

**Application of Fluorescent Antibody and Enzyme-Linked
Immunosorbent Assays for TCE and PAH Degrading Bacteria
(U)**

by

R. L. Brigmon

Westinghouse Savannah River Company

Savannah River Site

Aiken, South Carolina 29808

M. Franck

J. Brey

D. Scott

K. Landos

C. Fliermans

RECEIVED

JUN 21 1996

OSTI

A document prepared for 96TH AMERICAN SOCIETY FOR MICROBIOLOGY ANNUAL MEETING at New Orleans
from 05/19/96 - 05/23/96.

DOE Contract No. DE-AC09-89SR18035

This paper was prepared in connection with work done under the above contract number with the U. S.
Department of Energy. By acceptance of this paper, the publisher and/or recipient acknowledges the U. S.
Government's right to retain a nonexclusive, royalty-free license in and to any copyright covering this paper,
along with the right to reproduce and to authorize others to reproduce all or part of the copyrighted paper.

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

ds
MASTER

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

This report has been reproduced directly from the best available copy.

Available to DOE and DOE contractors from the Office of Scientific and Technical Information, P. O. Box 62, Oak Ridge, TN 37831; prices available from (615) 576-8401.

Available to the public from the National Technical Information Service, U. S. Department of Commerce, 5285 Port Royal Rd., Springfield, VA 22161

Application of Fluorescent Antibody and Enzyme-linked Immunosorbent Assays for TCE and PAH Degrading Bacteria. R.L. BRIGMON, M.M. FRANCK*, J. BREY, D. SCOTT, K. LANCIOS, AND C. B. FLIERMANS. Westinghouse Savannah River Company, Aiken, SC, Medical College of Georgia, Augusta, GA

Polyclonal antibodies (Pabs) were produced against 18 xenobiotic-degrading bacteria by immunizing New Zealand white rabbits. These include trichloroethylene (TCE) degrading methanotrophic and polycyclic aromatic hydrocarbon (PAH) degrading species. An enzyme-linked immunosorbent assay (ELISA) was used to test for specificity and sensitivity of the Pabs. Fluorescent antibodies (FA) were developed against select methanotrophic bacteria isolated from a TCE contaminated landfill at the Savannah River Site (SRS) and cultured from the American Type Culture Collection (ATCC). Analysis of cross reactivity testing data showed some of the Pabs to be group specific while others were species specific. Monitoring of controlled bioprocesses, environmental remediation and detection of environmental disturbance are some of the numerous applications of these community and species-level characterization methods. The threshold of sensitivity for the ELISA is 10^7 bacteria cells/ml depending on the Pab preparation. The FA can detect as few as one bacterium per ml. Analyses such as FA and ELISA can provide specific information about individual and community microbial populations. These methods can provide useful information on *in situ* community structure and function for bioremediation applications within 4 hours of sampling.

Keywords: Fluorescent antibody, ELISA, Trichloroethylene, PAH, methanotroph, bacteria, bioremediation, groundwater

INTRODUCTION

Historically, methods used to identify methanotrophic and polyaromatic hydrocarbon-degrading (PAH) bacteria in environmental samples have been inadequate because isolation and identification procedures are time-consuming and often fail to separate specific bacteria from other environmental microorganisms. Methanotrophic bacteria have been isolated and characterized from TCE-contaminated soils (Bowman et al. 1993; Fliermans et al., 1988). Fliermans et al., (1988) and others demonstrated that cultures enriched with methane and propane could cometabolically degrade a wide variety of chlorinated aliphatic hydrocarbons including ethylene; 1,2-cis-dichloroethylene (c-DCE); 1,2-trans-dichloroethylene (t-DCE); vinyl chloride (VC); toluene; phenol and cresol. Characterization of select microorganisms in the natural setting is important for the evaluation of bioremediation potential and its effectiveness. This realization has necessitated techniques that are selective, sensitive and easily applicable to soils, sediments, and groundwater (Fliermans, et al., 1994). Additionally these techniques can identify and quantify microbial types *in situ* in real time.

The number of organisms present in groundwater, soil and sediment samples can be determined directly using immunoassay procedures including enzyme-linked immunosorbent assay (ELISA) and fluorescent antibody (FA). Additionally, any potential cross reactions can be eliminated by employing a modified ELISA technique and carefully selecting antibodies as previously described (Brigmon et al., 1994). Immunoassay techniques capable of identification and quantification of bacteria in very complex environments. Specificity and sensitivity of the Pabs were evaluated with ELISA (Brigmon et al., 1995, Brigmon et al., 1994). ELISA can provide a rapid, reliable, sensitive and inexpensive methodology for monitoring a variety of systems for the methane-oxidizing bacteria capable of degrading TCE. This report describes ELISA and FA procedures for application of these techniques for selective enumeration of TCE and chlorobenzene (CB) degraders in environmental samples.

MATERIALS AND METHODS

Bacteria. The bacteria used in this project were obtained from the American Type Culture Collection (ATCC) as well as methanotrophic bacteria isolated from environmental samples at the SRS (Table 1). Methanotrophic bacteria were grown in minimal salts media with 10 % methane (Fogel et al., 1985). All other bacteria were grown on Peptone Yeast Extract (PTYG) (Difco, Detroit, MI).

Environmental Sampling. The Savannah River Site (SRS) is an 320 square mile facility located in a rural area along the Savannah River, principally in the Aiken and Barnwell counties of South Carolina. The SRS is owned by the U.S. Department of Energy and operated by Westinghouse Savannah River Company. In the early 1970's, solid waste was consolidated into a single Sanitary Landfill near the center of SRS Figure 1. Aerobic and anaerobic ground waters at the SRS Sanitary Landfill were contaminated with TCE, chlorobenzene (CB), and VC as a result of some of these activities. As part of an ongoing intrinsic bioremediation project work at the Sanitary Landfill soil samples were taken from drilling cores taken during monitoring well installation and evaluated with immunological and microbiological techniques for the presence of TCE and CB degraders.

TABLE 1 BACTERIA FOR IMMUNOASSAY DEVELOPMENT

ATCC Cultures	
43882	<u>Methylobacterium rhodiesianum</u>
35070	<u>Methylosinus trichosporium</u>
35068	<u>Methylomonas agile</u>
29832	Unidentified bacterium
31483	<u>Pseudomonas fluorescens</u>
12633	<u>Pseudomonas putida</u>
14347	<u>Rhodococcus rhodochrous</u>
35066	<u>Methylocystis parvis</u>
35069	<u>Methylosinus sporium</u>
43645	<u>Methylobacterium extorquens</u>
SRS Cultures	
S4A/1Bd	methanotroph
S3C/2AB	methanotroph
S3A/1Q	methanotroph
S2C/1b	methanotroph
S3A/1C	methanotroph
S4A/1BC	methanotroph
S3C/1b	methanotroph
S4B/1Aa	methanotroph

Polyclonal Antibodies. Polyclonal antibodies (Pabs) were developed in male New Zealand white rabbits at the Medical College of Georgia (MCG), Augusta, GA. Serum was collected and frozen at -70° C in 100 μ l aliquots.

ELISA For ELISA 100 μ l of a 10^7 organisms/ml suspension or media from MPN tubes, were added to wells in immunoassay plates previously treated with polylysine. ELISA plates were treated sequentially for 1 h each with PBS containing 1% bovine serum albumen (PBSA), 100 μ l of Pab, 100 μ l of a 1:1000 dilution in PBS of affinity-purified horse radish peroxidase goat anti-rabbit immunoglobulins. After incubation with each reagent, ELISA plates were washed 6X with PBS containing 0.05% Tween-20 on an ELISA plate washer reader. Negative controls included PBSA and normal rabbit serum. Bound conjugate was observed by addition of 100 μ l of substrate (1 mg/ml 2, 2'-Azino-bis(3-Ethylbenz-thiazoline-6-

Sulfonic Acid) (ABTS), in citrate buffer with 3% hydrogen peroxide). The plates were read on a ELISA plate reader at 405 nm after 30 min incubation at room temp.

FA. Immunoglobulins were isolated by ammonium sulfate precipitation from rabbit antisera having agglutination titers >1280 and conjugated with fluorescein isothiocyanate (FITC). The FAs were separated from unconjugated FITC on Sephadex G-25 columns, filtered with 0.2 μ m filter, and frozen in 100 μ l aliquots at -70°C. For analysis with FAs 10 μ l aliquots of bacteria suspension or environmental samples from media dilutions were pipetted onto slides and heat fixed for 10 min at 65°C. 10 μ l of a 2% hydrolyzed gelatin solution was then layered over the sample and allowed to dry for 10 min at 65°C. FAs were pipetted over the bacteria at room temperature in 10 μ l aliquots diluted 1:32 in PBS. The slides were incubated 30 min at room temperature in a wet chamber, rinsed with distilled water, washed in filtered PBS, dipped in 5% sodium pyrophosphate buffer (NPP) and dried. Slides were examined using a LSM 310 Laser Scanning Microscope (Carl Zeiss, Inc., Thornwood, NY) with a 488 nm filter.

Total heterotrophic bacteria were enumerated using the aerobic heterotrophic plate count technique that provides an estimate of the total number of viable aerobic, anaerobic and facultatively anaerobic bacteria in the soils. Low (1%) and high (100%) concentrations of Peptone-Trypticase-Yeast-extract-Glucose (PYTG) were used to indicate differences in bacteria adapted to oligotrophic and eutrophic conditions. Samples (1-2 grams) were weighed directly into 15 ml conical centrifuge tubes containing 9 ml of NPP. Subsequent serial dilutions were made in phosphate buffered saline. One tenth milliliter of each dilution was inoculated onto a plate of the appropriate medium. Each dilution (0.1 ml) was inoculated onto a corresponding plate of low and high strength medium of 1 % and full strength formulation of (PTYG), respectively (Balkwill 1989). The inoculum was evenly spread over the agar plates and incubated at room temperature for 1 week prior to counting.

Methanotrophic bacteria were counted in groundwater samples using the MPN Enumeration method. Minimal salts media (MSM) (Fogel et al. 1986) were supplied with 10 % methane 90 % air headspace in Balch tubes sealed with black butyl rubber stoppers. Triplicate tubes were run for each dilution. The first dilution contained 3 g of soil into 20 ml MSM with 3 subsequent 1 to 10 dilutions. Tubes were incubated for 6 weeks along with a set of 4 control tubes. The concentration of methane and carbon dioxide in the headspace of control tubes were averaged and the standard deviation represented the lower limit of methane removal and carbon dioxide production needed to count as a positive tube in the MPNs. After reading MPN tubes methane and carbon dioxide concentrations with the GC samples were taken in 100 μ l duplicates and tested with the ELISA.

Total bacterial counts were accomplished by the Acridine Orange Direct Count Method (AODC). One to 2 grams of soil was added to 5 mL Homogenizing Buffer, mixed well, sonicated for 30 sec. and vortexed for 30 sec. The mixture was then centrifuged at 3000 rpms for 5 min. The supernatant was diluted 1:10. One mL of this dilution was mixed with 0.5 ml acridine orange for 2 minutes, on a .2 micron nucleopore filter (this needs to be checked), and then vacuumed through the vacuum manifold. The filter was then mounted on the slide on top of 1 drop of immersion oil. One drop of immersion oil added on top of the filter and a cover slip applied.

Dichlorobenzene degraders were enumerated by a plate count procedure. This method provided an estimate of the number of viable aerobic and facultatively anaerobic bacteria capable of growth on chlorobenzene as a carbon and energy source. 1-3 g from each sample was diluted in 9 ml pyrophosphate buffered saline. Subsequent serial dilutions were made in PBS. Appropriate dilutions were spread on minimal salts medium solidified with 1.8 % agar (w/v) and supplemented with yeast extract (10 milligram per liter (mg/l)). Chlorobenzene was supplied in the vapor phase to cultures on the solid medium in desiccators at room temperature. Control plates with PBS Buffer were incubated in the presence of the chlorobenzene. Plates were incubated for 6-8 weeks or more prior to counting.

RESULTS

ELISA. The specificity of the ELISA utilizing the Pabs developed for the ATCC and SRS methanotrophic bacteria have been described previously (Brigmon et al., 1995b). The results of cross reactivity testing with the ELISA is shown in Table 2. Several of the Pabs appear to be group specific (i.e. SC12) while others only reacted primarily with immunogen (i.e. SC30).

TABLE 2. ELISA REACTIVITY

BACTERIA	ID	ANTIBODY PROBE												
		SC 11	SC 12	SC 13	SC 14	SC 15	SC 16	SC 17	SC 18	SC 19	SC 20	SC 22	SC 23	SC 24
S4A/1Bd	SC11	+	+	+	+	+	+	+	+	-	-	-	+	-
S3C/2AB	SC12	+	+	+	+	+	+	+	+	-	-	-	-	-
S3A/1Q	SC13	+	+	+	+	-	-	+	+	+	-	-	-	+
S2C/1b	SC14	+	+	+	+	+	+	+	+	+	-	-	-	+
S3A/1C	SC15	+	+	+	+	+	+	+	+	-	-	-	+	+
43882	SC16	-	+	+	+	-	+	+	+	-	-	-	-	+
35070	SC17	-	-	-	+	+	-	+	-	-	-	-	-	-
S4A/1BC	SC18	+	+	+	+	+	+	+	+	+	-	+	+	+
S3C/1b	SC19	+	+	+	+	+	+	+	+	+	-	+	+	+
35068	SC20	-	-	-	+	-	-	-	-	-	+	+	-	+
29882	SC22	-	-	-	+	-	-	-	-	-	-	+	-	-
31483	SC23	+	-	-	+	-	-	-	-	+	+	+	+	+
12663	SC24	-	-	-	+	-	-	-	-	+	-	+	+	+
14347	SC26	-	-	-	+	-	-	-	-	+	-	+	+	+
35066	SC27	-	-	-	-	-	-	-	-	-	-	-	-	-
35069	SC28	-	-	-	+	-	-	-	-	-	-	-	-	-
43645	SC29	-	-	-	+	-	-	-	-	+	+	+	+	+
S4B/1Aa	SC30	-	-	-	+	-	-	-	-	-	-	+	+	+

The detection limit of the ELISA is from 10^4 - 10^6 bacteria cells/ml depending on the Pab preparation. The sensitivity for SC17 the Pab against Methylosinus trichosporium is shown in figure 2. A panel of the antibodies was employed for the ELISA in testing environmental samples. Table 3 compares the MPN and ELISA method for methanotrophs with respect to positives and negatives. There was a 76% agreement between the two tests. The ELISA took 5 hours to complete while the MPN method takes 6 weeks.

Table 3

DETECTION OF METHANOTROPHS IN ENVIRONMENTAL SAMPLES BY CULTURE/BIOCHEMICAL AND ELISA METHODS

MICROBIOLOGICAL	
+	-
73	13
12	4

Samples were positive if absorbance \geq over controls

Samples were positive if growth on chlorobenzene as sole carbon and energy source

FA. The FAs to aromatic hydrocarbon degraders in landfill samples were tested on slides. Slides were made the same day samples were taken. AODC and landfill slides were made simultaneously. The results of cell counts calculation taken from slides were compared to plate counts. The slide counts were significantly higher for FAs against the Pseudomonas species tested compared to plate counts. The counts for FA for R. rhodochrous (ATCC 14347) was higher but not significantly different ($P > .05$) compared to the plate counts. Figure 3. shows a comparison of this FA (SC26) and microbiological data for the chlorobenzene degraders in this soil. Immunofluorescent binding to specific bacteria in samples from landfill from Pabs generated against Rhodococcus rhodochrous (ATCC 14347) (Figure 4) Pseudomonas fluorescens (ATCC 31483) (Figure 5), Pseudomonas putida (ATCC 12633) (Figure 6).

Figures 4,5, and 6. Pabs generated against Rhodococcus rhodochrous (ATCC 14347) (Figure 4) Pseudomonas fluorescens (ATCC 31483) (Figure 5), Pseudomonas putida (ATCC 12633) (Figure 6).

Table 4 summarizes the results from enumerating the total heterotrophic bacteria (1% and regular PYTG plates), total counts (AODC), chlorobenzene degraders from plate counts, and DFA counts.

Table 4. Results from enumerating the total heterotrophic bacteria (1% and regular PYTG plates), total counts (AODC), chlorobenzene degraders from plate counts, and FA counts.

MICROBIAL COUNT	N	BACTERIA COUNT	% of TOTAL COUNT
AODC (Cells/Gram Dry Weight)	215	1.67×10^6	100
Heterotrophs (1% PYTG) (CFU/ml)	105	3.18×10^4	0.1
Heterotrophs (PYTG) (CFU/ml)	105	1.42×10^4	0.1
Chlorobenzene Degraders (CFU/ml)	95	2.43×10^3	0.01
FA SC23	21	2.70×10^4	0.1
FA SC24	21	3.26×10^4	0.1
FA SC26	21	1.22×10^4	0.1
Methanotrophs	102	5.92×10^2	0.001

DISCUSSION

The immunological procedures described in this paper permit the selective enumeration of methanotrophic and chlorobenzene degrading bacteria. While these methods can be used for characterization of TCE and CB degrading bacteria and for monitoring the response of subsurface environments to contamination, present methods are tedious or incompatible for field applications.

The method for enumeration of CB degrading bacteria with plate counts employed here required use of chlorobenzene in the laboratory as a carbon source which required specific safety precautions and also generated additional hazardous waste. Both the CB and methanotroph microbial enumeration techniques took 6 weeks for completion as well as being labor intensive. The ELISA and FA techniques can be accomplished in 1 day.

There were some differences in enumeration results in comparing microbiological and immunological procedures tested here. These differences could be explained in part by the fact that FA and ELISA techniques react the same with live/active/inactive and dead bacterial cells while the microbial techniques measure live active microorganisms. In addition, the Pabs may not recognize certain bacteria in the environmental samples. However, in the ELISA for methanotrophs there were as many false negatives as positives compared to the MPN method (Table 3). The relative numbers of total bacteria to CB and TCE degraders are of interest regarding the structure of the microbial community in bioremediation applications (Table 4). This information indicates the potential for intrinsic bioremediation at this landfill.

In conclusion, the results shown here demonstrate the effective use of immunoassays for the purpose of studying select microorganisms in complex environments such as landfills. Preliminary work with these FAs and ELISA indicate they can be used to monitor methanotrophic bacteria in active TCE bioremediation work for bioreactors (Brigmon et al., 1995b). The fact the results comparing the MPN to ELISA for the methanotrophs were similar and the CB degraders matched one of the FAs is promising and a wider range of testing is ongoing at the landfill. A limitation of the ELISA is the sensitivity which may be improved by amplification techniques. The use of immunological criteria should facilitate the detection and quantification of specific bacteria in environmental samples. The method for enumeration of microbial chlorobenzene degraders generates hazardous waste and storage problems. The MPN method for methanotrophs is labor intensive and can take 6 weeks. The ELISA and DFA saves time over culture techniques, can be species specific, and reproducible. The immunological techniques presented here as well other antibody preparations under development will be used to evaluate the significance of these and other isolates in bioremediation efforts.

REFERENCES

- Balkwill, D. L., 1989, Numbers, Diversity, and Morphological Characteristics of Aerobic, Chemoheterotrophic Bacteria in Deep Subsurface Sediments From a Site in South Carolina, *Geomicrobiol. J.* 7:33-52.
- Bowman, J.P., L. Jimenez, I. Rosario, T.C. Hazen and G.S. Sayler. 1993. Characterization of the Methanotrophic Bacterial Community Present in a Trichloroethylene-Contaminated Subsurface Groundwater Site. *Appl. Environ. Microbiol.* 59:2380-2387.
- Brigmon, R.L., S.G. Zam, G. Bitton and S.R. Farrah. 1992. Detection of *Salmonella enteritidis* in environmental samples by monoclonal antibody-based ELISA. *J. Immunol. Methods.* 152: 135-142.
- Brigmon, R. L., G. Bitton, S. G. Zam, and B. O'Brien. 1995a. Development and application of a monoclonal antibody against *Thiothrix* spp. *Appl. Environ. Microbiol.* 61:13-20.
- Brigmon, R. L., D. P. Chynoweth, J. C. Yang, and S. G. Zam. 1994. An enzyme-linked immunosorbent assay (ELISA) for detection of *Clostridium aldrichii* in anaerobic digesters. *J. Appl. Bacteriol.* 77:448-455.
- Brigmon, R.L., M.M. Franck, J.S. Bray, R.B. Patel, C.J. Berry, and C.B. Fliermans. 1995b. Immunoassays for bacteria that degrade trichloroethylene. 95th General Meeting American Society for Microbiology, Washington, DC.
- DiSpirito, A.A., J. Gullledge, A.K. Shiemke, J.C. Murrell, M.E. Lidstrom and C.L. Krema. 1992. Trichloroethylene oxidation by the membrane-associated methane monooxygenase in the type I, type II and type X methanotrophs. *Biodegradation* 2: 151-164.
- Enzien, M.V., F. Picardal, T.C. Hazen, R.G. Arnold, and C.B. Fliermans. 1994. Reductive dechlorination of trichloroethylene and tetra chloroethylene under aerobic conditions in a sediment column. *Appl. Environ. Microbiol.* 60:220-2204.
- Fliermans, C.B., J.M. Dougherty, M.M. Franck, P.C. McKinsey and T. C. Hazen. 1994. Immunological Techniques as Tools to Characterize the Subsurface Microbial Community at a Trichloroethylene Contaminated Site. In: *Applied Biotechnology for Site Remediation* Eds. R. E. Hinchee, et al. Lewis Publishers. Boca Raton, FL. pgs 186-203.
- Fliermans, C.B., T.J. Phelps, D. Ringelberg, A.T. Mikell, and D.C. White. 1988. Mineralization of trichloroethylene by heterotrophic enrichment cultures. *Applied and Environmental Microbiology* 54: 1709-1714.
- Little, C.D., A.V. Palumbo, S.E. Herbes, M.E. Lidstrom, R.L. Tyndall and P.L. Gilmer. 1988. Trichloroethylene biodegradation by a methane-oxidizing bacterium. *Appl. Environ. Microbiol.* 54: 951-956.
- Nelson, M.J.K., S.O. Montgomery and P.H. Prichard. 1988. Trichloroethylene metabolism by microorganisms that degrade aromatic compounds. *Appl. Environ. Microbiol.* 54: 604-606.
- Vogel, T.M. and P.L. McCarty. 1985. Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride and carbon dioxide under methanogenic conditions. *Appl. Environ. Microbiol.* 49: 1080-1083.
- Wilson, J.T. and B.H. Wilson. 1985. Biotransformation of trichloroethylene in soil. *Appl. Environ. Microbiol.* 29:242-243.

Records Transfer to Archival Storage

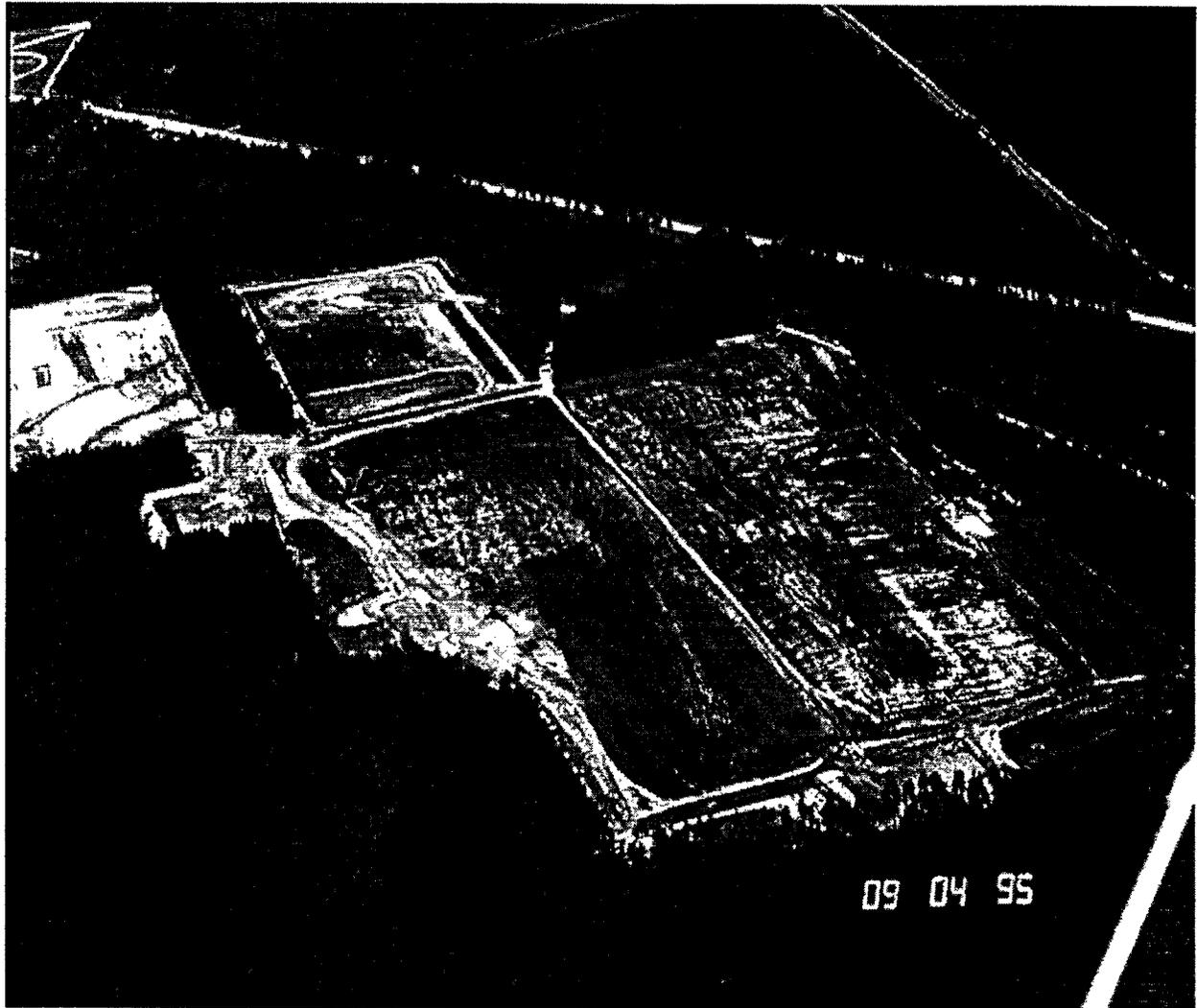
Submitter's Name Robin L. Brigmon	Building/Room 704-8T/13	Phone 7-77719	Organization Code L3640	Date Submitted 6-10-96	Please <input checked="" type="checkbox"/> Individually Indexed Record(s) Check <input type="checkbox"/> Box (Bulk) Indexed Record(s) One
Submitter's Signature	Classification of Records: <input type="checkbox"/> Secret <input type="checkbox"/> Confidential <input type="checkbox"/> Official Use Only <input type="checkbox"/> UCN <input checked="" type="checkbox"/> Unclassified	Access (Authorized to Refer or Withdraw) <input checked="" type="checkbox"/> Unlimited <input type="checkbox"/> Limited to Sender		No. of Boxes in Shipment _____	
Document ID/Revision (Addendum) Alternate ID/Task No./Project No. Sender's Box No.	Name/Title of Record (or Box Content Description)	Author(s) Addressee Keywords		Date of Record(s)	Retention Period Disposal Authority RIDS TRACK No.
WSRC-MS-95-0495		R.L. BRIGMON, M.M. FRANCK, J. BREY, D. 704-8T		5/10/96	Permanent
Intrinsic Bioremediation	Application of Fluorescent Antibody and Enzyme-Linked Immunosorbent Assays for TCE and PAH Degrading Bacteria.	Fluorescent Antibody, ELISA, Trichloroethylene, PAH, methanotroph, bacteria, bioremediation, groundwater			N1-434-89-8-7. B(1)(B) 1960
704-8T					

Shipment No. _____

Acknowledgement: The records listed above have been received by Records Administration.
 Signature _____ Date _____

Distribution: 1st and 2nd Copy—Sender forwards attached to records.
 3rd Copy—Sender retains until signed 2nd copy is returned, then destroys.

Figure 1. WSRC SANITARY LANDFILL



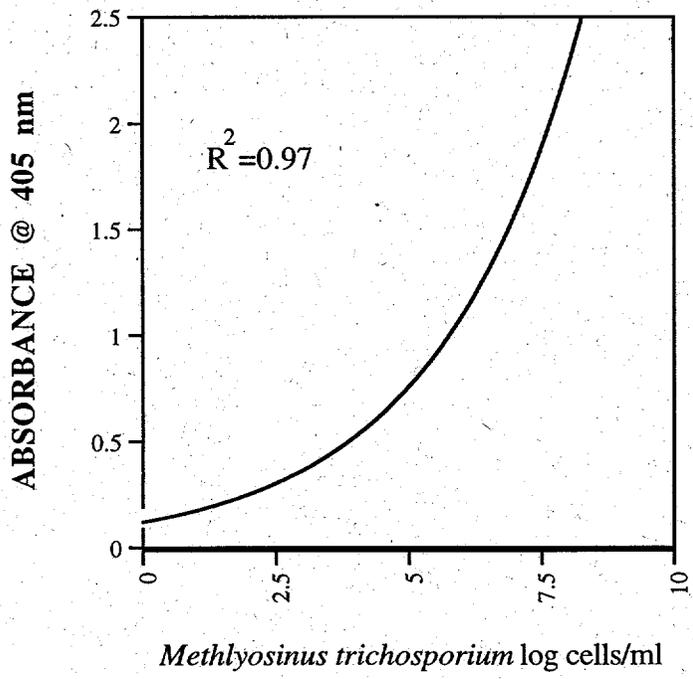


Figure 2. ELISA sensitivity of *Methylosinus trichosporium*

Figure 3. CHLOROBENZENE DEGRADERS IN SANITARY LANDFILL FA VS MICROBIOLOGICAL TECHNIQUE

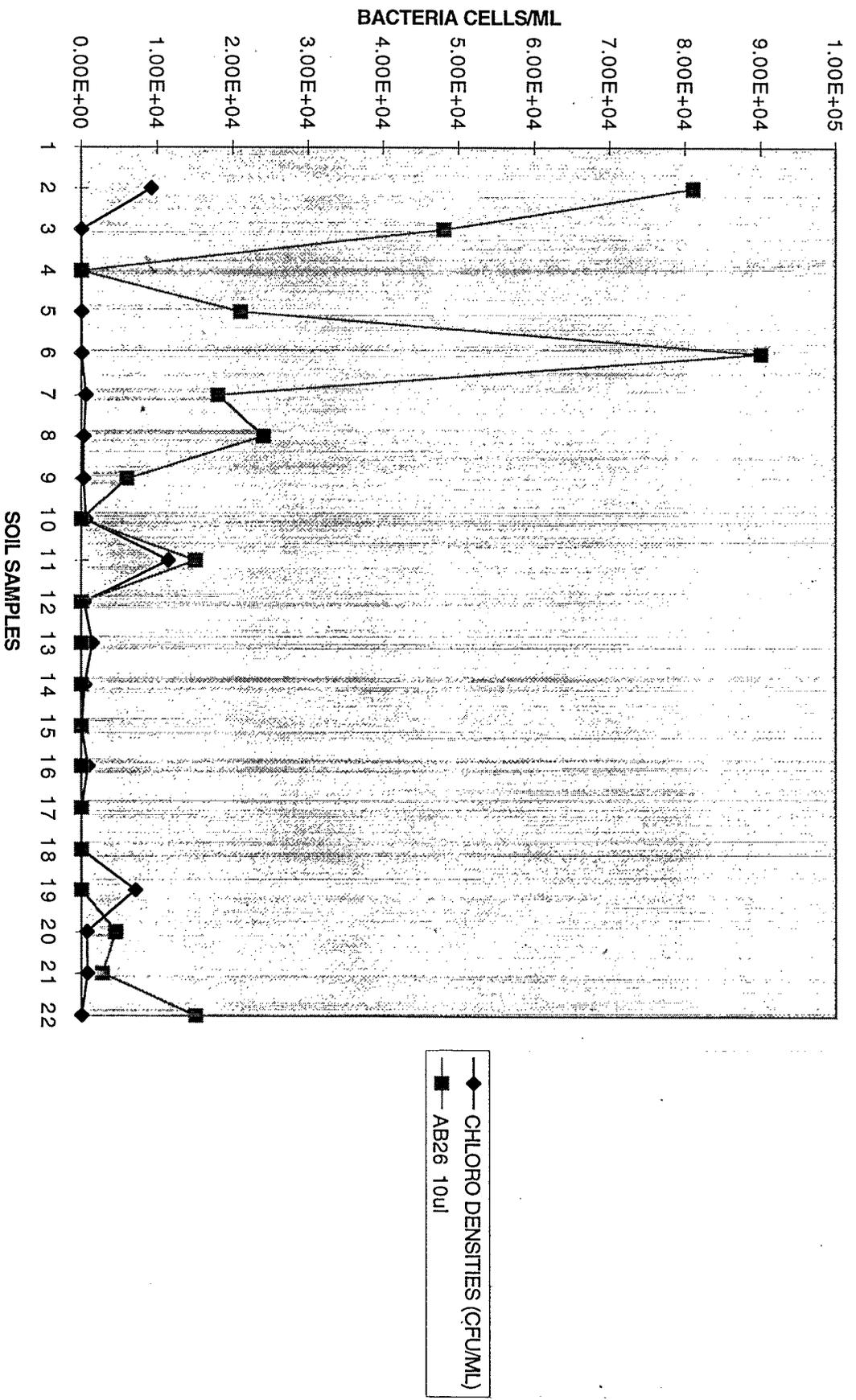


Figure 4

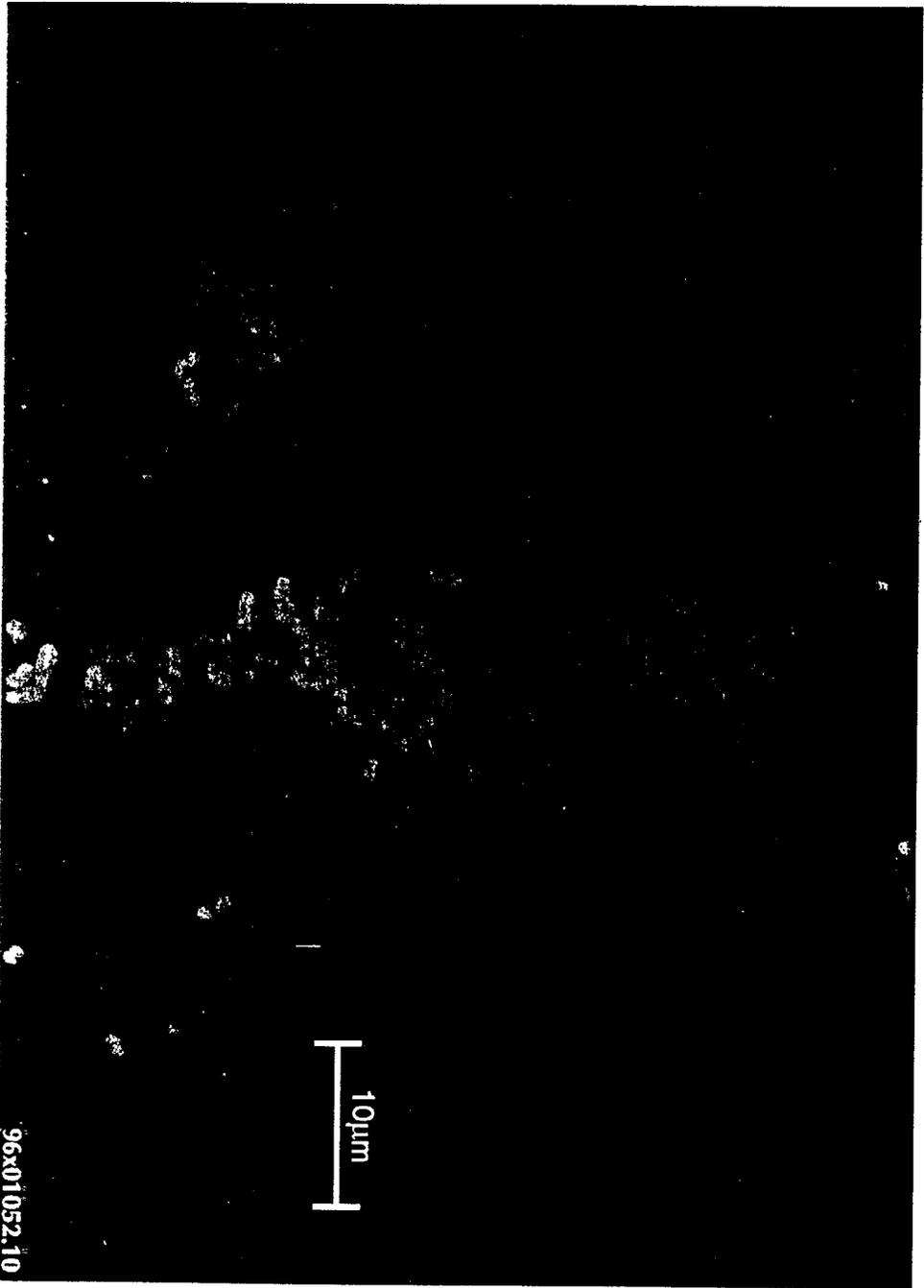
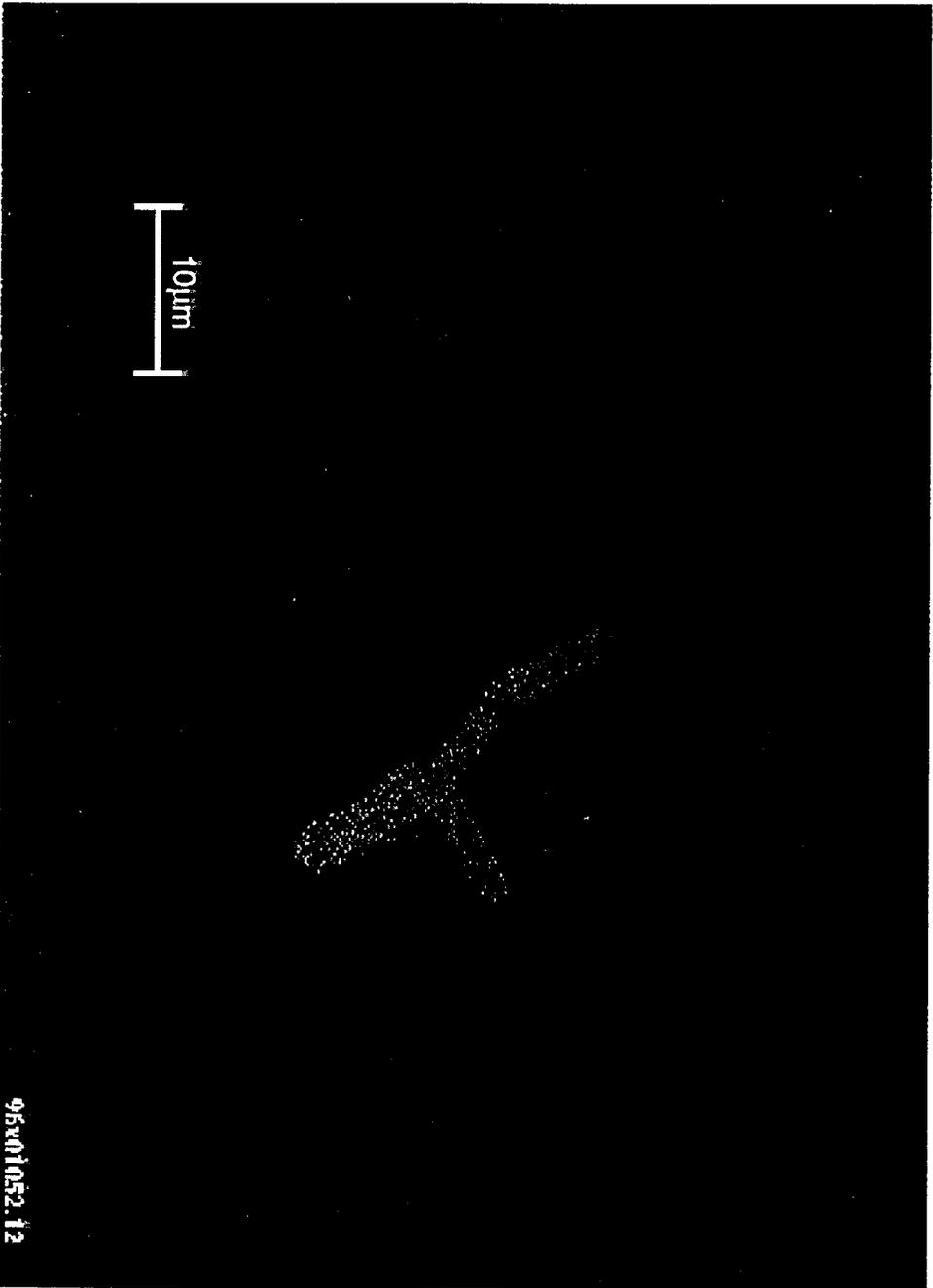


Figure 5



Figures 6

