Journal of Hazardous Materials, 22 (1989) 393–406
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

MECHANISMS RESPONSIBLE FOR THE BIODEGRADATION OF ORGANIC COMPOUNDS IN THE SUBSURFACE

MARK S. MORRIS and JOHN T. NOVAK

Department of Civil Engineering, Virginia Polytechnic Institute and State University, Blacksburg, VA 24060 (U.S.A.)

(Received November 15, 1988; accepted August 19, 1989)

Summary

This study was designed to establish the biodegradation rates of the C_1 through C_5 alcohols, phenol and 2,4-dichlorophenol (DCP) in two previously uncontaminated soils and to describe specific conditions which favor biodegradation.

Introduction

The contamination of groundwater has received widespread attention in recent years because of concern about the deterioration in the quality of groundwater supplies. Since groundwater comprises more than 95% of all available freshwater in the United States, subsurface contamination could pose significant health problems. Once in the groundwater, organic compounds may migrate with the flow of water or adsorb to the soil particles. Remediation of contaminated aquifers usually require costly treatment techniques such as activated carbon or aeration. In some cases, the aquifer must be abandoned in favor of alternate water supplies.

In recent years, researchers have searched for or attempted to culture bacteria capable of degrading man-made organic compounds as an inexpensive alternative to chemical and physical treatment of contaminated groundwater. While this type of research may eventually yield valuable results, it fails to address the fundamental question of why natural soil bacteria do not accomplish this task. The most widely held view is that soil bacteria are not acclimated to the organic compounds introduced into the environment. One would expect, however, that this acclimation period would not persist indefinitely and that a population of bacteria would develop in the subsurface which would eventually degrade xenobiotic compounds. This does not always appear to be the case. In reality, some compounds will degrade quite easily in the subsurface while others tend to persist almost indefinitely. The key questions, therefore, are why do these compounds persist and can the subsurface environment be

0304-3894/89/$03.50

manipulated to encourage or accelerate subsurface degradation? These questions cannot be answered with the information currently available.

This study was designed to establish the biodegradation rates of the C_1 through C_5 alcohols, tertiary butyl alcohol (TBA), phenol and 2,4-dichlorophenol (DCP) in two previously uncontaminated soils and to describe specific conditions which favor biodegradation. Methanol, ethanol and TBA are of interest because they are used as additives in some gasolines as octane boosters. These alcohols are highly soluble and do not adsorb as well to soil as do the more hydrophobic gasoline constituents, therefore, they have the potential to migrate from the source of contamination achieving significant levels in an aquifer. Also of concern is the ability of these alcohols to transport more toxic compounds such as benzene, toluene and xylene which are relatively insoluble in water but are highly soluble in alcohol. Phenol is a common industrial chemical used in resins, pharmaceuticals, fungicides, dyes, herbicides and germicides. Dichlorophenols (DCP) can be produced inadvertently by the chlorination of water containing phenol.

Materials and methods

Soil was obtained from two previously uncontaminated sites in Blacksburg, VA and Newport News, VA. The locations of these sites are shown in Fig. 1. The Blacksburg site was located on the dairy farm at the Virginia Polytechnic Institute and State University campus. This soil was unsaturated consisting mainly of a tightly packed clay. The Newport News site was located at the Harwood's Mill Water Treatment Plant below the spillway of the Harwood's Mill Reservoir. This soil was saturated just below the surface and was composed primarily of sand and silt. Soil was collected by using procedures similar to those developed by Dunlap et al. [1] and as modified by Wilson et al. [2] and Bengstsson [3]. Samples were collected at four to five foot intervals in Shelby tubes driven by a conventional drill rig. Soil was collected to a depth of 25 feet (7.5 m) at the Blacksburg site and 15 feet (4.5 m) at the Newport News site. The samples were extruded and parred with a flame sterilized spatula to eliminate any soil which was in contact with the Shelby tube. The remaining soil was transferred to sterilized mason jars and stored at 10°C.

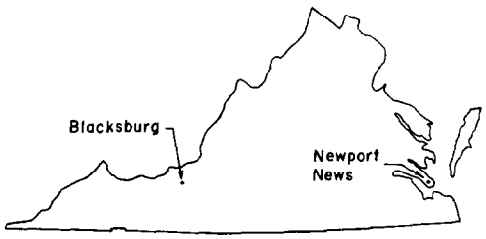

Fig. 1. Sampling site locations (State of Virginia).

To study the biodegradation of the test compounds, microcosms were constructed of 13 by 100 mm screw-capped test tubes with a 12 mm Teflon-coated septum for sampling. Each microcosm contained approximately 5–7 grams of soil. The test compounds were diluted to the desired concentration with sterilized distilled water and introduced as the only carbon source. Each microcosm was mixed once with a vortex mixer. Microcosms of each particular organic concentration were prepared in triplicate. All microcosms were stored in the dark at a constant temperature of 20°C. To assess the impact of non-biological processes such as adsorption, volatilization and chemical degradation on the loss of substrate, control microcosms were established. These contained soil which was autoclaved once a day for five consecutive days at 120°C and 15 psi (~1 bar) pressure. The static microcosm approach has several advantages including the ability to periodically monitor the samples easily, the use of native soil microorganisms, a small amount of soil is required for each microcosm which minimized the amount of soil that had to be collected in the field, and the ease in maintaining oxygen limiting conditions. The major disadvantage of static microcosms is that they do not simulate the flow through nature of a groundwater system.

Following the initial organic addition, numerous measurements of the residual organic concentration were performed in order to establish biodegradation rates for each compound in the two soils. Each microcosm was sampled by removing aqueous samples with a 10 μl syringe. To prevent the introduction of bacteria during the sampling process, the syringe needle was heat sterilized and the septum caps were cleaned with isopropyl alcohol. After sampling, the septum was covered with wax to prevent the loss of substrate through the hole in the septum created by the syringe needle. The concentration of each organic was measured by gas–liquid chromatography by using a flame ionization detector. A 6″ × 1/8″ stainless steel column packed with 0.2% Carbowax 1500 on 80/100 mesh Carbopak C in a Model 5880A Hewlet Packard gas chromatograph was used to identify the alcohols. Phenol and DCP were measured by using a 2 m × 2 mm glass column packed with 1% SP 1240 DP on 100/120 Supelcoport in a Model 560 Tracor gas chromatograph. Each compound was measured isothermally at temperatures selected to provide maximum detection. The injector port temperature was 150°C and the detector temperature was 225°C for each instrument. Sodium molybdate (1.0 m*M* as MoO_4^{-2}) was added in some cases to specifically inhibit sulfate reduction. Nitrate (0.8–1.6 m*M* as NO_3^-) was added in some cases to stimulate nitrate reduction and denitrification.

Results and discussion

Background

Only within the last few years have researchers attempted to characterize the subsurface biodegradation potential and explain the processes which con-

trol the fate of organic compounds in groundwater. McCarty and his coworkers have attempted to model biodegradation in the subsurface as a biofilm reactor. Since subsurface bacteria have been described as attached to the aquifer matrix, this model may be valid. According to Bouwer and McCarty [4], the biofilm model consists of four processes.

1. Substrate transport into the biofilm from the bulk liquid.
2. Substrate utilization with associated bacterial growth following Monod-type kinetics.
3. Substrate diffusion through the biofilm according to Fick's Law.
4. Biofilm growth and decay.

An interesting aspect of the biofilm model is the concept of a minimum substrate concentration (S_{min}) below which no degradation will occur [4–6]. S_{min} is defined as the concentration below which bacteria cannot obtain enough energy from utilization to support maintenance requirements. If valid, this concept would have significant effects on the ability of soil bacteria to degrade trace organic chemicals. Utilization of a compound below S_{min} may be possible if the limiting compound is used simultaneously with another more abundant compound which supports the energy requirements of the organism. This process has been termed secondary utilization. The limiting substrate is called the secondary substrate and the substrate which supports growth is called the primary substrate. Using the biofilm model, Bouwer and McCarty [4] reported that chlorobenzene, 1,3-dichlorobenzene and 1,4-dichlorobenzene were biodegraded in aerobic conditions but were persistent in a methanogenic environment. On the other hand, halogenated aliphatics such as chloroform, carbon tetrachloride and 1,1,1-trichloroethane were removed under methanogenic conditions but not under aerobic conditions. These experiments were conducted in a continuous flow column with glass beads to simulate the aquifer matrix. A mixed culture bacterial population was added and acetate was contained in the feed solution as a primary substrate to stimulate secondary utilization. While these experiments demonstrated the degradability of certain xenobiotic compounds, the relevance to actual subsurface conditions is debatable.

Simkins and Alexander [7] reported that the mineralization rates of compounds are related to the initial substrate concentration and the bacterial population density. Using radiolabeled benzoate ranging in concentration from 10 ng/ml to 100 μg/ml, the degradation kinetics followed first order, integrated Monod and logarithmic kinetics at low, intermediate and high substrate concentrations, respectively. Bacteria were obtained either from primary settling tank sewage or from cultures of *Pseudomonas*. The application of this type of information to the subsurface has not yet been demonstrated.

Only a very limited amount of information exists relating biodegradation to site specific conditions. Goldsmith [8] examined the degradation of two gasoline additives, methanol and tertiary butyl alcohol in subsurface samples col-

lected from three previously uncontaminated sites. One of the sites was aerobic while the other two were anoxic. Methanol degraded rapidly at all three sites. TBA, degraded slowly following zero order kinetics in individual microcosms, but indicated a first-order response with respect to the initial concentration. The presence of benzene, toluene and *m*-xylene did not affect the degradation rate of the alcohols. In each case, biodegradation in the saturated zone was greater than in the unsaturated zone.

White [9] determined that TBA would degrade rapidly in aquifer material obtained from a site previously contaminated with that compound. Biodegradation of TBA was accompanied by bacterial growth and could be modeled by the Monod equation. TBA degradation at an uncontaminated site was slow, exhibiting the same response as was observed by Goldsmith [8]. This slow rate could not be modeled adequately by Monod kinetics.

Smith and Novak [10] determined that phenol and four of its chlorinated derivatives were readily degraded in soil from two previously uncontaminated sites. In each case, biodegradation followed first-order kinetics with the rate of degradation proportional to the initial concentration. The degradation rates did not correlate well with the degree of chlorination.

In a study on biodegradation enhancement, Wilson [11] determined that the addition of nitrate to soil which did not contain an actively denitrifying bacterial population would inhibit the degradation of methanol due to the buildup of nitrite. This inhibition was relieved if the pH was raised above pH 6. The addition of sulfate inhibited methanol and TBA degradation at the site studied. Manipulation of the pH did not affect this condition. Variation of pH alone and the addition of organic substrates did not affect the rate of TBA degradation.

Suflita and Miller [12] found that chlorophenolic compounds degraded in soil from an actively methanogenic site, but was inhibited in soil from a non-methanogenic site. Evidence suggested that the non-methanogenic site was a sulfate reducing site. This study, however, did not demonstrate wether there was a relationship between the presence of sulfate reduction, absence of methane production and the inhibition of chlorophenolic degradation. There was speculation, however, that the three phenomena were interrelated. In a subsequent study, Gibson and Suflita [13] determined that 2,4,5-T would not degrade in an actively sulfate reducing site due to the presence of sulfate and not because there was a lack of suitable microorganisms. Smolenski and Suflita [14] determined that biodegradation of various cresol isomers was stimulated by the addition of sulfate and the reduction of sulfate but was inhibited in methanogenic conditions. This type of work raises questions concerning the effect of various reductive processes on biodegradation.

Methanogenesis involves a bacterial consortium of methanogenic and chemoheterotrophic non-methanogenic bacteria. In the first step, chemoheterotrophic non-methanogenic bacteria convert complex organics to volatile acids,

alcohols, hydrogen and carbon dioxide. Alcohols and volatile fatty acids which are longer than two carbons are converted to hydrogen and acetic acid by a class of bacteria called acetogens. Acetogens are obligate proton reducers, since they do not require an outside electron acceptor. Methanogenic bacteria utilize the acetic acid and hydrogen to produce methane. The acetogens and methanogens must exist in a symbiotic relationship to prevent the accumulation of fermentation intermediates [15].

Sulfate is reduced by two different methods. Assimilatory sulfate reduction involves the conversion of inorganic sulfate to organic sulfur for use by the organism in its metabolic process. In this process, the organism converts sulfate to hydrogen sulfide intracellularly for incorporation as sulfhydryl groups in the amino acids cysteine and methionine.

In dissimilatory sulfate reduction, on the other hand, sulfate is used as an electron acceptor during the oxidation of organic matter. Hydrogen sulfide is produced as an end product and released into the surrounding environment. In a study using waterlogged soil, Connell and Patrick [16] determined that no sulfide would accumulate until the redox potential was less than -150 mV. As the redox potential was decreased between -150 mV and -300 mV, the level of sulfide increased. They also determined that no sulfide was produced if the pH was less than 6.5 or greater than 8.5. In another study by Connell and Patrick [17], the addition of reducible iron resulted in a decrease in the H_2S concentration presumably by precipitation of iron sulfide. Furthermore, when H_2S was added to the system, the amount of H_2S removed by precipitation was equal to the ferrous iron released from the soil. The addition of nitrate to the soil inhibited sulfide production, probably as a result of an increased oxidation reduction potential. Sulfide production commenced after the nitrate had been exhausted. In many studies of sulfate reduction, molybdate is commonly used as an effective inhibitor of sulfate reduction. Molybdate is an analog of the sulfate ion and is taken up by sulfate reducing bacteria, which results in inhibiting the sulfate metabolic pathway at the initial activation stage.

An important aspect of dissimilatory sulfate reduction is the control it can exert on the carbon and electron flow in aquatic systems. In numerous studies, sulfate reduction has been shown to inhibit other forms of metabolism, in particular methanogenesis [18–25]. In each of these studies, the sulfate reducing bacteria successfully competed for the available H_2 and acetate with a resulting suppression of the methane producing bacteria. Winfrey and Zeikus [18] determined that methanogenesis involving H_2 and acetate was inhibited by as little as 0.2 m*M* sulfate. Once the sulfate was consumed, the flow of carbon and hydrogen was shifted to methanogenesis with the subsequent production of methane. Oremland and Polcin [23], on the other hand, reported that the methanogenesis of methanol, trimethylamine and methionine was not inhibited by the presence of sulfate ions. They described substrates such as acetate and H_2 for which methanogenesis was inhibited by sulfate reduction as com-

petitive; whereas, substrates such as methanol, trimethylamine and methionine were described as non-competitive.

Several theories have been proposed to explain the apparent inhibition of methanogenesis by sulfate reduction. Cappenberg [26] proposed that the presence of sulfide was toxic to methane producing bacteria. MacGregor and Keeney [27] contended that sulfate reduction increased the redox potential E_h of the sediment to a level inhibitory to methanogenesis. Winfrey and Zeikus [18] disputed each of these theories in their research. They contended that the small concentration of sulfate necessary to inhibit methanogenesis would not significantly alter the E_h. They also were unable to detect a significant amount of sulfide due to precipitation of metal sulfides and, therefore, concluded that there was not enough free sulfide in their system to affect the methanogenic bacteria. Though Winfrey and Zeikus do not propose a mechanism of their own, they do point out that sulfate inhibition of methanogenesis is compatible with the thermodynamics of the system. Their calculations are given as follows;

$$SO_4^{2-} + CH_3COO^- + H^+ \rightarrow H_2 + 2HCO_3^- \qquad \Delta G = 47.3\ \text{J/mol} \qquad (1)$$

$$CH_3COOH \rightarrow CO_2 + CH_4 \qquad \Delta G = 28.5\ \text{J/mol} \qquad (2)$$

Sulfate reduction of acetate as illustrated by eqn. (1) above yields a greater free enthalpy than does the methanogenic reaction shown in eqn. (2) Likewise, the reduction of sulfate by H_2 yields 154.0 J/mol, whereas, the reduction of CO_2 by H_2 to methane yields 135.1 J/mol.

Kristjansson et al. [28] and Schonheit et al. [29] attributed the competitive advantage of sulfate reducers over methane producers for H_2 and acetate to difference in substrate affinities as expressed by the value of the half-saturation constant (K_s) in the Monod kinetic model. Using pure cultures of *Desulfovibrio vulgaris* and *Methanobrevibacter arboriphilus*, the rate of H_2 usage was five times greater for the sulfate reducing bacteria than for the methane producer when the concentration of H_2 was limiting. The K_s was 1 μM for *D. vulgaris* and 6 μM for *M. arboriphilus*. Similarly, when acetate was used in limiting quantities, the K_s for the sulfate reducer was 0.2 mM and 3 mM for the methane producer.

Dissimilatory denitrification is a two step process in which certain facultative bacteria utilize organic carbon under anoxic conditions as an energy source and oxidized nitrogen as an electron acceptor. In the first step, nitrate is reduced to nitrite while organic carbon is oxidized. In the second step, nitrite is reduced to nitrogen gas. Denitrification yields the greatest free energy of the anaerobic processes. Consequently, reactions involving denitrifiers would take precedence over sulfate reducers and methane bacteria provided there is a sufficient source of electron acceptors [30,31].

This study

For each of the compounds examined in this study, the degradation rate was greater in the Newport News soil than in the Blacksburg soil. This comparison is shown in Fig. 2. The increase in rate from the Blacksburg soil to the Newport News soil ranged from 17% for phenol to 2150% for TBA. For the C_2 through C_5 alcohols, the degradation rate decreased as the chain length increased. The rate of methanol degradation did not fall into the same pattern exhibited by the other straight chain alcohols. Methanol, however, degraded faster than propanol in the blacksburg soil but slower in the Newport News soil. The most significant difference between the two soils is the response to TBA. The remainder of this discussion, therefore will focus on this difference.

The degradation pattern of TBA in the Blacksburg soil is shown in Fig. 3. In general, the rate of TBA degradation which was measured for 358 days was linear or zero order. As the initial concentration was increased, the degradation rate increased. For comparison, the degradation of methanol, ethanol and 1-butanol along with TBA is shown in Fig. 4. The response of the straight chain alcohols can be characterized as typical of a batch type reactor. The relationship between the TBA degradation rate and initial concentration is shown in Fig. 5 where the log degradation rate versus log initial concentration yields a straight line with a slope of approximately one. This same relationship was observed by Novak et al. [32] for previously uncontaminated soils from Wayland, NY and Dumfries, VA.

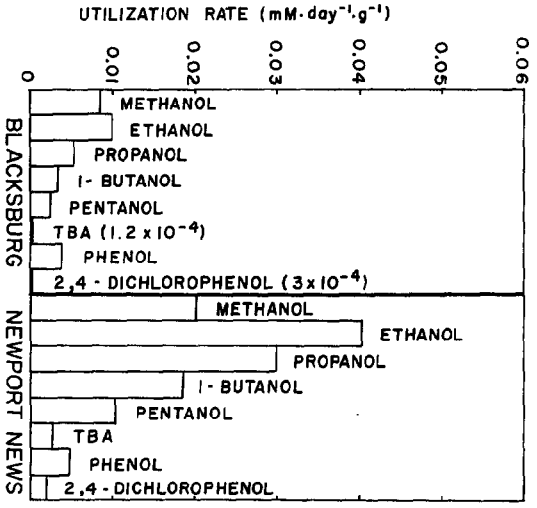

Fig. 2. Substrate utilization rate of the seven test organic compounds in the Blacksburg and Newport News soils.

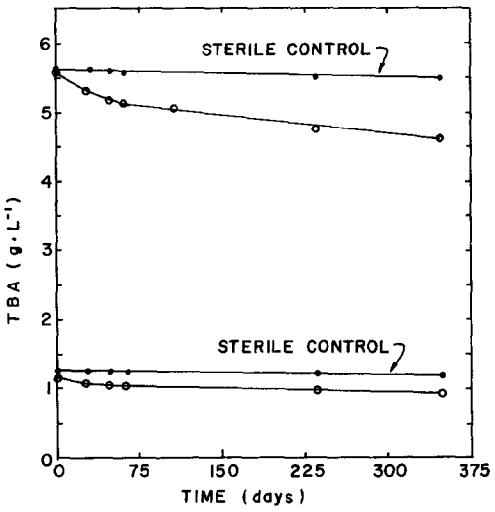

Fig. 3. Biological degradation of approximately 1200 mg/l and 5500 mg/l TBA in Blacksburg soil.

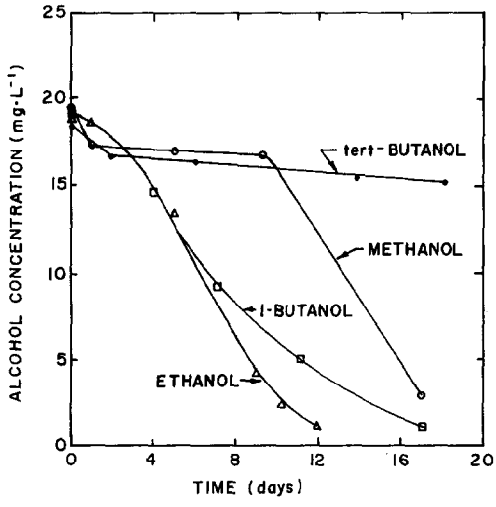

Fig. 4. Biological degradation of approximately 20 mg/l methanol, ethanol, 1-butanol and TBA in Blacksburg soil.

In the subsurface material from Newport News, TBA degraded rapidly. As shown in Fig. 6, 100 mg/l TBA degraded to an undetectable level in about 75 days in a pattern similar to the straight chain alcohols in Blacksburg soil. At higher concentrations, the rate of degradation appeared to be independent of the initial concentration. This is shown in Fig. 7 where a plot of log degradation rate versus log initial concentration indicates that the biodegradation rate was

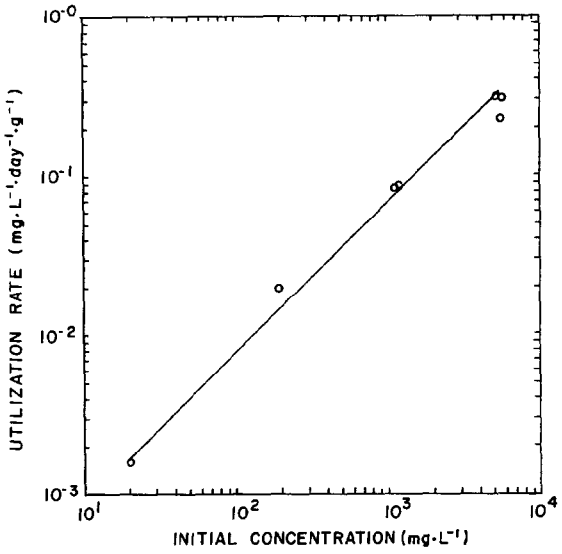

Fig. 5. Relationship between TBA utilization rate and initial concentration in Blacksburg soil.

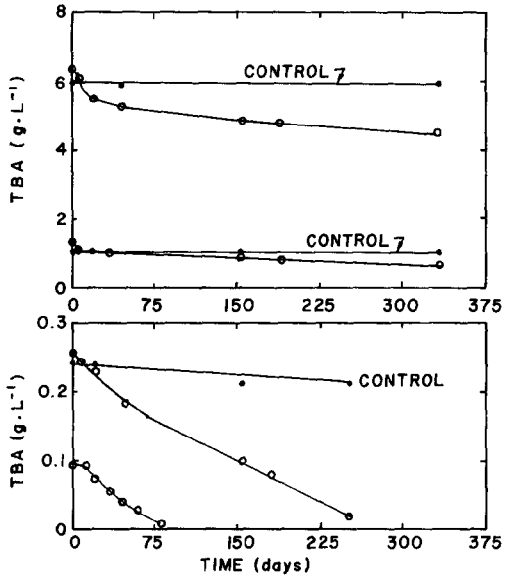

Fig. 6. Biological degradation of TBA in Newport News soil.

essentially constant in microcosms with an initial concentration between 10 mg/l and 500 mg/l. This rate decreased when the initial concentration was less than 10 mg/l. Since TBA readily degraded in the Newport News soil, but not in the Blacksburg soil, it would appear that environmental conditions, i.e. the

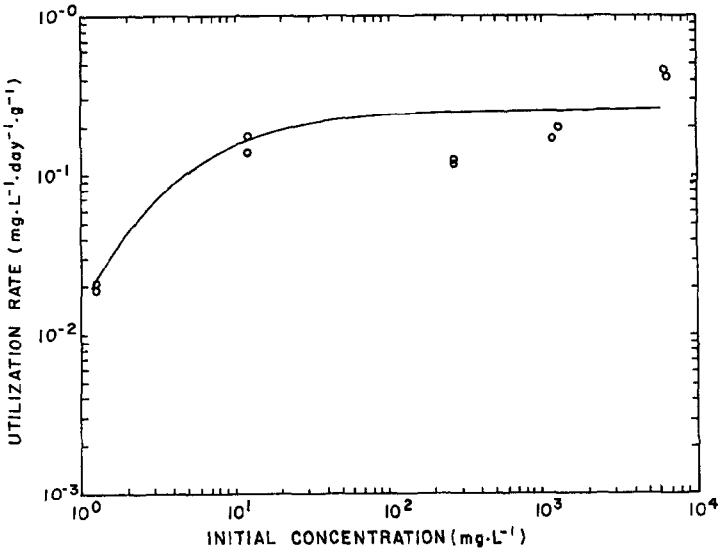

Fig. 7. Relationship between TBA utilization rate and initial concentration in Newport News soil.

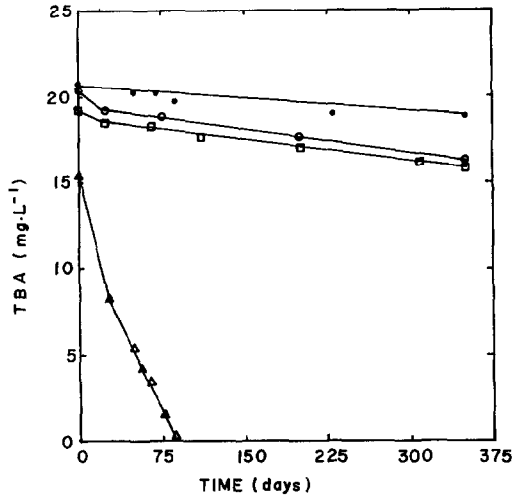

Fig. 8. Biological degradation of TBA in Blacksburg soil with the addition of nitrate and molybdate. ● Control, ○ TBA, □ 0.8 m*M* NO_3^-, and △ 1.0 m*M* MoO_4^{2-}.

lack of O_2, are not a factor in TBA degradation. Biological factors, therefore, may play a role in determining the degradability of TBA at the two sites. To examine this, molybdate and nitrate were added to influence the bacterial ecology of the systems. In the Blacksburg soil (Fig. 8), the addition of nitrate did not alter the degradation pattern of TBA. Evidently, a population of nitrate

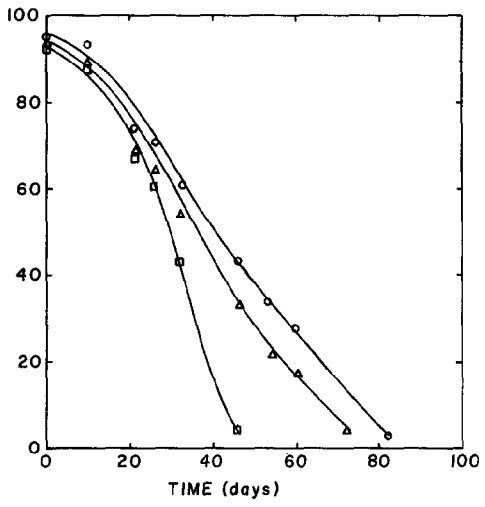

Fig. 9. Biological degradation of TBA in Newport News soil with the addition of nitrate and molybdate ○ TBA, △ 1.0 m*M* MoO_4^{2-}, and □ 1.6 m*M* NO_3^-.

reducing bacteria did not exist in the Blacksburg soil which could utilize TBA. The addition of molybdate to inhibit sulfate reduction, however, did affect TBA degradation. In the presence of 1 m*M* MoO_4^{2-}, 15 mg/l TBA degraded to an undetectable level in about 80 days.

In the Newport News soil (Fig. 9), the presence of MoO_4^{2-} did not significantly affect the degradation of TBA. Nitrate however, increased the rate of TBA degradation by almost a factor of two.

Discussion

From these results, it appears that ecological factors control the degradation rate of TBA rather than environmental factors although the ecology is an outgrowth of historical environmental conditions. The addition of nitrate to the Newport News soil stimulated degradation presumably because this soil contained an active denitrifying bacterial population. This was not the case in the Blacksburg soil, where nitrate addition did not affect degradation. By inhibiting sulfate reduction with molybdate, TBA readily degraded in the Blacksburg soil. At present, the role of sulfate on TBA degradation is not well understood and is the subject of further research, however, these results are consistent with what other researchers have reported for the biodegradation of chlorophenols. In these studies, chlorophenols readily degraded in soil from an actively methanogenic site but not at a sulfate reducing site. In addition, the control that sulfate reducing organisms exert over the flow of carbon and electrons in a mixed biological system has been documented.

Understanding interrelationships between the various reductive processes

and the microbial ecology, therefore, appear to be significant in several aspects of biological degradation. The biodegradation potential of organic compounds appears to be dependent on this relationship. As shown in this study, TBA was essentially a non-biodegradable compound in the Blacksburg soil under anoxic conditions. If, however, sulfate reduction was inhibited, TBA degraded to undetectable levels within a reasonable amount of time.

This relationship between reductive processes and the microbial ecology of a site is also important in determining wether a site is amenable to biodegradation. In the soil where denitrifiers were active (Newport News), TBA was easily degraded and in fact was stimulated by the addition of nitrate. Direct measurement of denitrifying organisms or the utilization of nitrate, therefore, may provide a good indicator that a site is a good candidate for biological degradation of compounds thought to be persistent.

Finally, biodegradation of organic compounds may be enhanced if a favorable microbial ecology can be produced at sites where biodegradation is inhibited. This study would suggest that conversion of a sulfate reducing site into a denitrifying site may promote the degradation of TBA in the subsurface.

References

1 W.J. Dunlap, J.F. McNabb, M.R. Scalf and R.L. Cosby, Sampling for organic chemicals and microorganisms in the subsurface, EPA-600/277-176, Robert S. Kerr Env. Res. Laboratory, Office of Res. and Dev., U.S. Environmental Protection Agency, Ada, OK, 1977.

2 J.T. Wilson, J.F. McNabb, D.L. Balkwill and W.C. Ghiorse, Enumeration and characterization of bacteria indigenous to a shallow water-table aquifer, Groundwater, 21 (1983) 134–142.

3 G. Bengtsson, Microcosm for groundwater Research, in: C.H. Ward, W. Giger and P.L. McCarty (Eds.), Ground Water Quality. John Wiley and Sons, New York, NY, 1985.

4 E.J. Bouwer, and P.L. McCarty, Modeling of trace organics biotransformation in the subsurface, Groundwater, 22 (1984) 433–440.

5 B.E. Rittmann, P.L. McCarty and P.V. Roberts, Trace Organics Biodegradation in Aquifer-recharge, Groundwater, 18 (1989) 236–243.

6 P.L. McCarty, M. Reinhard and B.E. Rittmann, Trace Organics in Groundwater, Environ. Sci. Technol. 15 (1981) 40–47.

7 S. Simkins and M. Alexander, Models for mineralization kinetics with the variables of substrate concentration and population density, Appl. Environ. Microbiol. 47 (1984) 1299–1306.

8 C.D. Goldsmith, Biodegradation of Methanol and Tertiary Butyl Alcohol in Previously Uncontaminated Subsurface Systems, Ph.D. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, VA, 1985.

9 K.D. White, A comparison of Subsurface Biodegradation Rates of Methanol and Tertiary Butanol in Contaminated and Uncontaminated Sites, Ph.D. Dissertation, Virginia Polytechnic Institute and State University, Blacksburg, VA, 1986.

10 J.A. Smith and J.T. Novak, Biodegradation of chlorinated phenols in subsurface soils, Water, Air Soil Pollut., 33 (1987) 29–42.

11 W.G. Wilson, Enhancement of Biodegradation of Methanol and Tertiary Butyl Alcohol in Groundwater Systems, Master of Science Thesis, Virginia Polytechnic Institute and State University, Blacksburg, VA, 1986.

12 J.M. Suflita and G.D. Miller, Microbial metabolism of chlorophenolic compounds in ground water aquifers, Environ. Toxicol. Chem., 4 (1985) 751–758.

13 S.A. Gibson and J.M. Suflita, Extrapolation of biodegradation results to groundwater aquifers: Reductive dehalogenation of aromatic compounds. Appl. Environ. Microbiol. 52 (1986) 681–688.

14 W.J. Smolenski and J.M. Suflita, Biodegradation of cresol isomers in anoxic aquifers, Appl. Environ. Microbiol., 53 (1987) 710–716.

15 R.E. Speece, Anaerobic biotechnology for industrial wastewater treatment, Environ. Sci. Technol., 17 (1983) 416A–427A.

16 W.E. Connell and P. Patrick, Sulfate reduction in soil: Effects of redox potential and pH, Science, 159 (1968) 86–87.

17 W.E. Connell and P. Patrick, Reduction of sulfate in waterlogged soils, Soil Sci. Soc. Am., 33 (1969) 711–715.

18 M.R. Winfrey, and J.G. Zeikus, Effect of sulfate on carbon and electron flow during microbial methanogenesis in freshwater sediments, Appl. Environ. Microbiol., 33 (1977) 257–281.

19 J.W. Abram and D.B. Nedwell, Inhibition of methanogenesis by sulfate reducing bacteria competing for transferred hydrogen, Arch. Microbiol. 117 (1978) 89–92.

20 D.O. Mountfort, R.A. Asher, E.L. Mays and J.M. Tiedje, Carbon and electron flow in mud and sandflat intertidal sediments at Delaware inlet, Nelson, New Zealand, Appl. Environ. Microbiol., 39 (1980) 686–694.

21 R.L. Smith and M.J. Klug, Electron donors utilized by sulfate reducing bacteria in eutrophic lake sediments, Appl. Environ. Microbiol., 42 (1981) 116–121.

22 J. Sorenson, D. Cristensen and B.B. Jorgensen, Volatile fatty acids and hydrogen as substrates for sulfate reducing bacteria in anaerobic marine sediments, Appl. Environ. Microbiol., 42 (1981) 5–11.

23 R.S. Oremland S. Polcin, Methanogenesis and sulfate reduction: Competitive and non-competitive substrates in estuarine sediments, Appl. Environ. Microbiol. 44 (1982) 1270–1276.

24 D.R. Lovley and M.J. Klug, Sulfate reducers can outcompete methanogens at freshwater sulfate concentrations, Appl. Environ. Microbiol. 45 (1983) 187–192.

25 M.R. Winfrey and D.M. Ward, Substrates for sulfate reduction and methane production in intertidal sediments, Appl. Environ. Microbiol. 45 (1983) 193–199.

26 T.E. Cappenberg, Interrelations between sulfate-reducing and methane producing bacteria in bottom deposits of a freshwater lake. I. Field observations, Antonie van Leeuwenhoek, 40 (1974) 285–295.

27 A.N. MacGregor and D.R. Keeney, Methane formation by lake sediments during *in vitro* incubation, Water Res. Bull., 9 (1973) 1153–1158.

28 J.K. Kristjansson, P. Schonheit and R.K. Thauer, Different K_s values for hydrogen of methanogenic bacteria and sulfate reducing bacteria: An explanation for the apparent inhibition of methanogenesis by sulfate, Arch. Microbiol. 131 (1982) 278–282.

29 P. Schonheit, J.K. Kristjansson and R.K. Thauer, Kinetic mechanism for the ability of sulfate reducers to out-compete methanogens for acetate, Arch. Microbiol., 132 (1982) 285–288.

30 L.D. Benefield and C.W. Randall, in: G.H. Sewell (Ed.), Biological Process Design for Wastewater Treatment. Prentice-Hall, Englewood Cliffs, NJ, 1980.

31 W. Stumm and J.J. Morgan, Aquatic Chemistry, 2nd ed. John Wiley and Sons, New York, NY, 1981.

32 J.T. Novak, C.D. Goldsmith, R.E. Benoît and J.H. O'Brien, Biodegradation of Methanol and Tertiary Butyl Alcohol in Subsurface Systems, Water Sci. Technol., 17 (1985) 71–85.