BIODEGRADATION OF METHANOL AND TERTIARY BUTYL ALCOHOL IN PREVIOUSLY UNCONTAMINATED SUBSURFACE SYSTEMS

by

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(ABSTRACT)

The objective of this study was to determine the potential for biodegradation in subsurface soils and groundwater from sites in Williamsport, PA, Wayland, NY, and Dumfries, VA. These subsurface systems were characterized both physically, chemically and biologically. Bacterial populations were substantial in all systems and ranged from 10^3 to 10^8 colony forming units per gram. Soil sampling was done in a quality-controlled aseptic manner using conventional drilling and sampling equipment. A matrix of test-tube microcosms was used to determine biodegradation rates of methanol and t-butyl alcohol at concentrations ranging from 1 to 1000 mg/L. Methanol degraded readily at all sites ranging from 0.8 mg/L/day to 20.4 mg/L/day and rates were generally greater in the saturated zone. TBA biodegraded at all sites, but was refractory in nature. Biodegradation rates for TBA in anaerobic subsurface systems were found to increase directly with initial concentration from 10^{-4} mg/L/day for 1 mg/L to 10^{-1} mg/L/day for 80

mg/L. TBA biodegradation in the aerobic system was essentially constant over all concentrations. Biokinetic coefficients were determined for methanol and TBA at each site based on plots of utilization rates versus substrate concentration and reciprocal plots of these values. The K values found suggest that aerobic subsurface systems can utilize alcohols at a greater rate than anoxic subsurface systems and can be used for comparative purposes. The K_s of anoxic subsurface systems were found to be large due to the low temperature (10°C) found in aquifers. The results indicate that methanol contamination in groundwater has much less associated risk to drinking water supplies due to the ease of biodegradation. However, TBA poses a much greater risk due to the very slow removal rates at low concentrations, which could result in a residual level for over a decade in some cases.

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INTRODUCTION

The potential for and occurrence of groundwater contamination due to gasoline leakage from pipelines and storage tanks is a major concern of the petroleum industry, as well as the general public. Gasoline spills of major proportions have been reported in the past (1,2) in addition to the small quantities that are continually being lost.

Groundwater comprises more than 95 percent of all available freshwater in the United States, and approximately 80 percent of all public water suppliers rely on this source. Groundwater has in the past been considered a pristine liquid. However, a survey of almost 35,000 water supplies revealed a widespread problem of contamination (3).

Of specific concern in this study is gasoline containing alcohol additives (gasohol) such as methanol and tertiary butyl alcohol (TBA). These alcohols present a unique situation after entry into a subsurface system. As gasohol travels through a groundwater aquifer, changes in component concentrations may occur. The mechanisms of disperson, sorption, and biodegradation are responsible for these changes. Dispersion is a function of hydraulics, while sorption is more complex. Predicted retention times of several organic compounds in the soil matrix of aquifers based on organic content of the matrix and the octanol-water partition coefficient were found to agree well with retention times in field studies (4). Sorption is responsible for separating the compounds of gasohol and producing a component wave effect as groundwater moves through the aquifer. Alcohols, particularly

methanol, are of concern since their presence in water is not easily detected by taste and odor until levels potentially detrimental to human health may be reached. Gasoline in general is not detectable until a concentration of 0.5 mg/L is attained. Due to the solubility of alcohols in water they would move ahead of the remaining gasoline components which are more easily detected. Dispersion and sorption result only in separation and concentration changes, but do not resolve the mode of removal of these toxics from the groundwater system.

Biodegradation would seem to be a mechanism of great importance in a groundwater aquifer, and little information of this kind is available. Therefore, the objectives of this investigation were to 1) quantify bacterial numbers as they vary with depth, 2) determine the potential for biodegradation by these microbes using an experimental microcosm matrix for several uncontaminated sites as it relates to depth and aquifer parameters, 3) determine the effect of benzene, toluene, and m-xylene (BTX) on alcohol biodegradation rates and 4) obtain a general risk assessment for groundwater contamination by gasohol by integrating the collected data.

BACKGROUND AND REVIEW OF LITERATURE

This study encompasses several scientific fields that impact the removal of subsurface contaminants. These are engineering, microbiology, geology, and biochemistry. An understanding of the macro- and micro-subsurface, both physically and biologically, is important. Therefore, the potential for natural assimilation of alcohols found in gasoline and the effects of multiple subsurface parameters will be discussed.

Subsurface System

Structure

Great heterogeneity can exist within the subsurface, but basically these systems consist of two major entities. There is an unsaturated and a saturated zone. The unsaturated zone is subject to influence by vadose water and lies above the water table, which may rise and fall within the unsaturated zone. The saturated zone, where water is held, may be homogeneous or consist of several layers, some of which can cause partial (aquitard or aquiclude) or complete (aquifuge) confinement of vertical water movement within the aquifer. These layers are currently referred to as leaky confining or confining aquifers. Groundwater flow may range from millimeters to meters in distance per year, but can

generally be said to be quite slow. Most aquifers are at some point underlain by bedrock and can be recharged by downward seepage through the unsaturated zone. Recharge can also occur by lateral flow or by upward seepage from underlying strata (5).

Environment

The nutrient status of an aquifer can be said to be ogliotrophic. Groundwater is generally low in organic carbon, low in temperature, anaerobic and of low pH. There are, of course, exceptions to these conditions at a given site. A major question with regard to subsurface biodegradability is; can organisms existing under such conditions cope with increased organic loading due to contamination? Ogliotrophic organisms can live under very low nutrient conditions, but can adapt to high nutrient conditions. The reverse adaptation is not thought to be as easily done. These organisms usually have a high surface to volume ratio and may have lower minimum substrate requirements to attain measurable growth, but the maximum growth rate is also lower. Subsurface bacteria prefer attachment and can often use multiple substrates (6). These factors along with increased pressure, spatial limitations, temperature, macro and micro nutrient limits, and others were evaluated.

A parameter considered to be of major significance was temperature. Soil temperature increases approximately 30°C/1000 meters in depth. This would preclude microbial growth at depths greater than 2500m if only thermophiles are considered. The average groundwater temperature, however, to depths of about 60 ft usually approximates the mean annual

air temperature. The conclusion was that organisms in the subsurface would take part in the assimilation of contaminants (7).

Biology

Until recently only the uppermost soil layers were believed to have significant bacterial populations. This in part was probably due to the expense and lack of methodology for obtaining aseptic samples from deep soil regions. Groundwater was considered to be relatively pure and bacteria-free.

Groundwater. Bacteria have been identified in groundwater and soils in several areas where spills of contaminating organics have occurred (2, 8-10) as well as uncontaminated areas (11). These are presented in Table 1 along with the type of contaminant for each case. It is probably safe to assume, therefore, that the groundwater offers a variety of microhabitats.

Soil. Several methods have been used to enumerate subsurface soil bacterial populations. Wilson et al. made direct measurements by utilizing epifluorescence microscopy with acridine orange staining (12). Counts were also obtained by culturing on soil extract agar. In addition transmission electron microscopy (TEM) was used to study organism morphology. Both gram-negative and positive cells were found. Most frequent were small rods (<1μm) and cocci. This is consistent with the findings of Ghiorse and Balkwill (13). TEM revealed that bacterial cells were most always coated with a dense clay-like material and an extracellular polymer matrix was found to be connecting cells to soil particles. Most bacteria were small in size relative to nutrient rich

Table 1. Microbes found in groundwater of contaminated and uncontaminated sites.

Organism(s)	Contaminant	Reference
Pseudomonas spp. Arthrobacter	Gasoline	2
Pseudomonas Nocardia Acinetobacter Flavobacterium Micrococcus	Gasoline	8
Pseudomonas	Gas oil	9
Pseudomonas spp. Pseudomonas putida Pseudomonas aeruginosa Pseudomonas stutzeri Alcaligenes spp. Alcaligenes denitrificans	Creosote	10
Microcyclus Prosthecomicrobium Gallionella Caulobacter Agrobacterium Hyphomicrobium Planctomyces Clostridium Nocardia Protozoa Yeasts	None	11

bacteria. This was not found to be true of bacteria observed in groundwater by Hirsch and Rades-Rohkohl (11). They did find, in agreement with Wilson et al., that groundwater bacteria carried hair-like surface polymers. The differences in reported size could be a function of living space, i.e., free floating in groundwater wells versus confinement in interstitial spaces.

While Wilson et al. (12) believed no organisms other than bacteria existed in the subsurface soils, Ghiorse and Balkwill found ellipsoid cells with pointed ends, narrow filaments, and some ovoid forms that were believed to have been fungal spores or yeast cells (13). Supporting this contention were ninety different organisms found in well water from Northern Germany. Seventy-two were bacteria, ten were protozoa, while eight were fungi, all of which grew well at 9°C(11).

The depths to which bacteria can exist seems to be great (7). At a site near Pensacola, Florida, bacteria were found to 410 m by estimates of biomass using five biochemical assays of fatty acids (14).

Biodegradation Studies

Many researchers have tried to estimate bioaccumulation of hazardous organic compounds using octanol/water partition coefficients (15,16) or estimates of microbial degradation rates by evaluation of chemical structure (17). Recent review papers have done a commendable job with respect to evaluating the potential for removal of hazardous compounds from the environment by biodegradation (6, 18), but these assessments cannot always be accurately compiled to predict actual removal rates for a given system.

No studies were found to exist dealing with the subsurface biodegradation of alcohols. However, several dealing with various other compounds in subsurface soils were available, as were investigations of the biodegradation in well waters of gasoline, gas oil, and creosote. Some of the original investigations in subsurface soil biodegradation can be subject to criticism. However, the work was done with no prior experiences upon which to draw. Therefore, considerable knowledge was gained and time saved by examination of these works.

The biodegradation of toluene, chlorobenzene, bromodichlormethane, 1,2-dichloroethane, 1,1,2-trichloroethane, trichloroethylene, and tetrachlorethylene were measured in subsurface soils from 1.2, 3.0, and 5.0 m at Lula, Oklahoma (12). This material was made into a slurry with distilled water and a sterile solution of the listed compounds was added and mixed before addition to 35 mL test tube microcosms. The slurry was assumed to be uniform. At given time intervals duplicate microcosms would be sacrificed for measurements of biodegradation. The initial concentrations were found to range from 85 to 666 µg/L indicating very poor mixing. All concentrations were normalized to the compound thought to be closer to the expected value. After incubation at 20°C for 16 weeks toluene was found to degrade rapidly at all three depths. The remaining compounds either degraded very slowly or not at all. The lack of degradation of these other compounds could have been due to the need for an extended incubation period, the use of sterile distilled water versus sterile groundwater, which may contain essential inorganic

nutrients, or the presence of toluene, which is easily degraded and may have been utilized preferentially.

A follow-up study of subsurface soils from Pickett, Oklahoma and Fort Polk, Louisiana used similar techniques to evaluate biodegradation (19). This time, however, the dose solution consisted of sterile well water containing the contaminants, and known amounts were added to the microcosm tubes containing the slurry to give a more reliable initial concentration of approximately 1 mg/L. Chloroform, 1,1-dichloroethane, 1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene, toluene, chlorobenzene, and styrene were the compounds tested. Toluene and styrene showed some evidence of biodegradation at both sites under incubation at 17°C, while the others were utilized very slowly or not at all.

The biodegradation rates of 47 water-soluble components of gas oil were determined in groundwater at 10°C (9). The substrate was obtained by shaking 1 ml of gas oil with 1 L of groundwater for 10 minutes to achieve a total aromatic hydrocarbon concentration of 2 mg/L in the water. All were identified by GC/MS. No compounds remained after 288 days. Of particular interest were ortho, meta, and para-xylene and toluene biodegradation. All were subject to complete degradation, but meta and para-xylene were degraded much faster than ortho-xylene. Of 30 isolates it was found that just 12 strains actually affected the composition of the hydrocarbons. Using GC/MS only four different degradation spectra were found implying that only four metabolically different strains existed. Studies with these four groups verified that

the ortho substitution was most resistant to degradation. Intermediate compounds that were identified agreed well with existing literature on aromatic metabolism, and suggested, to some extent, that pure culture studies are of predictive use.

At a creosote contaminated site in St. Louis Park, Minnesota, well studies down gradient from the source revealed that phenolic compounds and naphthalene were disappearing much faster than expected due to dispersion and sorption (20). Methane was found in the water from the contaminated area at 2-20 mg/L. Bacterial counts of 10^8-10^9 per 100 mL and isolates on creosote substrates confirmed the belief that bacterial action was occurring (10).

A pipeline loss of 100,000-250,000 gallons of gasoline occurred at Glendale, California (2). As a result, one of the first studies to suggest that bacterial degradation could be helpful in the natural cleanup of well waters was conducted at this site. The wells that had no gasoline taste or odor averaged 200/mL bacteria, while those exhibiting taste and odor averaged 50,000/mL.

As the result of another gasoline pipeline break a similar study was performed on groundwater in Ambler, Pennsylvania (1). Of the 3,186 barrels lost to the soil almost half remained after all means of physical extraction had been exhausted. To investigate the possibility of bacterial action, populations were enumerated by growth on minimal media salts agar incubated at 13°C in a dessicator containing gasoline (Sunoco 260) vapors and by nutrient agar plate counts. Counts in all

well waters ranged from 10^3-10^4 per mL. Enumerations done with toluene and n-paraffin (gasoline constituents) plates were found to yield 10^6 bacteria/mL well water.

Solutions to Aquifer Contamination

Physical

Generally only physical means have been considered and tested as a means of "cleaning up" an aquifer after a contaminant spill. Most of these methods are expensive and usually involve pumping water from the aquifer with subsequent removal or treatment and replacement. Treatment schemes consist of air stripping (20), granular activated carbon (3), or blending contaminated groundwater with other clean water sources (22). In some instances purging all the water from the aquifer, while simultaneously stopping the contaminating source has been successful (23).

Theoretical

Predictive models have been developed to aid in physical treatment schemes by estimating the size of the area to be affected by a contaminant plume. This is done by utilizing measurable groundwater and aquifer parameters. Soil profiles, hydraulic conductivity, groundwater velocity, and adsorptive factors of the contaminant are a few of the important inputs. Newer models have included first order biochemical decay in addition to these and other parameters (24,25). Models have been shown to be very sensitive to degradation rates when these are included (25), therefore, more recent attempts have recognized the importance of biodegradation by employing biofilm kinetics (4).

However, very little real world data of this kind exists. In addition, biodegradation rates may be site specific, but with enough data some generalizations on these rates could be assumed to hold true for aquifers that are similar in nature, i.e., soil type, anaerobic versus aerobic, etc. for modeling applications. Models may be of importance in the future for determining safe distances for spills near public water supplies.

Stimulation of Natural Biodegradation

Biodegradation, it seems, is a potent force in the eventual removal of unwanted compounds from the subsurface environment. There have been attempts to enhance biodegradation rates in well water studies.

It was found that by supplementing gasoline minimal media agar with 0.2 percent ammonium nitrate or sodium nitrate, sodium or potassium phosphates and magnesium sulfate that counts increased from 10^3-10^4 to 10^5-10^7 based on an increased concentration of electron acceptors for enhanced anaerobic respiration (1). It was decided to amend well waters with aerators as well as ammonium sulfate and mono and disodium phosphates (8). After addition, oxygen was believed to be utilized and the phosphate and nitrogen decreased, while microorganism counts increased to 10^7 at the spill site and decreased towards the perimeter to 10^4. Six months after nutrient treatment no gasoline was detectable in well waters by ultra-violet spectroscopy. However, it cannot be assumed that all gasoline was removed since each well only affects a given area. It is evident, though, that of the 36 wells treated bacterial action was accelerated.

In support of this contention lab studies on well-water gave similar results (9). When natural groundwater was given 2 mg/L of mixed aromatics from gas oil degradation proceeded, but stopped after 10 days resulting in an increase of 10^2 to 10^6/mL. The groundwater was found to be nitrogen deficient. The amount of inorganic nitrogen originally in the water stoichiometrically corresponded to the amount of hydrocarbon utilized. Upon the addition of ammonium chloride further degradation resulted.

Studies with Pure and Mixed Cultures

To thoroughly understand the biological processes that take place in the subsurface a review of research conducted with pure cultures is of utmost importance. The effects of pH, temperature, oxygen, and groundwater composition can be more effectively predicted when the routes by which biodegradation occur are known.

To obtain energy biological systems can utilize various sources. Both organic and inorganic molecules can serve as these sources (electron donors) and they can also serve as electron acceptors. In this study we are concerned with the utilization of the alcohols, methanol and tertiary butyl alcohol, as energy sources by subsurface organisms. Three routes of utilization should be considered. Aerobic respiration occurs in the presence of oxygen. Anaerobic respiration uses NO_3^-, SO_4^{2-} or CO_3^{2-} as electron acceptors. Fermentation occurs without any such electron acceptors. The energy yielded to the organism declines from the highest with aerobic respiration to the lowest with

fermentation. The thrust of all these reactions, of course, is to conserve energy in ATP.

From this general information it could be expected that a subsurface system would be subject to varying efficiencies of contaminant assimulation depending upon its oxygen and inorganic anion status.

Studies using pure or mixed cultures are valuable for determining the biochemical pathways for the diverse types of organisms that have already been shown to exist in the subsurface environment. There exists extensive literature, in most cases, to aid in defining the fate of biological contaminant molecules.

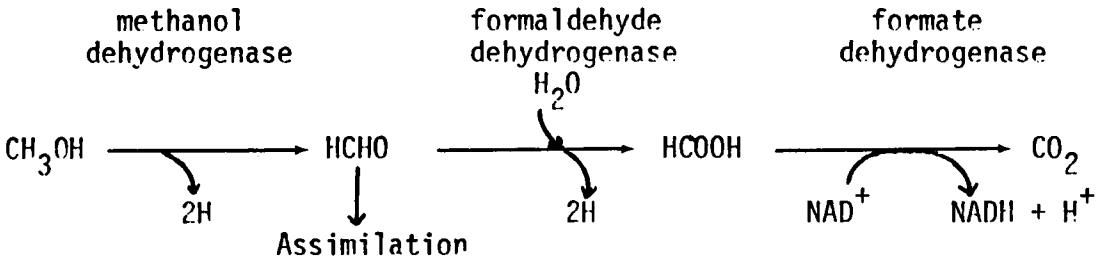
Alcohols

Methanol. The utilization of C_1 compounds by microbes at one time was considered to be obscure. It is now known that organisms capable of such metabolism are ubiquitous. Studies done on aerobic and anaerobic biological waste treatment have demonstrated the presence and usefulness of C_1 utilizers for the removal of methanol and formaldehyde (26-29).

Several excellent review papers exist on the utilization of C_1 compounds (30-33). These will be used to explain how methanol may be metabolized.

Generally, C_1 metabolizing microorganisms are recognized by their ability to utilize organic compounds that contain no carbon to carbon bonds and are more reduced than carbon dioxide. Three pathways of C_1 assimilation are known. These are the ribulose diphosphate pathway, the ribulose monophosphate pathway (RMP) and the serine pathway.

The general scheme of respiratory methanol utilization is as follows (30).



In the ribulose diphosphate pathway of CO_2 assimilation the CO_2 formed from methanol combines with ribulose diphosphate to yield phosphoglyceric acid (PGA) which in turn is converted to glyceraldehyde-3-phosphate. This pathway is used by only a few bacteria.

C_1 bacteria are presumed to assimilate most of their carbon by either the RMP or serine pathways. From studies of methane utilizing bacteria the RMP pathway has been designated as Type I, while the serine pathway has been designated Type II.

The RMP pathway found in Type I organisms is more efficient than the serine pathway in that it does not possess a complete tricarboxylic acid (TCA) cycle. All of the carbon atoms for cell material are derived from formaldehyde and there is no detectable 2-ketoglutarate dehydrogenase (30). In condensed form the reactions are as follows (34).

a) 3Formaldehyde + 3Ribulose-5-P → 3hexulose-6-P → 3Fructose-6-P
(synthase; isomerase)

b) 3Fructose-6-P + ATP → 3Ribulose-5-P + ADP + 3-phosphoglyceraldehyde
(cell material)

c) Overall: 3Formaldehyde + ATP → 3-Phosphogylceraldehyde + ADP

Variants of this cycle have been demonstrated (33).

The serine pathway is somewhat longer but can be briefly described. Formaldehyde is assimilated into cell material by the formation of Acetyl-CoA, then three, four, five and six carbon compounds are synthesized by the glyoxylate cycle (which regenerates oxaloacetate) and the reversal of glycolysis (34). The cyclic reactions in Figure 1 essentially demonstrate the serine pathway (30,32,34).

The first studies on the fermentation of methanol were conducted over thirty years ago. Work with Methosarcina explained the reaction involved for methanol utilization by ^{14}C labeling (35).

1) $CH_3OH + H_2O \rightarrow CO_2 + 6H$

2) $3CH_3OH + 6H \rightarrow 3CH_4 + 3H_2O$

3) $4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$

Subsequent work has served to support these findings with this and other organisms (36-38), but in addition concluded that growth yields must be limited by nutrients or some factor other than the energy source. A study on the anaerobic treatment of fuel oil consisting of 50 percent methanol and 50 percent higher alcohols, supportively found indications that trace elements were of great importance in preventing upset of the process.

Studies involving pure cultures have served to demonstrate the biochemical diversity of methylotrophic bacteria (39-41). A large

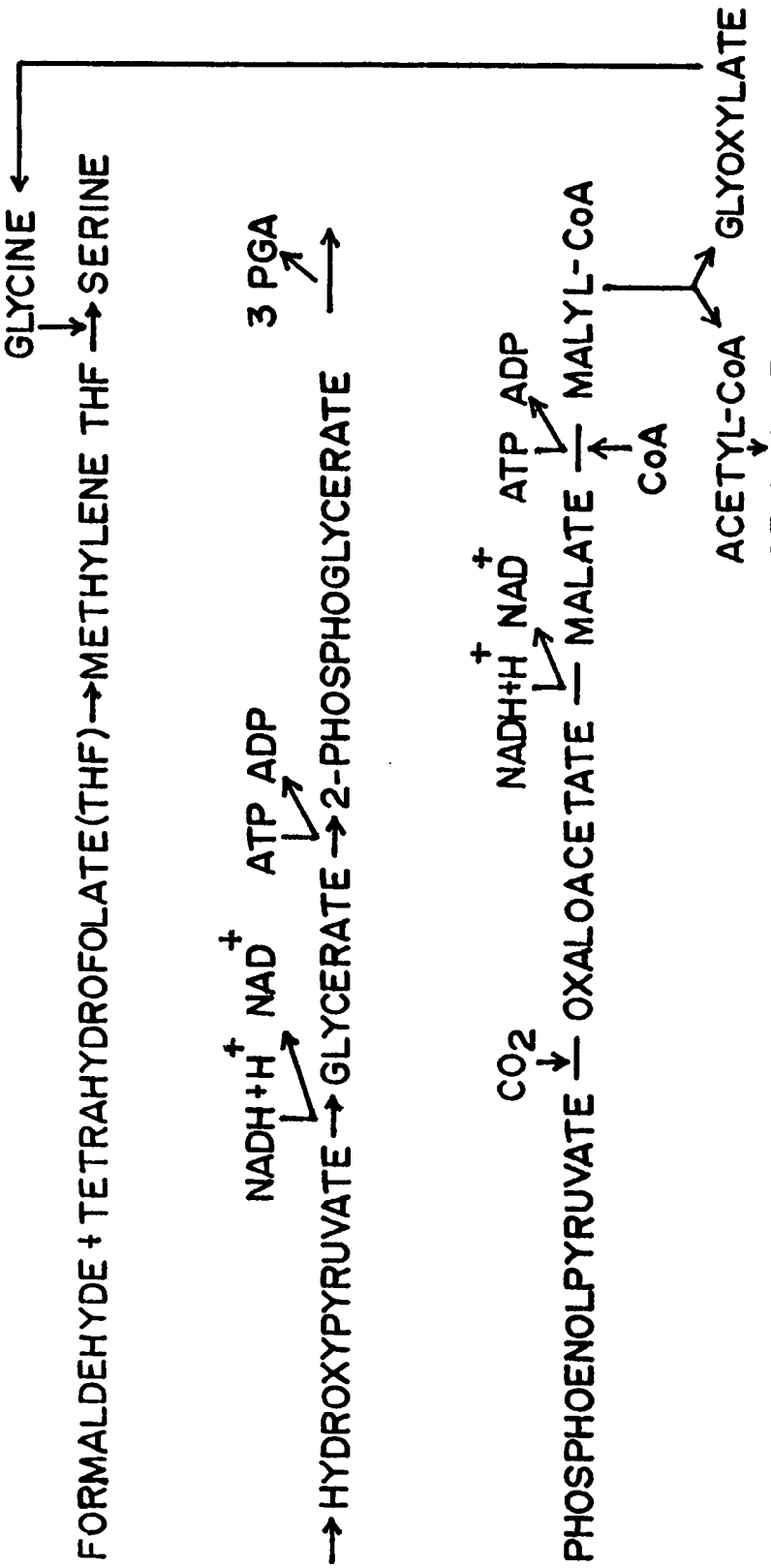


Figure 1. The serine pathway for methanol assimilation.

number of compounds can be oxidized, while not serving as a carbon or energy source (cometabolism). The compounds listed in Table 2 can be found in gasoline (Table 3) and are oxidizable by methylotrophs. From this partial list it is obvious that these organisms are important operating factors in the environment and could be of commercial importance due to their ability to create new products.

TBA. Tertiary butyl alcohol seems to be a compound that has been neglected in microbial research. No information is available concerning the metabolic pathways and very little exists for compounds of similar structure. They have been described as strongly resistant to biodegradation.

The quaternary alcohols, 2,2-dimethyl-1,3-propanediol and pentaerythritol, were not metabolized by acclimated activated sludge while quaternary dicarboxylic acids were easily metabolized (42).

A pathway for the degradation of 2,2-dimethylheptane to pivalic acid and tertiary butylbenzene to the diol by Achromobacter was reported (43) showing evidence for potential degradation of TBA based on similar refractory molecular structures.

Attack on the alcohol group of TBA may be hindered by the molecular structure, but it is feasible for enzymes to convert it to the corresponding aldehyde, ketone or acid.

A list of compounds that may be amenable to fermentation was proposed (44). These followed the conventional scheme of complex organics being converted to a higher organic acid or acetic acid which ultimately yields methane. TBA was found on this list based on the fact

Table 2. Compounds found in gasoline that are oxidizable by various methylotrophs and the resultant products.

Compound	Product
Hexane	1-hexanol 2-hexanol n-hexanol
2-butanol	2-butanone
Benzene	phenol hydroquinone
Toluene	p-cresol benzyl alcohol benzoic acid
Ethylbenzene	o-hydroxyethylbenzene p-hydroxybethylbenzene phenylethanol
Naphthalene	γ-(1,6)-naphthol β-naphthol

Table 3. Gasohol composition.*

Constituent	Volume %
Methanol	5
T-butyl Alcohol	5
Benzene**	2
Ethyl Benzene**	2
P-Xylene	2
O-Xylene	3
M-Xylene	4
C_9-C_{10} Aromatics	9
N-Hexane	3
Total Paraffins, Olefins & Naphthalenes	57
Toluene**	8

*Furnished by Atlantic Richfield Co.
**Priority pollutants

that it is present in industrial wastewaters that are treated by anaerobic processes. However, this is not conclusive proof of degradation.

Aromatics.

As listed in Table 3 gasohol contains several aromatic compounds, two of which are on the EPA priority pollutant list. Aromatics have long been considered hazardous to human health. They are also associated with "shock" loadings to wastewater treatment plants. For this reason water soluble concentrations of benzene, toluene, and m-xylene were investigated to determine their fate and impact on the biodegradation rates of methanol and TBA in the subsurface.

Similar work with mixed substrates of benzene and aliphatic hydrocarbons was done in the Chesapeake Bay giving site specific effects (45). Early investigators documented the decomposition of benzene and toluene in the soil (46), as well as the then known aromatic respiratory degradation mechanisms (47-49). These organisms actually use aromatics as a carbon source and therefore do more than just change the compound as was the case with several methylotrophs. It is believed that a substituted aromatic nucleus only serves to present an organism with a choice as to the mode of attack (50). The literature on the metabolism of aromatics is extensive and several papers will be utilized to illustrate aromatic biodegradation pathways. In general it appears as though there is a lack of substrate specificity by aromatic utilizing organisms.

Benzene. The biodegradation of benzene is illustrated in Figure 2. Most information obtained on this pathway was obtained from studies utilizing Pseudomonas sp. (50-53).

Toluene. Toluene also is utilized by a defined pathway of reactions very similar to benzene (51,54,55) as shown in Figure 3.

Xylene. The identification of the degradation products of meta and para-xylene was a difficult task, due to their instability, even with the aid of ultraviolet, infrared and proton magnetic resonance spectra (56,57). However, pathways have been discovered and are depicted in Figure 4.

Anaerobic Biodegradation. The method of fermentative ring fission is quite different from the aerobic pathway. It was realized that some hydrogen acceptor other than oxygen was used to rupture the benzene ring (58). The common requirement found for successful anaerobic utilization was the presence of nitrate (59,60). Degradation work done with Pseudomonas PN-1 grown anaerobically on benzoate was expressed as follows (60).

$$C_7H_6O_2 + 6KNO_3 \rightarrow 7CO_2 + 3N_2 + 6KOH$$

The anaerobic metabolism of several aromatics was studied using soil microorganisms in the presence of $KN^{18}O_3$ substantiated that nitrate was the electron acceptor (59).

The liberation of CO_2 as 60-70 percent of the carbon of benzoate indicated that the benzene ring had been disrupted.

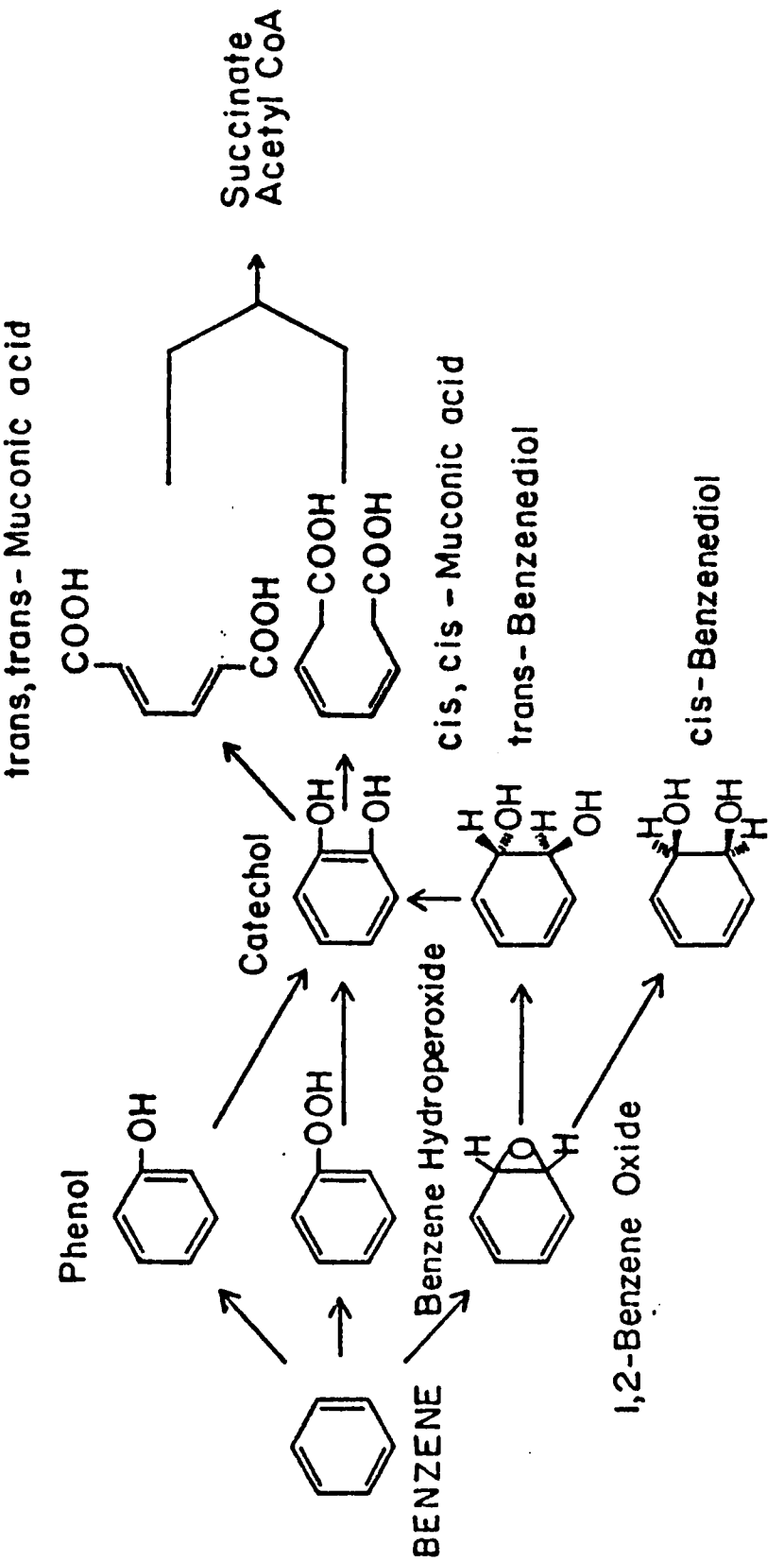


Figure 2. Benzene biodegradation pathway.

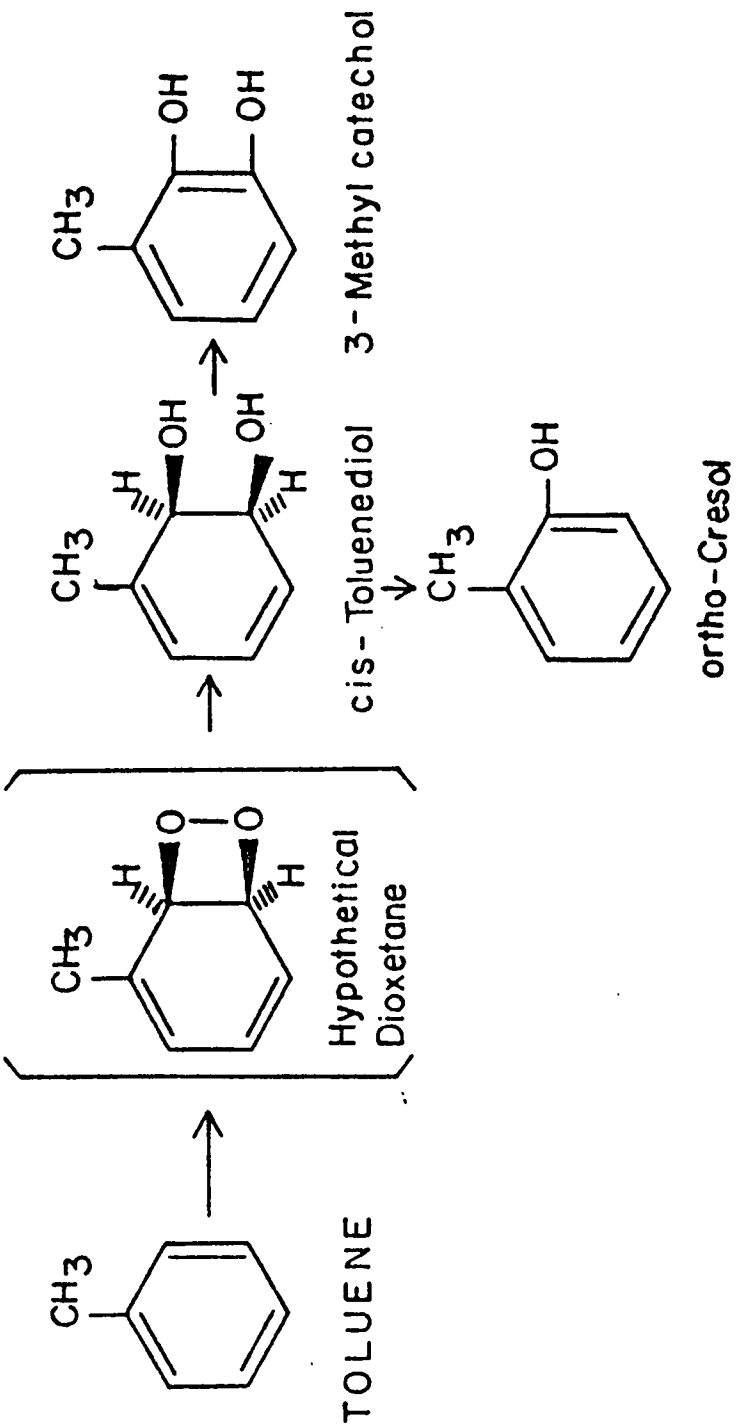


Figure 3. Toluene biodegradation pathway.

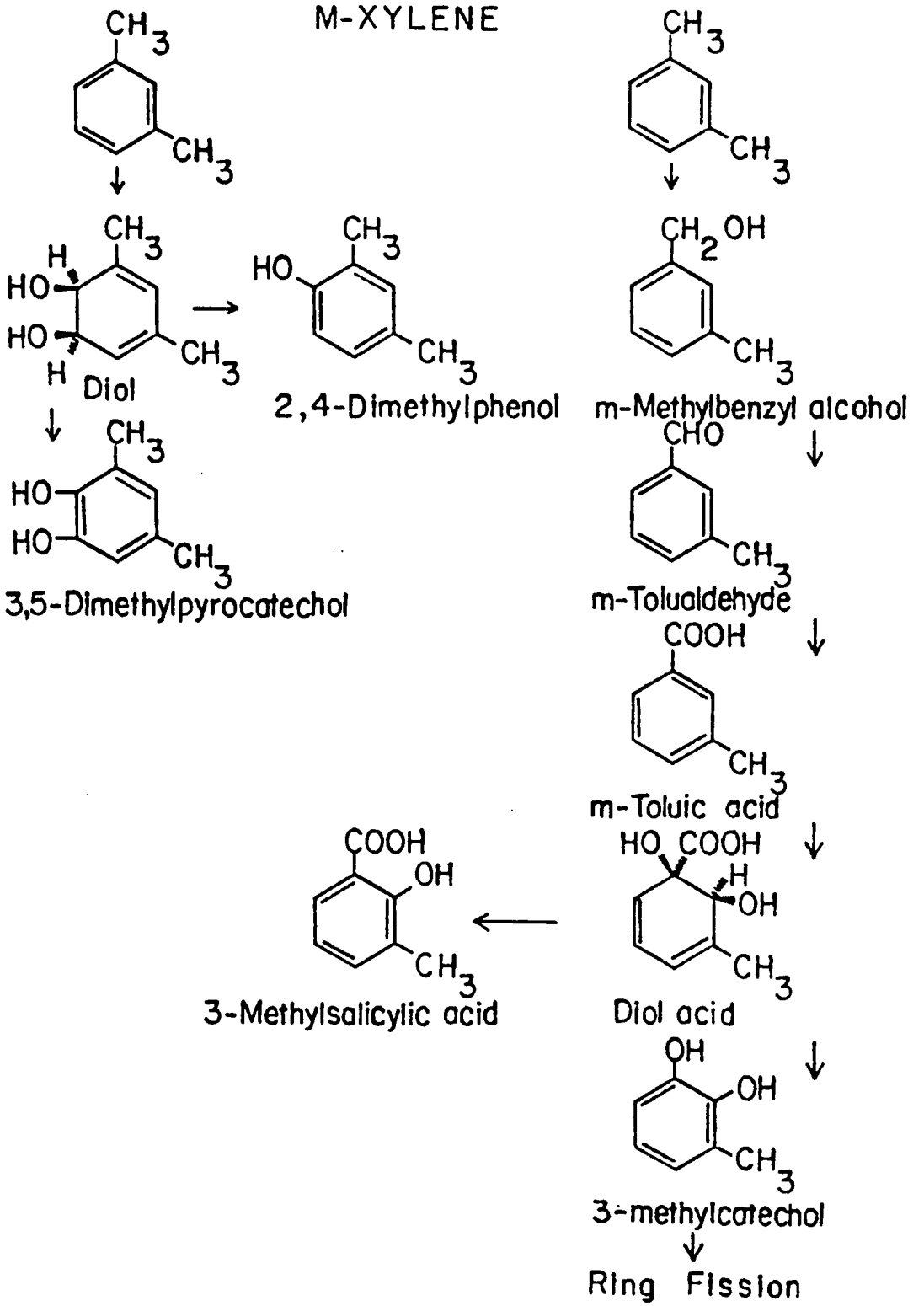


Figure 4. Two pathways for meta-xylene biodegradation.

Under strict anaerobic conditions eleven aromatic lignin derivatives indicated that 80 percent of the carbon was converted to CO_2 and CH_4 (61).

Employing ^{14}C labeling of the number one and seven carbon atoms of benzoate it was shown that the carboxyl of benzoate is only partly reduced to methane with the CH_4:CO_2 ratio being 1:5. The number one carbon of benzoate goes mostly to methane while very little is oxidized to CO_2 with the CH_4:CO_2 ratio being 25:1. It is clear that the benzene ring can be ruptured, but the mechanism is not certain (62).

Again working with ^{14}C benzoate in a sewage sludge enrichment $^{14}CH_4$ and $^{14}CO_2$ were produced (63). Without utilization of other substrates the stoichiometry would be:

$$4\,^{14}C_6H_6CO_2 + 18H_2O \rightarrow 15\,^{14}CH_4 + 9\,^{14}CO_2 + 4CO_2$$

The results proved to be within 3.5 percent for produced labeled methane.

METHODS AND MATERIALS

Study Areas

Selection

Potential sites were limited to Atlantic Richfield Company (ARCO) terminals in Virginia, Pennsylvania and New York. Basic geological conditions at these sites were obtained from general documents by Groundwater Technology, Inc. to aid in the selection of sites with principally sandy soils and a relatively high water table. Preliminary drilling was done in selected areas by local drillers under the supervision of James O'Brien of ARCO Petroleum Products using a small diameter, split-spoon sampler. At times, large gravels may not be returned to the surface by this method, even when present in subsoils, so some of the problems that could be encountered (i.e., gravelly sands) were not totally known. The number of potential sites was narrowed from these data and from discussions between ARCO personnel and Dr. G.W. Clough of Virginia Tech. Based upon all available information, the final selection process was made by ARCO. The four sites chosen to be most likely to permit successful sampling were Williamsport, PA; Corning, NY; Wayland, NY; and Dumfries, VA as shown in Figure 5.

Descriptions

Williamsport, PA was chosen as the first study area. The distribution terminal was located within 100 yards of the Susquehanna River on level terrain at the base of a mountain range. The water table was at approximately 14 feet and roughly corresponded to the level of

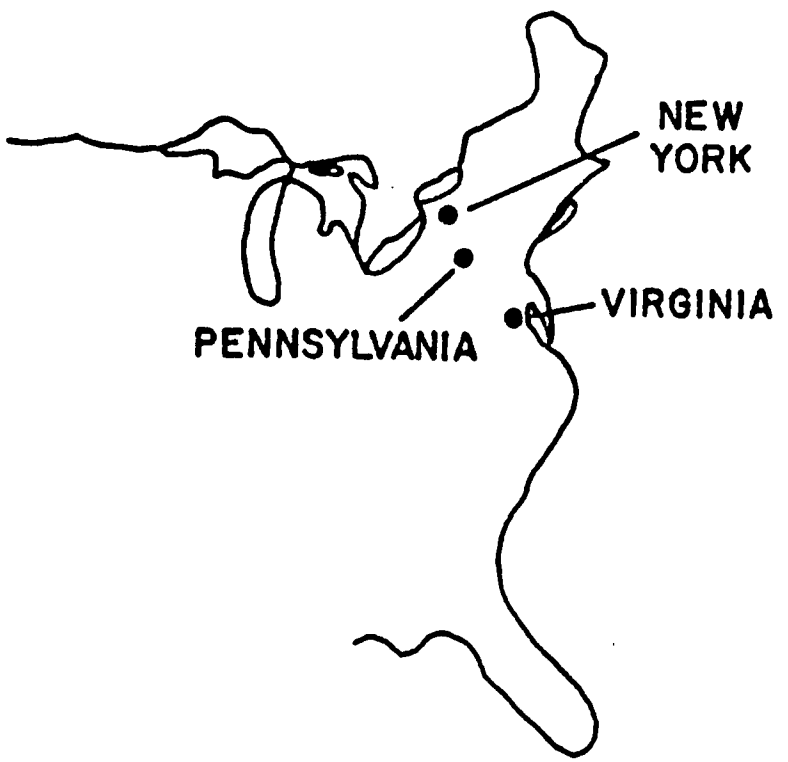


Figure 5. Location of study areas.

the river. Subsoils consisted of a loamy silt to a depth of 12 feet followed by a medium-dense, clean sand from 15-16 feet. Dense gravelly sand was found to 30 feet. At 36-38 feet, a relatively lean, dense sand, underlain by rock, was present .

The Corning, NY site proved to be an unsatisfactory sampling area due to large cobbles throughout the subsoil that prevented efficient drilling and sampling collection.

The Wayland, NY site was marginally more successful. Only a limited amount of material was sampled because of problems similar to those at the Corning area. Drilling was conducted within 400 yards of a small lake on flat terrain. The upper two feet of soil was dark and interspersed with marl. Below this, a continuous layer of marl (≅ 6 in) existed. The water table was at three to four feet.

The Dumfries, VA site was located adjacent to the Potomac River. Excellent samples were obtained here to 102 ft. This was due to the easily obtained alternating layers of sand and silty clay where iron deposits were observed in the denser layers. The water table was at 43 feet. However some samples above this level appeared saturated.

Sampling

Samples of subsurface material at each site were to be obtained from:

1) the unsaturated zone above the water table
2) the saturated zone below the water table

It was not necessary to maintain the in situ soil structure or density closely, an objective usually of primary concern in a geotechnical investigation. For the sampling work the following goals were applicable:

1) Apply minimum shock to the soil
2) Avoid introduction of any non-native agent into the soil being sampled.
3) Extrude the samples at the site by a rapid procedure during which only sterilized surfaces would be in contact with the soil.
4) Capture the samples in sterilized containers which could be sealed immediately.

To define the proper techniques for sampling, Professors G.W. Clough and J.T. Novak of VPI & SU traveled with Mr. J. O'Brien of ARCO Chemical to the EPA in Ada, Oklahoma to meet with researchers who had developed sterile, soil sampling procedures. The EPA methods are described by Wilson et al. (12). Basically, the sampling apparatus consisted of a heavy bronze cutting shoe fitted to a thick-walled sample tube. The shoe has a sharp edge at the penetration end and flares to a solid cylindrical shape into which the tube is inserted. The entire apparatus was designed to be pushed into the ground. Because the bronze cylindrical head is one inch larger than the tube, a gap is created around the tube as the unit is advance into the soil. To keep the specimen from falling out of the tube, a six-finger sample "catcher" is inserted between the bottom of the sample tube and the flared section of the cutting shoe. The particular sample catcher employed is used

conventionally to retain rock core during oil well drilling. EPA personnel indicated good success with their sampling apparatus. However, they had only used it in local clear river bed sands at relatively shallow depths.

For the ARCO sites, the EPA technique was felt to be inappropriate on the basis that:

1) The soils were dense, and simple pushing procedures would likely not penetrate enough distance for sampling.
2) Some of the soils were gravelly, and this would likely damage the sharp edge of the bronze cutting shoe.
3) Below the water table, the Williamsport soils would likely collapse into the gap created between the cutting shoe and the tube causing withdrawal problems and possible loss of the cutting shoe.

Alternative sampling techniques were investigated.

A conventional sampling procedure was adopted to fit the project requirements. The Warren George, Inc. drilling firm of Jersey City, NJ offered four alternatives: 1) an Osterberg hydraulic sampler; 2) a Denison sampler; 3) a Pitcher Barrel sampler, and, 4) a Dames and Moore sampler. A split spoon sampler was used in preliminary drilling.

The split spoon sampler is also known as the split barrel or split tube sampler. There are several modified versions of this sampler but basically it has a sample retainer located immediately above the barrel shoe and the barrel in which the sample is held is split longitudinally for easy sample removal. Split spoon sampling is accomplished by driving the sampler with a 140-300 lb hammer. It is common practice to

record the number of blows for each six inches of sampler penetration. The number of blows required for a 1.5 inch sampler to driven 12 inches with a given hammer weight can be used to determine the Standard Penetration Resistance (SPR) of the soil. Split-spoon sampling is generally used where it is necessary to determine stratification, identification, consistency, and density of soils at a site (64).

The Osterberg piston sampler (hydraulic sampler) consists of an actuating piston and a pressure cylinder. Introduction of fluid pressure on top of the actuating piston executes the sampling by pushing the Shelby tube into the soil (65).

A Denison sampler relies on a combination of jacking and coring to obtain the sample. It is made of an outer rotating core barrel with a bit, an inner stationary sample barrel with a cutting shoe, inner and outer barrel heads, an inner barrel liner and an optional core retainer (64).

The Pitcher sampler is basically a Denison sampler in which the inner barrel is spring loaded to provide automatic adjustment of the distance by which the cutting edge of the barrel leads the coring bit (64).

Shelby tubes were the sampling tubes employed and were made of brass or steel. Several types of samplers were used. The hydraulic sampler is pushed into place, the Denison and Pitcher Barrel samplers use rotary drilling and the Dames and Moore is driven. Table 4 lists the advantages and disadvantages of each sampling method. The Dames and Moore sampler was not used, however.

Table 4. Samplers considered for program.

Sampler	Insertion Method	Advantages	Disadvantages
Hydraulic	Hydraulic Push	Minimal disturbance	Limited to looser sands, no gravel
Denison	Rotary Drilling	Can drill into hard materials	Must use drilling mud
Pitcher Barrel	Rotary Drilling or Spring Push	Can drill into hard soil, push into soft soil	Must use drilling mud
Dames and Moore	Driven	Can penetrate hard soils	Must be driven by hammering

Drilling mud is used to maintain hole stability, especially in sands below the groundwater table, and is required for rotary drilling, as in the case of the Denison and Pitcher Barrel samplers, to return cuttings to the surface. While the mud serves to enhance the basic drilling process, a primary concern was that it might contaminate the soils. This was thought to be remote because the mud is very viscous and unlikely to flow freely. As a quality control measure, lithium chloride was added as a tracer to the mud.

Careful observations were also made for drilling mud penetration into the samples. No visible evidence of drilling was found inside any of the soil cores.

Soil Extrusion Apparatus

The extrusion device consisted of a hydraulically powered piston capable of generating in excess of 1000 psi, a dual clamp bed for holding the sampling tube, and a paring device (Figure 6). As the soil core was extruded from the sample tube, the first few centimeters were cut away with a flame sterilized spatula to remove any possibly contaminated material. The core was then forced through the sterilized, stainless steel, paring device, which trimmed away the outer one centimeter so that soil in contact with the sample tube walls was discarded. The final portion of the core sample was also discarded. The samples were extruded into acid-washed, sterile, one quart containers with Teflon-lined lids, and transported in iced coolers to the Virginia Tech labs for refrigeration.

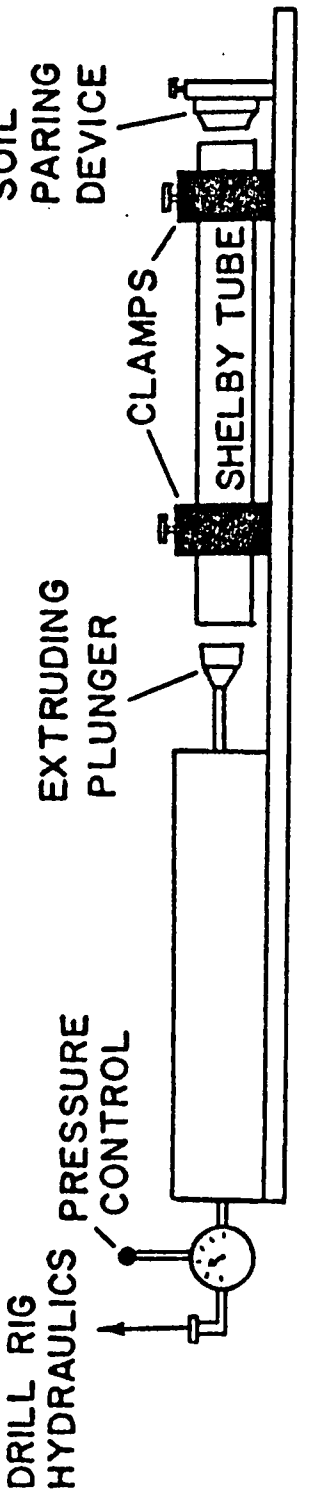


Figure 6. Soil extrusion apparatus.

Groundwater

Several methods of groundwater collection from wells are available. All of these are concerned with removing the water without contact with any adsorptive surface and maintaining sterility. Most employ either glass or Teflon tubing and a water lifting source, such as a peristaltic or vacuum pump. This necessitates having a power source and involves the transport of much equipment into the field. Problems were encountered with lifting water from significant depths, leaks, and the continual dismantling of the apparatus to empty the vacuum flask contents.

The most successful method was the most simple. Acid-washed, sterilized BOD bottles tied to a monofilament line were lowered down the well. The bottles were self-filling and could be removed and capped immediately constituting a sterile undisturbed 300 mL sample.

Field measurements were made on the temperature, dissolved oxygen level, and pH of the groundwater. Samples were shipped to Virginia Tech labs in ice-filled coolers.

Microcosms

Static Sacrificial Microcosms

A modification was made from the EPA procedure in that smaller test tubes were used (13 x 100 mm). This allowed more microcosms to be prepared from less subsurface material. The sacrificing of duplicate tubes with time as described by Wilson et al. (12) was the procedure followed with the exception that the organic concentration was measured

at zero time rather than calculated. The tube microcosms were filled with soil with a sterile, stainless steel spatula, then dosed with sterile, substrate-containing groundwater to simulate nutrient levels present in the subsurface system but, at the same time, avoid the introduction of additional bacteria. Soil and water were mixed in the tube microcosm with a vortex mixer so that approximately 1-1.5 mL of water remained at the surface for sampling by direct injection into a gas chromatograph.

The proposed Williamsport, PA test matrix for sole and mixed substrates is given in Table 5. Fifteen sacrificial-tube microcosms would be needed at each concentration, ten non-sterile and five sterile controls. This would provide for duplicate microcosms plus one control for five time intervals. By completing this matrix, 330 microcosms would need to be prepared. For this reason alternative microcosms were investigated.

Static Continuous Sampling Microcosms

The continuous microcosms were identical to the static microcosms except that the screw-top contained a Teflon-coated, silicone septum. A sterile syringe was used to remove a small sample (2-5 µL) from the liquid on the surface of the tube, so each microcosm unit could be monitored with time. This modification reduces the number of microcosms and permits a superior assessment of degradation rates.

Table 5. Proposed initial substrate matrix.

Microcosm	MEOH Substrate MEOH Conc., (mg/L)	TBA Substrate TBA Conc., (mg/L)	Alcohol Blend Substrate MEOH/TBA Conc. (mg/L)	Alcohol & Aromatic Substrate MEOH/TBA Conc. (mg/L)	Alcohol & Aromatic Substrate B/T/X Conc. (mg/L)
Material from Unsaturated Zone	100	50	100/50	100/50	7/18/54
	10	5	10/5	10/5	0.7/1.8/1.4
	1	1			
Material from Saturated Zone	100	50	100/50	100/50	7/18/14
	10	5	10/5	10/5	0.7/1.8/1.4
	1	0.5	1/0.5	1/0.5	0.07/0.18/0.14

MeOH = methanol
TBA = tertiary-butyl alcohol
B = benzene
T = toluene
X = meta-xylene

Circulating Microcosms

These microcosms consisted of 100 grams of subsurface soil sandwiched between two layers of acid-washed, sterile sand. The microcosm was filled with water from a bottom septum with a sterile syringe. Sampling was possible from this port as well. Circulation of the groundwater was achieved by inverting the flask daily, thus creating capillary action through the soil and sand.

Bacterial Enumeration

Bacterial populations were enumerated by Dr. R. E. Benoit and G. Allen of the VPI&SU Microbiology Department. Several methods were used. The direct counting method involved epifluorescence microscopy with acridine-orange staining (13). When excited by blue light, the dye fluoresces bright green when present on an organism and dim orange when on abiotic material. Plate counts were made using soil extract agar. Plate counts were also made with select media, i.e., formate and methyl amine, to encourage growth and isolation of C_1-utilizing organisms.

Analytical Methods

A method was needed to detect 50 ppb methanol to meet certain state standards for water. The problem encountered most often at low methanol concentrations was the difficulty in separating methanol from water because they are similarly polar. Purge and trap gas chromatography was thought to be the best method as had been shown by previous alcohol and water separations (65). This method is time-consuming and would limit

the number of microcosms that could be analyzed. Several months were spent by ARCO and Virginia Tech personnel in the development of methods that could measure the 50 ppb before actual microcosm studies could begin. Several options for the analysis of alcohols, as well as aromatics in water were found to be useful for the investigation.

Gas chromatography

The compounds to be measured by FID (Flame Ionization Detector); i.e. methanol, TBA, benzene, toluene and m-xylene; could be separated in the most efficient manner on a 7 ft x 1/8 inch stainless steel column packed with 0.2 percent Carbowax 1500 on 80/100 mesh Carbopak C. The instrument operating conditions as listed below:

Injection port - 100°C

FID - 250°C

N_2 carrier - 25 cc/min

Sample size - 2 µL

Oven temperature for separation of:

Methanol and TBA - 120°C isothermal

Alcohols + BTX - 120°C for 4 min. to 170°C at 20°C/min. and hold 35 min.

The separation of all five compounds, as it was performed on a Hewlett-Packard 5880A gas chromatograph, is shown in Figure 7. The detection limits with this column were 0.1 mg/L methanol, 0.05 mg/L TBA, and 0.03 mg/L for benzene, toluene, and m-xylene by direct injection.

Low Level Methanol Detection. A 6 ft x 1/8 inch stainless steel column packed with 150-200 mesh Porapak QS at an oven temperature of 180°C was

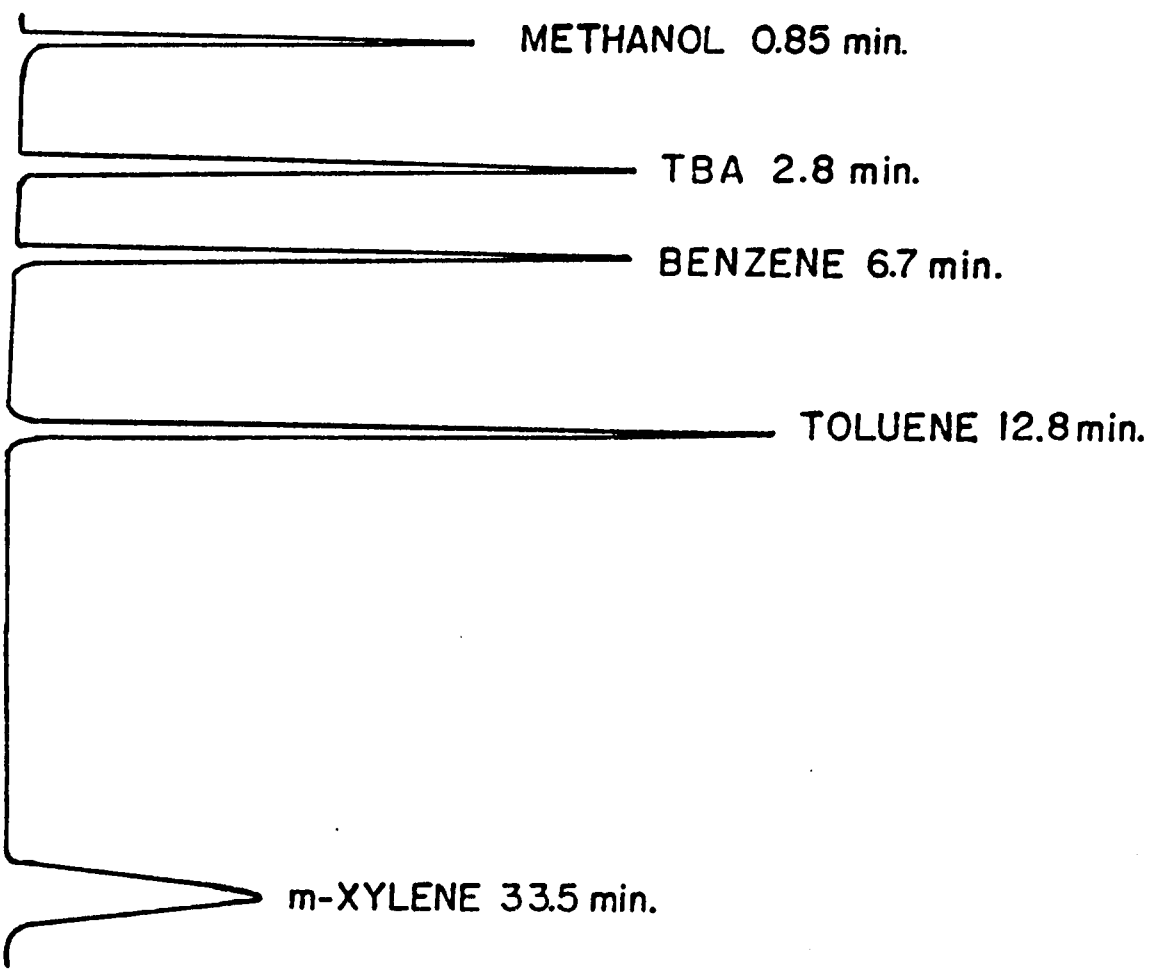


Figure 7. The separation of methanol, TBA, benzene, toluene, and m-xylene on a CW 1500 column.

used for the complete separation of methanol from water. This column is capable of handling water-sample volumes of 10 µL. When 5 µL samples were used, concentrations of 25 ppb could be detected but not consistently. At 100 ppb, reproducability was ± 2.7 percent and ± 10.9 percent at 50 ppb. Care must be taken to prevent "ghosting" after the injection of a high concentration sample. It can be eliminated by purging the column with pure water. To decrease analysis time, the column was shortened to 5.5 feet and the oven temperature was lowered to 120°C isothermal. Concentrations of 50 ppb in as little as 2 µL volumes could still be detected at the most sensitive attenuation setting. A tracing of the Hewlett-Packard 5880A gas chromatogram is shown in Figure 8. Instrument conditions were the same as with the previous mixture analysis, with the exception that the N_2 flowrate was 30cc/min.

ARCO Alternative Methods. Alcohols analysis can be performed as well on a 8 ft x 1/8 inch stainless steel column packed with 3% SP 1500 on 80/120 Carbopak B. Operating conditions were:

Injection port	- 180°C
FID	- 230°C
N_2 Carrier	- 21 cc/min.
Sample size	- 1 µL (direct injection)
	5 ml (purge and trap)

Oven temperature for separations by:

1) Direct injection of:

Alcohols - 40°C to 160°C at 20°/min and hold 2 min.

Alcohols and BTX - 40°C to 220°C at 20 min and hold 10 min.

Figure 8. The separation of 50 ppb methanol and water on a Porapak QS column.

2) Purge and trap - start program at "Desorb" on the LSC-2 model:

Alcohols - 2°C isothermal for 4 min to 180°C at 20°/min and hold 5 min.

Alcohols and BTX - 2°C isothermal for 4 min to 220°C at 20°/min d hold 10 min.

A 10 ft x 1/8 inch stainless-steel column packed with 5% SP 1200/1.75% Bentone 34 on 100/200 Supelcoport could be used for the aromatic compound separations using either direct injection or purge and trap. The instrument operating conditions are listed below.

Injection port	- 150°C
FID	- 150°C
N_2 carrier	- 30 cc/min.
Sample size	- 1 µL direct injection
	5 mL purge and trap

Oven temperature for separation by:

1) Direct injection - 80°C isothermal for 5 min. to 120°C at 5°/min. and hold 7 min.

2) Purge and Trap - start program at "Desorb" on LSC-2 50°C isothermal for 5 min. to 120°C at 5°/min.

Purge and Trap Operating Conditions for Tekmar Model LSC-2 Automatic Sample Concentrator:

Trap -	12 inch x 1/8 inch OD (0.010 inch wall) stainless steel, filled with 6 inch Tenax and 3-1/4" silica gel
Purging vessel -	5 mL firt type

Purging temperature -60°C (used Tekmar jacketed 5 mL sampler and Brinkman/Lauda RM3-S recirculating water bath)

Purge gas - N_2 at 40 cc/min.

Purge time - 12 min.

Desorb preheat - 180°C

Desorb - 180°C for 4 min.

Bake - 220°C for 7 min.

This same column was used for alternative direct injection BTX analysis at the Virginia Tech labs but the oven temperature program was modified to 70°C isothermal for 3 min. to 100°C at 6°/min and held for 3 min. The resulting chromatogram is shown in Figure 9.

Standards Preparation

Alcohols. Methanol and TBA stock primary standards were made by adding each to volumetric flasks by weight and diluting to volume with water. Dilutions to any concentration could then be made. Alcohols were found to be linear in area response from 0.1 to over 1000 mg/L when analyzed by gas chromatography.

Aromatics. Benzene, toluene, and m-xylene are all insoluble in water (66); thus, it is very difficult, if not impossible, to prepare accurate standards of these aromatics in water. All three, however, are infinitely soluble in each other and methanol. Since methanol elutes early in the chromatogram of these compounds it was used as the media to contain the matrix standard. Benzene, toluene, and m-xylene were desired in the concentrations of 7, 18 and 14 mg/L, respectively, in microcosms due to their extractability in water. In a small septum

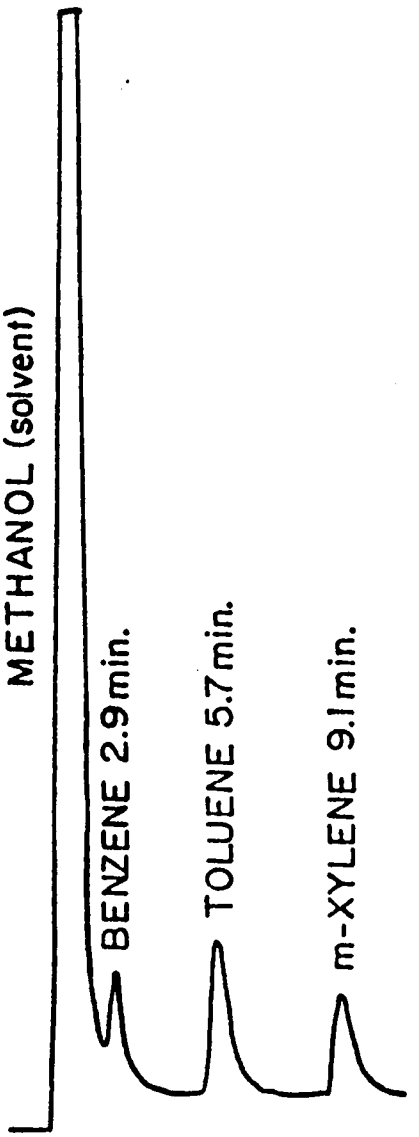


Figure 9. The separation of a benzene, toluene, and m-xylene standard in methanol on a SP1200/Bentone 34 column.

sealed vial a mixture of 0.7, 1.8, and 1.4 grams of BTX was added by syringe. If 0.39 g of this mixture is added to 100 mL methanol in a septum stoppered volumetric flask then 0.07 g benzene, 0.18 g toluene and 0.14 g m-xylene has been added or 700, 1800, and 1400 mg/L. Dilutions can be made to any desired concentration. Linear area response was found as with alcohol standards.

Lithium Analysis

Lithium chloride was used as a tracer in drilling mud to monitor potential contamination of soil core samples. Soil samples were dried in an oven at 150°C for 3 hours then placed in a dessicator until cool. lithium was extracted from the soil by digesting approximately one gram of each dried sample in 5 mL of concentrated nitric acid for three hours then mixing with 5 mL of distilled water. Each sample was filtered with a 10 mL Buchner funnel containing a glass-fiber filter to remove particulate matter. A blank was prepared with acid and water only. Samples were analyzed by atomic adsorption spectrophotometry.

Anion Analysis

Phosphates, chlorides, nitrates and sulfates in the groundwater were analyzed by ion chromatography in the Virginia Tech Biological Laboratories.

Microcosm Dosing

The weight of soil placed in each tube microcosm was known, as was the soil moisture content. After the microcosm was dosed the weight (ml) of added water was also known. The soil moisture (percent moisture x weight soil) plus the added (dose) water equals the total water

present in the microcosm. Then $C_1V_1 = C_2V_2$, where C = concentration and V = volume, can be used to determine the concentration of substrate needed in the dose water. For example, if the dose water added is 1.5 mL and soil water is 2.7 mL (i.e. 18% soil moisture x 15 g soil per tube microcosm) then total water is 2.7 + 1.5 = 4.2 ml. If the desired, final concentration in the microcosm water is 100 mg/L then solve the equation for C_1, or dosing water concentration.

Known parameters:

C_2 = 100 mg/L (desired concentration)

V_2 = 4.2 mL (total water in microcosm to be at 100 mg/L)

V_1 = 1.5 mL (dose water added)

Solving: $$C_1 = \frac{C_2V_2}{V_1} = \frac{(100 \text{ mg/L})(4.2 \text{ mL})}{1.5 \text{ ml}} = 280 \text{ mg/L}$$

Therefore, the concentration in the stock solution should be 280 mg/L so that when it is diluted by the soil mixture, the final concentration will be 100 mg/L.

RESULTS AND DISCUSSION

Soil Sampling

A hydraulic sampler was used to push a thin-wall, Shelby tube into the soil. Because a vacuum was generated at the head of the sample during the insertion of the tube using the hydraulic sampler, no retainer was necessary to hold the sample in the tube. Samples of sand were readily obtained by this method.

Sampling in the gravelly sands proved to be difficult, because the "gravels" ranged from small pebbles to sizes exceeding the diameter of the Shelby tube. These conditions ruled out the use of the hydraulic sampler, and made sample recovery, even with either the Denison or Pitcher Barrel, which work well for very dense sand and clays, impossible in some cases. In many areas, the sand component turned out to be very coarse and loosely packed, and even when using a retainer ring at the bottom of the sampler, the soil would flow out of the tubes resulting in sample loss. Only very limited success was achieved at sampling gravelly sand. Generally the best results were obtained where a sandy layer was encountered within the gravel or gravelly sand.

The field operations showed that all three of the samplers included in the work could be used to obtain suitable specimens for the research. The hydraulic sampler proved to be the best device because of its ease of use. However, it is limited to sands which are essentially free of gravel, and it may not be applicable in deeper holes were confining pressures make the sand too stiff for it to penetrate.

Extrusion Apparatus

Generally, the system developed by Warren George Co. performed well. However, two problems were encountered. First, because of frictional resistance of soil in the tube, the clamps were unable to hold the tube in place. Once the tube slipped, the extrusion device would no longer work. It appears that a retaining device should be added to this system.

A second problem occurred when the Pitcher Barrel sampler was used. This sampler uses a steel tube instead of brass. This tube is about 3/4 in. longer than the brass tube and would not fit into the bed of the extrusion device. Therefore, all tubes required hand cutting of the lower inch with a hacksaw before extrusion. Movement of the cutting device will correct this problem.

Lithium Tracer

It was found that lithium concentrations in the same sample could vary based on the extraction method employed. The tracer lithium in the drilling mud should have been adsorbed by the soil if exposure occurred. Lithium was added to the drilling mud at 10 mg/L at PA and 45-50 mg/L at VA and NY, respectively. However, naturally occurring lithium could be extracted to varying concentrations based on the contact time (digestion) in concentrated nitric acid causing some variation in the resultant concentration. The samples either had to be completely digested which requires extensive digestion time and the use of more hazardous acids or all extracted for an equal time. An equal extraction

time procedure was selected. Analyses indicated that no lithium contamination had occurred based on comparisons of split spoon samples where no mud was used, and center core and pared core samples taken with lithium dosed drilling mud. To further insure these results, samples were analyzed from various depths of dosed and non-dosed drillings. The results are shown in Table 6. The New York soil presented a unique problem. During acid digestion the high concentrations of marl in the soil reacted violently resulting in the loss of several samples. Minimal non-dosed soil was collected at New York and the remaining samples were required for microcosm preparation. Lithium was present in the ppb (< 1 mg/kg) range in most samples and no drilling mud contamination was evident.

Subsurface Bacterial Populations

Significant bacterial populations were found to be present in the subsurface systems of all sites. The results of acridine-orange direct counts and plate counts are listed in Tables 7-9. A general observation can be made for all sites based on Figures 10-12. Acridine-orange direct counts were consistently higher than the soil extract and soil extract plus formate and methylamine plate counts. A slight decrease in bacterial numbers is evident in the unsaturated zone by A-O direct counts for Pennsylvania, while a decrease of four orders of magnitude is shown for all plate counting methods in Figure 10. A similar response is seen for New York with one order of magnitude decrease shown for all counting methods from the surface through the saturated zone. The

Table 6. Lithium analyses for Williamsport, PA, Wayland, NY and Dumfries, VA soils.

Lithium Dosed			Non-dosed		
Hole No.	Depth(ft)	Conc.(mg/kg)	Hole No.	Depth(ft)	Conc.(mg/kg)
PA 1	7	0.31	PA 2	6.0	0.24
		0.21			0.21
PA 1	8.5	0.22	PA 2	8.0	0.16
		0.21			0.16
PA 1	9.0	0.33	PA 2	10.0	0.18
		0.40			0.17
PA 4	9.0	0.19	PA 2	12.0	0.32
		0.29			0.35
			PA 3	36.0	1.17
					1.08
			PA 5	8.0	0.18
					0.17
NY 1	4.0	1.06	NY 1	0.5	1.05
		1.29			
NY 1	4.0	1.06			
NY 1	6.0	1.98			
		1.86			
NY 2	3.0	0.77			
		0.94			
VA 1	17.0	0.14	VA 1	12.0	0.23
		0.11			0.29
			VA 1	17.0	0.14
VA 2	54.0	< 0.04			0.11
		< 0.04	VA 2	13.0	0.12
					0.10
			VA 2	32.0	0.10
					0.11
			VA 2	57.0	< 0.04
					< 0.04
			VA 2	102.0	< 0.04
					< 0.04

Table 7. Bacterial population in subsurface soil at Williamsport, PA.

	cfu/g soil+ Std. Dev.			
Depth ft(m)	AO	SE	SE + F	SE + MA
0	$5.6 \pm 1.9 \times 10^7$	$3.0 \pm 0.3 \times 10^7$	$6.7 \pm 0.2 \times 10^6$	$2.7 \pm 0.2 \times 10^7$
12 (3.7)	$3.9 \pm 1.4 \times 10^7$	$3.5 \pm 2.1 \times 10^3$	$9.3 \pm 0.6 \times 10^2$	$9.7 \pm 1.5 \times 10^2$
30 (9.1)	$4.6 \pm 2.7 \times 10^7$	$1.4 \pm 0.8 \times 10^5$	$6.6 \pm 4.6 \times 10^3$	$3.6 \pm 0.9 \times 10^4$

cfu/g - colony forming units/gram
AO - acridine-orange direct count
SE - soil extract agar
SE + F - soil extract + formate agar
SE + MA - soil extract + methyl amine agar

Table 8. Bacterial population in subsurface soil at Wayland, NY.

	cfu/g soil (+ SD)			
Depth ft(m)	AO	SE	SE + F	SE + MA
0	1.0×10^8	1.0×10^7	9.5×10^5	8.3×10^6
	$\pm 4.1 \times 10^7$	$\pm 4.0 \times 10^6$	$\pm 2.7 \times 10^5$	$\pm 6.5 \times 10^5$
6 (1.8)	7.6×10^7	9.3×10^5	9.0×10^5	8.5×10^5
	$\pm 3.8 \times 10^7$	$\pm 1.1 \times 10^5$	$\pm 8.4 \times 10^4$	$\pm 3.0 \times 10^4$
12 (3.7)	8.0×10^7	1.1×10^6	3.1×10^5	1.2×10^6
	$\pm 6.4 \times 10^7$	$\pm 8.5 \times 10^4$	$\pm 1.0 \times 10^5$	$\pm 2.1 \times 10^4$

Table 9. Bacterial Population in Subsurface Soil at Dumfries, VA.

	cfu/g soil + std. dev.			
Depth ft(m)	AO	SE	SE + F	SE + MA
0	$1.0 \times 10^8 \pm 4.1 \times 10^7$	$9.7 \times 10^6 \pm 5.7 \times 10^5$	$4.7 \times 10^3 \pm 3.5 \times 10^3$	$4.5 \times 10^6 \pm 4.0 \times 10^5$
11 (3.4)	$7.3 \times 10^7 \pm 3.7 \times 10^7$	$< 10^3$	$< 10^3$	$< 10^3$
14 (4.3)	$3.1 \times 10^7 \pm 2.2 \times 10^7$	$3.3 \times 10^6 \pm 4.0 \times 10^5$	$1.3 \times 10^4 \pm 3.6 \times 10^3$	$2.7 \times 10^6 \pm 8.0 \times 10^5$
30 (9.1)	$3.9 \times 10^7 \pm 3.4 \times 10^7$	$5.6 \times 10^5 \pm 7.1 \times 10^3$	$3.5 \times 10^5 \pm 8.5 \times 10^4$	$2.7 \times 10^5 \pm 1.7 \times 10^4$
57 (17.4)	$1.1 \times 10^8 \pm 6.4 \times 10^7$	$5.2 \times 10^6 \pm 9.2 \times 10^5$	$4.3 \times 10^6 \pm 7.2 \times 10^5$	$3.4 \times 10^6 \pm 4.7 \times 10^5$
80 (24.4)	$7.3 \times 10^7 \pm 5.6 \times 10^7$	$9.8 \times 10^5 \pm 5.3 \times 10^4$	$1.1 \times 10^6 \pm 1.1 \times 10^5$	$1.0 \times 10^6 \pm 8.1 \times 10^4$
102 (31.1)	$4.8 \times 10^7 \pm 4.5 \times 10^7$	$1.1 \times 10^5 \pm 2.8 \times 10^4$	$7.7 \times 10^4 \pm 4.9 \times 10^3$	$7.4 \times 10^4 \pm 2.0 \times 10^4$

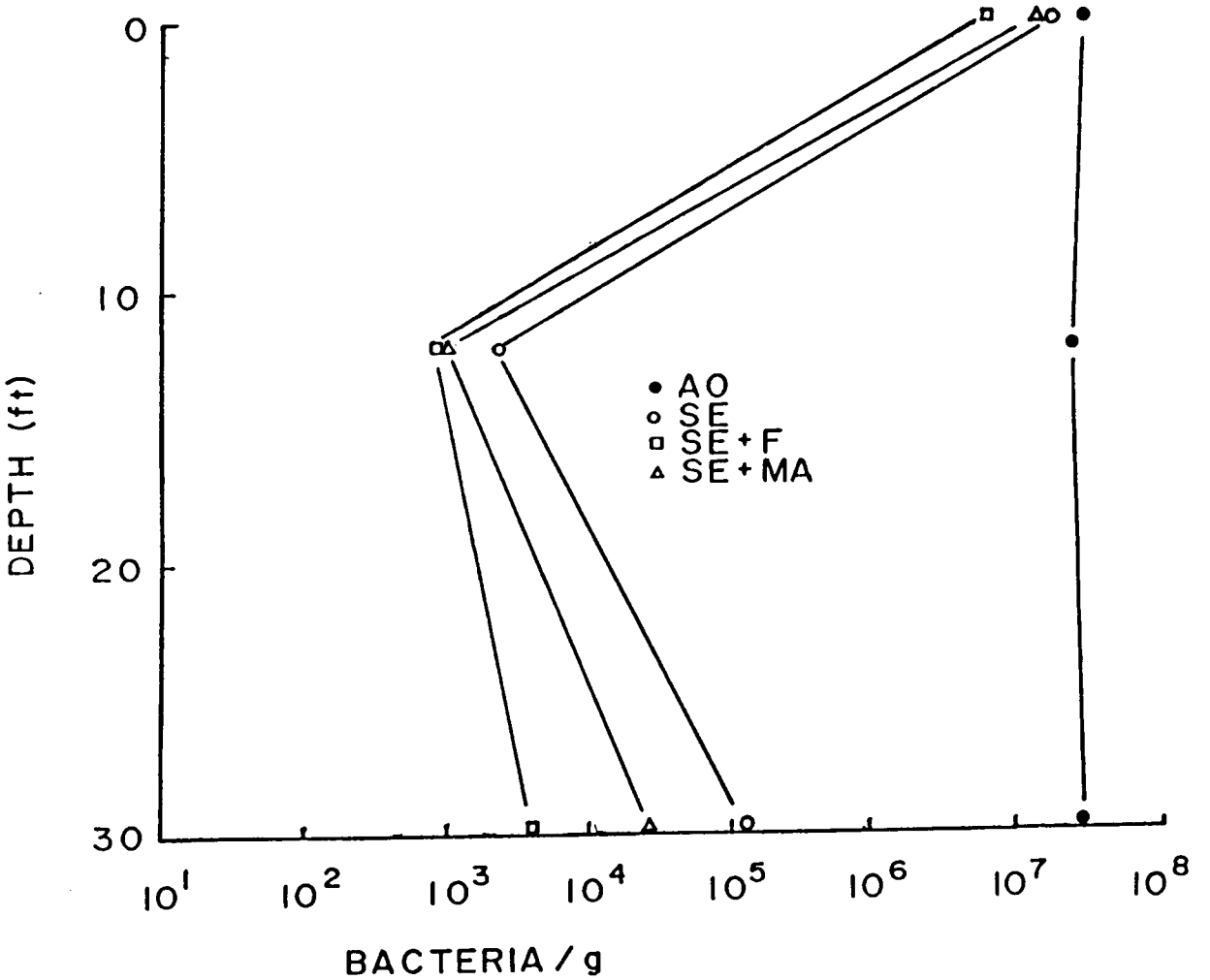


Figure 10. Bacteria found with depth by Acridine-Orange (AO) direct counts and soil extract (SE), SE plus formate and SE plus methyl amine (MA) agar plate counts of Pennsylvania soil.

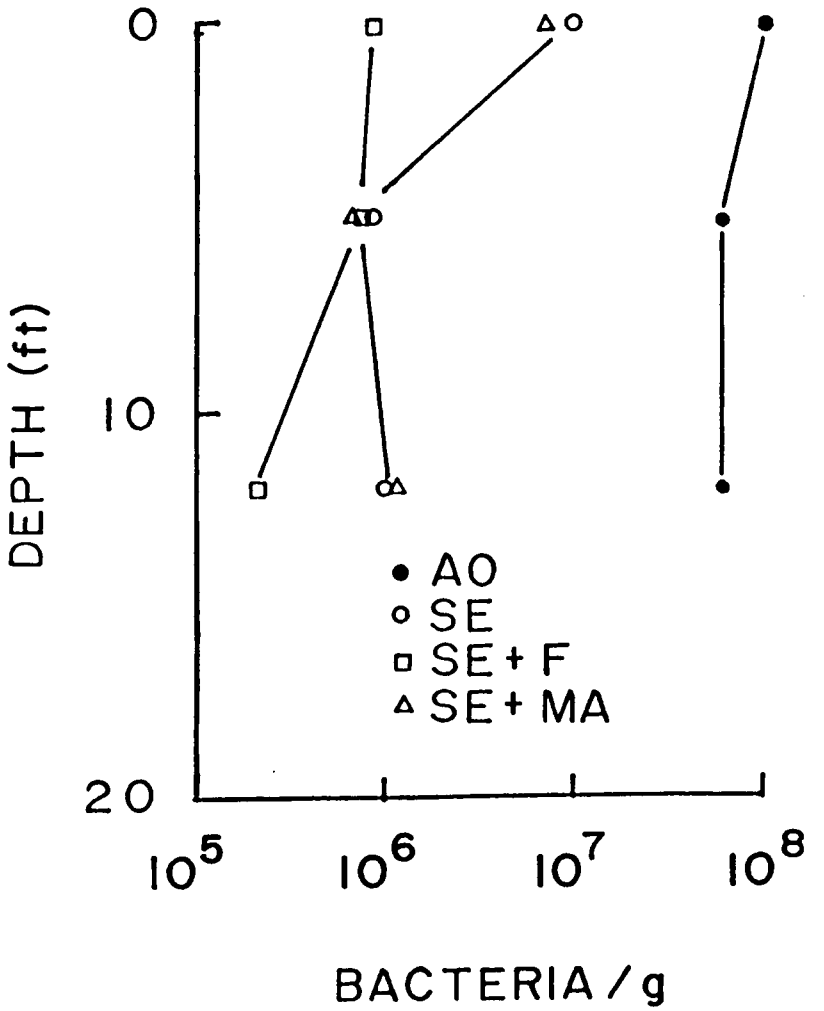


Figure 11. Bacteria found with depth by Acridine-Orange (AO) direct counts and soil extract (SE), SE plus formate, and SE plus methyl amine (MA) agar plate counts of New York Soil.

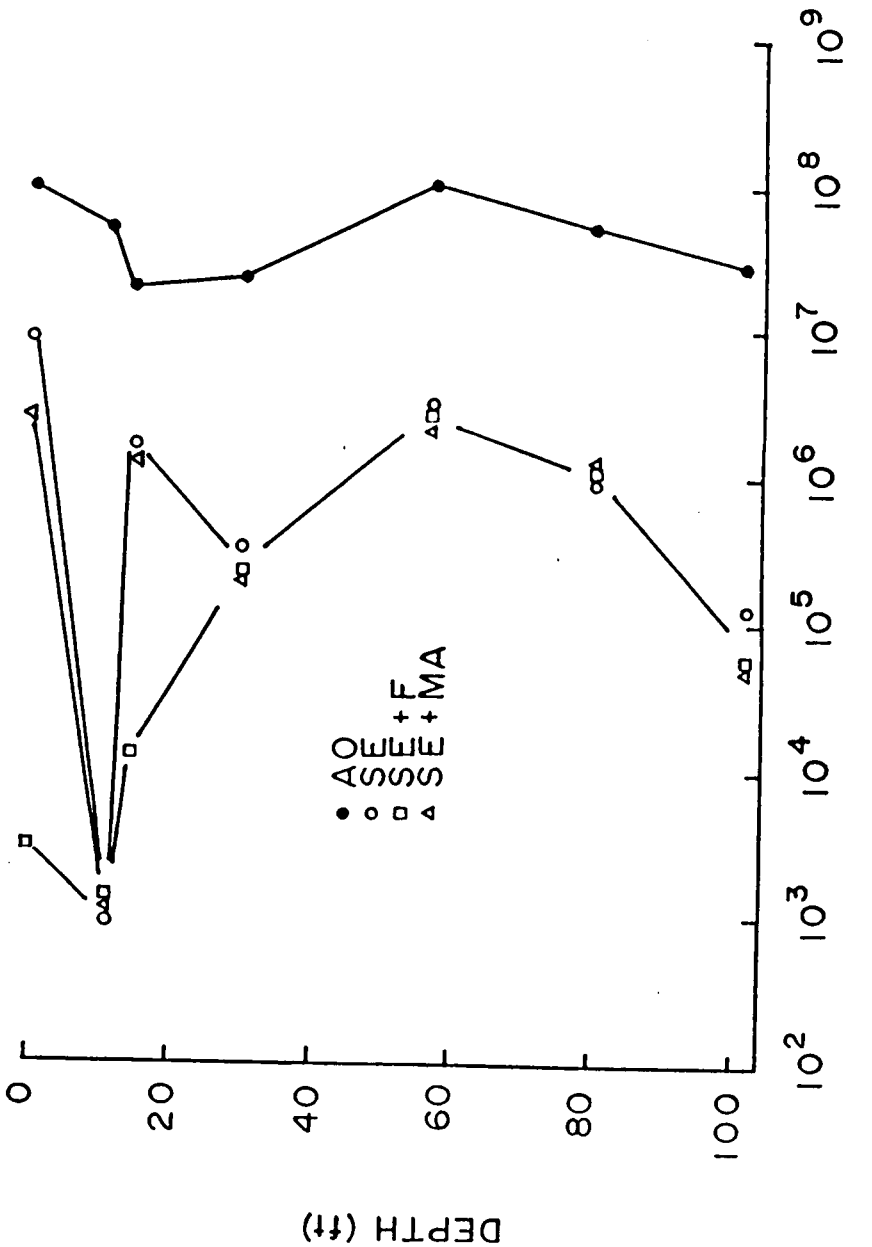


Figure 12. Bacteria found with depth by Acridine-Orange (AO) direct counts and soil extract (SE), SE plus formate, and SE plus methyl amine (MA) agar plate counts of Virginia soil.

Virginia site consisted of an upper unsaturated zone to 45 feet followed by a layered aquifer, i.e., alternating saturated and unsaturated soils. Figure 12 reflects this by all counting methods. The plate counts exhibited a dramatic decrease in the upper zone and this may be a reflection of the unnatural fill material present through this depth or an indication of the effect of dehydration upon bacterial numbers.

Acridine-orange counts seem to more accurately reflect the microbial populations of the subsurface. Plate counts may not provide the proper conditions for the growth of organisms that are adapted to a subsurface environment. This is shown by Figures 10-12 where plate counts follow the same trends as the direct counts except at a lower number.

Methylamine and formate were added to the soil extract agar to encourage the growth of C_1 utilizing organisms. This seemed to make little difference with the exception of the New York site where the deepest formate count was significantly lower.

Past incubation counts made on Pennsylvania microcosms that had been dosed with 100 mg/L methanol and 10 mg/L TBA demonstrated a 10 fold increase in bacteria numbers confirming the substrate loss in the microcosms as shown in Table 10. Had this not been done with saturated zone samples there exists the possibility that increases in bacteria numbers could be a result of rehydration.

Generally, it can be seen that in the Pennsylvania subsurface 10^6 - 10^7 bacteria exist per gram of soil by the A-O direct count throughout. In New york 10^8 bacteria per gram were present on the surface while only

Table 10. Bacterial population in Williamsport, PA saturated zone microcosms after a 30 day incubation.

Sample	Uncorrected AO Direct Count + SD	cfu/g SE Medium + SD
Original	$4.6 \pm 2.7 \times 10^{7}$	$1.4 \pm 0.8 \times 10^{5}$
Microcosm + 100 mg/L Methanol	$1.6 \pm 8.2 \times 10^{8}$	$2.4 \pm 0.3 \times 10^{7}$
Microcosm + 10 mg/L TBA	$1.9 \pm 9.5 \times 10^{8}$	$1.3 \pm 0.5 \times 10^{6}$

10^6 were found at lower depths. In Virginia, as in New York, the surface was 10^8, however, the subsequent profile was between 10^7 - 10^8. The types and numbers of bacteria present along with the aquifer characteristics can be used as a qualitative predictive tool for decontamination of an aquifer. There exists the future possibility of integrating microcosm data with direct counts and an aquifer characterization so that an approximate removal rate for various compounds can be more rapidly obtained.

Microcosm Dosing

Dosing experiments were performed using Williamsport, PA soil to determine if the theoretical concentration by the $C_1V_1 = C_2V_2$ formula would prove correct. The soil moisture was found to be approximately 18 percent. Triplicate tubes were dosed with solutions containing methanol and TBA to give theoretical concentrations based on soil weight and added water. Theoretical versus actual measured concentrations after mixing are listed along with margin of error (Table 11).

The experiment was repeated, based on the amount of water that should have been in the microcosm tubes to yield the measured concentrations. The dose solution was physically added, so the error must have been in the soil moisture value. The dose solution concentration was lowered to agree with a soil moisture content of 8-10 percent. The results are given in Table 12.

The measured and calculated concentrations agree well with an average difference between the two of 3.7 and 6.2 percent for methanol

Table 11. Theoretical and measured concentrations of Methanol and TBA in Williamsport, PA unsaturated zone soil.

Tube No.	Methanol/TBA (mg/L) Calculated Conc.	Measured Conc.	Percent Error
1	98.9/99.1	142.1/146.3	43.7/47.6
2	87.6/87.8	131.8/135.1	50.4/54.0
3	97.8/98.0	-- /160.5	-- /63.7

Table 12. Theoretical and measured concentrations of Methanol and TBA in Williamsport, PA unsaturated zone soil after lowering dose concentration.

Tube No.	Methanol/TBA (mg/L) Calculated Conc.	Measured Conc.	Percent Error
1	100.6/104.3	101.4/93.3	0.8/10.6
2	119.6/124.0	120.5/116.9	0.8/5.7
3	102.3/106.1	109.1/109.3	6.7/3.0
4	108.2/112.1	105.9/108.5	2.1/3.2
5	114.5/118.7	104.7/110.8	8.6/6.7
6	98.6/102.2	102.2/110.6	3.7/8.2

and TBA, respectively. Sandy soils from the saturated zone had a moisture content of approximately 20 percent, and calculated concentrations using actual soil moisture agreed well with measured concentrations as seen in the degradation data of dosed microcosms in the Appendix. It appeared that some moisture in the soil from the unsaturated zone must be "bound" in the silts and unavailable for mixing, while water in the sandy, saturated soil must be free. These results reaffirmed the belief that each microcosm must be analyzed initially to obtain a true zero time substrate concentration.

Groundwater Parameters

Groundwater parameters for both Williamsport, PA and the Wayland, NY and Dumfries, VA sites are listed in Table 13. The sites are quite different in most respects, which is useful for degradation rate comparisons.

Biodegradation

The subsurface systems investigated here are of varying nature as seen in Table 13 groundwater data. The Pennsylvania aquifer was of an aerobic nature and contained relatively high nitrate and sulfate concentrations. The New York and Virginia sites were anaerobic by comparison with only the New York aquifer containing significant sulfate levels. In considering the potential for degradation the aforementioned conditions are of importance since an aerobic reaction yields more energy in comparison to anaerobic respiration and fermentation. During

preparation and mixing of microcosms some air is inevitably introduced. However, because the average groundwater dose is 4 mL the total amount of oxygen in solution is minimal even if considered to be at saturation. The anions, nitrate and sulfate, then are important electron acceptors for the promotion of anaerobic respiration. Stoichiometric equations for the utilization of methanol by aerobic and anaerobic respiration, and fermentative pathways can be written if nitrate and sulfate are considered to be taken to nitrogen and sulfide, respectively.

The equations describing the metabolism of methanol are:

High Energy

$$CH_3OH + 1.4O_2 \xrightarrow{\text{aerobic}} CO_2 + 2\ H_2O$$

$$CH_3OH + NO_3^- \xrightarrow{\text{anaerobic}} CO_2 + 0.5\ N_2 + 2\ H_2O$$

$$CH_3OH + 0.75\ SO_4^{=} \xrightarrow{\text{anaerobic}} CO_2 + 0.75\ S^{=} + 2H_2O$$

$$2CH_3OH \xrightarrow{\text{fermentation}} 1.5\ CH_4 + CO_2 + H_2O$$

Low Energy

From the groundwater data and assuming saturation of the dose groundwater with oxygen at time zero the amount of methanol that can be utilized by aerobic and anaerobic respiration can be calculated. The Pennsylvania aquifer electron acceptors could stoichiometrically account for 40 mg/L of methanol utilization, while New York and Virginia could utilize 23 and 4 mg/L, respectively. The rate at which these reactions proceed can be quantified by the determination of the associated

Table 13. Chemical analyses of groundwater at the Pennsylvania, New York and Virginia sites.

Parameters	Location		
	Pennsylvania	New York	Virginia
Cl^-, mg/L	9.94	8.00	12.24
BR^-, mg/L	--	0.06	0.32
NO_3^-, mg/L	53.40	1.54	0.11
NO_2^-, mg/L	--	1.20	0.56
SO_4^{2-},mg/L	27.64	52.00	8.10
PO_4^{3-},mg/L	ND	ND	ND
Fe^{2+},mg/L	0.44	0.05	4.23
Ca^{2+},mg/L	22.60	73.80	2.88
Mg^{2+},mg/L	4.65	14.40	3.88
Na^+ ,mg/L	2.78	33.40	13.50
K^+ ,mg/L	1.09	3.50	4.54
TOC, mg/L	1.0	1.7	1.3
TOX	none	none	50.0 ppb
Alkalinity, mg/L as $CaCO_3$	none	180	none
Dissolved Oxygen, mg/L	6.7	0.7	0.2
Temperature (°C)	11.0	10.0	10.0
pH	4.73	7.81	4.50

biokinetic constants. The Michaelis-Menten equation is the basis for defining enzyme kinetics for reactions involving an enzyme and substrate. To describe an enzymatic reaction the coefficients K_m and V_{max} are required. These represent the Michaelis constant and the maximum reaction velocity, respectively. K_m and V_{max} can be determined from a plot of the velocity versus substrate concentration. K_m, also known as K_s, is equal to that concentration of substrate that will allow the reaction to proceed at one-half of its maximal velocity. Where [S] represents the substrate concentration, the velocity, v, is determined by the Michaelis-Menten equation.

$$v = \frac{V_{max}[S]}{[s] + K_m}$$

The reciprocal of both sides of this equation yields the Lineweaver-Burk modification.

$$\frac{1}{v} = \left(\frac{K_m}{V_{max}}\right)\left(\frac{1}{S}\right) + \left(\frac{1}{V_{max}}\right)$$

A plot of 1/v versus 1/s will permit a graphical determination of K_m and V_{max} and can verify the results of Michaelis-Menten plots when dealing with scattered data. An equation of the same form as the Michaelis-Menten equation was proposed by Monod to explain specific growth rate where μ and μ_{max} were substituted for the velocity terms and the K_s gave the substrate concentration that would result in one-half the maximum growth rate. This equation is not necessarily related to a specific

biochemical mechanism but represents an observed response for either mixed substrates or mixed biological populations or both.

The modified Monod equation, commonly used to describe wastewater treatment kinetics describes the specific substrate utilization rate as a function of the limiting nutrient concentration (70). The general equation form of the equation is:

$$q = \frac{k\,[S]}{K_s + [S]}$$

where:

[S] = substrate concentration; mg/L

k = maximum utilization rate; mg/L/day

K_s = half-saturation constant

The value of q may be calculated for steady state systems as

$$q = \frac{\Delta S/\Delta T}{X}$$

where:

ΔS = substrate utilized; mg/L

ΔT = time; days

X = biomass concentration; mg/L

In this study, an X value specific for the substrates being studied could not be determined so values of q are taken as $\Delta S/\Delta T$ are from batch microcosm data. For cases where a lag period occurred, this portion of time was not included in the rate determinations. It should be recognized that variations in q may reflect differences not only in

substrate responses but also in substrate specific organism numbers and these are considered in the discussion of results.

Pennsylvania

Methanol. Methanol as a sole substrate at 1 and 10 mg/L was completely degraded within one week in both the saturated and unsaturated zones. At 100 mg/L, methanol was also readily degraded in both zones as shown in Figures 13 and 14. The biodegradation of methanol does not appear to be inhibited by the addition of 1, 5, and 10 mg/L TBA nor by 10 mg/L TBA with 7, 18, 14 mg/L BTX. It has been shown that several methylotrophic organisms are capable of oxidizing aromatic compounds. This may contribute to the fact that no inhibition of alcohol biodegradation was seen in the presence of the aromatics. The levels of benzene, toluene and m-xylene were chosen based on their extractability from gasoline by water as found by Atlantic Richfield. At 500-1000 mg/L methanol was biodegraded as shown in Figures 15 and 16. The addition of 50 mg/L TBA showed no inhibitory effect on methanol biodegradation.

Both low and high methanol concentrations using sacrificed versus septum-capped microcosms gave similar biodegradation results as seen in the previously discussed figures and by examination of the rates as listed in Table 14. Therefore, the remaining sites were studied by septum-capped microcosms for several reasons. These are: less soil was required which permitted the use of soil from sites where only small quantities of soil were obtained, and more consistent data were provided by continuously monitoring the same microcosms over time. Also, the addition of BTX was difficult with sacrificial microcosms due to the

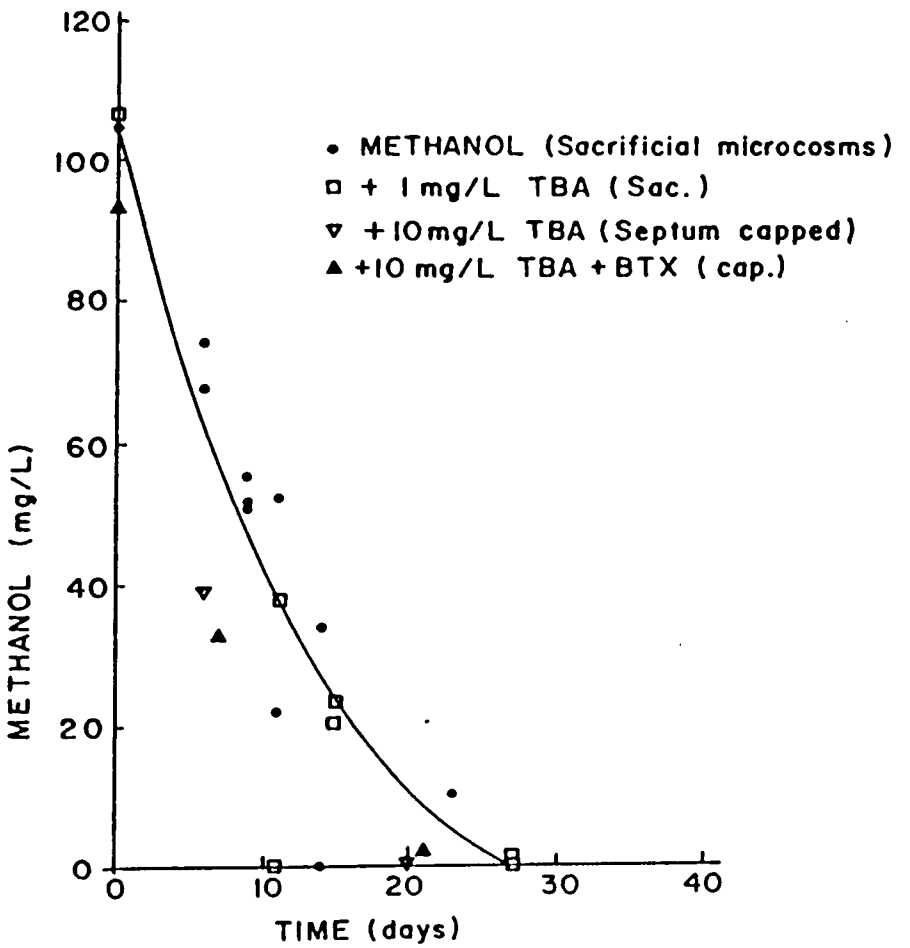


Figure 13. Methanol biodegradation in the Pennsylvania unsaturated zone.

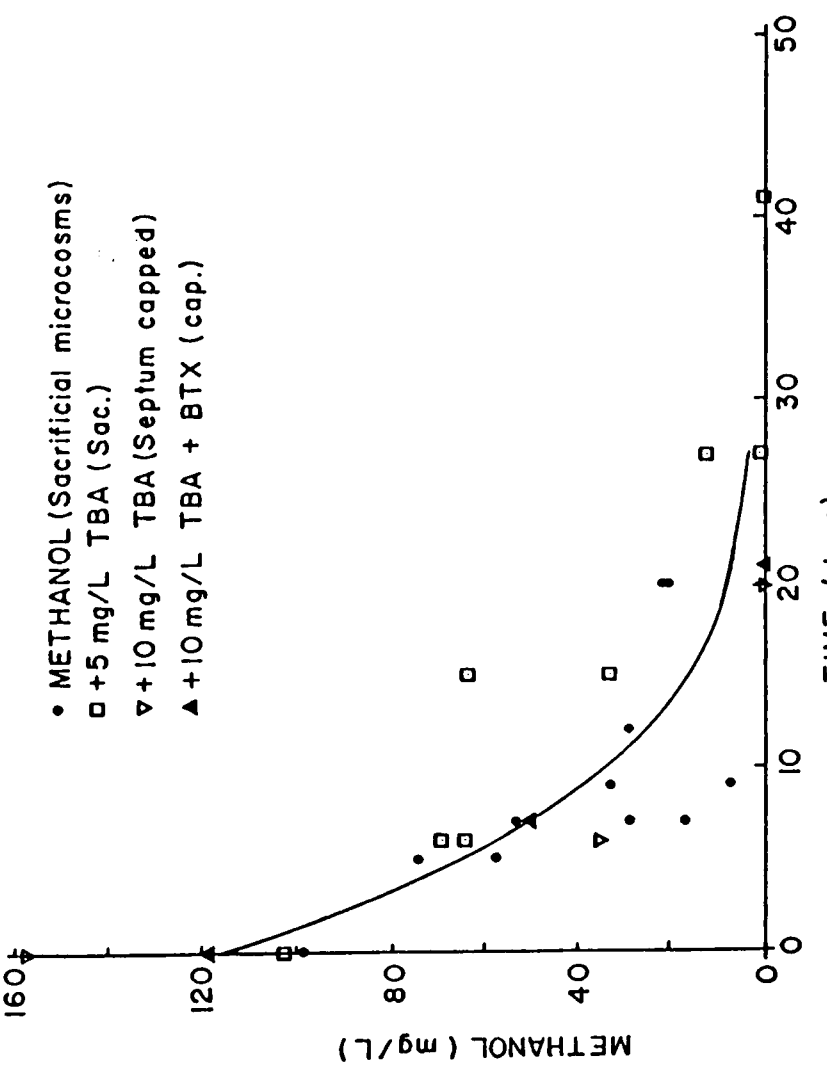


Figure 14. Methanol biodegradation in the Pennsylvania saturated zone.

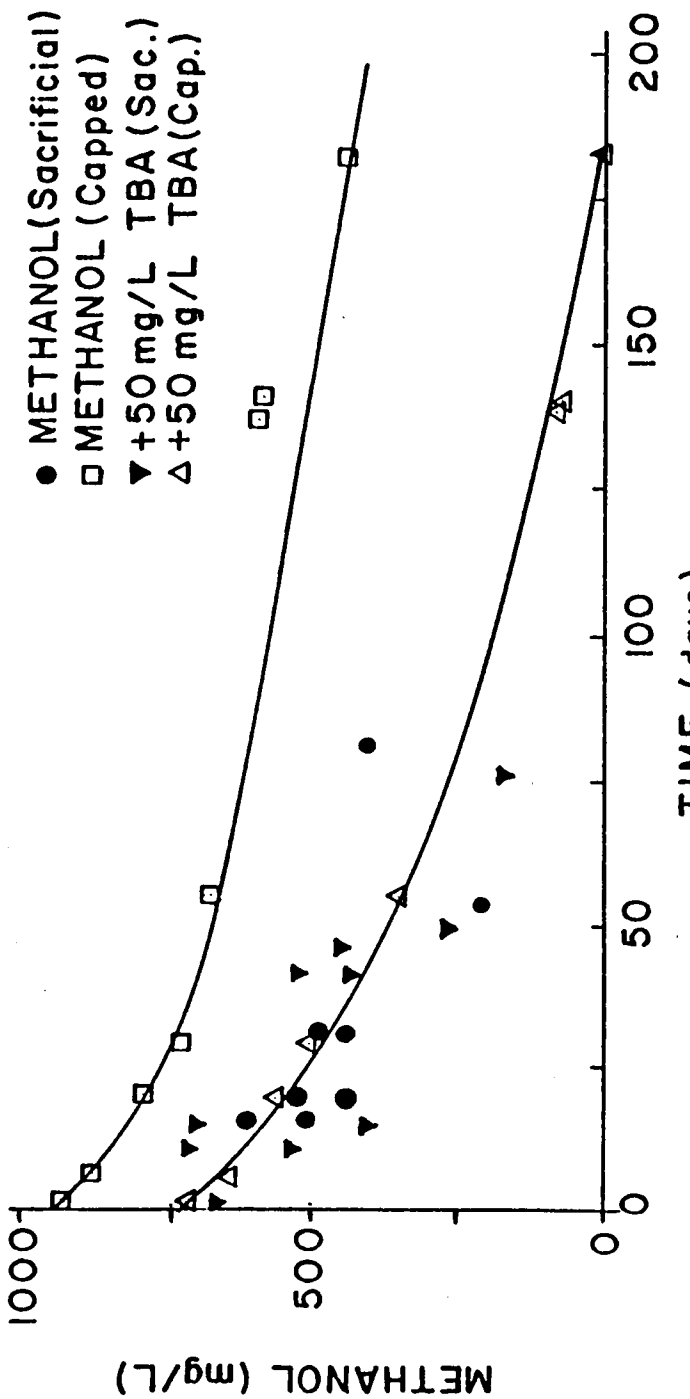


Figure 15. Methanol biodegradation in the Pennsylvania unsaturated zone.

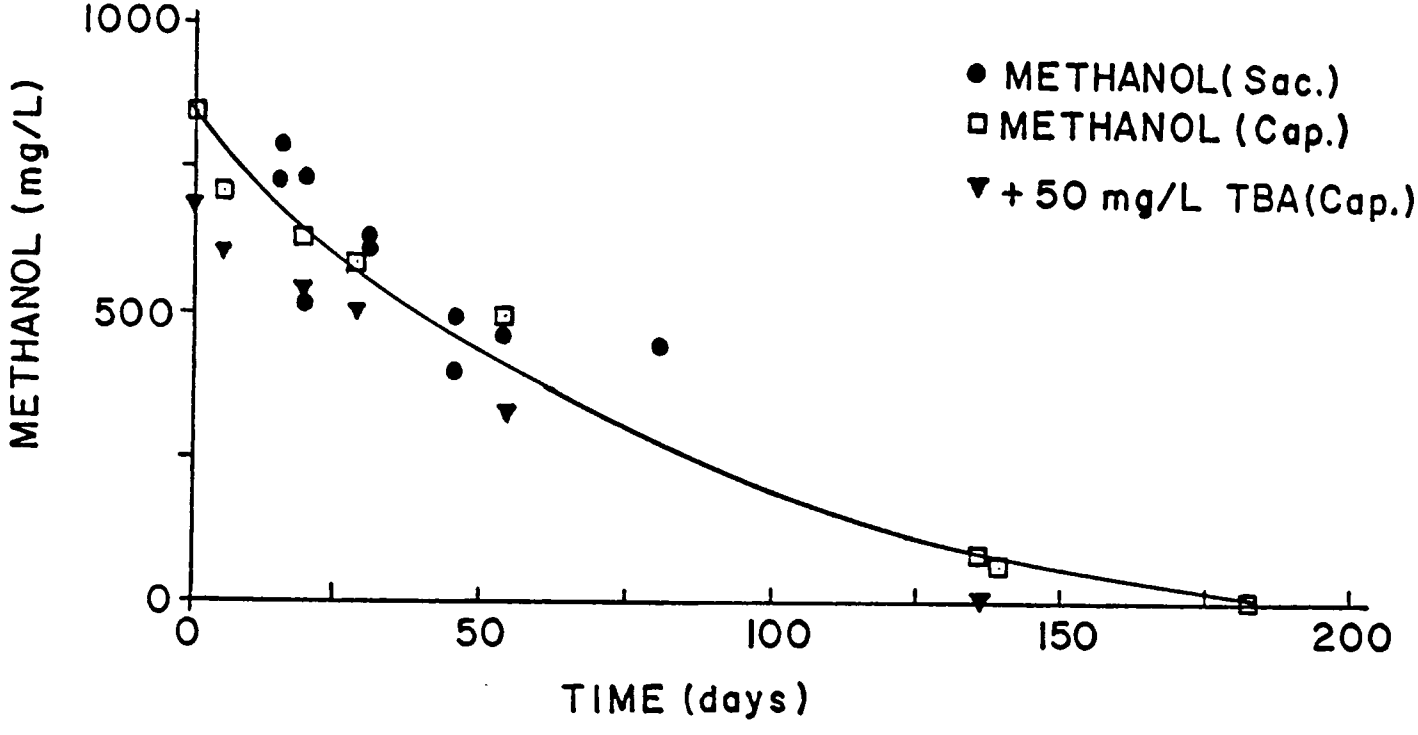


Figure 16. Methanol biodegradation in the Pennsylvania saturated zone.

Table 14. Utilization rates (mg/L/day) for the Pennsylvania subsurface

Methanol			
Unsaturated Initial Concentration (mg/L)	Rate	Saturated Initial Concentration (mg/L)	Rate
106.2	4.19	115.6	4.51
112.0 + 1TBA	6.01		
116.1 + 5TBA	1.32	111.0 + 5 TBA	3.87
111.1 + 10TBA	11.88*	157.9 + 15 TBA	20.38*
92.9 + 10TBA + BTX	4.34*	118.5 + 10 TBA + BTX	5.62*
577.0	2.11	1212.0	9.41
929.0	2.63*	843.0	5.61*
738.0 + 50TBA	7.49		
717.0 + 50TBA	4.54*	683 + 50 TBA	6.44*
TBA			
1.14	0.21	0.97	0.04
2.15	0.38		
3.36	0.58		
3.71	0.38		
4.42	0.39		
4.86	0.48		
5.12	0.52		
5.55	0.47		
6.23	0.45	5.39	0.11
		10.17	0.27
1.01 + 100 MeOH	0.01		
6.20 + 100 MeOH	0.01	5.14 + 100 MeOH	0.11
9.61 + 100 MeOH	0.27*	15.1 + 100 MeOH	0.14*
7.46 + 100 MeOH + BTA	0.20*	7.75 + 100 MeOH + BTX	0.02*
70.8	0.26	100.8	1.77
80.1	2.22*	128.6	0.67*
49.4 + 700 MeOH	0.31		
48.3 + 700 MeOH	0.21*	45.3 + 700 MeOH	0.28*

*septem-capped microcosms

volatility and low solubility of these compounds in water. However, they could be injected directly using a septum cap minimizing any chance for loss due to volatilization.

There are several observations that can be made concerning the plotted biological utilization rates. Methanol biodegradation begins immediately in both the unsaturated and saturated zones and the data can be represented as a zero order reaction rate or as a first order decay. There is also considerable scatter in the rate data and this may reflect organism variations, particularly since some organism clumping was observed microscopically. In order to resolve some of the variations in reaction rate and data scatter, a kinetic analysis was conducted.

A plot of q versus S (Figure 17) was used to evaluate the kinetic response. Both unsaturated and saturated data are indicated. No obvious rate order could be seen from the data in Figure 17. For aerobic systems the maximum substrate utilization rate, k and K_s, are depressed at lower temperatures (70). This indicates that methanol would be removed at a much greater rate from aquifers with a higher temperature since these values approach those for municipal activated sludge. The data shown in Figure 17 exhibits some scatter. This could be a reflection of differences in organism populations or changes that occurred in the microcosm over time. Initially, aerobic conditions prevailed. A slow conversion to an anaerobic state then took place as the oxygen was depleted and nitrate and sulfate were used as electron acceptors. If any microcosms contained an undetected air space, continuing aerobic conditions may have existed. This is a possible

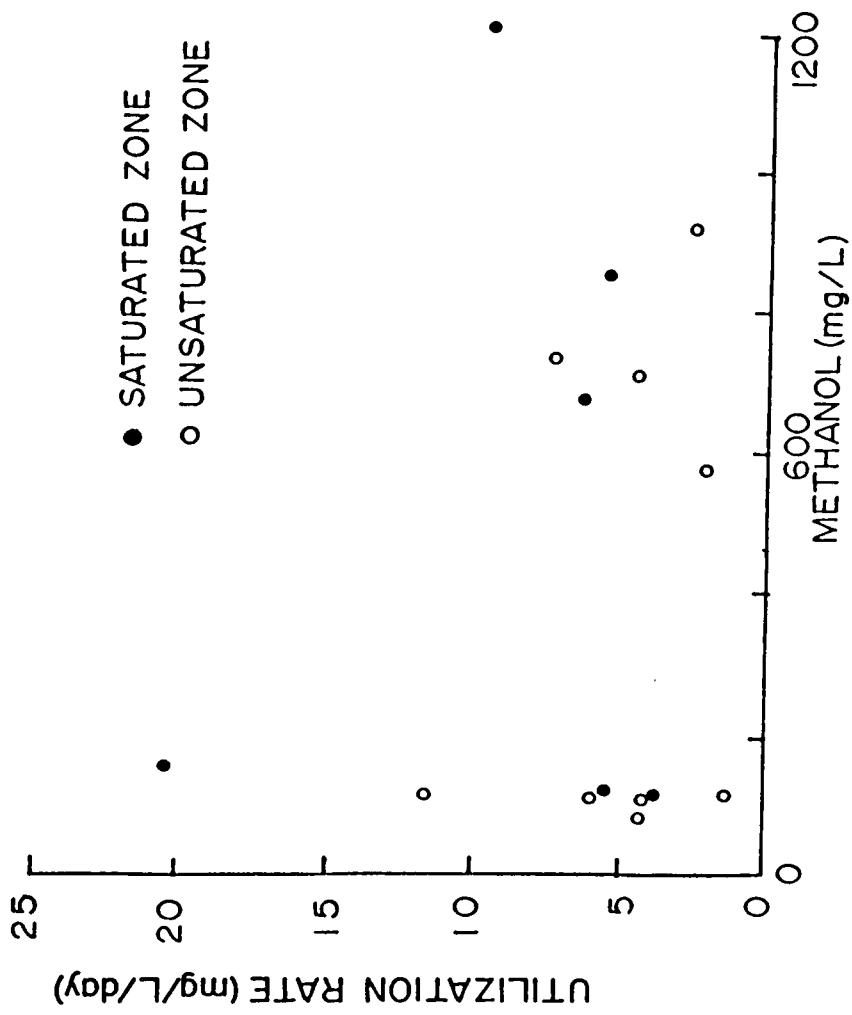


Figure 17. Determination of kinetic constant K for methanol in Pennsylvania soil.

explanation for the outlying data. Evidence of anaerobic conditions was seen in most microcosms towards the complete utilization of the substrate in that black deposits formed throughout the soil. Based on the sulfate and iron analysis of the groundwater these were most likely iron sulfide. It would be logical to assume that if the microcosms were maintained aerobically for the duration of the study that the time involved for complete degradation would have been much less.

The rate order for methanol utilization was determined by a plot of log rate versus log concentration and was found to be zero order as seen in Figure 18. This is in agreement with the general slopes exhibited in the arithmetic plots.

Circulating Microcosms. The Pennsylvania continuous sampling sand/soil microcosms were incubated for one month. As can be seen in Figure 19 the degradation rates here were much slower than in the static microcosms. These units were to be used originally as a monitor for utilization rates to determine when static microcosms should be sacrificed. Their slower rates could be attributed to two facts. The ratio of substrate to soil was much greater than in the tube microcosms, and the unsaturated zone soil was very compact and may have allowed limited water percolation. Therefore, the use of these microcosms was discontinued.

TBA.

Tertiary butyl alcohol biodegradation was complete within 50 days at concentrations from 1-10 mg/L as shown in Figures 20 and 21. At concentrations from approximately 50-130 mg/L TBA, complete biodegrad-

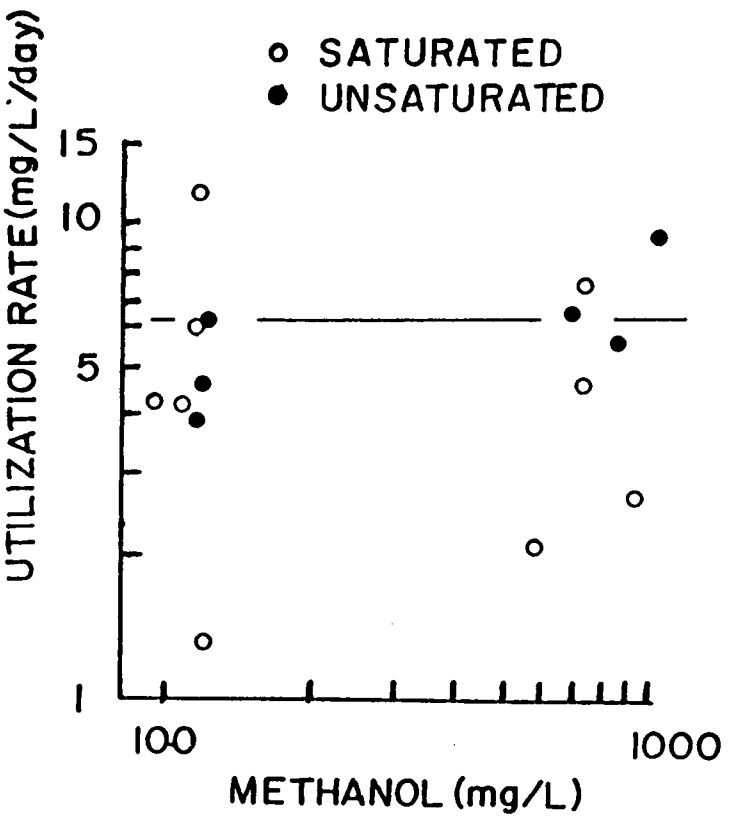


Figure 18. Mean value for methanol utilization rates in Pennsylvania soil.

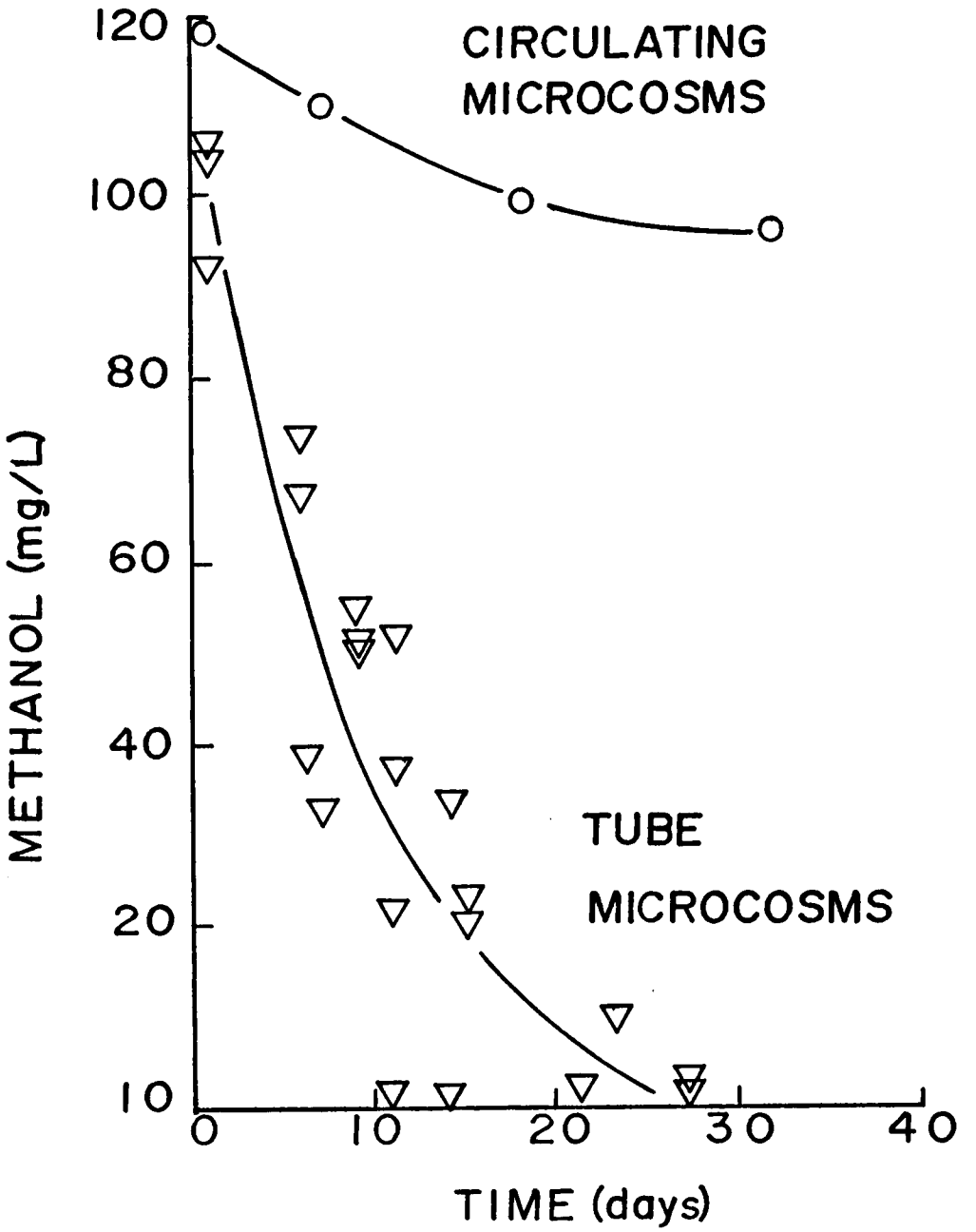


Figure 19. Comparison of rates obtained with tube and circulating microcosms.

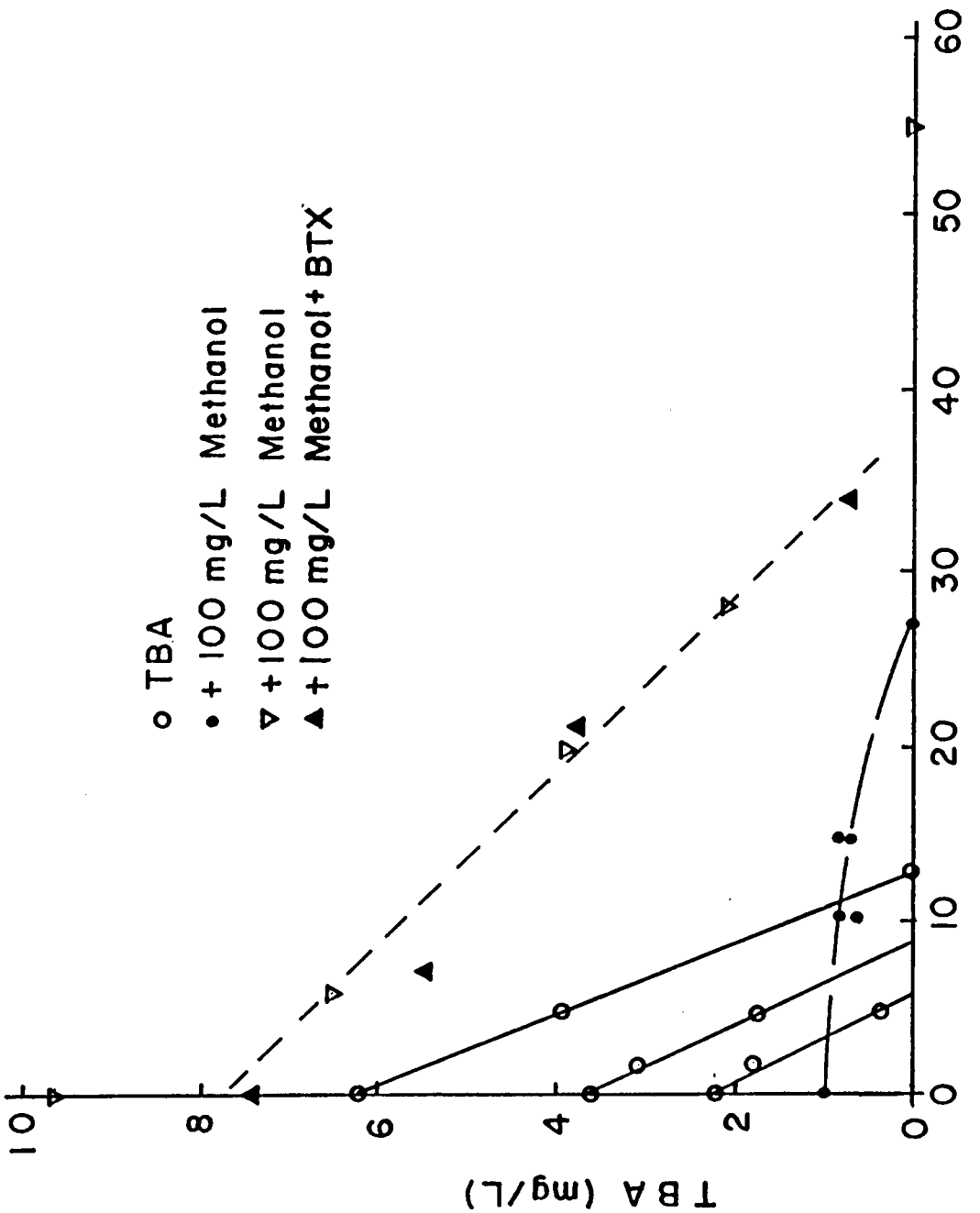


Figure 20. TBA biodegradation in the Pennsylvania unsaturated zone.

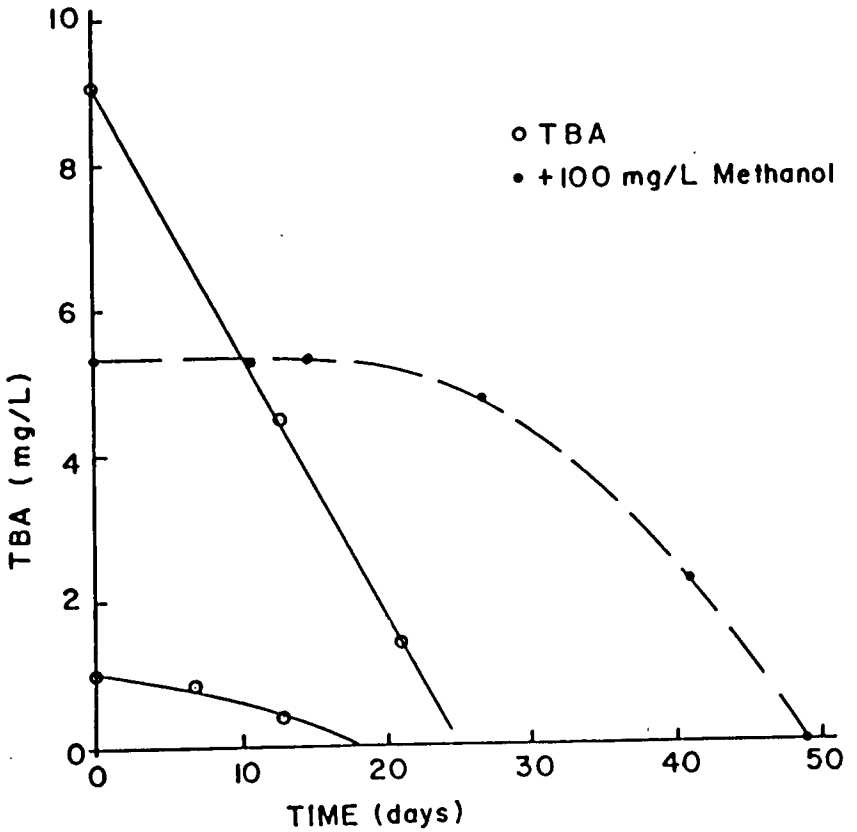


Figure 21. TBA biodegradation in the Pennsylvania saturated zone.

ation required more than 200 days in the unsaturated zone and approximately 180 days in the saturated zone as shown in Figures 22 and 23. The utilization rates (mg/L/day) exhibited variability in a few cases (Table 14). This may be due to the infrequent occurrence of TBA degraders in soil or a reflection of their acclimation time for this particular site.

At the Pennsylvania study area, both methanol and TBA are degradable to levels below the analytical detection limit. The biological removal of methanol and TBA from the groundwater in this area appears to be quite likely if given enough time and continuing contamination does not occur. TBA, however, is more refractory than methanol and would therefore persist for a longer time.

In the Pennsylvania unsaturated and saturated zones TBA biodegradation sometimes exhibited a retarded rate when added with 100 mg/L methanol. At the 1 mg/L TBA concentration a definitive change in the degradation rate occurred in the unsaturated zone in the presence of methanol as compared to TBA alone. However, in the saturated zone a similar rate was noted for 1 mg/L TBA only. A 20 day lag occurred for the degradation of 5 mg/L TBA in the presence of 100 mg/L methanol in the saturated zone supporting the initial observation at 1 mg/L in the unsaturated zone that the presence of methanol may retard TBA degradation. This phenomenon was also observed to occur at the higher levels of TBA. At approximately 50 mg/L TBA with 700 mg/L methanol an extended lag of greater than 140 days was seen for TBA biodegradation.

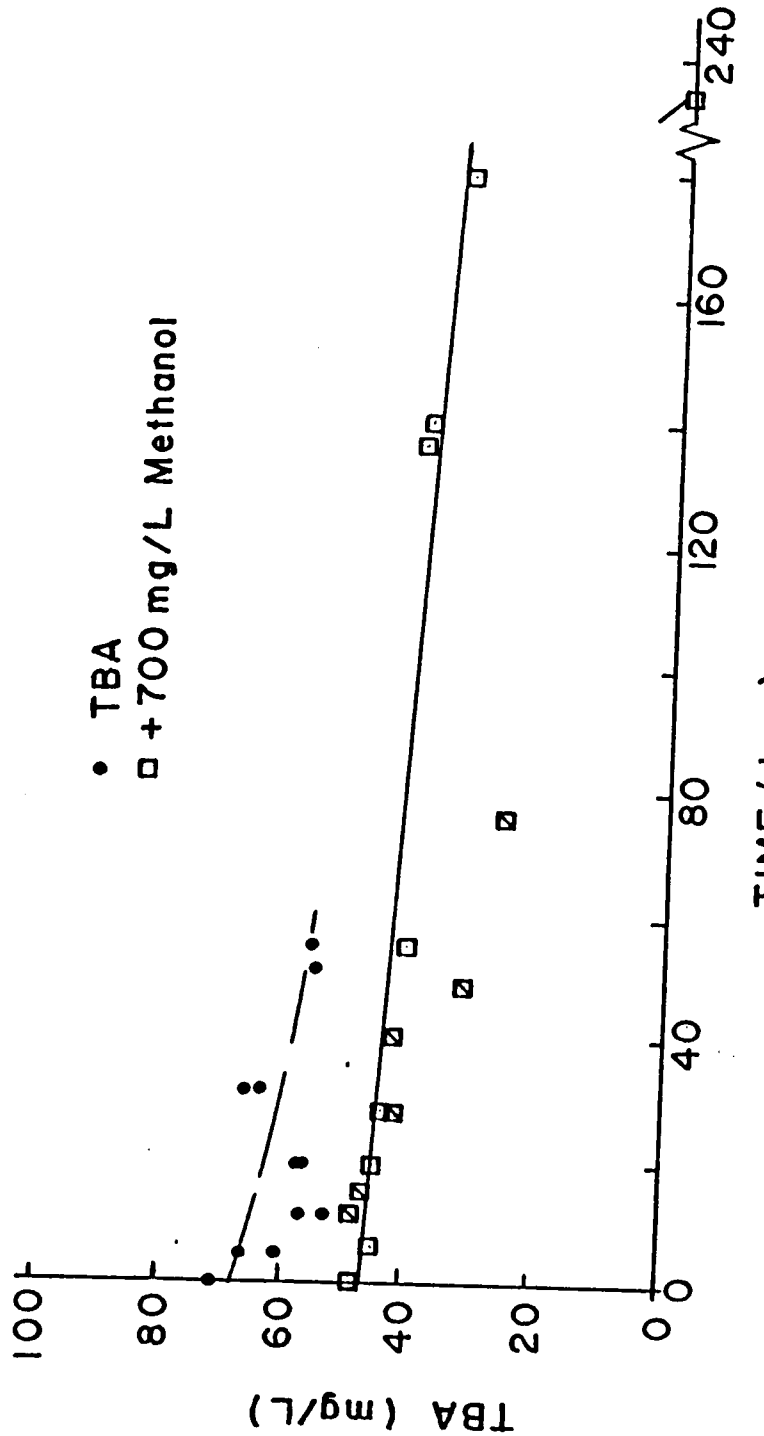


Figure 22. TBA biodegradation in the Pennsylvania unsaturated zone.

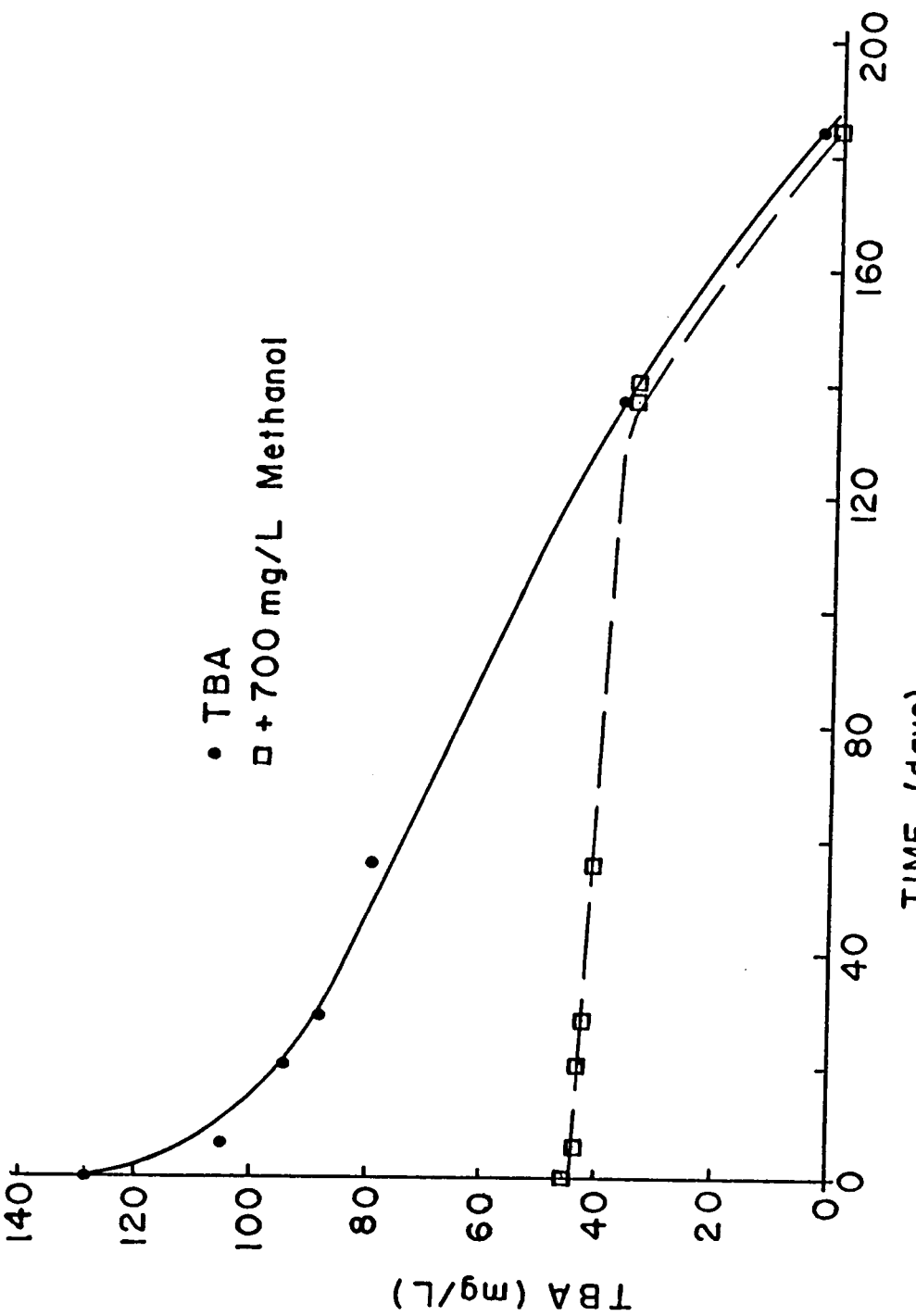


Figure 23. TBA biodegradation in the Pennsylvania saturated zone.

Plots of both methanol and TBA biodegradation when simultaneously added to the same microcosm are shown in Figures 24-26. It appears in some cases that TBA biodegradation begins only after the removal of most of the methanol. This is demonstrated at both high and low concentrations of each compound. Sequential growth seem to most often occur at higher concentrations of TBA and methanol. This is demonstrated in Figure 26 where TBA biodegradation does not begin until methanol levels are significantly lowered. Several mechanisms have been used to describe the type of reaction shown in Figures 24-26. The kinetics of enzymatic reactions having two or more substrates are generally more complex than single substrate reactions since there may be several enzyme-substrate complexes. Since more than one enzyme is also being dealt with here it would be mere speculation to suggest which type of sequential substrate utilization reaction is occurring.

Determination of kinetic coefficients was made using the utilization rate data for TBA as shown in Figure 27. The response was as expected for aerobic systems at low temperatures. The data shown suggest that Monod kinetics may be used to describe saturated zone kinetics but not the unsaturated response. The k, K_s and K for the saturated zone were found to be 0.6 mg/L/day, 23.5 mg/L and 0.026 day^{-1}, respectively. A log-log plot of rate versus concentration suggests a zero order rate for the unsaturated zone as shown in Figure 28 with a k of 0.4 mg/L/day. Since bacterial numbers were found to be constant by A-O direct counts with depth it may be postulated that there is some difference in activity of the bacterial populations in the unsaturated

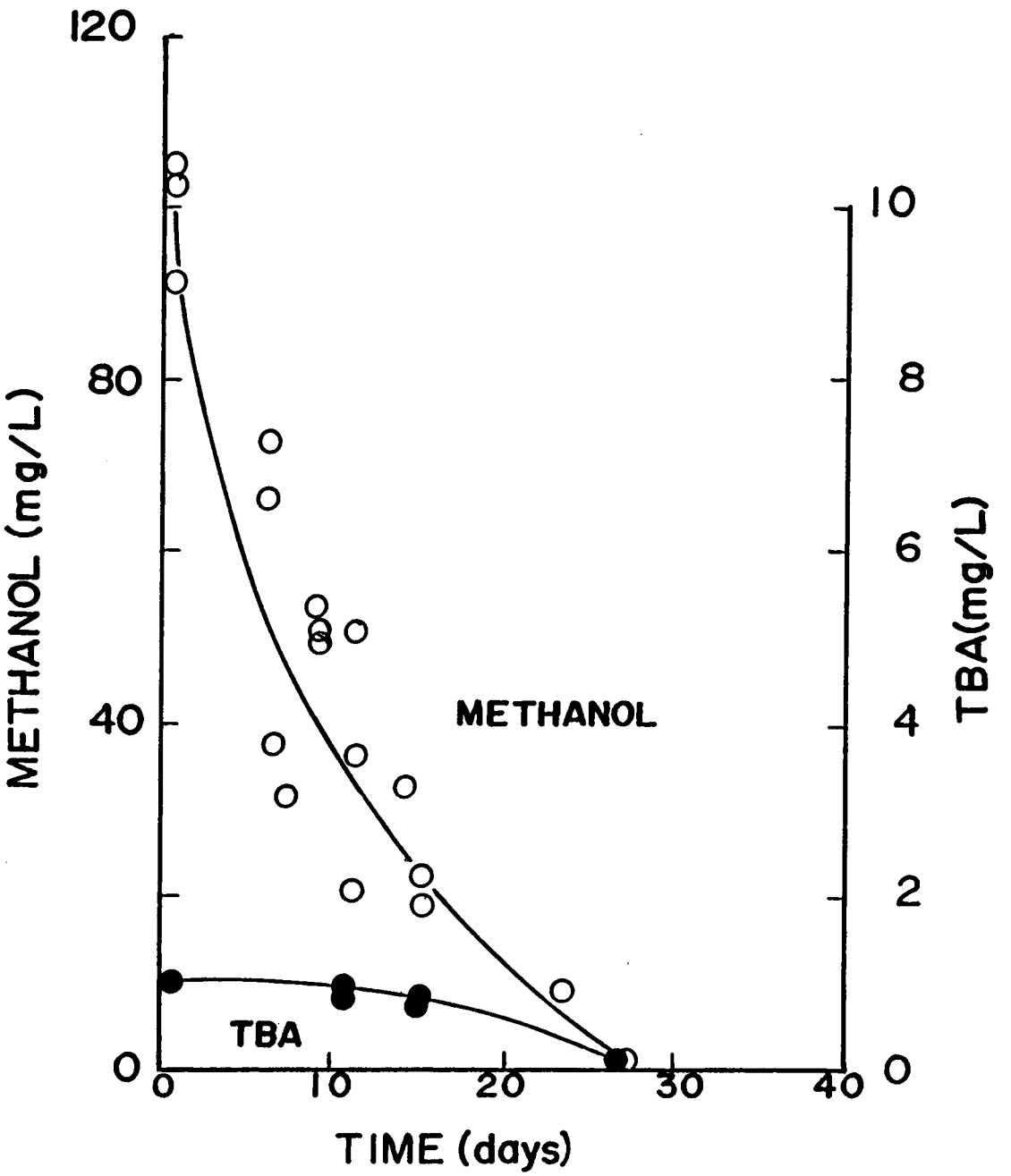


Figure 24. TBA biodegradation in the presence of methanol in the Pennsylvania unsaturated zone.

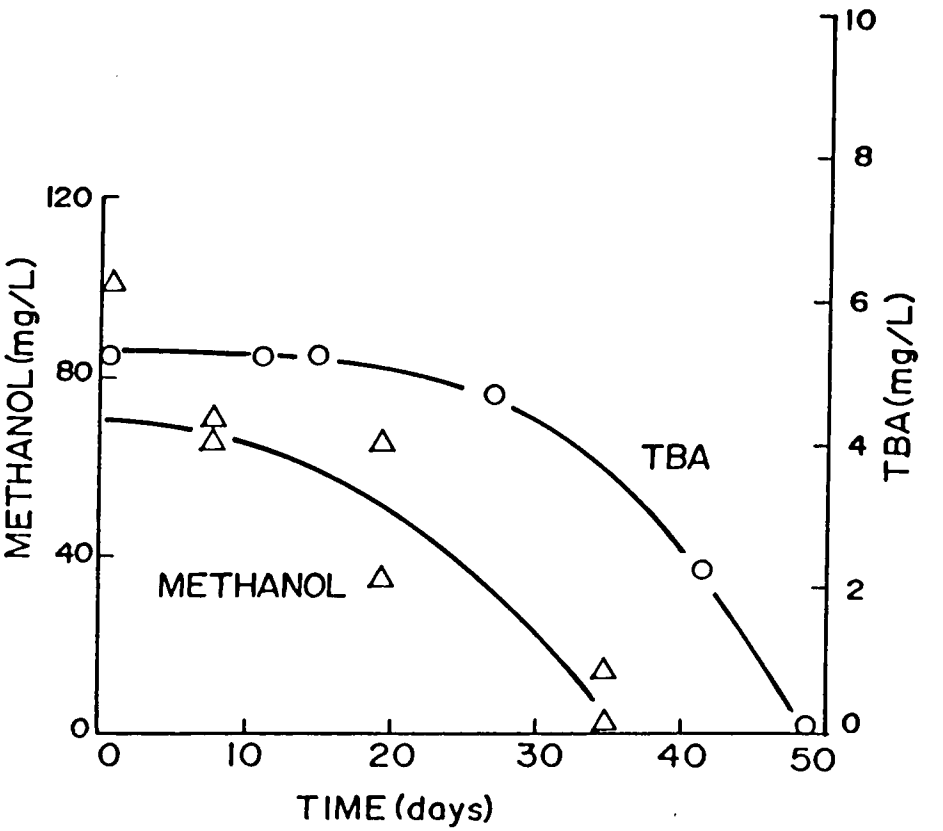


Figure 25. TBA biodegradation in the presence of methanol in the Pennsylvania saturated zone.

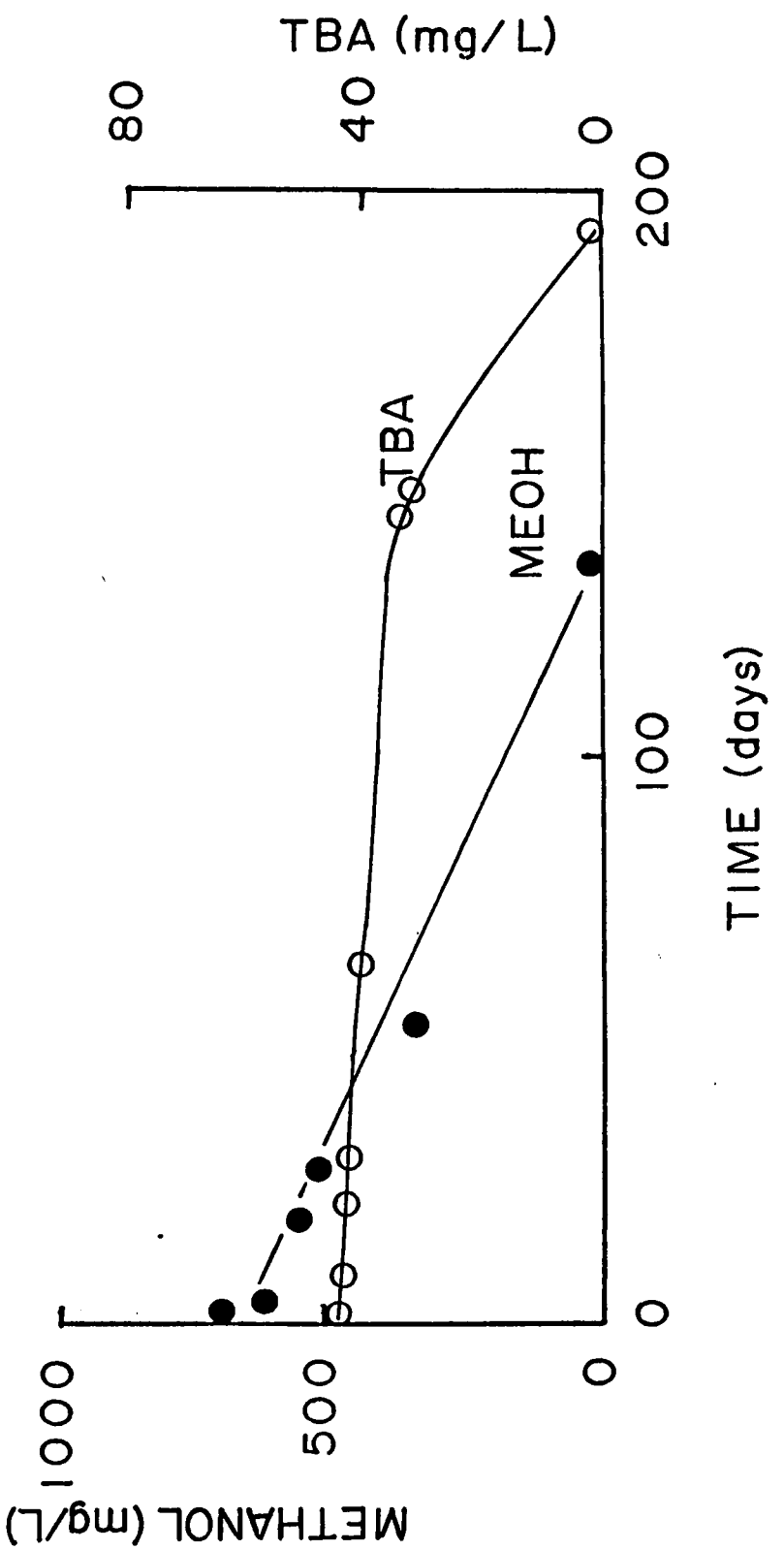


Figure 26. TBA biodegradation in the presence of 700 mg/L methanol in the Pennsylvania saturated zone.

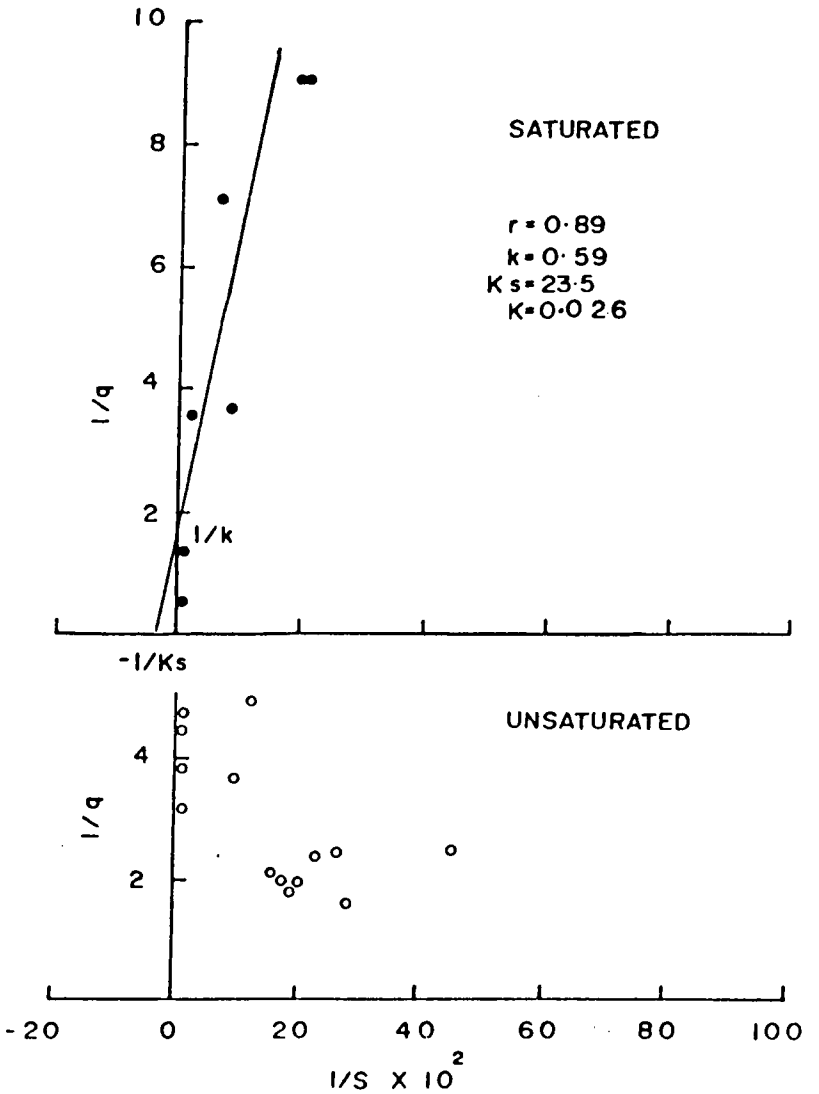


Figure 27. Determination of TBA kinetic constants using Lineweaver-Burk plot for the Pennsylvania soil.

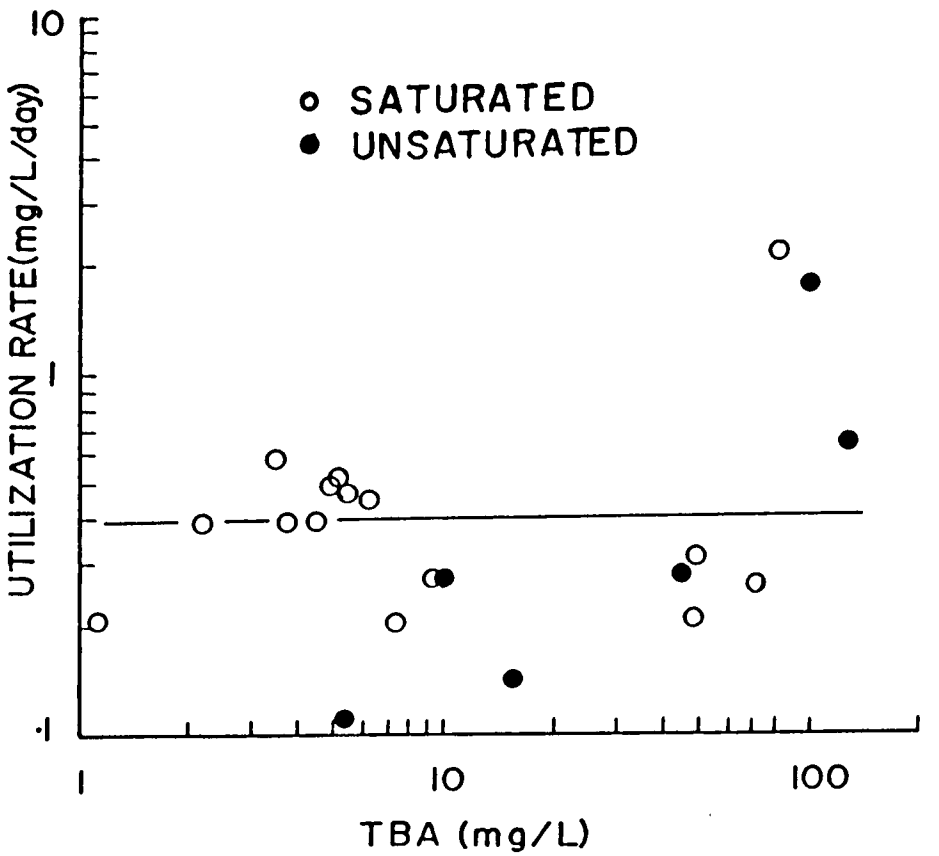


Figure 28. Determination of rate order for TBA utilization in Pennsylvania soil.

and saturated zones towards TBA. There is further evidence of this in the arithmetic plots of concentration against time in Figures 22 and 23.

Control Microcosms

Problems were encountered initially with substrate loss in the control static microcosms. Experimental results with Pennsylvania soil are shown in Tables 15 and 16. It was determined by actual cell counts that the controls were not rendered sterile by 45 minutes of autoclaving. A study was conducted to determine the optimum sterilization time. The various treatments are shown in Table 17. Extensive autoclaving was required to maintain sterile controls for any length of time. Even soil placed in a muffle furnace for 3 hours showed some substrate loss after an extended period of time. This most likely was due to contamination by the needle introduced through the septum cap to obtain samples. This problem was corrected by using a heat sterilizer for the gas chromatographic syringe after solvent rinsing, and by wiping the microcosm sampling surface with isopropanol. From the controls, it can be seen, however, that in the case of alcohols that disappearance is due to biodegradation and not to adsorption.

A plot of BTX removal in sterile versus non-sterile microcosms is shown in Figure 29. It can be seen that when equally dosed microcosms are followed with time that there is essentially no difference in the rates. It is conceivable that BTX in the percentage found in gasoline could be rapidly adsorbed allowing the more soluble constituents to move ahead with the water front. The adsorption rate would be expected to

Table 15. Residual methanol concentrations in controls from the Pennslyvania saturated and unsaturated zones autoclaved for 45 minutes.

	Concentration (mg/L)					
Time (Days)	0	7	9	11	14	23
Unsaturated	10.4	11.6				
	10.0		9.1			
	12.7			9.5		
	12.8					11.1
	11.7					10.0
	111.8	108.4				
	100.2		82.7			
	83.0			73.7		
	124.2				87.2	
	101.9					67.5
Saturated	13.5	14.4				
	13.3		16.5			
	13.8			ND		
	8.9					ND
	6.0					ND
	121.7	104.5				
	172.4		64.1			
	104.5			73.5		
	150.4				63.0	
	86.6					80.2

Table 16. Residual tertiary butyl alcohol concentrations in controls from the Pennsylvania unsaturated and saturated zones autoclaved for 45 minutes.

	Concentration (mg/L)					
Time (Days)	0	5	11	19	32	52
Unsaturated	0.9	0.8				
	0.9		0.9			
	0.8			0.7		
	0.6					0.5
	0.7					0.8
	5.2	5.2				
	4.8		4.7			
	5.4			5.4		
	5.2				5.3	
	5.5					5.6
	78.9	70.6				
	67.7		53.9			
	61.8			47.8		
	65.4				52.9	
Time (Days)	0	7	13	21	34	54
Saturated	1.2	1.2				
	0.6		0.8			
	1.1			0.7		
	15.0	12.7				
	10.4		7.1			
	14.4			10.0		
	8.0				6.7	
	14.5					10.8
	146.1	115.5				
	149.1		113.5			
	192.7			123.9		
	171.0				129.1	
	151.3					96.9

Table 17. Determination of optimum sterilization of controls by various treatments.

	Methanol Concentration (mg/L)			
Time (Days)	0	11	26	47
Autoclaving time (min.)				
45	70.3	71.9		
	99.9	99.3		
	89.5		89.0	
	89.9		7.9	
	114.2			124.6
120	99.3	99.2		
	110.6	110.8		
	92.4		91.5	
	88.1		88.9	
	115.1			49.6
120 + 180	135.9	136.4		
	104.6	104.9		
	170.5		153.5	
	143.8		137.9	
	106.2			83.1
Muffle Furnace (septum caps)	229.4	229.7	231.3	162.5
	226.8	227.7	228.3	217.7

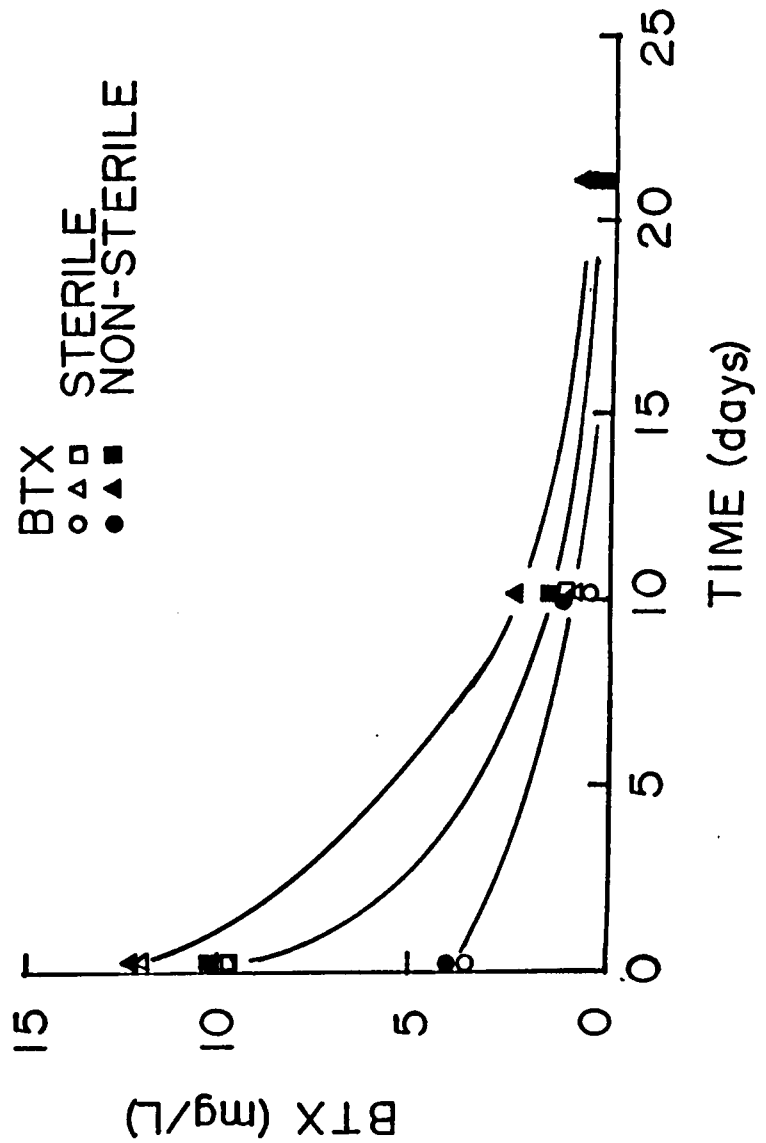


Figure 29. BTX removal in sterile and non-sterile Pennsylvania microcosms.

vary from site to site based on the chemical characteristics of the soil.

The adsorption of aromatic hydrocarbons has been investigated by others. While one study demonstrated minimal adsorption (67), others showed that sorption could occur and is related to soil properties, particularly organic content. Xylene has been found to be sorbed more readily than toluene and toluene more readily than benzene (68,69). One investigator demonstrated that the moisture content of the soil aided in the formation of a pellicular film of gasoline on soil.

New York

Biodegradation was studied only in the saturated zone at New York due to a shallow water table and a soil structure which made sample collection difficult.

Methanol. Figures 30 and 31 show methanol utilization at initial concentrations of approximately 80 and 500 mg/L, respectively. The utilization rates (Table 18) were lower than at Pennsylvania, and initially exhibited a lag period. The utilization rate beyond the lag was quite rapid. A lag of approximately 20 days occurred with methanol as a sole substrate at 80 mg/L. At the same concentration, however, only a 10 day lag occurred when TBA or TBA and BTX were present. It is interesting to note that complete biodegradation was accomplished in 35 days when TBA and BTX were added, while 60-75 days were required for methanol or methanol and TBA. This appears to be an enhancement effect which could again be related to the methylotrophs ability to oxidize aromatics. Similarly, for 500 mg/L initial concentrations, methanol

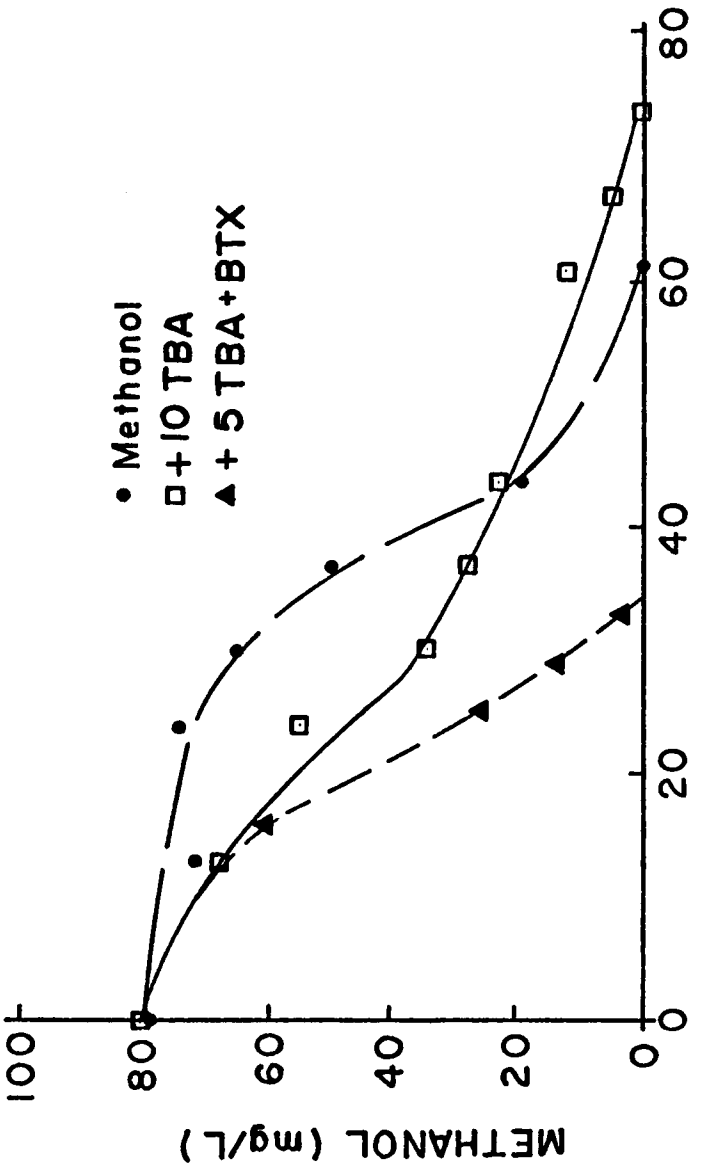


Figure 30. Methanol biodegradation in the New York saturated zone.

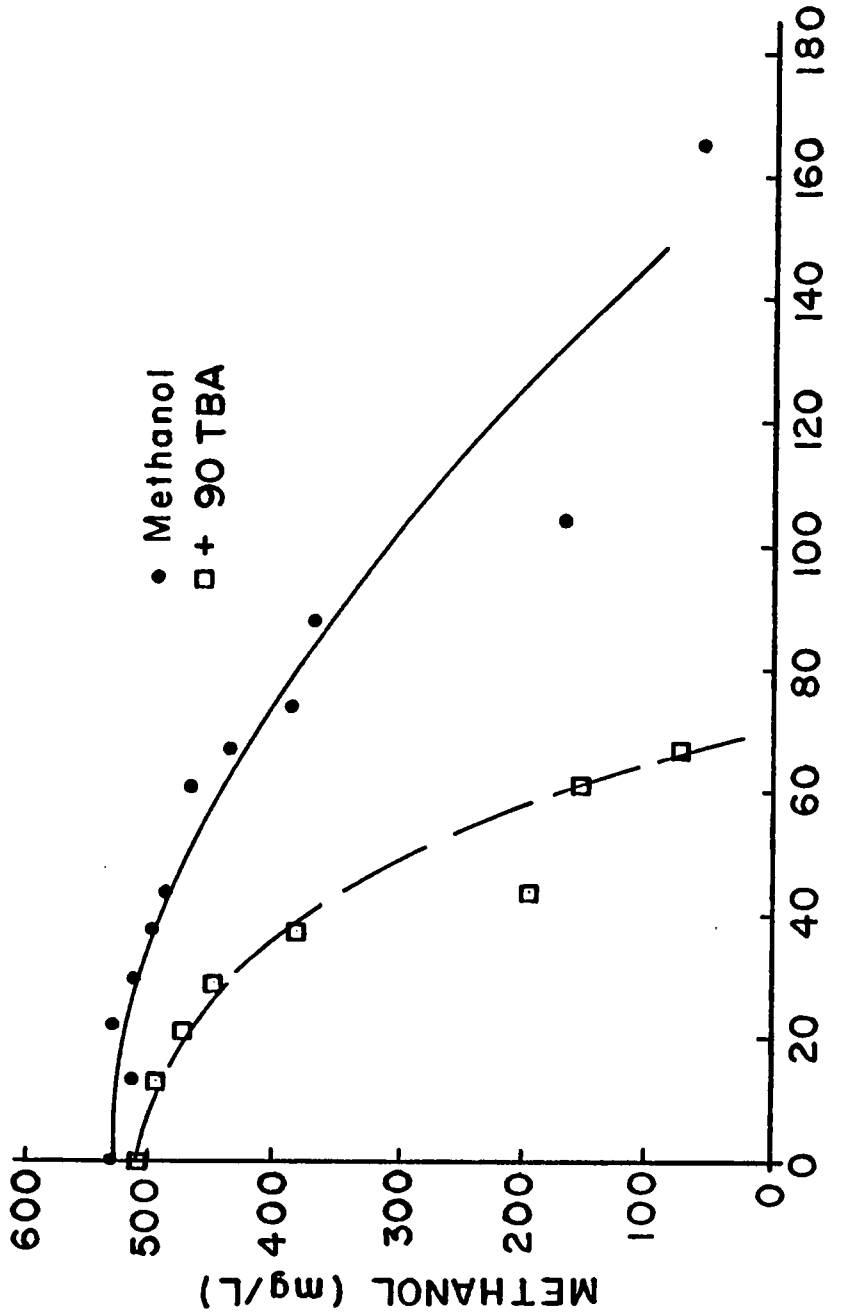


Figure 31. Methanol biodegradation in the New York saturated zone.

Table 18. Saturated zone utilization rates (mg/L/day) for New York.

Initial Concentration, mg/L	Rate, mg/L/day
Methanol	
79.2	1.58
80.2 + 10 TBA	1.15
79.1 + 5 TBA + BTX	2.08
531	2.84
512 + 90 TBA	6.92
TBA	
1.3	1.90×10^{-3}
10.5	1.65×10^{-2}
77.8	2.23×10^{-1}
11.8 + 100 MeOH	5.05×10^{-2}
5.7 + 100 MeOH + BTX	6.52×10^{-2}
88.9 + 500 MeOH	1.40×10^{-1}

degradation was greatly increased by the presence of TBA. The presence of 90 mg/L TBA resulted in complete biodegradation in 70 days compared to 180 days for methanol alone. Enzyme inducement is accomplished by the presence of a molecule similar in structure to the substrate being degraded. Since TBA is an alcohol, but more refractory than methanol, enzyme inducement could be occurring.

Kinetic plots were made for these data and are shown in Figures 32 and 33. The k, K_s, and K were 5.4 mg/L/day, 200.3 mg/L, and 0.027 $days^{-1}$, respectively. The large K_s is indicative of the increase in K_s associated with low temperatures and anaerobic systems, as is the low K value (70). Limited data were available due to minimal soil samples.

TBA. TBA biodegradation from the New York site as shown in Figures 34 and 35 is slower than that observed for the Pennsylvania site. The specific rates are listed in Table 18. Once again there is an indication of enhanced biodegradation due to the presence of methanol and BTX. There is no indication of methanol preference over TBA as was seen in Pennsylvania. At higher TBA concentrations the utilization rates were nearly equal with and without methanol and as a second substrate. A log-log plot in Figure 36 of rate versus concentration shows that the degradation rate is approximately first order. Due to the low temperature (10°C) and anaerobic conditions it would be expected that the K_s would be large (71). The K_s value of 463 mg/L (Figure 37) supports this contention. This would make extremely low levels of TBA very difficult to remove. Kinetic plots support this. Figure 38 shows the K value to be 0.0018 day^{-1}. This is much smaller than the K value

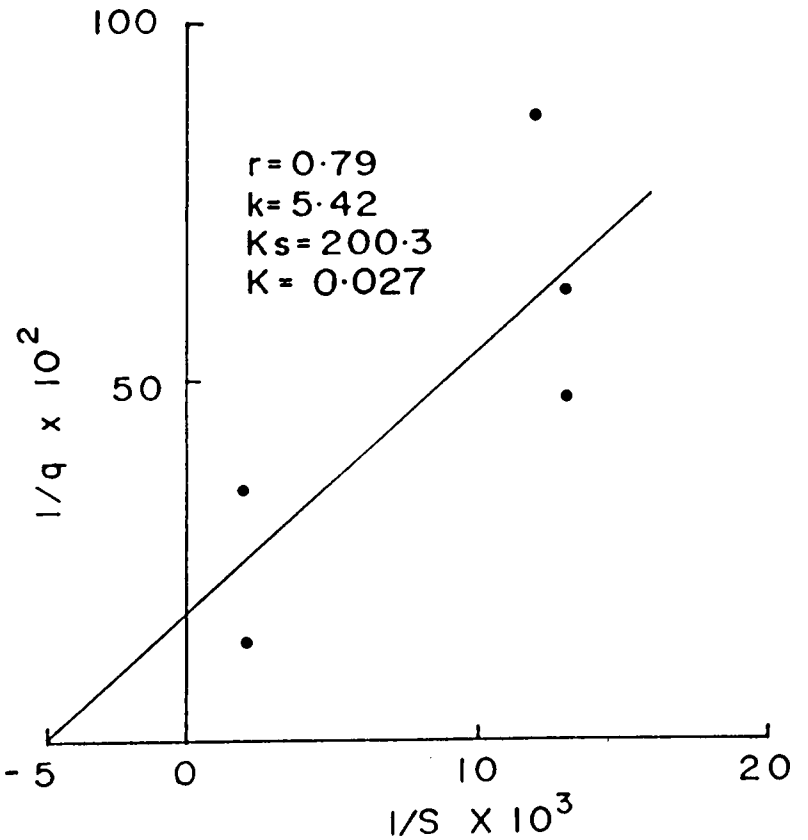


Figure 32. Determination of the kinetic constants k, K_s and K for methanol in saturated New York soil.

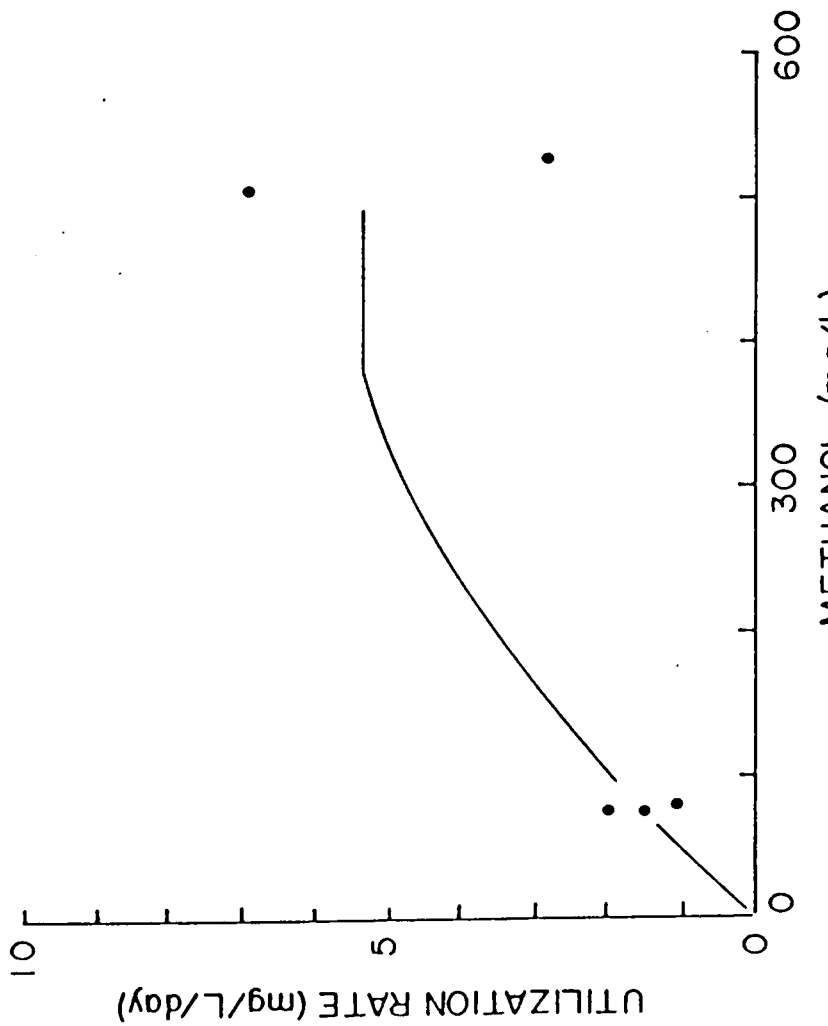


Figure 33. Determination of the kinetic constant K for methanol in saturated New York soil.

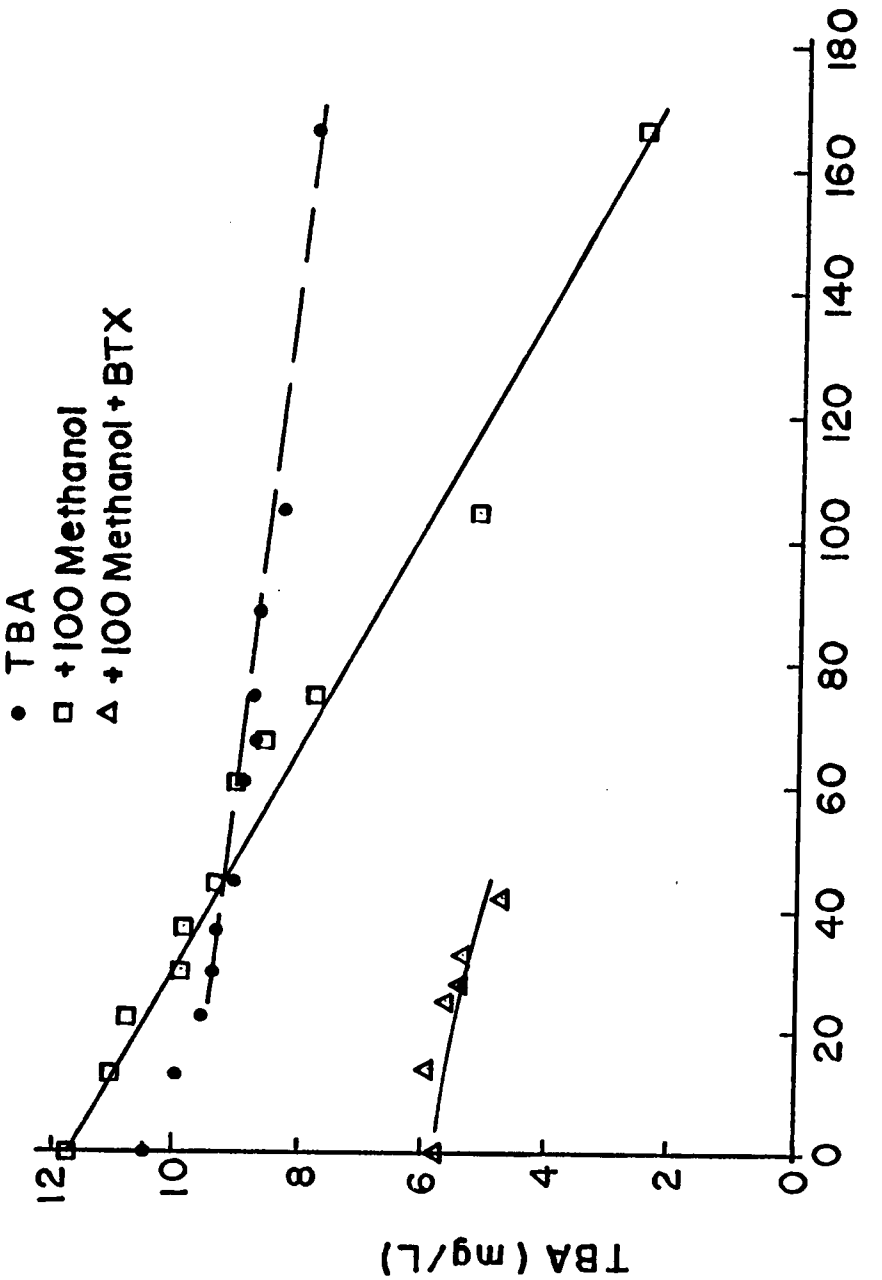


Figure 34. TBA biodegradation in the New York saturated zone.

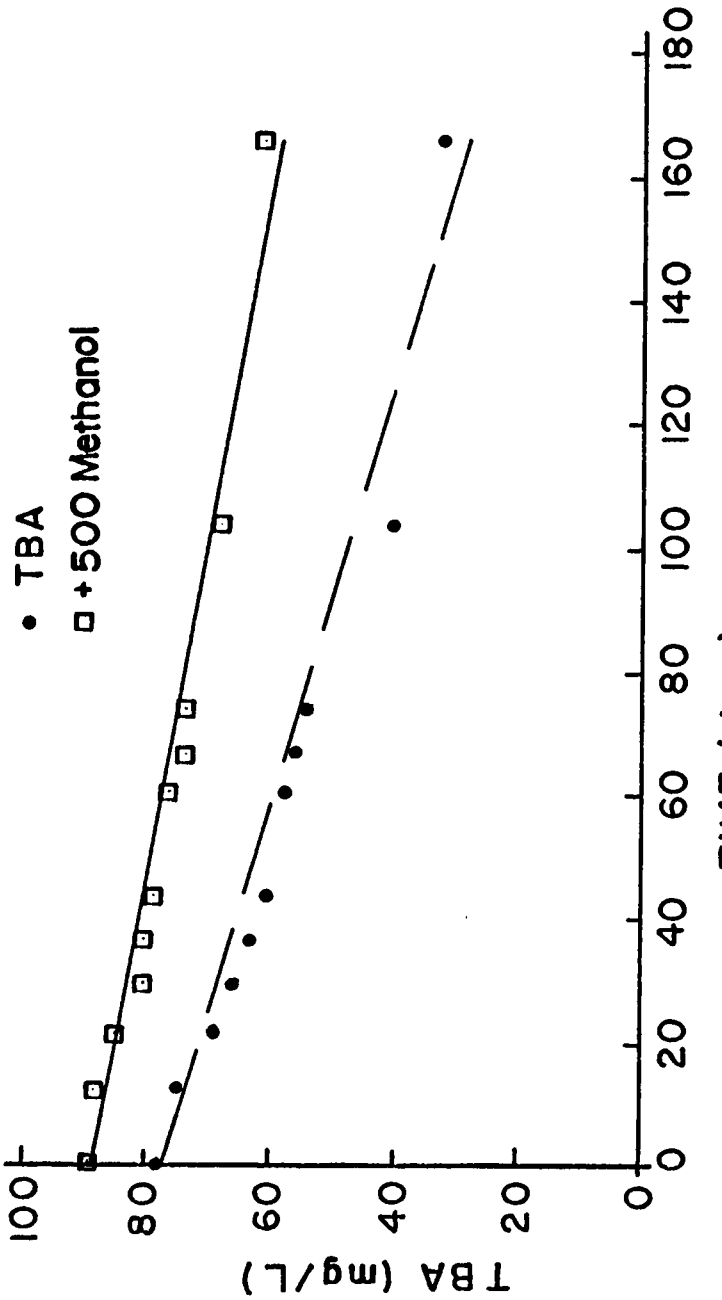


Figure 35. TBA biodegradation in the New York saturated zone.

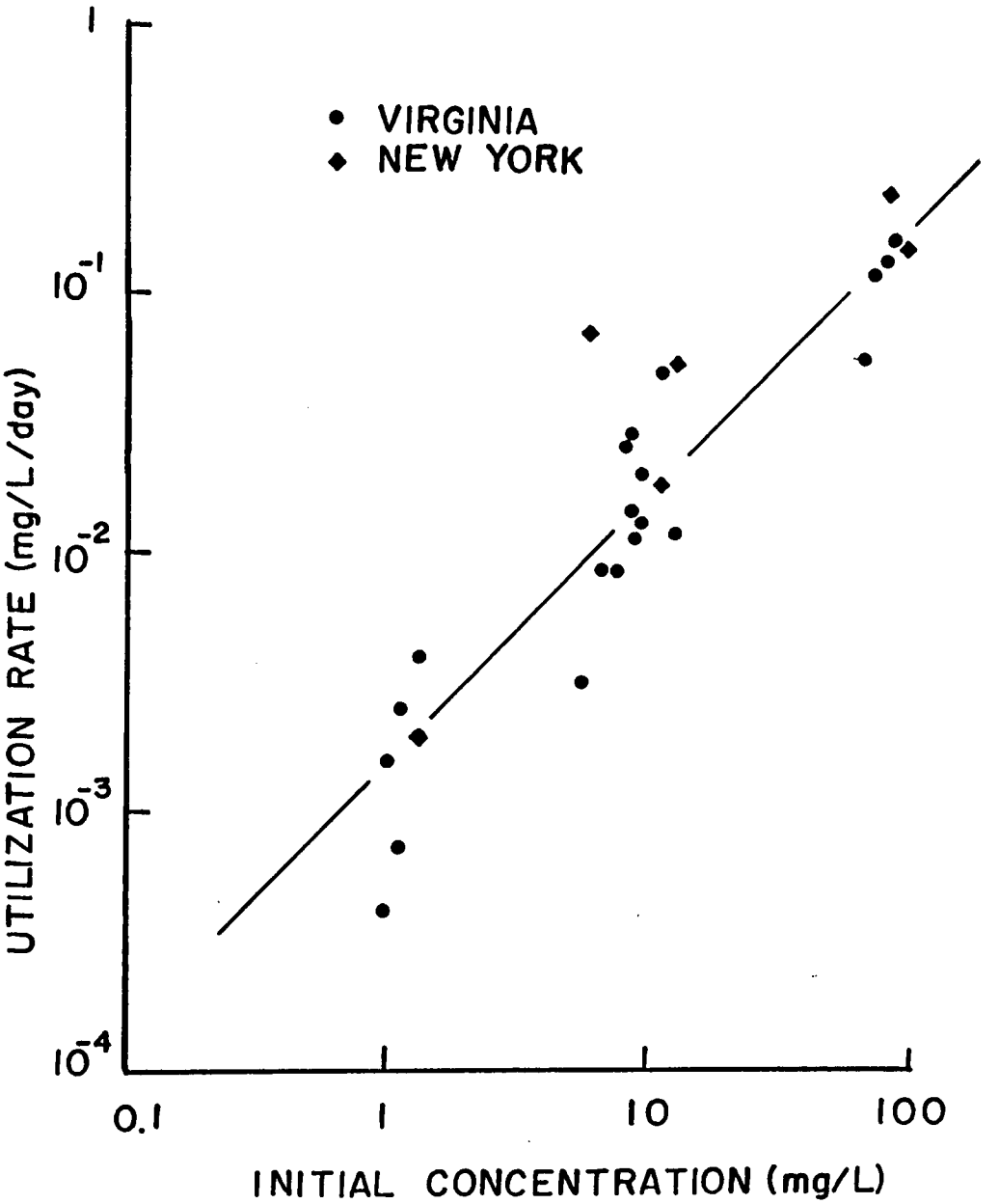


Figure 36. Determination of rate order for TBA utilization in the New York and Virginia soils.

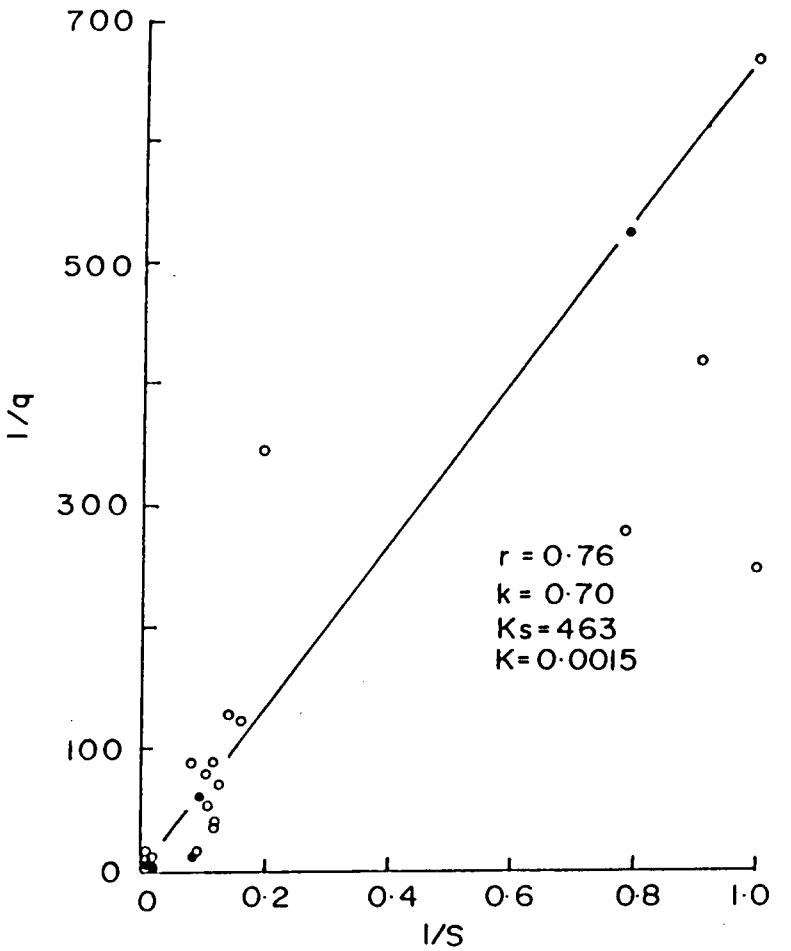


Figure 37. Determination of kinetic constants k, K_s, and K for TBA in New York and Virginia soils.

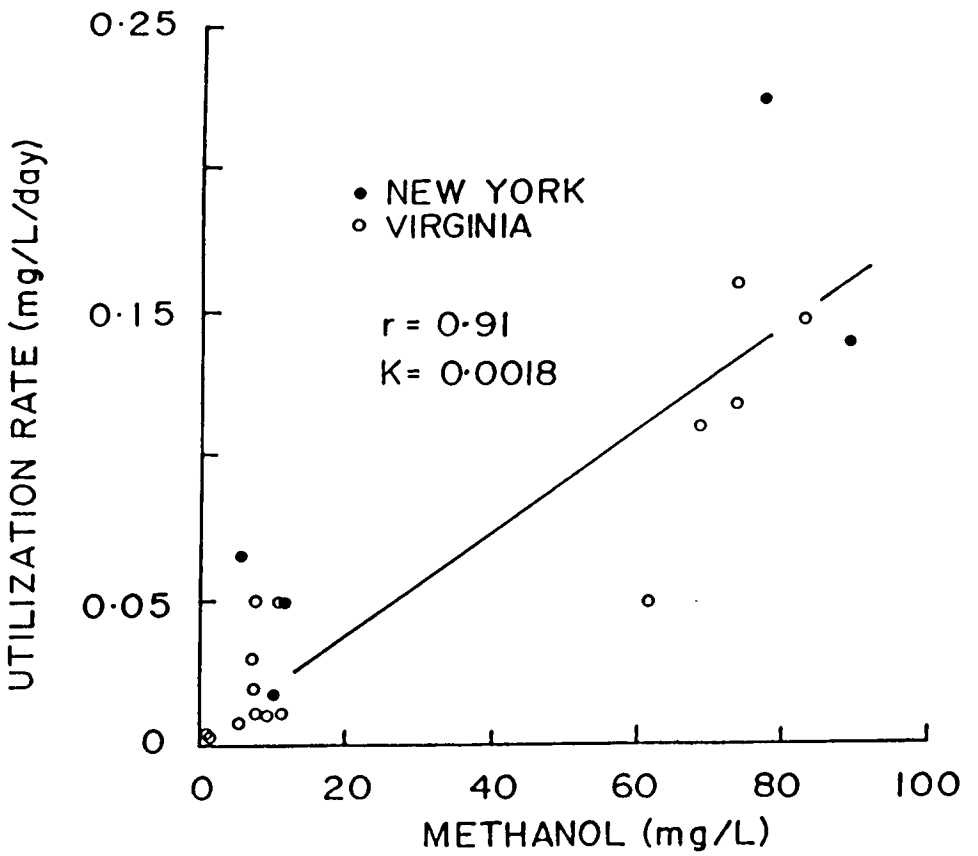


Figure 38. Determination of the kinetic constant K for TBA in New York and Virginia soils.

found for methanol. TBA appears to be much less biodegradable in anaerobic systems than methanol.

Groundwater. Because of questions relating to the relative distribution of organisms in soil versus free floating in groundwater, microcosms consisting only of groundwater were dosed with methanol and TBA. Tubes containing groundwater only, when dosed with concentrations of methanol and TBA equivalent to those studied in soil microcosms, exhibited very slow degradation rates. Results are given in Table 19 for a 180 day period. Minimal loss was found in sterile controls. This suggests that biodegradation in the water matrix is insignificant.

Control Microcosms. New York controls were autoclaved every four to five days for 25 minutes each of five autoclavings. Table 20 shows that these microcosms were effectively sterilized, since no appreciable loss of methanol or TBA was found. BTX, however, once again was lost due to adsorption as shown in Figure 39.

Virginia

The Virginia site provided a large quantity of data since drilling was successful to 31 m. Five depths were investigated because the aquifer seemed to be a layered one. Utilization rates are given in Table 21. The data have been presented in several ways. Plots of the effects of depth upon methanol and depth upon TBA biodegradation are presented. Treatment effects, that is, single and multiple substrates at individual depths are also presented.

Methanol. Methanol biodegradation rates in the saturated zone are similar to the New York site except no lag occurs. It is evident in

Table 19. Biodegradation in New York groundwater.

	Concentration (mg/L) at time (days)		
Compounds	0	92	180
Methanol	103.1	72.2	50.9
	997	757	685
TBA	0.6	0.4	0.1
	8.1	8.1	7.6
	73.7	75.1	73.5
Methanol/TBA	88.6/7.9	69.7/7.9	50.8/7.9
	900/100.8	745/79.6	697/77.9
Methanol Controls	83.4	83.6	76.8
TBA Controls	10.0 62.4	10.1 64.7	9.7 61.0

Table 20. Methanol, TBA, benzene, toluene, and m-xylene control microcosms for New York.

	Concentration (mg/L) at time (days)			
Compounds	0	14	25	69
Methanol/TBA	91.5/11.8	88.8/11.9	86.5/11.6	88.3/11.7
BTX	3.1,4.1,3.3	0.7,1.1,0.5	ND,ND,ND	
	101.9/88	100.6/8.6	92.9/8.4	91.7/8.7
	3.2,5.0,4.2	0.3,0.3,0.2	ND,ND,ND	

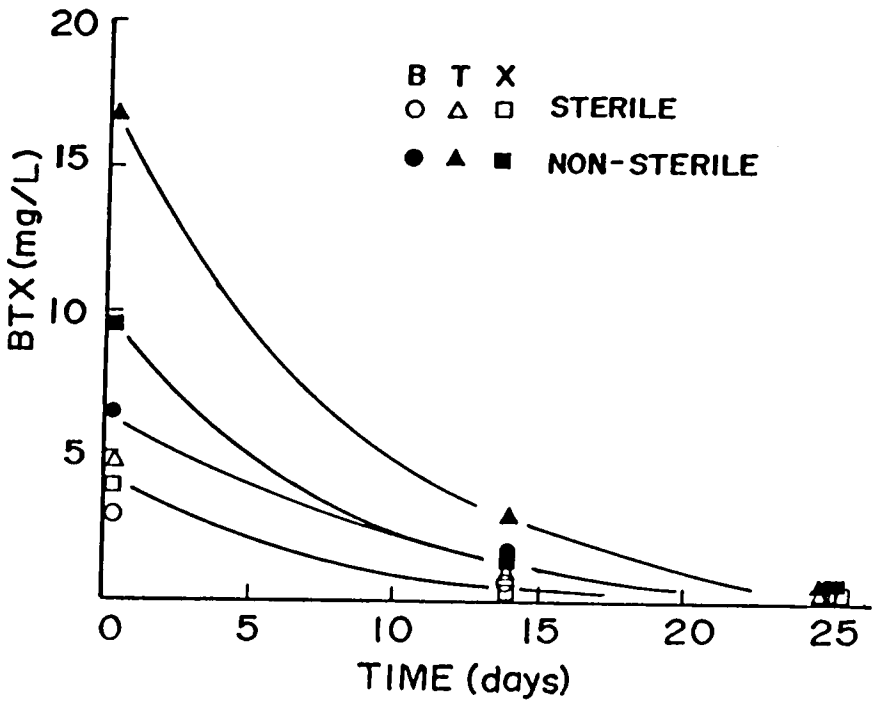


Figure 39. BTX removal in sterile and non-sterile New York microcosms.

Table 21. Utilization rates (mg/L/day) for Virginia.

Initial Concentration	Depth (ft) 11	30	57	80	102
Methanol (mg/L)					
95.1	0.93				
87.3 + 10 TBA	0.86				
81.7 + 7 TBA + BTX	0.88				
724.0	1.89				
79.7		0.84			
878.0		2.12			
68.5			1.03		
68.2 + 5 TBA			0.97		
80.2 + 7 TBA + BTX			0.79		
691.0			2.36		
87.5				2.36	
996.0				7.89	
86.6					2.34
99.9 + 8 TBA					2.67
94.1 + 8 TBA + BTX					1.47
799.0					3.57

(Continued)

Table 21. Utilization rates (mg/L/day) for Virginia (cont'd).

Initial Concentration	Depth (ft) 11	30	57	80	102
TBA (mg/l)					
1.0	4.00×10^{-3}				
6.4	8.00×10^{-3}				
8.8 + 100MeOH	1.90×10^{-2}				
8.5 + 100MeOH + BTX	1.10×10^{-2}				
81.8	1.49×10^{-1}				
1.1		7.0×10^{-4}			
11.9		1.08×10^{-2}			
74.4		1.19×10^{-1}			
1.0			1.50×10^{-3}		
8.2			2.45×10^{-2}		
5.4 + 100 MeOH			2.90×10^{-3}		
7.2 + 100 MeOH + BTX			7.80×10^{-3}		
62.7			4.90×10^{-2}		
1.1				2.40×10^{-3}	
8.9				1.20×10^{-2}	
74.2				1.59×10^{-1}	
1.3					3.80×10^{-3}
11.0					4.61×10^{-2}
8.1 + 100 MeOH					2.69×10^{-2}
7.8 + 100 MeOH + BTX					1.35×10^{-2}
69.2					1.09×10^{-1}

Figure 40 that at 24 and 31 m methanol utilization is greater than in the unsaturated zone. Biodegradation of approximately 800 mg/L methanol, however, only showed an increased rate at 24 m as shown in Figure 41. Since bacterial counts showed no significantly greater population at 24 or 31 m than on the surface the explanation must be elsewhere. The groundwater analyses for Virginia did not reveal a reason for the increased rates in the saturated zone. It must be that the bacterial population is more active since it is not subject to dehydration from the rise and fall of the groundwater table or there exists a greater proportion of the population that is amenable to methanol biodegradation. This postulation is supported to some degree by soil extract plus formate or methylamine agar plate counts. An increase in the population is seen through this area. However, it is interesting to note the increased rate when 24 and 31 meters are considered the saturated zone. Kinetic data for methanol further substantiates the activity of the saturated zone. As seen in Figures 42 and 43 the kinetics are different for the two aquifer zones. The k, K_s and K for the unsaturated zone were 2.3 mg/L/day, 118.9 mg/L and 0.019 $days^{-1}$, while they were 5.7 mg/L/day, 158.3 mg/L and 0.036 day^{-1} for the saturated zone.

Treatment effects as seen in Figures 44 and 45 at individual depths showed no effect at 3.4 and 17 m on methanol utilization rates. However, Figure 46 demonstrates at 34 M it required approximately 62 days to degrade 100 mg/L methanol in the presence of TBA and BTX.

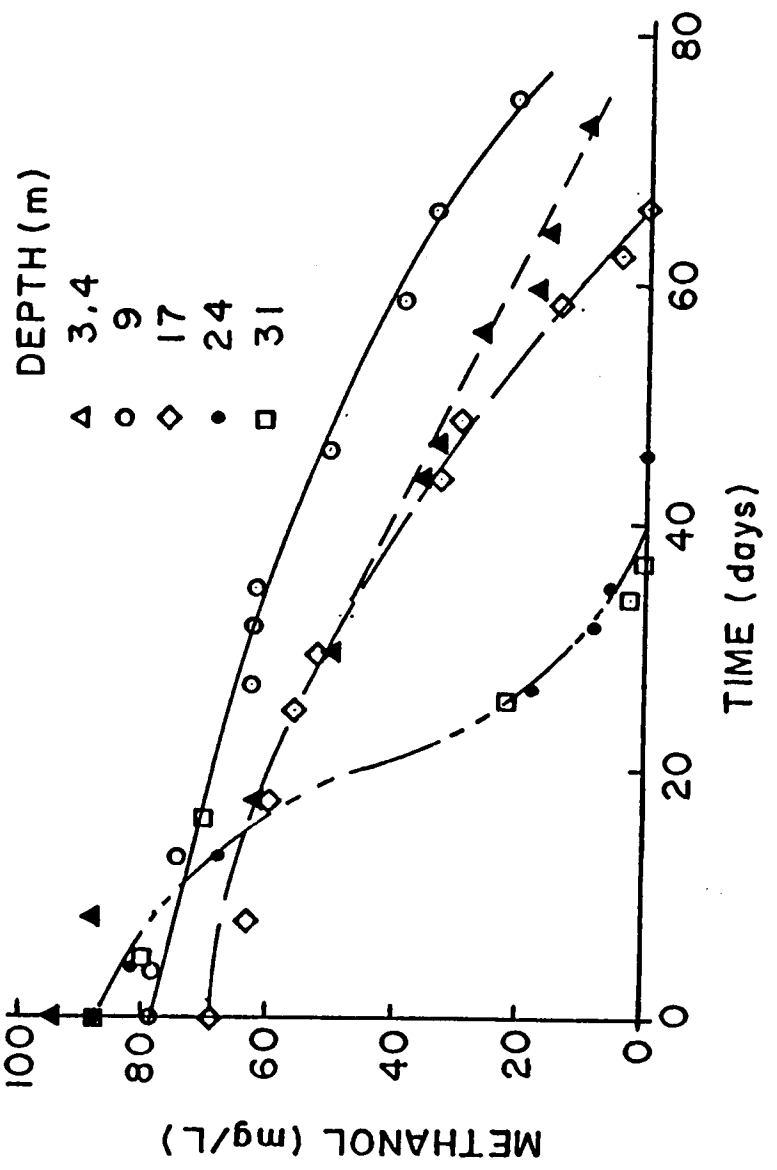


Figure 40. Methanol biodegradation at various depths in the Virginia subsurface.

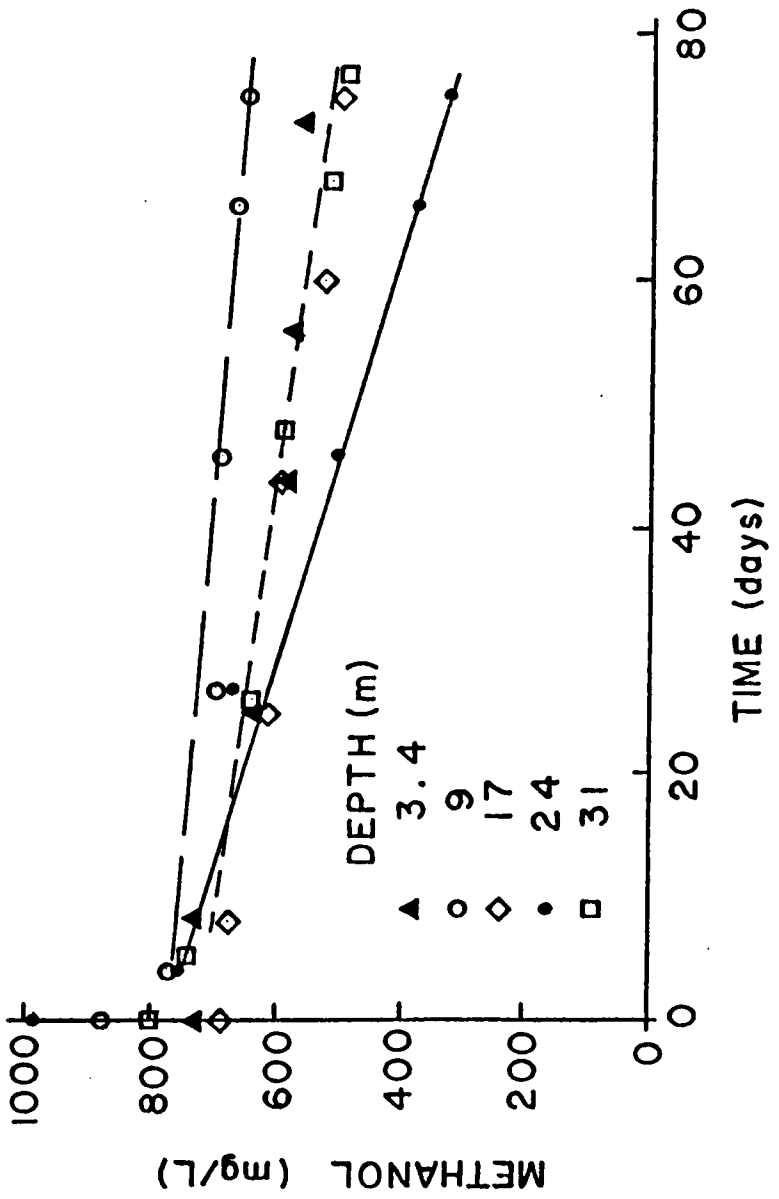


Figure 41. Methanol biodegradation at various depths in the Virginia subsurface.

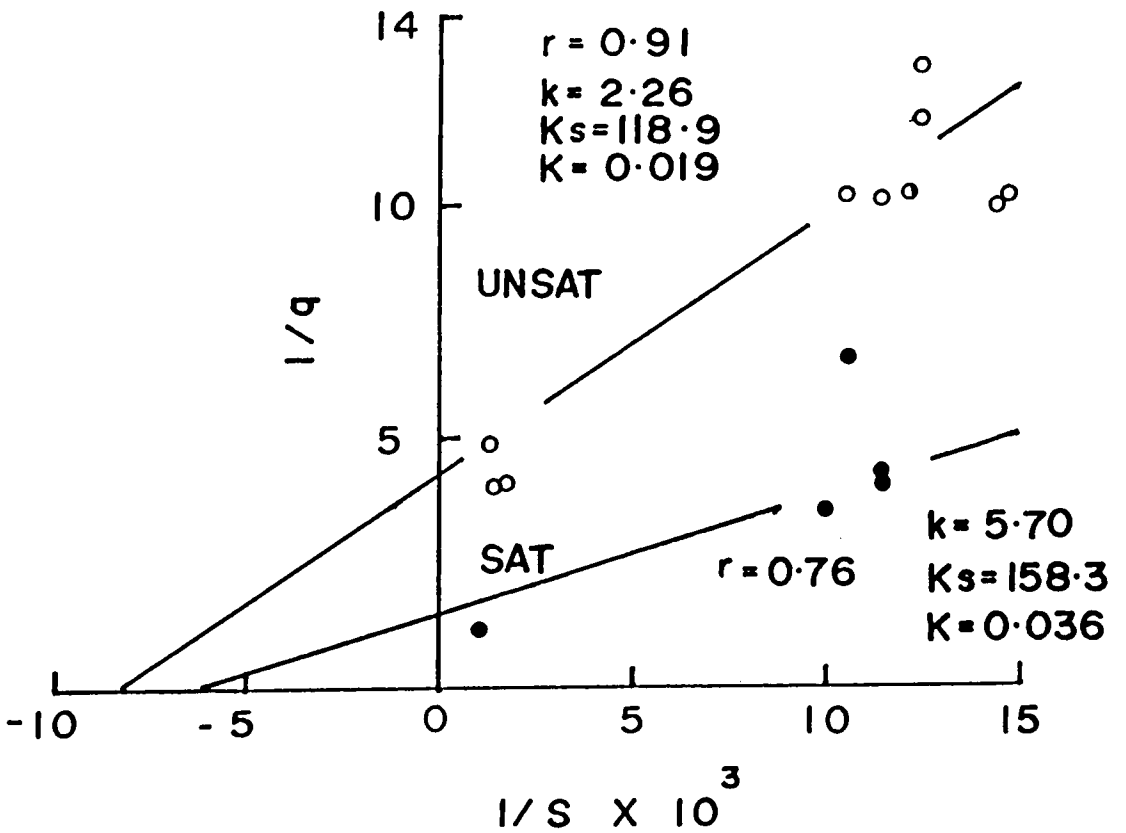


Figure 42. Determination of kinetic constants k, K_s, and K for methanol in Virginia soil.

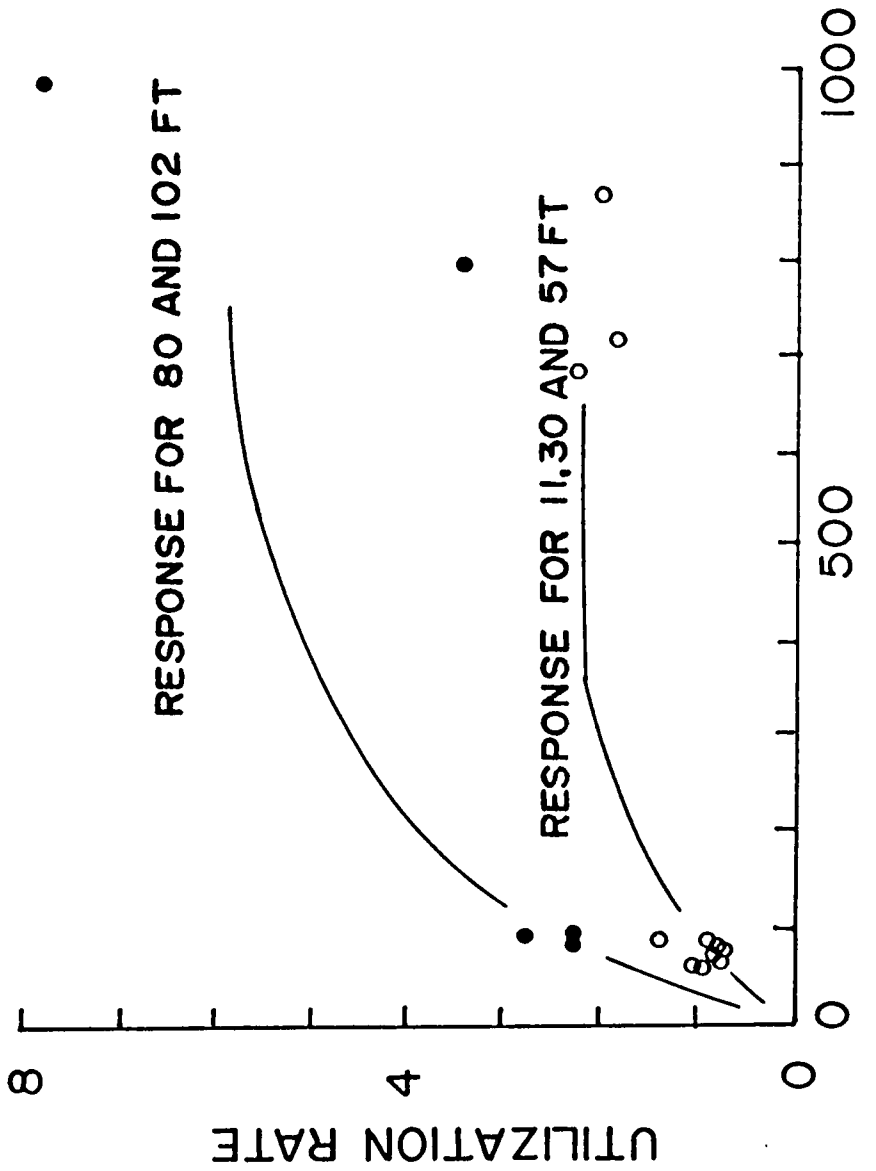


Figure 43. Determination of the kinetic constant K for methanol in Virginia soil.

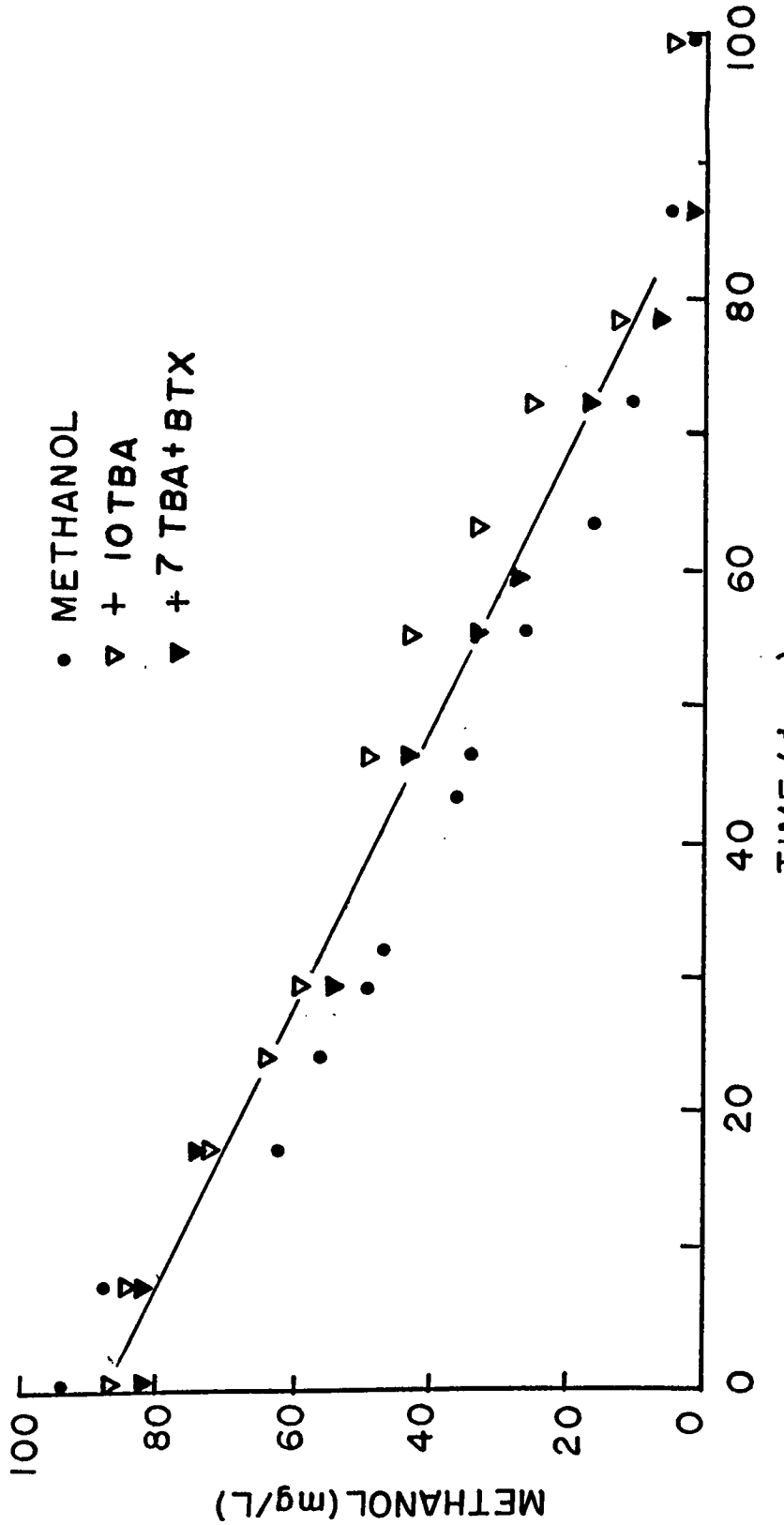


Figure 44. The effect of TBA and TBA + BTX on methanol biodegradation in Virginia soils at 3.4 M (11 ft.).

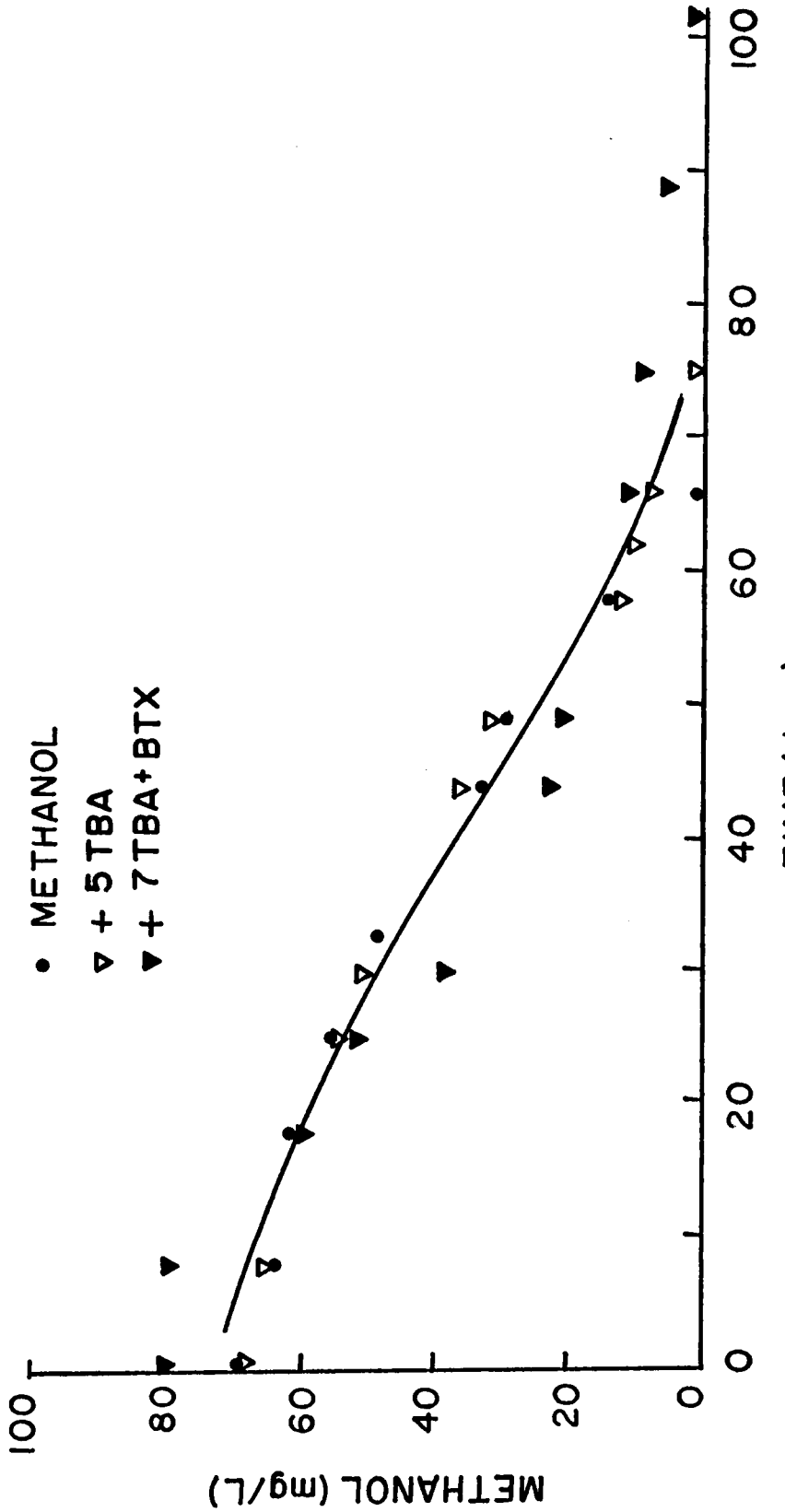


Figure 45. The effect of TBA and TBA + BTX on methanol biodegradation in Virginia soils at 17 M (57 ft).

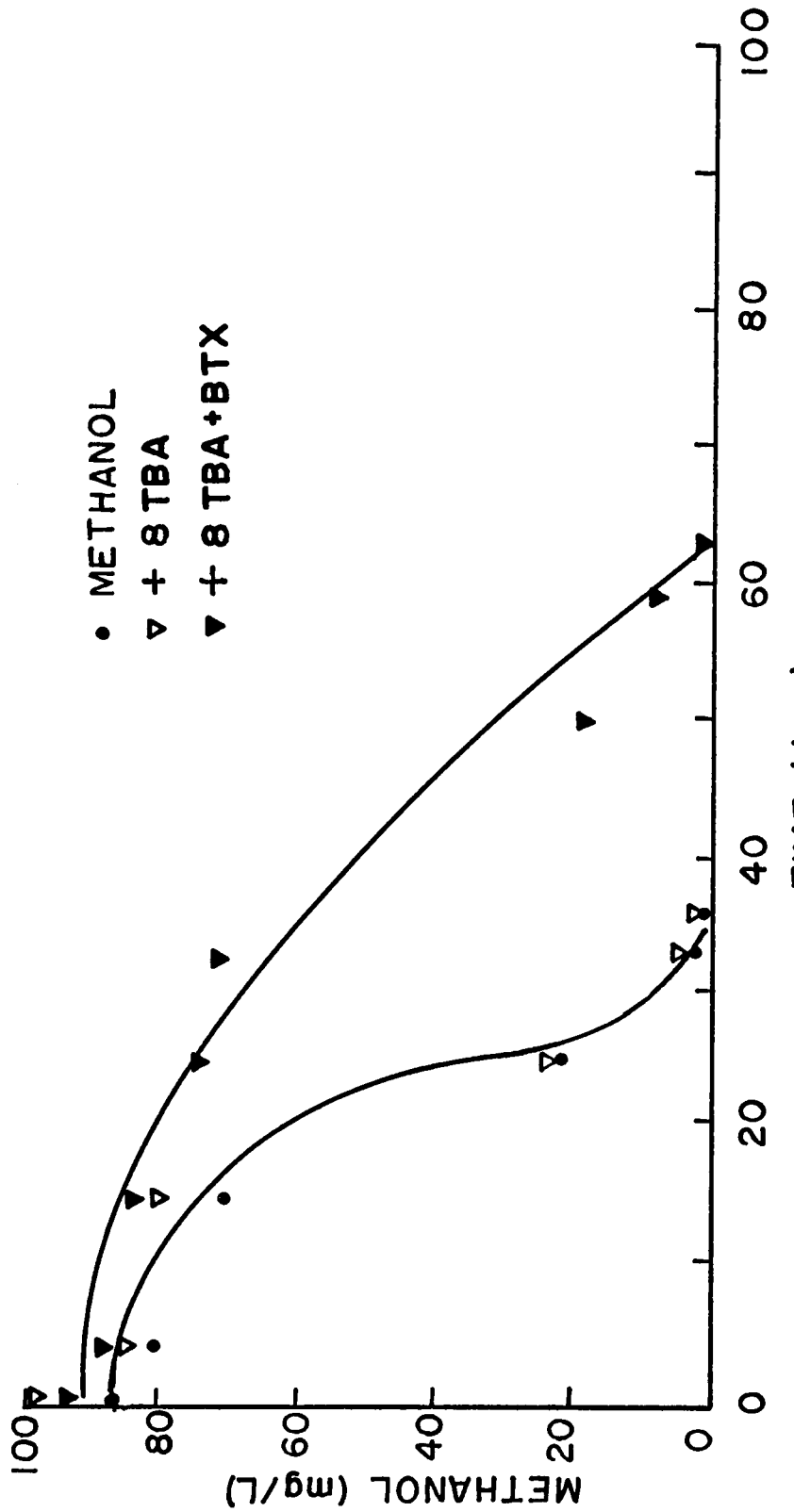


Figure 46. The effect of TBA and TBA + BTX on methanol biodegradation in Virginia soils at 31 M (102 ft).

Methanol alone or methanol and TBA required only 35 days. This could be a reflection of population dynamics, i.e., mostly alcohol degraders or may reflect microcosm differences since the same differences are demonstrated in Figure 40.

TBA. TBA biodegradation at the Virginia site was very slow. Figure 36 demonstrates that Virginia and New York had the same utilization rates for TBA and exhibited a first-order reaction rate. The exact rate values are given in Table 21. The biodegradation of TBA at 60-80 mg/L for all depths studied is shown in Figure 47. Lower concentrations of TBA exhibit the same line slope as shown in Figure 47. Examination of the utilization rates reveals no treatment effects as was found in New York. The kinetic data for TBA was shown to be the same as New York in Figures 37 and 38.

Similarities in kinetic data for both methanol and TBA at the New York and Virginia sites are excellent indications that anaerobic aquifers may react similarly to the same compounds. A change from optimum pH reduces bacterial activity. Based on the mechanism of non-competitive inhibition, the K_s, though, does not change. The maximum rate is reduced at non-optimum pH. The pH of the New York site was 7.8, while at Virginia it was 4.5. Only if these were the optimum pH for these systems would no difference in the maximum rates be expected. Temperatures were equal and do not need to be considered in the comparison.

Control Microcosms. Virginia controls were autoclaved in the same manner as New York. Table 22 shows that these microcosms were

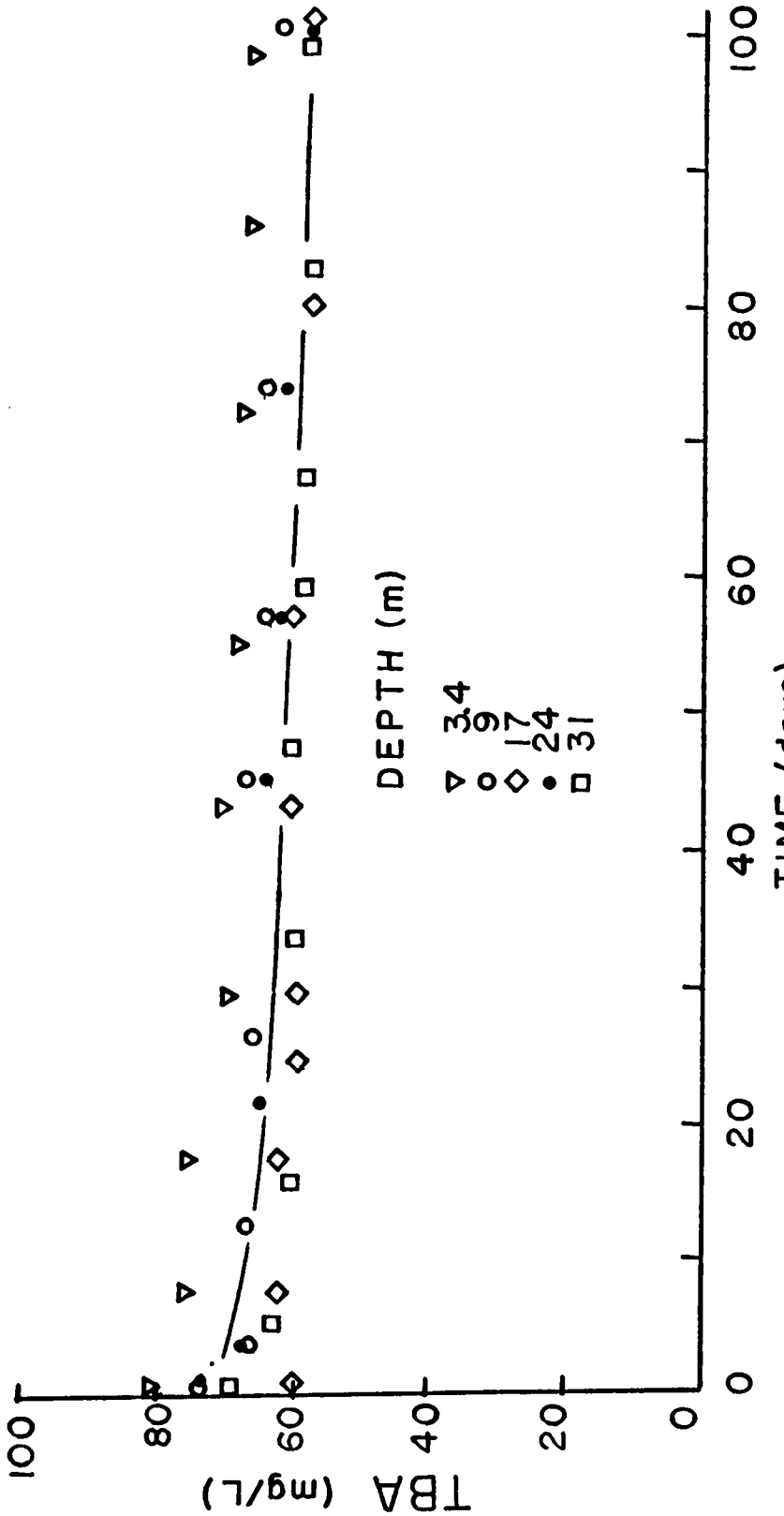


Figure 47. TBA biodegradation at various depths in the Virginia subsurface soils.

Table 22. Methanol, TBA, benzene, toluene, and m-xylene control microcosms for Virginia.

	Concentration (mg/L) at time (days)			
Compounds	0	14	25	69
Methanol/TBA	74.4/7.8	72.4/7.8	70.0/7.6	73.2/7.8
BTX	7.4,16.5,7.0	1.1,2.3,1.0	0.6,1.0,0.3	ND,ND,ND
	82.2/7.4	67.2/7.5	66.2/7.4	66.5/7.2
	5.5,16.4,13.5	1.5,3.5,3.5	0.7,1.0,0.5	ND,ND,ND

effectively sterilized. BTX was lost due to adsorption as shown in Figure 48.

Risk Assessment

Models are based upon proven scientific principles and are generally expanded to include various correction factors to give a more accurate prediction. This is the case in aquifer predictive equations also.

Several mathematical models have been published which describe the movement of contaminant compounds with groundwater through aquifers. These generally deal with the factors of dispersion, adsorption and biodegradation. These factors can only be accurately utilized when specific data are available. For example, the organic content of the soil has been demonstrated to directly correlate with the retention of many compounds. The heterogeneity of aquifer material then complexes the determination of adsorption factors. The total adsorption would be the weighted sum of each aquifer layer down to the aquifer base. More importantly, the values for biodegradation rates are not readily available and may be site specific.

Even the simplest of several equations presented by Hoeks requires that extensive data for an aquifer be available. Hoeks used the equation

$$\frac{C}{C_o} = e^{-\gamma t/(1+R)}$$

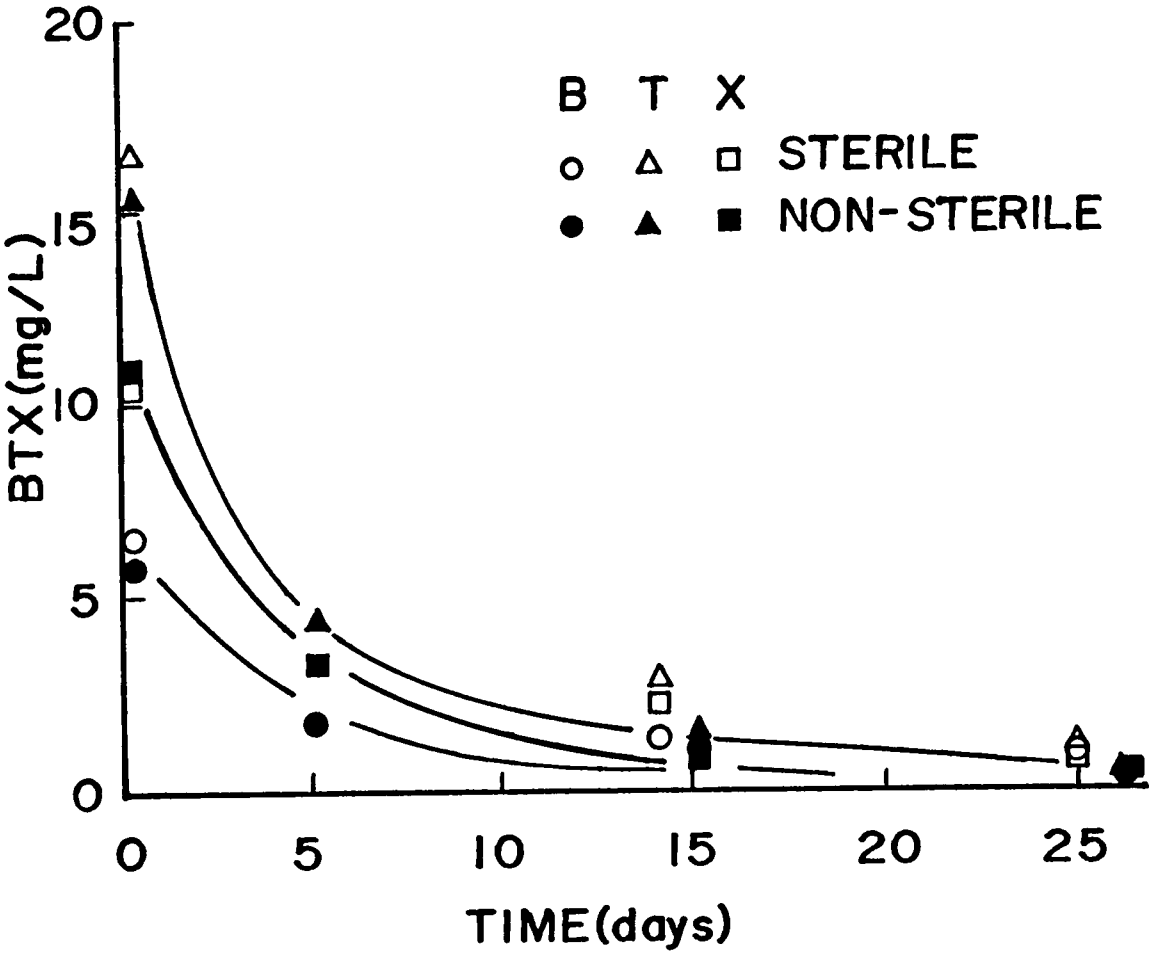


Figure 48. BTX removal in sterile vs. non-sterile microcosms for Virginia soil.

as a means of predicting the concentration of a degradable contaminant at a given time (distance). Where if first order, γ = decay coefficient (yr^{-1}), t = time, R = Kd/e (K_d = adsorption coefficient; e = effective water filled pore volume) or distribution factor and directly affects the retardation term (1+R). If no retardation is assumed

$$\frac{C}{C_o} = e^{-\gamma t}$$

This is simply the expression for first-order reactions.

Simplifying assumptions, therefore, can be stated to aid in the solution of contaminant transport and biodegradation. Groundwater flow can be assumed to be unidirectional, whether it be horizontal, downward or upward, and to have some flow velocity which can be expressed in terms of distance per time. Alcohols are infinitely soluble in water and since they are not readily adsorbed, the velocity of the groundwater and the alcohol contaminants can be assumed equal. Dispersion must be either gotten from field observations or estimated from empirical relationships. Recent aquifer models have considered dispersion to be not fully understood and consistently present approaches that do not include dispersion as a factor in modeling. Deletion of dispersion would only seem to make predictions liberal in nature.

It is reasonable to use basic plug flow reactor equations to predict the time required to remove a substrate since reaction order and biokinetic constants are known and summarized in Table 23. For zero order reactions:

Table 23. Summary of kinetic data from Pennsylvania, New York and Virginia at 10°C.

		Parameter		
Location	Substrate	k(mg/L/day)	K_s(mg/L)	K(day^{-1})
Pennsylvania	MeOH	6.3	--	--
	TBA saturated	0.6	23.5	0.026
	unsaturated	0.4	--	--
New York	MeOH	5.4	200.3	0.027
	TBA	0.7	463.0	0.0018
Virginia	MeOH saturated	5.7	158.3	0.036
	unsaturated	2.3	118.9	0.019
	TBA	0.7	463.0	0.0018

$$t_{PF} = \frac{1}{K}(C_o - C_e)$$

For first-order reactions:

$$t_{PF} = \frac{1}{K} \ln\left(\frac{C_o}{C_e}\right)$$

The amount of time calculated can be directly converted into distance from a point spill where no contamination will remain, if the groundwater velocity is known. Assume the effective water-filled pore volume in the aquifer to be 35%. An initial dilution of the contaminant can be calculated as the concentration at C_o based on an estimate of aquifer volume initially affected. For example, if a surface area of 25 x 25 meters had 5000 L of alcohol spilled and the aquifer extended from the surface to 30 meters there would be 18750 m^3 affected or (18750 x 0.35) 6562.5 m^3 of groundwater available for dilution at time zero. The initial concentration would be (5.0×10^3 L/6.56×10^6 L) 762 ppm by volume. The length of time required for removal is obviously controlled by the magnitude and nature of the spilled compound, percentage of water in the aquifer, and the bacterial population.

To compare the effects of spills at the three sites investigated the time required to remove 1000 mg/L alcohol was calculated for each saturated zone and is reported in Table 24. It is evident from these data that methanol is readily biodegradable at all sites. Only a very high groundwater velocity would present problems. TBA, however, is biodegraded quite slowly and could exist in an anaerobic aquifer for many years. The aerobic Pennsylvania aquifer required approximately 0.4

Table 24. Time and distance requirements for the complete biodegradation of 1000 mg/L methanol and TBA in the saturated zone moving at one m/day.

	Time(yrs)/Distance(m)	
Location	Methanol	TBA
Pennsylvania	0.43^{0}/158.7	1.21/ 442.0
New York	1.17^{1}/426.4	17.52^{1}/6396.0
Virginia	0.88 /319.8	17.52^{1}/6396.0

^{0} - zero order
^{1} - first order

years for TBA biodegradation. In either case for TBA a very slow groundwater velocity would be desired.

The impact of groundwater velocity upon the contaminated distance is alarmingly evident when a velocity of 1 M/day is assumed. An initial spill of only 1000 mg/L methanol would impact 159-427 M. An equal amount of TBA would affect 443 M in the best case and 6396 M in the worst case. The distances were derived, of course, assuming that these compounds are completely degradable. A very important consideration is whether or not a minimum contaminant level exists at which bacterial utilization can no longer occur. This certainly appears to be the case with TBA at anaerobic sites. The low temperature (10°C) increases the K_s, i.e., the amount of substrate needed to achieve half the maximum utilization rate. For example, the TBA K_s for New York and Virginia is 463 mg/L. Therefore, the lower the concentration, the lower the utilization rate becomes. At 1 mg/L TBA the utilization rate was generally in the $2x10^{-3}$ mg/L/day range. For 0.1 mg/L TBA (100 ppb) the rate would be $2x10^{-4}$ mg/L/day, at 0.01 mg/L TBA the resulting rate is $2x10^{-5}$ and so on. A minimum biodegradable concentration may or may not be a level fit for human consumption. These levels have yet to be determined.

SUMMARY AND CONCLUSIONS

Subsurface systems at Williamsport, PA, Wayland, NY, and Dumfries, VA were characterized physically, chemically and biologically. Physical parameters of the groundwater were determined in the field and laboratory. Subsurface soil sampling was done in a quality-controlled aseptic fashion using conventional drilling equipment and samplers. The quantification of bacterial numbers at all depths sampled to 31 m was done by acridine-orange stain direct counts, soil extract agar plate counts and C_1 agar plate counts to determine if the potential for biodegradation existed in subsurface systems. The biodegradation rates of the gasoline additives, methanol and TBA, were studied using test tube microcosms as was the effect of the gasoline components benzene, toluene, and m-xylene upon the alcohol biodegradation. These rates were used to estimate the residence times of the two alcohols in the three systems investigated.

The results described in this dissertation warrant the following conclusions:

1. Bacterial populations exist in substantial numbers to 31 m with minimal depth effects on these numbers by Acridine-orange direct counts, however, soil extract agar plate counts exhibited some population decrease in the unsaturated zone.
2. Methanol was readily biodegradable in all subsurface soils examined both aerobic and anoxic.

3. Methanol biodegradation rates were greater in the saturated zones of anoxic systems.
4. In aerobic aquifer systems TBA was biodegraded more rapidly.
5. Methanol exhibited a retardation of TBA biodegradation in some cases. However, TBA demonstrated no such effect on methanol.
6. Benzene, toluene and m-xylene exhibited no adverse effect on methanol or TBA biodegradation.
7. Kinetic data were found to be similar for anoxic aquifers and both aerobic and anoxic aquifers followed established temperature-substrate utilization reactions.
8. Groundwater contamination by TBA may persist because of its recalcitrant nature, while methanol can be rapidly assimilated by subsurface microorganisms.

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APPENDIX A

Williamsport, PA Biodegradation Data

Table A-1. Methanol biodegradation in the unsaturated zone at 1 mg/L.

Time (days)	Concentration (mg/L) 0	7	24
	1.3	ND*	
	1.0	ND	
	1.2		ND
	1.2		ND
	1.0		ND
	1.0		ND
	1.2		ND
	1.1		ND
	1.3		ND
	1.0		ND

*ND = non-detectable (less than 0.05 mg/L)

Table A-2. Methanol biodegradation in the unsaturated zone at 1 mg/L.

Time (days)	Concentration (mg/L) 0	5	21
	1.4	ND	
	1.0	ND	
	1.6		ND
	1.4		ND
	1.4		ND
	1.2		ND
	1.2		ND
	1.6		ND
	1.1		ND

Table A-3. Methanol biodegradation in the unsaturated zone at 10 mg/L.

Time (days)	Concentration (mg/L) 0	7	24
	11.9	ND	
	14.2	ND	
	11.0		ND
	10.1		ND
	10.5		ND
	11.7		ND
	9.7		ND
	13.4		ND
	9.6		ND
	10.4		ND

Table A-4. Methanol biodegradation in the unsaturated zone at 10 mg/L.

Time (days)	Concentration (mg/L) 0	5	21
	9.2	ND	
	10.9	ND	
	11.6		ND
	10.1		ND
	11.8		ND
	7.4		ND
	7.8		ND
	12.1		ND
	14.3		ND
	8.3		ND

Table A-5. Methanol biodegradation in the unsaturated zone at 100 mg/L.

Time (days)	Concentration (mg/L)					
	0	6	9	11	14	23
	103.9	73.4				
	101.4	67.9				
	118.6		51.2			
	106.2		51.6			
	108.6		55.2			
	109.4			52.0		
	102.2			22.5		
	87.7				ND	
	108.4				33.8	
	106.2					9.8

Table A-6. Methanol biodegradation in the unsaturated zone at 100 mg/L.

Time (days)	Concentration (mg/L)					
	0	5	7	9	12	21
	99.3	57.5				
	94.5	74.0				
	81.7		28.5			
	100.6		16.4			
	94.2		53.1			
	92.5			7.4		
	89.7			33.0		
	103.5				28.1	
	113.6					20.6
	117.5					21.2

Table A-7. Methanol biodegradation in the unsaturated zone at 700 mg/L.

Time (days)	Concentration (mg/L)						
	0	16	20	32	46	54	82
	691	620					
	778	499					
	705		525				
	657		444				
	603			442			
	594			448			
	680				456		
	673				451		
	622					204	
	577						403

Table A-8. Methanol biodegradation in the unsaturated zone at 1000 mg/L.

Time (days)	Concentration (mg/L)						
	0	16	20	32	46	54	82
	1100	732					
	1124	777					
	1386		730				
	926		508				
	1225			609			
	1495			616			
	1209				400		
	1185				486		
	1179					463	
	1282						440

Table A-9. Methanol Biodegradation in the saturated and unsaturated zones at ~1000 mg/L using septum capped microcosms for continuous sampling.

	Concentration (mg/L)								
Time (days)	0	6	20	28	55	137	140	183	232
Unsaturated	1033	938	819	754	685	660	643	495	325
	933	876	791	744	669	654	648		373
	815	789	733	703	671	471	450	403	254
Saturated	923	765	634	574	494		21.2	ND	-
	862	715	645	613	516		89.2	ND	-
	744	649	606	560	480	76.6	64.1	ND	-

Table A-10. Tertiary butyl alcohol biodegradation in the unsaturated zone at 1 mg/L.

Time (days)	Concentration (mg/L) 0	5	11	19	32	52
	0.8	ND*				
	0.7	ND				
	0.6		ND			
	2.0		ND			
	0.7			ND		
	1.0			ND		
	0.8				ND	
	0.7				ND	
	0.6					ND
	0.7					ND

*ND = non-detectable (less than 0.4 mg/L)

Table A-11. Tertiary butyl alcohol biodegradation in the unsaturated zone at 1 mg/L.

Time (days)	Concentration (mg/L)					
	0	7	13	21	34	54
	0.8	0.7				
	1.1	0.9				
	1.0		0.4			
	1.0		0.4			
	1.2			ND		
	1.0			ND		
	1.3				ND	
	1.0				ND	
	1.1					ND
	0.9					ND

Table A-12. Tertiary butyl alcohol biodegradation in the unsaturated zone at 5 mg/L.

Time (days)	Concentration (mg/L)					
	0	5	11	19	32	52
	5.0	5.0				
	4.7	4.8				
	5.0		5.0			
	5.4		5.3			
	5.2			6.0		
	5.3			5.5		
	5.1				5.5	
	4.8				5.1	
	5.3					ND
	5.7					ND

Table A-13. Tertiary butyl alcohol biodegradation in the unsaturated zone at 1-6 mg/L.

Time (days)	Concentration (mg/L) 0	2	5	13
	1.1	0.9	ND	
	1.1	0.6	ND	
	1.0		ND	
	1.1		ND	
	2.3	1.9	0.4	
	2.1	1.8	ND	
	2.8		ND	
	2.3		0.6	
	3.4	2.8	0.8	ND
	3.4		0.8	ND
	3.7	3.1	1.9	ND
	3.7		1.8	ND
	4.4		2.5	ND
	4.9	3.9	2.5	ND
	5.1	4.1	2.8	ND
	5.1		2.2	ND
	5.2	4.7	2.6	ND
	5.5		3.0	ND
	5.6	4.7	3.4	ND
	6.2		4.8	ND

Table A-14. Tertiary butyl alcohol biodegradation in the unsaturated zone at 10 mg/L.

Time (days)	Concentration (mg/L)					
	0	7	13	21	34	54
	11.0	8.8				
	9.9	7.3				
	10.9		6.3			
	6.5		2.5			
	11.5			0.6		
	7.9			2.3		
	8.0				1.7	
	12.3				ND	
	5.7					ND
	7.2					ND

Table A-15. Tertiary butyl alcohol biodegradation in the unsaturated zone at 70 mg/L.

Time (days)	Concentration (mg/L) 0	5	11	19	32	52	56
	70.9	65.7					
	70.7	60.1					
	71.9		52.9				
	61.3		56.8				
	72.2			57.0			
	72.8			57.5			
	74.2				64.1		
	71.4				66.5		
	69.5					55.5	
	72.7						56.1

*ND = non-detectable (less than 0.4 mg/L)

Table A-16. Tertiary butyl alcohol biodegradation in the unsaturated zone at 100 mg/L.

Time (days)	Concentration (mg/L) 0	6	12	20	33	53	57
	126.6	104.0					
	78.2	57.4					
	113.2		71.9				
	71.0		33.3				
	103.7			58.0			
	95.7			56.4			
	112.3				27.7		
	114.9				14.5		
	121.2					36.5	
	71.3						0.4

Table A-17. Tertiary butyl alcohol biodegradation in the saturated zone at 100 mg/L.

	Concentration (mg/L)							
Time (days)	0	6	20	28	55	137	183	232
Unsaturated	74.7	69.7	5.6	ND	-	-		
	85.2	80.9	10.0	ND	-	-		
	80.3	77.4	31.0	11.4	0.5	ND		
Saturated	176.1	135.5	121.7	112.4	96.3	35.7	ND	-
	123.7	101.2	80.3	82.4	78.6	65.2	10.2	ND
	86.1	78.9	71.5	69.9	63.3	9.3	ND	

Table A-18. Methanol/tertiary butyl alcohol biodegradation in the unsaturated zone at 100 mg/L.

Time (days)	Concentration (mg/L)						
	0	11	15	27	41	49	76
	106.0/1.0	ND/0.6					
	122.8/1.1	37.3/0.8					
	109.2/0.9		22.9/0.7				
	114.9/0.9		20.8/0.8				
	115.8/1.0			ND/ND			
	122.0/1.2			ND/ND			
	113.5/0.9				ND/ND		
	79.1/0.7				ND/ND		
	82.2/0.9					ND	
	92.0/0.9						ND

Table A-19. Methanol/tertiary butyl alcohol biodegradation in the unsaturated zone at 100/5 mg/L.

Time (days)	Concentration (mg/L) 0	15	27	41	49	76
	117.1/6.1	63.5/6.8				
	125.6/6.9	62.0/7.4				
	118.4/6.2		40.9/3.6			
	111.1/5.8		65.3/3.1			
	114.1/6.1			52.7/5.7		
	115.5/6.2			24.4/5.7		
	107.7/6.0				30.8/5.2	
	118.2/6.2					16.2/5.3

Table A-20. Methanol/tertiary butyl alcohol biodegradation in the unsaturated zone at 100/5 mg/L.

Time (days)	Concentration (mg/L) 0	11	15	27	41	49	76
	125.7/6.8	63.6/5.6					
	93.7/4.6	68.5/4.7					
	99.7/5.0		32.9/4.5				
	112.2/5.9		62.9/6.2				
	111.0/6.8			11.9/6.3			
	69.4/3.2			ND/3.0			
	103.5/5.3				ND/4.3		
	93.5/4.7				ND/0.1		
	103.6/5.4					ND/0.1	
	105.5/5.7						ND/ND

Table A-21. Methanol/tertiary butyl alcohol biodegradation in the saturated and unsaturated zone at 100/10 mg/L using septum-capped microcosms for continuous sampling.

Time (days)	Concentration (mg/L) 0	6	20	28	55
Unsaturated	119.4/9.8	44.0/7.0	ND/5.1	ND/0.9	ND/0.2
	126.7/11.8	53.8/7.5	ND/6.6	ND/5.4	ND/ND
	87.3/17.3	21.7/5.0	ND/ND	-	-
Saturated	152.8/12.3	17.7/8.3	ND/7.0	ND/6,6	ND/6.9
	153.1/13.0	35.0/8.3	ND/8.3	ND/7.9	ND/7.1
	167.8/20.0	54.1/9.1	ND/9.1	ND/8.6	ND/7.7

Table A-22. Methanol/tertiary butyl alcohol biodegradation in the unsaturated zone at ~700/50 mg/L.

Time (days)	Concentration, mg/L						
	0	11	15	27	41	49	76
	644/42.7	546/44.0					
	823/55.1	727/55.4					
	669/43.8		339/39.4				
	789/52.6		705/55.5				
	666/44.9			503/38.7			
	740/49.3			475/45.4			
	859/57.7				444/40.5		
	902/60.8				525/46.0		
	695/46.8					265/32.1	
	596/40.0						168/25.9

Table A-23. Methanol/tertiary butyl alcohol biodegradation in the saturated and unsaturated zones at 700/50 mg/L using septum capped microcosms for continuous sampling.

Time (days)	Concentration, mg/L 0	6	20	28	55	137	140	183	232
Unsaturated	661/44.7	605/42.2	526/41.9	474/40.5	377/40.8	94.1/39.7	80.8/40.1	ND/37.5	-/ND
	761/50.8	678/48.3	598/49.3	532/46.9	275/43.0	163 /41.9	162 /41.2	ND/29.5	-/ND
	729/49.5	651/46.6	556/46.5	502/44.6	314/41.2	2.9/39.3	ND/37.8	ND/ND	-/ND
Saturated	694/45.9	618/43.0	538/42.5	476/41.8	287/38.8	ND/37.6	-/37.3	ND/ND	
	684/45.7	605/42.9	560/43.5	528/42.9	356/40.9	ND/39.3	-/39.2	ND/ND	
	673/44.4	605/42.2	549/42.5	508/41.5	347/41.2	ND/39.3	-/39.2	ND/ND	

Table A-24. Methanol/tertiary butyl alcohol, benzene, toluene, and m-xylene biodegradation using septum-capped microcosms for continuous sampling.

Time (days)	Concentration, mg/L 0	7	21	34	71
Unsaturated					
MeOH/TBA	99.1/6.7	34.3/6.1	ND/5.6	ND/2.0	ND/ND
BTX	4.6,7.1,3.3	ND,0.7,ND	0.1,0.5,ND	0.1,ND,ND	ND,ND,ND
	89.2/9.8	35.2/5.1	4.5/5.1	ND/0.04	ND/ND
	4.4,6.6,2.9	1.8,1.3,0.6	1.0,0.08,ND	1.3,0.06,ND	ND,ND,ND
	90.5/5.9	31.4/5.3	1.0/0.7	ND/ND	-
	3.2,5.2,3.0	0.5,0.6,ND	0.08,0.4,ND	ND,ND,ND	-
Saturated					
	119.4/9.2	54.3/7.3	ND/7.2	ND/6.0	ND/6.0
	8.2,18.9,17.0	0.7,1.4,2.2	0.1,0.9,ND	ND,ND,ND	ND,ND,ND
	109.8/6.8	31.0/6.5	ND/6.4	ND/6.4	ND/6.1
	2.5,3.4,1.9	0.3,0.7,ND	0.2,0.5,ND	0.8,ND,ND	ND,ND,ND
	126.4/7.2	31.0/6.5	1.2/6.7	ND/6.1	ND/6.3
	8.8,13.6,11.4	1.2,5.6,1.8	0.1,0.9,ND	0.3,0.1,ND	ND,ND,ND

Table A-25. Benzene, toluene, and m-xylene loss from the saturated and unsatruated zones using septum-capped microcosms.

Time (days)	Concentration, mg/L 0	7	21	34	71
Unsaturated	1.9,5.0,2.9	1.4,0.2,ND	0.8,0.2,ND	0.1,ND,ND	ND,ND,ND
	2.0,42.,2.3	0.4,0.6,0.2	1.0,0.7,ND	0.1,ND,ND	ND,ND,ND
	4.3,7.8,4.4	1.0,0.8,0.1	0.2,0.7,ND	0.05,ND,ND	ND,ND,ND
Saturated	5.0,9.7,5.9	1.3,0.8,0.1	0.2,0.7,ND	0.03,ND,ND	ND,ND,ND
	6.9,14.4,9.0	1.4,1.2,0.1	0.1,0.9,ND	0.03,ND,ND	ND,ND,ND
	4.2,7.9,4.5	0.3,1.6,0.2	0.1,0.8,ND	ND,ND,ND	ND,ND,ND

ND = non-detectable (less than 0.03 mg/L)

Table A-26. Biodegradation of methanol and tertiary-butyl alcohol in the unsaturated zone in duplicate continuous sampling sand/soil microcosms.

Time (days)	Concentration (mg/L) 0	7	18	32
Dose				
MeOH	118.6	112.0	105.1	90.9
MeOH	118.0	108.3	94.5	85.7
TBA	5.1	5.0	5.1	3.2
TBA	5.1	5.1	5.8	3.9
TBA	51.2	49.9	47.9	44.7
TBA	50.5	50.5	46.5	43.2
MeOH/TBA	108.2/54.2	110.0/56.1	84.0/51.2	68.9/49.8
MeOH/TBA	102.9/54.8	106.5/55.0	87.1/51.9	82.6/47.9

APPENDIX B

Wayland, NY Biodegradation Data

Table B-1. Methanol and TBA biodegradation in the Wayland, NY saturated zone.

Time (days)	Concentration (mg/L) 0	13	22	30	34	37	44	61
Methanol	104.7	81.9	81.4	75.6	74.4	65.6	27.0	ND
	64.8	64.7	71.4	62.4	-	49.7	23.8	ND
	68.1	69.3	73.3	56.9	-	33.5	7.3	ND
	539	537	577	555	-	568	564	564
	574	568	552	553	548	533	504	475
	479	445	462	440	-	418	394	364
TBA	1.7	-	1.0	1.0	0.94	1.0	0.9	0.9
	1.2	1.0	1.1	1.1	-	1.1	1.1	1.0
	0.9	0.9	1.0	1.0	-	1.0	1.0	1.0
	12.7	12.0	11.8	11.3	11.2	11.0	10.5	10.5
	8.6	8.4	8.2	7.9	-	8.0	7.9	7.6
	10.3	9.6	8.9	9.0	-	9.3	9.0	8.7
	46.7	46.8	45.3	44.6	-	45.2	44.7	42.9
	65.7	61.9	54.4	53.4	-	52.4	48.7	46.5
	121.0	116.4	106.7	101.3	95.2	93.0	88.5	83.1
Methanol/TBA	77.5/11.8	63.2/10.9	54.6/9.8	43.9/9.7	-	36.4/9.8	32.3/9.1	24.7/9.0
	84.1/13.0	65.7/11.9	41.2/12.2	ND/10.2	-	ND/10.0	ND/9.6	ND/8.7
	78.9/10.5	74.1/10.5	70.0/10.3	59.5/9.91	-	48.1/9.9	36.9/9.4	10.91/9.4
	540/114.1	526/114.9	483/107.5	433/101.3	-	267/101.5	ND/94.8	ND/91.1
	380/70.4	371/68.9	371/68.3	361/66.3	-	357/66.8	71.3/70.0	ND/67.2
	615/82.3	585/80.7	572/78.5	546/76.4	-	518/74.8	512/74.6	470/70.2

Table B-1. (Cont.)

Time (days)	Concentration (mg/L) 67	74	88	104	166	206
Methanol						
	561	527	453	410	178	ND
	420	345	165	ND	ND	ND
	334	302	211	88	ND	ND
TBA	0.9	0.8	0.9	0.9	0.9	0.9
	1.0	1.0	0.9		1.0	1.0
	0.9	0.9	0.9	0.8	0.9	0.9
	10.3	10.3	10.3	9.5	9.0	9.3
	7.6	7.5	7.6	7.4	7.1	7.3
	8.6	8.5	8.2	8.1	7.7	7.7
	43.6	42.9	42.8	42.2	39.0	40.6
	44.7	41.3	-	24.8	2.9	0.4
	80.4	77.1	-	65.8	56.1	54.7
Methanol/TBA	15.9/8.7	ND/8.8	-	ND/7.9	-/6.1	/4.1
	ND/8.3	ND/7.0	-	ND/ND	ND/ND	/ND
	ND/9.2	ND/8.9	-	ND/7.7	ND/1.6	/ND
	ND/87.9	ND/86.1	-	ND/76.3	ND/64.8	/58.0
	ND/66.5	ND/67.7	-	ND/65.3	ND/60.8	/61.1
	233/69.2	ND/69.1	-	ND/64.1	ND/60.1	/61.3

Table B-1. (Cont.)

Time (days)	Concentration (mg/L) 0	14	25	29	33	42	69
Methanol/TBA	71.5/5.3	69.4/5.6	22.0/5.3	10.3/5.1	ND/5.0	ND/4.1	
+ BTX	6.6,18.6,8.2	0.3,0.6,0.2	ND,ND,ND				ND/ND
	86.6/6.1	50.9/6.2	27.4/5.8	15.8/5.7	7.3/5.7	ND/5.3	
	5.9,14.9,10.7	2.6,5.1,1.9	ND,ND,ND				ND/2.4

APPENDIX C

Dumfries, VA Biodegradation Data

Table C-1. Biodegradation in Dumfries, VA soil from 11 feet.

Time (days)	Concentration (mg/L) 0	8	18	25	30	33	44	47
Methanol	123.2	103.9	78.7	59.1	49.5	45.2	30.7	29.5
	48.4	49.5	47.2	45.1	45.6	44.8	38.6	35.3
	66.9	71.6	65.6	55.1	54.2	52.3	41.4	38.6
	652	656	661	621	623		603	
	763	768	759	651	637		594	
	757	749	716	621	618		589	
TBA	1.1	1.1	1.1	1.1	1.0		1.0	
	1.1	1.1	1.1	1.1	1.1		1.1	
	0.8	0.7	0.8	0.8	0.8		0.8	
	7.7	7.9	7.9	7.3	7.1		6.9	
	5.0	5.2	5.2	4.9	5.0		4.8	
	77.0	72.3	72.4	68.8	68.3		68.1	
	87.4	76.3	75.6	70.5	69.7		70.5	
	80.9	78.6	79.3	73.3	71.1		72.9	
Methanol/TBA	79.6/8.1	83.8/8.1	72.2/8.0	60.2/7.6	56.1/7.4		50.0/7.3	48.1/7.2
	105.5/10.8	94.7/10.2	85.8/10.5	75.4/10.2	69.7/9.8		62.2/9.3	56.1/9.0
	76.7/7.5	73.6/7.4	60.0/7.4	58.4/7.0	53.2/7.6		49.2/6.7	46.3/6.8

Table C-1. (Cont.)

Time (days)	Concentration (mg/L) 56	60	64	73	79	87	100
Methanol	20.3	16.7	14.2	5.9	2.7	ND	ND
	29.3	23.2	22.4	7.0	6.8	5.9	2.1
	30.9	26.1	25.5	19.3	15.9	12.5	5.5
	592			566		533	532
	588			564		534	534
	561			561		532	539
TBA	1.0			1.0		1.0	1.0
	1.1			1.1		1.0	1.1
	0.8			0.8		0.8	0.8
	6.8			6.7		6.4	6.4
	4.6			4.7		4.7	4.7
	67.3			67.3		64.6	64.7
	69.2			68.7		68.9	68.9
	69.6			67.8		67.7	67.0
Methanol/TBA	40.5/6.9	36.6/6.9	32.6/6.8	23.8/6.8	12.1/6.4	0.8/6.5	ND/6.5
	51.1/8.8	44.2/8.3	43.6/8.7	31.7/8.6	19.5/8.2	12.5/7.9	4.2/8.0
	41.1/6.8	35.4/6.5	33.8/6.6	22.5/6.4	3.3/6.5	ND/6.2	ND/6.3

Table C-1. (Cont.)

Time (days)	Concentration (mg/L) 0	8	18	25	30	33	44	47
Methanol/TBA + BTX	76.4/9.0 4.6,12.5,9.8	77.1/10.4 0.9,3.6,2.1	76.8/10.5 0.6,1.1,0.9	53.2/10.0 0.3,0.1,0.6	50.6/10.1 0.2,ND,ND		44.8/9.7	43.7/10.0
	79.8/6.3 6.0,18.4,14.1	83.9/7.1 0.9,1.9,0.8	59.1/7.0 0.9,1.5,1.4	58.4/6.9 0.1,0.1,0.1	54.8/6.9 ND,ND,ND		48.7/6.3	46.4/6.8
	89.0/6.1	90.6/8.1	86.7/8.5	81.4/8.1	57.6/7.5		47.8/7.5	41.8/7.5

Table C-1. (Cont.)

Time (days)	Concentration (mg/L) 56	60	64	73	79	87	100
Methanol/TBA + BTX	36.8/9.7	31.2/9.3	30.0/9.3	23.9/9.5	14.1/9.4	3.5/9.2	0.1/9.3
	40.6/6.7	29.8/5.9		22.5/6.4	7.3/6.6	ND/6.5	ND/6.5
	29.7/7.0	22.5/6.6	18.7/6.8	5.5/6.9	ND/6.5	ND/6.3	ND/6.4

Table C-2. Biodegradation in Dumfries, VA soil from 30 feet.

Time (days)	Concentration (mg/L) 0	4	13	27	32	35	46	49
Methanol	77.1	77.9	70.1	52.3	49.3	49.0	22.4	16.3
	80.6	79.4	76.8	66.8	67.2	67.1	64.1	64.5
	81.4	80.8	78.5	71.5	71.5	71.4	69.4	67.2
	1032	875	815	731	731		710	
	845	781	754	706	710		703	
	756	710	691	668	671		671	
TBA	1.1	1.0	0.9	0.9	1.0		1.1	
	1.5	1.3	1.3	1.3	1.3		1.3	
	1.4	1.2	1.1	1.1	1.1		1.2	
	16.5	16.4	16.1	15.9	15.6		15.5	
	9.6	10.0	10.0	9.9	9.9		10.0	
	9.5	9.1	9.2	9.1	9.2		9.1	
	76.5	70.3	70.2	67.8	67.3		67.0	
	68.7	60.2	63.2	62.3	63.2		63.9	
	77.9	67.9	68.1	67.9	68.2		69.2	

Table C-2. (Cont.)

Time (days)	Concentration (mg/L) 58	62	66	75	81	89	102
Methanol	ND	ND	ND	ND	ND	ND	ND
	58.4	55.9	52.2	40.3	1.2	ND	ND
	62.8	56.0	53.6	27.1	13.0	4.7	ND
	689		674	664			664
	685		686	671			668
	655		656	654			655
TBA	1.0			1.0			1.0
	1.1			1.1			1.1
	1.1			1.1			1.1
	15.0			15.0			14.6
	9.2			9.6			9.2
	8.7			8.6			8.5
	64.8			64.3			60.3
	62.3			62.3			61.6
	65.8			66.4			65.1

Table C-3. Biodegradation in Dumfries, VA soil from 57 feet.

Time (days)	Concentration (mg/L) 0	8	18	25	30	33	44	49
Methanol	61.9	55.7	55.6	49.6	44.4	37.3	ND	-
	78.2	74.3	71.4	62.9	58.7	56.1	50.9	46.0
	65.3	63.3	62.5	56.2	54.6	53.5	49.5	43.9
	658	666	667	618	622		602	
	730	691	673	604	608		607	
	684	692	697	650	634		625	
TBA								
	0.9	0.9	0.9	0.9	0.8		0.9	
	1.1	1.0	0.9	1.0	1.0		1.0	
	3.7	3.9	3.8	3.5	3.5		3.6	
	8.7	8.2	8.1	7.6	7.3		7.6	
	7.8	7.3	7.3	6.7	6.7		6.7	
	58.1	62.5	61.5	59.9	59.5		59.9	
	60.7	65.1	65.8	62.8	62.0		62.8	
	61.9	60.5	61.9	57.9	57.7		59.5	
Methanol/TBA	72.5/5.6	71.1/5.4	66.9/5.4	59.6/5.3	56.1/5.4		44.8/5.0	37.4/5.0
	65.6/5.7	62.8/6.1	61.9/6.3	55.6/6.2	52.3/6.0		45.6/5.9	42.7/6.1
	66.4/4.5	61.3/4.7	58.1/4.7	51.5/4.7	45.6/4.6		33.1/4.7	28.4/4.5

Table C-3. (Cont.)

Time (days)	58	62	66	75	81	89	102
				Concentration (mg/L)			
Methanol	-	-	-	-			
	27.9	14.1	2.3	ND			
	17.0	ND	-	-			
	523			448			401
	575			524			441
	596			571			509
TBA				0.77			
	0.8				0.8		0.9
	0.9				0.9		0.8
	3.4				3.2		3.2
	7.0				6.7		6.8
	6.3				6.1		6.1
	59.4				58.4		58.4
	61.9				58.6		58.7
	58.3				56.0		56.1
Methanol/TBA	ND/5.0		ND/5.0		ND/5.0		/5.0
	38.1/6.0	32.5/6.0	27.2/6.2	ND/6.01	ND/5.8		/5.9
	0.1/4.6	ND/4.6	-/4.5		ND/4.5		/4.6

Table C-3. (Cont.)

Time (days)	Concentration (mg/L) 0	8	18	25	30	33	44	49
Methanol/TBA + BTX	86.4/6.9 3.4,8.1,3.7	87.2/8.6 1.5,3.9,2.1	62.0/8.8 0.6,0.9,0.5	60.8/8.2 0.3,0.6,0.4	49.2/7.8 ND,ND,ND		44.8/8.0	45.0/7.9
	73.2/5.3 7.9,19.0,6.2	72.7/6.8 3.1,8.1,4.5	55.5/6.9 0.9,1.6,1.0	35.6/6.7 0.4,0.8,0.6	32.8/6.5 ND,0.1,ND		25.4/6.4	26.0/6.7
	81.0/5.5 4.4,10.2,6.9	81.1/6.2 3.9,8.8,4.5	61.9/6.0 0.4,0.5,0.7	60.7/5.5 0.2,0.4,0.2	32.0/5.8 ND,ND,ND		ND/5.9	

Table C-3. (Cont.)

Time (days)	Concentration (mg/L) 58	62	66	75	81	89	102
Methanol/TBA + BTX	39.7/7.7	33.7/7.2	34.0/7.5	32.5/7.5	32.3/7.0	23.8/7.1	0.1/7.0
	22.3/6.6	18.9/6.4	17.0/6.6	ND/6.7	ND/6.4		/6.5
	ND/5.7		-/5.5		ND/5.5		/5.5

Table C-4. Biodegradation in Dumfries, VA soil from 80 feet.

Time (days)	Concentration (mg/L) 0	4	13	27	32	35	46	58
Methanol	97.3	84.7	64.2	ND	ND	ND	ND	
	83.5	82.4	68.6	ND	ND	ND	ND	
	81.7	78.1	75.0	54.1	39.5	16.7	ND	
	828	787	748	647	631		566	545
	1031	803	766	715	681		557	442
	1129	744	729	657	562		426	314
TBA	1.2	0.9	1.1	1.0	1.0		1.0	1.0
	1.2	1.1	1.0	1.1	1.1		1.1	1.0
	0.8	0.8	0.8	0.9	0.9		0.9	0.8
	13.5	9.5	10.0	9.8	9.4		9.7	9.3
	12.4	8.8	8.1	8.1	8.1		8.0	7.5
	9.1	8.5	8.8	8.7	8.6		8.6	7.9
	72.7	66.1	68.2	63.5	63.8		63.1	59.8
	75.7	67.9	66.8	68.2	67.1		65.6	62.2

Table C-4. (Cont.)

Time (days)	Concentration (mg/L) 66	75	81	89	102
Methanol					
	518	519	502	483	479
	378	287	198	148	4.9
	250	191	144	137	90.5
TBA		0.9			0.8
		1.0			0.9
		0.9			0.9
		8.7			8.7
		7.6			7.4
		7.6			7.2
		59.4			55.0
		62.7			61.0

Table C-5. Biodegradation in Dumfries, VA soil from 102 feet.

Time (days)	0	5	16	26	34	37	48	51
				Concentration (mg/L)				
Methanol	69.8	68.2	64.1	24.6	6.8	0.6	ND	
	91.4	90.4	72.4	22.7	ND			
	98.5	85.4	77.8	19.4	0.7	ND		
	883	865	826	684	661		648	
	732	690	641	599	584		560	
	783	727	718	643	626		585	
TBA	1.2	1.3	1.2	1.1	1.1		1.2	
	1.3	1.3	1.2	1.2	1.2		1.1	
	1.4	1.1	1.0	1.2	1.0		1.2	
	8.8	7.6	7.4	7.2	6.9		7.1	
	12.5	11.2	11.4	10.6	10.9		10.9	
	11.7	10.5	10.2	10.3	10.2		10.2	
	66.3	62.7	58.9	59.3	60.4		59.5	
	70.2	61.3	60.3	60.0	59.2		60.1	
	71.1	63.6	61.3	61.7	61.0		62.1	
Methanol/TBA	111.9/7.9	90.0/7.2	86.0/6.7	16.7/7.0	ND/6.9	ND/6.7	ND/6.3	
	93.4/8.3	81.7/7.8	75.4/7.3	24.7/7.8	5.1/7.6	ND/7.4	ND/7.4	
	94.5/8.0	84.5/7.1	81.3/6.8	29.4/7.1	10.2/6.9	2.9/6.8	ND/6.4	

Table C-5. (Cont.)

Time (days)	60	64	68	77	83	104
Methanol						
	557		554	516	499	383
	505		494	479	478	429
	542		528	509	475	472
TBA	1.1		1.1		1.1	0.9
	1.1		1.0		1.0	0.9
	1.1		1.1		1.1	1.0
	6.8		6.8		6.4	5.4
	10.2		10.3		9.7	6.9
	9.6		9.4		8.8	6.4
	57.6		57.3			57.2
	57.2		57.1			57.2
	59.6		59.0			59.3
Methanol/TBA	ND/6.7		-/6.3		/6.1	/5.1
	ND/7.4		-/7.5		/7.2	/6.1
	ND/6.7		-/6.8		/6.3	/4.7

Table C-5. (Cont.)

Time (days)	0	5	16	26	34	37	48	51
				Concentration (mg/L)				
Methanol/TBA + BTX	89.8/8.9 6.3,17.3,12.1	85.3/7.7 2.7,6.3,5.0	80.6/7.0 0.9,1.5,0.6	59.1/7.5 0.5,0.9,0.4	58.3/7.6 ND,ND,ND	53.7/7.6	50.3/7.3	51.7/7.4
	107.1/7.6 5.8,15.8,12.3	101.2/7.2 1.8,4.1,3.2	98.3/6.3 0.9,1.5,0.5	92.6/6.2 0.4,0.6,0.3	89.2/6.6 ND,ND,ND		44.1/6.4	4.7/6.7
	85.4/6.7 4.9,12.0,7.6	79.6/6.6 1.1,2.5,2.0	75.1/6.3 0.5,1.1,0.3	74.3/6.2 0.2,0.3,0.1	71.9,6.3 ND,ND,ND	1.75/6.4	ND/6.1	

Table C-5. (Cont.)

Time (days)	Concentration (mg/L) 60	64	68	77	83	104
Methanol/TBA + BTX	40.6/7.6	ND/7.5	-/7.5		ND/7.4	/7.2
	ND/6.4		-/6.3		ND/6.3	/6.1
	ND/6.0		-/6.1		ND/6.0	/5.8

Table C-6. Methanol Biodegradation at Dumfries, VA for all depths.

Time (days)	Concentration (mg/L)														
Depth (ft.)	0	8	18	25	30	33	44	47	56	60	64	73	79	87	100
11	123.2	103.9	78.7	59.1	49.5	45.2	30.7	29.5	20.3	16.7	14.2	5.9	2.7	ND	ND
	48.4	49.4	47.2	45.1	45.6	44.8	38.6	35.3	29.3	23.2	22.4	7.0	6.8	5.9	2.1
	66.9	71.6	65.6	55.1	54.2	52.3	41.4	38.6	30.9	26.1	25.5	19.3	15.9	12.5	5.5
	0	4	13	27	32	35	46	49	58	62	66	75	81		
30	77.1	77.9	70.1	52.3	49.3	49.0	22.4	16.3	ND	-	-	ND	ND	ND	ND
	80.6	79.4	76.8	66.8	67.2	67.1	64.1	64.5	58.4	55.9	52.2	40.3	1.2	ND	ND
	81.4	80.8	78.5	71.5	71.5	71.4	69.4	67.2	62.8	56.0	53.6	27.1	13.0	4.7	ND
	0	8	18	25	30	33	44	49	58	62	66	75			
57	61.9	55.7	55.6	49.6	44.4	37.3	ND	ND	ND	ND	ND	ND			
	78.2	74.3	71.4	62.9	58.7	56.1	50.9	46.0	27.9	14.1	2.3	ND			
	65.3	63.3	62.5	56.2	54.6	53.5	49.5	43.9	17.0	ND	ND	ND			
	0	4	13	27	32	35	46								
80	97.3	84.7	64.2	ND	ND	ND	ND								
	83.5	82.4	68.6	ND	ND	ND	ND								
	81.7	78.1	75.0	54.1	39.5	16.7	ND								
	0	5	16	26	34	37	48								
102	69.8	68.2	64.1	24.6	6.8	0.6	ND								
	91.4	90.4	72.4	22.7	ND	ND	ND								
	98.5	85.4	77.8	19.4	0.7	ND	ND								

Table C-7. Methanol Biodegradation at Dumfries, VA for all depths.

Time (days) / Depth (ft.)	Concentration (mg/L)											
	0	8	18	25	30	44	56		73		87	100
11	652	656	661	621	623	603	592		566		533	532
	763	768	759	651	637	594	588		564		534	534
	757	749	716	621	618	589	561		561		532	539
	0	4	13	27	32	46	58	66	75			102
30	1032	875	815	731	731	710	689	674	664			664
	845	781	754	706	710	703	685	686	671			668
	756	710	691	668	671	671	655	656	654			655
	0	8	18	25	30	44	58		75			102
57	658	666	667	618	622	602	523		448			401
	730	691	673	604	608	607	575		524			441
	684	692	697	650	634	625	596		571			509
	0	4	13	27	32	46	58	66	75	81	89	102
80	828	787	748	647	631	566	545	518	519	502	483	479
	1031	803	766	615	681	557	442	378	287	198	148	4.9
	1129	744	729	657	562	426	314	250	191	144	137	90.5
	0	5	16	26	34	48		68	77	83		104
102	883	865	826	684	661	648	557	554	516	499		383
	732	690	641	599	584	560	505	494	479	478		429
	783	727	718	643	626	585	542	528	509	475		472

Table C-8. TBA Biodegradation at Dumfries, VA for all depths.

Time (days)	Concentration (mg/L)									
Depth (ft.)	0	8	18	25	30	44	56	73	87	100
11	1.1	1.1	1.1	1.1	1.0	1.0	1.0	1.0	1.0	1.0
	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.0	1.1
	0.8	0.7	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
	0	4	13	27	32	46	58	75		102
30	1.1	1.0	0.9	0.9	1.0	1.1	1.0	1.0		1.0
	1.5	1.3	1.3	1.3	1.3	1.3	1.1	1.1		1.1
	1.4	1.2	1.1	1.1	1.1	1.2	1.1	1.1		1.1
	0	8	18	25	30	44	58	81		102
57										
	0.9	0.9	0.9	0.9	0.8	0.9	0.8	0.8		0.9
	1.1	1.0	0.9	1.0	1.0	1.0	0.9	0.9		0.8
	0	4	13	27	32	46	58	75		102
80	1.2	0.9	1.1	1.0	1.0	1.0	1.0	0.9		0.8
	1.20	1.1	1.0	1.1	1.1	1.1	1.0	1.0		0.9
	0.8	0.8	0.8	0.9	0.9	0.9	0.8	0.9		0.9
	0	5	16	26	34	48	60	68	83	104
102	1.2	1.3	1.2	1.1	1.1	1.2	1.1	1.1	1.1	0.9
	1.3	1.3	1.2	1.2	1.2	1.1	1.1	1.0	1.0	0.9
	1.4	1.1	1.0	1.2	1.0	1.2	1.1	1.1	1.1	1.0

Table C-9. TBA Biodegradation at Dumfries, VA for all depths.

Time (days)	Concentration (mg/L)									
Depth (ft.)	0	8	18	25	30	44	56	73	87	100
11	7.7	7.9	7.9	7.3	7.1	6.9	6.8	6.7	6.4	6.4
	5.0	5.2	5.2	4.9	5.0	4.8	4.6	4.7	4.7	4.7
	0	4	13	27	32	46	58	75		102
30	16.5	16.4	16.1	15.9	15.6	15.5	15.0	15.0		14.6
	9.6	10.0	10.0	9.9	9.9	10.0	9.2	9.6		9.2
	9.5	9.1	9.2	9.1	9.2	9.1	8.7	8.6		8.5
	0	8	18	25	30	46	58	81		102
57	3.7	3.9	3.8	3.5	3.5	3.6	3.4	3.2		3.2
	8.7	8.2	8.1	7.6	7.3	7.6	7.0	6.7		6.8
	7.8	7.3	7.3	6.7	6.7	6.7	6.3	6.1		6.1
	0	4	13	27	32	46	58	75		102
80	13.5	9.5	10.0	9.8	9.4	9.7	9.3	8.7		8.7
	12.4	8.8	8.1	8.1	8.1	8.0	7.5	7.6		7.4
	9.1	8.5	8.8	8.7	8.6	8.6	7.9	7.6		7.2
	0	5	16	26	34	48	60	68	83	104
102	8.8	7.6	7.4	7.2	6.9	7.1	6.8	6.8	6.4	5.4
	12.5	11.2	11.4	10.6	10.9	10.9	10.2	10.3	9.7	6.9
	11.7	10.5	10.2	10.3	10.2	10.2	9.6	9.4	8.8	6.4

Table C-10. TBA Biodegradation at Dumfries, VA for all depths.

Time (days) / Depth (ft.)	Concentration (mg/L)									
	0	8	18	25	30	44	56	73	87	100
11	77.0	72.3	72.4	68.8	68.3	68.1	67.3	67.3	64.6	64.7
	87.4	76.3	75.6	70.5	69.7	70.5	69.2	68.7	68.9	68.9
	80.9	78.6	79.3	73.3	71.1	72.9	69.6	67.8	67.7	67.0
	0	4	13	27	32	46	58	75		102
30	76.5	70.3	70.2	67.8	67.3	67.0	64.8	64.3		60.3
	68.7	60.2	63.2	62.3	63.2	63.9	62.3	62.3		61.6
	77.9	67.9	68.1	67.9	68.2	69.2	65.8	66.4		65.1
	0	8	18	25	30	44	58	81		102
57	58.1	62.5	61.5	59.9	59.5	59.9	59.4	58.4		58.4
	60.7	65.1	65.8	62.8	62.0	62.8	61.9	58.6		58.7
	61.9	60.5	61.9	57.9	57.7	59.5	58.3	56.0		56.1
	0	4	13	27	32	46	58	75		102
80	72.7	66.1	68.2	63.5	63.8	63.1	59.8	59.4		55.0
	75.7	67.9	66.8	68.2	67.1	65.6	62.2	62.7		61.0
	0	5	16	26	34	48	60	68		104
102	66.3	62.7	58.9	59.3	60.4	59.5	57.6	57.3		57.2
	70.2	61.3	60.3	60.0	59.2	60.1	57.2	57.1		57.2
	71.1	63.6	61.3	61.7	61.0	62.1	59.6	59.0		59.3

Table C-11. Methanol with TBA biodegradation at Dumfries, VA for all depths.

Time (days) / Depth (ft.)	Concentration (mg/L)							
	0	8	18	25	30	44	47	56
11	79.6/8.1	83.8/8.1	72.2/8.0	60.2/7.6	56.1/7.4	50.0/7.3	48.1/7.2	40.5/6.9
	105.5/10.8	94.7/10.2	85.8/10.5	75.4/10.2	69.7/9.8	62.2/9.3	56.1/9.0	51.1/8.8
	76.7/7.5	73.6/7.4	60.0/7.4	58.4/7.0	53.2/7.0	49.2/6.7	46.3/6.8	41.1/6.8
	0	8	18	25	30	44	49	58
57	72.5/5.6	71.1/5.4	66.9/5.4	59.6/5.3	56.1/5.4	44.8/5.0	37.4/5.0	ND/5.0
	65.6/5.7	62.8/6.1	61.9/6.3	55.6/6.2	52.3/6.0	45.6/5.9	42.7/6.1	38.1/6.0
	66.4/4.5	61.3/4.7	58.1/4.7	51.5/4.7	45.6/4.6	33.1/4.7	28.4/4.5	0.1/4.6
	0	5	16	26	34	37	48	60
102	111.9/7.9	90.0/7.2	86.0/6.7	16.7/7.0	ND/6.9	ND/6.7	ND/6.3	/6.7
	93.4/8.3	81.7/7.8	75.4/7.3	24.7/7.8	5.1/7.6	ND/7.4	ND/7.4	/7.4
	94.5/8.0	84.5/7.1	81.3/6.8	29.4/7.1	10.2/6.9	2.9/6.8	ND/6.4	/6.7

Table C-11. (Cont.)

Time (days)	Concentration (mg/L)					
Depth (ft.)	60	64	73	79	87	100
11	36.6/6.9	32.6/6.8	23.8/6.8	12.1/6.4	0.8/6.5	ND/6.5
	44.2/8.3	43.6/8.7	31.7/8.6	19.5/8.2	12.5/7.9	4.2/8.0
	35.4/6.5	33.8/6.6	22.5/6.4	3.3/6.5	ND/6.2	ND/6.3
	62	66	75	81		102
57		-/5.0		ND/5.0		/5.0
	32.5/6.0	27.2/6.2	ND/6.01	ND/5.8		/5.9
	ND/4.6	-/4.5		ND/4.5		/4.6
		68		83		104
102		/6.3		/6.1		/5.1
		/7.5		/7.2		/6.1
		/6.8		/6.3		/4.7

Table C-12. Methanol, TBA and BTX biodegradation at Dumfries, VA for all depths.

Time (days)	Concentration (mg/L)							
Depth (ft.)	0	8	18	25	30	44	47	56
11	76.4/9.0 4.6,12.5,9.8	77.1/10.4 0.9,3.6,2.1	76.8/10.5 0.6,1.1,0.9	53.2/10.0 0.3,0.1,0.6	50.6/10.1 0.2,ND,ND	44.8/9.7	43.7/10.0	36.8/9.7
	79.8/6.3 6.0,18.4,14.1	83.9/7.1 0.9,1.9,0.8	59.1/7.0 0.9,1.5,1.4	58.4/6.9 0.1,0.1,0.1	54.8/6.9 ND,ND,ND	48.7/6.3	46.4/6.8	40.6/6.7
	89.0/6.1 3.7,9.3,4.6	90.6/8.1 1.0,2.2,1.2	86.7/8.5 0.3,0.4,0.2	81.4/8.1 ND,ND,ND	57.6/7.5	47.8/7.5	41.8/7.5	29.7/7.0
	0	8	18	25	30	44	49	58
57	86.4/6.9 3.4,8.1,3.7	87.2/8.6 1.5,3.9,2.1	62.0/8.8 0.6,0.9,0.5	60.8/8.2 0.3,0.6,0.4	49.2/7.8 ND,ND,ND	44.8/8.0	45.0/7.9	39.7/7.7
	73.2/5.3 7.9,19.0,6.2	72.7/6.8 3.1,8.1,4.5	55.5/6.9 0.9,1.6,1.0	35.6/6.7 0.4,0.8,0.6	32.8/6.5 ND,0.1,ND	25.4/6.4	26.0/6.7	22.3/6.6
	81.0/5.5 4.4,10.2,6.9	81.1/6.2 3.9,8.8,4.5	61.9/6.0 0.4,0.5,0.7	60.7/5.5 0.2,0.4,0.2	32.0/5.8 ND,ND,ND	ND/5.9		ND/5.7

Table C-12. (Cont.)

Time (days)	Concentration (mg/L) 60	64	73	79	87	100
Depth (ft.)						
11	31.2/9.3	30.0/9.3	23.9/9.5	14.1/9.4	3.5/9.2	0.1/9.3
	29.8/5.9		22.5/6.4	7.3/6.6	ND/6.5	ND/6.5
	22.5/6.6	18.7/6.8	5.5/6.9	ND/6.5	ND/6.3	ND/6.4
	62	66	75	81	89	102
57	33.7/7.2	34.0/7.5	32.5/7.5	32.3/7.0	23.8/7.1	0.1/7.0
	18.9/6.4	17.0/6.6	ND/6.7	ND/6.4		/6.5
		-/5.5		ND/5.5		/5.5

Table C-12. (Cont.)

Time (days)	Concentration (mg/L) 0	5	16	26	34	37	48	51
Depth (ft.)								
102	89.8/8.9	85.3/7.7	80.6/7.0	59.1/7.5	58.3/7.6	53.7/7.6	50.3/7.3	51.7/7.4
	6.3,17.3,12.1	2.7,6.3,5.0	0.9,1.5,0.6	0.5,0.9,0.4	ND,ND,ND			
	107.1/7.6	101.2/7.2	98.3/6.3	92.6/6.2	89.2/6.6		44.1/6.4	4.7/6.7
	5.8,15.8,12.3	1.8,4.1,3.2	0.9,1.5,0.5	0.4,0.6,0.3	ND,ND,ND			
	85.4/6.7	79.6/6.6	75.1/6.3	74.3/6.2	71.9/6.3	1.75/6.4	ND/6.1	
	4.9,12.0,7.6	1.1,2.5,2.0	0.5,1.1,0.3	0.2,0.3,0.1	ND,ND,ND			

Table C-12. (Cont.)

Time (days) / Depth (ft.)	Concentration (mg/L) 60	64	68	83	104
102	40.6/7.6	ND/7.5	/7.5	/7.4	/7.2
	ND/6.4		/6.3	/6.3	/6.1
	ND/6.0		/6.1	/6.0	/5.8

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