

Biodegradation of Tertiary Butyl Alcohol by an Introduced
Pseudomonas sp. in Subsurface Soil Microcosms

by

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Abstract

Microcosm studies determined that the indigenous microflora of the subsurface Groseclose clayey loam did not metabolize TBA, but TBA degradation was stimulated by the addition of the Pseudomonas sp., VT100. The indigenous subsurface microflora of the Fredrick clay metabolized TBA in soil microcosms that contained 10 to 500 mg/liter TBA. The addition of Pseudomonas sp., VT100, to the Fredrick clay enhanced the rate of TBA degradation in the 10 and 500 mg/liter TBA treatments, and reduced the time required for the initiation of TBA degradation. The indigenous Fredrick clay microflora reduced the growth and TBA degrading potential of the Pseudomonas sp.. The addition of yeast extract and acetate to the Groseclose clayey loam allowed the indigenous microflora to initiate TBA degradation and enhanced TBA degradation in the sterile Groseclose clayey loam inoculated with Pseudomonas sp., VT100. We hypothesize that the native TBA-degrading microflora competed with the Pseudomonas sp. in both soils. A 1.0 mM molybdate amendment stimulated TBA degradation by the indigenous micorflora but reduced the

rate of TBA degradation by Pseudomonas sp., VT100, in subsurface soil microcosms. A TBA-degrading Alcaligenes sp. was isolated from the Fredrick clay. Limited TBA degradation was observed in one of three deep subsurface coastal plain soils after 220 days of incubation.

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INTRODUCTION

Tertiary butyl alcohol (TBA) is a recalcitrant compound that is used as a gasoline additive (18). It is also a by-product in the production of a number of industrial chemicals (11). TBA is soluble in water, therefore, it represents a potential threat to groundwater environments in the event of a chemical spill. The xenobiotic nature of TBA was first demonstrated by Horn (11). Subsequently, Novak et al. (18) showed that TBA can be degraded in subsurface soil microcosms, but that the rate of TBA degradation was dependent on the abiotic characteristics and environmental history of the soil. Recently, a Pseudomonas sp., which could use TBA as a carbon and energy source, was isolated from subsurface soil obtained from a site with a history of hydrocarbon contamination (3). This culture was used in this study.

Well developed microbial populations have been found to be present in both shallow (7,8) and deep subsurface (7,25) environments. These microbial communities may have the potential to degrade some commonly encountered groundwater pollutants (28). While indigenous aquatic and soil microorganisms have a demonstrable capacity to degrade a number of recalcitrant compounds, activity is often preceded by an extended adaptation or lag period (4,19)

The use of introduced microorganisms has been proposed as an alternative to physical and chemical methods to enhance the remediation of contaminated subsurface environments (6,17). The introduction of non-indigenous microorganisms into a new habitat to promote the biodegradation of recalcitrant compounds has had varying success. A strain of Pseudomonas cepacia, capable of utilizing trichlorophenoxyacetic acid (2,4,5-T), degraded 90% of the 2,4,5-T present in a heavily contaminated soil (12). The mineralization of four polycyclic aromatic hydrocarbons (PAH) in pristine freshwater sediment was enhanced by an introduced (PAH)-degrading Mycobacterium sp. (10). In contrast, a 10^8 cells per g soil inoculum of an oil degrading bacterium failed to enhance the biodegradation of light and heavy oil in soil (14). Competition with indigenous microflora can inhibit the colonization and activity of introduced microorganisms in some soils. A 4-(2,4-dichlorophenoxy) butyric acid (4-(2,4-DB)-degrading Flavobacterium sp., which actively degraded 4-(2,4-DB) in sterile soil, did not degrade 4-(2,4-DB) in nonsterile soil (15). Goldstein et al. (9) hypothesized that the success of an introduced microorganism is dependent on: the ability of the microorganism to initiate degradation even at low pollutant concentrations, withstand biotic and abiotic

stresses of the given environment, selectively degrade the organic pollutant as opposed to other organic compounds, and move through soil to contaminated sites.

This study utilized subsurface soil microcosms to determine if an introduced Pseudomonas sp. could initiate TBA degradation in two pristine subsurface soils. We ascertained the TBA degrading potential of the indigenous microflora in several shallow and deep subsurface soils and attempted to isolate another TBA-degrading microorganism from these soils. The most-probable-number (MPN) technique was used to measure the growth and survival of the introduced bacterium in the subsurface soils. Finally, the effect of inoculum size, organic supplements and a molybdate amendment on TBA degradation was studied. In this study, we report the first successful stimulation of TBA degradation in uncontaminated subsurface soil by an introduced Pseudomonas sp.

Materials and Methods

Chemicals and reagents. TBA (purity, >99%) was purchased from Fisher Scientific Co. (Raleigh, N. C.). Pure ethyl alcohol U. S. P. was procured through the Warner-Graham Co. (Cockeysville, MD.). All other chemicals were reagent grade and obtained from either Fisher Scientific Co. or J. T. Baker Chemical Co. (Phillipsburg, N.J.). All media and agar were obtained from Difco Laboratories (Detroit, Michigan).

Cultures. The Pseudomonas sp. VT100, used in this study, was isolated from subsurface soil obtained from a site with a long term history of hydrocarbon contamination (3). Chaddock (3) demonstrated that this bacterium could utilize TBA as a sole carbon and energy source, but growth was inhibited when the TBA concentration exceeded 500 mg/liter. Pseudomonas sp. VT100 was grown and maintained under static conditions at 25°C in 500 ml Erlenmeyer flasks containing 200 ml of a defined TBA medium. This defined TBA medium contained (per liter): K_2HPO_4 , 166.7 mg; KH_2PO_4 , 83.3 mg; $(NH_4)_2SO_4$, 1.0 g; $CaCl_2 \cdot 2H_2O$, 83.3 mg; $MgSO_4 \cdot 7H_2O$, 83.3 mg; ferric citrate, 1.7 mg; and TBA, 250 mg. The K^{1+} salts and TBA were prepared as a single filter sterilized stock solution (pH 7.5) and added to the remaining autoclaved solution after it had cooled. Prior to autoclaving, the pH of the NH_4^+ , Ca^{2+} , Mg^{2+} salts

solution was adjusted to 8.0 with 1.0 N NaOH. The following succinate medium was used to check culture purity (per liter): succinic acid, 1.0 g; sodium acetate, 1.0 g; yeast extract, 1.0 g; peptone, 0.5 g; agar, 15.0 g (pH 7.5).

A second TBA degrading culture was isolated from the Fredrick clay. Enrichment cultures were established by adding 2 to 3 g of Fredrick clay to 500 ml Erlenmeyer flasks containing 150 ml of defined TBA medium amended with 1 ml/liter of a trace element solution (13). The culture was incubated at 25°C for 3 months, then streaked to purity on defined TBA medium amended with 15 g agar. Small, well defined colonies were visible after 3 to 5 days incubation at 25°C. Well isolated colonies were transferred to and cultured in 125 ml Erlenmeyer flasks containing 75 ml of the defined TBA medium plus trace element solution. To determine culture purity, the soil isolate was grown on succinate agar, typticase soy agar, plate count agar, yeast extract agar, and egg yolk agar. The N/F system (Flow Laboratories, Inc., McLean, Virginia) was used to tentatively identify this soil isolate.

Soil Samples. The 2 soils used in this study were obtained from a wooded area on the Virginia Tech cattle research farm. This location was assumed to have no prior exposure to hydrocarbon contamination. Soil samples were

collected using a hand auger and transferred to sterile screw cap jars. The soil was transported back to the laboratory within 1 hour of collection and stored at 4°C. The 2 soil samples, collected from 3 and 4 meters depth, belonged to the Frederick and Groseclose series respectively. The Frederick sample was a fine clay and the Groseclose sample was a clayey loam (5). The pH of the soils were 4.7 and 4.5 respectively, as determined using a 1:1 soil water slurry (20). The organic content of the fine clay was 0.19% while the clayey loam had an organic content of 0.15% (22). The heterotrophic aerobic microflora of the two soils were enumerated by a serial dilution/agar plate count technique using yeast extract and 0.1% yeast extract-0.1% peptone-0.1% glucose agar plates. The indigenous TBA degrading populations were enumerated using a most probable number (MPN) technique. A serially diluted 10 g sample of each soil served as the inoculum for the MPN tubes, which contained the defined TBA medium. The sulfate reducing populations of the 2 soils were enumerated by a MPN procedure which utilized Postgate's medium E (21). All MPN determinations of each soil were completed in duplicate.

Deep subsurface soil samples were obtained from the Allendale, South Carolina drill site from Dr. T. J. Phelps, Institute of Applied Microbiology, Knoxville,

Tenn. These soils belonged to the following formations: the Middendorf sand (416 m depth), the Middendorf clay (458 m depth), and the Cape Fear sand (463 m depth). The viable count of these soils did not change significantly during storage (data not shown).

Laboratory Microcosms. Laboratory microcosms were initiated at four concentrations of TBA (mg/liter): 10, 100, 250, and 500. TBA mineralization was monitored by measuring the change in TBA concentration over time in soil microcosms containing: indigenous microorganisms, sterile and nonsterile soil inoculated 8.6×10^5 and 8.6×10^6 Pseudomonas sp. VT100 cells per g soil (dry weight) inoculums, and sterile uninoculated control soil. Sterile, acid cleaned 13 x 100 mm culture tubes were filled with 5.5 g (dry weight) of subsurface soil. The soil was amended with a filter sterilized stock solution of TBA and a 1 ml washed Pseudomonas sp., VT100, inoculum, where applicable, to establish a total liquid volume of 6.5 ml. The microcosms were sealed with open top screw caps fitted with teflon backed silicon septa (Pierce Co., Rockford, Ill.) to accommodate sampling. The Pseudomonas sp., VT100, was harvested from the defined TBA medium after 4 weeks of incubation. The cell suspension was prepared by centrifugation at 12,500 Xg for 15 minutes and the pellet was resuspended in 0.01 M phosphate buffer (pH

7.5). To determine the TBA degrading potential of deep subsurface bacteria, sterilized Groseclose clayey loam (250 mg/liter TBA) was inoculated with 1.0 ml from 10^{-1} dilutions of the Middendorf sand, the Middendorf clay, and the Cape Fear sand. Sterilized soil used throughout this study was prepared by repeated autoclaving. All experimental microcosms were established in triplicate and incubated in the dark at 25°C , except for the Middendorf sand, the Middendorf clay, and the Cape Fear sand samples which were established as single treatments. The rate of TBA degradation was determined within the linear phase of the TBA degradation curve. All reported rates of TBA degradation and percent losses were corrected by subtracting the rate or amount TBA volatilization in the sterile controls over the entire incubation period.

The effect of molybdate on TBA mineralization was determined by amending a series of microcosms containing the Fredrick fine clay, and four concentrations of TBA, with 1.0 mM molybdate in the form of sodium molybdate. A subset at each TBA concentration was inoculated with 8.6×10^6 Pseudomonas sp., VT100, cells per g soil (dry weight). The effect of a readily degradable carbon supplement on TBA mineralization was determined by amending microcosms (250 mg/liter TBA) containing the Groseclose clayey loam with either yeast extract, ethyl alcohol or acetate in the

form of sodium acetate, at 10 and 100 mg/liter concentrations. The supplementary carbon sources were added as autoclaved stock solutions. Additional treatments included: the addition of 8.6×10^6 Pseudomonas sp., VT100, cells per g soil (dry weight), and 1.0 mM molybdate additions to inoculated and uninoculated microcosms. TBA degradation in inoculated and uninoculated microcosms that did not receive any supplemental carbon source was also monitored. Sterilized control microcosms amended with each organic supplement were also monitored for TBA losses.

Quantitative Analysis. The TBA concentration in the subsurface soil microcosms was monitored with a Hewlett-Packard 5880A Gas Chromatograph (Hewlett Packard, Avondale, PA.) fitted with a flame ionization detector and a stainless steel column packed with 0.2% Carbowax 1500 on 80/100 mesh Carbopak C (Supleco Inc., Bellefonte, PA.). The oven was operated isothermally at 110°C , the injector temperature was 125°C , and the detector temperature was 185°C .

Enumeration of TBA Degraders. The Fredrick clay microcosms were sacrificed at the end of the experiment, and a known quantity of subsurface soil was removed from each replicate to form a pooled sample. The pooled sample was serially diluted and used to inoculate MPN tubes

containing defined TBA medium. The inoculated MPN tubes were incubated for 3 months at 25°C. MPN tubes were scored positive when the TBA concentration in the inoculated tubes was significantly below the average of 10 uninoculated control tubes. The TBA mineralizing population of each pooled sample was calculated by the method of Alexander (1). The reported populations in the inoculated nonsterile Fredrick clay included the Pseudomonas sp., VT100, and the native TBA degrading microflora.

RESULTS

Microbiological characteristics of subsurface soils.

TBA-degrading microorganisms were present in both of the Blacksburg soils. The Fredrick clay contained 45 indigenous TBA-degrading microorganisms per g soil (dry weight) while the Groseclose clayey loam had 9 TBA degraders per g soil (dry weight). There were $2.2 \pm 0.2 \times 10^6$ aerobic, heterotrophic microorganisms and $6.1 \pm 0.4 \times 10^2$ sulfate reducing bacteria present per g soil (dry weight) in the Fredrick clay. The respective populations in the Groseclose clayey loam were $1.3 \pm 0.8 \times 10^5$ and 6.6×10^1 cells per g soil (dry weight).

The TBA-degrading bacterium isolated from the Fredrick fine clay was oxidase positive, catalase positive, urease positive, lipase negative, gram negative, rod shaped and a nonfermenter. It was able to oxidize acetimide but not glucose, xylose, mannitol, lactose, maltose, ONPG, or esculin. Also no H_2S , or indole production, and no visible or fluorescent pigment production was observed. This organism was named VT200 and tentatively identified as an Alcaligenes sp., based on the results of the N/F System tests (Flow Laboratories, McLean, VA.). This bacterium had 5 different biochemical traits from the Pseudomonas sp. VT100. The TBA degradation trait appears to be unstable in aqueous culture. When the bacteria obtained

from a TBA enrichment are transferred, their ability to degrade TBA may be lost, although cell turbidity may be observed in the defined TBA medium. During the culture purification process, the TBA degrading trait may be lost much more frequently than in enrichment cultures (data not shown). During this study, Alcaligenes VT200 lost the ability to utilize TBA.

TBA degradation in different soils At an initial TBA concentration of 250 mg/liter, TBA degradation was initiated in the Fredrick clay after a 49 day lag period and 64% of the TBA was degraded after the 136 day incubation (Fig. 1). No TBA degradation was detected in the Groseclose clayey loam during 140 days of incubation. The deep subsurface microflora of the Middendorf clay initiated TBA degradation after 77 days of incubation and degraded 14% of the TBA over a 220 day incubation period, at a rate of 0.66 mg/liter/day. The native microflora in the Middendorf sand, and the Cape Fear sand did not initiate TBA degradation over this same incubation period.

TBA degradation in the Fredrick clay was enhanced by the addition of 10^6 Pseudomonas sp. VT100 cells per g soil (dry weight). After 115 days of incubation, TBA was mineralized to below detectable concentrations (Fig. 1). The addition of 10^6 Pseudomonas sp. cells per g soil (dry weight) to the Groseclose clayey loam promoted the

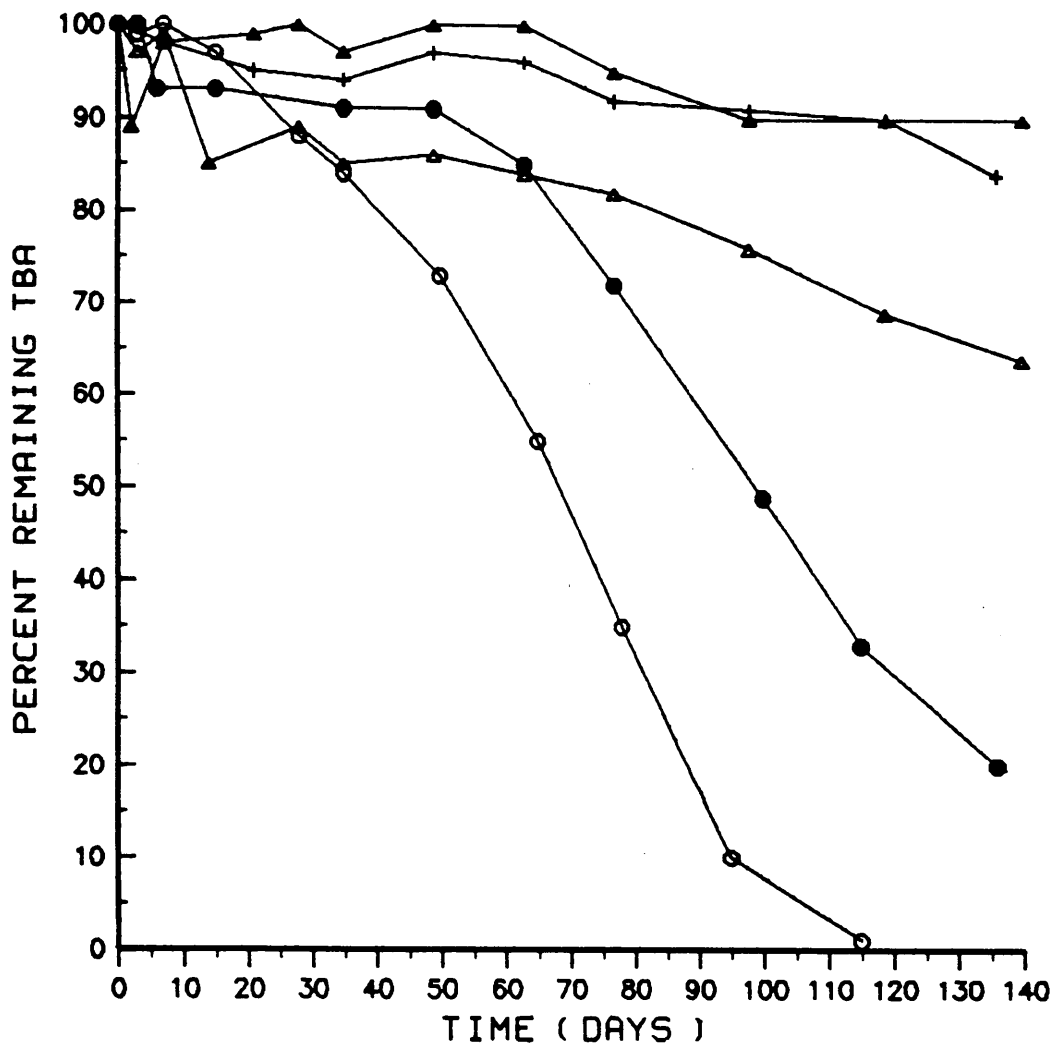


Figure 1. Degradation of tertiary butyl alcohol (TBA) in two subsurface soils amended with 250 mg/liter of TBA. Treatments include: ● uninoculated Fredrick clay, ○ Fredrick clay inoculated with 10^6 *Pseudomonas* sp. VT100 cells per g soil (dry weight), ▲ uninoculated Groseclose clayey loam, △ Groseclose clayey loam inoculated with 10^6 *Pseudomonas* sp. VT100 cells per g soil (dry weight), and + sterile control soil.

initiation of TBA degradation. The rate of TBA degradation was slower than in the inoculated Fredrick clay and only 20% of the TBA was degraded after 140 days of incubation. In both inoculated sterile soils, the Pseudomonas sp. VT100 alone promoted TBA degradation (Table 1).

Effect of initial TBA concentration on TBA biodegradation. The indigenous TBA-degrading microflora in the Fredrick clay was functional at all four initial concentrations of TBA. The maximum rate of TBA degradation of the indigenous microflora was observed at the intermediate concentration of 100 mg/liter TBA (Table 1). At this concentration, the rate of TBA degradation was more pronounced than in the same soil inoculated with the TBA-degrading Pseudomonas sp., VT100, culture. As the initial concentration of TBA was increased or decreased, the rate of TBA degradation in the Fredrick clay decreased (Table 1). The addition of 10^6 Pseudomonas sp., VT100, cells per g soil (dry weight) to the Fredrick clay microcosms amended with either 100 or 250 mg/liter TBA did not accelerate the rate of TBA degradation (Table 1). However, the 10^6 Pseudomonas sp., VT100, cells per g soil (dry weight) inoculum did accelerate the rate of TBA degradation in the Fredrick clay, dosed with 10 and 500 mg/liter TBA, as compared to the uninoculated soil

Table 1. Rates of TBA degradation by indigenous microflora, and 10^5 or 10^6 Pseudomonas sp. per g soil (dry weight) in sterilized and nonsterilized soil. Degradation rates corrected for abiotic TBA losses in sterile soil controls.

Treatment	Degradation rate(mg/l/day)	
	Inoculum(cells/g soil(dry weight))	
	None	10^6
Soil: Fredrick clay.		
10 mg/liter TBA		
soil w/ indigenous flora	0.4	
sterile soil + Strain VT100		1.1
indigenous flora + Strain VT100		1.2
100 mg/liter TBA		
soil w/ indigenous flora	2.5	
sterile soil + Strain VT100		4.6
indigenous flora + Strain VT100		2.2
250 mg/liter TBA		
soil w/ indigenous flora	2.0	
sterile soil + Strain VT100		2.7
indigenous flora + Strain VT100		2.2
500 mg/liter TBA		
soil w/ indigenous flora	1.8	
sterile soil + Strain VT100		3.0
indigenous flora + Strain VT100		2.8
Soil: Groseclose clayey loam.		
250 mg/liter TBA		
soil w/ indigenous flora	0	
sterile soil + Strain VT100		0.7
indigenous flora + Strain VT100		0.6

microcosms. This effect was more pronounced in the 10 mg/liter treatment (Fig. 2) than in the 500 mg/liter treatment (Fig. 3). Since the highest rates of TBA degradation observed in this experiment were in the sterile soil inoculated with the Pseudomonas sp. and the Pseudomonas sp., VT100, is least effective where the indigenous microflora are most active, it is our hypothesis that the indigenous TBA degrading microflora may directly compete with the Pseudomonas sp., VT100, inoculum. This competition may reduce the potential of the introduced Pseudomonas sp. to degrade TBA, at TBA concentrations of 100 mg/liter or above, in soil environments.

The 10^5 Pseudomonas sp., VT100, per g soil (dry weight) inoculum exhibited a lower rate of TBA degradation than the larger Pseudomonas sp., VT100, inoculum in the 10 and 100 mg/liter TBA treatments. In the sterile soil amended with 10 mg/liter, the 10^5 Pseudomonas sp. cells per g soil (dry weight) inoculum did not initiate TBA degradation over an 80 day incubation period while the same size inoculum only degraded TBA at a rate of 1.6 mg/liter/day in the 100 mg/liter sterile soil treatment. This smaller Pseudomonas sp. inoculum was also less effective in nonsterile soil amended with 10 mg/liter TBA. In the 250 and 500 mg/liter TBA treatments, both inocula of the

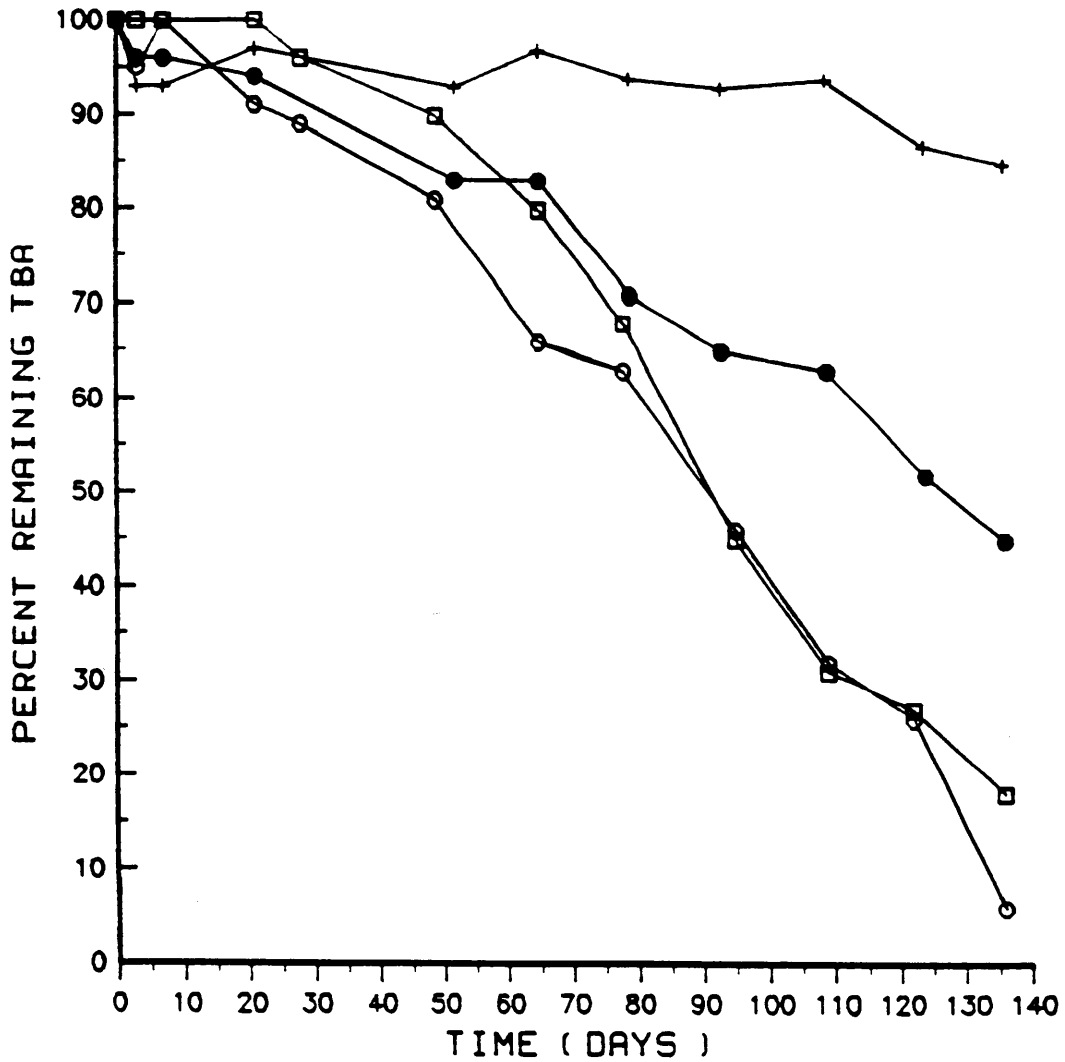


Figure 2. Degradation of tertiary butyl alcohol (TBA) in Fredrick clay amended with 500 mg/liter TBA. Treatments include: ● uninoculated Fredrick clay, ○ Fredrick clay inoculated with 10^6 *Pseudomonas* sp. VT100 cells per g soil (dry weight), ◻ Fredrick clay inoculated with 10^5 *Pseudomonas* sp. VT100 cells per g soil (dry weight), and + sterile control soil.

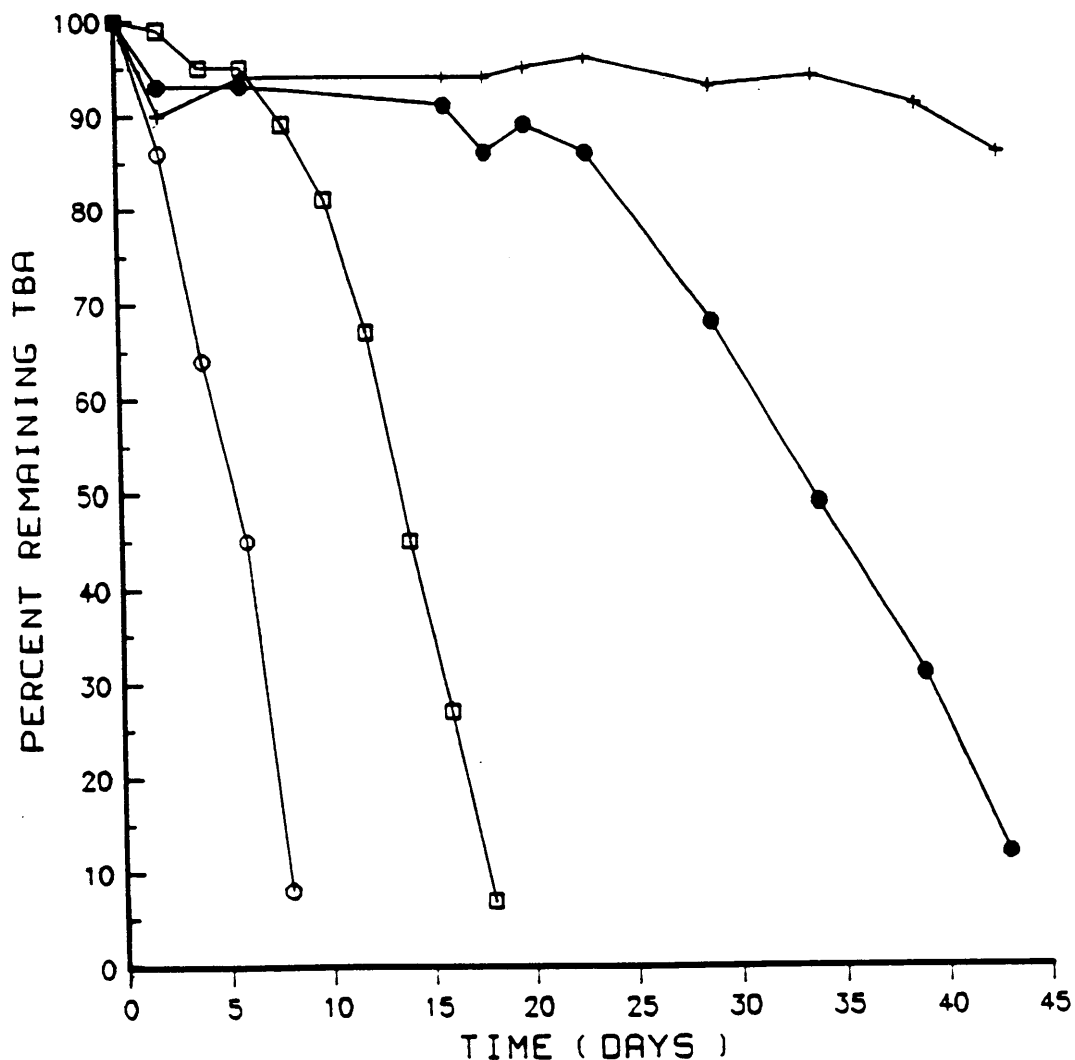


Figure 3. Degradation of tertiary butyl alcohol (TBA) in Fredrick clay amended with 10 mg/liter TBA. Treatments include: —●— uninoculated Fredrick clay, —○— Fredrick clay inoculated with 10^6 *Pseudomonas* sp. VT100 cells per g soil (dry weight), —□— Fredrick clay inoculated with 10^5 *Pseudomonas* sp. VT100 cells per g soil (dry weight), and —+— sterile control soil.

Pseudomonas sp., VT100, exhibited similar rates of TBA degradation in sterile and nonsterile soil. At concentrations of TBA below 100 mg/liter, 10^6 Pseudomonas sp. cells per g soil (dry weight) may be necessary to affect the maximum rate of TBA degradation.

There was an initial period where little or no measurable TBA degradation was observed in the uninoculated Fredrick clay microcosms amended with the four concentrations of TBA. The 10^6 Pseudomonas sp. cells per g soil (dry weight) inoculum reduced the time required to reach the maximum rate of TBA degradation in the Fredrick clay microcosms amended with 100, 250, and 500 mg/liter TBA. In the 10 mg/liter Fredrick clay microcosms, TBA was degraded at the maximum rate immediately upon the addition of 10^6 Pseudomonas sp., VT100, cells per g soil (dry weight). The 10^6 Pseudomonas sp., VT100, cells per g soil (dry weight) inoculum was more effective than the 10^5 Pseudomonas sp. cells per g soil (dry weight) inoculum at reducing the time required to reach the maximum rate of TBA degradation in the Fredrick clay when amended with the four concentrations of TBA. This suggests that the time required to reach the maximum rate of TBA degradation is a growth related response and that the indigenous Fredrick clay microflora and the 10^5 Pseudomonas sp. cells per g soil (dry weight)

Table 2. TBA degrading populations in Fredrick clay after 140 days incubation. Populations include: 10^5 and 10^6 *Pseudomonas* sp. VT100 cells per g soil (dry weight) in sterile and nonsterile soil and the indigenous microflora alone.

Treatment	Population (cells/g soil(moist weight)) x 10^5		
	Inoculum(cells/g soil(dry weight))		
	None	10^5	10^6
Soil: Fredrick clay.			
10 mg/liter TBA			
soil w/ indigenous flora	0.011		
sterile soil + Strain VT100		0.14	1600
indigenous flora + Strain VT100		3.9	61
100 mg/liter TBA			
soil w/ indigenous flora	0.13		
sterile soil + Strain VT100		14	180
indigenous flora + Strain VT100		1.1	1.4
250 mg/liter TBA			
soil w/ indigenous flora	0.75		
sterile soil + Strain VT100		270	150
indigenous flora + Strain VT100		52	22
500 mg/liter TBA			
soil w/ indigenous flora	0.74 ^a		
sterile soil + Strain VT100		260	2700
indigenous flora + Strain VT100		5.8	2.6

^a Soil amended with 1.0mM molybdate.

inoculum require more time to attain a population large enough to affect the maximum rate of TBA degradation. The indigenous microflora of the Groseclose clayey loam did not initiate TBA degradation over the entire incubation period, whereas the addition of 10^6 Pseudomonas sp. cells per g soil (dry weight) to the sterile and nonsterile soil exerted a measurable effect on TBA degradation after 77 days of incubation. Some typical TBA degradation curves in the inoculated and uninoculated Fredrick clay microcosms are shown in figures 1 and 3.

MPN estimations of TBA degrading microbial populations.

These experiments were conducted to determine: if the small indigenous TBA degrading population in the Fredrick clay changed during the incubation, and if the introduced Pseudomonas sp., VT100, populations were still present at the end of the experiment. The native TBA degrading microflora in the Fredrick clay increased up to three orders of magnitude in the 100, 250, and 500 mg/liter treatments and two orders of magnitude in the 10 mg/liter treatment (Table 2).

In the presence of the indigenous microflora, the combined populations of the indigenous TBA degrading microflora and Pseudomonas sp. were the same or smaller than the original 10^6 cells per g soil (dry weight) Pseudomonas sp., VT100, inoculum (Table 2.). However, the

Pseudomonas sp., VT100, population increased one to two orders of magnitude in the sterile soil microcosms (Table 2.). It appears that the indigenous microflora reduced the capacity of introduced pseudomonad to proliferate in the Fredrick clay during the experiment. In general, the growth of the 10^5 Pseudomonas sp. VT100 cells per g soil (dry weight) inoculum paralleled that of the larger inoculum, as discussed above (Table 2.).

The effect of supplemental carbon sources on TBA degradation. The addition of yeast extract and acetate stimulated the initiation of TBA degradation in the uninoculated Groseclose clayey loam, whereas the corresponding unamended microcosm showed no activity (Table 3). Yeast extract and acetate also enhanced the TBA-degrading activity of the Pseudomonas sp. in the same subsurface soil. Neither concentration of ethyl alcohol enhanced TBA degradation in the inoculated or uninoculated Groseclose clayey loam. The indigenous TBA degrading microflora successfully competed with the Pseudomonas sp. , VT100, in the amended microcosms and reduced the amount of TBA degraded by the introduced pseudomonad in nonsterile soil as compared to the inoculated sterile soil microcosms (Table 3). This effect was most pronounced following the addition of both concentrations of acetate and 10 mg/l of yeast extract.

Table 3. Effect of organic supplements on TBA degradation in Groseclose clayey loam. Treatments include: sterile and nonsterile soil inoculated with 10^6 Pseudomonas sp. VT100 cells per g soil (dry wt.), and uninoculated soil. Data corrected for abiotic TBA losses in sterile soil controls.

Treatment ¹	% TBA degradation		
	Day 35	Day 63	Day 140
<u>Pseudomonas</u> sp. VT100.			
TBA	0%	7%	21%
TBA+10 mg/l Y. E.	20%	31%	45%
TBA+100 mg/l Y. E.	7%	15%	32%
TBA+10 mg/l Acetate	22%	41%	57%
TBA+100 mg/l Acetate	10%	11%	36%
TBA+10 mg/l ETOH	8%	15%	21%
TBA+100 mg/l ETOH	0%	6%	26%
Indigenous flora.			
TBA	0%	0%	0%
TBA+10 mg/l Y. E.	4%	9%	13%
TBA+100 mg/l Y. E.	0%	8%	26%
TBA+10 mg/l Acetate	2%	11%	11%
TBA+100 mg/l Acetate	7%	8%	16%
TBA+10 mg/l ETOH	4%	2%	2%
TBA+100 mg/l ETOH	2%	7%	2%
Indigenous flora + <u>Pseudomonas</u> sp. VT100.			
TBA	9%	11%	20%
TBA+10 mg/l Y. E.	3%	13%	25%
TBA+100 mg/l Y. E.	17%	22%	35%
TBA+10 mg/l Acetate	22%	21%	26%
TBA+100 mg/l Acetate	3%	14%	19%
TBA+10 mg/l ETOH	0%	12%	17%
TBA+100 mg/l ETOH	16%	16%	24%

¹ Y. E., yeast extract; ETOH, ethyl alcohol.

Effect of molybdate on TBA degradation. The effect of a 1.0 mM molybdate amendment on the lag period, and the rate of TBA degradation is summarized in Table 4. In both soils, molybdate consistently stimulated the rate of TBA degradation and reduced the initial lag period of the indigenous microflora. In contrast, the molybdate addition did not stimulate the TBA-degrading potential of the Pseudomonas sp.. In most instances, the lag period of the Pseudomonas sp. was unaffected or slightly increased and the rate of TBA degradation was decreased as compared to the unamended subsurface soils. The size of the indigenous and introduced TBA-degrading populations in the molybdate amended microcosms were not different than the respective populations in the unamended microcosms.

Table 4. Effect of 1.0 mM molybdate amendment on degradation rates, lag period, and population growth of 10^6 Pseudomonas sp. VT100 cells per g soil (dry wt) and indigenous TBA degrading microflora. Change in lag period and degradation rates in molybdate amended soil. Contrasted with corresponding unamended soil, as shown in Tables 1 and 2.

Treatment	Change in Lag (days)	Change in Degradation rate (mg/liter/day)
Soil: Fredrick clay.		
10 mg/liter TBA		
indigenous flora	-15	+0.6
indigenous flora	0	+0.3
+ Strain VT100		
100 mg/liter TBA		
indigenous flora	-11	+0.5
indigenous flora	+7	-0.7
+ Strain VT100		
250 mg/liter TBA		
indigenous flora	-42	+0.5
indigenous flora	-7	-1.0
+ Strain VT100		
500 mg/liter TBA		
indigenous flora	-14	+0.5
indigenous flora	+7	-0.9
+ Strain VT100		
Soil: Groseclose clayey loam.		
250 mg/liter TBA		
indigenous flora	>-77	+0.3
indigenous flora	-14	0
+ Strain VT100		

Discussion

The Pseudomonas sp., VT100, increased TBA degradation in two pristine subsurface soils. Whereas the indigenous Groseclose clayey loam microflora did not respond to TBA additions, the inoculation of the Pseudomonas sp. to the Groseclose clayey loam produced an active TBA degrading population. The indigenous microflora of the Fredrick clay did respond to the TBA additions, but the introduced pseudomonad enhanced the rate of TBA degradation and reduced the initial lag period. In the latter case, the Pseudomonas sp., VT100, was most effective at the lowest (10 mg/liter) and highest (500 mg/liter) concentrations of TBA, where the TBA degrading activity of the indigenous microflora was reduced. There was no significant difference in TBA degradation between the native Fredrick clay community and the Pseudomonas sp. at intermediate TBA concentrations. An introduced pentachlorophenol (PCP) degrading Flavobacterium sp. produced comparable effects in a pristine soil amended with 100 ppm of PCP. Although the indigenous microflora initiated PCP degradation after a pronounced lag period, a 1×10^7 Flavobacterium sp. cells per g soil (dry weight) inoculum accelerated PCP mineralization by reducing the initial lag period (4).

TBA is not a natural product, but the native microflora in a number of pristine soils could degrade TBA (16,18).

Novak et al. (18) found that the rate of TBA degradation in two pristine anaerobic aquifer soils ranged between 0.01 and 0.1 mg/liter/day at TBA concentrations from 1 to 100 mg/liter. The rate of TBA degradation in an aerobic subsurface soil was higher and remained constant with changing TBA concentration. The indigenous microflora in another soil degraded 0.7 mg/liter/day of TBA, at an initial TBA concentration of 100 mg/liter (16). These rates of TBA degradation are similar to the maximum rate observed in the Groseclose clayey loam when amended with organic supplements, while the 2.5 mg/liter/day TBA degradation rate seen in the Fredrick clay is the highest yet observed for native soil microorganisms. It is significant that the indigenous microflora, which included Alcaligenes sp. VT200, exhibited a similar rate of TBA degradation at intermediate concentrations of TBA as the Pseudomonas sp., which was isolated from an area of chronic hydrocarbon contamination. Also significant, was the ability of the indigenous microflora of the Middendorf clay, which was collected from a depth of 458 m, to initiate TBA degradation. Hybridization experiments, using a ³²P-labeled whole-TOL-plasmid probe, found homology with plasmid and chromosomal DNA from some bacteria isolated from pristine subsurface environments at depths from 40 to 245 m. This suggests that

microorganisms in natural environments may have the capacity to mineralize organic compounds normally not present in the given locale (7). The other deep subsurface soil samples, the Middendorf sand and the Cape Fear Sand, tested in this study did not decompose any of the added TBA. These subsurface samples either have no indigenous TBA degrading microflora, or there is a very long lag period.

Recalcitrant organic chemicals may persist in the environment for long periods of time if they are present at low concentrations. Boethling and Alexander (2) found that 2,4-dichlorophenoxyacetate (2,4-D) or 1-naphthyl-N-methylcarbamate mineralization by a natural aquatic community was curtailed when the concentrations were decreased from 22,000 and 30,000 ng/ml to 3 ng/ml or less. The rate of p-chlorobenzoate and chloroacetate degradation also decreased sharply as the concentrations were decreased from ug/ml to pg/ml values. While the indigenous microflora of the Fredrick clay maintained activity in the 10 mg/liter TBA treatment, the rate of TBA degradation was 4 times lower than at the higher concentrations of TBA. The TBA degrading capacity of the Pseudomonas sp., VT100, is an induced trait (3). The pseudomonad was induced when inoculated to the subsurface soil microcosms and therefore readily metabolized low concentrations of TBA.

Increases in indigenous pollutant degrading microbial populations often parallel the initiation of organic pollutant degradation. Wiggins and Alexander (27) found that the disappearance of p-nitrophenol (PNP) in lake water coincided with an increase in a PNP mineralizing population from 10 PNP degraders per ml to over 10^5 PNP degraders per ml. They proposed that the adaptation period in PNP degradation reflected the time period during which the initially small population increased to a size where it could affect PNP degradation. The time required to reach the maximum rate of TBA degradation in the uninoculated Fredrick clay was accompanied by a three orders of magnitude increase in the indigenous TBA-degrading microflora. This initial period of inactivity was observed at all four concentrations of TBA. While the addition of the 10^5 Pseudomonas sp., VT100, cells per g soil (dry weight) inoculum shortened the time required to reach the maximum rate of TBA degradation, there was still a pronounced period where no TBA degradation was detected. Given the induced state of the Pseudomonas sp. inoculum when added to the soil microcosms, it was surprising to see any initial period of no detectable TBA degradation in the inoculated soil micorcosms. The addition of 10^6 Pseudomonas sp. cells per g soil (dry weight) was correlated with the most rapid initiation of TBA

degradation , thus suggesting that a minimum Pseudomonas sp., VT100, population is required to affect TBA degradation in the Fredrick clay, especially at low initial concentrations of TBA.

The native microflora of the Fredrick clay limited the growth of introduced Pseudomonas sp., VT100. The Pseudomonas sp., VT100, populations were up to three orders of magnitude larger in sterile soil than the combined populations of the Pseudomonas sp. and the indigenous TBA-degrading microflora in the corresponding nonsterile soil treatments. An introduced population of a 2,4-dichlorophenol (DCP)-mineralizing Pseudomonas sp. declined from 1.6×10^6 to approximately 10^4 cells per ml in nonsterile sewage, with or without added DCP, over a 12 day incubation (9). In sterile soil, this DCP-mineralizing population increased to over 10^7 cells per ml and was unaffected by the presence of DCP. A different effect was noted with a PAH-mineralizing Mycobacterium sp.. The growth of this bacterium was unaffected in nonsterile soil and the population increased from 1.5×10^5 to 2.8×10^5 Mycobacterium cells per g (wet weight) of sediment over a 10 week incubation (10).

The rate of TBA degradation was reduced in the soil which contained the Pseudomonas sp., VT100, and the indigenous microflora, as compared to the inoculated

sterile soil. This rate difference favors the hypothesis that some components of the indigenous microflora, which included the Alcaligenes sp., VT200, competed with the Pseudomonas sp., VT100, inoculum. The population data cited above supports this observation. A 4-(2,4-DB)-degrading Flavobacterium sp., that was very active in sterile soil, did not degrade 4-(2,4-DB) in the presence of native soil microorganisms (15). Similarly, a DCP-degrading Pseudomonas sp. that readily initiated DCP degradation in sterile soil, did not confer any enhanced DCP degradation in nonsterile soil (9). The function of introduced microorganisms is not always limited by presence of indigenous soil and aquatic microflora. An introduced PAH-degrading Mycobacterium sp. exhibited similar pyrene mineralization rates were in both sterilized and nonsterilized soil (10).

The abiotic characteristics of a soil environment, which include soil moisture, inorganic nutrient content, and soil temperature as well as the actual soil type, influence the initiation and extent of recalcitrant organic pollutant degradation (4,25). The TBA-degrading capacity of the Pseudomonas sp. was limited in the Groseclose clayey loam, as contrasted with the Fredrick clay. The decreased activity of the Pseudomonas sp. VT100, even in the absence of the indigenous microflora,

suggests that some abiotic parameter of the Groseclose clayey loam limited the pseudomonad's ability to degrade TBA. A 4-(2,4-DB)-degrading Flavobacterium sp., that was extremely active in a sterilized silt loam, degraded little 4-(2,4-DB) in a second unique sterile silt loam (15). This suggests that a physical or chemical parameter of the second soil limited the catabolic potential of the Flavobacterium sp..

The addition of readily degradable organic compounds can have a stimulatory effect on the mineralization of recalcitrant organic pollutants. The degradation of three monosubstituted phenols, by an aquatic microbial community, was enhanced following the addition of amino acid, carbohydrate, and fatty acid supplements. This effect was more pronounced as the concentration of these organic supplements was increased (23). A chemical supplement, provided by yeast extract and acetate, stimulated TBA degradation by the native microflora in the Groseclose clayey loam, whereas no activity was observed in the unamended soil. A similar effect was seen with the Pseudomonas sp., VT100, in the yeast extract and acetate amended sterile soil microcosms. In contrast, the introduced pseudomonad degraded less TBA in the nonsterile Groseclose clayey loam microcosms, supplemented with yeast extract and acetate, than in the respective sterile soil

microcosms that lacked an indigenous TBA-degrading population. This strengthens the hypothesis that some components of an active indigenous TBA degrading microflora can limit TBA degradation by the Pseudomonas sp. in soil environments. In liquid culture, increasing concentrations of yeast extract inhibited TBA degradation but augmented growth of the Pseudomonas sp., VT100. In the oligotrophic Groseclose clayey loam, the yeast extract and acetate amendments may have provided the Pseudomonas sp., VT100, and the indigenous microflora with a chemical that was otherwise limited. Organic supplements can also inhibit introduced and indigenous pollutant degrading microbial populations in soil and aquatic environments. The Pyrene-mineralizing capacity of an introduced Mycobacterium sp., in freshwater sediments, was limited following 0.1% glucose and peptone amendments (10). It was proposed that the amendments stimulated the rapid growth of the native microorganisms, which outgrew the introduced Mycobacterium sp.. Degradation of ethylene dibromide, p-nitrophenol, phenol and toluene by a microbial community, native to a pristine aquifer, was limited by the addition of glucose and amino acids (24).

The addition of 1.0 mM of molybdate stimulated TBA degradation by the otherwise inactive microflora of the Groseclose clayey loam. It also increased TBA degradation

by an already active Fredrick clay microflora. Morris (16) also noted an increase in TBA degradation in a number of soils following the addition of 0.2 mM molybdate. But this effect was only observed in soil devoid of an active indigenous TBA degrading microflora.

While the Pseudomonas sp., VT100, consistently stimulated TBA degradation in a number of pristine soils, the native microflora also demonstrated the ability to degrade TBA after an extended incubation period. The Pseudomonas sp. was most effective at reducing the lag in TBA degradation and stimulating TBA degradation in the 10 and 500 mg/liter TBA treatments. concentrations of TBA. In situations where the indigenous microflora can degrade TBA, manipulation of the native microbial population may be a more desirable means for removing TBA from the environment. The inoculation of the Pseudomonas sp., VT100 may find use as a method to stimulate TBA degradation in soils that lack a native TBA-degrading microflora or in contained and semi-contained bioreactors.

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Methyl tertiary butyl ether Induction Experiments.

Introduction

Methyl tertiary butyl ether (MTBE) is a five carbon compound with a molecular weight of 88.15. It has a melting point of -109°C , a boiling point of 55.2°C , and is completely soluble in H_2O (2). In 1988, 4.68 billion pounds of MTBE was produced in the United States and it ranked 31st in tonnage of the top 50 chemicals manufactured (1). Due to the similarity of the chemical structures of MTBE and tertiary butyl alcohol (TBA) it was hypothesized that Pseudomonas sp. VT100 might have the ability to degrade MTBE.

Methods

Enrichment cultures were established by adding approximately 100 ml of a defined MTBE medium to 500 ml screw cap Erlenmeyer flasks. This defined MTBE medium contained the same reagents as the defined TBA medium except MTBE was substituted for TBA. These enrichment cultures were inoculated with 1.0 ml of a washed Pseudomonas sp. strain VT100 cell suspension. Enrichment cultures were established at four MTBE concentrations (mg/liter): 50, 100, 500, and 1000. All incubations were done in the dark at 25°C .

In an attempt to limit MTBE volatilization, the

enrichment cultures were transferred to 20 x 150 mm screw cap culture tubes filled with the defined MTBE medium, at four concentrations of MTBE (mg/l): 10, 50, 100, and 250. The culture tubes were inoculated with 1.0 ml from the first series of enrichment cultures and sealed with parafilm. A third attempt to establish a stable enrichment culture utilized 500 ml side arm Erlenmeyer flasks filled with 200 ml of the defined MTBE medium dosed with four concentrations of MTBE (mg/liter): 10, 50, 100, and 250. The sidearms were filled with sterile MTBE in an effort to saturate the headspace and limit MTBE volatilization.

Analysis for MTBE mineralization was performed on a Hewlett Packard 5880A Gas Chromatograph linked to a flame ionization detector. The oven was operated isothermally at 125°C, the injector temperature was set at 150°C and the detector temperature was set at 225°C.

Results

The first set of MTBE enrichment cultures inoculated with the Pseudomonas sp. VT100 became turbid after approximately one month incubation. Due to the volatility of MTBE, there was no difference between the MTBE concentrations in the enrichment cultures and the controls, and MTBE utilization could not be conclusively demonstrated. The same problem was encountered with the

enrichment cultures in the side arm Erlenmeyer flasks.

A decrease in the MTBE concentration in the culture tube enrichment cultures at the 50, 100, and 250 mg/l MTBE concentrations was observed (Table 1). Sampling for MTBE utilization had to be limited to prevent the creation of any headspace in the culture tubes. There was a dramatic drop in the MTBE concentrations in the enrichment cultures and controls after the second sampling, presumably due to volatilization.

Discussion

Although the change in the turbidity of the enrichment cultures was not measured, the decrease in the MTBE concentration in the culture tube enrichments as compared to the controls, suggests that the Pseudomonas sp. degraded MTBE. This observation was not correlated to any increase in cell density or turbidity. To establish the relationship between MTBE utilization and growth, a culturing method that limits MTBE volatilization must be devised.

Table 5. Methyl tertiary butyl ether (MTBE) concentrations in enrichment cultures amended with four MTBE concentrations (mg/liter): 10, 50, 100, and 250. The MTBE concentration was determined on days 28 and 42.

Culture	Initial MTBE Concentration (mg/liter)							
	10		50		100		250	
	Time(Days)							
	28	42	28	42	28	48	28	48
MTBE Concentration (mg/liter)								
Control	19.1	16.3	54.4	54.0	129.8	99.5	217.7	171.9
1000-1	18.5	15.2	46.3	20.2	123.2	32.8	235.4	159.6
1000-2	19.1	15.2	50.1	22.6	103.0	68.7	233.0	130.4
500-1	19.5	14.1	50.0	24.9	88.7	43.6	232.0	67.0
500-2	21.1	16.4	51.6	43.5	119.9	43.5	235.4	196.7
100-1	17.8	17.7	53.3	52.5	125.1	107	165.8	119.3
100-2	18.1	17.5	53.0	32.9	125.4	88.4	135.4	70.5
50-1	17.9	17.3	56.4	46.2	120.0	82.4	164.8	43.9
50-2	18.0	15.9	55.0	49.3	132.1	97.7	153.0	130.7

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**Determination of Antibiotic Resistance of
Pseudomonas sp. VT100.**

Introduction

This study was initiated to determine if Pseudomonas sp. VT100 harbored any antibiotic resistance.

Methods

All media and reagents were obtained from either Fisher Scientific Co. (Raleigh, N.C.) or J. T. Baker Co. (Phillipsburg, N. J.). The antibiotics were purchased through the Sigma Co. (St. Louis, Mo.)

The entire surface of succinate agar plates was inoculated with a turbid Pseudomonas sp. VT100 cell suspension, using a sterile cotton swab. Succinate agar included (per liter): 1.0 g succinic acid, 1.0 g yeast extract, 1.0 g sodium acetate, 0.5 g peptone and 15.0 g agar. Resistance to antibiotics was determined by using Sensi-discs (BBL Microbiology Systems, Cockeysville, Md.). The effect of erythromycin, kanamycin, sulfamethoxazole/trimethoprim, novobiocin, nitrofurazone, colistin, lincomycin, cephalothin and oxacillin on the viability of Pseudomonas sp. VT100 was determined. Following one week of incubation at 30°C, the inoculated succinate/antibiotic agar plates were checked for zones of inhibition around the Sensi-disc (BBL) antibiotic disks.

The level of antibiotic resistance was determined by growing the Pseudomonas sp. on succinate agar plates amended with increasing concentrations of the antibiotics to which the Pseudomonas sp. VT100 demonstrated resistance.

Results

The Pseudomonas sp. VT100 was found to be resistant to 15 ug/ml lincomycin and 30 ug/ml cephalothin.

Discussion

This study attempted to find a method to selectively enumerate the Pseudomonas sp. VT100 population in mixed cultures. This antibiotic plate method was used in conjunction with the most probable number (MPN) technique to enumerate the Pseudomonas sp. VT100 populations in the laboratory microcosms at the end of the experimental incubations. Succinate agar plates amended with 15 ug/ml lincomycin and 30 ug/ml cephalothin were inoculated with the same dilution series used to inoculate the MPN tubes. A number of microorganisms in the native Fredrick clay community were resistant to these antibiotics, thus limiting the effectiveness of the antibiotic plate method for enumerating the introduced Pseudomonas sp. VT100 populations.

Literature Review

Tertiary butyl alcohol (TBA) is a four carbon alcohol with a molecular weight of 74.12, a melting point of 25°C, boiling point of 82-83°C, and is soluble in H₂O (27). The reported LD50 in rats is 3.5 g/kg (26). TBA is a potentially serious groundwater pollutant, that can reach high concentrations in water before without being detectable to taste or odor (22). TBA is a by product from the production of propylene oxide and isobutylene (13). It is also used as a gasoline additive and is found at concentrations of up to 5% in some gasoline (22). Approximately 35% of the reported 796,000 underground petroleum storage tanks in the contiguous United States would not pass the Environmental Protection Agency 0.10 gallon/hour tightness test (19). Petroleum products leaking from these storage facilities pose a potential threat to groundwater, which supplies a significant amount of the potable water in the United States (4, 20).

Presently air stripping, charcoal filtration, and chemical degradation are the only practical methods for decontaminating groundwater (8). These methods are expensive, technologically difficult to execute, and create alternative environmental threats (8). Biological remediation may represent an alternative for degrading environmental pollutants. Three approaches to biological

remediation have been considered and they include: enhancement of indigenous microorganisms, introduction of degrader microorganisms into the contaminated environment, and the use of microorganisms in contained or semicontained bioreactors (21).

While TBA is resistant to biological degradation, TBA degradation has been observed in a number of subsurface soils (20, 22). Novak et al. (22) found that the indigenous microflora in three subsurface soils had the ability to degrade TBA at concentrations up to 100 mg/liter. TBA degradation was more pronounced in soil obtained from an aerobic aquifer than in anaerobic aquifer soils. The addition of 0.2 mM molybdate in the form of NaMOO_4 stimulated TBA degradation in soil with no prior exposure to any hydrocarbon contamination (20). However, this effect was only seen in soil devoid of any active indigenous TBA degrading population. Horn et al. (13) claimed that 99.5% TBA degradation was achieved in a bioreactor, using a heterotrophic enrichment culture. This study did not include the appropriate controls to account for volatilization of TBA from the bioreactors. Chadduck (5) isolated the first bacterium capable of utilizing TBA as a carbon and energy source in pure culture. This Pseudomonas sp., designated strain VT100, was isolated from subsurface soil that had experienced a

long term exposure to hydrocarbon contamination.

Indigenous microbial populations, as large as 10^7 cells per g soil (dry weight), were found in shallow aquifer material, using acridine-orange fluorescent direct counting (10). White et al. (30) utilized a gas chromatography/mass spectrometry technique, which measured 4 components of the lipopolysaccharide and ribitol of teichoic acid, to estimate the gram-negative and gram-positive bacterial populations in aquifer material obtained from 410 m depth. The estimated bacterial populations, at this depth, were 10^7 bacteria per g soil (dry weight) (30). Bacterial populations in 50 uncontaminated subsurface soil samples, from 0.2 to 260 m depth, ranged from nondetectable numbers to 10^8 CFU per g soil (dry weight). The populations did not decrease with depth and the lowest populations were found in soil with greater than 20% clay (9). This study also investigated the occurrence of plasmids and found there to be less in surface soil bacterial isolates than in isolates from deeper sediments. Approximately 33% of the bacterial isolates contained plasmids, with the majority being between 150 and 400 kb in size. There was homology with four of these plasmids to the TOL plasmid (9). These plasmids may encode traits allow these oligotrophic bacteria to reside in low-nutrient environments and

degrade resistant organic compounds that filter down to subsurface environments (9). This is contrasted with Wilson et al. (32) who proposed that, as compared to surface soil bacteria, subsurface populations have adapted to oligotrophic environments and may have difficulty degrading some common organic groundwater pollutants. Ghiorse and Balkwill (10) hypothesized that many organic groundwater pollutants will persist in subsurface environments because the indigenous bacteria are unable to tolerate elevated concentrations of pollutants. The potential exists for these pollutants to accumulate over time and limit the possibility for recovery. Introduced microorganisms with enhanced degradative capacities may accelerate the mineralization of many of these pollutants in subsurface environments.

The mineralization of organic pollutants by indigenous soil and aquatic microorganisms has been reported by a number of researchers (1, 14, 17, 24, 26, 31). However, even in the presence an indigenous population capable of using such compounds, an extended adaptation period often precedes the initiation of degradation of many common environmental pollutants (1, 8, 14, 17, 23, 24, 25, 31). The adaptation or lag period is defined as the time required to initiate or increase the rate of degradation following exposure to a chemical (1). The length of the

adaptation period is often related to the time required for a specific population to increase to a size capable of effecting biodegradation. At one site the indigenous p-nitrophenol (PNP)-degrading population increased from 10^1 to 10^5 PNP mineralizers per g soil prior to the initiation of degradation (31). A 15-fold increase in m-cresol, m-aminophenol, and p-chlorophenol utilizing microbial populations was reported following adaptation to these mono-substituted phenols (24). The adaptation period was further reduced when these mixed aquatic communities were preincubated with added phenol, suggesting a growth related response to mono-substituted phenol mineralization. A natural pentachlorophenol (PCP)-degrading microbial population was found to increase three orders of magnitude following adaptation to PCP (6). Aelion et al. (1) found that the adaptation of mixed subsurface microbial communities to chlorobenzene, 1,2,4-trichlorobenzene, m-cresol, m-aminophenol, aniline, phenol, p-chlorophenol, ethylene dibromide, and PNP was accompanied by increases in the specific degrader populations.

Nutrient limitations have been cited as a factor inhibiting the mineralization of organic pollutants in subsurface environments. Thornton-Manning et al. (28) found that together the addition of nitrogen and

phosphorous accelerated phenol degradation in subsurface soil. In contrast, phenol degradation was not enhanced in surface soils by the addition of the same concentrations of these inorganic nutrients. Similar effects were seen with field collected periphyton and bacterial isolates. Together, phosphorous and nitrogen reduced the lag period in p-cresol utilization. These inorganic nutrients were more effective at reducing the lag with periphyton samples collected from sites low in these two nutrients (17). Accelerated PNP degradation has been reported following the addition of 10mM sulfate as well as comparable phosphorous and nitrate amendments (14). Readily degradable carbon source such as amino acids, carbohydrates, and fatty acids enhanced the degradation of several mono-substituted phenols by a mixed aquatic community (25). In another study, the addition of brewery waste yeast was found to be more effective at enhancing the biodegradation of light and heavy oils than the addition of 10^8 oil degrading bacteria per g soil (wet weight) (16). This is contrasted with another study that found that 1% glucose and peptone amendments inhibited a polycyclic aromatic hydrocarbon degrading Mycobacterium sp. (12). Additions of glucose, and amino acids inhibited the mineralization of ethylene dibromide, PNP, phenol, and toluene by a natural aquatic community (26). Even the

concentration of the recalcitrant pollutant has been found to effect the extent of degradation by natural microbial communities. Boethling and Alexander (3) found the rate of degradation for several environmental pollutants fell off markedly when the concentration was decreased below a particular threshold concentration. They proposed that a low substrate concentration may be very important in limiting biodegradation in natural environments.

The inoculation of microorganisms, with enhanced degradative capacities, to increase the degradation of environmental pollutants has produced variable results. The addition of nearly 10^8 oil degrading bacteria per g soil (wet weight), had no statistically significant influence on the biodegradation of both light and heavy oils in a soil environment (16). A pure culture of Mucor alternans, a common soil fungi which could degrade dichlor-diphenyl-trichloro-ethane (DDT) in culture, failed to maintain activity when incubated in DDT contaminated soil for 11 weeks (2). Seed inoculation of 2,4-dichlorophenoxyacetic acid (2,4-D) sensitive alfalfa seeds with a 2,4-D-utilizing Flavobacterium failed to protect the seeds when planted in nonsterile 2,4-D contaminated soil (18). It was proposed that the indigenous microflora limited the activity of the introduced 2,4-D-degrading Flavobacterium. Goldstein et al. (11) hypothesized four

possible explanations for the failure of introduced microorganisms to enhance biodegradation in the environment: the pollutant degrading microorganisms may fail to grow because concentration of the organic pollutant is too low to support growth, the organism may be susceptible to toxins or predators in the environment, the introduced microorganisms may preferentially use other organic compounds over the target pollutant, and finally the introduced microorganism may be unable to move through soils to sites containing the pollutant.

A number of studies have succeeded in enhancing the degradation of organic xenobiotics by the addition of specific degrader microorganisms to the polluted environment. A PCP-degrading Arthobacter (ATCC 33790) successfully degraded PCP from a soil system contaminated with 120-150 mg/liter of PCP. A 10^6 cell per g soil (wet weight) inoculum reduced the halflife from two weeks to one day (7). In a later but more complete study, attempts to degrade PCP from contaminated soil with a PCP-degrading Flavobacterium produced variable results. Repeated inoculations of 1×10^7 degraders per g soil (dry weight) were required to initiate substantial PCP degradation in a soil environment (6). No mineralization was observed in a second highly contaminated soil, even following a 10-fold dilution with an noncontaminated soil (6). It was

hypothesized that the indigenous microflora or a high concentration of PCP inhibited the Flavobacterium sp. in this second soil. A Pseudomonas sp., strain AC1100, successfully reduced 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) concentrations in contaminated nonsterile soil. At 2,4,5-T concentrations below 5000 μg per gram soil (wet weight), repeated inoculation of 5×10^7 AC1100 cells per g soil (wet weight) resulted in the complete degradation of 2,4,5-T. At 2,4,5-T concentrations above 10000 μg per g soil (wet weight) more than 90% degradation was observed (15). Another study found that inoculation of soil slurries with a consortium of four adapted Pseudomonas spp. reduced the lag prior to the initiation of degradation and stimulated the more complete degradation of benzene, toluene, o-, m-, and p-xylene, chlorobenzene, and o-dichlorobenzene as compared to uninoculated soil (23).

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Table 6. Biodegradation of tertiary butyl alcohol (TBA) in Fredrick clay dosed with 500 mg/liter TBA. Treatments include: nonsterilized soil inoculated with 10^5 and 10^6 *Pseudomonas* sp. VT100 cells per g soil (dry weight), uninoculated soil and sterilized control soil. All treatments performed in triplicate. Refer to figure 4.

Time (days)	Inoculum (cells per g soil (dry weight))			
	Uninoculated	10^5	10^6	Control
Concentration TBA (mg/liter)				
0	486.6+10.4	440.1+4.7	467.7+0.0	460.0+0.6
3	464.9+7.0	441.6+0.0	445.9+21.5	428.3+1.0
7	454.0+5.0	454.5+8.4	469.6+26.6	427.1+8.0
21	457.8+12.8	438.8+8.9	425.1+20.3	446.0+11.5
28	-	423.6+14.0	418.0+20.6	-
35	432.6+5.5	-	-	426.0+7.5
42	-	398.8+11.6	384.5+20.4	-
49	-	395.9+9.7	381.1+21.6	-
52	406.1+12.5	-	-	429.1+5.0
56	-	369.0+14.7	351.4+25.5	-
65	401.8+8.6	350.6+16.8	308.2+16.2	446.9+1.4
70	-	348.1+19.7	326.6+29.4	-
78	-	301.4+28.9	294.1+34.7	-
79	344.3+18.9	-	-	432.8+11.1
86	347.9+13.2	-	-	430.1+2.5
89	-	237.0+24.8	247.3+25.0	-
93	314.9+14.9	-	-	429.9+4.4
95	-	-	211.8+24.0	-
100	283.9+22.1	-	-	446.4+13.7
102	-	163.2+40.9	181.5+31.6	-
109	306.3+25.9	138.0+39.0	149.7+39.7	433.5+9.7
115	278.2+26.4	115.5+29.0	131.1+40.6	-
122	-	117.4+35.2	122.2+36.1	-
124	251.5+20.0	-	-	401.8+27.7
129	-	90.3+32.2	86.3+28.5	-
130	230.7+29.8	-	-	399.2+29.4
136	218.2+31.1	80.4+34.5	73.8+23.5	392.2+36.1

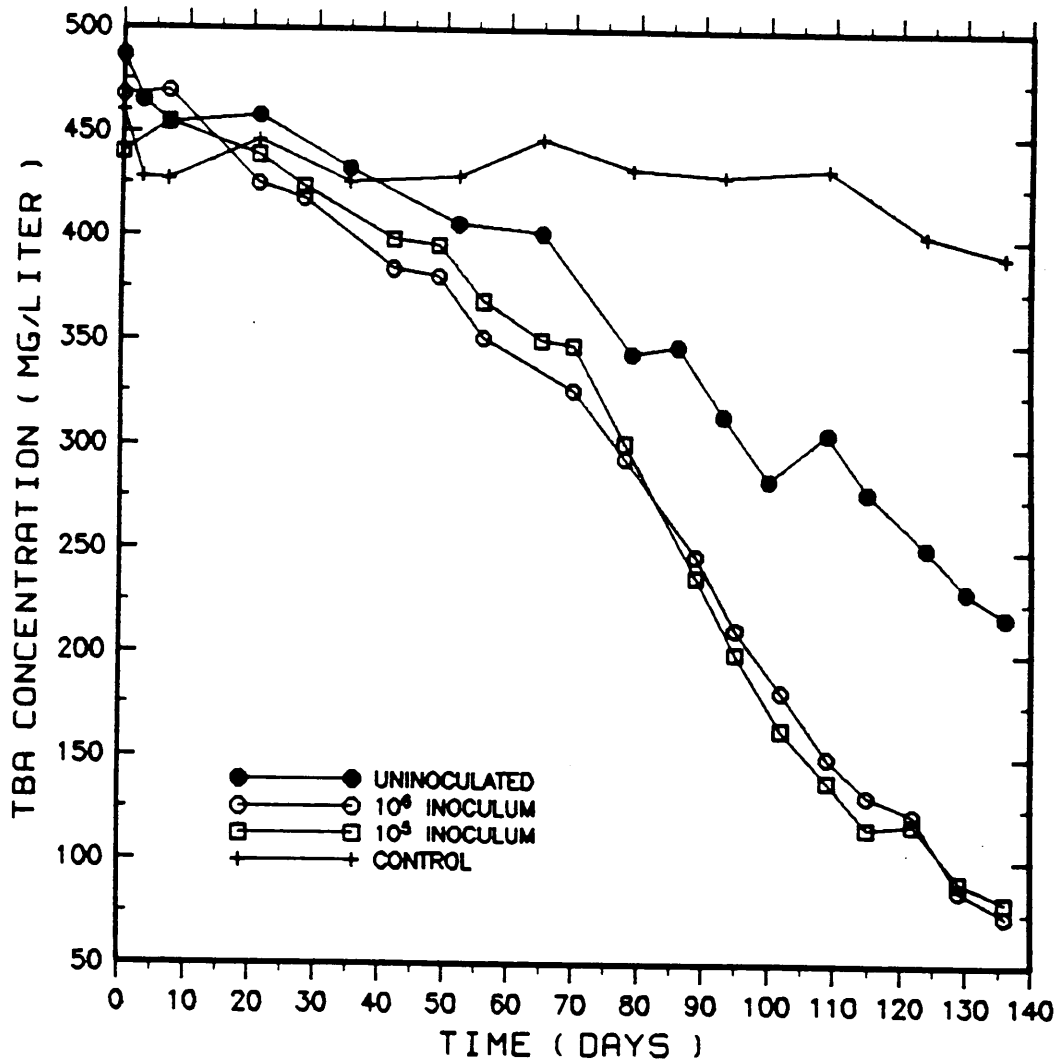


Figure 4. Concentration of tertiary butyl alcohol (TBA) over time in Fredrick clay amended with 500 mg/liter TBA. Treatments include: nonsterile soil, nonsterile soil inoculated with 10^5 and 10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100, and sterilized soil control. All the treatments were established in triplicate.

Table 7. Biodegradation of tertiary butyl alcohol (TBA) in Fredrick clay dosed with 500 mg/liter TBA. Treatments include sterilized soil inoculated with 10^5 and 10^6 *Pseudomonas* sp. VT100 cells per g soil (dry weight) and sterilized control soil. All treatments performed in triplicate. Refer to figure 5.

Time (days)	Inoculum (cells per g soil (dry weight))		
	10^5	10^6	Control
	TBA Concentration (mg/liter)		
0	429.7+9.6	443.6+3.6	460.0+0.6
3	414.3+8.0	431.2+12.4	428.3+1.0
7	446.2+22.0	466.7+8.4	427.1+8.0
21	436.0+5.8	455.5+9.4	446.0+11.5
28	436.7+2.4	437.8+12.1	-
35	-	-	426.0+7.5
42	431.0+11.1	391.6+4.6	-
49	422.4+19.1	367.7+12.6	-
52	-	-	429.1+5.0
56	402.8+19.4	351.0+22.3	-
65	375.5+32.9	333.1+32.0	446.9+1.4
70	392.5+40.8	320.7+29.2	-
78	358.2+31.4	283.0+21.2	-
79	-	-	432.8+11.1
86	-	-	430.1+2.5
89	310.0+32.7	242.4+26.3	-
93	-	-	429.9+4.4
95	291.6+36.9	217.5+35.6	-
100	-	-	446.4+1.4
102	267.3+42.9	193.4+40.3	-
109	229.4+55.5	168.7+50.7	433.5+9.7
115	202.8+60.2	144.9+56.0	-
122	184.2+53.8	128.9+59.4	-
124	-	-	401.8+27.7
129	134.8+50.1	101.4+58.1	-
130	-	-	399.2+29.5
136	95.9+46.9	79.4+55.2	392.2+36.1

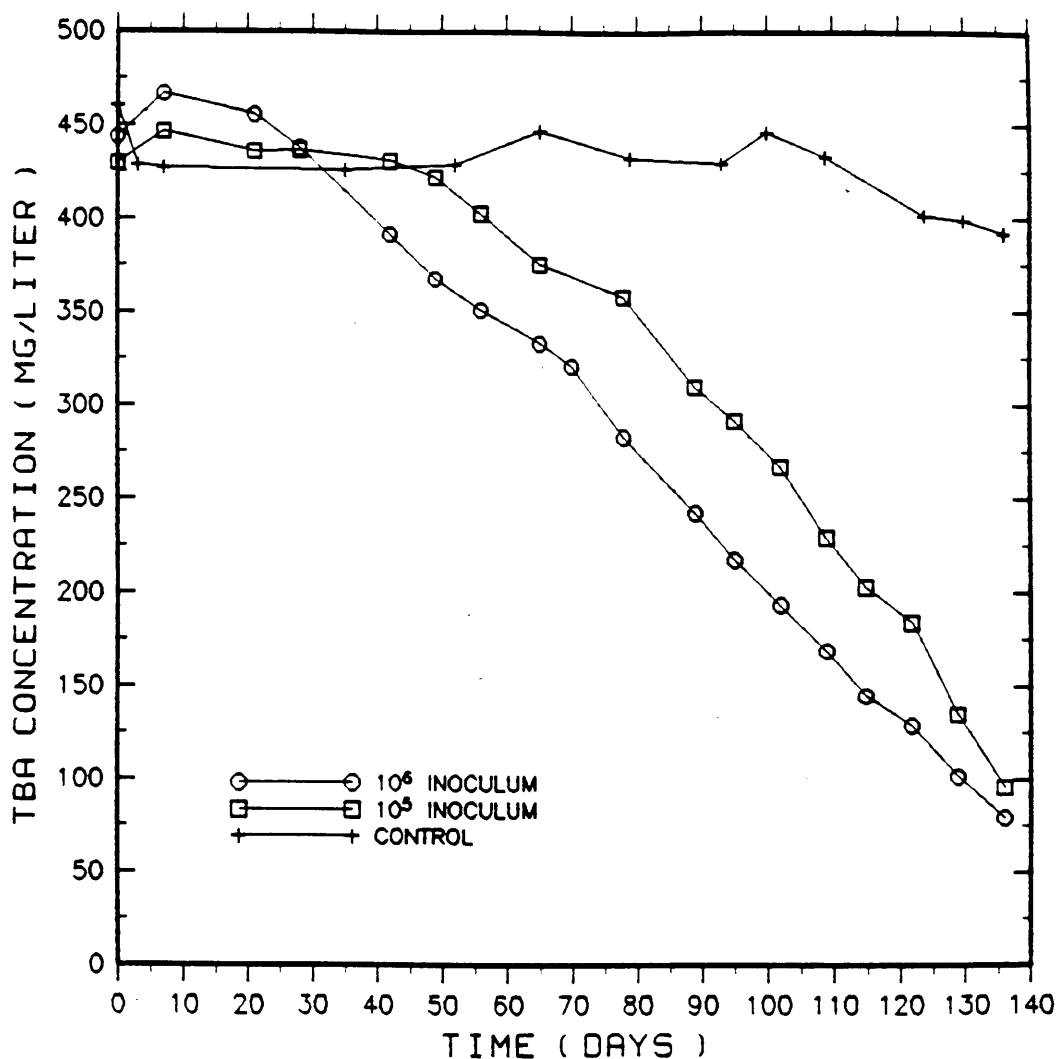


Figure 5. Concentration of tertiary butyl alcohol (TBA) over time in Fredrick clay amended with 500 mg/liter TBA. Treatments include: sterilized soil inoculated with 10^5 and 10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100, and sterilized soil control. All the treatments were established in triplicate.

Table 8. Degradation of tertiary butyl alcohol (TBA) in Fredrick clay amended with 1.0 mM molybdate and 500 mg/liter TBA. Treatments include: soil inoculated with 10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100 and uninoculated soil. All treatments performed in triplicate. Refer to figure 6.

Time (days)	TBA concentration (mg/liter)		Time (days)	TBA Concentration (mg/liter)	
	Inoculated	Uninoculated		Inoculated	Uninoculated
0	449.7+17.1	467.0+0.0	58	-	259.4+14
2	471.5+5.4	458.8+9.2	59	370.1+17.4	-
6	-	453.7+1.2	65	-	231.7+4.3
7	468.0+8.5	-	68	348.7+19.4	-
11	450.2+0.4	441.9+1.3	72	-	215.3+10.4
16	-	425.9+4.6	75	331.8+31.8	-
18	424.1+3.0	-	78	-	205.4+15.1
20	-	407.2+1.4	82	306.7+24.7	-
22	425.7+1.8	-	85	-	194.2+19.1
26	-	382.8+1.1	89	276.2+15.0	-
27	414.5+5.8	-	92	-	172.4+20.0
32	403.5+15.0	-	95	251.9+8.9	-
33	-	374.2+2.2	99	-	152.9+18.3
36	398.6+0.0	-	102	235.3+6.5	-
37	-	351.0+3.7	106	-	134.8+8.5
40	-	337.1+2.3	120	-	129.1+16.4
42	386.8+10.8	-	123	168.5+10.1	-
43	-	321.7+0.5	134	-	120.1+26.2
45	369.9+10.6	-	137	162.9+12.7	-
51	-	285.7+4.2			

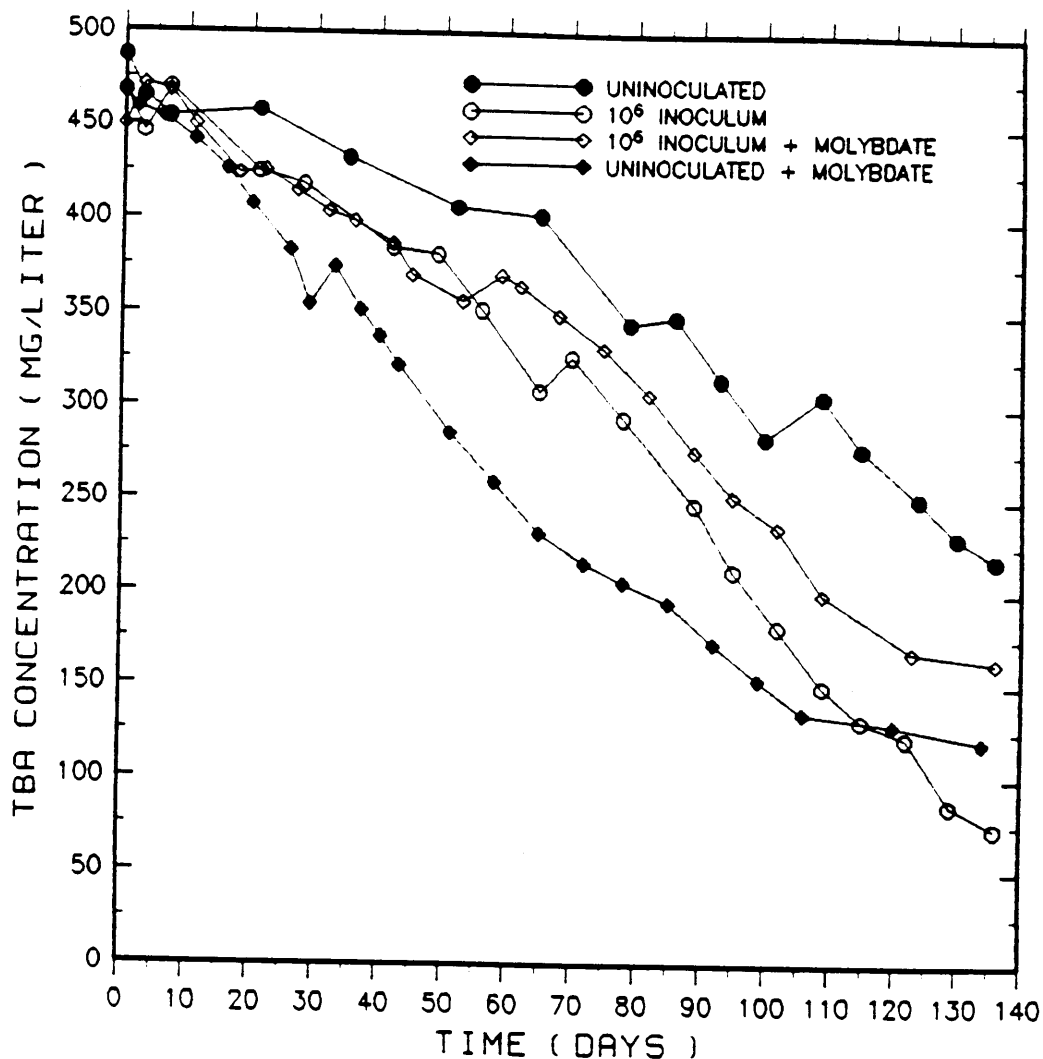


Figure 6. Effect of molybdate on the biodegradation of tertiary butyl alcohol (TBA). Fredrick clay amended with 1.0 mM molybdate and 500 mg/liter TBA. Treatments include: nonsterile soil with and without a 1.0 mM molybdate amendment, and nonsterile soil inoculated with 10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100 with and without a 1.0 mM molybdate amendment. All the treatments were established in triplicate.

Table 9. Biodegradation of tertiary butyl alcohol (TBA) in Fredrick clay dosed with 250 mg/liter TBA. Treatments include: nonsterilized soil inoculated with 10^5 and 10^6 cells per g soil (dry weight) of Pseudomonas VT100, uninoculated soil and sterilized control soil. All treatments performed in triplicate. Refer to figure 7.

Time (days)	Inoculum (cells per g soil (dry weight))				
	Uninoculated	10^5	10^6	Control	
		TBA Concentration (mg/liter)			
0	283.6+5.5	235.0+1.6	228.0+13.3	255.8+0.8	
2	252.8+11.4	-	-	235.3+8.6	
3	-	231.1+5.8	220.7+9.4	-	
7	264.4+2.8	241.8+5.8	240.4+5.1	243.2+4.8	
15	263.4+4.1	229.2+6.8	223.7+2.9	254.8+0.7	
28	-	212.7+7	201.5+7.4	-	
31	248.9+14.9	-	-	249.3+4.1	
35	-	199.5+13.8	190.9+12.1	-	
42	-	191.9+17.0	181.0+13.2	-	
49	257.8+6.1	-	-	270.6+3.5	
50	-	180.5+16.7	167.5+15.2	-	
57	-	162.3+19.5	150.9+22.2	-	
63	239.4+20.1	-	-	254.6+2.3	
65	-	145.8+21.5	125.4+24.4	-	
70	230.3+15.4	113.2+26.6	115.6+24.3	251.3+2.9	
77	204.2+35.8	-	-	254.8+1.3	
78	-	72.4+55.2	80.4+29.2	-	
85	185.7+42.6	-	-	248.6+7.1	
89	-	46.4+44.5	37.0+32.8	-	
95	-	31.2+43.9	22.4+31.1	-	
100	139.2+44.4	-	-	243.7+4.4	
102	-	27.3+36.8	16.8+23.5	-	
109	115.6+37.8	20.7+29.2	9.3+12.8	237.1+5.8	
115	93.8+30.5	16.6+23.4	2.9+4.1	-	
122	-	12.1+17.2	ND	-	
125	68.8+23	-	-	215.0+16.2	
129	-	7.8+11.1	-	-	
136	57.5+19.9	2.6+3.7	-	207.3+8.3	

ND: TBA degraded below detectable concentrations.

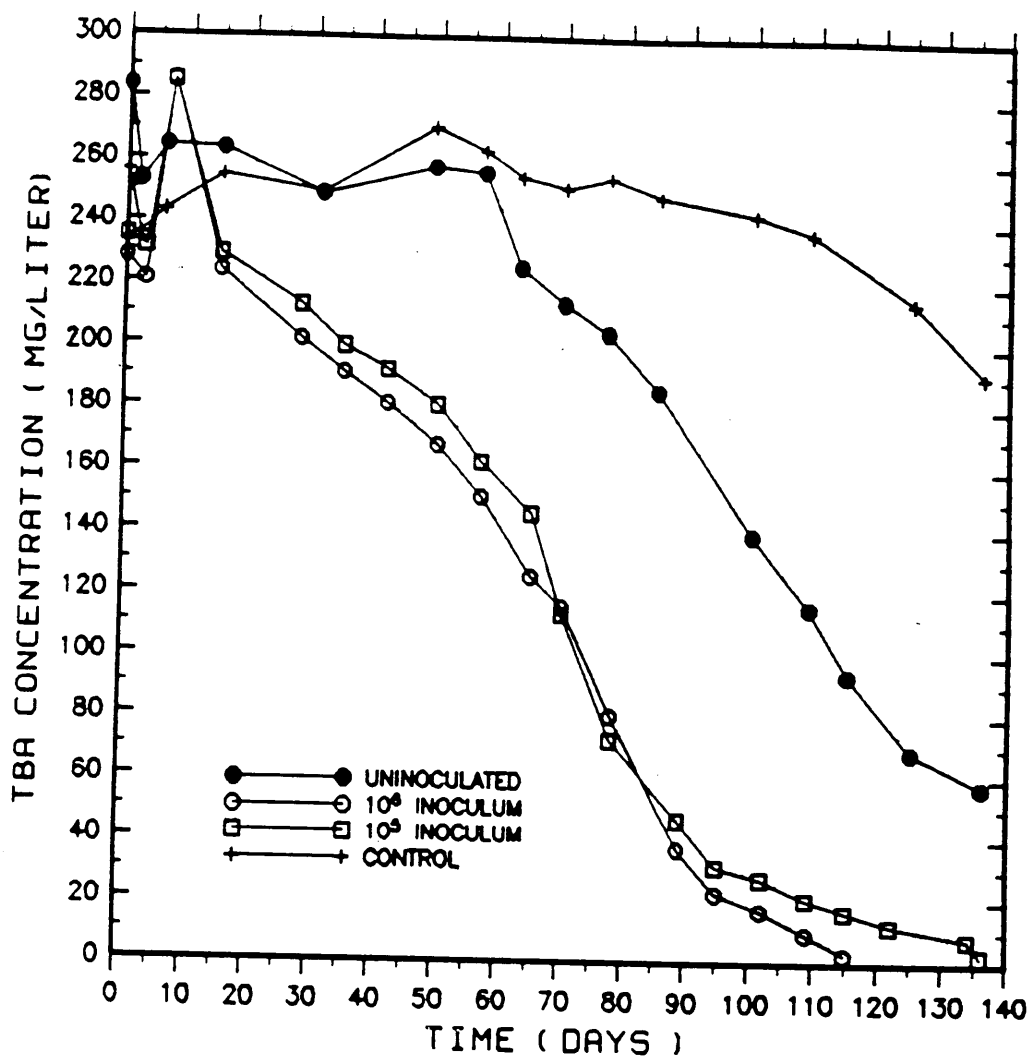


Figure 7. Concentration of tertiary butyl alcohol (TBA) over time in Fredrick clay amended with 250 mg/liter TBA. Treatments include: nonsterile soil, nonsterile soil inoculated with 10^5 and 10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100, and sterilized soil control. All the treatments were established in triplicate.

Table 10. Biodegradation of tertiary butyl alcohol (TBA) in Fredrick clay dosed with 250 mg/liter TBA. Treatments include: sterilized soil inoculated with 10^5 and 10^6 cells per g soil (dry weight) of *Pseudomonas* VT100, and sterilized control soil. All treatments performed in triplicate. Refer to figure 8.

Time (days)	Inoculum (cells per g soil (dry weight))		
	10^5	10^6	Control
	Concentration TBA (mg/liter)		
0	272.0 \pm 1.5	260.5 \pm 1.1	255.8 \pm 10.8
2	-	-	235.3 \pm 8.6
3	263.6 \pm 5.1	260.0 \pm 1.9	-
7	285.4 \pm 2.5	285.7 \pm 2.4	243.2 \pm 4.8
15	268.6 \pm 9.3	272.3 \pm 6.1	254.8 \pm 0.7
28	271.1 \pm 6.1	267.4 \pm 7.8	-
31	-	-	249.3 \pm 4.1
35	267.5 \pm 4.5	251.7 \pm 13.2	-
42	261.0 \pm 4.3	226.9 \pm 21.6	-
49	-	-	270.6 \pm 3.5
50	263.4 \pm 6.1	205.3 \pm 9.3	-
57	-	214.8 \pm 23.2	-
63	-	-	254.6 \pm 2.3
65	247.8 \pm 3.7	203.6 \pm 26.9	-
70	234.3 \pm 6.0	178.7 \pm 25.4	251.3 \pm 2.9
77	-	-	254.8 \pm 1.3
78	201.6 \pm 9.3	150.5 \pm 41.9	-
85	-	-	248.6 \pm 7.1
89	156.2 \pm 42.1	107.7 \pm 48.5	-
95	133.6 \pm 55.6	68.7 \pm 46.8	-
100	-	-	243.7 \pm 4.4
102	113.4 \pm 67.2	42.3 \pm 29.8	-
109	87.8 \pm 71.4	19.3 \pm 18.6	237.1 \pm 5.8
115	84.3 \pm 69.5	6.7 \pm 4.9	-
122	74.8 \pm 66.4	ND	-
125	-	-	215.0 \pm 16.2
126	-	-	215.0 \pm 16.2
129	54.6 \pm 48.4	-	-
136	47.3 \pm 45.5	-	207.3 \pm 8.3

ND: No TBA detected

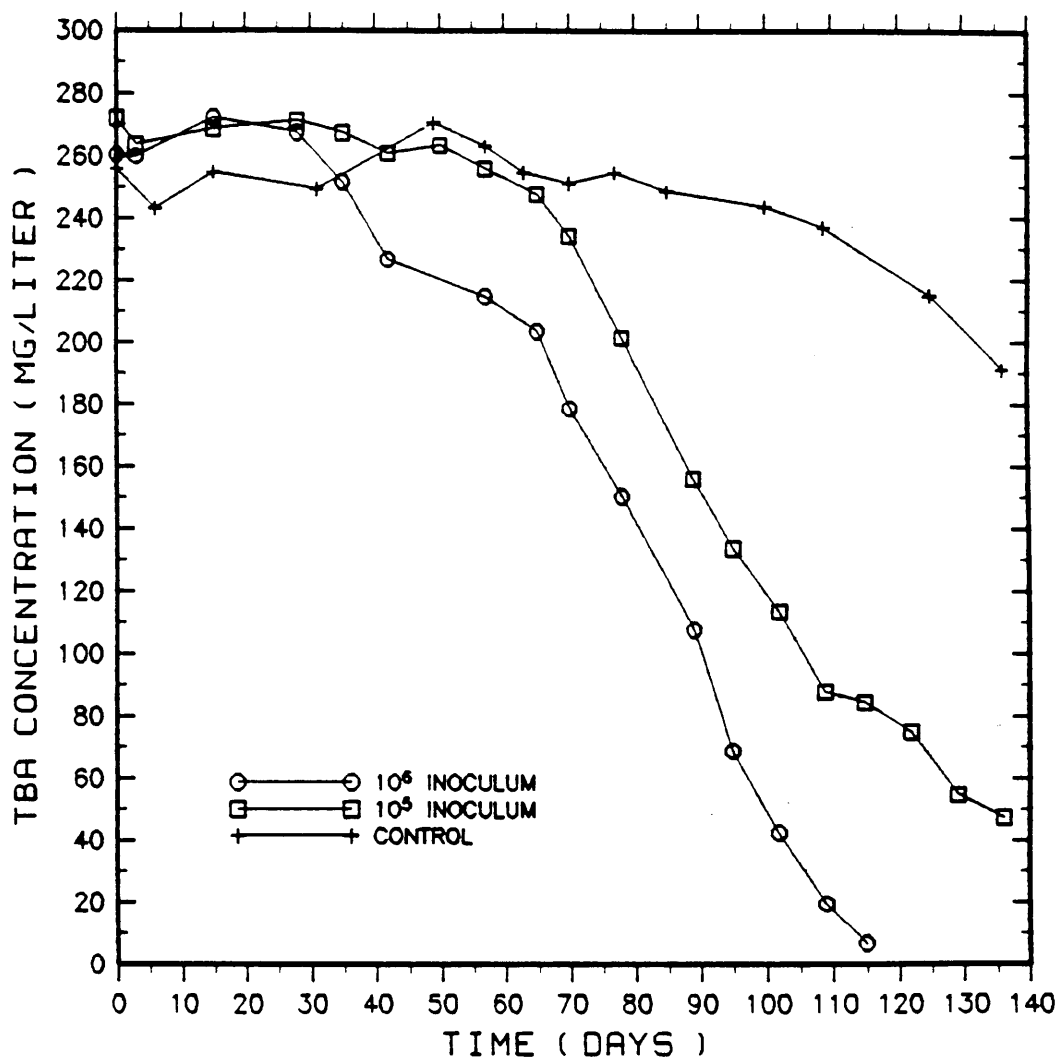


Figure 8. Concentration of tertiary butyl alcohol (TBA) over time in Fredrick clay amended with 250 mg/liter TBA. Treatments include: sterilized soil inoculated with 10^5 and 10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100, and sterilized soil control. All the treatments were established in triplicate.

Table 11. Degradation of tertiary butyl alcohol (TBA) in Fredrick clay amended with 1.0 mM molybdate and 250 mg/liter TBA. Treatments include: soil inoculated with 10^6 cell per g soil (dry weight) *Pseudomonas* sp. VT100 and uninoculated soil. All treatments performed in triplicate. Refer to figure 9.

Time (days)	TBA Concentration (mg/liter)		Time (days)	TBA Concentration (mg/liter)	
	Inoculated	Uninoculated		Inoculated	Uninoculated
0	254.4+12.2	268.2+0.5	58	-	80.4+7.9
2	240.8+4.9	268.6+1.3	59	145.3+7.8	-
6	-	271.8+5.0	65	-	67.6+8.4
7	238.6+12.9	-	68	113.0+9.2	-
11	227.6+8.4	262.5+6.7	72	-	53.6+8.3
16	-	253.6+1.9	75	90.8+13.1	-
18	210.3+10.9	-	78	-	42.8+9.6
22	203.7+11.2	-	82	58.2+27.3	-
26	-	196.8+1.3	85	-	35.6+10.1
27	195.7+7.3	-	89	48.4+14.1	-
32	184.3+5.4	-	92	-	22.0+7.2
33	-	153.7+5.4	95	28.7+17.2	-
37	-	130.1+6.9	102	15.7+15.7	-
40	-	122.2+7.1	106	-	15.1+10.6
42	180.3+6.7	-	109	6.5+5.7	-
43	-	119.0+6.6	120	ND	6.9+6.9
45	175.3+6.5	111.9+5.7	134	-	1.9+1.9
51	-	93.9+7.6			
53	151.9+8.4	-			

ND: TBA degraded below detectable concentrations.

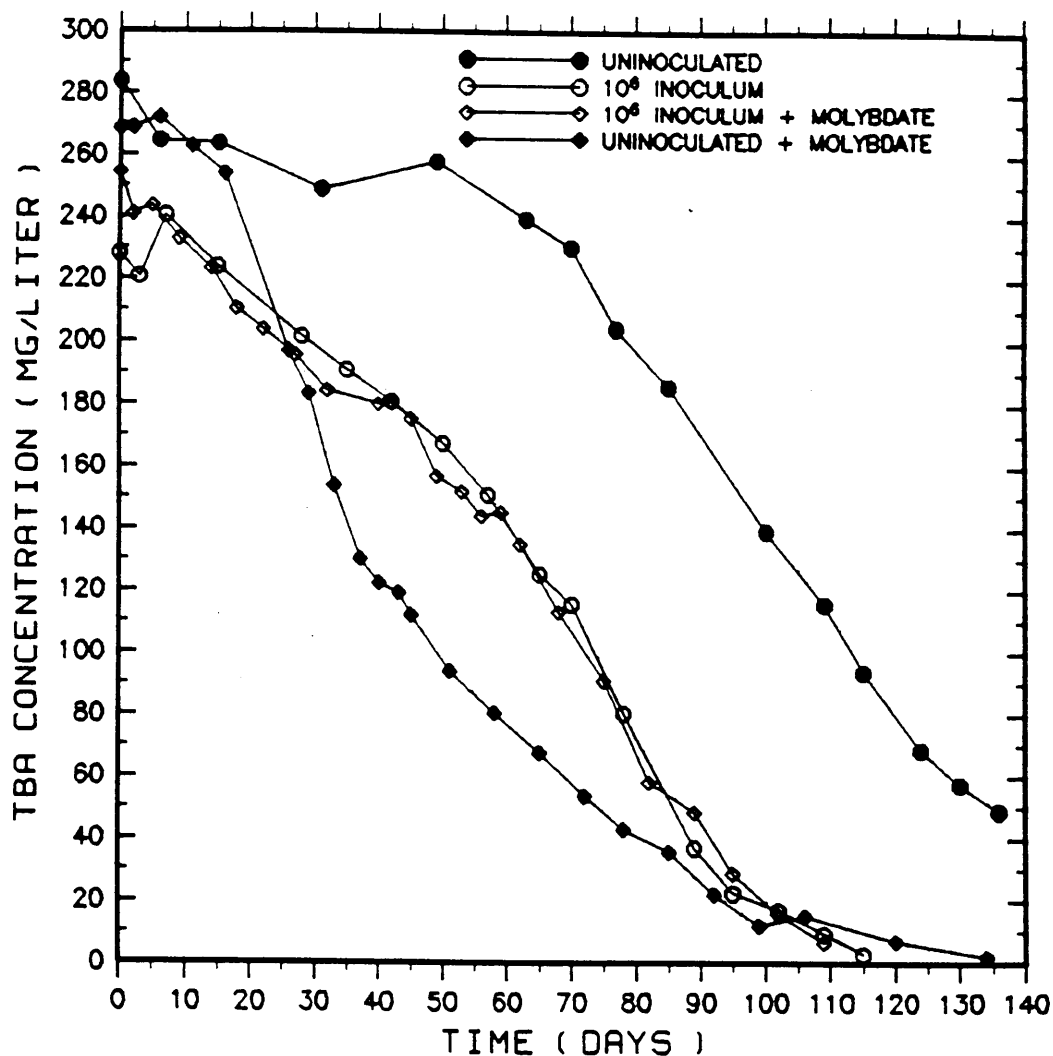


Figure 9. Effect of molybdate on the biodegradation of tertiary butyl alcohol (TBA). Fredrick clay amended with 1.0 mM molybdate and 250 mg/liter TBA. Treatments include: nonsterile soil with and without a 1.0 mM molybdate amendment, and nonsterile soil inoculated with 10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100 with and without a 1.0 mM molybdate amendment. All the treatments were established in triplicate.

Table 12. Degradation of tertiary butyl alcohol (TBA) in Fredrick clay dosed with 100 mg/liter TBA. Treatments include: nonsterilized soil inoculated with 10^5 and 10^6 cells per g soil (dry weight) of *Pseudomonas* sp., VT100, uninoculated soil and sterilized control soil. All treatments performed in triplicate. Refer to figure 10.

Time (days)	Inoculum (cells per g soil(dry weight))			
	Uninoculated	10^5	10^6	Control
	Concentration TBA (mg/liter)			
0	107.4+5.6	107.3+4.3	127.9+4.9	107.3+4.3
3	112.8+1.8	-	-	-
4	-	107.4+4.9	121.1+5.6	113.4+1.2
8	119.2+3.9	100.0+3.3	103.0+13.6	103.8+3.9
13	106.1+3.8	-	-	-
17	-	103.1+1.2	100.3+3.3	115.4+1.3
18	104.5+5.5	-	-	-
27	85.3+9.5	70.1+8.7	65.8+4.2	98.5+0.9
33	62.6+11.9	59.5+14.5	56.0+10.4	106.0+0.3
36	48.9+8.9	56.4+16.3	53.5+10.4	109.3+0.8
39	43.2+12.5	50.0+17.0	50.9+12.7	106.3+3.8
41	-	46.0+11.8	47.2+16.3	106.2+0.6
43	29.3+12.9	41.3+17.4	43.3+12.0	103.6+0.9
45	-	36.8+18.1	38.8+10.9	101.9+1.3
47	21.8+14.2	-	-	-
48	-	32.9+18.4	34.0+9.7	103.0+0.2
49	13.5+13.8	-	-	-
51	-	25.6+20.1	26.7+9.0	101.3+1.4
53	7.3+9.7	-	-	-
54	-	20.8+18.6	21.7+7.6	100.4+2.7
57	2.7+3.8	-	-	-
58	-	13.3+12.8	7.0+6.7	98.7+2.6
61	ND	8.8+9.4	2.9+3.3	99.9+3.3
63	-	7.0+5.9	ND	-
67	-	2.7+3.7	-	99.0+2.1

ND: Degraded below detectable concentrations.

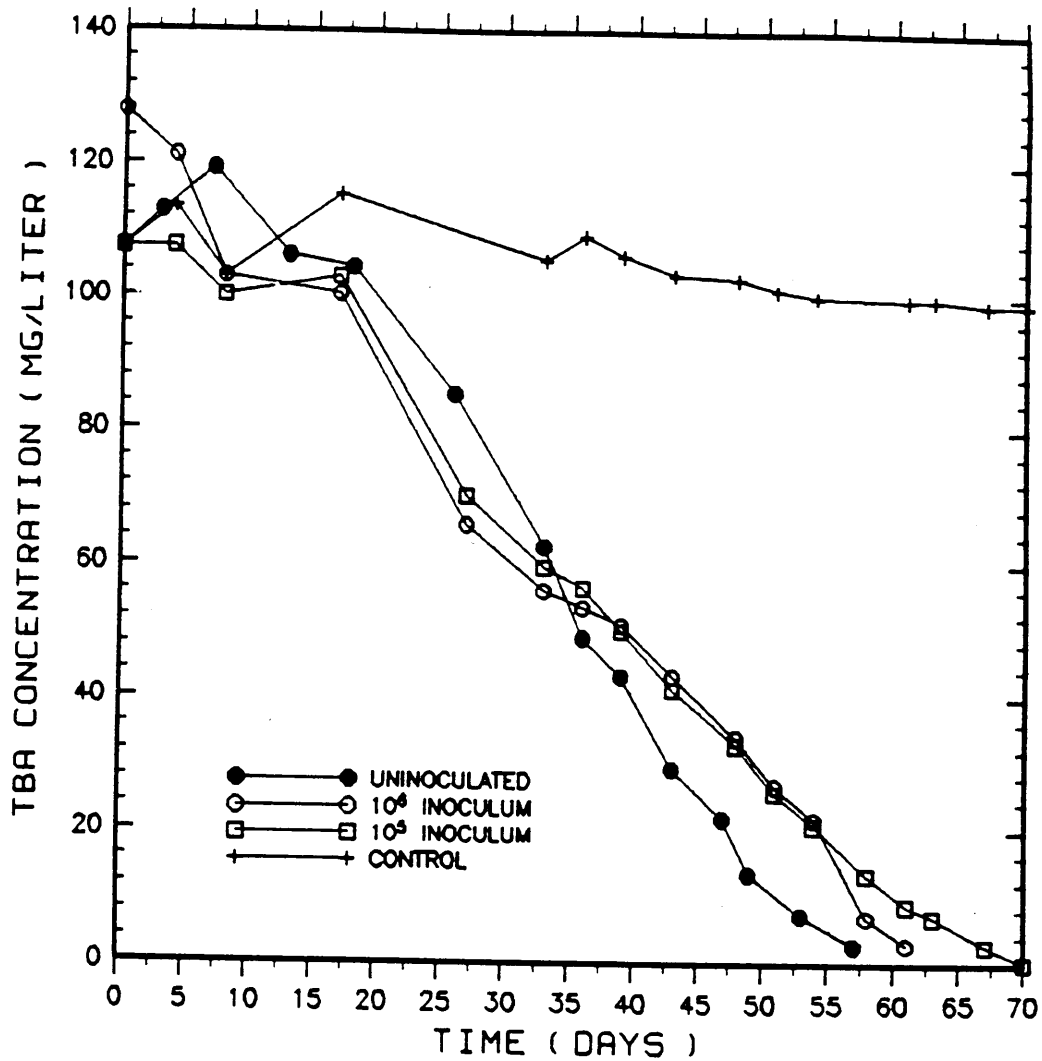


Figure 10. Concentration of tertiary butyl alcohol (TBA) over time in Fredrick clay amended with 100 mg/liter TBA. Treatments include: nonsterile soil, nonsterile soil inoculated with 10^5 and 10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100, and sterilized soil control. All the treatments were established in triplicate.

Table 13. Degradation of tertiary butyl alcohol (TBA) in Fredrick clay dosed with 100 mg/liter TBA. Treatments include: sterilized soil inoculated with 10^5 and 10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100. and sterilized control soil. All treatments performed in triplicate. Refer to figure 11.

Time (days)	Inoculum (cells per g soil(dry weight))		
	10^5	10^6	Control
	TBA Concentration (mg/liter)		
0	99.0+1.1	123.6+3.7	107.3+4.3
4	97.2+3.4	123.4+1.2	113.4+1.2
8	99.3+1.0	114.7+0.5	103.8+3.9
17	105.1+1.5	122.9+0.7	115.4+1.3
27	95.5+1.9	99.6+3.3	98.5+0.5
33	91.7+1.3	45.3+10.1	106.0+0.3
36	91.6+2.5	20.6+13.7	109.0+0.8
39	83.3+3.4	8.5+3.7	106.3+0.6
41	82.3+3.9	0.7+1.0	106.2+0.5
43	76.4+5.2	0.2+0.3	103.6+0.9
45	71.4+6.9	0.3+0.4	101.9+1.3
48	68.9+8.9	ND	103.0+0.2
51	62.0+11.0	-	101.3+1.4
54	56.6+13.3	-	100.4+2.6
58	47.7+19.0	-	98.7+2.6
61	42.5+22.2	-	99.9+3.3
63	37.2+23.6	-	-
67	30.7+22.4	-	99.0+2.1
70	26.1+19	-	99.0+1.4
79	18.2+18.1	-	-
87	4.2+5.5	-	91.0+6.4

ND: Degraded below detectable concentrations.

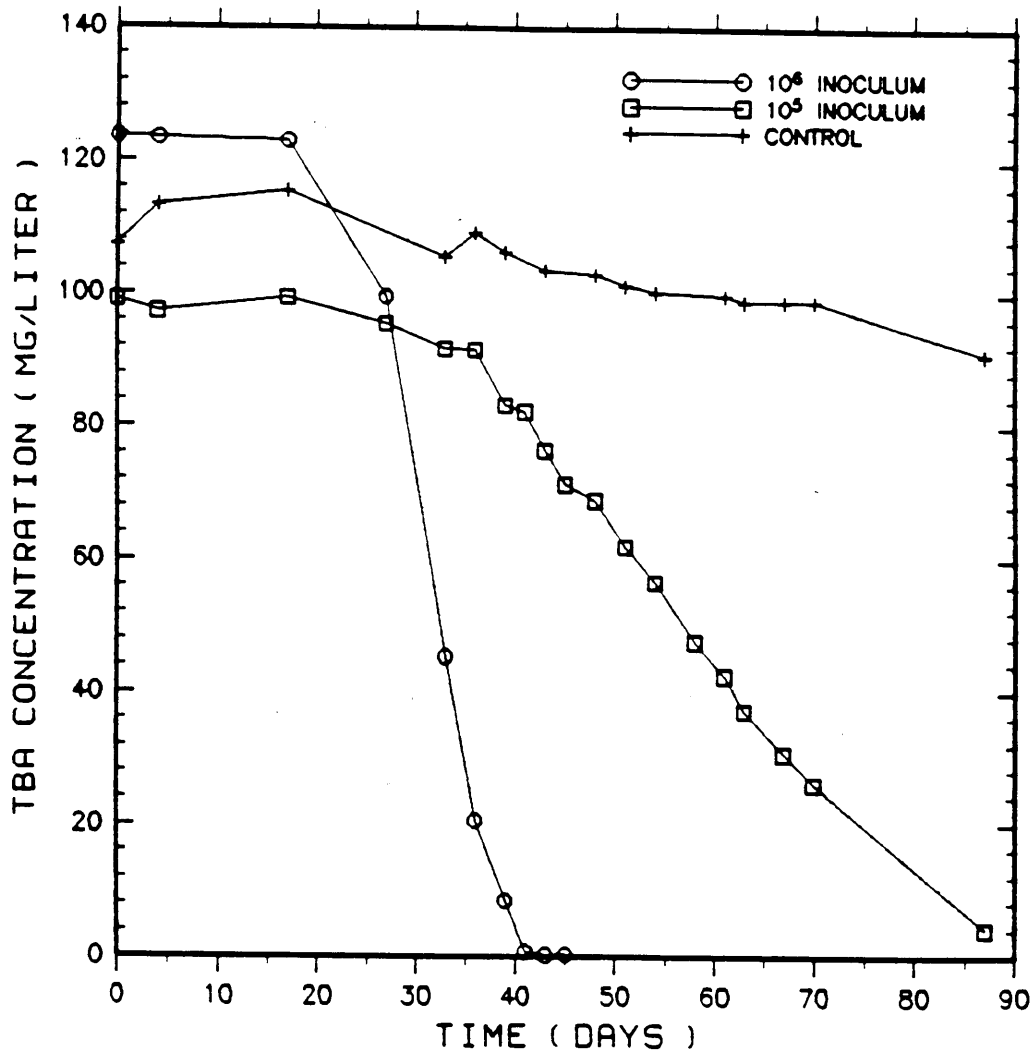


Figure 11. Concentration of tertiary butyl alcohol (TBA) over time in Fredrick clay amended with 100 mg/liter TBA. Treatments include: sterilized soil inoculated with 10^5 and 10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100, and sterilized soil control. All the treatments were established in triplicate.

Table 14. Degradation of tertiary butyl alcohol (TBA) in Fredrick clay amended with 1.0 mM molybdate and 100 mg/liter TBA. Treatments include: soil inoculated with 10^6 cell per g soil (dry weight) *Pseudomonas* sp. VT100 and uninoculated soil. All treatments performed in triplicate. Refer to figure 12.

Time (days)	TBA Concentration (mg/liter)		Time (days)	TBA Concentration (mg/liter)	
	Inoculated	Uninoculated		Inoculated	Uninoculated
0	105.1+1.4	124.9+4.6	32	56.1+4.0	-
2	100.1+0.2	124.7+3.2	33	-	29.2+24.7
3	101.9+1.7	-	37	-	18.7+18.7
5	100.6+2.3	-	40	-	14.5+14.5
6	-	122.2+5.8	42	43.7+8.2	-
7	98.4+1.5	-	43	-	11.8+10.8
9	90.8+0.8	-	45	36.5+8.0	10.2+10.2
11	89.6+2.1	118.1+3.8	49	31.0+8.5	-
14	85.1+3.2	-	51	-	3.0+3.0
16	-	106.2+4.4	53	24.8+10.2	1.9+1.9
18	74.7+2.8	-	56	18.3+9.6	ND
22	63.6+10.5	-	59	13.9+11.7	-
26	-	63.5+16.1	61	9.4+9.4	-
27	61.8+4.7	-	63	3.0+3.0	-
29	-	41.1+15.1			

ND: TBA degraded below detectable concentrations

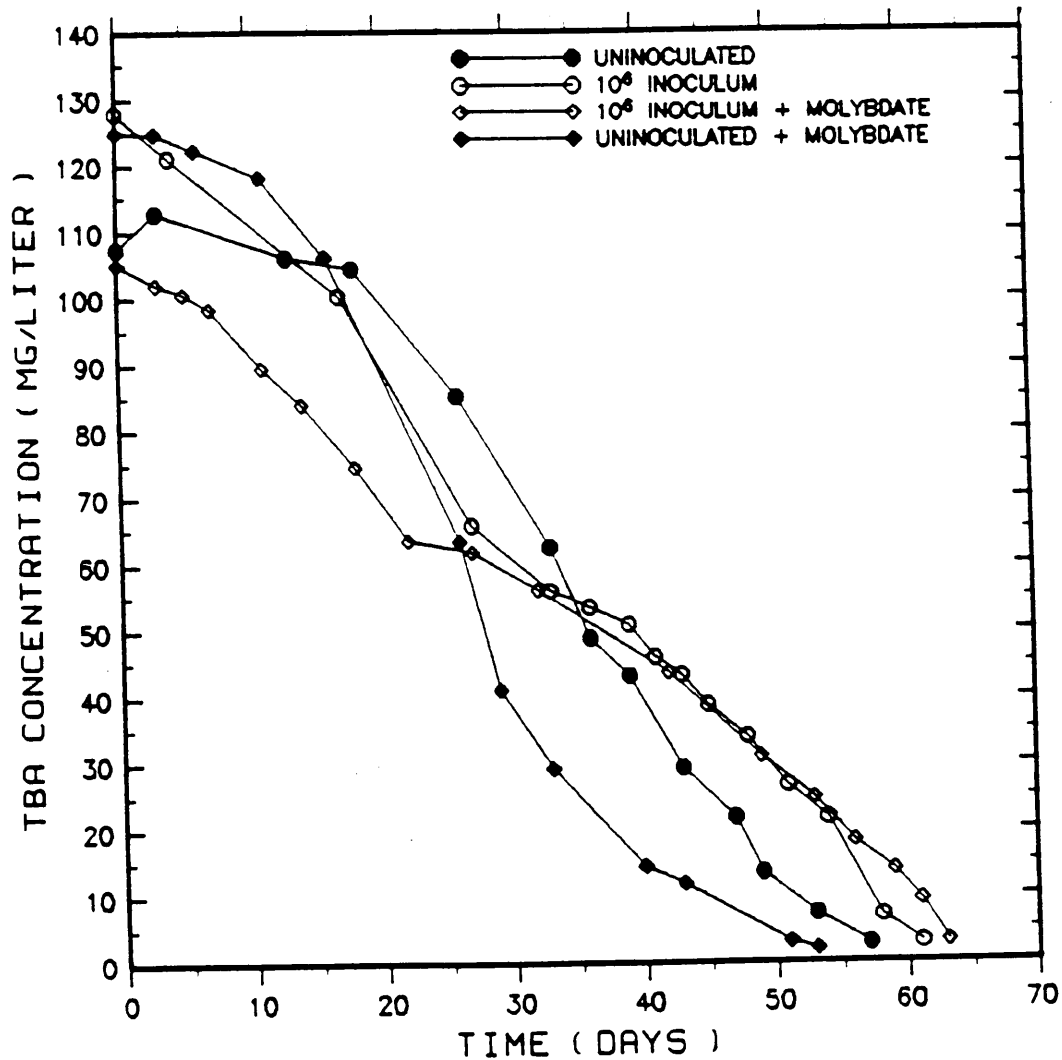


Figure 12. Effect of molybdate on the biodegradation of tertiary butyl alcohol (TBA). Fredrick clay amended with 1.0 mM molybdate and 100 mg/liter TBA. Treatments include: nonsterile soil with and without a 1.0 mM molybdate amendment, and nonsterile soil inoculated with 10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100 with and without a 1.0 mM molybdate amendment. All the treatments were established in triplicate.

Table 15. Degradation of tertiary butyl alcohol (TBA) in Fredrick clay dosed with 10 mg/liter TBA. Treatments include nonsterilized soil inoculated with 10^5 and 10^6 cells per g soil (dry weight) of Pseudomonas VT100, uninoculated soil and sterilized control soil. All treatments performed in triplicate. Refer to figure 13.

	Inoculum (cells per g soil(dry weight))			
	Uninoculated	10^5	10^6	Control
Time (days)	TBA Concentration (mg/liter)			
0	13.4+0.1	15.2+0.1	10.6+0.2	18.8+1.0
2	12.5+0.4	15.0+0.1	9.1+0.2	16.9+1.0
4	-	14.5+0.1	6.8+0.5	-
6	12.5+0.0	14.2+0.1	4.8+0.5	17.6+0.5
8	-	13.5+0.3	0.8+0.5	-
10	-	12.3+0.6	ND	-
12	-	10.3+0.7	-	-
14	-	6.9+0.1	-	-
15	12.3+0.1	4.2+1.0	-	17.7+0.3
18	11.6+0.4	1.1+1.0	-	-
20	12.0+1.3	ND	-	17.9+0.3
23	11.6+0.5	-	-	18.0+0.3
29	9.1+1.4	-	-	17.5+0.2
34	6.6+3.9	-	-	17.7+0.3
39	4.2+3.0	-	-	17.0+0.2
43	1.7+1.2	-	-	16.1+0.1
49	ND	-	-	16.3+0.6

ND: Degraded below detectable concentrations

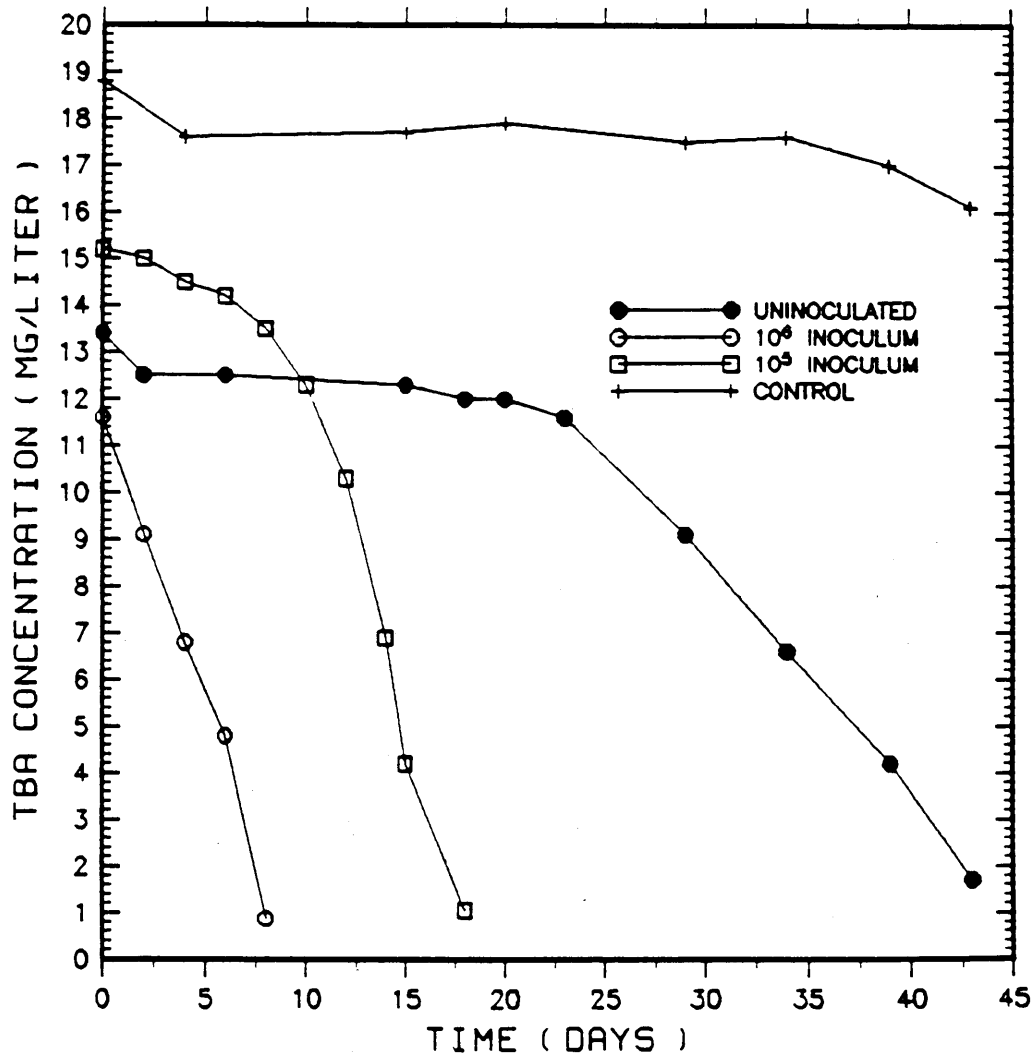


Figure 13. Concentration of tertiary butyl alcohol (TBA) over time in Fredrick clay amended with 10 mg/liter TBA. Treatments include: nonsterile soil, nonsterile soil inoculated with 10^5 and 10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100, and sterilized soil control. All the treatments were established in triplicate.

Table 16. Degradation of tertiary butyl alcohol (TBA) in Fredrick clay dosed with 10 mg/liter TBA. Treatments include sterilized soil inoculated with 10^5 and 10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100 and sterilized control soil. All treatments performed in triplicate. Refer to figure 14.

Time (days)	Inoculum (cells per g soil(dry weight))		
	10^5	10^6	Control
TBA concentration (mg/liter)			
0	12.5+0.6	10.3+0.3	18.8+1.0
2	-	6.8+0.3	16.9+1.0
3	-	6.0+0.7	-
4	12.0+0.8	-	-
5	-	3.6+3.5	-
6	-	-	17.6+0.5
7	-	3.3+3.3	-
8	12.0+1.0	-	-
9	-	1.1+1.0	-
12	-	ND	-
15	11.3+0.4	-	17.7+0.3
20	-	-	17.9+0.3
23	11.7+0.5	-	18.0+0.3
29	10.3+1.0	-	17.5+0.2
34	9.4+2.1	-	17.7+0.3
39	8.2+3.6	-	17.0+0.2
43	7.4+4.5	-	16.1+0.1
49	7.0+4.9	-	16.3+0.6
53	6.9+4.8	-	15.8+0.1
65	6.9+4.8	-	15.4+0.2
75	6.9+4.8	-	15.2+0.4

ND: Degraded below detectable concentrations

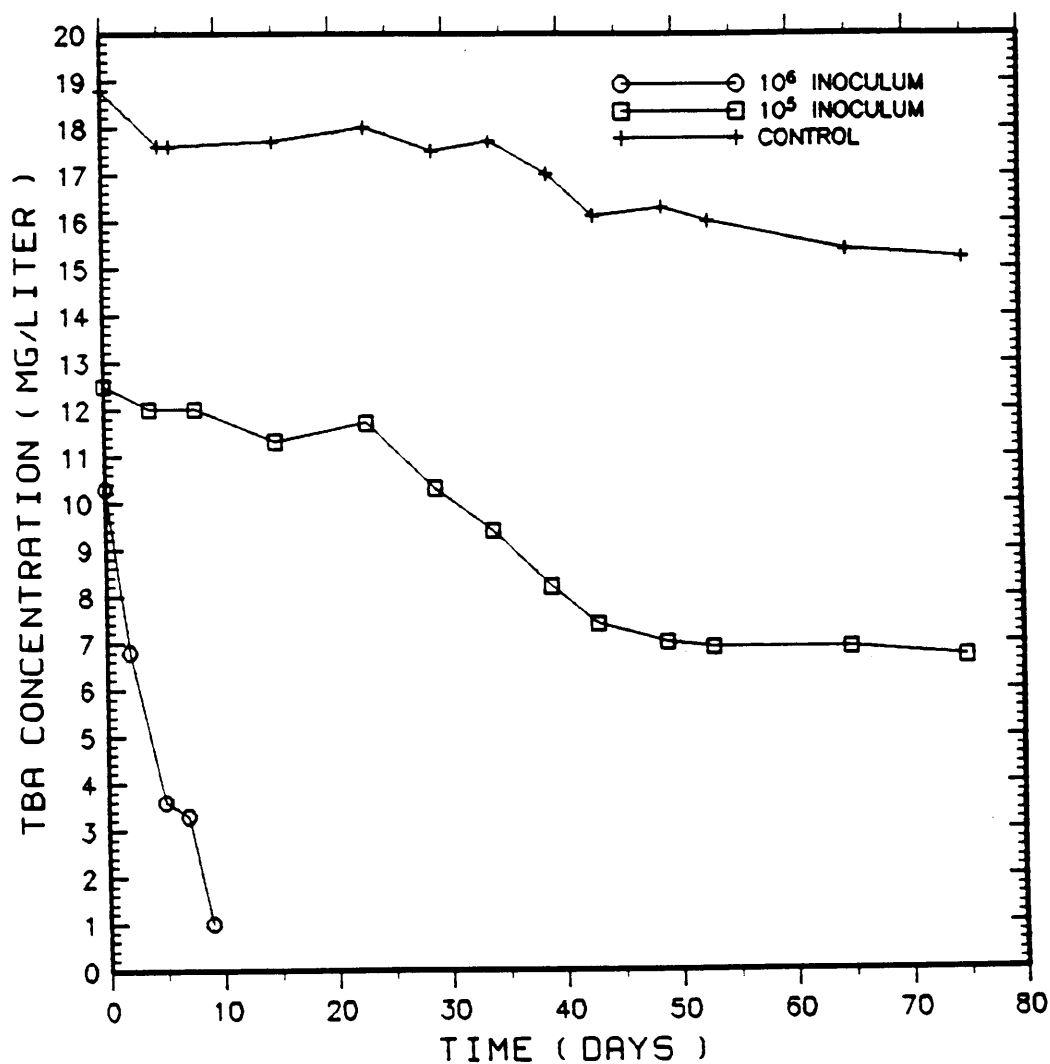


Figure 14. Concentration of tertiary butyl alcohol (TBA) over time in Fredrick clay amended with 10 mg/liter TBA. Treatments include: sterilized soil inoculated with 10^5 and 10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100, and sterilized soil control. All the treatments were established in triplicate.

Table 17. Degradation of tertiary butyl alcohol (TBA) in Fredrick clay amended with 1.0 mM molybdate and 10 mg/liter TBA. Treatments include soil inoculated with 10^6 cell per g soil (dry weight) *Pseudomonas* sp. VT100 and uninoculated soil. All treatments performed in triplicate. Refer to figure 15.

Time (days)	TBA Concentration (mg/liter)		Time (days)	TBA Concentration (mg/liter)	
	Inoculated	Uninoculated		Inoculated	Uninoculated
0	16.5+0.2	11.5+0.2	8	-	10.9+0.1
2	15.0+0.5	11.5+0.1	9	4.6+0.5	-
3	14.1+0.2	-	11	0.3+0.3	10.1+0.2
4	-	11.1+0.3	13	ND	8.1+0.7
5	11.8+0.0	-	16	-	3.5+0.2
6	-	11.2+0.2	17	-	2.4+0.1
7	9.0+0.4	-	18	-	1.1+0.2

ND: TBA degraded below detectable concentrations

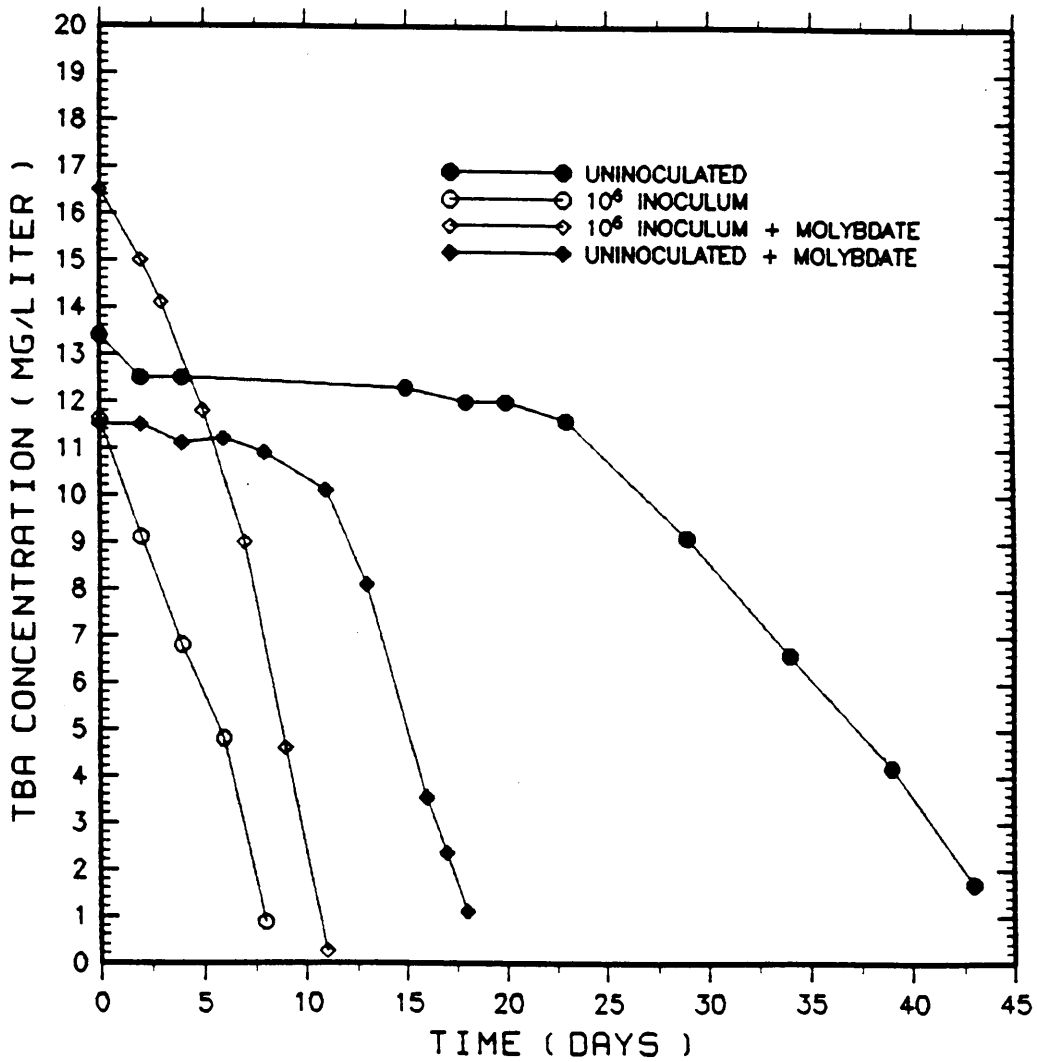


Figure 15. Effect of molybdate on the biodegradation of tertiary butyl alcohol (TBA). Fredrick clay amended with 1.0 mM molybdate and 10 mg/liter TBA. Treatments include: nonsterile soil with and without a 1.0 mM molybdate amendment, and nonsterile soil inoculated with 10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100 with and without a 1.0 mM molybdate amendment. All the treatments were established in triplicate.

Table 18. Degradation of tertiary butyl alcohol (TBA) in Groseclose clayey loam dosed with 250 mg/liter TBA. Treatments include: sterile and nonsterile soil inoculated with 10^6 cells per g (dry weight) soil of *Pseudomonas* VT100 and indigenous microflora, and sterilized control. Molybdate (1.0 mM) added to inoculated and uninoculated treatments. All treatments performed in triplicate. Refer to figure 16.

Time (days)	TBA Concentration (mg/liter)				
	Nonsterile Soil		Sterile Soil		
	Inoculated		Uninoculated		Inoculated
	No MoO_4	MoO_4	No MoO_4	MoO_4	No MoO_4
0	288.7+4.7	260.0+12.9	245.9+12.6	265.0+5.9	252.2+19.4
3	286.0+5.0	257.5+8.0	250.0+12.1	265.0+13.7	257.7+18.6
7	286.5+8.6	243.5+9.8	241.6+9.4	254.2+10.1	255.2+11.5
14	246.1+0.9	217.3+15.2	-	-	236.6+18.4
21	-	-	244.3+13.4	230.2+22.7	-
28	257.6+3.4	217.3+15.1	245.6+10.3	230.2+22.7	221.6+13.5
35	244.5+8.2	193.7+15.6	238.4+14.4	229.2+17.5	243.4+21.3
49	248.1+7.6	180.4+12.6	245.6+8.7	227.6+20.7	230.9+11.3
63	243.0+11.8	190.0+33.1	241.5+7.6	227.6+32.2	225.7+10.5
77	237.5+16.8	184.9+30.9	250.6+2.3	229.2+34.6	220.0+13.4
98	218.4+17.7	160.7+35.5	233.0+5.2	208.4+42.8	198.0+15.7
119	199.2+18.9	137.5+36.4	222.0+3.6	185.1+45.1	175.8+10.6
140	183.5+20.0	121.0+36.1	221.3+3.2	189.4+53.5	159.0+4.1

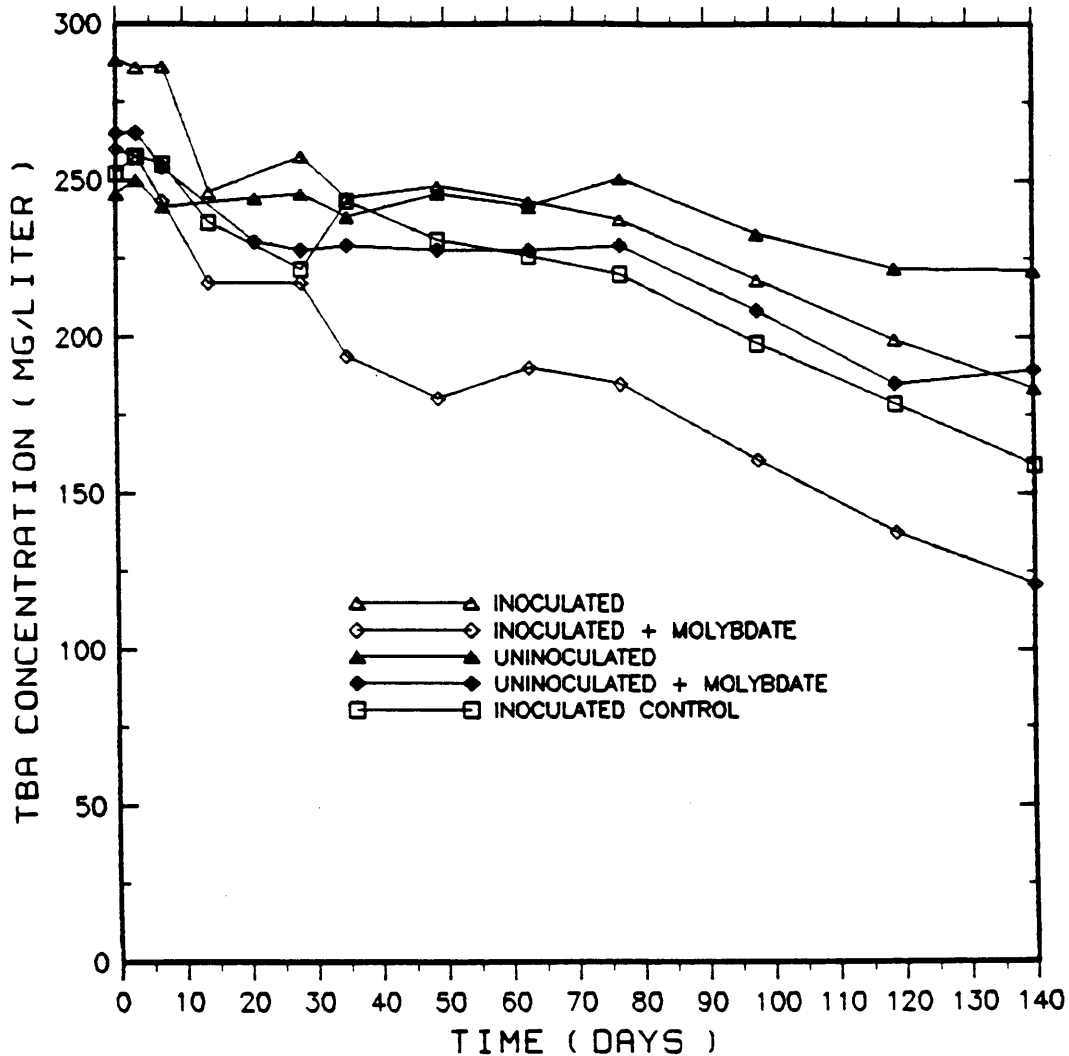


Figure 16. The concentration of tertiary butyl alcohol (TBA) over in Groseclose clayey loam amended with 250 mg/liter TBA. Treatments include: sterile and nonsterile soil inoculated with 8.6×10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100, uninoculated soil, and inoculated and uninoculated soil amended with 1.0 mM molybdate. All the treatments were established in triplicate.

Table 19. Degradation of tertiary butyl alcohol (TBA) in Groseclose clayey loam amended with 10 mg/liter yeast extract and 250 mg/liter TBA. Treatments include: sterile and nonsterile soil inoculated with 10^6 cells per g soil (dry weight) of *Pseudomonas* VT100, indigenous microflora and sterilized control. Molybdate (1.0mM) added to inoculated and uninoculated treatments. All treatments performed in triplicate. Refer to figure 17.

Time (days)	TBA Concentration (mg/liter)					
	Nonsterile Soil			Sterile Soil		
	Inoculated		Uninoculated		Inoculated	
	No MoO ₄	MoO ₄	No MoO ₄	MoO ₄	No MoO ₄	MoO ₄
0	267.1+9.1	264.4+12.5	281.8+8.0	297.3+3.6	253.6+9.9	
3	257.9+2.8	256.1+8.7	275.9+6.5	278.4+13.0	239.4+4.4	
7	249.0+18.4	260.0+14.9	241.2+0.4	262.7+11.8	231.4+15.0	
14	-	-	-	-	220.7+5.3	
21	237.5+3.3	212.0+13.3	253.3+20.8	254.2+31.8	-	
28	233.3+10.0	229.8+26.6	263.4+7.6	255.5+25.1	197.4+4.0	
35	234.6+4.1	239.4+10.1	248.6+14.6	250.4+4.6	188.0+13.1	
49	224.6+10.1	221.9+16.2	238.5+10.4	241.9+20.7	167.1+18.5	
63	215.2+8.6	211.0+22.1	241.2+10.8	228.2+20.8	165.1+13.1	
77	209.1+10.6	202.4+25.4	236.0+8.2	226.1+25.8	153.5+10.1	
98	181.9+10.1	166.8+29.5	215.2+14.4	201.6+29.5	129.6+12.7	
119	164.3+10.1	145.7+33.5	192.4+18.2	176.7+24.6	111.4+10.0	
140	152.5+14.8	134.5+30.3	197.2+17.5	173.9+34.4	99.6+12.8	

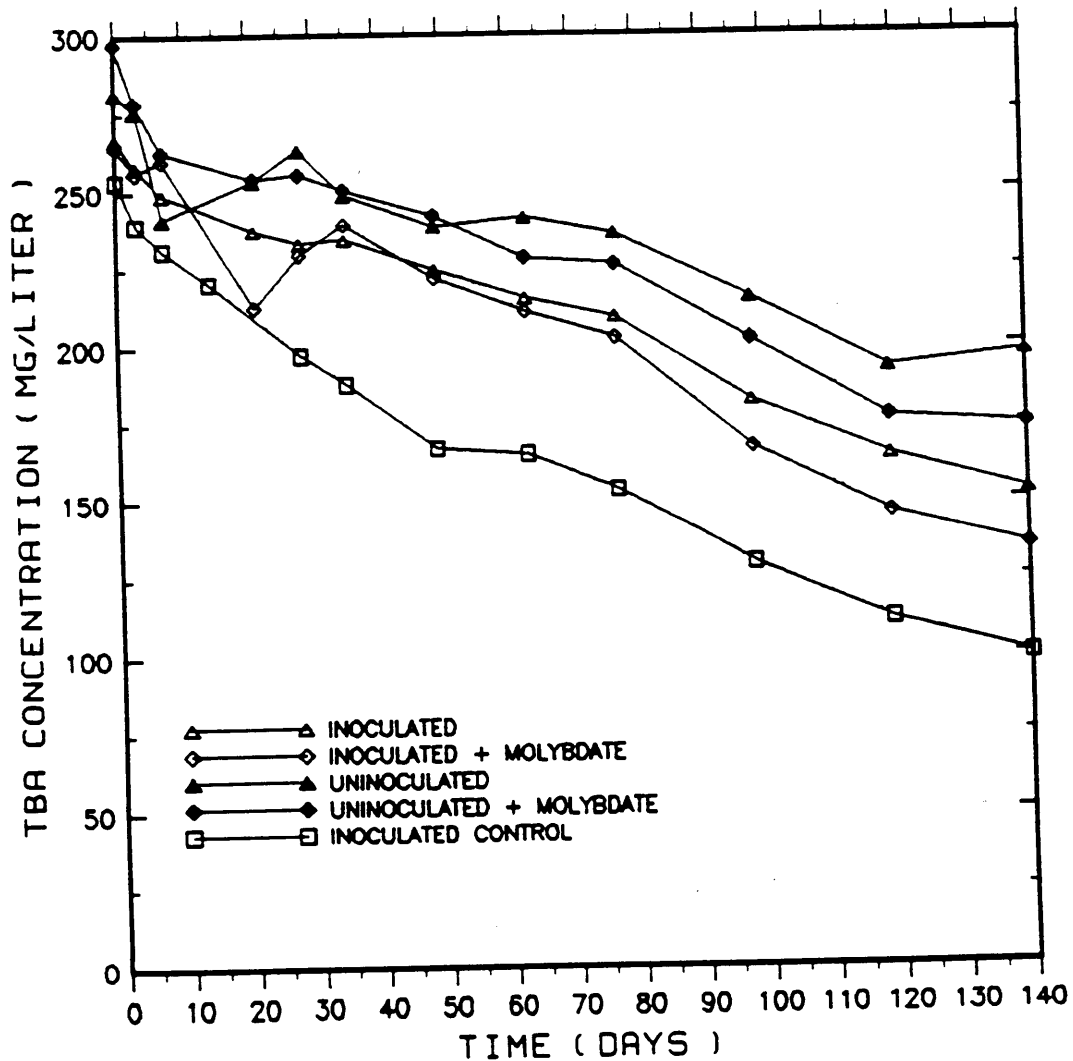


Figure 17. The concentration of tertiary butyl alcohol (TBA) over time in Groseclose clayey loam amended with 250 mg/liter TBA and 10 mg/liter yeast extract. Treatments include: sterile and nonsterile soil inoculated with 8.6×10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100, uninoculated soil, and inoculated and uninoculated soil amended with 1.0 mM molybdate. All the treatments were established in triplicate.

Table 20. Degradation of tertiary butyl alcohol (TBA) in Groseclose clayey loam amended with 100 mg/liter yeast extract and 250 mg/liter TBA. Treatments include: sterile and nonsterile soil inoculated with 10^6 *Pseudomonas* sp. cells per g soil (dry weight), indigenous microflora and sterilized control. Molybdate (1.0 mM) added to inoculated and uninoculated treatments. All treatments performed in triplicate. Refer to figure 18.

Time (days)	TBA Concentration (mg/liter)				
	Nonsterile Soil				Sterile Soil
	Inoculated		Uninoculated		Inoculated
	No MoO ₄	MoO ₄	No MoO ₄	MoO ₄	No MoO ₄
0	277.5+14.6	264.3+14.6	295.8+1.5	241.3+8.5	241.6+4.0
3	284.6+7.9	268.2+8.0	275.3+20.7	240.3+21.6	246.6+2.9
7	285.0+7.4	256.3+14.0	238.4+12.2	208.8+10.3	245.9+10.4
14	241.2+13.0	222.8+14.6	-	-	210.7+15.5
21	-	-	257.7+12.2	235.8+16.5	-
28	228.4+7.2	208.9+15.2	269.9+9.5	232.1+10.4	210.9+12.3
35	219.4+1.1	205.9+14.6	260.9+15.5	239.1+5.4	215.0+8.3
49	213.3+18.5	192.9+32.2	250.4+10.6	227.4+16.0	210.2+7.4
63	209.9+9.8	174.4+28.6	242.8+11.5	202.1+12.3	200.3+13.6
77	202.4+6.9	166.4+37.0	234.9+10.0	196.0+11.1	187.2+17.5
98	178.9+7.9	142.1+41.8	199.4+14.3	162.3+21.6	166.3+18.6
119	158.0+4.0	128.3+35.8	183.1+14.3	140.6+20.0	143.3+20.0
140	140.5+2.9	107.9+35.5	159.8+16.6	124.4+20.6	130.4+20.4

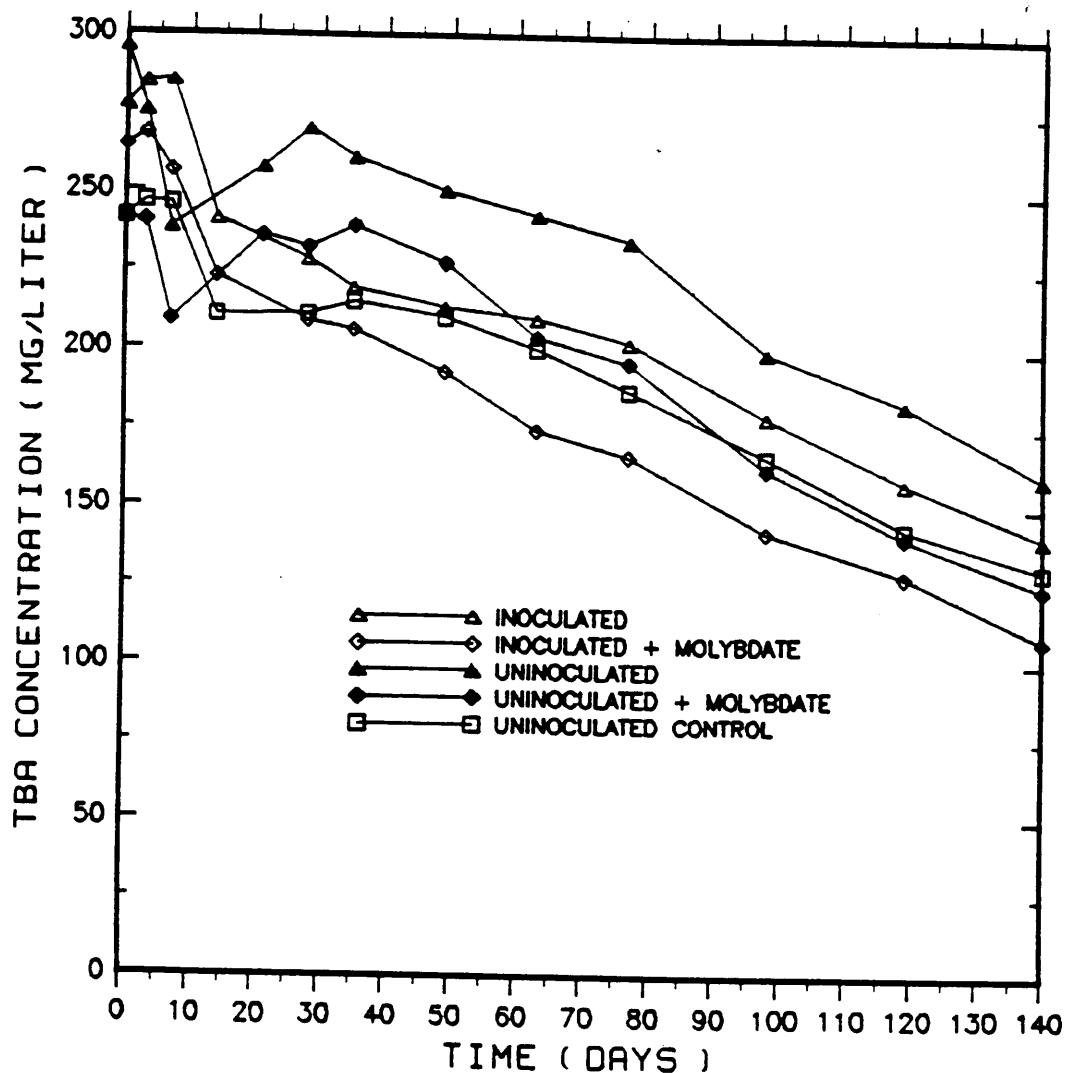


Figure 18. The concentration of tertiary butyl alcohol (TBA) over time in Groseclose clayey loam amended with 250 mg/liter TBA and 100 mg/liter yeast extract. Treatments include: sterile and nonsterile soil inoculated with 8.6×10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100, uninoculated soil, and inoculated and uninoculated soil amended with 1.0 mM molybdate. All the treatments were established in triplicate.

Table 21. Degradation of tertiary butyl alcohol (TBA) in Groseclose clayey loam amended with 10 mg/liter acetate and 250 mg/liter TBA. Treatments include: sterile and nonsterile soil inoculated with 10^6 *Pseudomonas* sp. cells per g soil (dry weight), indigenous microflora, and sterilized controls. Molybdate (1.0 mM) added to inoculated and uninoculated treatments. All treatments performed in triplicate. Refer to figure 19.

Time (days)	TBA Concentration (mg/liter)				
	Nonsterile Soil				Sterile Soil
	Inoculated		Uninoculated		Inoculated
	No MoO ₄	MoO ₄	No MoO ₄	MoO ₄	No MoO ₄
0	265.0+10.6	272.9+11.1	259.9+11.4	268.3+14.8	246.3+2.1
3	277.0+24.8	268.4+9.0	245.1+5.0	267.6+7.2	239.9+5.5
7	278.6+26.3	255.6+15.5	237.1+6.9	250.5+6.8	241.6+4.0
14	259.8+1.2	243.0+20.6	-	-	218.5+10.2
21	-	-	214.0+24.3	246.8+23.5	-
28	206.1+8.4	199.8+33.3	225.7+16.5	237.1+9.9	175.1+13.4
35	198.0+6.7	193.5+41.7	224.1+21.7	245.6+7.3	177.4+10.0
49	212.5+5.0	200.6+45.1	228.6+23.6	264.7+5.1	141.6+6.0
63	207.3+6.0	199.5+31.2	208.1+29.9	258.4+4.5	136.2+2.4
77	200.3+14.4	193.2+45.3	219.5+31.1	259.1+8.0	125.5+4.3
98	180.6+10.2	174.3+43.5	197.3+30.2	244.6+10.0	100.8+3.7
119	169.4+12.8	140.9+47.0	182.8+38.7	233.4+8.4	81.5+6.7
140	159.0+11.1	142.0+53.1	180.9+39.9	226.1+12.0	67.2+4.5

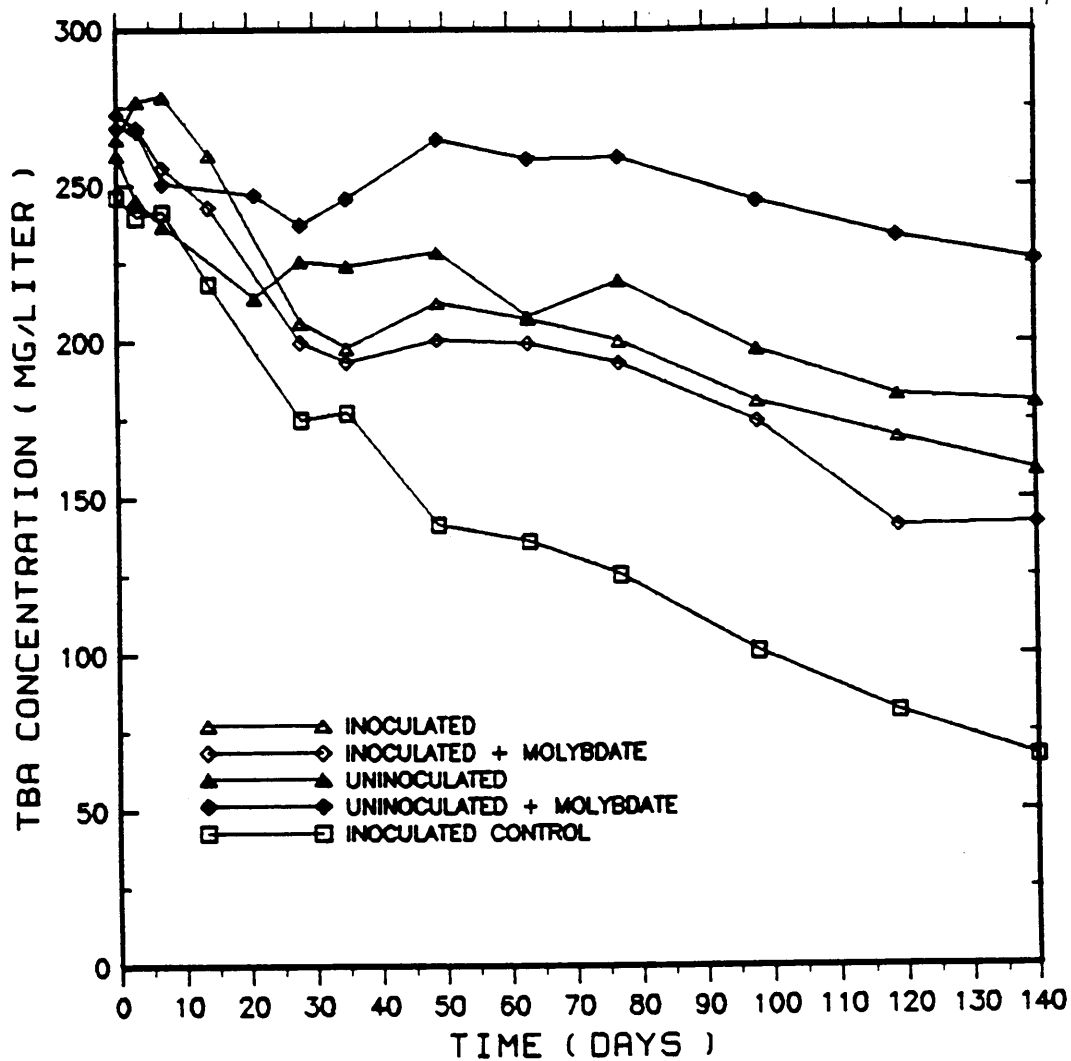


Figure 19. The concentration of tertiary butyl alcohol (TBA) over time in Groseclose clayey loam amended with 250 mg/liter TBA and 10 mg/liter acetate. Treatments include: sterile and nonsterile soil inoculated with 8.6×10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100, uninoculated soil, and inoculated and uninoculated soil amended with 1.0 mM molybdate. All the treatments were established in triplicate.

Table 22. Degradation of tertiary butyl alcohol (TBA) in Groseclose clayey loam amended with 100 mg/liter acetate and 250 mg/liter TBA. Treatments include: sterile and nonsterile soil inoculated with 10^6 *Pseudomonas* sp. cells per g soil (dry weight), indigenous microflora, and sterilized control. Molybdate (1.0 mM) added to inoculated and uninoculated treatments. All treatments performed in triplicate. Refer to figure 20.

Time (days)	TBA Concentration (mg/liter)					
	Nonsterile Soil				Sterile Soil	
	Inoculated		Uninoculated		Inoculated	
	No MoO ₄	MoO ₄	No MoO ₄	MoO ₄	No MoO ₄	MoO ₄
0	275.3+10.4	240.2+14.3	298.6+14.0	280.8+3.6	243.9+2.6	
3	262.7+8.8	234.5+13.8	265.0+11.7	259.0+10.4	246.9+4.1	
7	265.7+7.6	232.0+15.5	272.3+8.2	252.3+19.4	245.3+4.7	
14	226.5+3.0	211.5+10.0	254.0+12.7	250.7+4.6	222.7+11.0	
28	231.1+4.9	222.4+18.0	247.5+15.1	225.3+3.2	235.4+8.5	
35	240.0+1.5	209.7+3.3	231.3+23.0	242.6+2.5	206.5+11.2	
49	220.0+22.0	200.0+23.4	223.6+30.8	239.2+8.7	216.5+10.9	
63	214.8+19.7	201.6+18.5	234.2+37.2	238.8+4.8	210.0+7.2	
77	222.1+20.3	201.1+17.6	236.1+46.3	242.6+6.8	193.7+13.0	
98	200.1+24.2	178.3+17.4	209.1+59.0	223.8+4.5	166.0+17.4	
119	186.1+33.1	156.7+23.4	193.9+66.5	222.2+3.9	140.7+23.5	
140	170.2+28.1	144.3+22.5	179.5+72.6	210.1+1.1	117.8+26.4	

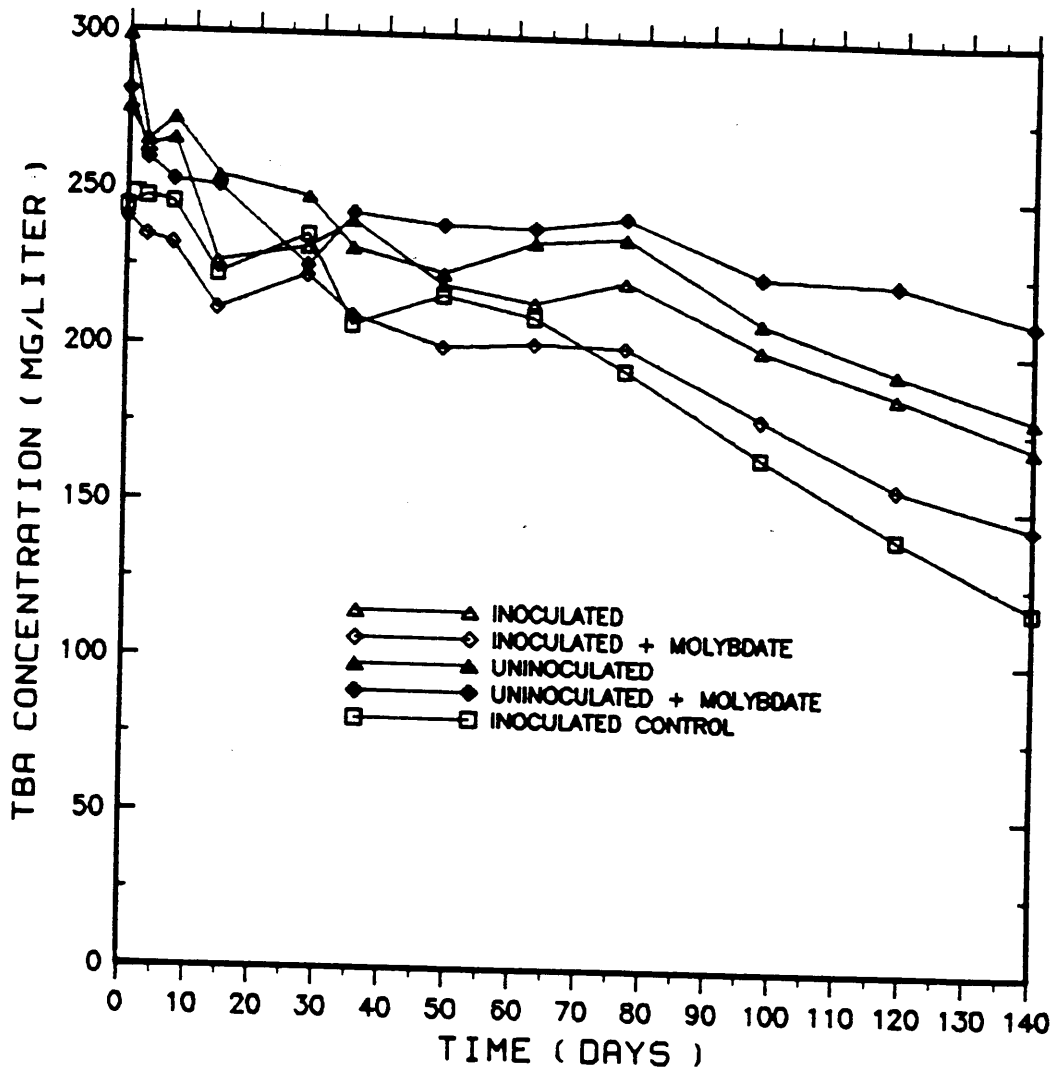


Figure 20. The concentration of tertiary butyl alcohol (TBA) over time in Groseclose clayey loam amended with 250 mg/liter TBA and 100 mg/liter acetate. Treatments include: sterile and nonsterile soil inoculated with 8.6×10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100, uninoculated soil, and inoculated and uninoculated soil amended with 1.0 mM molybdate. All the treatments were established in triplicate.

Table 23. Degradation of tertiary butyl alcohol (TBA) in Groseclose clayey loam amended with 10 mg/liter ethyl alcohol and 250 mg/liter TBA. Treatments include: sterile and nonsterile soil inoculated with 10^6 cells per g soil (dry weight) of *Pseudomonas* VT100, indigenous microflora and sterilized control. Molybdate (1.0 mM) added to inoculated and uninoculated treatments. All treatments performed in triplicate. Refer to figure 21.

Time (days)	TBA Concentration (mg/liter)				
	Nonsterile Soil				Sterile Soil
	Inoculated		Uninoculated		Inoculated
	No MoO ₄	MoO ₄	No MoO ₄	MoO ₄	No MoO ₄
0	251.9+22.5	255.1+9.8	280.1+14.3	280.7+8.0	259.1+5.2
3	249.0+20.0	242.5+20.3	259.1+16.2	276.1+8.3	239.6+8.4
7	241.7+14.3	229.4+23.7	255.5+20.7	253.4+3.6	209.7+12.0
14	231.9+19.7	227.6+10.4	263.3+19.1	258.4+9.5	230.3+24.8
28	240.1+13.7	221.0+5.1	232.9+19.1	239.3+18.2	211.4+0.7
35	235.9+17.9	227.2+11.6	249.4+10.4	247.6+11.2	223.0+7.9
49	214.9+11.4	207.9+10.1	233.6+21.3	237.6+21.2	216.5+7.2
63	210.1+25.5	219.4+9.9	242.7+20.5	238.9+15.1	209.0+8.7
77	215.2+29.6	214.3+15.6	250.6+16.4	253.1+13.3	199.0+12.7
98	194.9+22.8	185.5+16.3	237.0+16.1	241.9+17.0	185.7+24.4
119	179.3+30.4	184.6+19.8	222.4+19.3	230.7+21.6	168.4+12.6
140	166.9+34.2	169.1+21.7	212.3+16.6	217.5+7.6	162.7+24.4

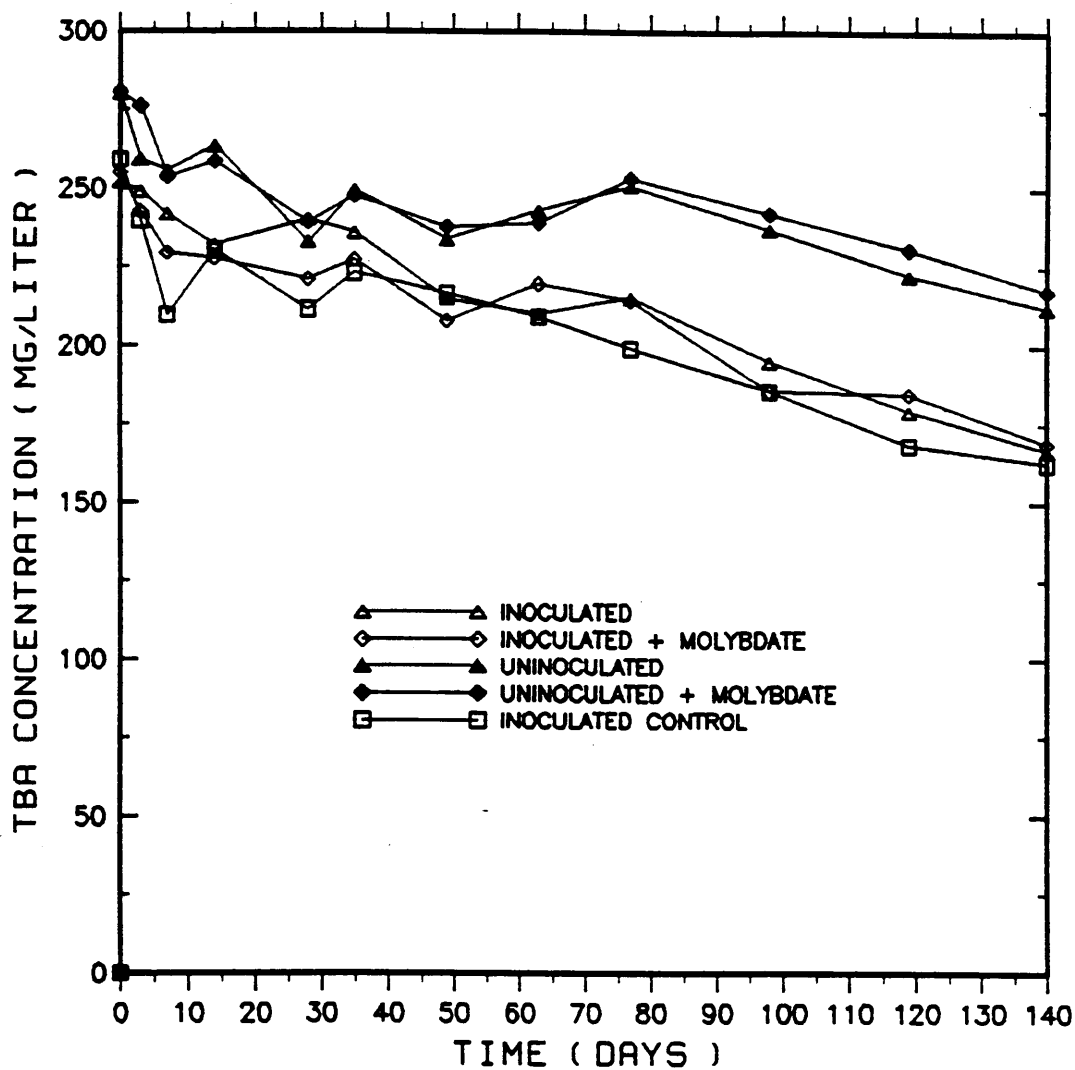


Figure 21. The concentration of tertiary butyl alcohol (TBA) over time in Groseclose clayey loam amended with 250 mg/liter TBA and 10 mg/liter ethyl alcohol. Treatments include: sterile and nonsterile soil inoculated with 8.6×10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100, uninoculated soil, and inoculated and uninoculated soil amended with 1.0 mM molybdate. All the treatments were established in triplicate.

Table 24. Degradation of tertiary butyl alcohol (TBA) in Groseclose clayey loam amended with 100 mg/liter ethyl alcohol and 250 mg/liter TBA. Treatments include: sterile and nonsterile soil inoculated with 10^6 *Pseudomonas* sp. cells per g soil (dry weight), indigenous microflora and sterilized control. Molybdate (1.0 mM) added to inoculated and uninoculated treatments. All treatments performed in triplicate. Refer to figure 22.

Time (days)	TBA Concentration (mg/liter)					
	Nonsterile Soil				Sterile Soil	
	Inoculated		Uninoculated		Inoculated	
	No MoO ₄	MoO ₄	No MoO ₄	MoO ₄	No MoO ₄	MoO ₄
0	271.1+16.3	238.5+14.8	256.4+8.9	259.2+4.6	243.1+11.6	
3	267.7+15.9	243.2+11.0	-	-	244.4+10.2	
7	225.1+6.0	205.6+7.7	248.0+7.8	224.2+3.0	230.7+19.4	
14	233.2+15.8	212.4+13.5	238.8+3.6	228.5+16.8	220.7+10.6	
28	215.5+16.3	230.8+23.1	237.5+9.3	226.7+10.4	227.8+18.1	
35	208.8+1.8	216.0+26.4	235.2+6.5	229.6+13.1	229.4+18.6	
49	206.0+8.4	225.8+9.2	230.4+3.5	227.3+6.8	236.1+9.1	
63	214.4+22.2	226.2+9.4	229.6+5.4	227.1+7.4	218.5+14.6	
77	213.0+24.4	206.7+13.1	234.6+1.8	229.2+7.7	207.2+17.7	
98	195.2+26.0	206.7+13.2	223.2+3.0	213.8+7.0	176.8+18.6	
119	172.1+23.0	182.9+8.6	218.2+4.7	204.2+8.7	150.9+16.6	
140	160.3+33.4	173.0+8.4	210.0+6.5	207.2+9.0	137.8+16.6	

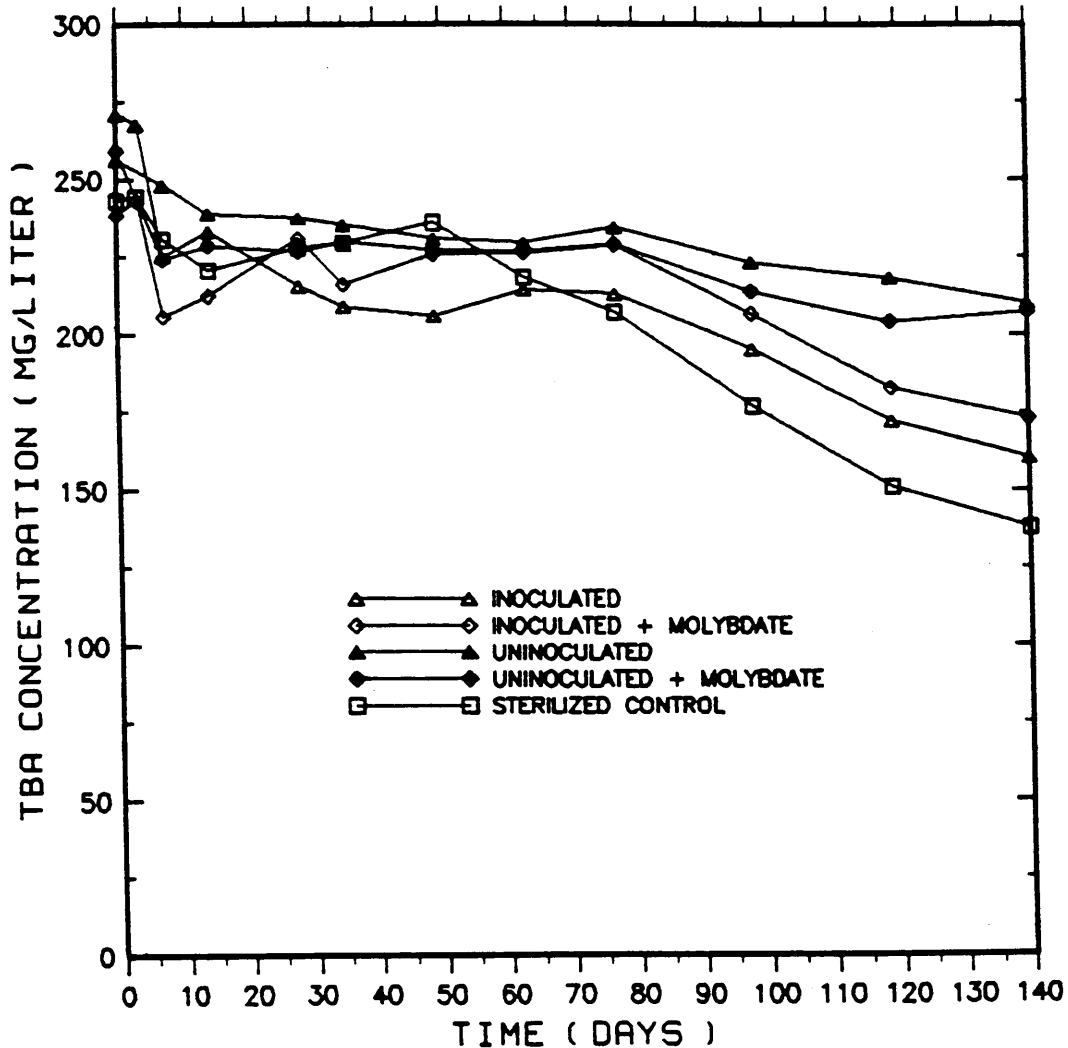


Figure 22. The concentration of tertiary butyl alcohol (TBA) over time in Groseclose clayey loam amended with 250 mg/liter TBA and 100 mg/liter ethyl alcohol. Treatments include: sterile and nonsterile soil inoculated with 8.6×10^6 cells per g soil (dry weight) of *Pseudomonas* sp. VT100, uninoculated soil, and inoculated and uninoculated soil amended with 1.0 mM molybdate. All the treatments were established in triplicate.

Table 25. Tertiary butyl alcohol (TBA) concentration over time in sterilized Groseclose clayey loam control microcosms. Served as controls for soil systems amended with three organic supplements: yeast extract, ethyl alcohol, and acetate. Unamended control established in triplicate, all other controls established as single treatment. Refer to figure 23.

Time (days)	TBA Concentration (mg/liter)				
	Unamended	Unamended + MoO ₄ ⁴⁻	Yeast Ext.	ETOH ^a	Acetate
1	306.3+14.7	229.2	360.4	345.6	311.8
7	305.3+6.0	236.5	300.7	236.6	256.5
21	291.4+4.4	243.1	304.1	261.6	267.8
35	283.1+3.5	234.7	298.1	263.5	264.6
49	286.9+0.6	228.4	288.2	272.9	263.6
63	285.5+3.0	230.1	296.5	275.3	257.1
77	273.3+0.9	229.4	296.2	274.5	255.0
98	269.3+0.7	221.4	283.0	264.1	248.8
119	269.3+2.2	215.6	282.5	254.2	246.5
140	263.7+1.6	210.1	277.4	260.7	239.5

^a ETOH: Ethyl Alcohol

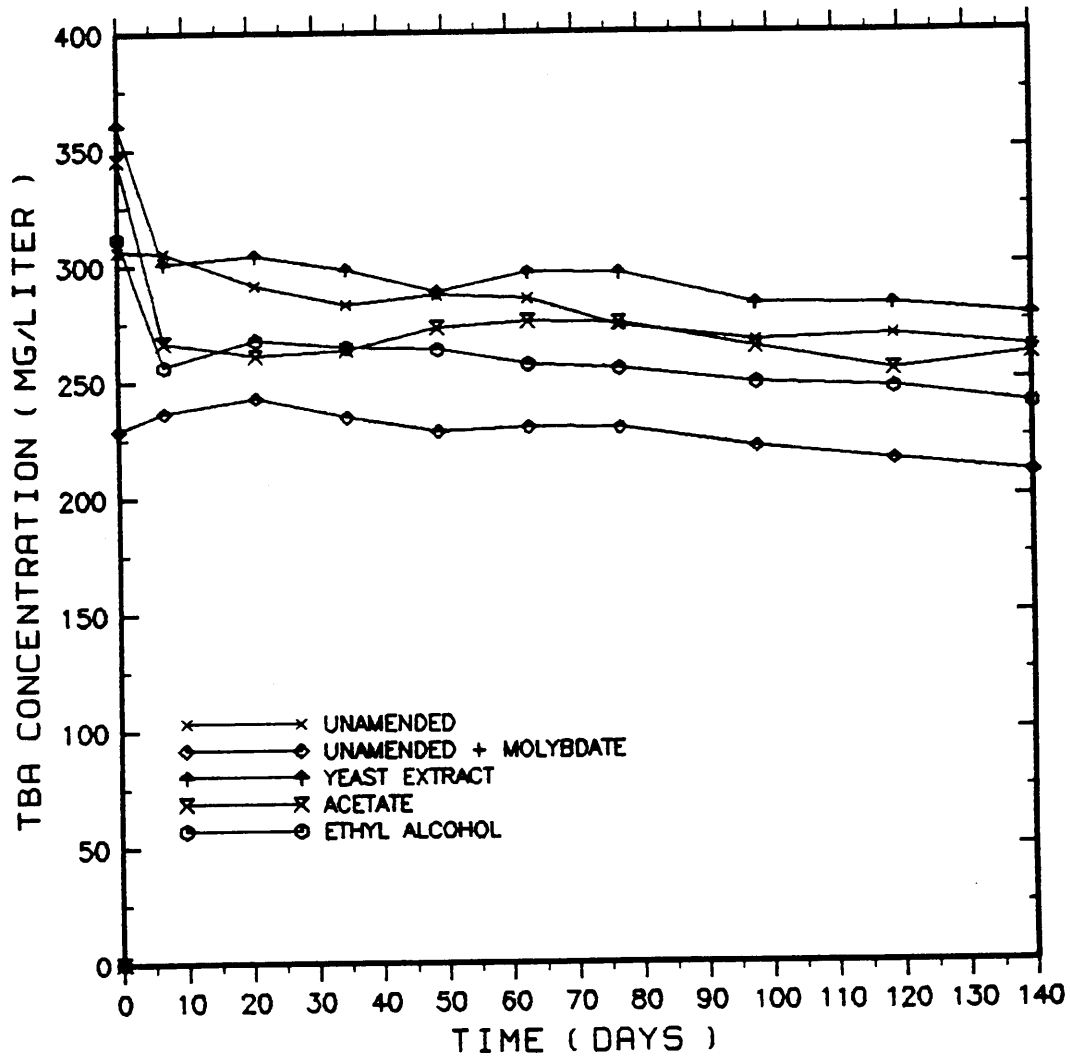


Figure 23. Concentration of Tertiary butyl alcohol (TBA) in sterile Groseclose clayey loam control microcosms. In addition to unamended control microcosms, control microcosms amended with (100 mg/liter): yeast extract, acetate in the form of sodium acetate, and ethyl alcohol were monitored for TBA volatilization. The unamended control microcosms were established in triplicate.

Table 26. Concentration of tertiary butyl alcohol (TBA) in sterilized Groseclose clayey loam when inoculated with 10^{-1} dilutions of three deep subsurface aquifer soils. Treatments include: Middendorf clay, Middendorf sand, and Cape Fear sand, and sterile control soil. Aquifer samples collected from the coastal plain of South Carolina. Refer to figure 24.

Time (days)	TBA concentration (mg/liter)			
	Middendorf clay	Middendorf sand	Cape Fear sand	Control
0	348.8	213.2	250.1	306.3+14.7
7	396.7	201.9	306.5	305.3+6.0
21	370.2	201.1	255.3	291.4+4.4
35	-	-	-	283.1+3.5
49	351.9	216.0	280.3	286.9+0.6
63	355.8	201.6	282.3	285.5+3.0
77	350.4	203.7	280.0	273.3+0.9
91	321.5	191.5	233.1	-
98	-	-	-	269.3+0.7
112	309.6	179.7	249.0	-
119	-	-	-	269.3+2.2
133	292.1	188.8	243.4	-
140	-	-	-	263.7+1.6
154	277.3	182.7	232.8	-
220	197.4	141.3	168.2	-
227	-	-	-	215.1+1.2

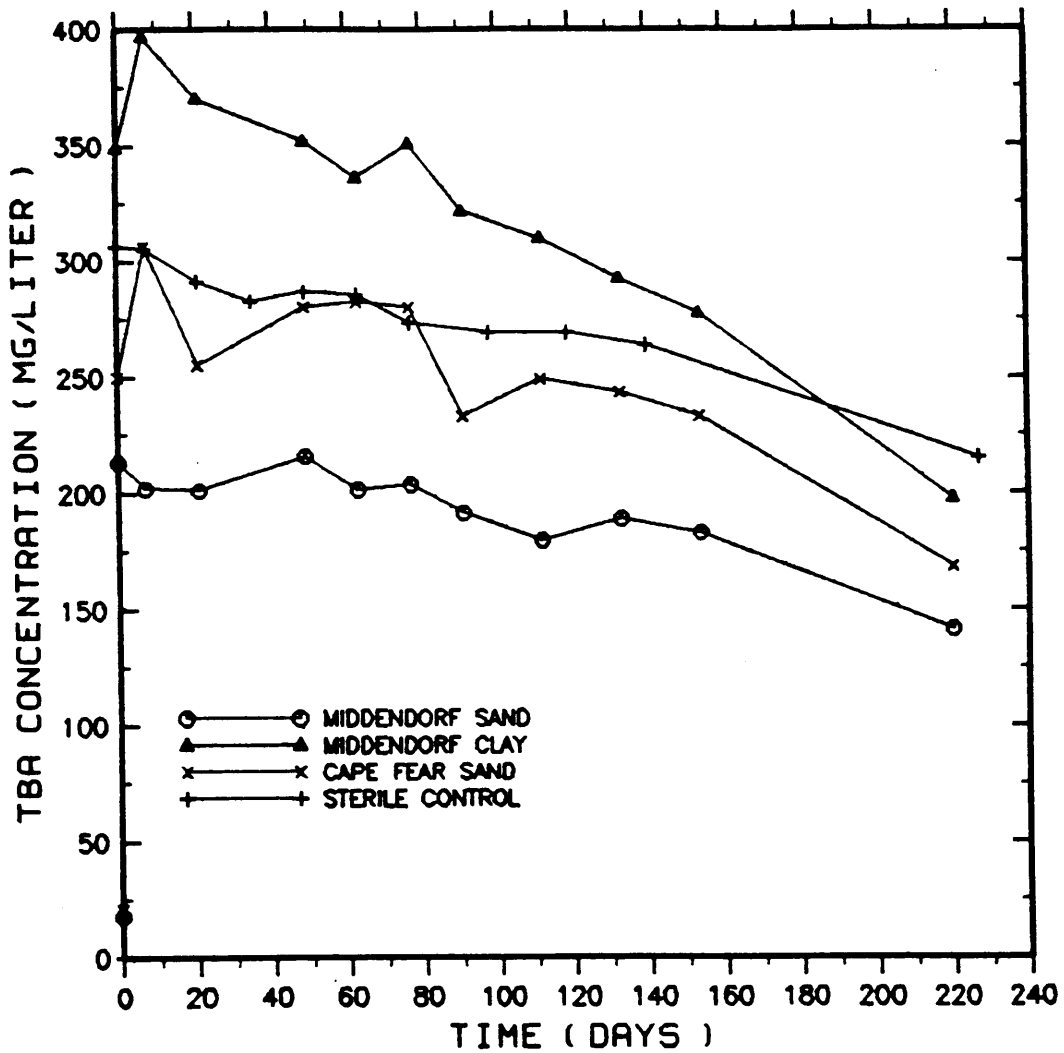


Figure 24. The concentration of tertiary butyl alcohol (TBA) over time in sterile Groseclose clayey loam inoculated with 10^{-1} dilutions of three deep subsurface aquifer samples. Samples collected from the coastal plain region of South Carolina. Treatments include: Middendorf sand, Middendorf clay, Cape Fear sand, and sterile control soil.

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