ADVANCES IN TCE-DEGRADING THERMOPHILIC, MESOPHILIC, AND ALKALINE-RESISTANT ORGANISMS

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INTRODUCTION

Trichloroethylene (TCE) is a chlorinated organic solvent that has been widely used in metal processing, electronics, dry cleaning, paint and many other industries. The methylotrophic bacteria have been shown to co-metabolize TCE. These organisms have been utilized as degraders of TCE in aquifers (Semprini et al. 1987) which suggests that the use of methylotrophs is a practical solution for TCE contamination.

Electroosmosis is a relatively old technique used to dewater soils (Casagrande, 1949). Electroosmosis uses a direct-current electric field to induce a motion of a liquid and dissolved ions that transports the contaminants in the electric field. This technique is limited to fairly soluble contaminants like benzene, toluene, xylene, phenol, and chlorinated solvents (Bruell et al. 1992; Acar et al. 1992; Shapiro and Probstein, 1993). This technique creates highly acidic and basic zones in the soil. The process can also raise the temperature of the soil substantially. We are selecting methylotrophs to survive and degrade TCE in this environment.

METHODOLOGY

Soil samples were taken at 10 major locations in Yellowstone Park and shipped back to the laboratory the next day. When the samples arrived in the laboratory, they were placed in water baths at 50, 60, 70, or 80^{o}C, whichever temperature was closest to the temperature from which they came. Five grams of sample were placed in 20 ml of "L" medium (ATCC, 1984) in serum stoppered bottles. These were then purged with 0.6% methane in air. The bottles were then placed in water baths at the appropriate temperature. After 10 days, the bottles were sub-sampled by placing 1 ml of the aqueous phase on L agar plates containing 2% agar. These plates were placed in desiccators which were sealed then purged with the methane:air mixture for 30 minutes. The desiccators were then placed in incubators at the appropriate temperature. As different colonies appeared they were picked and placed in L medium or L medium diluted 1:10 with water and returned to serum stopped bottles. These were grown-up in the methane in air mixture and restreaked for purity. These isolates were then ready for screening for TCE degradation

Each isolate was grown in duplicate 60 ml serum stoppered bottles for at least one month in 20 ml of L medium, L medium diluted 1:10 with deionized water, or AMS medium (Whittenbury et al. 1970) or AMS medium diluted 1:10 with deionized water. The medium was adjusted to pH 2, 4, 8 or 10 with concentrated NaOH or HCl. The bottles were periodically purged with a mixture of 2.5% methane in air to provide a carbon source.

After the growth period of at least one month, each isolate was tested for TCE degradation. The methane was first removed by placing them in the laminar flow hood for 1 hour. At that point, the serum bottle was resealed. Two microliters of a TCE stock solution were added to each bottle so that the final concentration of TCE added was approximately 10 ppm. On the same day that the serum bottles received the TCE, the starting concentrations of TCE were determined. TCE concentration was measured using a GC-FID. Headspace samples (0.5ml) were removed from the serum bottles and injected into the GC. On the fifth day, the bottles were resampled for TCE concentration. The concentration of TCE obtained from the second analysis was then subtracted from the initial value of TCE to indicate the level of TCE degradation or disappearance.

A model electroosmosis microcosm (EM) was developed to test the bioremediation process. This design now allows for maintenance free operation for at least a month. This design also allows for the manipulation of various components of the EM to test concepts for field applications. The EM chamber is 20 cm long and 10 cm wide and is made of polyethylene phthalate (PET). (The chamber is a two liter soft drink bottle). The bottom of each EM is filled with 400 grams of sand. This acts as a reservoir for water. On top of the sand, a mixture of 100 grams of granular graphite and 50 grams of granular activated carbon is layered. This makes up the anode. Next a soil slurry is made. It contains 1500 grams of soil obtained from subsurface soil environment. The soil is mixed for one hour in a Hobart mixer with 600 ml of tap water. The whole soil slurry can be added to make a complete soil column. If a remediation zone

is to be added, half of this mixer is then gently poured onto the anode electrode. The remediation zone is created by adding 150 grams of granular activated carbon on top of the first layer of soil plus 50 ml of tap water. Then the rest of the soil is added gently on top of the remediation layer. Another electrode, identical to the first, is then poured on top of this soil. This is the cathode. On top of the cathode is added 400 grams of sand and then the rest of the chamber is filled with tap water. A Mariotte Bottle is used to control the water level. A gas relief valve is added to the anode to remove oxygen gas generated process. Tests were made using the EM. Some tests were run at constant 100 volts for 6 hrs. All other tests were run at a constant 25 volts. (The soil temperature does not exceed the ambient temperature at this voltage.) At the end of the test, the pH of the soil is taken with pH paper every 2 cm along the soil column length.

RESULTS

The screening program to date has identified 20 (Table 1) isolates that appear to have degraded more than 20 % of the TCE in 5 days; two isolates showed more than 30% degradation in 5 days. Thirteen of the isolates came from 50°C (total of 59) and seven came from 60°C (total of 27). Most isolates came from more basic pH's. Ten came from pH 8 and 3 from pH 10 but six came from pH 4 conditions. We have no pH 2 isolates that reached 20 % degradation within 5 days but we have one pH 2 isolate that was close to 20 %.

In general the low pH isolates (pH 2 and 4) degraded more TCE when grown in the AMS medium at full strength (data not given) . Similarly, the high pH isolates (pH 8 and 10) degraded more TCE in full strength L medium. However, there were exceptions in each case.

Some EMs were run for 6 hrs at 100 volts to simulate a long field experiment. The temperature rose as high as 82°C in these chambers. At 25 volts, the chambers stay in equilibrium with the ambient temperature. When the EM is run at 25 V, the current and monitoring voltage both started high but dropped quickly. The purge water started flowing immediately but slowed after about 400 hours. It took nearly 600 hours to move 2 pore volumes (pore volume is 969 ml).

If an activated carbon layer was added in the middle of the soil column, the current started out higher than in the soil alone configuration but the current rapidly dropped to about 35 mA. Voltage also started very high, declined then rose again. About 3 pore volumes were moved in 300 hours. The results of the pH analysis of the soil at the end of the experiments indicated that the pH near the anode was 4 or higher but near the cathode the pH was as high as 12 (data not given).

CONCLUSIONS

It must be noted that we have not proven that any of these isolates are methylotrophs nor that the TCE was biodegraded. These questions will await further analysis. It does appear that the isolates we have obtained have the capacity to survive the extreme pH and high temperatures expected in the electroosmosis process. So far these organisms grow very slowly and we have much to learn about the optimal growth conditions. The design of the EM has allowed us to create conditions expected in the field

TABLE 1. SUMMARY OF ISOLATES TESTED FROM YELLOWSTONE NATIONAL PARK, WYOMING

LOCATION	# ISOLATES	# DEGRADATION > 20%	% TOTAL
A. Mammoth Hot Springs (MHS)	16	2	13
B. Norris Geyser Basin (NGB)	8	1	13
C. Madison Junction (MAD)	6	1	17
D. Paint Pot (PP)	7	1	14
E. V Road Site (VRS)	0	0	0
F. Near Obsidian Cliff (OBS)	2	1	50
G. Mud Volcano Site (MVS)	4	0	0
H. Above Sulfur Caldron (ASC)	4	0	0
I. Calcite Spring Site (CSS)	19	7	37
J. Artist Paint Pots Site (APPS)	20	7	35
Total	86	20	24

and this should be a useful laboratory model to test electroosmosis. Next, the isolates will be tested in the EMs for biodegradation of TCE.

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