INFLUENCE OF SORPTION / DESORPTION PROCESSES ON THE BIOAVAILABILITY OF ORGANIC CONTAMINANTS

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The environmental fate for many non-ionic organic contaminants is a function of both their transport (partitioning between solid and aqueous phases) and their transformation (primarily biological degradation). While these two processes have received much individual attention, few studies have sought to integrate them. This is most likely the result of the complexity of the experimental systems, requiring a simultaneous understanding of both the physical chemistry and microbiology of contaminant behavior. During the first eight months of this project, we have taken a systematic approach to addressing the question of how sorption of organic contaminants to soils affects their bioavailability to microbial degraders.

Perhaps the most definitive, but by no means complete, study to assess the bioavailability of sorbed contaminants, was the work of Ogram et al (1985) involving the herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D). These authors developed a series of mathematical models to describe conditions in which soluble and/or soil-sorbed 2,4-D was degraded by free-living and/or attached bacteria. The application of these models involved several assumptions and required that numerous experimental conditions be met. Among these were:

1. That the test contaminant attains equilibrium between the aqueous and solid phases very rapidly upon its addition.
2. That the proportions of free-living and attached bacteria are known.
3. That degradation follows first order kinetics, i.e., that degradation rate is a function only of the effective substrate concentration.
4. That organisms mineralize the contaminant to CO_2 and H_2O without the accumulation of intermediates which would partition differently between the solid and aqueous phases than the parent compound.
5. That the numbers of organisms do not change over the course of the bioavailability assay.

An additional assumption, not explicitly stated by Ogram et al, is that:

6. Desorption kinetics are slow relative to biodegradation kinetics.

In our project, we are proposing to rigorously test Ogram's models for 2,4-D bioavailability and apply them to studies of the bioavailability of non-ionic organic compounds, a more abundant and toxic group of organic contaminants. Each of the assumptions/conditions outlined above is being tested to provide a fundamental basis for bioavailability studies with three compounds covering a broad range of sorptive and biodegradative properties (toluene, naphthalene and phenanthrene). We are employing four soils differing in their textural characteristics, sorptive capacities, and organic carbon contents. A diversity of bacterial

strains differing in their motility, gram reaction, surface properties, degradative capacities and attachment characteristics are being screened to provide controlled variability in bioavailability assays. In this way, we hope to identify the important parameters which limit the biodegradation of sorbed organic contaminants in the environment.

Working with four soils of varying organic carbon contents, we have studied the extent and kinetics of naphthalene sorption and desorption using batch equilibration techniques. These studies have shown that naphthalene approaches sorptive equilibrium rapidly at first (24 h), but that partitioning from solution to the solid phase continues to increase for many days thereafter (Figure 1). As a result, we routinely pre-equilibrate soil-water systems for a week or more prior to the initiation of bioavailability studies. Similarly, the initial rate of naphthalene desorption is rapid but declines upon the continuous depletion of the aqueous phase concentration. Furthermore, the kinetics of desorption become slower as the initial equilibration times increase (Figure 2). This observation has caused us to reevaluate Ogram's conclusions and to modify our interpretations of bioavailability data to account for desorption kinetics. This aspect of our work will be discussed more fully below.

We have developed procedures for measuring the extent and kinetics of bacterial attachment to soils. Attachment is quantified by incubating cells in a soil slurry for predetermined times, after which the slurry is diluted and filtered through a 3 um filter. This pore size allows passage of free-living bacteria while retaining those attached to particles greater than 3 um in diameter. Unattached cells are enumerated by plate counts and direct epifluorescence microscopic counts and are compared to soil-free controls. We have found sorption to be extremely rapid and to be bacteria- and soil-specific. The rapid attachment of cells to soils (Figure 3) obviates the need for a pre-equilibration period before bioavailability studies are initiated. In an analogous fashion to contaminant sorption, as soil organic carbon content (and specific surface area) increases, so does microbial attachment (Figure 4). The extent of attachment varies among the four strains of naphthalene-degrading bacteria being studied and is apparently a function of the relative cell surface hydrophobicity. The latter has been determined by measuring the binding of radiolabeled cells to a hydrophobic gel matrix. Since the degree of physical association between the biodegradative cells and the sorbent (and sorbed contaminant) may be an important factor governing bioavailability, we will include in our studies bacterial strains ranging widely in their propensities for attachment.

We are also developing an independent method to study microbial attachment to soils employing flow cytometry. This method, which relies on the differential fluorescence and light scattering properties of soil particles and bacteria has the advantage of not requiring a separation step in the analysis (Figure 5). Particles traveling through a high velocity flow cell are illuminated by spot-focused lasers. Detection of laser light scatter provides a measure of soil particle size and number. Bacteria are detected by measuring the fluorescence emission from DNA-stained cells. By looking at the light scattering properties of fluorescent (bacterial) particles, we can determine the number of attached bacteria and the particle sizes to which they are attached. Flow cytometry has been used successfully in the analysis of aquatic microbial populations, but this is its first application to soil microbial populations to our knowledge. We have obtained excellent agreement between the flow cytometric and filtration assays for attachment (Figure 6). Because of the speed of flow cytometric analysis (~10^4 particles/second) and its applicability to other questions of relevance to microbial activities in subsurface environments, we plan to develop this method during the continuation of this project.

Using high performance liquid chromatography (HPLC), radiorespirometric and colorimetric methods, we have characterized several strains of bacteria with regard to their mode, extent and kinetics of biodegradation. Two important conditions of the models are that

degradation follows first order kinetics (i.e., that rates are limited only by the effective substrate concentration) and that primary metabolites do not accumulate in the medium. We have identified for our studies bacterial strains which mineralize the test compounds to CO_2 and H_2O without the accumulation of metabolites by examining culture fluids using HPLC. Figure 7 shows HPLC chromatograms for (a) a 1 d culture of naphthalene-degrading Pseudomonas putida (ATCC 17484) in which no aromatic intermediates are present, and (b) a 1 d culture of naphthalene-degrading isolate NP-2c in which a large metabolite peak at 13.26 minutes and an array of smaller metabolite peaks between 9 and 12 minutes retention time are evident. Most of our studies to date have focused on strain 17484.

By examining naphthalene mineralization rates over a range of bacterial densities, we have found that a cell density of 10^8 cells/ml or greater is sufficient to provide first order degradation kinetics over a range of naphthalene concentrations (Figure 8). To date, we have used linear (ln) transformations of exponential rate data to derive values for first order degradation rate constants (K_1). To avoid inaccuracies in error estimations by this method, we are currently examining non-linear regression programs for calculating kinetic rate constants. This will enable us to ascribe statistical significance to differences in degradation rates as influenced by sorption of contaminants.

To ensure that cell numbers do not change over the course of a bioavailability assay, we have assessed the effect of the antibiotic, chloramphenicol, on naphthalene mineralization by P. putida 17484. At concentrations as low as 17 ppm, protein synthesis and cell division are arrested. The mineralization of naphthalene in pre-induced cells is uninhibited, however, up to a chloramphenicol concentration of 34 ppm (Figure 9). Interestingly, above this concentration no mineralization occurs but naphthalene is metabolized to the intermediate, salicylate.

In our studies of naphthalene degradation by various bacterial strains, we have noted that these differ in both their growth rates on naphthalene and in their efficiencies of converting the hydrocarbon into cell biomass. If bioavailability of sorbed hydrocarbon is limited by the rate of desorption from the solid phase, these organism-specific (Monod) growth parameters could greatly influence the results observed. An organism with a high substrate affinity and which is able to maintain a high growth rate at low dissolved substrate concentrations, will be more effective in driving the desorption process by extensively depleting the dissolved substrate. Conversely, an organism with low substrate affinity, and which grows sub-optimally at low dissolved substrate concentrations, may be unable to maintain growth as dissolved substrate is only slowly replenished through desorption. Our studies, therefore, will include interspecies comparisons of bioavailability in identical soil-water-contaminant systems. Our expectation is that sorbed substrate availability will vary among organisms characterized by different Monod kinetic parameters. We have begun substrate affinity and biodegradation kinetic characterizations of our naphthalene-degrading isolates using radiorespirometric, fluorimetric and other methods.

Having gained an understanding of the fundamental components of our model systems (contaminant-sorbent interactions, bacteria-sorbent interactions and bacteria-contaminant interactions), we have begun to assemble the components and to conduct bioavailability studies. Our initial results suggest that degradation is limited to compounds in solution and that desorption from the solid phase is required before degradation can occur. Figure 10 shows that the percent mineralization of naphthalene by P. putida 17484 in one experiment was directly related to the percent of naphthalene in solution. The results also suggest that soluble naphthalene is equally available to free-living and attached bacteria.

During the remainder of the first year of our project and, pending approval, the second year, we will continue bioavailability studies using naphthalene, phenanthrene and toluene as test compounds. We will systematically vary the other components of the experimental systems

to identify variables important in bioavailability, i.e., the compound-specific magnitude of sorption, desorption kinetics, bacterial attachment, organism-specific growth parameters, etc.

Having elucidated the factors controlling bioavailability in these model systems, we will then examine the bioavailability of aged contaminants in actual field soils and subsurface materials. As pointed out above, desorption rates tend to decrease as equilibration time of contaminants increases. The slow desorption of aged contaminants has been postulated to account for the persistence and recalcitrance of biodegradable compounds in soil systems. As we move into the third year of this project, we will apply our fundamental understanding of the relation between sorption / desorption and bioavailability gained from laboratory model systems described above, to study subsoils containing aged residues. With the experimental measures of the sorption / desorption equilibria and biodegradation kinetics of contaminants from previously exposed sites, we will gain a better understanding of what limits biodegradation of contaminants in nature, and under what circumstances limited bioavailability may be an important factor in the implementation of biological remediation technologies. We would like to have access to actual site materials of interest to DOE for this purpose. Hopefully, this will provide us with the information we need to identify the parameters which control biodegradation in natural systems and enable us to more accurately predict the fate of contaminants in the subsurface environment.

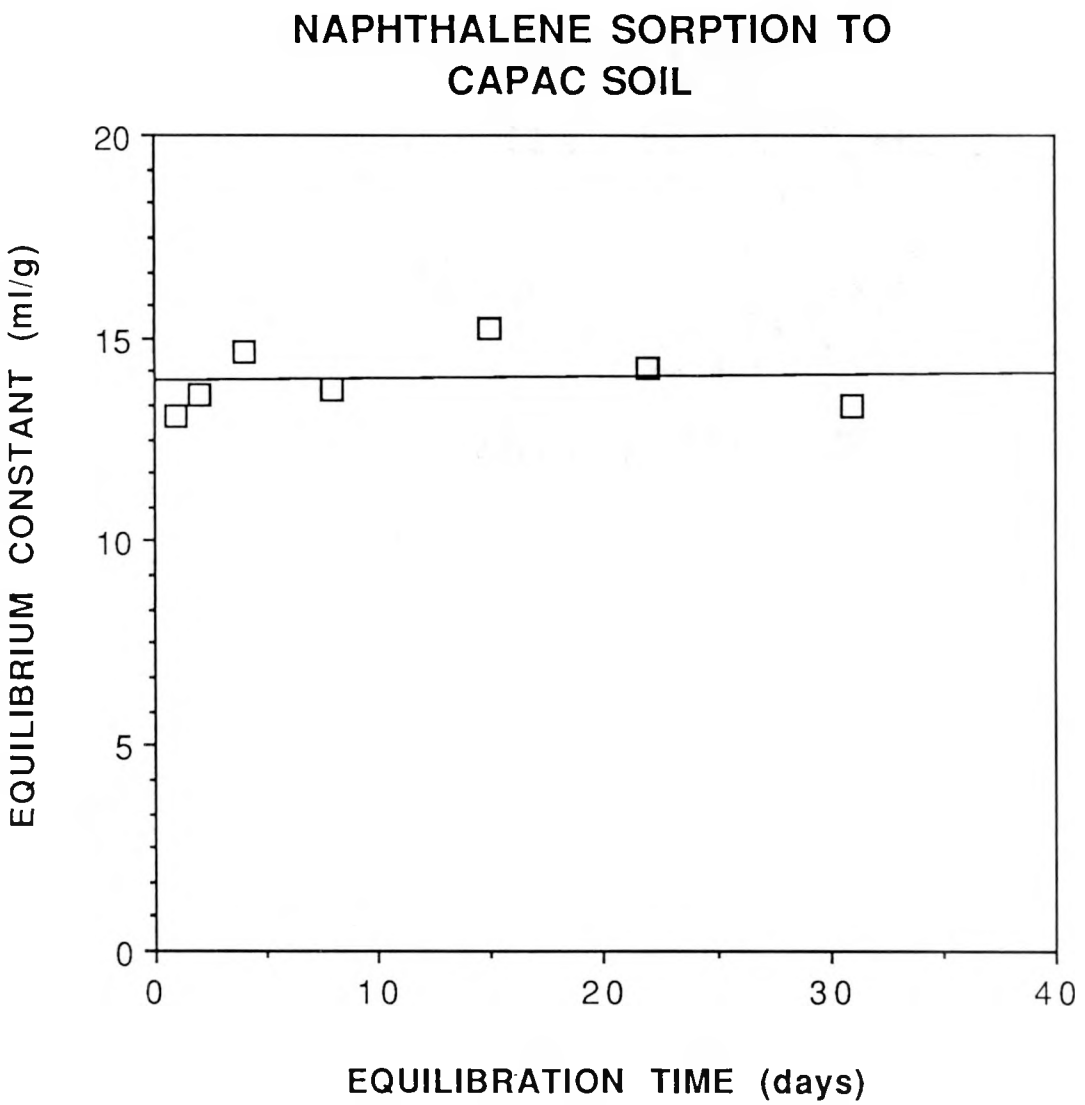
NAPHTHALENE SORPTION TO
CAPAC SOIL
EQUILIBRIUM CONSTANT (ml/g)
20
15
10
5
0
0
10
20
30
40
EQUILIBRATION TIME (days)

FIGURE 1.

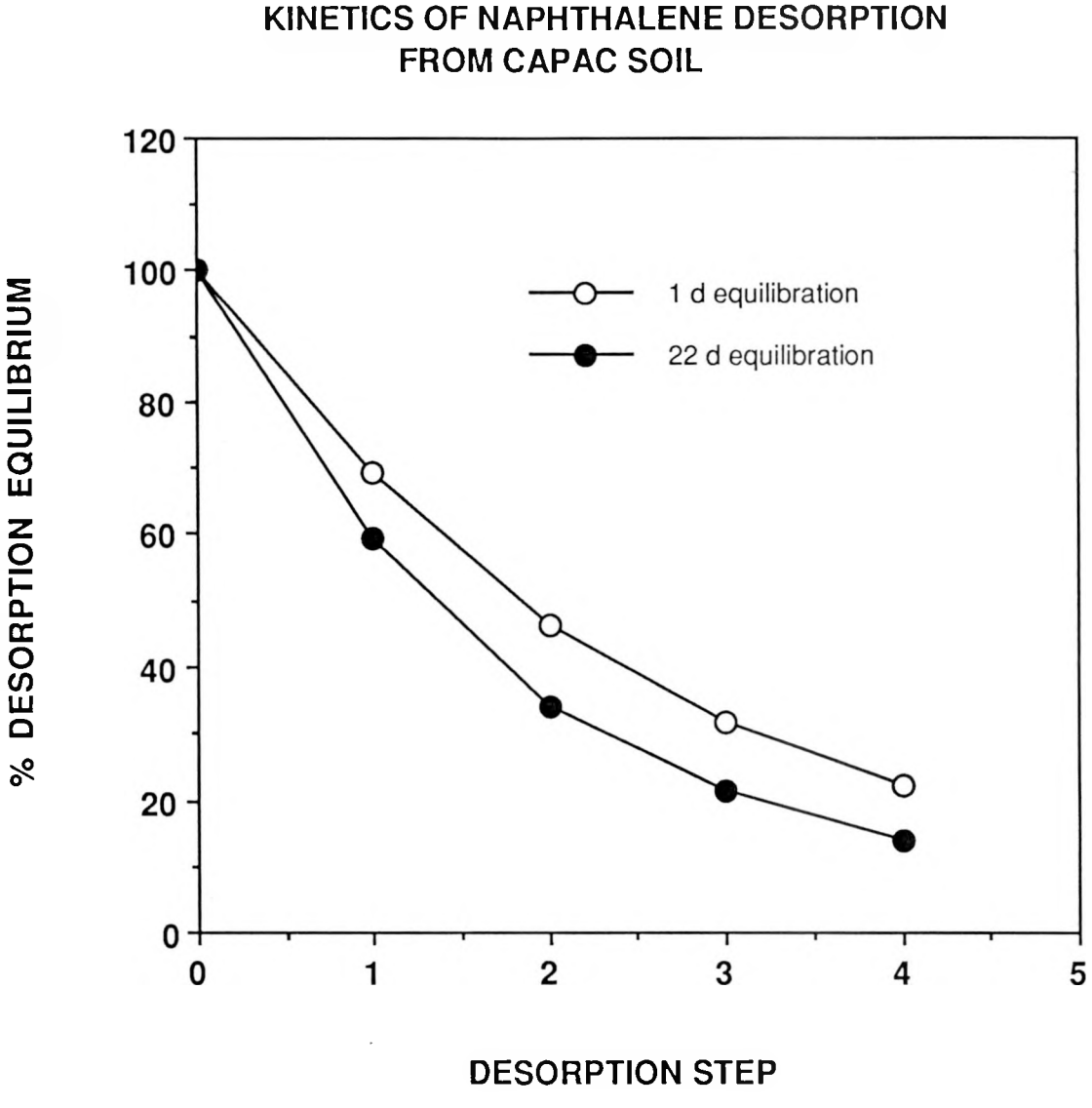
KINETICS OF NAPHTHALENE DESORPTION
FROM CAPAC SOIL
% DESORPTION EQUILIBRIUM
120
100
80
60
40
20
0
0
1
2
3
4
5
1 d equilibration
22 d equilibration
DESORPTION STEP

FIGURE 2.

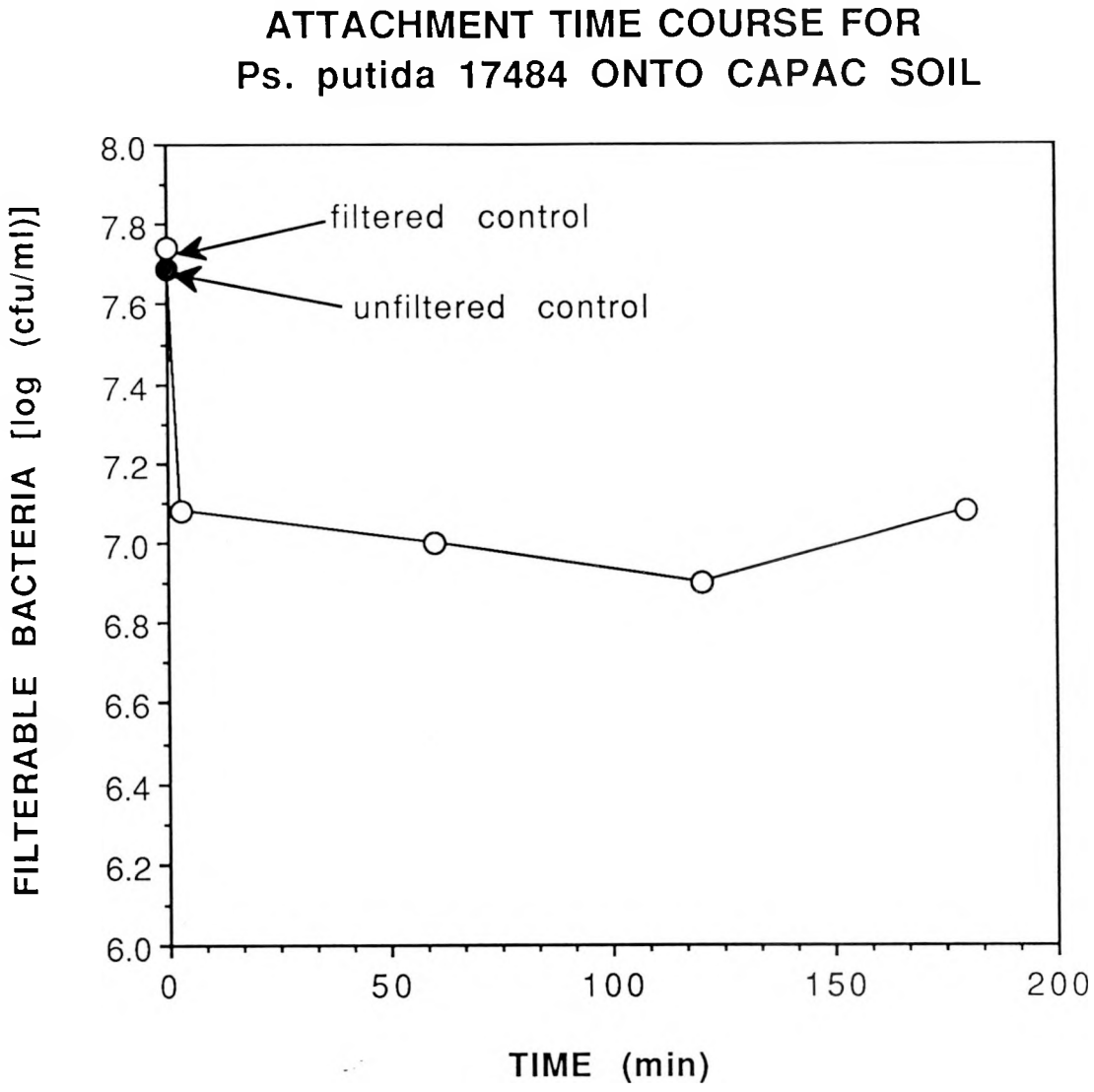
ATTACHMENT TIME COURSE FOR
Ps. putida 17484 ONTO CAPAC SOIL
FILTERABLE BACTERIA [log (cfu/ml)]
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
filtered control
unfiltered control
0
50
100
150
200
TIME (min)

FIGURE 3.

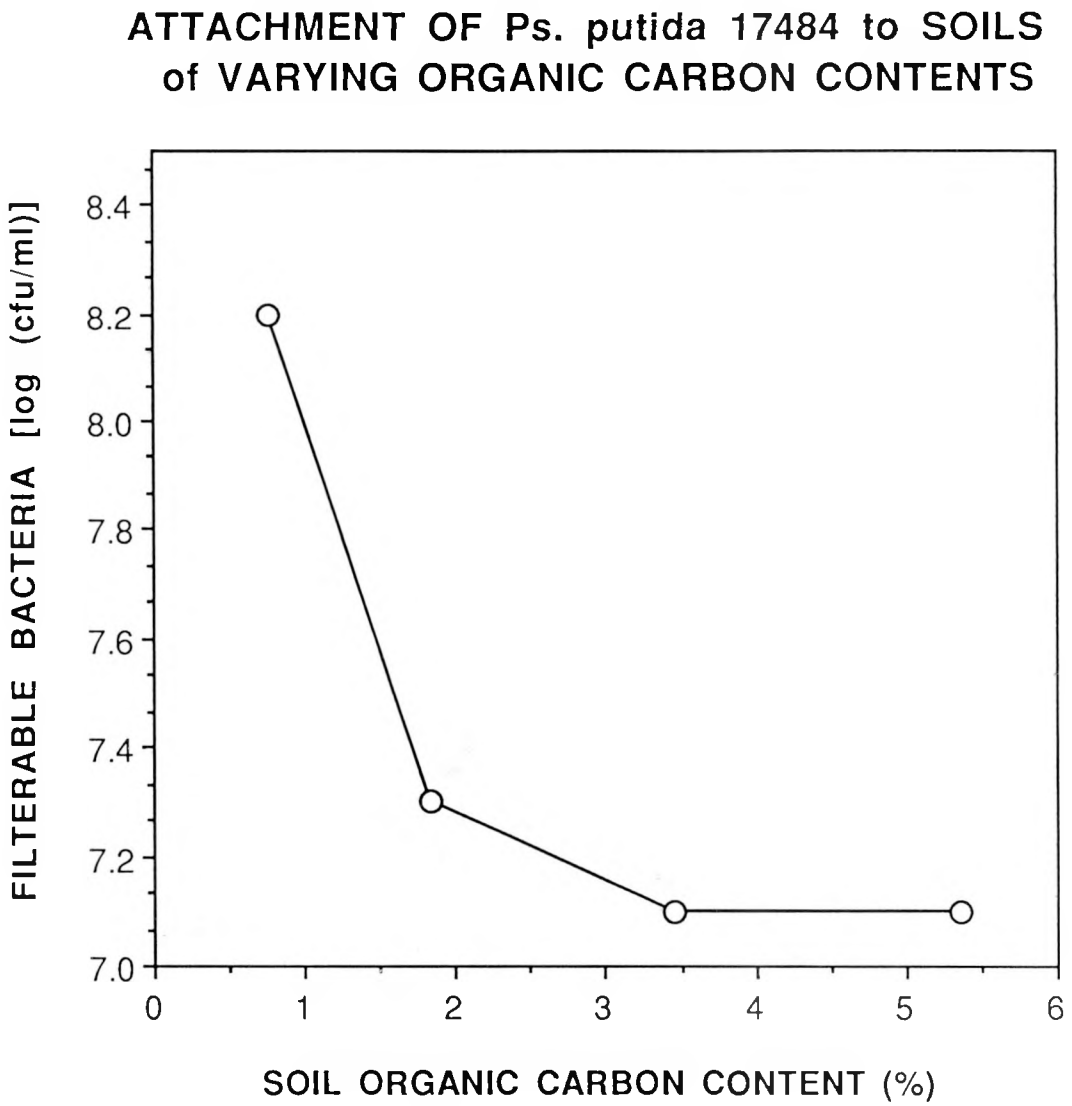
ATTACHMENT OF Ps. putida 17484 to SOILS
of VARYING ORGANIC CARBON CONTENTS
FILTERABLE BACTERIA [log (cfu/ml)]
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
0
1
2
3
4
5
6
SOIL ORGANIC CARBON CONTENT (%)

FIGURE 4.

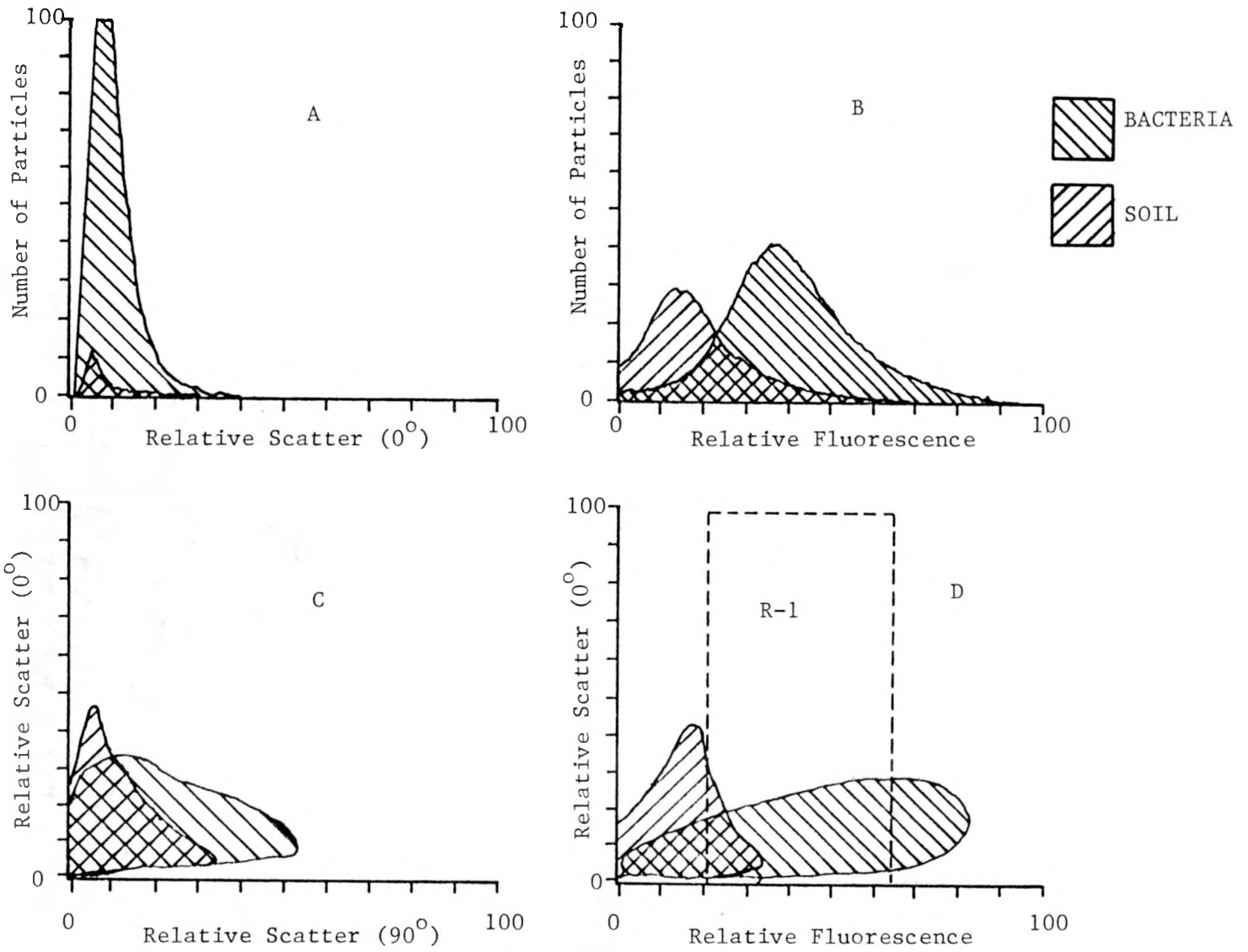

FIGURE 5. Flow cytometric analysis of Capac soil and naphthalene-degrading bacteria showing their different light scattering and fluorescence characteristics. Panel C, right angle scatter vs. forward scatter; panel B, fluorescence histograms; panel D, forward scatter vs. fluorescence, and; panel A, Scatter histograms of particles falling within R-1 of panel D.

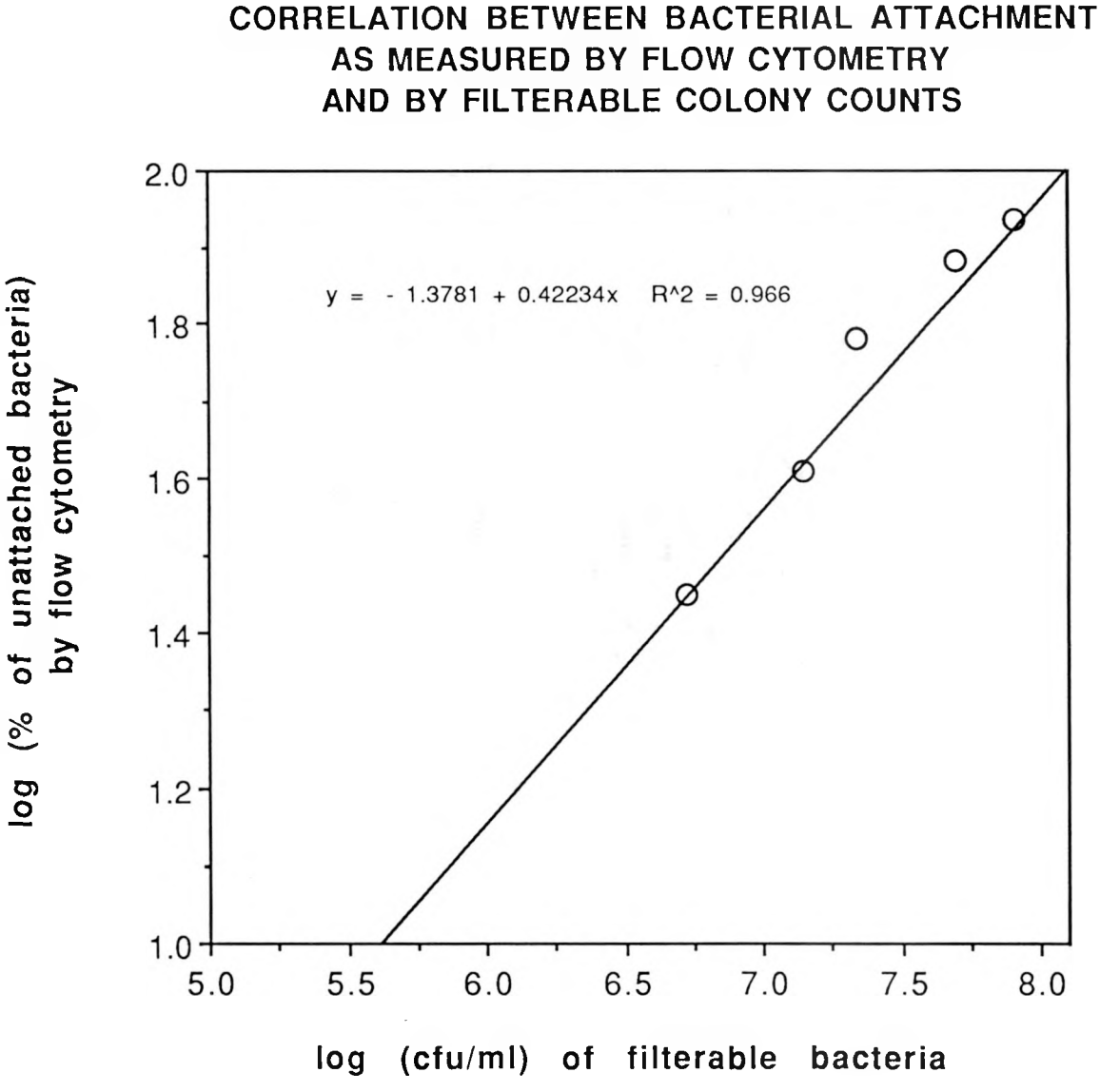
CORRELATION BETWEEN BACTERIAL ATTACHMENT
AS MEASURED BY FLOW CYTOMETRY
AND BY FILTERABLE COLONY COUNTS
y = - 1.3781 + 0.42234x R^2 = 0.966
log (% of unattached bacteria)
by flow cytometry
2.0
1.8
1.6
1.4
1.2
1.0
5.0
5.5
6.0
6.5
7.0
7.5
8.0
log (cfu/ml) of filterable bacteria

FIGURE 6.

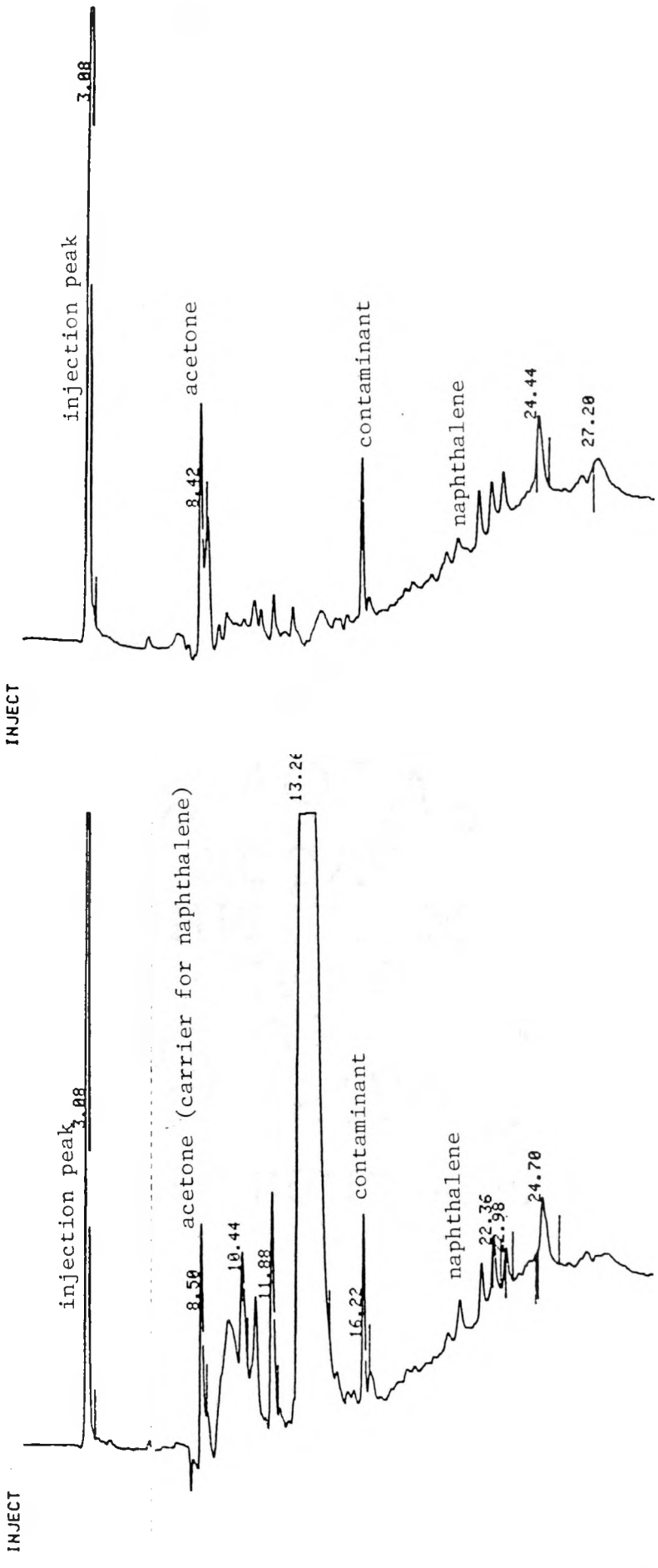

FIGURE 7. High performance liquid chromatograms for (a) Ps. putida (17484) and (b) organism NP-2c grown in naphthalene broth.

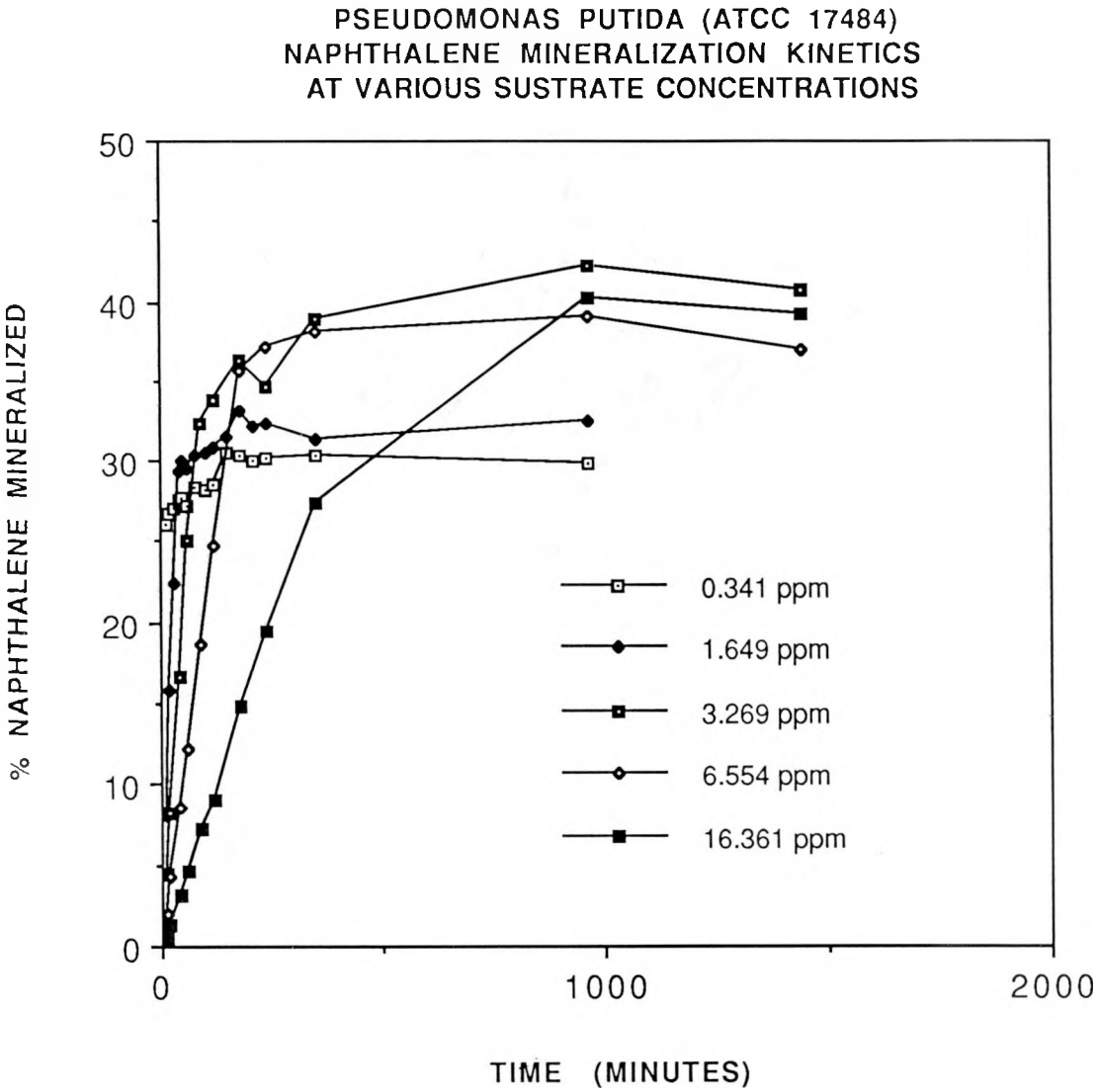
PSEUDOMONAS PUTIDA (ATCC 17484)
NAPHTHALENE MINERALIZATION KINETICS
AT VARIOUS SUSTRATE CONCENTRATIONS
% NAPHTHALENE MINERALIZED
50
40
30
20
10
0
0
1000
2000
TIME (MINUTES)
0.341 ppm
1.649 ppm
3.269 ppm
6.554 ppm
16.361 ppm

FIGURE 8.

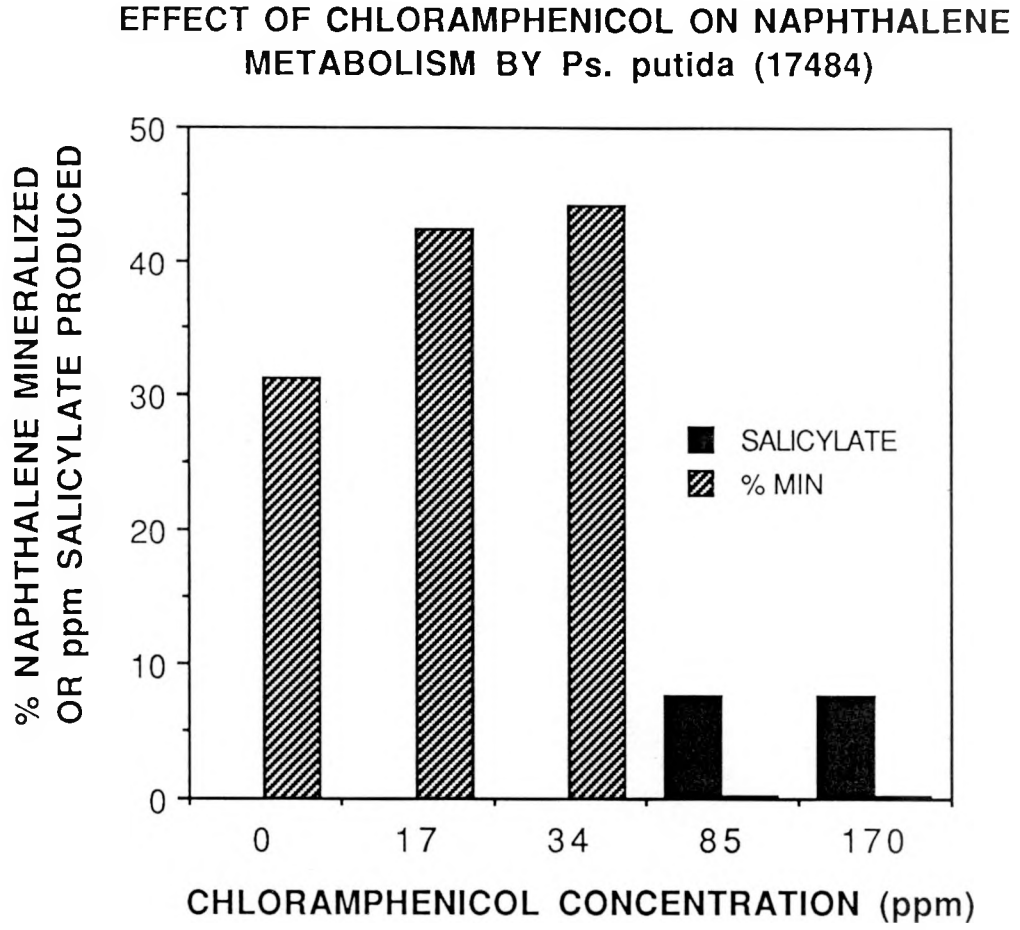
EFFECT OF CHLORAMPHENICOL ON NAPHTHALENE METABOLISM BY Ps. putida (17484)
% NAPHTHALENE MINERALIZED OR ppm SALICYLATE PRODUCED
50
40
30
20
10
0
SALICYLATE
% MIN
0
17
34
85
170
CHLORAMPHENICOL CONCENTRATION (ppm)

FIGURE 9.

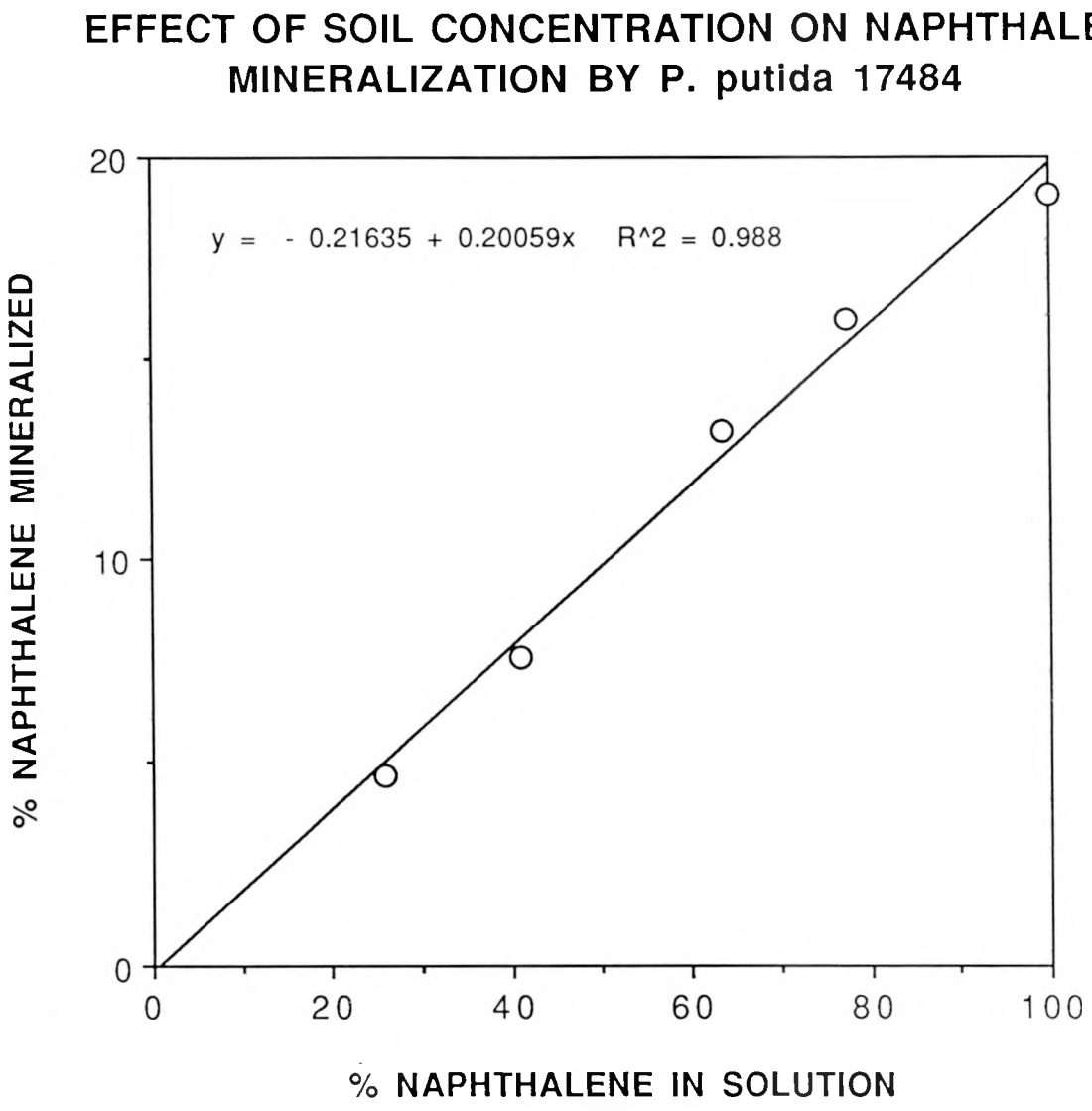
EFFECT OF SOIL CONCENTRATION ON NAPHTHALENE MINERALIZATION BY P. putida 17484
y = - 0.21635 + 0.20059x R^2 = 0.988
% NAPHTHALENE MINERALIZED
% NAPHTHALENE IN SOLUTION
0
10
20
0
20
40
60
80
100

FIGURE 10.