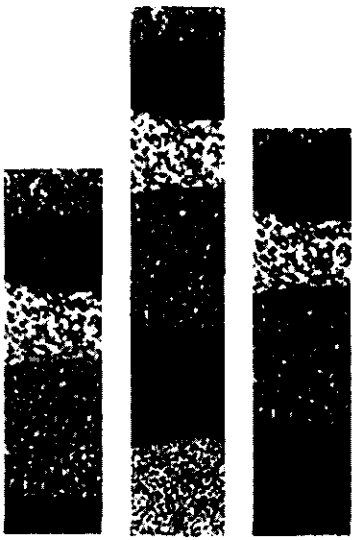
In Situ Aeration and Aerobic Remediation



Editors

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BIODEGRADATION OF ETHYLENE DIBROMIDE THROUGH COMETABOLIC MECHANISMS

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ABSTRACT: Three laboratory-scale batch reactor studies were conducted to determine whether soil microbes could degrade ethylene dibromide (EDB) through cometabolic mechanisms. The cometabolic growth substrates investigated were propane, methane, and natural gas. The bacterial consortium was isolated from soils in a mineral salts medium. Reactors were constructed using 160 mL sterile serum bottles with initial EDB concentrations of 200 µg/L. Each reactor was amended with propane, methane, or natural gas, then sealed and incubated. Controls were prepared without the addition of the consortium. In all active batch reactors, the ethylene dibromide concentration was reduced by >99% within 11 days of incubation. The laboratory results indicated that EDB could be biodegraded in the presence of propane, methane, and natural gas. Therefore, a technology such as cometabolic air sparging may show promising results for in situ bioremediation when applied in the field.

INTRODUCTION

Ethylene dibromide (1,2-dibromoethane [EDB]) is a persistent pollutant in groundwater due to improper disposal and spillage. EDB has been used as a pesticide and as an additive to leaded gasoline, but its use is declining due to increased regulation. EDB is extremely toxic and is a known carcinogen (Long et al., 1982). Since the discovery that EDB present in drinking water at barely detectable levels was related to increased risk of cancer, the need for effective means to treat contaminated waters has grown more urgent. The current drinking water standard for EDB is 0.02 µg/L and sites such as those at the Massachusetts Military Reserve (MMR) have EDB plumes containing over 100 µg/L. Current treatment of such plumes has been pump-and-treat, a relatively expensive option, and one that does not remediate the plume, but controls it from spreading off-site.

In a previous study, under anaerobic conditions, a methanogenic mixed culture removed >99% of initial EDB concentrations within two weeks of incubation (Bouwer et al., 1985). It has also been shown that mixed bacterial cultures, obtained from selective enrichment with EDB and vitamin-supplemented mineral salts medium in shake flasks, were capable of aerobically degrading EDB (Freitas et al., 1996). Freitas et al. (1996) also indicated that the culture completely converted EDB to bromide, H_2O, carbon dioxide, and biomass. Native soil and groundwater microbes collected from Windsor Locks, Connecticut readily degraded EDB with initial concentrations of 6 to 8 µg/L within 1 week under aerobic conditions (Pignatello, 1985).

Few studies have addressed the issue of cometabolic degradation of EDB. Cometabolic air sparging is a promising technology that has recently been proven successful at McClellan AFB, CA (Magar et al., 2001). Cometabolic air sparging has the advantage of being relatively simple to implement and inexpensive in comparison to pump-and-treat. The objective of this research was to determine if microorganisms were capable of degrading EDB under aerobic cometabolic conditions. A variety of primary substrates were tested, including methane, propane, and natural gas. For field applications, natural gas or propane were the preferred substrates since they are less expensive; however, previous studies had only shown cometabolic degradation with methane as the primary substrate (Leeson and Bouwer, 1986), so methane was also included in the testing.

MATERIALS AND METHODS

A bacterial consortium was isolated from soils collected from the Department of Defense Housing Facility in Novato, California. Mixed cultures were grown continuously in 160 mL serum bottles using 1 gram of soil and 25 mL of a mineral salts media (Foster and Davis, 1966). Each serum bottle was sealed with a Mininert™ valve (Dynatech Precision). Cultures were amended with 20 mL of propane, methane, or natural gas by injection through the Mininert™ valve using a Hamilton Gas-tight syringe fitted with a 22½-gauge needle. Making successive transfers with the mineral salts medium selectively enriched mixed cultures able to degrade propane, methane, or natural gas.

Sacrificial batch reactors were prepared in triplicate for each sampling event and consisted of 160-mL sterile serum bottles. A 10-mL aliquot of the consortium was added to 60-mL of mineral salts medium in each batch reactor. An EDB stock solution was then injected into each serum bottle to achieve initial EDB concentrations of 200 µg/L. The reactor headspace was sparged for two minutes with ultra high purity (UHP) oxygen and each reactor was sealed with a Mininert™ valve. A volume of 20-mL of the appropriate gaseous substrate was injected into each reactor using a Hamilton Gas-Tight syringe. Controls were prepared identically to the active reactors except without the addition of the consortium. The batch reactors were incubated in the dark at 28°C and 150 RPM.

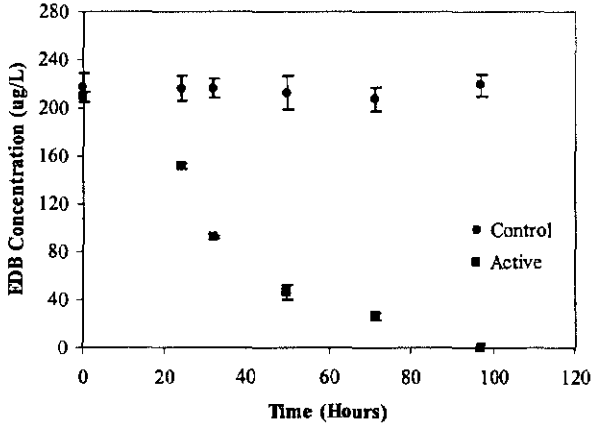
Concentrations of oxygen, carbon dioxide, primary substrate (propane, methane, or natural gas), cell density, and EDB were measured in all batch reactors initially and in each sacrificed batch reactor during a sampling event. The active and control batch reactors were sampled in triplicate every 24 to 72 hours depending on percent EDB removal. Concentrations of EDB were measured using an HP 5890 Series II gas chromatograph (GC) with an electron capture detector (ECD) and split/split less injection. The detection limit of EDB was 0.029 µg/L. The samples were extracted and tested by following EPA SW846 Method 8011. Optical density was measured at each sampling point using a Milton-Roy Spectronic 21D. The optical density was correlated to cell density. Propane, methane, and natural gas concentrations also were measured at each sampling event using a Varian Star 3400 equipped with a flame ionization detector (FID) by drawing off 5 mL of the headspace of each serum bottle with a gastight syringe and directly injecting into the GC. Oxygen and carbon dioxide

were tested by using an SRI 8610A gas chromatograph equipped with a thermal conductivity detector (TCD).

RESULTS AND DISCUSSION

Propane Batch Reactors. Microorganisms in active batch reactors amended with propane degraded >99% of initial EDB (to below the detection limit) after four days of incubation. Control batch reactors amended with propane showed no significant loss of EDB (Figure 1A). Simultaneous degradation of propane, oxygen utilization, and cell growth strongly suggest that the microorganisms are responsible for EDB degradation. Microorganisms in the active batch reactors

A)



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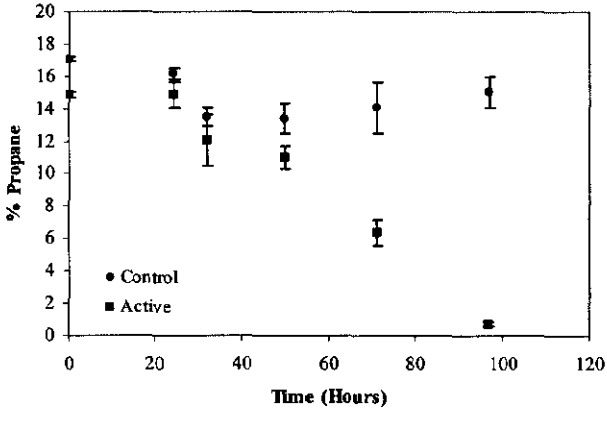

FIGURE 1. EDB (A) and propane (B) degradation in batch reactors. The average of triplicate samples with ± standard deviation is shown.

degraded >95% of initial propane within four days of incubation. In contrast, the control reactors showed a loss of <10% initial propane concentrations (Figure 1B).

Methane Batch Reactors. Microorganisms in active batch reactors amended with methane degraded >99% of initial EDB (to below reporting limit) after six days of incubation (Figure 2A). However, the control reactors showed no significant loss of EDB after 13 days of incubation. The active reactors degraded >95% of initial methane and the controls showed a 31% loss of initial methane (Figure 2B). As with the results in the propane-amended batch reactors, these results indicate that EDB is degraded by the microorganisms in the presence of oxygen and methane.

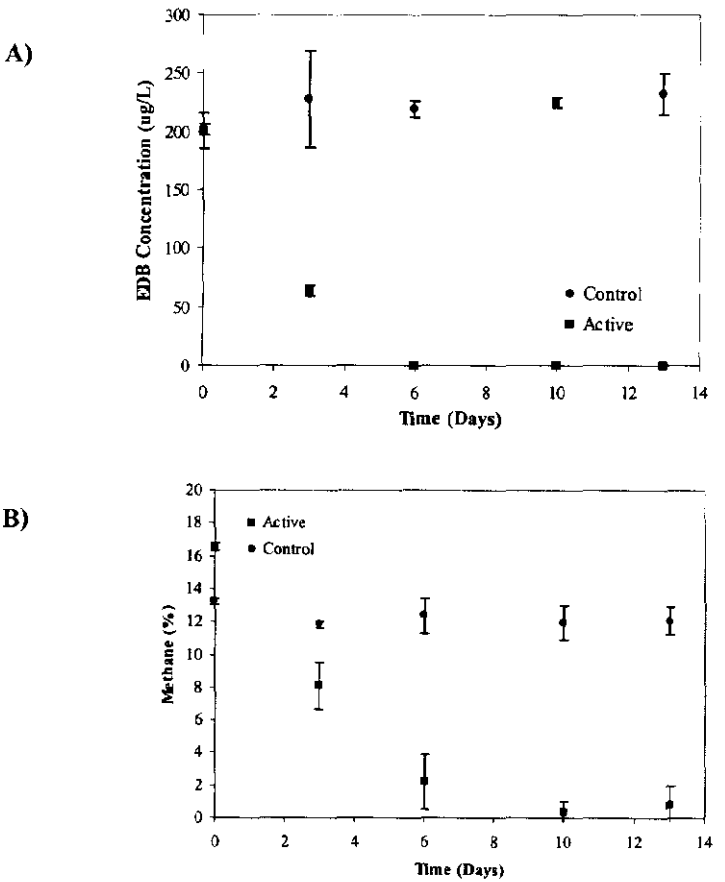

FIGURE 2. EDB (A) and methane (B) degradation in batch reactors. The average of triplicate samples with ± standard deviation is shown.

Natural Gas Batch Reactors. Microorganisms in active batch reactors amended with natural gas degraded >99% of initial EDB (to below reporting limit) after six days of incubation. Control batch reactors amended with natural gas showed no significant loss of EDB (Figure 3A). The active batch reactors degraded 100% of the initial natural gas after 10 days of incubation while the controls showed no significant loss (Figure 3B). These results confirm those from the propane- and methane-amended batch reactors, demonstrating that EDB is degraded by the microorganisms in the presence of a primary growth substrate and oxygen.

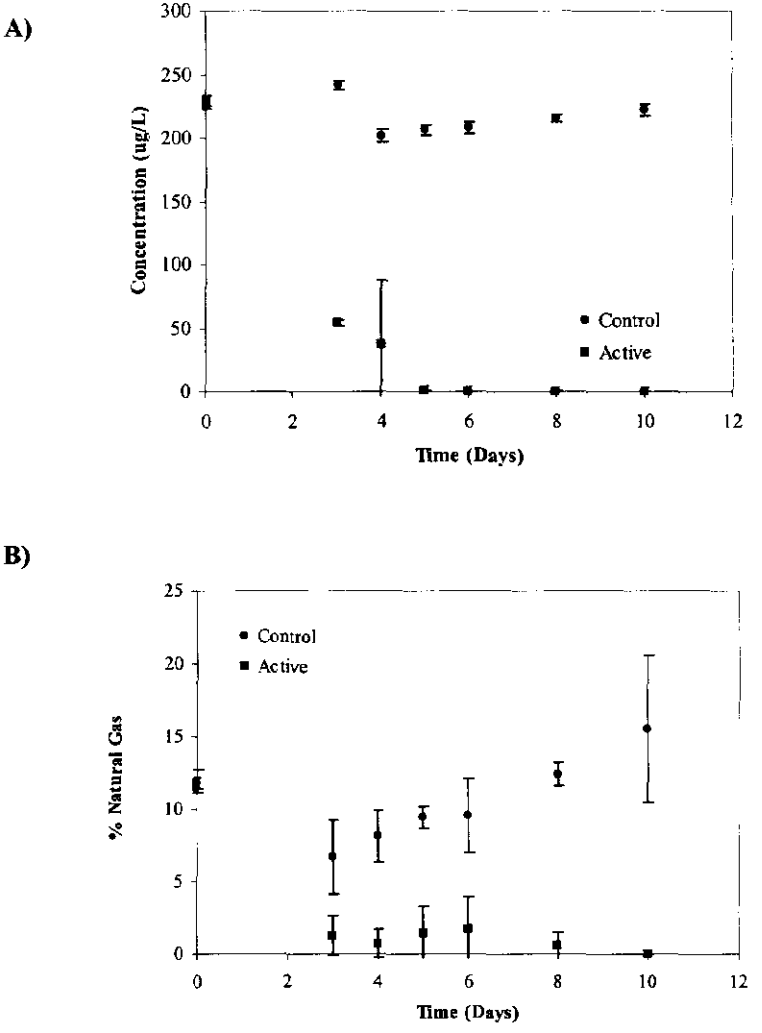

Figure 3. EDB (A) and natural gas (B) degradation in batch reactors. The average of triplicate samples with ± standard deviation is shown.

These results with natural gas are in contrast to previous studies that demonstrated that EDB could not be degraded in the presence of natural gas, possibly due to additives that inhibited growth (Leeson and Bouwer, 1986). The ability to use natural gas rather than methane or propane is desirable, since it is a cheaper and more readily available product.

CONCLUSIONS

The batch reactor experiments resulted in three mixed cultures, isolated using propane, methane, or natural gas as growth substrates, which were capable of degrading EDB. The results provide evidence of biodegradation of EDB through cometabolic mechanisms. Further studies are necessary to confirm this result.

Currently, regulations require that EDB concentrations in groundwater be reduced to 0.02 µg/L. At sites with EDB concentrations above MCLs, pump-and-treat is typically applied, followed by granular activated carbon (GAC) treatment of the extracted groundwater. This is a relatively expensive option given the length of time the systems must be operated (+30 years) and is unsatisfactory in that it does not result in destruction of EDB. The results from this research indicate that EDB can be degraded relatively quickly by microorganisms, suggesting that in situ bioremediation of EDB-contaminated groundwater is possible. In situ bioremediation of EDB could be accomplished through implementation of cometabolic air sparging, a technology that has been proven successful in the field. Cometabolic air sparging to treat EDB-contaminated groundwater has the potential to achieve contaminant concentrations below MCLs within a relatively short time frame.

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