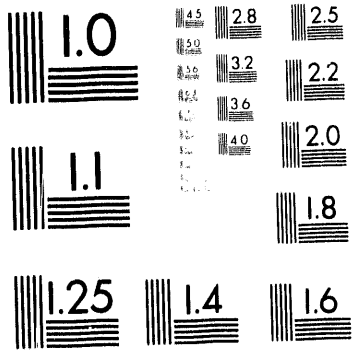

1.0
1.1
1.25
1.4
1.6
1.8
2.0
2.2
2.5
2.8
3.2
3.6
4.0
4.5
5.0
5.6

1 of 1

APPLICATION OF MICROBIAL BIOMASS AND ACTIVITY MEASURES TO ASSESS IN SITU BIOREMEDIATION OF CHLORINATED SOLVENTS

T. J. Phelps[1], S. M. Pfiffner[2], R. Mackowski[2], D. Ringelberg[2], D. C. White[1,2], S. E. Herbes[1], and A. V. Palumbo[1]. [1] Environmental Sciences Division, Oak Ridge National Laboratory[3], Oak Ridge, TN 37831 [2] Center for Environmental Biotechnology, University of Tennessee, Knoxville, TN 37932-2567.

DISCLAIMER

Key words: bioremediation, activity, monitoring, in situ, biotechnology, microbiology

---

[3] ORNL is managed by Martin Marietta Energy Systems, Inc. for the U. S. Department of Energy under contract DE-AC05-84 OR21400. This research was funded by the DOE Office of Technology Development.

INTRODUCTION

Evaluating the effectiveness of chlorinated solvent remediation in the subsurface can be a significant problem given uncertainties in estimating the total mass of contaminants present. If the remediation technique is a biological activity, information on the progress and success of the remediation may be gained by monitoring changes in the mass and activities of microbial populations.

The in situ bioremediation demonstration at the U.S. Department of Energy (DOE) Savannah River Site (SRS) is designed to test the effectiveness of methane injection for the stimulation of trichloroethylene (TCE) degradation in sediments. Past studies have shown the potential for TCE degradation by native microbial populations (Fliermans et al. 1988). The design and implementation of the SRS Integrated Demonstration is described in this volume (Hazen et al. 1993). A control phase without treatment was followed by a phase withdrawing air. The next phase included vacuum extraction plus air injection into the lower horizontal well located below the water table. The next period included the injection of 1% methane in air followed by injection of 4% methane in air. Based on the literature (Little et al. 1988, Wilson and Wilson 1985), it was hypothesized that the injection of methane would stimulate methanotrophic populations and thus accelerate biological degradation of TCE. Measuring the success of bioremediation is a complex effort that includes monitoring of changes in microbial populations associated with TCE degradation. These monitoring efforts are described in this paper and in related papers (Brockman et al. 1993, etc) in this volume.

METHODS

The primary source of samples for monitoring bioremediation was groundwater obtained from a series of 12 wells (Figure 1, Hazen et al. 1993) located at and around the site at Savannah River.

Wells were sampled twice monthly and experiments were initiated onsite or samples were stored on ice until processed at the University of Tennessee (UT) or Oak Ridge National Laboratory (ORNL).

Microbial biomass was evaluated using turbidimetric most probable number (MPN) techniques for methanotrophic and methylotrophic populations. A phosphate- and bicarbonate-buffered mineral salts medium, supplemented with 5% methane (vol/vol, headspace), was used to enumerate methanotrophs exhibiting turbidity. The methylotrophic medium was supplemented with 3 mg L^{-1} of yeast extract, 5 mM methanol, and 3% headspace propane. Microbial activity was assessed by measuring TCE mineralization in time course experiments and enrichments as described previously (Phelps et al. 1989). Assays used 10 mL groundwater, 1.0 mL sterile water and 0.5 μCi of carrier-free [1,2-^{14}C]TCE and were incubated 30 days. Methane and CO_2 were assayed using a Shimadzu GC-8A gas chromatograph (GC) with a thermal conductivity detector (TCD). Radioactive $C0_2$, VC, TCE, and PCE were quantified using a gas proportional counter (GPC) analyzing the effluents from the gas chromatograph (GC-GPC). Groundwater samples (1 L) were filtered through 0.2μm inorganic filters and analyzed for biomass and community structure. Microbial phospholipids were extracted (Phelps et al. 1989) and methyl esters of the phospholipid fatty acids (PLFAs) were analyzed by GC.

Specific nitrogen transformations were evaluated by measuring urease activity (adding urea and measuring ammonia production) and ammonia uptake (by measurement of the loss of ammonia after addition) in well water. Ammonia concentrations were measured using a Technicon autoanalyzer.

RESULTS AND DISCUSSION

The numbers of methanotrophs and methylotrophs increased significantly during the treatments (Table 1). During the control period, 19% of the analyses indicated presence of methanotrophs in

10mL samples. None of the analyses from the control, vacuum extraction or air injection periods indicated $> 10^3$ methanotrophs/mL. Methanotrophic populations increased dramatically with the initiation of methane injection. Wells exhibiting $> 10^1$ methanotrophs/ml increased from < 10% to 47% with 1% methane additions and to 85% during the 4% methane injections. During the final 2 months of 1% methane injection, 53% of the samples indicated $> 10^3$ methanotrophs/mL. During the control period, 61% of the samples indicated the presence of $> 10^1$ methylotrophs/mL. Between the control and air injection periods, the percentage of wells with $> 10^3$ methylotrophs increased from 11% to 81%. These results indicated that methanotrophic and methylotrophic populations increased > 100-fold during the treatments. Increased methanotrophic densities within several individual wells were 4 to 5 orders of magnitude (data not shown). Interestingly, both methanotrophic and methylotrophic densities decreased in many wells during the 4% methane injection phase, suggesting that nutrients or other constraints may have become limiting.

Further evidence of increased methanotrophic densities was shown by the PLFA analyses (i.e. well MHT-2C, Figure 1). Well MHT-2C showed an increase in lipid biomass from < 5 pmol PLFA/mL to values often > 30 pmol/mL shortly after the onset of methane injection. Increased methanotrophic populations were detected by signature PLFAs of type II methanotrophs resembling *Methylosinus trichosporium* OB3b. When total lipid biomass was compared to mole percent PLFA for type II methanotrophs, the increases in methanotrophic PLFA corresponded to the increases in lipid biomass. By the end of the 1% methane injection, lipids typical of type II methanotrophs, including 18:1w8c, 18:1w7c, 18:2a, and 18:2b, represented more than half of the total PLFA present.

Enrichment experiments examining $^{14}CO_2$ production from [1,2- ^{14}C]TCE showed potential for TCE degradation throughout the operations at the SRS. For example, during the 1% methane injection, the amount of TCE degradation in the enrichments typically was < 10% (Figure 2) but there was substantially more TCE degradation (> 20%) in some of the analyses (6C, 7C, 8C).

Maximum TCE degradation in the enrichments was 56% after 30 days. If bioremediation were optimized, most analyses should reveal mineralization within 10 days without the need for enriching nutrients, a criterion that fewer than 5% of the analyses met.

SUMMARY

There have been substantial changes in biological activity and biomass with the increasingly aggressive measures to promote TCE degradation. The data indicate the success in stimulating TCE-degrading populations. Other nutrients may be limiting, and a further phase (methane injection with nutrient addition) is currently being implemented to further increase critical population levels and degradative activity.

REFERENCES

Brockman, F., D.Workman, W. Sun, A. Ogram, and L. Bucey, 1993. "Baseline characterization of TCE degradative potential by enrichment techniques and DNA probe analysis." In R. E. Hinchee (Ed.), In situ and On-site bioreclamation: The Second International symposium Proceedings.

Fliermans, C. B., T. J. Phelps, D. Ringelberg, A. T. Mikell, and D. C. White. 1988. "Mineralization of trichloroethylene by heterotrophic enrichment cultures." Appl. Environ. Microbiol. 54: 1709-1716.

Hazen, T., J. Doughtery, M. Enzien, M. Franck, C. Fliermans, and C. Eddy. 1993. "DOE/SRS integrated demonstration: in situ bioremediation of soil and groundwater at a chlorinated solvent-

contaminated site using horizontal wells to inject air and methane. In R. E. Hinchee (Ed.), In situ and On-site bioreclamation: The Second International symposium Proceedings.

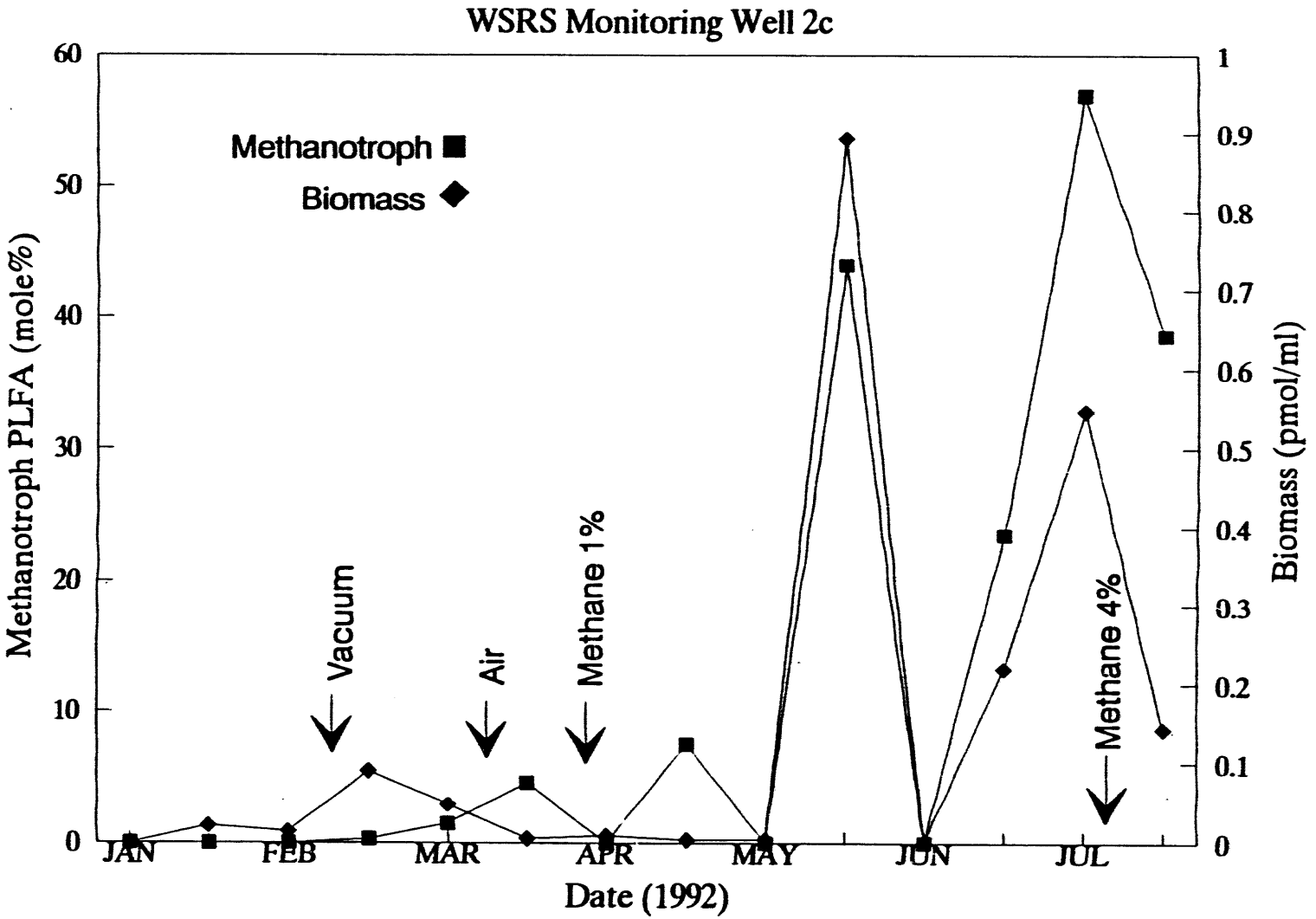
Little, C. D., A. V. Palumbo, S. E. Herbes, M. E. Lindstrom, R. L. Tyndall, and P. J. Gilmer. 1988. "Trichloroethylene biodegradation by pure cultures of a methane-oxidizing bacterium." Appl. Environ. Microbiol. 54:951-956.

Phelps, T. J., D. Ringelberg, D. Hedrick, J. Davis, C. B. Fliermans, and D.C. White. 1989. "Microbial activities and biomass associated with subsurface environments contaminated with chlorinated hydrocarbons." Geomicrobiology J. 6: 157-170.

Wilson, J. T., and B. H. Wilson. 1985. Biotransformation of trichloroethylene in soil. Appl. Environ. Microbiol. 49:242-243.

Table 1. Average percent of wells with presumptive methanotrophs (methane) and methylotrophs (methy.) at greater than 0.1/mL, 10^1/mL and 10^3/mL.

Treatment	> 0.1/mL Presumptive		> 10^1/mL Presumptive		> 10^3/mL Presumptive	
	Methy.	Methane	Methy.	Methane	Methy.	Methane
Control	100	19	61	3	11	0
Vacuum Extraction	100	13	100	8	42	0
Air Injection	100	67	100	8	83	0
1% Methane	100	82	100	47	79	32
4% Methane	100	92	100	85	81	38


WSRS Monitoring Well 2c
Methanotroph
Biomass
Vacuum
Air
Methane 1%
Methane 4%
Methanotroph PLFA (mole%)
Biomass (pmol/ml)
Date (1992)
JAN
FEB
MAR
APR
MAY
JUN
JUL
0
10
20
30
40
50
60
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1

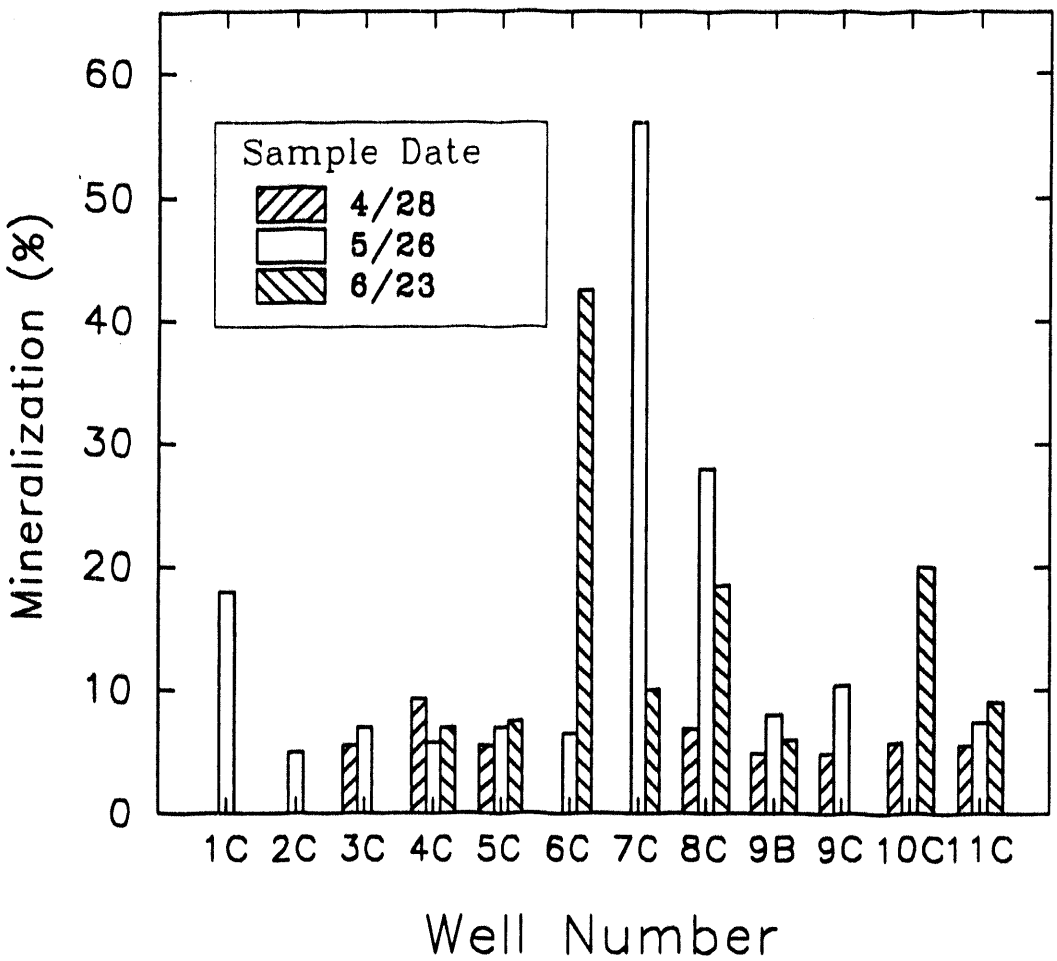


Fig 2 Phelps et al

LIST OF FIGURE AND TABLE CAPTIONS

TABLE 1. Average percent of wells with presumptive methanotrophs (methane.) and methylotrophs (methy.) at greater than 0.1/mL, 10^1/mL and 10^3/mL.

FIGURE 1. Methanotrophic PLFA and total biomass over time. Mole percent of methanotrophic PLFA (squares) was compared to biomass (diamonds) in well MHT-2C. Type II methanotrophic PLFA included 18:1w8c, 18:1w7c, 18:2a, and 18:2b.

FIGURE 2. Mineralization of [1,2-^{14}C]TCE to $^{14}CO_2$ by microorganisms enriched from groundwater. $^{14}CO_2$ in blanks and control tubes after 30-d experiments were 1.4 $\pm$ 1.0% .

DATE

FILMED

12/20/93

END