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Kinetics of Mixed Microbial Assemblages Enhance Removal of Highly Dilute Organic Substrates

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Our experiments with selected organic substrates reveal that the rate-limiting process governing microbial degradation rates changes with substrate concentration, *S*, in such a manner that substrate removal is enhanced at lower values of *S*. This enhancement is the result of the dominance of very efficient systems for substrate removal at low substrate concentrations. The variability of dominant kinetic parameters over a range of *S* causes the kinetics of complex assemblages to be profoundly dissimilar to those of systems possessing a single set of kinetic parameters; these findings necessitate taking a new approach to predicting substrate removal rates over wide ranges of *S*.

In the past, mathematical models designed to predict the rates at which microorganisms mediate the uptake and degradation of dissolved organic compounds, and especially anthropogenic chemicals, have been based on the assumption that a single set of kinetic parameters governs the processes (3, 4, 7, 11, 13, 14, 16, 18). These kinetic parameters include a maximum reaction velocity, V_{max}, and half-saturation constant, K, representing the substrate concentration, S, at which half of V_{max} is attained. Some researchers, however, have demonstrated that microorganisms, especially in complex assemblages, are kinetically heterogeneous: they possess diverse substrate removal systems, each having its own characteristic set of kinetic parameters (2, 6, 9, 10, 17). For a particular substrate, kinetic heterogeneity may result from the presence of multiple transport, degradative, or assimilative systems or other rate-limiting, substrate-concentration-dependent phenomena that follow rectangular hyperbolic functions.

Models incorporating the assumption that a single V_{max} and K govern the substrate removal rate, v, are based on the Michaelis-Menten equation: $v = (V_{max} \cdot S)/(K + S)$. For substrate concentrations at which uptake is undersaturated (concentrations well below K), v can be considered proportional to V_{max}/K. Because natural microbial assemblages commonly possess a variety of substrate removal systems, each operating optimally at different substrate concentrations, it is important that constancy of V_{max}/K should not be assumed over a wide range of S.

MATERIALS AND METHODS

Water samples were collected from the Oconee River and Lago Lake, Athens, Ga.; Okefenokee Swamp, Folkston, Ga.; mangrove swamps near Chub Cay, Bahamas; and Fresh Creek, Andros Island, Bahamas. According to previously published procedures using laboratory microcosms (11), the Oconee River samples were used to grow biofilm, which was subsequently detached and blended to provide replicate samples of a diversity of concentrated microflora. Kinetic studies were done by amending replicate water samples or blended biofilm with a wide range of initial concentrations of either D-[U-^{14}C]glucose (11.4 mCi/mmol), [U-^{14}C]phenol (118 mCi/mmol), *p*-[U-^{14}C]cresol (10.33 mCi/mmol), [^{14}C]methanol (8.7 mCi/mmol), *n*-[^{14}C]butanol (5.22 mCi/mmol), *p*-[U-^{14}C]chlorophenol (8.9 mCi/mmol), [1,3-^{14}C]acetone (3.14 mCi/mmol), or 2,4-dichlorophenoxyacetic acid methyl ester (2,4-DME). ^{14}C-labeled substrates were used to study mineralization (respiration to $^{14}CO_2$), and 2,4-DME was used for studying only parent compound loss. Kinetic studies included substrate amendments over a range of initial concentrations as much as 9 orders of magnitude. Rates were determined using triplicate water samples in 160-ml Pyrex bottles containing 50 ml of water incubated at 25 ± 1°C on a shaker. On the basis of acridine orange direct counts (8), bacterial numbers were constant during the incubation periods, which lasted 4 to 7 h, except for the *p*-chlorophenol and glucose studies, which lasted for approximately 24 h. On the basis of control samples containing 0.4% Formalin, abiotic transformation of the chemicals was negligible. Kinetic experiments with these and other chemicals are described in detail elsewhere (2, 10). Rate data were analyzed using both linear and nonlinear regressions, and values of V_{max} and K at various S values were derived from the slopes and intercepts of biodegradation data plotted according to modified Lineweaver-Burk linearizations developed by Wright and Hobbie (18). Pseudo-first-order rate coefficients, k_1, were calculated as the slopes of the linear regression lines (method of least squares) of the plots of natural logarithm of S versus time.

RESULTS AND DISCUSSION

As with previous experiments (2), our Wright-Hobbie plots were not linear, but were hyperbolic, having multiple slopes and intercepts, indicating the existence of higher K and V_{max} values at higher ranges of S (data not shown). For these kinetically heterogeneous assemblages, apparent K values were generally 10- to 100-fold higher than the initial S. Substrate removal systems, therefore, were undersaturated even at the highest concentrations tested. V_{max}/K ratios were relatively constant for several of the highest amended substrate concentrations tested; however, they were increasingly higher at successively lower substrate concentrations (Fig. 1 and 2). Consequently, substrate removal rates were increasingly enhanced at successively lower amended substrate concentrations. Using rate data obtained at high ranges of S, other researchers have also noted that degrada-

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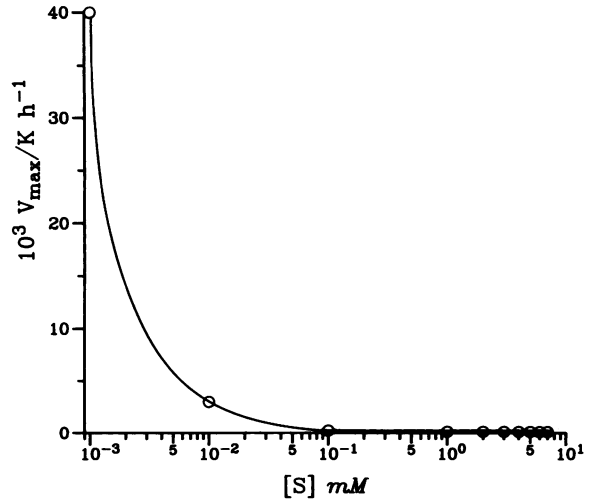

FIG. 1. Enhancement of phenol mineralization in replicate freshwater samples from Lago Lake as systems with different kinetic parameters dominated the kinetics at successively lower amended phenol concentrations, [S]. Enhancement was indicated by an increase in V_{max}/K, which determines reaction velocity below K.

tion rates of organic chemicals are faster than expected at low substrate concentrations (17).

In our mineralization studies, enhancement of substrate removal was evident over a wide range of S (Fig. 1), but was especially pronounced at S values of <10 μM (Fig. 2). Moreover, the magnitude of enhancement was compound specific. Acetone mineralization, for example, exhibited no measurable velocity enhancement over the same concentration range over which *p*-cresol exhibited about a 10-fold enhancement.

On the basis of linear transformations of data, estimated values of V_{max} and K contain various degrees of error, depending on the method of data analysis (1, 5, 12, 15). The enhancement effects that we observed, however, spanned

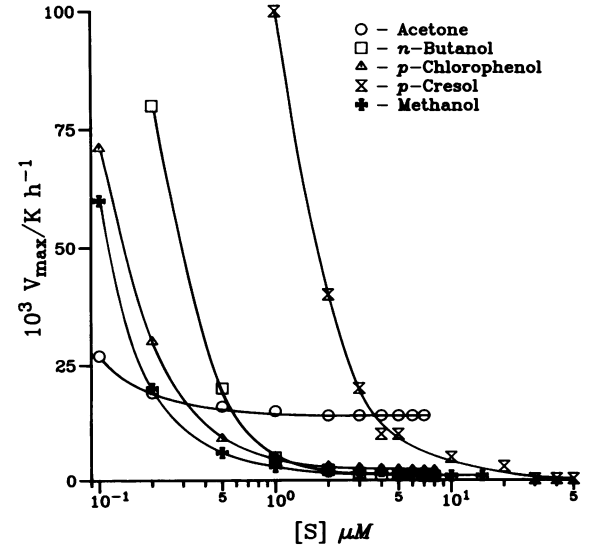

FIG. 2. Mineralization kinetic parameters (V_{max}/K) for low concentrations of various organic substrates added to replicate marine and freshwater samples from Chub Cay and Lago Lake.

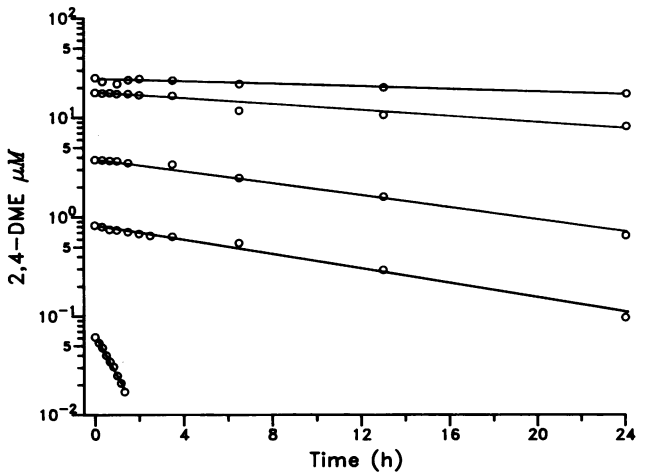

FIG. 3. Enhancement of removal of 0.06 μM 2,4-DME in replicate freshwater samples from Andros Island.

orders of magnitude of variation in kinetic parameters and were evident even by direct comparison of plots of S versus time. Relative to rates at higher values of S, a 100-fold increase in the specific rate of 2,4-DME degradation (i.e., the slope of plots of the natural logarithms of substrate concentrations versus time), for example, occurred at or below 0.06 μM in samples from Andros Island (Fig. 3), and a 3.5-fold increase in the specific rate of glucose removal occurred at concentrations at or below 1 μM in water from Lago Lake (Fig. 4). (Additional short-term experiments [<5 h] confirmed that the rate enhancement observed with glucose was concentration dependent rather than time dependent.) However, only a twofold increase in the specific rate of 2,4-DME removal occurred between 0.66 and 0.02 μM in samples of Oconee River microflora (Fig. 5). The magnitude of enhancement, therefore, was specific to the microbial assemblage (Fig. 3 and 5) as well as to the substrate and its concentration (Fig. 1 and 2).

The kinetic pattern of complex microbial assemblages described above produces an interesting twist with regard to

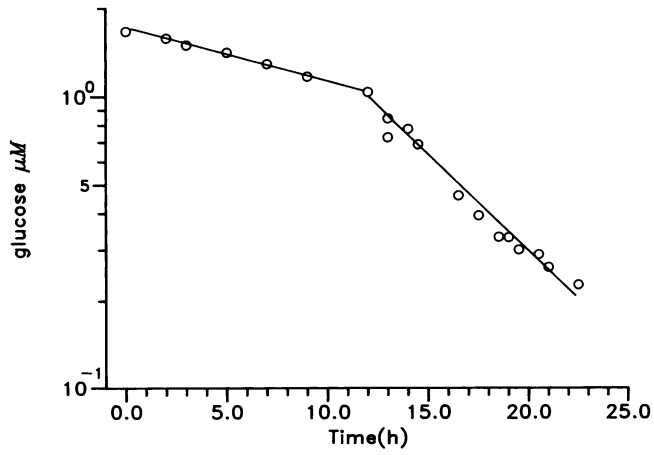

FIG. 4. Enhancement of D-glucose removal in a water sample from Lago Lake. The two slopes represent k_1 values of (0.04 ± 0.001) and (0.14 ± 0.007) h^{-1}. Glucose concentrations were calculated from the amount of glucose amended to the sample minus the sum of the amounts of $^{14}CO_2$ respired plus the amounts incorporated in the organisms.

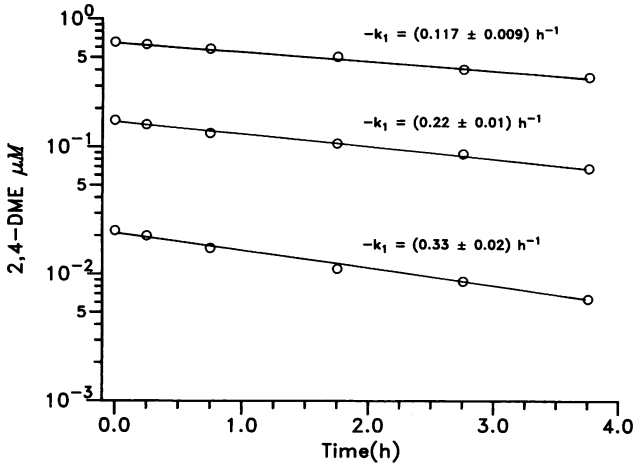

FIG. 5. Enhancement of 2,4-DME removal in replicate blended biofilm samples of Oconee River microflora.

using values of K and V_{max} to estimate v over wide ranges of S. In systems exhibiting only a single K and V_{max}, v can be estimated as $S(V_{max}/K)$ when $S < K$, with increasing accuracy at diminishingly lower values of S. [At $S = K$, $S(V_{max}/K)$ overestimates v by a factor of 2.] When $S > K$, $S(V_{max}/K)$ is increasingly inaccurate for increasingly higher values of S. The increasing degree of error results from the true v approaching a constant maximum value, V_{max}, while $S(V_{max}/K)$ continues to yield values of v increasingly higher than V_{max}. Except at very low S, however, complex assemblages exhibit K and V_{max} values, increasing somewhat proportionally with S as different kinetic systems dominate. As K and V_{max} increase, v may indeed exceed the lower V_{max} values of the complex system. By determining K and V_{max} at low S and using those values in the Michaelis-Menten equation, one may erroneously predict v to be nearly equal to the low-range V_{max} at high S in a complex assemblage. Because systems with higher K and V_{max} values dominate the kinetics at the higher S, v may be considerably higher than the V_{max} of the system that dominated at the low S value where K and V_{max} were determined. Also, because V_{max}/K is relatively constant except at very low S values, $S(V_{max}/K)$ may yield a better estimate of v than will the Michaelis-Menten equation when K and V_{max} determined at low S are used to estimate v at very high S (where systems having higher K and V_{max} values dominate). We have concluded, therefore, that although $S(V_{max}/K)$ is a good predictor of v when $S < K$ in systems exhibiting a single K and V_{max}, the opposite condition is true for kinetically heterogeneous systems: $S(V_{max}/K)$ is a better predictor of v than the Michaelis-Menten equation when $S > K$, for K and V_{max} determined at low S and used to predict v at very high S.

If the Michaelis-Menten equation is used for predicting v at very low S, where V_{max}/K is highly variable, and if changes in V_{max}/K are disregarded, very large errors can occur in predicted substrate removal rates. In one study, for example, V_{max} and K were 0.5 μg liter^{-1} h^{-1} and 0.01 μg liter^{-1} for mineralization of 0.1 μg liter^{-1} of phenol and 4.1×10^3 μg liter^{-1} h^{-1} and 3.6×10^7 μg liter^{-1} for 10^6 μg liter^{-1} of phenol. Using the Michaelis-Menten equation and kinetic parameters determined at the high concentration, one would expect a v of 1.1×10^{-5} μg liter^{-1} h^{-1} at an S value of 0.1 μg liter^{-1}. Because of substrate removal enhancement at lower concentrations, however, the measured v was 0.5 μg liter^{-1} h^{-1}, representing a 45,000-fold error in the predicted rate. Therefore, at very low concentrations of S, where V_{max}/K is dynamic, neither the Michaelis-Menten equation nor $S(V_{max}/K)$ is a good predictor of v, and changes in V_{max}/K versus S must be accounted for to accurately predict v over wide ranges of S.

When S was greater than 10 μM in our experiments, the similarity of V_{max}/K over a wide range of S made disparities in rates obvious only when v was measured over S ranges of several orders of magnitude. As a result, kinetic experiments involving amendments of cultures with only a narrow range of S may not reveal the potential kinetic diversity of microorganisms.

As was shown in studies of methyl parathion degradation by a *Flavobacterium* sp. (10), substrate removal rates may follow, to substrate extinction, kinetics indicative of a single set of kinetic parameters, while subsamples of the same culture amended with a range of initial substrate concentrations exhibit kinetics indicative of a range of kinetic constants. Lewis et al. (10) suggested that a metabolite formed in the metabolism of the substrate prevented the operation of the other substrate removal system that normally dominated the kinetics at low substrate concentrations. For the compounds reported in our current experiments, however, none of the environmental samples "locked in" to the initial kinetics of the higher substrate concentrations. The results obtained with *Flavobacterium* sp., therefore, do not appear to be representative of mixed microbial assemblages and may be compound and organism specific.

In summary, microbial assemblages displaying a diversity of kinetic parameters exhibit increases in V_{max} and K with increases in S, so that saturation of the combined systems does not occur over a wide range of S values. Furthermore, the increasingly disproportionate changes in V_{max} and K with decreasing S cause the kinetics of complex assemblages to be profoundly dissimilar to those of systems possessing a single set of kinetic parameters. Consequently, for heterogeneous kinetic systems, models based upon the Michaelis-Menten equation are unsatisfactory for predicting v at low S by directly using kinetic parameters determined at high S, and vice versa.

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