CONF-9110749--1

PNL-SA--21341

DE93 004407

THE EFFECT OF SORPTION ON THE DEGRADATION OF AROMATIC ACIDS AND BASES

C. C. Ainsworth
J. K. Fredrickson
S. C. Smith

October 1992

Presented at the
Sorption and Degradation of
Agrichemicals Symposium
October 27 - January 1 1991
Denver, Colorado

Prepared for
the U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
Richland, Washington 99352

Abstract

The availability and degradation of selected ionizable organic compounds sorbed to pure mineral phases are discussed. Substrates sorbed to mineral surfaces may or may not be protected from microbial attack; the degree of protection appears to be dependent on the type and cell density of the microorganism involved. The currently available data, however, demonstrate that there is little, if any, consensus on the types of reactions or interactions that facilitate sorbed substrate utilization. Rates of degradation of organic bases and cations that sorb to clay minerals via an exchange reaction are suggested to be directly related to substrate binding intensity and conformation on the clay surface. Similarly, rates of degradation of organic acids sorbed to the surface of oxides are suggested to be related to their interaction with the surface and the type of oxide sorbent. Although the rate-limiting step in microbial utilization of sorbed acids and bases is apparently a desorption process, the rate of desorption is itself linked to the compound's binding intensities on a given sorbent. Thus, as the binding intensities of compounds increase, chemical kinetic reactions, rather than mass-transfer processes, appear to limit the rate of desorption.

Introduction

The rate of biodegradation of xenobiotic organic chemicals in soils is often observed to be dependent on the aqueous substrate concentration, and a substrate's interaction with a solid phase can significantly affect its degradation rate. Several investigators have suggested that desorption from soils and sediments tends to limit the biodegradation rate of pesticides and organic contaminants (Stevens et al., 1990; Heitkamp et al., 1984; Mihelcic and Luthy, 1988; Subba-Rao and Alexander, 1982). Rijnaarts et al. (1990) concluded that desorption is the rate-limiting factor controlling degradation of α-hexachlorocyclohexane, and that intraparticle mass-transfer processes control desorption. The sorption/desorption kinetics of hydrophobic organic compounds (HOCs) from soils, sediments, and surfaces in general are often characterized by a rapid initial uptake (or release) followed by a slow approach toward equilibrium (Wu and Gschwend, 1986; Leenheer and Ahlrichs, 1971; Karickoff, 1981; Brusseau and Rao, 1989). This slow reaction (sorption or desorption) can occur on a time scale of days to months or longer (Coates and Elzerman, 1986). The long equilibration and release times observed in sorption/desorption studies have been characterized as indicating diffusion-controlled processes, such as micropore diffusion in aggregated materials (Wu and Gschwend, 1986; Steinburg et al., 1987) or intraorganic matter diffusion (Brusseau and Rao, 1989). Hence, diffusion or mass-transfer processes are often suggested to limit biodegradation of HOCs.

Unlike HOCs, the reactions that ionizable organic compounds undergo at the solid/water interface are more chemical in nature than the entropy-driven partitioning associated with HOC sorption; these interactions include van der Waals forces, H-bonding, dipole-dipole interactions, ion exchange, covalent bonding, protonation, ligand exchange, cation bridging, and water bridging (Koskinen and Harper, 1990). Depending on the strength of binding, sorbate-solid interactions could cause desorption kinetics to limit biodegradation or, as in the case of diquat, to inhibit degradation totally (Weber and Coble, 1968).

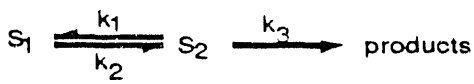
Desorption is a combination of chemical kinetic and mass-transfer processes, and thus, it is difficult to unambiguously distinguish which is the slow step in controlling the overall desorption rate, given a heterogeneous system such as soil. This difficulty primarily arises

from the fact that, for a given compound, sorption to soil may be related to a continuum of sorption mechanisms and sorption sites (Koskinen and Harper, 1990). For instance, sorption mechanisms for the triazine herbicides have been suggested to include hydrophobic interactions, cation exchange, van der Waals forces, cation bridging, and charge transfer, and both organic carbon and clay minerals have been viewed as sorption sites (Hayes, 1970; Koskinen and Harper, 1990). Therefore, to experimentally test hypotheses concerning chemical kinetic effects on biodegradation rates, it is necessary to minimize the mass-transfer processes inherent in multiphase systems. Obtaining information regarding a particular sorption mechanism is best accomplished by minimizing the number of other sorption mechanisms controlling the solid/water partitioning of the target compound. This requirement is best served by investigations conducted in mono-mineralogic systems. However, as one might expect, there is only limited information in the literature regarding sorbate degradation in such systems.

The purpose of this discussion is to illustrate the possible effects that sorption has on the biodegradation of selected ionizable organic compounds sorbed to single, pure mineral phases. In turn, the effects of sorption on degradation kinetics will be related to mechanisms of sorption. This is accomplished using both published data and data from our laboratory. This discussion is separated into four sections: 1) availability of sorbed compounds, 2) organic cation sorption/degradation, 3) organic acid sorption/degradation, and 4) conclusions. Because of the paucity of data on this subject, particularly with regard to pure phase studies, few concrete conclusions may be drawn; hence the discussion is largely illustrative. Reactions of particular interest that are discussed are cation exchange of organic bases to clay minerals and surface complexation to γ-Al_2O_3 by several organic acids. In an attempt to isolate the desorption process from the degradation process, fluorescence spectroscopy was used in studies with the organic bases quinoline and 2-hydroxyquinoline sorbed to hectorite and with salicylic acid in the presence of γ-Al_2O_3. The use of fluorescence spectroscopy as a tool to isolate desorption processes from biodegradation processes is discussed, as is the use of this technique in conjunction with $^{14}CO_2$ evolution studies.

Availability of Sorbed Substrates

A common perception is that sorption of a substrate to soil inhibits or at least reduces its availability to microbial attack. Most often, reduced availability of a substrate for microbial utilization is the conclusion drawn from estimates of the aqueous biodegradation rate and observed decreases in that rate in the presence of a solid phase; if a decreased degradation rate is observed as well, desorption is considered limiting. If one assumes that the sorbed species is unavailable, then a simplified model of the sorption/degradation process is



(1)

where S_1 and S_2 are substrate in the unavailable (sorbed) and available (aqueous) forms, respectively (Hamaker and Goring, 1976), and k_1, k_2, and k_3 are first-order rate constants for sorption, desorption, and mineralization, respectively. If this model is accurate, then desorption must occur prior to degradation. However, this does not mean that desorption will necessarily control the degradation rate. On the other hand, however, if this model is accurate, 1) sorption could retard biodegradation rates by decreasing the aqueous concentration of the substrate (S_2) to levels below the threshold level necessary for microbial utilization, or 2) the rate of desorption (k_2) could be rate-limiting with respect to the mineralization process (k_3).

Studies in soils and sediments with isopropylphenyl phosphate (Heitkamp et al., 1984), chlorproham and di-*n*-butyl phthalate (Steen et al., 1980), and hexachlorohexane (Rijnaarts et al., 1990) suggest that the sorbed substrate is protected from microbial attack. For hexachlorohexane, Rijnaarts et al. (1990) concluded that intraparticle mass-transfer processes controlled desorption and biodegradation because the degrading microorganism could not penetrate the interior of the soil aggregates. From model evaluations of (2,4-dichlorophenoxy)acetic acid (2,4-D) soil desorption/biodegradation rates, Ogram et al. (1985) concluded that sorbed 2,4-D was completely protected from microbial attack. Furthermore, they demonstrated that both sorbed and solution-phase bacteria were able to utilize aqueous-phase 2,4-D only; in addition, the organisms (whether sorbed or in the aqueous phase) were

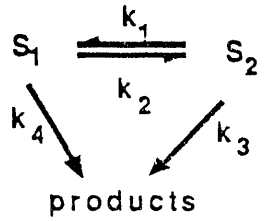
equally efficient in degrading solution-phase 2,4-D. In general, these studies suggest that sorption protects the substrate from microbial attack and that desorption limits biodegradation. Remberger et al. (1986), however, suggested that sediment-sorbed chloroguaiacols, chlorocatechols, and chloroveratroles may be accessible to biological transformations. Similar results have been observed in pure mineral studies in which the substrate was sorbed to the surface via mechanisms other than hydrophobic partitioning and was apparently accessible to microorganisms. Thus, there are conflicting conclusions with regard to the bioavailability of sorbed organic compounds.

Weber and Coble (1968) found that no degradation of diquat occurred in an aqueous suspension of montmorillonite when diquat was in the sorbed state, indicating that microorganisms could not utilize the sorbed substrate. In this study, sorption probably decreased the aqueous concentration of diquat to levels below the threshold level necessary for microbial utilization; clearly, sorbed diquat was protected from microbial attack. However, even when the aqueous concentration of diquat was above the threshold level for microbial utilization, degradation was so slow that no conclusions could be drawn regarding the role of desorption from either montmorillonite or kaolinite. In other pure clay-sorbate systems, dextran (Olness and Clapp, 1972), quinoline (Smith et al., 1992), aspartate and cysteine (Dashman and Stotzky, 1986), alkylamines (Wszolek and Alexander, 1979), and benzylamine (Miller and Alexander, 1991) sorbed to montmorillonite were apparently at least partially protected from microbial attack. The last two of these investigations, however, demonstrated that the effect of sorption on the rate of biodegradation was dependent on inoculum size. Similarly, degradation of salicylic acid, phthalic acid, and catechol was significantly reduced when they were sorbed to γ-Al_2O_3 (Ainsworth et al., unpublished).

Wszolek and Alexander (1979) observed that at population densities of approximately $2x10^8$ cells/mL, degradation of n-decylamine sorbed to montmorillonite was about 32 times slower than for the aqueous phase. However, at greater cell densities (6 and $20x10^9$ cells/mL), degradation was faster in the presence of montmorillonite than in its absence (Fig. 1). Ainsworth et al. (unpublished) studied the degradation of phthalate by Pseudomonas putida NMH102-2 and Pseudomonas cepacia PHK in the presence and absence of γ-Al_2O_3. The rate of phthalate mineralization differed not only between the presence or absence of γ-Al_2O_3, but also

between microorganisms. The latter observation was particularly intriguing, given that the numbers of organisms were the same [approximately 10^7 colony-forming units (cfu) per milliliter] and that both microorganisms utilize the same metabolic pathway to mineralize phthalate (i.e., mineralization proceeds through a 4,5-dihydroxyphthalate intermediate). In solution, the P. cepacia mineralized the phthalate much faster than did P. putida. Phthalate mineralization by P. putida (as measured by $^{14}CO_2$ evolution) showed evidence of desorption-limited microbial utilization and hence protection of the sorbed substrate (Fig. 2a); however, phthalate sorption to γ-Al_2O_3 did not greatly affect the rate of phthalate utilization by P. cepacia PHK (Fig. 2b). Similarly, Guerin and Boyd (1992) observed that the rate and extent of naphthalene mineralization by P. putida (ATCC 17484) and a Gram-negative soil isolate were vastly different. The rate and extent of naphthalene mineralization by P. putida were indicative of an enhanced soil desorption rate, but the soil isolate exhibited desorption-limited degradation with regard to both rate and extent of mineralization.

Based on the criteria stated earlier and the data noted above, a substrate in the sorbed state may be protected from microbial utilization under all conditions. A simplified two-compartment model of this phenomenon is



(2)

where S_1, S_2, k_1, k_2, and k_3 have the same definitions as in reaction 1 and k_4 is the first-order rate constant for sorbate utilization directly from the surface (Scow et al., 1986). In fact, this model provided the best estimate of nitrilotriacetic acid and phenol mineralization in soil (Scow et al., 1986). This model has also been utilized in other investigations (see Alexander and Scow, 1989). While the model may provide the best fit of data under certain conditions, no data have been published that substantiate the physical nature of the model. Wszolek and Alexander (1979) speculated that at high population densities of the n-decylamine degrader used in their

study, extracellular enzymes or microbially mediated pH change facilitated n-decylamine desorption from the bentonite. Although the mechanism(s) responsible for the observations in the phthalate study also have not been determined, pH changes mediated by either microorganism in the supernatant were minimal (<0.5 pH units). Also, phthalate mineralization by P. cepacia was so rapid that it is unlikely that extracellular enzymes were involved. However, the phthalate results would suggest that 1) significant differences exist between different microorganisms and their ability to utilize sorbed substrate, and 2) while both phthalate degraders utilize the same pathways for phthalate metabolism, their ability to scavenge substrate differs significantly. Guerin and Boyd (1992) concluded that the observed differential bioavailability of soil-sorbed naphthalene for the two microorganisms is the result of organism-specific properties. From the few examples discussed here and others (see Stotzky, 1986), it is evident that little is known mechanistically about coupled processes (chemical and biological) and their interactions regarding sorbed substrate utilization by microorganisms.

Sorption-Degradation of Organic Bases

Sorption - Exchange reactions between inorganic cations or anions have been characterized as rate-limited mass-transfer reactions with either diffusion through a water film (film diffusion; FD) or intraparticle diffusion (PD) being the rate-limiting process (Sparks, 1989). The chemical reaction(s) associated with the exchange/desorption process has never been definitively demonstrated in soils or in clay systems. Nor have chemical reactions or kinetics been suggested to be rate-limiting, as it has always been assumed that exchange reactions are too rapid to affect the overall process rate. However, Liberti and Passino (1985) reported that the rate of exchange is affected by chemical interactions between a sorbate and the exchanger phase in gel-type resins that exhibit high selectivity.

High selectivity for clay surfaces is a characteristic of a variety of organic cations and bases (Zachara et al., 1990; Ainsworth et al., 1987; Hayes et al., 1978; Thompson and Brindley, 1969; Lailach et al., 1968; Weber and Weed, 1968; Weber et al., 1965). Sorption of paraquat

and diquat to the surface of clay minerals occurs via cation exchange, and both are sorbed up to the exchange capacity of the clay. These pesticides exhibit a high affinity for the surfaces of montmorillonite, vermiculite, and kaolinite (Hayes et al., 1978; Weed and Weber, 1969; Weber and Weed, 1968; Weber et al., 1965). For fully exchanged montmorillonite and vermiculite, only about 5% of the sorbate is desorbed by 1M $BaCl_2$ (Weber and Weed, 1968). Kaolinite, on the other hand, releases approximately 80% of the sorbate in 1M $BaCl_2$ (Weber and Weed, 1968). The difference in paraquat and diquat desorbability between montmorillonite or vermiculite and kaolinite may be due to adsorption location (basal plane sorption for the 2:1 clays versus edge sorption with kaolinite) and the sorbate's flat orientation parallel to the basal plane in the 2:1 clay systems. In addition, lamellar collapse of montmorillonite and vermiculite, at high surface loading, onto the flattened organocation further increased their binding intensity. Sorption of a series of n-alkylammonium ions (Theng et al., 1967; Vansant and Uytterhoeven, 1972) and α, ω – bistrimethylammoniumalkanes (Maes et al., 1980) to montmorillonite exhibited increased selectivity with increasing molecular weight. Similarly, the selectivity (as denoted by the calculated selectivity coefficient [Kv]) of a series of N-heterocyclic compounds with Na-montmorillonite was observed to increase as ring number increased (K_V increases in the order acridine > quinoline > pyridine; Zachara et al., 1990).

Maes et al. (1980) observed a decrease in the enthalpy and free energy of exchange (ΔH°_{ex} and ΔG°_{ex}, respectively) with increasing chain length for α,ω – bistrimethylammoniumalkanes exchange to Camp Berteau Na^+- or Ca^{2+}-montmorillonite. For every additional CH_2 group, the ΔH°_{ex} and ΔG°_{ex} decreased by approximately -0.6 and -0.88 kJ/mol, respectively. Maes et al. (1980) attributed the observed change in ΔH°_{ex} and ΔG°_{ex} to delocalization of charge from the nitrogen to the alkyl chain. CNDO/2 calculations (Maes et al. 1980) showed that the formal charge on the NH_x head group was increasingly delocalized over the alkyl tail as the number of CH_2 groups increased. Maes et al. (1980) cited charge delocalization as the cause for the observed correlations between enhanced selectivity and 1) molecular weight and 2) polarizability for exchange of straight-chain ammoniumalkanes on montmorillonite. Likewise, surface stability of pyridine over selected alkylammonium cations on the surface of montmorillonite is hypothesized to be caused by the greater polarizability of pyridine and delocalization of positive charge through the π-ring system (Maes et al. 1978). Similar

molecular calculations demonstrate that formal positive charge is distributed through the ring structure of the N-heterocyclic compounds pyridine, quinoline, and acridine and that the increase in selectivity with ring number was closely correlated with formal charge on the ring N and with solvation energy (Zachara et al., 1990). Similar interactions have been suggested to stabilize paraquat and diquat on the surface of montmorillonite (Hayes et al., 1978). The high selectivity associated with these interactions clearly results from forces other than simple coulombic interaction. However, the high binding affinity of organic cations and bases to clays and the effect of binding intensity on sorbate degradation rate is not well understood.

Degradation - Wszolek and Alexander (1979) followed microbial mineralization rates, via evolution of ^{14}C, from a homologous series of n-alkylamines in aqueous solution and compared them to the mineralization rates of the same series of n-alkylamines sorbed to bentonite. The data suggest that as the molecular weight of the sorbate increased, so did its resistance to microbial attack. The correlation between resistance to microbial attack and molecular weight would appear to be similar to the correlations noted by Maes et al. (1980) and by Cowan and White (1958), who demonstrated that ΔG^0_{ex} decreased linearly with increasing chain length (molecular weight) for the sorption of a series of n-alkylamines to bentonite. Unfortunately, selectivity coefficients were not determined in the Wszolek and Alexander (1979) study, and hence, no definitive relationship can be determined between sorption selectivity of the n-alkylamines and their degradation.

The degradation of n-decylamine sorbed to bentonite was observed to be about 32 times slower in the presence of bentonite than in the absence of bentonite at high bacterial densities ($2x10^8$ cells/mL; Fig. 1). Similarly, Miller and Alexander (1991) found that desorption limited degradation of benzylamine that was sorbed to montmorillonite only at high population densities ($>10^7$ cells/mL). The presence of bentonite did not appear to affect the microbial population in a physical sense (e.g., it did not hinder uptake) or to cause metabolic problems, given that rates of glucose mineralization rates by the same microorganism in the presence and absence of the clay were identical.

The results of Wszolek and Alexander (1979) and Miller and Alexander (1991) suggest that desorption probably limited degradation at high isolate densities and that sorbed substrate was

protected from microbial attack. However, it is only possible to speculate whether mass transfer or chemical kinetics was the rate-limiting process. The decreasing rate of degradation with increasing chain length observed by Wszolek and Alexander (1979) suggests that the strength of binding is related to the rate of degradation and that a chemical kinetic process becomes increasingly important with increasing chain length. However, an interparticle diffusion mass-transfer process analogous to that hypothesized for HOC compounds (Brusseau and Rao, 1989), where the sorbate must go through a series of adsorption/desorption steps prior to arrival in the bulk solution (and subsequent utilization), could explain the observed results just as well as a chemical kinetic process.

Quinoline, an ionogenic N-heterocyclic compound (pk_a = 4.92), is strongly sorbed to smectite surfaces via cation exchange of the protonated quinolinium ion (Ainsworth et al., 1987; Zachara et al., 1990). Quinoline (Q) is further stabilized on the surface as a result of its parallel orientation to the clay surface (Greene-Kelly, 1955) and exhibits increased surface stability because of to its π-electron ring structure. The thermodynamic exchange constant (K_{ex}) for Na $\Rightarrow$ Q exchange is 340 (Ainsworth et al., 1987).

Smith et al. (1992) used a quinoline-degrading bacterium at relatively high population densities ($\approx 10^7 - 10^8$ cfu/mL) and five concentrations of Na-montorillonite (0.0 to 1.14 $cmol_{(-)}/L$) to probe the effects of sorption on quinoline mineralization. Quinoline is rapidly converted to a 2-hydroxyquinoline intermediate by the bacterium (Brockman et al., 1989). In the absence of clay, both Q and 2-hydroxyquinoline were observed in solution after 30 min. In the presence of Na-montmorillonite, 2-hydroxyquinoline was observed only within the first 30 min and only in the least dense clay suspensions (Smith et al., 1992). In addition, the overall Q mineralization rate was reduced, with the reduction being dependent on clay concentration and hence a reflection of the Q solid/aqueous phase partitioning (Fig. 3); the initial total Q concentration was constant, but as clay concentration was increased, the aqueous concentration decreased from 89 to 6.9% of the total Q and surface coverage decreased from 0.32 to 0.013 cmol(QH+)/kg. The evolution of $^{14}CO_2$ apparently proceeded at two distinct rates. The initial rates appeared to be directly related to the aqueous Q concentration (and therefore the clay concentration), and a much slower rate became apparent after aqueous Q was depleted. The latter rate decreased slightly with increasing clay concentration. Modeling the $^{14}CO_2$ data as first-

order rates yielded a rate constant (k_{obs}) that was about 20 to 30 times lower in the presence of clay than the rate constant for aqueous mineralization.

The observations of Smith et al. (1992), Miller and Alexander (1991), and Wszolek and Alexander (1979) strongly suggest that desorption from the clay surface limits mineralization of organic bases sorbed through a cation exchange mechanism. However, conclusions about the actual rate of desorption and its impact on mineralization cannot be made because such conclusions would be drawn from observations of the end product (i.e., $^{14}CO_2$), which is produced by a series of metabolic reactions that are predominantly internal to the cell.

In an attempt to delineate more clearly the effect of desorption on the overall process of Q degradation, Smith et al. (1992) employed fluorescence spectroscopy to take advantage of the fluorescence properties of quinoline, the quinolinium ion, and its microbial transformation product, 2-hydroxyquinoline. The quinolinium ion and 2-hydroxyquinoline are both fluorophores whose fluorescence maxima differ by about 40 nm. Quinoline does not fluoresce. Hectorite (a trioctahedral smectite) was used in these studies instead of montmorillonite because hectorite does not quench the quinolinum or 2-hydroxyquinoline fluorescence. In addition, the Q-degrading bacterium does not fluoresce and the fluorescence of both fluorophores is totally quenched when they are taken up by the bacterium. Because of these qualities, the transformation of quinolinium to 2-hydroxyquinoline and its uptake from aqueous solution could be traced directly by simultaneously placing aqueous Q and the bacterium into a cuvette and repeatedly acquiring spectra with time. The same process can be used to trace the changes in concentration of quinolinium ion on the surface of hectorite as the bacterium utilizes the aqueous-phase Q, causing desorption of the surface-bound substrate.

Using this technique, Smith et al. (1992) observed that the transformation of quinolinium to 2-hydroxyquinoline was extremely rapid, and that loss of 2-hydroxyquinoline from solution was approximately 5 to 8 times slower than the transformation of quinolinium to 2-hydroxyquinoline (Fig. 4). In a hectorite suspension (pH 6.0) previously equilibrated with Q (>99% of the Q partitioned to the surface), the disappearance of quinolinium differed from the pattern in the aqueous system in three major aspects (Fig. 5): 1) the rate of quinolinium disappearance from the surface of hectorite was about 30 times slower than in solution, 2) the

transient build-up of 2-hydroxyquinoline observed in the aqueous system did not occur in the presence of clay, and 3) examination of the aqueous phase showed the Q and 2-hydroxyquinoline concentrations were below detection limits ($<10^{-8}$ M) over the entire course of the study. Smith et al. (1992) conclude that, in the absence of clay, the uptake of 2-hydroxyquinoline is the rate-limiting step in Q mineralization, but in the presence of clay, desorption is rate-limiting. Put in the context of the Hamaker and Goring (1976) model (Rx. 1), $k_3 \gg k_2$ and the back reaction, represented by k_1, does not occur. Interestingly, the difference in the rates of quinolinium disappearance between the aqueous and clay suspensions, as determined by fluorescence, was about the same as that determined using $^{14}CO_2$ mineralization (i.e., about a factor of 30) (Smith et al., 1992). This observation suggests that the biological processes are not affecting the observed rates; that is, microbial processes after uptake are not rate-limiting. This is not always true, however, as will be discussed with regard to salicylate biodegradation in the next section.

The disappearance of Q from the surface of hectorite, as a function of surface loading, yielded a family of parallel curves whose slopes appeared to be the same (Fig. 6; Smith et al., 1992). Additionally, the rate of quinolinium disappearance from the clay surface was not affected by the saturating cation (Na or Ca) nor by stirring rate. Smith et al. (1992) concluded that desorption was the rate-limiting step in these studies, and that a chemical process rather than a mass-transfer process controlled desorption. Also, data from the fluorescence studies indicate that either 1) Q at surface concentrations $\leq$0.004 cmol(QH+)/kg is irreversibly sorbed, or 2) the aqueous Q concentration supported by such surface concentrations is less than the critical level necessary for microbial utilization. These conclusions reflect the fact that the quinolinium surface concentration appears to asymptotically approach 0.004 cmol(QH+)/kg at all initial concentrations. Even after 17 h, the quinolinium surface concentration was still slightly above this value.

One of the major determining factors associated with identifying desorption as a rate-limiting step in the biodegradation of an organic cation or base is the interaction between the sorbate and the surface. For instance, the investigations of Wszolek and Alexander (1979) with a homologous series of n-alkylamines indicate that there is a relationship between desorption, microbial utilization, and binding intensity. Similarly, there appears to be a considerable

difference between the utilization of sorbed Q and that of 2-hydroxyquinoline (Ainsworth et al., unpublished data). Sorption of 2-hydroxyquinoline to the hectorite surface occurs via a cation exchange reaction, but the selectivity coefficient is about three to four times less than that of Q. Using fluorescence spectroscopy and the procedure outlined by Smith et al. (1992), the disappearance of quinolinium and 2-hydroxyquinoline in the presence of Na-hectorite and the Q-degrading bacterium was followed with time (Fig. 7). 2-hydroxyquinoline was observed to disappear at a faster rate than Q. These data are in direct contradiction to those for aqueous solution, where Q transformation to 2-hydroxyquinoline was approximately 5 to 8 times faster than the uptake of 2-hydroxyquinoline. Interestingly, in the sorbed state, the quinolinium surface complex exhibits a 15- to 20-nm blue shift, whereas the 2-hydroxyquinoline-surface complex exhibits no such shift in the maximum wavelength emission. While it is not possible to conclusively demonstrate that this lack of shift is related to binding intensity, Lakowicz (1986) suggests that a blue shift in the emission spectra of a fluorophore bound to a macro molecule can be caused by emission from unrelaxed fluorophores, typically due to a strong interaction between the fluorophore and its surrounding environment.

Sorption-Degradation of Organic Acids

Salicylate, phthalate, and catechol sorb strongly to γ-Al_2O_3 [salicylic (2-hydroxybenzoic acid) pK_{a1}= 2.97, pK_{a2} = 13.40; pyrocatechol (1,2-dihydroxybenzene) pK_{a1}= 9.2, pK_{a2} = 13.0; phthalic (1,2-benzenedicarboxylic acid) pK_{a1}= 2.8, pK_{a2} = 4.9] (Fig. 8). Sorption of catechol, salicylate, and phthalate (or other organic acids) to Fe and Al oxide surfaces occurs via a surface ligand exchange reaction (Kung and McBride, 1989; McBride and Wesselink, 1988; Zeltner et al., 1986; Kummert and Stumm, 1980; Parfitt et al. 1977; Nagarajah et al., 1970). The degree of sorption varies in response to the pH, surface charge, aqueous speciation of the sorbates, and other aqueous- and solid-phase phenomena. Hence the fractional adsorption of the acids with changes in pH is a reflection of the interactions of these phenomena. All experiments reported here were conducted at pH 6.5. Prior to use, the γ-Al_2O_3 (100 m^2/g) was washed in 0.1 M NaOH and eluted with distilled water to pH 7.5. Bacteria used in all of the studies were grown in mineral media that contained the specific organic acid substrate as the sole carbon

source. Bacterium were washed 3 times in a 0.9% saline solution just prior to use, to remove substrate from the cell suspension. For the sorption/degradation experiments, the substrate was equilibrated with γ-Al_2O_3 for 48 h, during which time the pH was adjusted to value of 6.5. A fresh cell suspension was used for each experiment.

Pseudomonas putida 1901, a methionine auxotroph of P. putida P_pG1 (Yen and Gunsalus, 1982) was used in experiments to assess the influence of sorption on the biodegradation of salicylate and catechol. P. putida 1901 degrades naphthalene through salicylate and catechol intermediates. The mineralization of salicylate by P. putida 1901 (10^7 cfu/mL) may be simulated using a first-order rate whose rate constant does not change over a wide range of substrate concentrations. In the presence of γ-Al_2O_3, where about 80 to 90% of the substrate is sorbed (Ainsworth et al., unpublished), the rate of salicylate mineralization is substantially decreased (Fig. 9). In addition, $^{14}CO_2$ evolution studies of salicylate mineralization with and without γ-Al_2O_3 and over a range in P. putida (1901) cell densities (10^7 to $10^{9.4}$ cfu/mL) have demonstrated that 1) the salicylate mineralization rate constant in solution is dependent on cell density, and 2) the rate of salicylate mineralization in the presence of γ-Al_2O_3 is slower than in the absence of γ-Al_2O_3 by about a factor of 5, regardless of cell density (Table 1). Catechol degradation by the same bacterium shows a similar response to cell density and the presence of γ-Al_2O_3, but degradation of the sorbed substrate is slightly more rapid than that observed for salicylate.

Direct observation of desorption and microbial uptake of salicylate was obtained using fluorescence spectroscopy as described by Smith et al. (1992). Unlike the $^{14}CO_2$ evolution studies, however, these studies used a salicylate aqueous concentration of 5 $\times 10^{-5}$ M carbon and 1 g/L γ-Al_2O_3. At this concentration, only about 25% of the salicylate was partitioned to the solid surface. Interestingly, during the first 200 min of the experiment, the rate of salicylate disappearance due to microbial uptake was the same in either the presence or the absence of γ-Al_2O_3 (Fig. 10). However, in the presence of γ-Al_2O_3, the rate of salicylate disappearance decreased by more than an order of magnitude (about a factor of 10.3) in comparison to the aqueous-phase-only salicylate at times greater than 200 min. The 200-min time frame corresponds to the time required for the depletion of aqueous-phase salicylate (Fig. 10).

Although it is difficult to precisely determine the actual concentrations in these studies, extrapolation of the data for the later times (>200 min) back to t = 0 yields an intensity (in arbitrary units) that is approximately equal to that associated with the surface concentration at t = 0. These observations suggest that the initial rate of disappearance is the result of microbial uptake of the aqueous-phase salicylate, and the later rate is the direct result of desorption controlling the rate of microbial uptake of salicylate. The fluorescence and $^{14}CO_2$ evolution data suggest that 1) salicylate is at least partially protected from microbial attack in the sorbed state, 2) desorption limits degradation in the absence of or in limited amounts of aqueous-phase substrate, and 3) desorption may be limited by a surface, chemical-controlled desorption reaction.

The difference in the utilization rate of salicylate in the presence and absence of γ-Al_2O_3 as determined by fluorescence spectroscopy (Fig. 10) is greater than the corresponding difference determined for complete mineralization (Fig. 9). Salicylate utilization as measured by ^{14}C evolution is about one half that determined by fluorescence, which would suggest that there are rate-limiting steps in the salicylate-to-CO_2 pathway other than salicylate desorption that impact the overall rate as determined by $^{14}CO_2$ evolution. In the quinoline example, the effect of sorption on uptake was a decrease in rate of about a factor of 30, regardless of whether $^{14}CO_2$ evolution or fluorescence was used in determining the rate, suggesting that desorption is substantially slower than the microbial mineralization process. While this difference is not great, it does suggest that in addition to $^{14}CO_2$ evolution, other techniques must also be utilized to understand the role of sorption in substrate utilization by microorganisms.

As stated earlier, adsorption of many organic acids to oxide surfaces occurs through the formation of inner-sphere complexes as a result of replacement of surface-coordinated OH groups via ligand exchange reaction. The "ligand-like" adsorption of these acids is typical of many anionic species on oxides, and although the sorption kinetics and mechanisms of these acids have not been investigated, inorganic "ligand-like" sorbates may serve as a model. Molybdate adsorption to goethite has been described as the formation of an inner-sphere "ligand-like" surface complex (Zhang and Sparks, 1989). From pressure-jump studies, Zhang and Sparks concluded that the molybdate adsorption mechanism is a two-step process:

Step 1

$$SOH_2^+ + MoO_4^{2-} \rightleftharpoons SOH_2^+ -- MoO_4^{2-} \quad (3)$$

Step 2

$$SOH_2^+ -- MoO_4^{2-} \rightleftharpoons SMoO_4^- + H_2O \quad (4)$$

where the SOH represents the oxide surface hydroxyl sites. The first reaction (3) represents the formation of an ion-pair complex on the protonated surface site (outer-sphere complex). The second reaction (4) is the formation of the inner-sphere complex. The first step is very rapid, and the second step is slow by comparison and is the rate-limiting reaction. The second step involves the breaking and forming of bonds (Zhang and Sparks, 1989). It is believed that a similar mechanism is involved in the sorption of salicylate to γ-Al_2O_3 and that the inner-sphere complex may be bidentate or binuclear; also we believe it is the reversal of the second step that is the rate-limiting step that affects the rate of salicylate utilization in the present study.

Salicylate, phthalate, and catechol have been hypothesized to sorb to the γ-Al_2O_3 surface as a monodentate surface complex at pH 6.5 (Kummert and Stumm, 1980). Catechol, however, has also been suggested to form bidentate or binuclear complexes on Al oxides depending on the oxide surface structure (McBride and Wesselink, 1988). Further, Zeltner et al. (1986) concluded from cylindrical internal reflection-Fourier transform infrared spectroscopy (CIR-FTIR) that salicylate forms a bidentate six-membered ring structure at the surface of goethite. Another type of complex suggested from infrared spectroscopic investigations is a binuclear complex between oxalate and the goethite surface (Parfitt et al., 1977). The formation of these different types of complexes appears to be dependent on the solid surface structure, and we suspect that for a given compound the strength of the surface complexes would vary accordingly. For example, infrared investigations of *p*-hydroxybenzoate sorbed to different Fe oxides suggested that although bidentate binding of the carboxylate formed the dominant surface complex, goethite formed the strongest complex (Kung and McBride, 1989). It is our belief that the magnitude of the effect of desorption on microbial utilization will vary in direct response to the type of

surface complex formed (i.e., monodentate, bidentate, or binuclear) and the strength of the bond(s) formed.

Conclusions

Sorption of organic compounds to inorganic surfaces and organic matter can protect the compound from microbial attack. This protection may arise from 1) inaccessibility of the micropores to microbes, 2) surface stabilization against desorption of the compound, or 3) reduction of aqueous-phase concentrations to levels below that necessary for microbial utilization. Clearly there are biologically mediated reactions that facilitate sorbed substrate utilization, and these are organism- and cell-density-dependent. It is not known whether these interactions are related to a biologically induced shift in the controlling environmental parameters (e.g., pH), production of extracellular enzymes, or direct microbial utilization of a substrate from the surface. These reactions that facilitate substrate utilization must, however, be investigated more thoroughly and at a mechanistic level. From a scientific viewpoint, these interactions are likely to play a significant role in the behavior of xenobiotic compounds in the natural environment. From a purely applied point of view, these interactions may be of benefit in facilitating the remediation of contaminated soils and vadose and saturated zones.

From the few examples discussed here, it is evident that surface-substrate interactions can diminish, enhance, or have no effect on the observed rate of aqueous biodegradation of a substrate. In those systems where degradation of ionizable compounds is significantly reduced by sorption (and the sorbate is protected from microbial attack), it appears that the binding intensity and the surface conformation of the sorbate are important, as suggested for a homologous series of n-alkylamines (Wszolek and Alexander, 1979) or for quinoline compared to 2-hydroxyquinoline sorbed to montmorillonite (Smith et al., 1992). For those compounds that interact with oxides through ligand exchange, binding intensity may be related to the surface structure of the sorbent, as suggested by Kung and McBride (1989) for *p*-hydroxybenzoate. However, there is a dearth of data that relate binding at the mechanistic level to biodegradation rates. The available data, however, do indicate that the interactions between substrate, surfaces, and microbial utilization are important in our understanding of organic compound fate and transport, and therefore more studies linking sorption and degradation at a basic level are needed.

Acknowledgments

This research was supported by the Subsurface Science Program, Office of Health and Environmental Research, U. S. Department of Energy (DOE). The continued support of Dr. F. J. Wobber is greatly appreciated. Pacific Northwest Laboratory is operated for DOE by Battelle Memorial Institute under Contract DE-AC06-76RLO 1830.

Literature Cited

Ainsworth, C. C., J. M. Zachara and R. L. Schmidt. 1987. Quinoline sorption on Na-montmorillonite: contributions of the protonated and neutral species. Clays Clay Miner. 35:121-128.

Alexander, M., and K. M. Scow. 1989. Kinetics of biodegradation in soil. *In* "Reactions and Movement of Organic Chemical in Soils" (eds. B. L. Sawhney and K. Brown). SSSA Special Publication No. 22; SSSA, and ASA, Madison, WI. pp. 243-270.

Brockman, F. J., B. A. Denovan, R. J. Hicks, and J. K. Fredrickson. 1989. Isolation and characterization of quinoline-degrading bacteria from subsurface sediments. Appl. Environ. Microbiol. 55:1029-1032.

Brusseau, M. L., and P. S. C. Rao. 1989. Sorption nonideality during organic contaminant transport in porous media. CRC Crit. Rev. Environ. Contr. 19:33-99.

Coates, J. T., and A. W. Elzerman. 1986. Desorption kinetics for selected PCB congeners from river sediments. J. Contam. Hydrol. 1:191-198.

Cowan, C. T., and D. White. 1958. The mechanism of exchange reactions occurring between sodium montmorillonite and various n-primary aliphatic amine salts. Trans. Faraday Soc. 54: 691-697.

Dashman, T., and G. Stotzky. 1986. Microbial utilization of amino acids and a peptide bound on homoionic montmorillonite and kaolinite. Soil Biol. Biochem. 18:5-14.

Greene-Kelly, R. 1955. Sorption of aromatic organic compounds by montmorillonite. Part I. Orientation studies. Faraday Soc. Trans. 51:412-424.

Guerin, W. F., and S. A. Boyd. 1992. Differential bioavailability of soil-sorbed naphthalene to two bacterial species. Appl. Environ. Microbiol. 58:1142-1152.

Hamaker, J. W., and C. A. I. Goring. 1976. Turnover of pesticide residues in soil. *In* "Bound and Conjugated Pesticide Residues" (ed. Kaufman, D. D., et al.), Am. Chem. Soc., Washington, D. C. pp. 219-243.

Hayes, M. H. B. 1970. Adsorption of triazine herbicides on soil organic matter, including a short review on soil organic matter chemistry. Residue Rev. 31:131-174.

Hayes, M. H. B., M. E. Pick, and B. A. Toms. 1978. The influence of organocation structure on the adsorption of mono- and bipyridinium cations by expanding lattice clay minerals, I. Adsorption by Na^+-montmorillonite. J. Colloid Interface Sci. 65:254-265.

Heitkamp, M. A., J. N. Huckins, J. D. Petty, and J. L. Johnson. 1984. Fate and metabolism of isopropylphenyl diphenyl phosphate in freshwater sediments. Environ. Sci. Technol. 18:434-439.

Karickoff, S. W. 1981. Semi-empirical estimation of sorption of hydrophobic pollutants on natural sediments and soils. Chemosphere 10: 833-846.

Koskinen, W. C. and S. S. Harper. 1990. The retention process: Mechanisms. *In* "Pesticides in the Soil Environment: Processes, Impacts, and Modeling" (ed. H. H. Cheng). SSSA Book Series:2, SSSA, Madison WI. pp. 51-78.

Kummert, R., and W. Stumm. 1980. The surface complexation of organic acids on hydrous γ-Al_2O_3. J. Colloid Interface Sci. 75:373-385.

Kung, K-H., and M. B. McBride. 1989. Coordination complexes of *p*-hydroxybenzoate on Fe oxides. Clays Clay Miner. 37:333-340.

Lailach, G. E., T. D. Thompson, and G. W. Brindley. 1968. Adsorption of pyrimidines, purines, and nucleosides by Li-, Na-, Mg-, and Ca-montmorillonite (clay-organic studies XII). Clays Clay Miner. 16:285-293.

Lakowicz, J. R. 1986. "Principles of fluorescence spectroscopy". Plenum Press, New York.

Leenheer, J. A., and J. L. Ahlrichs. 1971. A kinetic and equilibrium study of the adsorption of carbaryl and parathion upon soil organic matter surfaces. Soil Sci. Soc. Am. Proc. 35:700-705.

Liberti, L. and R. Passino. 1985. Ion-exchange kinetics in selective systems. *In* "Ion Exchange and Solvent Extraction" (eds. J. Marinsky and Y. Marcus). Marcel Dekker, New York. pp. 175-207.

Maes, A., L. V. Leemput, A. Cremers, and J. Uytterhoeven. 1980. Electron density distribution as a parameter in understanding organic cation exchange in montmorillonite. J. Colloid. Interface Sci. 77:14-20.

Maes, A., P. Marynen, and A. Cremers. 1978. Thermodynamic stability of the ammonium ions of ethylenediamine and histamine in montmorillonite. Progr. Colloid Polymer Sci. 65:245-250.

McBride, M. B., and L. G. Wesselink. 1988. Chemisorption of catechol on gibbsite, boehmite, and noncrystalline alumina surfaces. Environ. Sci. Technol. 22:703-708.

Mihelcic, J. R., and R. G. Luthy. 1988. Microbial degradation of acenaphthene and naphthalene under denitrification conditions in soil-water systems. Appl. Environ. Microbiol. 54:1188-1198.

Miller, M. E., and M. Alexander. 1991. Kinetics of bacterial degradation of benzylamine in a montmorillonite suspension. Environ. Sci. Technol. 25:240-245.

Nagarajah, S., A. M. Posner, and J. P. Quirk. 1970. Competitive adsorption of phosphate with polygalacturonate, and other organic anions on kaolinite and oxide surfaces. Nature 228:83-84.

Ogram, A. V., R. E. Jessup, L. T. Ou, and P. S. C. Rao. 1985. Effects of sorption on biological degradation rates of (2,4-dichlorophenoxy)acetic acid in soils. Appl. Environ. Microbiol. 49: 582-587.

Olness, A., and C. E. Clapp. 1972. Microbial degradation of a montmorillonite-dextran complex. Soil Sci. Soc. Am. Proc. 36:179-181.

Parfitt, R. L., V. C. Farmer, and J. D. Russell. 1977. Adsorption on hydrous oxides. I. Oxalate and benzoate on goethite. J. Soil Sci. 28:29-39.

Remberger, M., A-S. Allard, and A. H. Neilson. 1986. Biotransformations of chloroguaiacols, chlorocatechols, and chloroveratroles in sediments. Appl. Environ. Microbiol. 51:552-558.

Rijnaarts, H. H. M., A. Bachmann, J. C. Jumelet, and A. J. B. Zehnder. 1990. Effect of desorption and intraparticle mass transfer on the aerobic biomineralization of α-hexachlorocyclohexane in a contaminated calcareous soil. Environ. Sci. Technol. 24:1349-1354.

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.

Smith, S. C., C. C. Ainsworth, S. J. Traina, and R. J. Hicks. 1992. The effect of sorption on the biodegradation of quinoline. Soil Sci. Soc. Am. J. 56:737-746.

Sparks, D. L. 1989. "Kinetics of Soil Chemical Processes." Academic Press, New York.

Steen, W. F., D. F. Paris, and G. L. Baughman. 1980. Effects of sediment sorption on microbial degradation of toxic substances. *In* "Contaminants and Sediments Vol. 1. Fate and Transport, Case Studies, Modeling, Toxicity" (ed. R. A. Baker). Ann Arbor Science, Ann Arbor, MI. pp. 477-482.

Steinburg, S. M., J. J. Pignatello, and B. L. Sawhney. 1987. Persistence of 1, 2-dibromoethane in soils: Entrapment in intraparticle micro pores. Environ. Sci. Technol. 21:1201-1206.

Stevens, T. O., R. L. Crawford, and D. L. Crawford. 1990. Biodegradation of Dinoseb (2-*sec*-butyl-4,6-dinitrophenol) in several Idaho soils with various Dinoseb exposure histories. Appl. Environ. Microbiol. 56:133-139.

Stotzky, G. 1986. Influence of soil mineral colloids on metabolic processes, growth, adhesion, and ecology of microbes and viruses. *In* "Interactions of Soil Minerals with Natural Organics and Microbes" (eds. P. M. Huang and M. Schnitzer). SSSA Special Publication No. 17; Soil Sci. Soc. Am., Madison, WI. pp. 305-428.

Subba-Rao, R. V., and M. Alexander. 1982. Effect of sorption on mineralization of low concentrations of aromatic compounds in lake water samples. Appl. Environ. Microbiol. 44: 659-668.

Theng, B. K. G., G. J. Greenland, and J. P. Quirk. 1967. Adsorption of alkylammonium cations on montmorillonite. Clay Miner. 7:1-17.

Thompson, T. D., and G W. Brindley. 1969. Adsorption of pyrimidines, purines, and nucleosides by Na-, Mg-, and Cu(II)-illite (clay-organic studies XVI). Amer. Miner. 54:858-868.

Vansant, E. F., and J. B. Uytterhoeven. 1972. Thermodynamics of the exchange of n-alkylammonium ions on Na-montmorillonite. Clays Clay Miner. 20:47-54.

Weber, J. B., and H. D. Coble. 1968. Microbial decomposition of diquat adsorbed on montmorillonite and kaolinite clays. J. Agric. Food Chem. 16:475-478.

Weber, J. B., and S. B. Weed. 1968. Adsorption and desorption of diquat, paraquat, and prometone by montmorillonitic and kaolinitic clay minerals. Soil Sci. Soc. Am. Proc. 32:485-487.

Weber, J. B., P. W. Perry, and R. P. Upchurch. 1965. The influence of temperature and time on the adsorption of paraquat, diquat, 2,4-D, and prometone by clays, charcoal, and an anion exchange resin. Soil Sci. Soc. Am. Proc. 29:678-688.

Weed, S. B., and J. B. Weber. 1969. The effect of cation exchange capacity on the retention of diquat^{2+} and paraquat^{2+} by three-layer clay minerals. I. Adsorption and release. Soil Sci. Soc. Am. Proc. 33:379-385.

Wszolek, P. C., and M. Alexander. 1979. Effect of desorption rate on the biodegradation of n-alkylamines bound to clay. J. Agric. Food Chem. 27:410-414.

Wu, S., and P. M. Gschwend. 1986. Sorption kinetics of hydrophobic compounds to natural sediments and soils. Environ. Sci. Technol. 20:725-730.

Yen, K.-M., and I. C. Gunsalus. 1982. Plasmid gene organization: Naphthalene/salicylate oxidation. Proc. Natl. Acad. Sci. USA. 79:874-878.

Zachara, J. M., C. C. Ainsworth, and S. C. Smith. 1990. The sorption of N-heterocyclic compounds on reference and subsurface smectite clay isolates. J. Contam. Hydrol. 6:281-305.

Zeltner, W. A., E. C. Yost, M. L. Machesky, M. I. Tejedor-Tejedor, and M. A. Anderson. 1986. Characterization of anion binding on goethite using titration calorimetry and cylindrical internal reflection-Fourier transform infrared spectroscopy. *In* "Geochemical Processes at Mineral Surfaces" (eds. J. A. Davis and K. F. Hayes), ACS Symposium Series 323, Am. Chem. Soc., Washington, D.C. pp. 142-161.

Zhang, P. C., and D. L. Sparks. 1989. Kinetics and mechanisms of molybdate adsorption/desorption at the goethite/water interface using pressure-jump relaxation. Soil Sci. Soc. Am. J. 53:1028-1034.

Table 1. First-order rate constants for the mineralization of salicylate from aqueous solution (Aq. rate constant), and in the presence of γ-Al_2O_3 (Sorbed rate constant) as a function of P. putida 1901 cell density (log cfu/mL).

Cell density (log cfu/mL)	Aq. rate constant (h^{-1})	Sorbed rate constant (h^{-1})	Aq./Sorbed
9.36	-0.819	-0.175	5.9
8.52	-0.257	-0.069	3.8
7.49	-0.032	-0.011	2.9
7.08	-0.015	-0.003	5.0

Figure Captions

1. Mineralization of n-decylamine in the presence or absence of Na-bentonite and at three different cell densities (after Wszolek and Alexander, 1979).

2. Phthalate mineralization over time in the presence or absence of γ-Al_2O_3 (1.0 g/L) by a) P. putida NMH102-2 and b) P. cepacia PHK at a cell density of 10^7 cfu/mL and a total concentration of $10^{-6.5}$ M phthalate (Ainsworth et al., unpublished).

3. Mineralization over time of 10^{-6} M quinoline by a quinoline-degrading bacterium ($\approx 10^7$ cfu/mL) as a function of Na-montmorillonite suspension density (after Smith et al., 1992).

4. Fluorescence emission spectra with time of aqueous quinolinium transformation to 2-hydroxyquinoline and its subsequent utilization ($10^{-4.30}$ M initial quinoline; 10^8 cfu/mL cell density; after Smith et al., 1992).

5. Fluorescence emission spectra with time of quinolinium disappearance from the surface of hectorite as a result of aqueous quinoline biodegradation by a quinoline-degrading bacterium (after Smith et al., 1992).

6. Hectorite-surface concentration of quinolinium over time as a function of initial surface loading. The open and closed symbols represent duplicate runs; data were collected from fluorescence emission spectra of a suspension over time (after Smith et al., 1992).

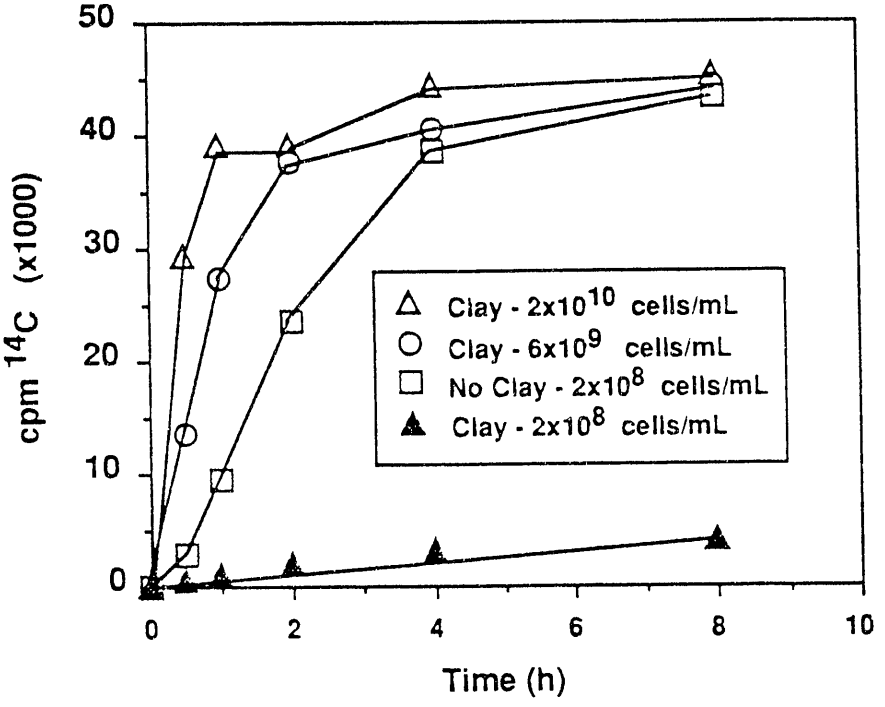
7. Results from two separate with hectorite-suspensions equilibrated with 1) quinoline and 2) 2-hydroxyquinoline in the presence of a Quinoline-degrading bacterium at equal cell densities and total concentrations of quinoline and 2-hydroxyquinoline. Data were collected from fluorescence emission spectra of the suspension with time (Ainsworth et al., unpublished data).

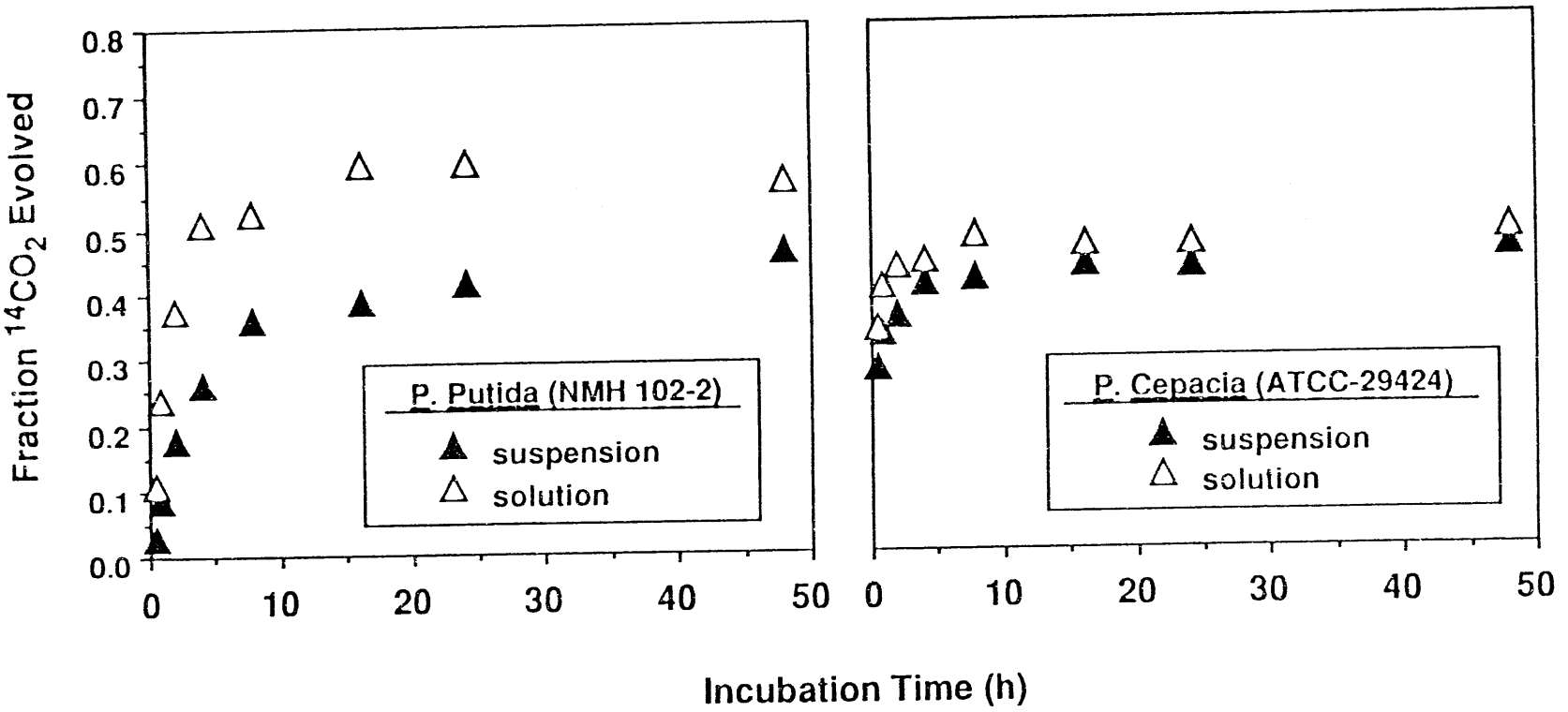
8. Fractional adsorption of three organic acids ($10^{-6.5}$ M) on γ-Al_2O_3 suspension (1.0 g/L) as a function of pH.

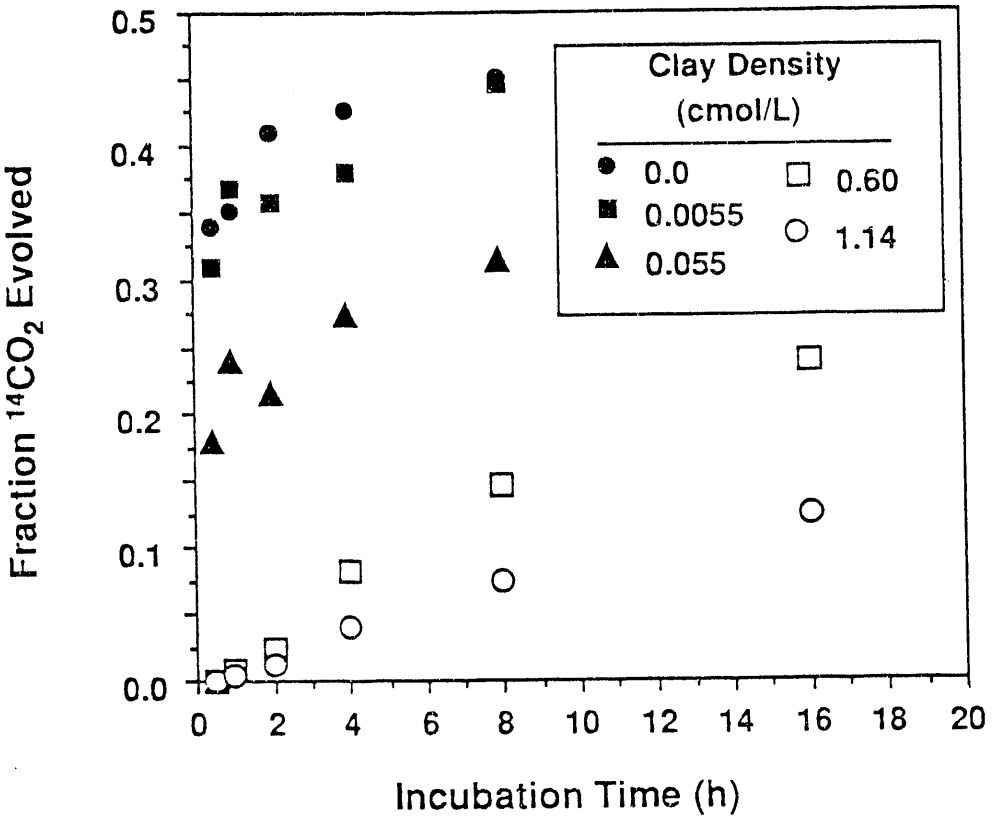
9) Salicylate mineralization over time in the presence or absence of γ-Al_2O_3 at pH 6.5 and as a function of P. putida 1901 population density.

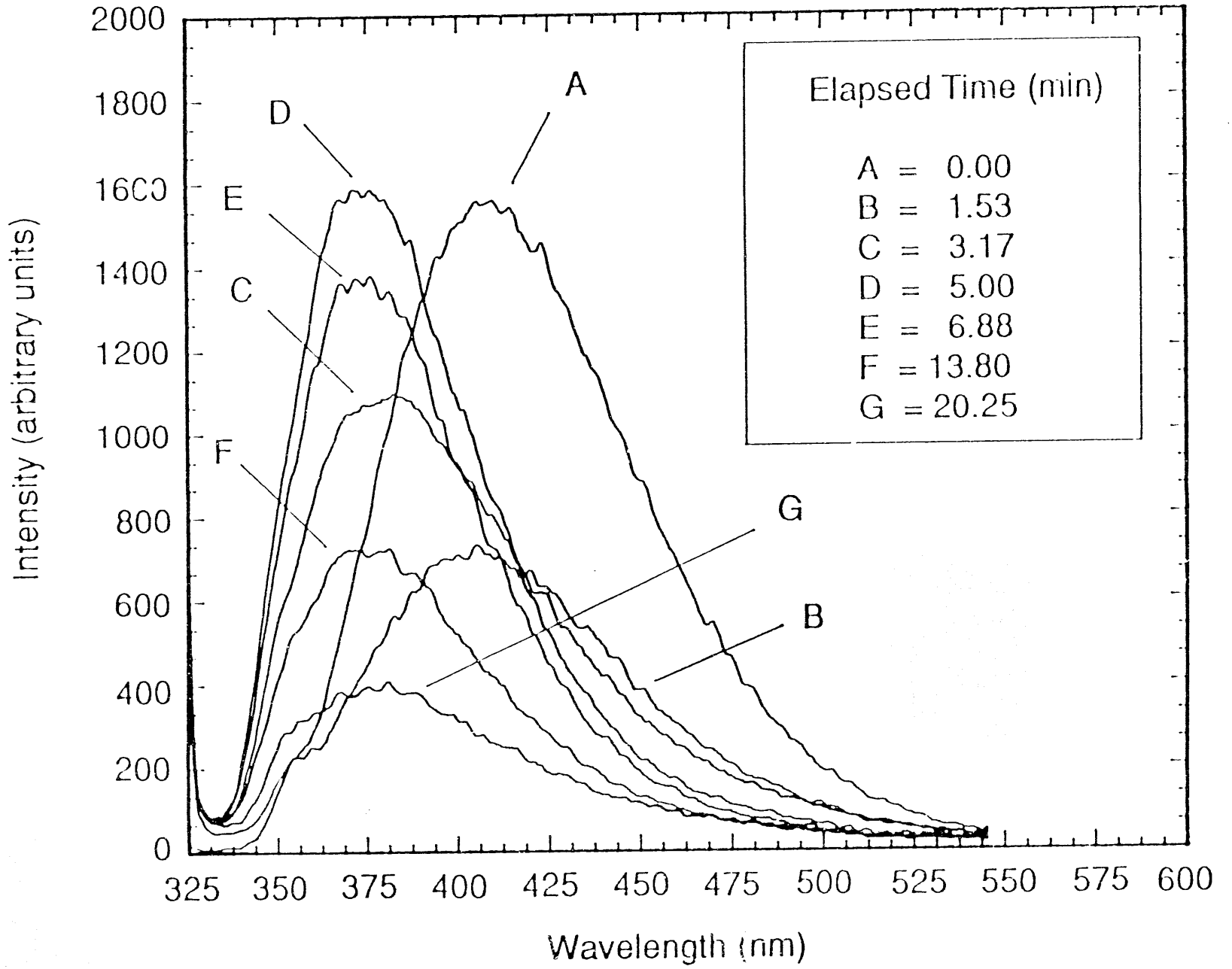
10) Disappearance of salicylate from aqueous solution and γ-Al_2O_3 suspension (total salicylate = 10^{-5} M; pH 6.5; γ-Al_2O_3 1.0g/L; P. putida density = 10^7 cfu/mL). Data were collected from fluorescence emission spectra taken over time.

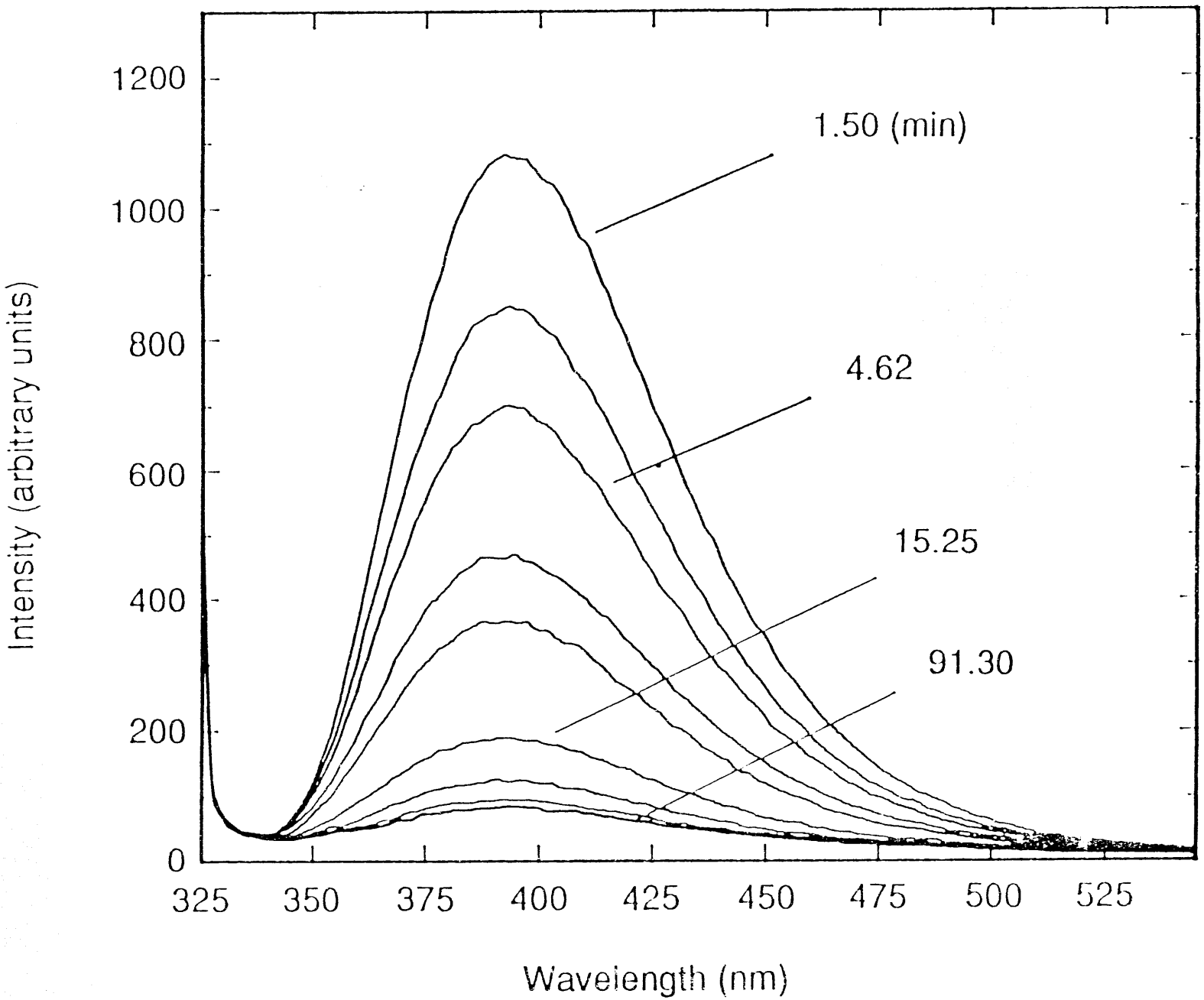
Fig 1

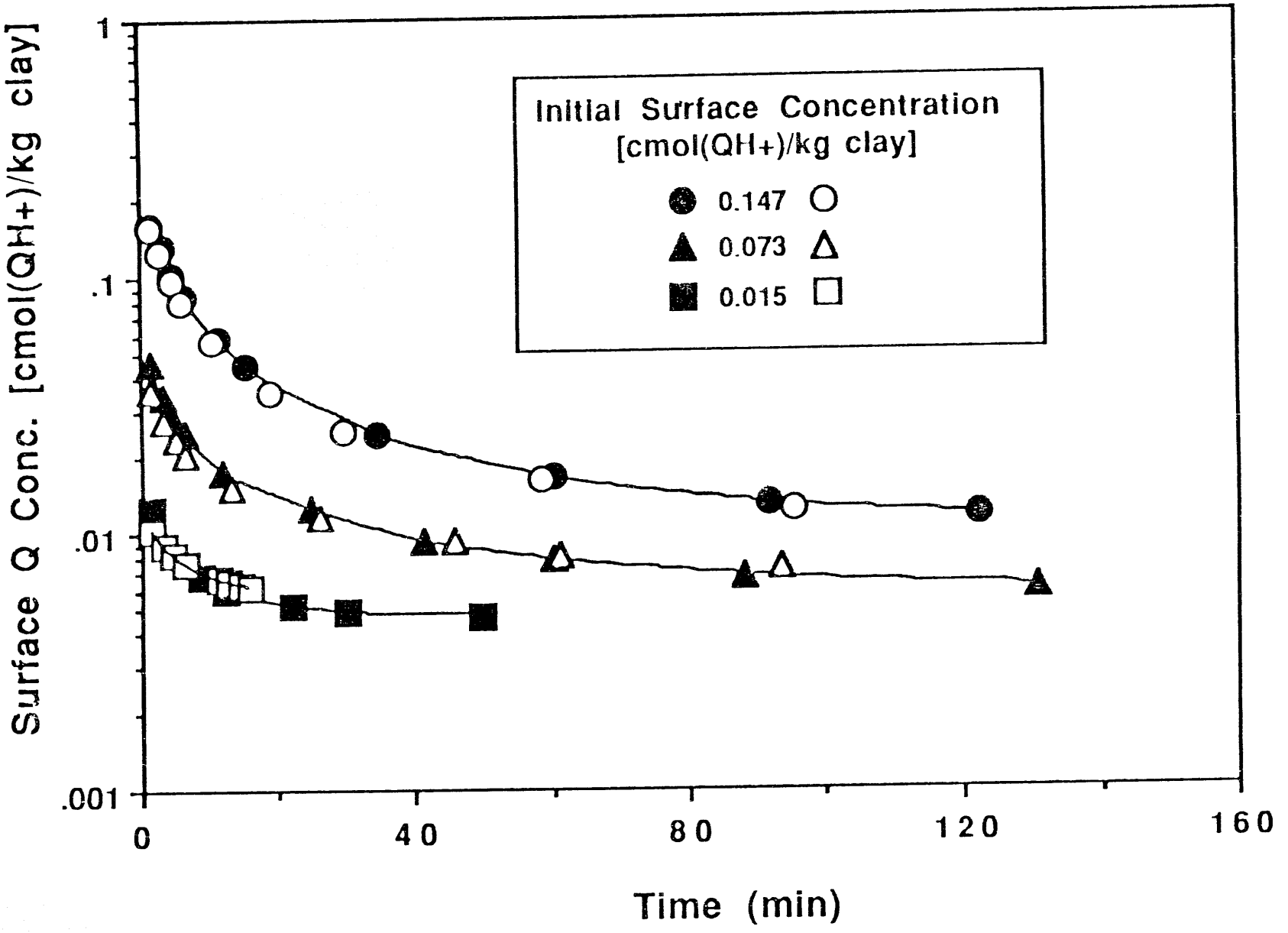

50
40
30
20
10
0
cpm ^{14}C (x1000)
0
2
4
6
8
10
Time (h)
Clay - 2x10^{10} cells/mL
Clay - 6x10^{9} cells/mL
No Clay - 2x10^{8} cells/mL
Clay - 2x10^{8} cells/mL

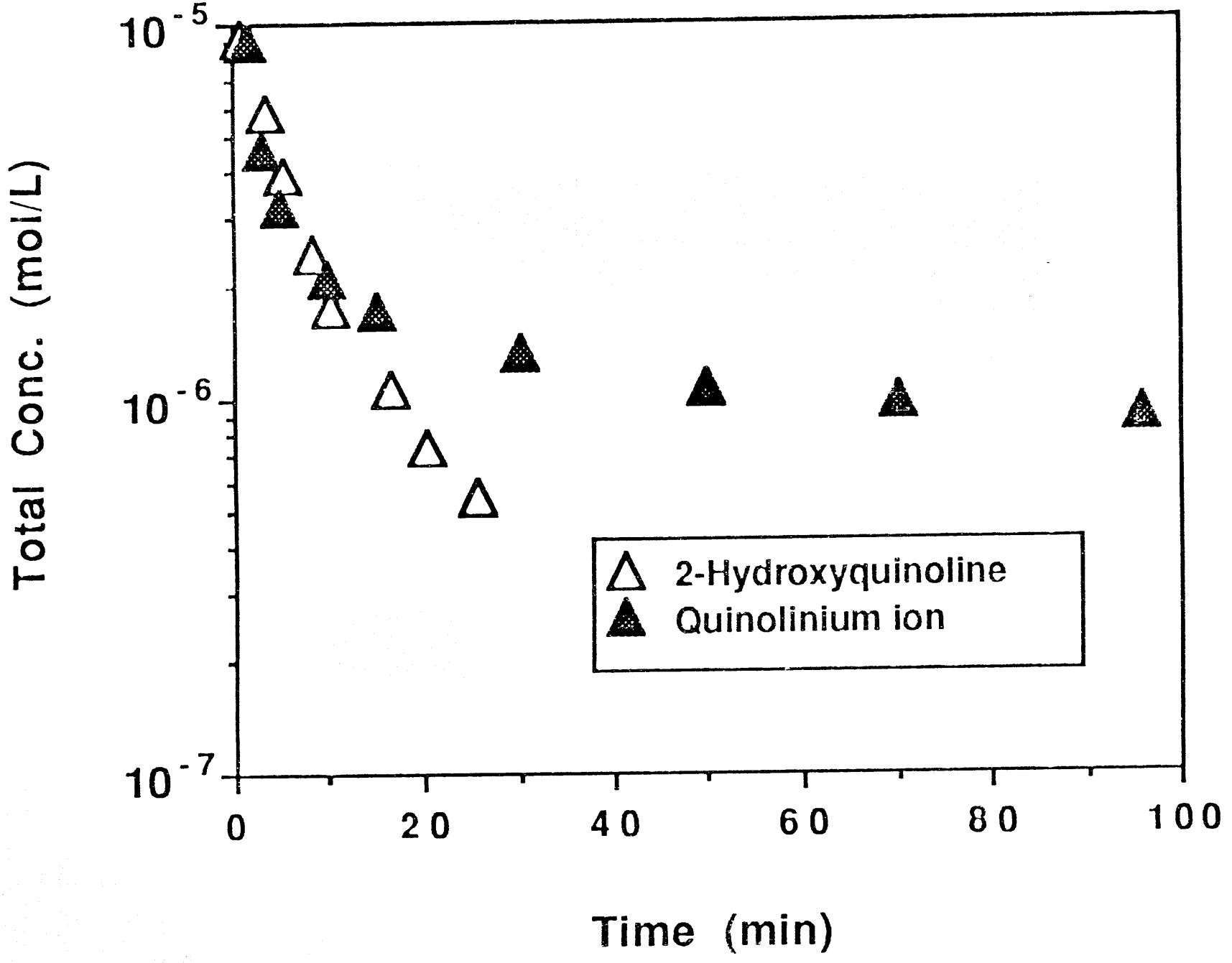

Fraction $^{14}CO_2$ Evolved
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0
10
20
30
40
50
P. Putida (NMH 102-2)
suspension
solution
P. Cepacia (ATCC-29424)
suspension
solution
Incubation Time (h)

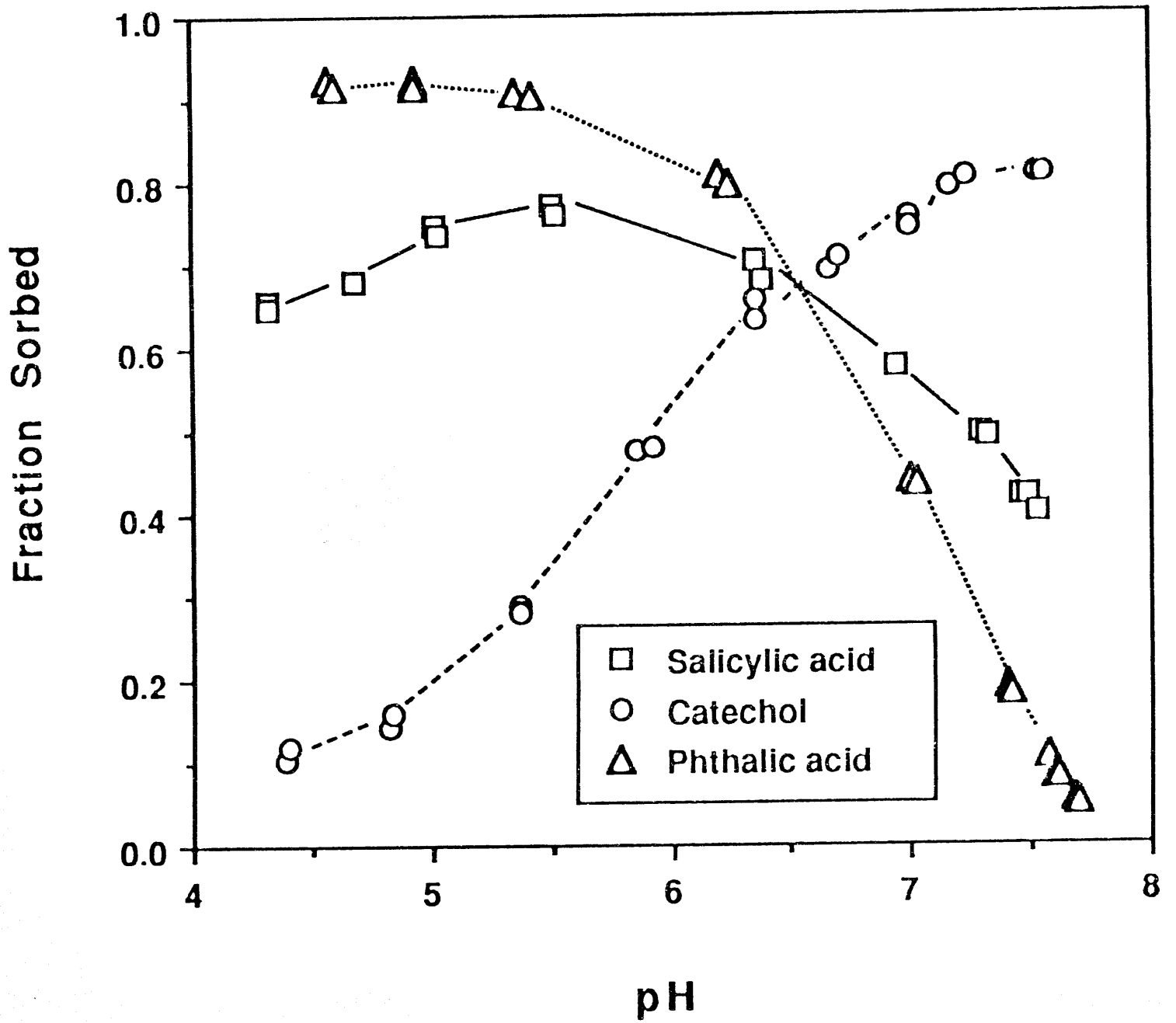

Fraction $^{14}CO_2$ Evolved
0.5
0.4
0.3
0.2
0.1
0.0
0
2
4
6
8
10
12
14
16
18
20
Incubation Time (h)
Clay Density
(cmol/L)
0.0
0.0055
0.055
0.60
1.14

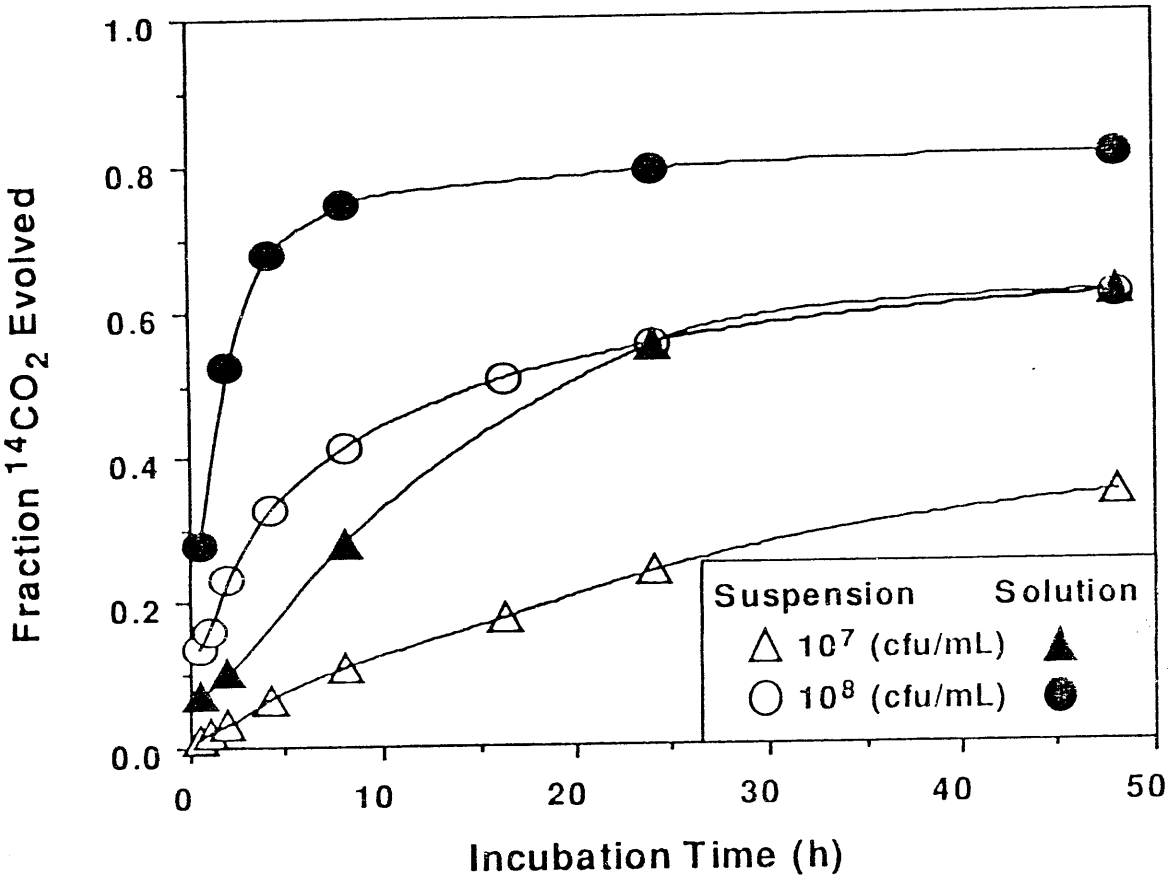

Intensity (arbitrary units)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
325
350
375
400
425
450
475
500
525
550
575
600
Wavelength (nm)
A
B
C
D
E
F
G
Elapsed Time (min)
A = 0.00
B = 1.53
C = 3.17
D = 5.00
E = 6.88
F = 13.80
G = 20.25

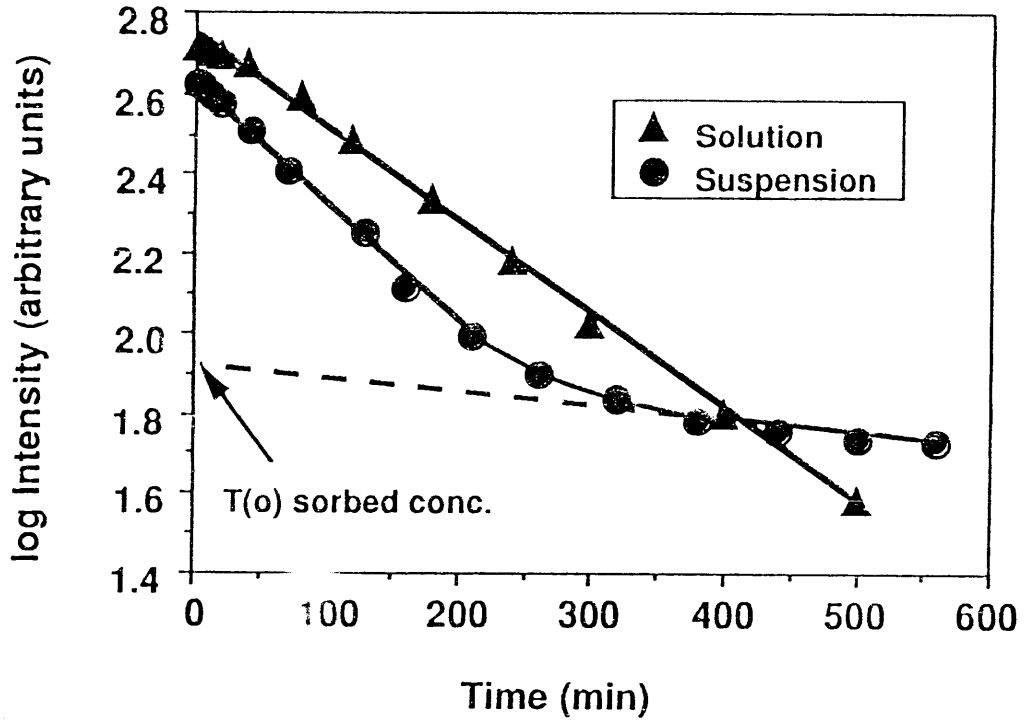

1200
1000
800
600
400
200
0
Intensity (arbitrary units)
1.50 (min)
4.62
15.25
91.30
325
350
375
400
425
450
475
500
525
Wavelength (nm)


Surface Q Conc. [cmol(QH+)/kg clay]
1
.1
.01
.001
0
40
80
120
160
Time (min)
Initial Surface Concentration
[cmol(QH+)/kg clay]
0.147
0.073
0.015


10^{-5}
10^{-6}
10^{-7}
Total Conc. (mol/L)
0
20
40
60
80
100
Time (min)
2-Hydroxyquinoline
Quinolinium ion


1.0
0.8
0.6
0.4
0.2
0.0
Fraction Sorbed
4
5
6
7
8
pH
Salicylic acid
Catechol
Phthalic acid


1.0
0.8
0.6
0.4
0.2
0.0
Fraction $^{14}CO_2$ Evolved
0
10
20
30
40
50
Incubation Time (h)
Suspension
Solution
10^7 (cfu/mL)
10^8 (cfu/mL)


log Intensity (arbitrary units)
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
0
100
200
300
400
500
600
Time (min)
Solution
Suspension
T(o) sorbed conc.

END

DATE FILMED

2 / 19 / 93