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Bioavailability of sorbed- and separate-phase chemicals

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Introduction

Two areas of interest to environmental scientists and engineers are understanding the environmental fate of hazardous hydrophobic pollutants and optimizing the remediation of soil contaminated by these compounds. Biological treatment processes are a potential, cost effective technology for efficient remediation of some contaminated soils. Hydrophobic organic compounds may be sorbed to soils and sediments or present in a separate phase (e.g., oil and coal tar). Consequently, the effectiveness of bioremediation of soil contaminated with organics may be affected by physiochemical processes that control phase partitioning between solid and liquid, and subsequent solute accessibility to microorganisms. Thus, the bioremediation of soils contaminated with hydrophobic solutes, may depend on the rate and extent of desorption from a solid surface or dissolution from a separate phase. This phenomenon, termed bioavailability, has been identified as one of four research priorities for bioremediation technologies (Alexander 1991).

The purpose of this paper is to review information on the effect of phase-partitioning on biodegradation of hydrophobic pollutants. This will include a review of evidence which suggests the importance of improved understanding of rate controlling phase partitioning mechanisms, studies examining the bioavailability of sorbed- and separate-phase pollutants, and models which consider the effect of phase-partitioning on biodegradation.

Field and laboratory observations

Long-term observations of fate and remediation of hydrophobic organics which have contaminated soils suggest that phase partitioning may control the rate and extent of microbial degradation. The persistence of 1,2-dibromoethane in soils suggests that some physicochemical process may prevent biological degradation (Steinberg et al. 1987; Pignatello et al. 1987). This phenomenon may be due to the presence of an irreversible sorbed or desorption resistant fraction (Isaacson & Frink 1984; DiToro & Horzempa 1982), which has been explained as both an experimental artifact (Gschwend & Wu 1985), and/or intraorganic matter diffusion or retarded intraparticle diffusion (Wu & Gschwend 1988; Brusseau et al. 1991).

Studies with soil contaminated with polycyclic aromatic hydrocarbons (PAHs) provide additional examples which indicate the importance of phase partitioning on biodegradation. These studies indicate that PAH dissolution and desorption from soil may be a limiting factor in achieving successful bioremediation (Smith et al. 1989).

A number of experimental studies have been conducted to investigate the biological treatment of 2- through 6-ring PAHs present in liquid cultures and solid matrices (Barnhardt & Julian 1989; Wang et al. 1990; Park et al. 1990). The results of these efforts have indicated that a range of 2- through 6-ring PAHs may biodegrade when present in aqueous solution; however, removal from the solid matrix is

less predictable and generally much less efficient. In the past, this was attributed to the higher molecular weight PAHs being more recalcitrant to biodegradation than the lower molecular weight PAHs. However, more recent information suggests that lower removal rates observed for higher molecular weight PAHs reflect their low concentrations in the aqueous phase. Moreover, biodegradation of PAH in soil typically approaches a limiting value, after which little change in PAH concentration is observed. This indicates that mass transfer difficulties may have to be overcome in order to develop efficient biological treatment processes for many hydrophobic compounds in soil, sediment, and aqueous systems.

Association of microorganisms with surfaces

Microorganisms are known to occur as aqueous suspensions, attached on solid surfaces, and inside pores of granular activated carbon (GAC) (Stucki & Alexander 1987; Moersen & Rehm 1987). Kilbertus (1980) found that the mean diameter of pores occupied by soil bacteria is approximately 2 μm which is slightly larger than the average size (0.5 to 0.8 μm) of most soil bacteria (Casida 1971). It is also reported that 6 percent of the inner volume of soil aggregates are occupied by microorganisms (Kilbertus 1980).

It is well known that many microorganisms associate themselves with solid surfaces. The movement of cells in suspension may be active if they are motile, or by brownian motion. This random movement yields random collisions with solid surfaces which, if the geometry of the collision is suitable, may result in attachment or adhesion to the surface, followed possibly by growth and reproduction. This latter attachment or adhesion may be reversible or irreversible. Movement to a surface may also occur by buoyancy forces or movement of the surface itself.

The location to which microorganisms attach is affected by surface properties. For example, Gray and Parkinson (1968) report that 60 percent of the total bacteria in soil were located on organic matter coated particles. These particles made up only 15 percent of the total particle surface area while it was estimated that bacteria colonized only 0.02 percent of the sand grain surface.

Microbial utilization of sorbed- and separate-phase substrate

Before biodegradation of a target substrate can occur, the substrate must be available, typically as a free solute, to the initial binding site of the cellular mechanism responsible for its catabolism. In some instances, this mechanism may consist of non-specific, passive diffusion of the substrate through the cellular envelope followed by specific interaction of the substrate with the enzyme responsible for the first step of its catabolism. However, if the catabolic mechanism requires preliminary interaction with an extracellular enzyme, then the target substrate must be freely available in terms of geometry to bind to the enzyme's active site. If the initial step in substrate catabolism is transport into the cell involving a specific transport system, then the substrate must be freely available for binding to the active site of the protein required for its transport. The following sections present information concerning the microbial utilization of substrates which are initially associated with solid and separate phases.

Sorbed-phase substrate

Some sources report that only aqueous-phase substrate is available to microorganisms (Chakravarty et al. 1972; Ogram et al. 1985). Chakravarty et al. (1972) found that a model to describe growth on solid n-eicosane, which assumed that only the aqueous-phase substrate is used by cells, fit experimental data well. However, the conclusions of this study are equivocal because the values of many parameters in this model, including the concentration of growth substrate that supports one half of the specific growth rate of the organism observed at saturating levels of substrate (i.e. Ks), were assumed. Ogram et al. (1985) found that of three models, the model which best fit the experimental data with logarithmic phase adapted *Flavobacterium*-like cells

growing on (2,4-dichlorophenoxy)acetic acid, was one that assumed that sorbed substrate was not degraded. This model also assumed that both suspended and attached cells could degrade soluble substrate and that desorption of sorbed 2,4-dichlorophenoxy acetic acid was instantaneous, i.e. that the soil-water distribution of 2,4-dichlorophenoxy acetic acid was always at equilibrium.

Shimp and Young (1988) measured the kinetics of biodegradation of dodecyltrimethylammonium chloride (TMAC) and phenol in sediment cores and sediment-water slurries. It was observed in sediment cores that TMAC was degraded more quickly in overlying water than in sediments. This suggested that sorption lowered the degradation rate of TMAC. In contrast, the authors suggested that a portion of the sorbed phenol was bioavailable. However, no conclusive proof was provided to support this suggestion.

It has been reported that diffusive transport resistance limits biodegradation of sorbed chemicals (Speitel and Digiano 1987) while diffusive transport out of clay and polyacrylamide gel-exclusion beads has been shown to control the rate of biododegradation in the bulk aqueous phase (Scow & Alexander 1992). Accordingly, studies have found that sorbed-phase substrate which can readily desorb is available to microorganisms in addition to aqueous-phase substrate (Subbu-Rao & Alexander 1984; Erhardt & Rehm 1985; Speitel & Digiano 1987; Robinson et al. 1990).

Robinson et al. (1990) found that *P. putida* was capable of degrading readily desorbed and aqueous-phase toluene. The study noted that it may be possible that *P. putida* utilizes sorbed toluene directly; however, due to the fast desorption of the extractable portion of toluene, this was not likely. Speitel and Digiano (1987) found that phenol and p-nitrophenol adsorbed on GAC can be biodegraded upon desorption. In this study, biodegradation was assumed by measuring CO_2 evolution from a GAC column which had been seeded with an ill-described population of microorganisms. Erhardt and Rehm (1985) reported that most of the phenol adsorbed onto GAC was available to immobilized *Candida sp.* and *Pseudomonas sp.* after diffusion from the GAC. Subba-Rao and Alexander (1982) found that, initially, benzylamine biodegradation was independent of desorption from montmorillonite in lake water. However, at later stages, after most aqueous-phase benzylamine was degraded, it was suggested that biodegradation may have been desorption limited and sorption prevented complete biodegradation. As with all four of these articles, no detailed information (e.g., cell identity, cell preparation, initial concentration) was provided on the microorganisms used in this study.

Though the majority of published studies suggest that sorption reduces bioavailability, other studies suggest that sorbed compounds are available to microorganisms without prior desorption (Gordon & Millero 1985; Remberger et al. 1986; Griffith & Fletcher 1991; Guerin & Boyd 1992). Remberger et al. (1986) suggest that substrates such as chloroquaiacals, chlorocatechol, and chloroveratriles are available for biodegradation, though it was not proven if biodegradation occurred in sorbed state or after desorption.

Gordon and Millero (1985) experimentally determined that low molecular weight organic acids and sugars, adsorbed on hydroxypatite, were still available for biodegradation. However, adsorbed substrate was less available than aqueous substrate. The effect of adsorption on decreasing substrate mineralization was greatest for the most strongly adsorbed substrate, citrate. It was unclear in this study whether the attached bacteria utilize the adsorbed material while it is adsorbed or only after it is desorbed. Though not discussed by Gordon and Millero, the distinction between organisms that can utilize citrate and those that can not appears to be due to the presence of a specific transport system referred to as the citrate permease. Most organisms have the potential to metabolize citrate because it is a prominent intermediate in the Kreb's cycle. However, not all organisms can transport citrate into the cell. Because transport is required, it seems reasonable that the citrate would have to desorb prior to its utilization in order to render it available for binding to the permease.

Guerrin and Boyd (1992) reported that the type of microorganism present was important in determining whether sorbed naphthalene was bioavailable in a soil-water system. The authors reported

that for an unidentified soil isolate, the initial rate of naphthalene degradation was proportional to the aqueous-phase naphthalene concentration. Naphthalene degradation was thus limited by the concentration of dissolved substrate. However, another microorganism, *Pseudomonas putida* 17484, could degrade both sorbed and aqueous phase naphthalene as the initial naphthalene degradation rate exceeded that predicted by degradation of the aqueous phase localized naphthalene only. Based upon measured initial rates of mineralization at limiting concentrations of substrate (i.e. naphthalene), the authors suggest that organism-specific procees may enhance substrate desorption resulting in higher rates of mineralization than would be predicted by inspection of independently determined kinetic constants for substrate utilization by the organism. The precise basis for the differential utilization of sorbed and separate-phase substrate by the two organisms was not determined.

Griffith and Fletcher (1991) have indicated that aqueous-phase methyl-coumarinyl-amide (MCA) leucine is utilized by non-attached *Pseudomonas sp.* strain NCIMB 2021 while surface-associated MCA-leucine was utilized by surface-associated microorganisms. The surface was prepared from marine diatomaceous earth. Attached cells were assumed to have similar physiology compared to non-attached cells. Of interesting note, was a comparison of the degradation of two different substrates, MCA-leucine and bovine serum albumin (BSA) (molecular weights of 385 and 68,000, respectively) by the suspended and attached microorganisms. Non-attached cells had a higher rate of hydrolysis of MCA-leucine versus attached cells. However, attached cells had a much higher rate of hydrolysis of the higher molecular weight BSA.

The results reported for the utilization of BSA and MCA-leucine by attached versus non-attached cells are interesting. BSA is a large globular protein that is a polyelectrolyte and is capable of ionic and hydrophobic interactions with a complex surface. Griffith and Fletcher reported that greater than 95 percent of the initial BSA was sorbed onto the surface after 30 minutes. Procaryotic organisms utilize large proteins by excreting proteases that hydrolyze the protein to small peptides and free amino acids. These products are then transported and metabolized by pathways that exist in many microorganisms. Assuming that both organisms in the study of Griffith and Fletcher could produce proteases, it is expected that the attached organism would utilize BSA more effectively because its excreted protease would be immediately available to the BSA. This however, does not mean that the unattached cells cannot use BSA. Their proteases would simply have to diffuse to the substrate if it is attached.

The effect of solid surfaces upon microbial activity has long been recognized (Zobell 1943). In experimental systems, it has been observed that the presence of a solid can stimulate, inhibit, or have no effect on biological degradation of a target substance. System parameters such as type of solid surface, microorganism, and substrate, are some important considerations which may affect microbial activity.

It is known that bacteria have different abilities to attach to surfaces. However, the relationship of microorganisms with solid surfaces is complex. For example, some organisms, such as cells of the genus *Caulobacter*, require attachment to a solid surface for cell growth and reproduction (Shapiro 1976). They also become immotile, via the lost of flagella, following attachment to a surface. In addition, the virulence of many pathogenic organisms is known to be enhanced by their ability to adhere to the surfaces of cells lining the respiratory tract (Gaastra & de Graaf 1982). Furthermore, the specific attachment of cells of the chemoautotrophic organism *Thiobacillus ferrooxidans* to crystalline lattice structures of pyrite has been implicated in the process of pyrite oxidation and bioleaching of precious metals (Bennet & Tributsch 1978).

Surface attachment has also been shown to promote spore formation, as well as influence cell size, shape, and metabolic activities (Fletcher 1985). However, not all surface associations exhibited by microorganisms are necessarily meaningful. Cells possessing adhesive pili, or charged or hydrophobic surfaces, may display random, non-specific associations with surfaces which are neither required for, not alter their metabolic activities. Thus, for studies on the catabolism of sorbed pollutants, the relationship of cell attachment, if present, to pollution dis-

solution, must be carefully evaluated. Furthermore, it is known that the type of surface may influence bacterial attachment, bacterial activity, and nutrient accumulation.

A good example which illustrates the complexity of interpreting surface effects on microbial activity is the many studies investigating biological activity in the presence of clay particles. These studies indicate that organic compound metabolism can be enhanced, inhibited, or negligibly affected by the presence of clays. For example, the presence of kaolinite has been shown to either have little or no effect on degradation of glucose, starch, and aldehydes while montmorillonite enhanced degradation of these substrates (Stotzky 1966; Stotzky & Rem 1966; Stotzky & Rem 1967; Novakova 1972; Filip 1973). However, montmorillonite has been shown to inhibit degradation of dextran (Olness & Clapp 1972) and diquat, a cationic pesticide, (Weber & Coble 1968) while the presence of kaolinite has been shown to have no effect on diquat degradation (Weber & Coble 1968). Dashman and Stotzky (1986) reported that a portion of amino acids and peptides bound to clays were utilized as a carbon or nitrogen source while another portion was not utilized. Smith et al. (1992) found through experiments that quinoline degradation was reduced in the presence of montmorillonite and hectorite. This work suggests that desorption limited the biodegradation rate of quinoline. However, degradation of acetate, a poorly adsorbing solute, was also reduced which suggested that the presence of clay particles may physically interfere with the uptake of a substrate.

The above information on pure clay systems is extensive relative to literature on complex multicomponent soil systems. However, even the information from pure clay systems is difficult to interpret and illustrates the difficulty in studying microbial activity in complex systems such as soil and sediment environments. However, it appears that the binding strength between a clay and a solute strongly affects the solute's bioavailability. For example, montmorillonite is known to more strongly adsorb diquat than kaolinite (Weber et al. 1969). Montmorillonite has a much larger cation exchange capacity than kaolinite and also swells, thus exposing its inner layers to organic compounds. Thus, montmorillonite is thought to sequester diquat in its inner layers, which causes difficult chemical extraction by traditional methods. Furthermore, it is felt that clay particles may also locally buffer pH changes at the clay-water-microbial interface which can affect microbial populations and also affect the moisture activity which can then influence enzyme activity or bacterial movement (Marshall 1976).

The strength of bacterial association with surfaces may also affect microbial activity. For example, Kefford et al. (1982) used three strains of bacteria with different adhesive properties to study the scavenging of stearic acid coated on a glass surface. Organisms grown to stationary phase were added to 50-ml aqueous-phase systems. All three organisms scavenged the fatty acid. However, a more hydrophobic, more adhesive, pigmented strain of *Serratia marcescens* was more efficient at scavenging stearic acid than the nonpigmented strain. These results suggested that firmer adhesion allowed closer interaction to solid surface associated stearic acid. However, while the irreversibly adsorbed (firm adhesion where subsequent Brownian motion is not observed) pigmented strain of *Serratia marcescens* utilized stearic acid more quickly in the first hour than a reversibly adsorbed (still exhibits Brownian motion and may be removed by shear forces) Leptospira biflexa patoc, the Leptospira biflexa patoc was more efficient at scavenging over the next 49 hours. It was suggested that while Serratia marcescens could more rapidly colonize the surface but could not move to other surface sites after depleting substrate near its locality while leptospira biflexa patoc could readily move to other surface areas as substrate was depleted in its vicinity.

Fletcher (1986) reported that attached *P. fluorescens* cells used glucose twice as fast as cells in suspension. It was also found that the surface composition (i.e., glass and plastic) had no effect on utilization of leucine. This phenomenon has also been observed for leucine utilization by a marine bacterium (Bright & Fletcher 1983a 1983b).

Desorption of sorbed substrate may also prolong the availability of the substrate, thus sustaining the microorganisms for a longer period of time (Erhardt & Rehm 1985; Remberger et al. 1986; Speitel & Digiano 1987; Robinson et al. 1990; van Loos-

drecht et al. 1990). Erhardt and Rehm (1985) reported that GAC can act as a depot for adsorbed phenol which, when released, is biodegraded by microorganisms. This phenomenon has also been postulated for naphthalene released from soil (Mihelcic & Luthy 1991). Robinson et al. (1990) reported that extended incubation of their experimental microcosm provided for a greater mass of substrate to be biodegraded as long as the substrate concentration stayed above a minimum threshold required by the microorganisms.

Another reported effect of sorption is the ability of sorption to lower the aqueous phase level of chemicals which may be toxic. For example, Erhardt and Rehm (1985) reported that rapid uptake of phenol by activated carbon allowed immobilized cells to survive exposure to toxic aqueous-phase phenol concentrations.

Van Loosdrecht et al. (1990) reported that a high substrate concentration and a fast mass transfer of the substrate are benefits to a microorganism growing on a solid surface with a desorbing substrate. The enhanced mass transfer is due to a shorter diffusion distance. Furthermore, it is believed that facilitated nutrient uptake at a solid surface may enhance degradation, especially in systems where nutrient concentrations are low (Bright & Fletcher 1983a 1983b).

Separate-phase substrate

The two general types of interactions postulated for describing the mechanism of substrate cell interaction are: 1) substrate uptake through direct contact of cells with hydrocarbon droplets larger than the cells; and 2) uptake from the bulk aqueous phase of fine droplets of substrate less than 1 μm in diameter or solubilized substrate (Gutnick & Rosenberg 1977; Reddy et al. 1982; Singer & Finnerty 1984).

It has been found that microorganisms can grow on the soluble portion of water-insoluble compounds after dissolution from a solid phase (Thomas et al. 1986; Stucki & Alexander 1987). Microorganisms have also been shown to grow only on aqueous-phase biphenyl, phenanthrene, and naphthalene and not on solid-phase substrates (Wodzinski & Bertolini 1972; Wodzinski & Coyle 1974; Stucki & Alexander 1987). For example, when biphenyl and phenanthrene were initially added as solids, phase-contrast microscopy could not detect microorganisms growing on the solid surfaces (Stucki & Alexander 1987). However, it was observed that dissolution rates of solid substrate may have limited biodegradation of the organic. Thomas et al. (1986) conducted studies of the effects of dissolution on the mineralization of naphthalene and 4-chlorobiphenyl and found that microorganisms may use substrate as it spontaneously dissolves. It was observed that biodegradation rates were directly related to the surface area of solid substrates. This observation was hypothesized to be due to solubilization enhancement by the microorganisms or physical contact with the insoluble substrate because octadecane was mineralized at a faster rate than its dissolution rate.

Furthermore, some bacteria have been observed to partition into an oil phase and orient themselves perpendicularly at the oil-water interface (Marshall & Cruickshank 1973). This is believed to be attributed to the nonpolar nature of portions of some bacterial surfaces. For example, direct cell contact to n-hexadecane has been shown to result in microbial degradation (Nakahara et al. 1977; Rosenberg & Rosenberg 1981). Efroysom and Alexander (1991) showed that cells of the genus *Arthrobacter* attached to the surface of a heptamethylnonane-water interface in order to degrade naphthalene and n-hexadecane dissolved in the organic solvent.

Also, Koch et al. (1991) found that some induced mutants of *Pseudomonas aeroginosa* PG201 which lacked the ability to produce rhamnolipids could not grow in a minimal medium with hexadecane as a carbon source; however, after addition of purified rhamnolipids, the organism grew on hexadecane.

These results suggest that separate-phase pollutants may not be used as a substrate in instances where physical processes or microorganisms can not facilitate dissolution of a solute into the aqueous phase or microorganisms can not attach onto the surface of a separate phase.

Effect of surfactants on biodegradation

Because surfactants can increase the interfacial surface area between water and a substrate, it is believed that this may also increase substrate bioavailability in soil systems. Typically, surfactant concentrations must be greater than the critical micelle concentration (CMC) before significant solubilization occurs (Edwards et al. 1991), especially in soil systems where some of the surfactant may adsorb onto soil (Liu et al. 1991). Furthermore, because natural sorbents consist of various charged surfaces, the success of a surfactant in reducing sorption depends not only on the surfactant type, but also on the sorbent (Brickell & Keinath 1991). A few studies have been conducted to investigate the effect of surfactant addition on biodegradation of hydrophobic contaminants. However, in many instances these studies lack adequate characterization of both the microorganisms and surfactants utilized in experiments which makes interpretation difficult.

Rittmann and Johnson (1989) investigated the effect of an unidentified dispersant on biodegradation of lubricating oil in soil. In that study it was observed that adequate mixing was required to enhance hydrocarbon availability. The effect of the dispersant on soil-water slurry systems where vigorous mixing was applied, was much more significant than that in static soil plots where adequate mixing was not possible. However, though the dispersant enhanced the initial oil degradation rate, this initial advantage was reduced over the 10-day experimental duration. Laha and Luthy (1991) assessed the effects of several nonionic surfactants (i.e., Brij 30, Tergitol NP-10, and Triton X-100) at concentrations of 0.01 to 1.0 percent (v/v) on the degradation of phenanthrene in soil-water suspensions. The types and concentrations of microorganisms utilized for these experiments were not reported. It was found that surfactant concentrations greater than approximately 0.01 to 0.05 percent completely inhibited biological degradation of phenanthrene. Sub CMC levels of surfactant did not appear to have an inhibitory effect on phenanthrene degradation, but neither did such levels enhance the degradation rate. Further studies which substituted glucose for phenanthrene as a growth substrate suggested that the surfactant inhibition effect on biodegradation was not attributed to toxicity of the surfactants. It was also postulated that the surfactant was being used as a competitive substrate so the degradation of phenanthrene was repressed in degradation experiments. However, when glucose was substituted for surfactant, there was no effect on the rate of phenanthrene degradation. The utilization of the phenanthrene in the presence of glucose is somewhat difficult to interpret in this study. In most instances, the presence of glucose would completely catabolite repress the utilization of a poor carbon source such as phenanthrene. The continued metabolism of phenanthrene in these experiments may suggest some experimental problems and additional pure culture studies using defined medium should be conducted to confirm that the organisms employed are capable of using phenanthrene in the presence of glucose.

Arostein et al. (1991) studied the effect of nonionic surfactants, Alfonic 810–60 and Novel II 1412–56, on the degradation of phenanthrene and biphenyl by an uncharacterized microbial population in a soil-water suspension. The results of these experiments indicated that the presence of surfactants could enhance the catabolism of aromatics from contaminated soils, even in the absence of surfactant-induced desorption of the target substrates. However, a biochemical or physiological basis for these results was not forwarded. Unfortunately, these investigators provided no information on the identity of the organisms responsible for the observed catabolism of biphenyl and phenanthrene and apparently did not conduct separate studies to evaluate the effect of the test surfactants on the growth of the experimental organisms in defined medium. In this regard, we have observed (unpublished observations) that low concentrations of the nonionic surfactant Triton X-100 stimulated the growth of a PAH-utilizing *Pseudomonas fluorescens* isolate when grown in a glycerol-based minimal medium. This effect may be due to a generalized influence of the surfactant on cellular permeability. Thus, for the studies reported by Arostein et al. (1991), it is possible that the observed enhanced utilization of biphenyl and phenanthrene was the result of a nonspecific effect of the surfactants on

the permeability of the indigenous organisms toward the aromatics, or other nutrients whose rate of transport into the cell limited cell growth.

For studies involving surfactants, an evaluation of the influence of the surfactant on the growth of the experimental organism(s) in defined medium on the target substrate as well as other carbon sources should be conducted. This would allow a clear distinction to be made between influences on growth resulting from specific surfactant-substrate interactions and generalized, non-specific influences on cellular permeability mediated by the intrinsic chemical properties of the surfactant.

The inhibitory effect of chemical surfactant on a yeast strain growing on hydrocarbon was observed by Aiba et al. (1969) and Mimura et al. (1971). It was believed that the presence of surfactant interfered with the direct interaction between cells and substrate. This explanation was also supported by a study by Efroymson and Alexander (1991). Efroysom and Alexander assessed the role of an organic solvent in determining the means by which bacteria degrade two hydrophobic substrates. It was found that in the presence of the nonionic surfactant, Triton X-100, the degradation of hexadecane that had been dissolved in heptamethylnonane was completed prevented. It was suggested that the surfactant prevented adherence of the bacteria to the heptamethylnonancewater interface and hence prevented direct contact of the cells with hexadecane. This was supported by showing that the number of bacteria in the aqueous phase decreased just prior to the rapid phase of degradation in the absence of Triton X-100, whereas with the Triton X-100 present, the number of bacteria in the aqueous phase remained essentially constant. In addition to altering the solvent-water interface, Triton X-100 may have also had some effect on cell membranes.

Biologically produced surfactants have also been investigated for their potential to enhance biodegradation. Falatko and Novak (1992) investigated the addition of uncharacterized biosurfactants on the degradation of toluene, m-xylene, naphthalene, and 1,2,4-trimethylbenzene by a mixed culture of microorganisms. The authors did not report any enhanced biodegradation of any of the four chemicals and observed inhibition effects with some of the surfactants. However, the results of this study are very inconclusive due to the omission of important information. The number and type(s) of organisms used in the degradation studies were not identified in this study. Furthermore, the supposed surfactant (s) was never characterized; therefore, the reader is never exactly sure what was added to the biodegradation experiments. Oberbremer et al. (1990) added glyclolipids at a concentration of 200 mg/L to investigate degradation of C14 to C18 hydrocarbons and naphthalene. They found that the addition of surfactant in excess of the CMC shortened the adaption phase and increased hydrocarbon removal from 81 percent to 93–99 percent over 79 hours.

Models which combine sorption and biodegradation

Several approaches have been used to interpret results of laboratory studies of microbial degradation of sorbed organic solutes in soil or clay suspensions. The approaches make different assumptions regarding local equilibrium with the solid surface and the time scale for diffusive transport through an immobile fluid relative to the time scale of microbial degradation. Furthermore, the kinetics of biodegradation are not always fully considered during interpretation of data and model development. For example, if solute transport is required as the initial step in catabolism, the mechanism of transport must be carefully distinguished. For example, if transport is only via the mechanism of simple diffusion, then the rate of transport and possibly coupled catabolism would display nonsaturable kinetics with transport being first order with respect to the aqueous concentration of the pollutant. However, if the mechanism of transport is via a facilitated or active transport system, then the rate of transport would display saturable kinetics reflecting the kinetic properties of the proteins comprising the transport systems. Although this type of information is important in the development of kinetic models, it is often unavailable or simply ignored.

The simplest model to describe the degradation of sorbed organic compounds assumes rapid desorption with degradation of aqueous-phase solute.

The basic assumption is that sorbed-phase solute is not degraded and that sorption is reversible and rapid compared to the rate of microbial degradation. In this type of approach, sorption affects the biodegradation kinetics only by decreasing the aqueous contaminant concentration. This approach has been utilized to describe a mixed population of microorganisms degrading chlorpropham and phthalate ester in aquatic sediments assuming no growth of the microorganisms (Steen et al. 1980).

A similar approach was used to describe benzylamine adsorption to montmorillonite and subsequent degradation (Miller & Alexander 1991). Experiments showed that desorption equilibration was reached in less than 2 minutes and degradation was much slower; therefore, an assumed instantaneous sorption equilibrium was described by a Langmuir isotherm. Other assumptions were first-order degradation of the substrate and sorbed substrate being unavailable to microorganisms. The model was found to fit the experimental data very well.

Another approach that recognizes that sorbed phase solute is protected from degradation and that sorption is reversible and rapid compared to the biodegradation rate, incorporates both attached and suspended bacteria in the degradation of aqueous-phase solute. In this study, Ogram et al. (1985) verified the very rapid sorption kinetics of 2,4-D in their experimental systems and used this to describe the degradation of 2,4-D in soil in short-term experiments. The model may not be applicable for organic solutes that require long contact times to attain desorption equilibrium. In typical soils, the surface associated microbial concentration may be much more significant than the suspended microbial concentration, in which case, the form of the equation reverts to one similar to that of Steen et al. (1980).

Guerin and Boyd (1992) used a three parameter model based on an enzyme-catalyzed reaction with the addition of a slowly-binding inhibitor, to describe microbial utilization of naphthalene in a soil-water system. This model assumes that aqueous-phase naphthalene is utilized according to first-order kinetics which is followed by zero-order utilization of desorbing naphthalene.

While these simple models appear to describe experimental results for many environmental contaminants reasonably well, they can not be easily applied to all hydrophobic pollutants. Furthermore, these models may not provide a realistic picture of phenomena which occurs in natural and engineered environments. Therefore, more complex models, which include intra-aggregate diffusion, have been developed to provide a more realistic conceptual understanding of the mechanism(s) which affect biodegradation. Several researchers have modeled intra-aggregate diffusion of pollutants in soil-water systems (Crittenden et al. 1986; Wu & Gschwend 1988). These models can be conceptually visualized by imagining a sorbate which is slowly incorporated into a porous aggregated particle. These models predict that for a given solute, large particles show a slower approach to equilibrium than smaller particles. Thus, biodegradation rates could be enhanced by reducing the diffusive path length and increasing the sorbent surface area, thus increasing sorption-desorption rates. Also the rate of uptake is predicted to be much slower for more hydrophobic compounds because a hydrophobic pollutants' movement is retarded as it diffuses inside an aggregate. Furthermore, compounds with higher molecular weights will penetrate slower because of lower diffusivities though this effect is expected to be less pronounced than soil size or solute hydrophobicity.

Brusseau et al. (1991) proposed that sorption-related nonequilibrium may result from chemical nonequilibrium or from rate-limiting diffusive mass transfer. Major physical differences between intraorganic matter diffusion and retarded intraparticle diffusion are that: 1) the pore size associated with organic matter is similar to sorbate molecular size, where particle pore sizes are much larger than the diffusing molecule and 2) the particle pores are fixed and rigid whereas, the pore network associated with organic matter changes constantly. Intra-aggregate diffusion may restrict the availability of organic solute to microorganisms and control biodegradation through diffusion-limited mass transfer (Rijnaarts et al. 1990; Mihelcic & Luthy 1991; Scow & Hudson 1992). This approach couples rates

of sorption-desorption and biodegradation into an overall model.

These coupled desorption-degradation models assume that organic solute is degraded by suspended and sorbed microorganisms at similar rates, that solute desorption is described by a retarded intra-aggregate diffusion process, and that sorbed solute is not degraded. This type of model has been used to simulate microbial degradation of naphthalene in soil-water suspensions (Mihelcic & Luthy 1991). In this system, experiments and modeling showed that sorption was reversible and the rate of desorption was rapid compared to biodegradation. A similar model has been used to show how intraparticle mass transfer kinetics of alphahexachlorocyclohexane may limit biodegradation in soil-water suspensions (Rijnaarts et al. 1990). In that study, stirred samples with an associated smaller particle size distribution showed faster biodegradation than end-over-end samples which had larger particle size distributions. Finally, this type of coupled desorption-degradation model has been used to describe phenol and glutamate diffusing from clay spheres and polyacrylamide gel exclusion beads (Scow & Alexander 1992). These models have been shown to fit experimental data well. However, though the kinetics of sorption and desorption are modeled in a complex manner with current modeling approaches, the kinetics of biodegradation are typically modeled as first or mixed order. Furthermore, experimental results to support model predictions are also typically obtained in undefined systems.

Summary

The investigation of the physical, chemical, and biological parameters affecting the biodegradation of sorbed- and separate phase contaminants in soil-water systems is admittedly complex and presents an investigator with numerous experimental challenges. The analysis of the data reported from both natural and engineered systems clearly shows that the results obtained by any individual investigator are highly dependent upon the target substrate being examined, the identity and concentration of the organism(s), and the nature of the sorbent. To date, there seems to be few common principles which govern the rate of degradation of a selected pollutant. However, the diversity of the sorbent-water systems examined and the variations in experimental design employed by investigators renders the existing studies virtually impossible to compare. Models to predict experimental observations are sophisticated in their approach to describing mass transfer kinetics yet are simplistic in their approach to describing biodegradation and cell growth.

This situation is best exemplified by the few studies conducted on the effect of synthetic surfactants on the biodegradation of sorbed, hydrophobic pollutants. In these studies, the presence of surfactants has been shown to have no effect, a stimulatory effect, or an inhibitory effect on the degradation of target substrates depending upon the nature of the surfactant and organism(s) employed and whether the surfactant was utilized at concentrations below or above its CMC.

In addition, the vague descriptions, or complete omission, of the biological elements of many studies precludes any serious attempt to evaluate, or reproduce, published studies. For instance, in studies where nonindigenous organisms are employed in the presence of surfactants, confusion surrounding questions of surfactant toxicity, surfactant degradation, efficiency of substrate utilization, and surfactant-substrate specific effects on cell growth could be substantially minimized by conducting conventional microbiological studies in defined media. In addition, attention by investigators to the growth, adaptation, and enumeration of the experimental organism(s), where possible, would alleviate numerous experimental uncertainties. This will increase our understanding of bioavailability and assist the engineering of more efficient, cost effective biological treatment systems.

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