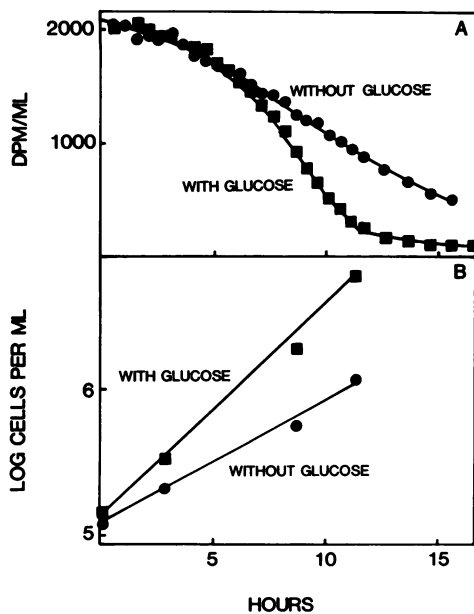
TABLE 3. Parameters and standard deviations of three models of the kinetics of substrate disappearance fit to data on the metabolism of [^{14}C]PNP in the presence and absence of unlabeled glucose

Substrate (concn [μg/ml])	Model	Rate constant	μ_{max} (h^{-1})	K_m (μg/ml)	X_0 (dpm/ml)	S_0 (dpm/ml)	ζ
[^{14}C]PNP (1.0)	Logistic	$k_4 = 0.0002 \pm 0.00001^a$	NA^b	NA	141 ± 22	$1,899 \pm 16$	0.09 ± 0.01
[^{14}C]PNP (1.0)–[^{12}C]glucose (20)	IV	$k_1 = 0.01 \pm 0.001^c$	0.39 ± 0.007	NA	NA	$2,061 \pm 7$	0.06 ± 0.003
[^{14}C]PNP (3.0)–[^{12}C]glucose (3.0)	V	$k_0 = 41 \pm 3.4^d$	0.23 ± 0.02	0.41 ± 0.23	NA	$1,772 \pm 15$	0.05 ± 0.005

^{a} Constant k_4 has units of milliliters per hour per microgram.
^{b} NA, Not applicable.
^{c} First-order rate constant, k_1, with units of hours^{-1}.
^{d} The constant $k_0 = V_{max}B_0$, with units of micrograms per milliliter per hour. B_0 is the initial population density.

test substrate, and K_m is to the half-saturation constant for mineralization of the test substrate by nongrowing cells or by cells that are growing on other substrates. Values for K_m and K_s for a given organism should be equivalent if the rate of mineralization is limiting the rate of growth. When a single substrate is present at a concentration approximately equal to K_s, the kinetics of its mineralization should be best described by the Monod equation (15). At initial PNP concentrations approximately equal to K_s, however, the curves of substrate disappearance were best fit by the logistic equation rather than by the Monod equation. The sets of data that were best fit by the Monod equation were from tests in which the initial PNP levels were approximately six times higher than the estimated K_s values for that substrate. In the case of phenol, the initial substrate concentration for the curve that was best fit by the Monod equation was 15 times higher than the estimated value for K_s. This is similar to the factor of approximately 16 reported by Robin-

son and Tiedje (11) for data that were fit by the Monod equation.

A similar concentration effect seems to apply for models that describe the simultaneous use of two substrates (13). In the presence of an additional carbon source, the kinetics of mineralization of the substrate at a level close to the K_m should be best described by a model that includes the parameter K_m (13). When provided with PNP at a concentration in the vicinity of the K_m (1 μg/ml) and glucose at a concentration of 20 μg/ml, *Pseudomonas* sp. grew exponentially and mineralized PNP. The model of best fit for PNP mineralization under these circumstances was model IV described by Schmidt et al. (13), which incorporates parameters for exponential growth and first-order degradation but not the parameter K_m. Thus, as with the Monod family of models, the model that was formulated to be applied at substrate concentrations well below the K_m was the model of best fit for substrate concentrations in the vicinity of the K_m. These examples indicate that the parameter K_m or K_s is only necessary to describe mineralization kinetics when the initial substrate concentration is high enough above the K_s so that the initial rate of mineralization or growth approaches the
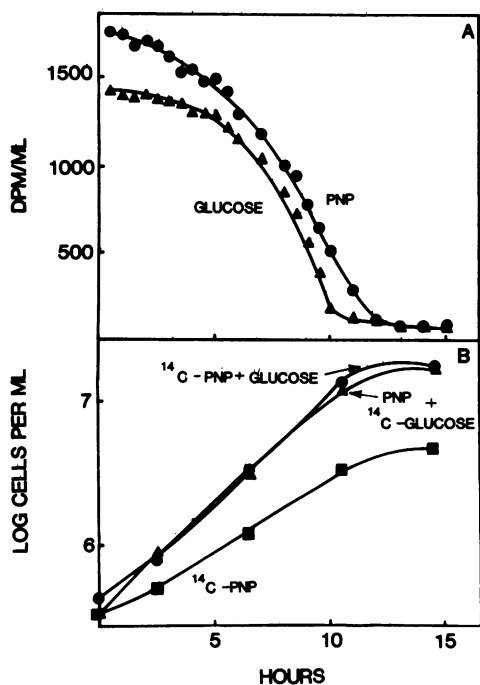

FIG. 4. (A) Simultaneous mineralization of 3 μg of PNP and 10 μg of glucose per ml; (B) growth of *Pseudomonas* sp. in media with PNP and glucose or with PNP only.
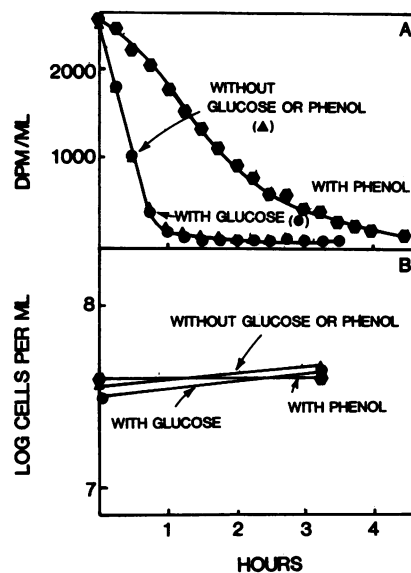

FIG. 5. (A) Mineralization of 4 μg of PNP per ml by *Pseudomonas* sp. in the presence and absence of glucose or phenol; (B) sizes of the bacterial population.

TABLE 4. Parameters and standard deviations of model XI (Michaelis-Menten equation) fit to data on the mineralization of 4 μg of PNP per ml in the absence of a second substrate or in the presence of 100 μg of glucose or phenol per ml

Second substrate	$k_0{}^a$	K_m (μg/ml)	S_0 (dpm/ml)	ζ
None	4,182 ± 129	0.67 ± 0.08	2,604 ± 12	0.035 ± 0.002
Glucose	3,942 ± 331	0.67 ± 0.21	2,487 ± 30	0.051 ± 0.004
Phenol	1,344 ± 90	1.28 ± 0.23	2,672 ± 22	0.043 ± 0.006

^{a} $k_0 = V_{max} B_0$, with units of disintegrations per minute per milliliter per hour.

maximum rate. If the initial substrate concentration is much greater than K_s, then logarithmic or zero-order kinetics apply, and K_s is, again, not necessary to describe mineralization kinetics (15).

An experiment was therefore conducted to determine if a dual-substrate model that includes the parameter K_m would fit curves of PNP mineralization at initial PNP concentrations above the K_m. The data also demonstrate that PNP and glucose were mineralized simultaneously (Fig. 4). Simultaneous use of two substrates by bacteria has been shown to occur when both substrates are present at limiting concentrations in batch cultures (13) or at low dilution rates in chemostat cultures (2). At high concentrations or dilution rates, diauxic utilization is common, but Pseudomonas sp. used glucose and PNP simultaneously even when both compounds were present at high (nonlimiting) concentrations. Reber and Kaiser (9) also have demonstrated the simultaneous use of high concentrations of glucose and aromatic compounds by Pseudomonas putida growing in batch cultures.

The curve depicting mineralization of 3 μg of PNP in the presence of glucose (Fig. 4) was fit by model V described by Schmidt et al. (13). Model V should apply when the active population is growing exponentially while simultaneously mineralizing a second compound present at a concentration near the K_m. It was not possible statistically to determine whether model V or the Monod model, which also provided a good fit to these data, best represents the data; however, based on the theory underlying the models and an examination of the growth curves (Fig. 4), a distinction between the two models can be made. Implicit in the Monod equation is the assumption that only the substrate being measured contributes substantially to the growth of the population. This condition was not met in this experiment because the concentration of glucose was high enough to stimulate the growth of the population metabolizing PNP. Thus, the use of the Monod equation in this instance is unjustified, even though it and model V fit the data equally well.

The applicability of model V to these data can be further tested by comparing the model predictions for some of the parameters of model V with values for these same parameters obtained by independent means. Thus, an estimate of the growth rate of 0.27 h^{-1} was obtained by running a linear regression (correlation coefficient, $r = 0.97$) on the data for growth of the bacterium in a medium with 3 μg of PNP and 10 μg of glucose per ml. This estimate is a little higher than the growth rate estimated by model V ($\mu_{max} = 0.23 \pm 0.02$ h^{-1}). In addition, a value for K_m of 0.4 ± 0.2 μg/ml was estimated by model V from these data. This compares quite favorably with the estimate of 0.7 ± 0.01 μg/ml obtained from the experiment involving nongrowing cells, but it is much lower than the estimate of K_s for growing cells that were using PNP as their sole substrate (1.1 ± 0.2 μg/ml). The

slight discrepancy between the parameter estimates from model V and those obtained by independent means may indicate that glucose influences the kinetics of PNP utilization in ways not accounted for by the model, for example, by lowering the K_m for PNP mineralization.

Glucose did not have a detectable effect on PNP mineralization at high cell densities (3 × 10^7 cells per ml), possibly because the cells were not growing and because the PNP was mineralized very rapidly by the large numbers of bacteria. This is a further indication that the stimulatory effect of glucose on PNP mineralization, described above, results from the increased rate of growth of the PNP-mineralizing population when it is also metabolizing glucose.

The Michaelis-Menten equation best described the mineralization of PNP by nongrowing cells in the presence or absence of glucose. The similarity in the parameter estimates for PNP mineralization in the presence and absence of glucose further indicates that glucose does not affect PNP mineralization, except when glucose stimulates the growth of the metabolizing population. Phenol, however, had a marked influence on the kinetics of PNP mineralization by nongrowing cells. The curve of PNP mineralization in the presence of 100 μg of phenol per ml was best fit by the logistic equation, which implies that growth occurred. It is evident from the results presented in Fig. 5, however, that no growth occurred during the experiment, and therefore, the Michaelis-Menten model was used to fit the data. On the other hand, phenol caused a threefold decrease in the rate constant (k_0) and a twofold increase in the value of K_m for PNP mineralization by nongrowing cells, possibly because phenol interferes with the uptake or mineralization of PNP. Thus, while the curve of substrate disappearance was sigmoidal, as would be expected of a curve reflecting population growth, no increase in cell number was evident.

This study demonstrates the effects of both a stimulatory substrate (glucose) and an inhibitory substrate (phenol) on the kinetics of PNP mineralization by Pseudomonas sp. The shapes of the curves describing biodegradation of a substrate can change markedly in response to a second substrate, and the correct interpretation of such curves is dependent on knowledge of whether the metabolizing population is growing. Knowledge gained from such studies should aid in the interpretation of kinetic data from natural ecosystems as well as in finding means by which to enhance the degradation of organic toxicants at waste disposal sites.

LITERATURE CITED

1. Bard, Y. 1974. Nonlinear parameter estimation. Academic Press, Inc., New York.
2. Harder, W., and L. Dijkhuizen. 1982. Strategies of mixed substrate utilization in microorganisms. Philos. Trans. R. Soc. Lond. Ser. B 297:459–479.
3. Imada, A., K. Kitano, K. Kintaka, M. Muroi, and M. Asai. 1981. Sulfazecin and isosulfazecin, novel β-lactam antibiotics of bacterial origin. Nature (London) 289:590–591.
4. 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.
5. Lechner, U., and G. Straube. 1984. Influence of substrate concentration on the induction of amidases in herbicide degradation. Z. Allg. Mikrobiol. 24:581–584.

6. **Martin, P., and R. A. MacLeod.** 1984. Observations on the distinction between oligotrophic and eutrophic marine bacteria. Appl. Environ. Microbiol. **47:**1017–1022.
7. **McCarty, P. L., M. Reinhard, and B. E. Rittmann.** 1981. Trace organics in groundwater. Environ. Sci. Technol. **15:**40–51.
8. **Palleroni, N. J.** 1984. *Pseudomonas* Migula 1894, p. 141–199. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of determinative bacteriology, 9th ed., vol. 1. The Williams & Wilkins Co., Baltimore.
9. **Reber, H. H., and P. Kaiser.** 1981. Regulation of the utilization of glucose and aromatic substrates in four strains of *Pseudomonas putida*. Arch. Microbiol. **130:**243–247.
10. **Robinson, J. A.** 1985. Determining microbial kinetic parameters using nonlinear regression analysis: advantages and limitations in microbial ecology. Adv. Microb. Ecol. **8:**61–114.
11. **Robinson, J. A., and J. M. Tiedje.** 1983. Nonlinear estimation of Monod growth kinetic parameters from a single substrate deple-

tion curve. Appl. Environ. Microbiol. **45:**1453–1458.
12. **Schmidt, S. K., and M. Alexander.** 1985. Effects of dissolved organic carbon and second substrates on the biodegradation of organic compounds at low concentrations. Appl. Environ. Microbiol. **49:**822–827.
13. **Schmidt, S. K., S. Simkins, and M. Alexander.** 1985. Models for the kinetics of biodegradation of organic compounds not supporting growth. Appl. Environ. Microbiol. **50:**323–331.
14. **Scow, K. M., S. Simkins, and M. Alexander.** 1986. Kinetics of mineralization of organic compounds at low concentrations in soil. Appl. Environ. Microbiol. **51:**1028–1035.
15. **Simkins, S., and M. Alexander.** 1984. Models for mineralization kinetics with the variables of substrate concentration and population density. Appl. Environ. Microbiol. **47:**1299–1306.
16. **Stratton, R. G., E. Namkung, and B. E. Rittmann.** 1983. Secondary utilization of trace organics by biofilms on porous media. J. Am. Water Works Assoc. **75:**463–469.