CONF-920307--7

COMETABOLIC BIOTREATMENT OF TCE-CONTAMINATED GROUNDWATER

Laboratory and Bench-Scale Development Studies

CONF-920307--7

DE92 007252

T. L. Donaldson, H. L. Jennings, A. J. Lucero,
G. W. Strandberg, and M. I. Morris

Chemical Technology Division
Oak Ridge National Laboratory*
Oak Ridge, Tennessee 37831

A. V. Palumbo, P. A. Boerman, and R. L. Tyndall

Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee 37831

For presentation at the
Waste Management '92 Conference, Tucson, AZ
March 1-5, 1992

*Managed by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under contract DE-AC05-84OR21400.

COMETABOLIC BIOTREATMENT OF TCE-CONTAMINATED GROUNDWATER

Laboratory and Bench-Scale Development Studies

T. L. Donaldson, A. V. Palumbo, P. A. Boerman, H. L. Jennings, A. J. Lucero, R. L. Tyndall, G. W. Strandberg, and M. I. Morris

Oak Ridge National Laboratory
Oak Ridge, Tennessee

ABSTRACT

The Oak Ridge National Laboratory is conducting a demonstration of two cometabolic technologies for biotreatment of groundwater contaminated with trichloroethylene (TCE) and other organics. Technologies based on methanotrophic (methane-utilizing) and toluene-degrading microorganisms will be compared side-by-side on the same groundwater stream. Laboratory and bench-scale bioreactor studies have been conducted to guide selection of microbial cultures and operating conditions for the field demonstration. This report presents the results of the laboratory and bench-scale studies for the methanotrophic system.

BACKGROUND

The Oak Ridge National Laboratory (ORNL) is conducting a demonstration of cometabolic technology for biotreatment of TCE-contaminated groundwater at a seep from a classified burial ground at the Oak Ridge K-25 Site. The seep water contains trichloroethylene (TCE), perchloroethylene (PCE), benzene, toluene, chlorinated ethanes, and other volatile organic chemicals at a total concentration of several parts per million (ppm). This seep water is currently released through a National Pollution Discharge Elimination System (NPDES) permitted discharge point. Treated effluent from the demonstration will be collected in a tanker trailer and transported to the Central Neutralization Facility, a licensed treatment facility at the K-25 Site.

The performance of two different types of cometabolic technology will be evaluated. In both cases the TCE is cometabolized in the sense that metabolism of a different primary substrate is necessary to obtain the simultaneous (co)metabolism of TCE. Trichloroethylene alone is unable to support growth of the microorganisms.

The first technology being demonstrated utilizes methanotrophic (methane-utilizing) microorganisms. Degradation of TCE is accomplished by a nonspecific enzyme, methane monooxygenase (MMO), whose principal function is to oxidize methane to provide energy for the microbial cells. Toluene-degrading microorganisms will be used in the second cometabolic technology to be demonstrated. These microorganisms also have a nonspecific enzyme, toluene dioxygenase, that can degrade TCE. Both technologies will be demonstrated in side-by-side tests.

A bioreactor skid unit has been loaned to ORNL by the Air Force Civil Engineering Support Agency at Tyndall Air Force Base. The skid unit has been installed in a van-type trailer for protection from inclement weather. The two stainless steel columnar bioreactors will be operated as trickle-flow bioreactors using a structured packing material to support the methanotrophic biofilms. Addition of the toluene-based system for side-by-side testing is scheduled for later in the project. The toluene-based technology is not covered in this report.

LABORATORY AND BENCH-SCALE DEVELOPMENT STUDIES

Experimental laboratory and bench-scale studies of the methanotrophic technology for TCE biodegradation were carried out to meet two principal technical objectives:

1. Provide data for a comparative evaluation of several different methanotrophic cultures in order to select a culture for use in the demonstration; and

2. Determine satisfactory operating conditions for the bioreactor for the methanotrophic technology demonstration.

In order to select a microbial culture from among the several available methanotrophic cultures and to choose satisfactory operating conditions, experimental data are needed to address the following uncertainties and issues:

- TCE degradation rates
- Methane requirements and utilization
- Culture stability and biofilm formation
- Rate enhancement using formate
- Micronutrient requirements and limitations
- Pathogenicity of microbial cultures

Tests have been carried out in batch and continuous modes to pursue the objectives. The results from both types of experiments have been used to reach decisions regarding the best microbial culture and operating conditions for scale-up to the technology demonstration at the K-25 Site.

The original plans included investigation of the degradation of other organics in addition to TCE and treatability studies with actual seep water from the K-25 Site. However, the resources and time were not available to complete this work, and thus these factors will be evaluated at the pilot scale during the field demonstration.

Batch Experiments

Cultures were obtained by enrichments of water samples from several contaminated sites. One culture, designated KCMC, came from a DOE site in Kansas City. Two cultures, designated DT1 and DT2, came from waste disposal areas at the Y-12 Plant in Oak Ridge. Two additional cultures were obtained from the Oak Ridge K-25 Site by incubating a mixture of 50% mineral salts medium and 50% seep water, and by filtering seep water through 0.45-μm filters and incubating the filter in mineral salts medium (1).

Cultures were maintained on a mineral salts agar, which is a modification of NATE medium (2,3), in desiccator jars under a 30% (vol/vol) methane-air atmosphere. Desiccator jars contained ambient concentrations of oxygen minus the volume displaced by methane, which produced a final oxygen concentration of about 15% (vol/vol).

Starter cultures were inoculated by transfer of cells from stock plates to 100 mL of NATE in 250-mL bottles with Teflon-lined septum caps. A 30-mL volume of sterile-filtered methane gas was injected to yield a 10% (vol/vol) methane headspace, and cultures were incubated at 295°K on a shaker table at 75 rpm. During late exponential phase, the starter cultures were used as inocula to grow cells for degradation assays. Subsamples (2 mL) of culture were transferred into 250-mL bottles containing NATE medium and grown as described above.

In preparation for degradation experiments, cultures were transferred into 250-mL centrifuge bottles and spun at 3000 x g for 1 hour at 283°K. Pellets were resuspended in a smaller volume of NATE medium which increased the cell concentration twofold or more. Cell suspension aliquots of 12 mL were transferred to 40-mL vials and sealed with Teflon-lined septum caps. In most cases, water saturated with TCE and 5 mL of sterile-filtered methane were injected through the septa, and the vials were inverted and incubated under the same conditions as those described for cell growth. For formate experiments, TCE and either methane, methane plus 20 mM sodium formate, or formate alone were present in the vials. Sterile controls contained heat-killed cells, TCE, and methane.

Experiments were terminated by injection of hexane (4 mL) followed by thorough mixing, which consistently extracted greater than 99% of the remaining TCE. Triplicate vials were sacrificed at intervals and TCE was quantified by gas chromatography after a minimum 24-h extraction period. One μL of each hexane extract was analyzed using a Perkin Elmer Sigma 2000 (Norwalk, CT) gas chromatograph. The instrument was equipped with a 2 ft x 1/8 in. i.d. glass column packed with 1% SP-1000 60/80 Carbopack B (Supelco, Bellefonte, PA) and an electron capture detector with nitrogen as the carrier gas (30 mL/min). The oven temperature was 373°K, the detector temperature was 623°K, and the injector was held at 523°K.

Protein concentrations were measured using a Bio-Rad protein determination kit (Bio-Rad, Richmond, CA) with an ultrafast centrifuge analyzer (COBAS-FARA model, Roche Diagnostic Systems, Nutley, NJ).

Continuous Bioreactor Experiments

The laboratory-scale bioreactor system was comprised of six 5-cm i.d. glass columns and one 10-cm i.d. glass column. The bottoms of the columns were sealed by stainless steel plates with Viton rubber gaskets. The plates and gaskets were held in place by bolted flanges. Each plate had a single 0.9-cm port for both the liquid and gas effluent. Five of the columns (columns 2 through 6) were packed with 1.6-cm polypropylene flexirings to a depth of about 45 cm. Column 1 was packed with 0.48-cm i.d. polypropylene tubing chopped into 0.3-cm to 1-cm lengths, also with a depth of approximately 45 cm. Column 7, which was 10-cm i.d., contained one 16.8-cm tall element of Koch/Sulzer type BX structured packing made of a mixture of polypropylene and polyacrylonitrile.

The five cultures tested were DT1, DT2, KCMC, OB3b, and one of the cultures isolated from the K-25 Site, designated K-25E. All of the cultures are mixed cultures of microorganisms isolated from contaminated sites, except for OB3b, which is a pure culture of Methylosinus trichosporium (strain OB3b) used previously at ORNL (4). Columns 4 and 7 contained the DT1 culture; column 1 initially contained the K-25E culture, but was changed to DT1 on June 13, 1991; column 2 contained the OB3b culture; column 3 contained the K-25E culture; column 5 contained the KCMC culture; and column 6 contained the DT2 culture. Column 7 was later reinoculated with the KCMC culture.

The liquid feed to the bioreactors was a synthetic waste that consisted of mineral nutrients and TCE dissolved in distilled water. The mineral nutrients were contained in a 200-liter stainless steel drum, and were mixed with a concentrated TCE solution to form the liquid feed. A multiple-head Masterflex peristaltic pump was used to feed the columns. With this pump the six 5-cm i.d. columns received the same flow rate and the 10-cm column received approximately four times the flow rate of the other columns. A mixture of 3% methane in air was fed cocurrently with the liquid flow to all of the columns. Metering valves were used to maintain the gas flow rates equal.

Sampling and Analysis

The concentration of TCE was monitored using a Hewlett Packard (HP) Model 5890A gas chromatograph equipped with an electron capture detector. The response of the detector was integrated and plotted with a HP Model 3396A recording integrator. Separation was achieved with a Megabore DB+1 capillary column (J&W Scientific Corp).

Samples of both the influent and effluent liquids were taken using a 25-mL gastight syringe. The syringe was first rinsed with a sample, then emptied. A 20-mL sample was then taken by slowly pulling the plunger so that no air was drawn into the syringe with the sample, thus preventing volatilization of TCE. The samples were then injected into 63-mL amber bottles containing 20 mL of hexane. The bottles were sealed with screw cap closures having a Teflon-faced, silicone rubber seal. The bottles were then placed on a rotating mixer for 16 to 24 hours to allow extraction of the TCE to the hexane. Approximately 1.5 to 1.8 mL of the hexane was then pipetted into a 2-mL autosampler vial and sealed with a crimp-type septum seal. The vials were then placed in the autosampler tray from a HP Model 7673A automatic sampler/injector. The integrator was programmed to calculate the concentration of TCE based on calibration with known standards. The gas chromatograph was recalibrated periodically.

Effluent gas samples were taken through a septum-sealed tee using a 10-μL gastight syringe. The syringe needle was inserted through the septum at a sufficient depth to place the needle tip in the center of the off-gas stream. The syringe was then flushed a minimum of five times by raising and lowering the plunger. A 5-μL sample was then taken and immediately injected into the gas chromatograph for analysis. This procedure was repeated until the results were within 20 μg/L for consecutive samples.

Calibration standards for gas-phase TCE measurements were generated using aqueous standards prepared to contain known concentrations (0.1 to 1.0 mg/L) of TCE. A measured volume of the standard solution was placed in Teflon-faced septum-sealed bottles of a known volume. Based on the Henry's Law Constant for TCE, the concentration of TCE in the gas phase can be calculated.

The calibration was then compared with standards of TCE in hexane as a check on the value of the Henry's Law Constant. The integrator was programmed to calculate the concentration of TCE present in the sample in μg/L.

The amount of methane used by the bioreactors was measured by sampling the inlet and exit gases with a 10-μL syringe. These samples were analyzed using a Hewlett Packard Model 5890 gas chromatograph equipped with a flame ionization detector. Separation was achieved with a Megabore DB+1 capillary column (J&W Scientific Corp).

Bioreactor Operation

Variables investigated for their effects on TCE degradation include liquid flow rate, gas flow rate, micronutrient concentration, and carbon source. The liquid flow rates to the 5-cm columns were varied from approximately 5 mL/min to 15 mL/min, and the flow rate to the 10-cm column was varied from approximately 20 mL/min to 60 mL/min. The methane/air mixture was fed cocurrently with the liquid at approximately 2.5 to 5.0 mL/min to the 5-cm columns and about 10 mL/min to the 10-cm column. The influent TCE concentration was 0.7 to 1.0 ppm.

For most experiments, the nutrient concentrations fed to the bioreactors were approximately one tenth those used in the batch studies. This is believed to be satisfactory because the bioreactor columns were operated in a continuous mode in which both the nutrients and the TCE were replenished continuously. In contrast, the batch experiments must have all the nutrients added at the beginning. For continuous operation, it is desirable to minimize the quantity of nutrients needed, especially for field operations.

Experiments were also conducted with the bioreactors to evaluate the use of formate as an alternative carbon source. The rationale for these experiments is the competitive substrate model for TCE and methane access to the methane monooxygenase enzyme. Removal of the methane and replacement by formate as the source of reducing power might increase the TCE degradation rate since methane would no longer be competing with TCE for the enzyme.

These formate experiments were conducted by first replacing the methane/air feed with an air-only feed. The effluent air was then analyzed by gas chromatograph repeatedly until no methane was detected. Then the liquid feed was switched to an otherwise identical feed except that it also included 20 mM formate. The column was then sampled hourly for several hours before returning to normal operation.

RESULTS

The experimental results are summarized below. Further details may be found in Reference 1.

TCE Degradation Rates

Cultures DT2, KCMC, and OB3b produced comparable TCE degradation rates in batch tests at 295°K. Under appropriate nutrient conditions, these cultures were able to degrade about 75% of the TCE in 24 hours when the initial concentration was approximately 3 to 4 μM (400 to

500 ppb). The TCE degradation rate by DT1 was significantly slower. The two cultures isolated from the seep water at K-25 did not degrade TCE nearly as well as the others.

In the continuous bioreactors, the highest degradation rate was observed in column 2 with the pure OB3b culture. The K-25E culture in column 4 appeared to have the lowest degradation rate, which is consistent with the batch results. Of the mixed cultures, the DT1 culture in column 4 appeared to do slightly better than the others; however, the DT2 and KCMC cultures performed essentially as well.

No significant effects of liquid and gas flow rates were observed over the ranges of 5 to 15 mL/min for liquid and 2.5 to 5 mL/min for gas. These results differ from earlier ORNL studies (5) in which the TCE degradation rate increased with increasing liquid flow rate (although the percent degradation decreased). In the present case, the effects of liquid flow rate may be masked by the relatively low degradation and the experimental variability.

Calculations indicate that the TCE reaction rates observed in batch tests and in continuous columnar bioreactors are comparable (1). The batch rates were somewhat higher, as would be expected because the effectiveness of cells in suspension is expected to be greater than cells in a biofilm. (However, fixed-film processes offer many operational advantages over suspended-growth batch processes.) Also, it was visually observed that the liquid distribution over the packing and biofilms was not uniform, which would lead to inefficient utilization of biofilms.

A significant failure of the TCE degradation capabilities of all bioreactors was experienced in mid-May 1991. This failure is believed to have resulted from experimental manipulations: In an effort to select for cultures capable of high TCE degradation rates, the nitrate was removed from the nutrients formula, producing a condition in which no nitrogen was available to the cultures. During the same general time period, a switch was made to ORNL process water in place of distilled water for makeup of the nutrients. This stream comprised the vast majority of the liquid flow to the bioreactors. It was observed subsequently that the pH of this nutrient stream increased gradually. At one point, the columns were exposed to a pH of at least 7.8. Very shortly thereafter, the TCE degradation fell to zero in all columns.

Various efforts were made to determine the cause of the loss of TCE degradation and to rejuvenate the bioreactors. The nitrate was replaced in the nutrient formula approximately three weeks later, and distilled water was reinstated for makeup of nutrients. A pH control system was added to hold the pH of the nutrient tank at pH 7. The bioreactor columns continued to consume methane, and samples of the DT2 and OB3b cultures from the bioreactors showed good TCE degradation in batch tests. However, recovery of the TCE degradation activity in the columns was not observed until approximately four months later.

pH Effects Over the pH range of 6.0 to 7.2, the TCE degradation rate appeared to be relatively insensitive to pH variations in the ORNL batch experiments. A decrease in TCE degradation rate above pH 7 has been observed by T. J. Phelps at the University of Tennessee (personal communication); 90% of the activity was lost at pH 7.5, and recovery took days to weeks. Thus, the loss of TCE degradation in the bioreactors described above may have been caused by high pH (at least 7.8 at one time). However, the nitrate was also absent at the same time.

Methane Requirements and Utilization

For batch experiments, the cultures were grown in the presence of approximately 30% (vol/vol) methane in air. About 15% methane in air was used for the batch TCE degradation tests. No measurements were made to determine methane consumption.

Methane consumption was measured in the bioreactors. At a feed concentration of 3% methane in air, the concentration typically was reduced to 1 to 0.5% in the off-gas. The gas residence time in the bioreactors was about 400 min. In earlier studies (5), 3% methane feed was found to be satisfactory for TCE degradation; a decrease in TCE degradation was observed when the influent methane was less than 2% and when it was greater than about 10%.

Culture Stability and Biofilm Formation

Under batch conditions, cultures DT2, KCMC, K-25E, and OB3b all required a temperature below 310°K for growth. In contrast, culture DT1 grew quite well at 310°K, but it would not grow satisfactorily at 295°K.

Over the course of preparing consortia for the various TCE batch degradation tests, it was found that KCMC was the most reliable consortia to culture, and would grow to higher densities more easily. All the cultures would occasionally fail to grow satisfactorily; however, the KCMC culture was less prone to this problem than were the other cultures.

All of the cultures DT1, DT2, KCMC, K-25E, and OB3b were observed to form satisfactory stable biofilms on the polypropylene packing in the continuous bioreactors. Visual observations suggested that in biofilms the OB3b culture grew the slowest, while the K-25E and KCMC cultures grew the fastest.

A major failure of the TCE degradation activity of all the biofilm cultures was experienced during the continuous bioreactor tests, as described earlier. Since the cause of the failure has not been determined, there is no evidence to suggest that any one of the cultures may be more robust with respect to this failure mode than the others.

Rate Enhancement Using Formate

Batch tests indicated that the TCE degradation rate was essentially unchanged when 20 mM formate was used in the absence of methane. However, the relatively long batch reaction times of 24 to 48 hours may have precluded observation of short-time effects. In the bioreactors, where the residence time is on the order of 1 hour, it was found that formate did increase the TCE degradation rate for short periods of time, but after approximately 3 to 4 hours the degradation rate fell to zero. The degradation rate was not enhanced by removal of methane alone without addition of formate.

Micronutrient Requirements and Limitations

The influence of copper and ammonia was investigated in these studies because of evidence from previous studies at ORNL and elsewhere that the TCE degradation rate is sensitive to these species. Copper and ammonia are common constituents in nutrient media for microbial growth;

copper is an important constituent in some enzyme molecules, and ammonia is a readily available source of nitrogen.

Copper is believed to affect the relative amounts of the soluble and particulate forms of MMO. Evidence from other investigators indicates that lower copper concentrations favor higher TCE degradation rates. Ammonia is a competitive substrate for the MMO, as are methane and TCE as described earlier. Therefore, removal of ammonia may lead to increased TCE degradation rates.

Two batch experiments were conducted to evaluate the effects of copper. Elimination of copper from the normal trace metals formulation had no effect on the performance of the KCMC culture. In the other experiment, the concentrations of the other trace metals were increased five-fold while the copper was maintained at the baseline level. This test was designed to look for competitive effects among the trace metals. Again, no effect on TCE degradation was seen for the DT2 culture. Although these experiments did not show a definitive response to copper, the copper was omitted from other batch nutrients and the continuous bioreactor nutrients on the basis of the results from other investigators.

The usual ammonium chloride was omitted from the nutrient media for batch tests with the K-25 and DT2 cultures. Improvement in TCE degradation was observed in both cases; the improvement was more significant with DT2. On this basis, ammonium chloride was not used thereafter in batch and bioreactor tests; only nitrate was provided as a nitrogen source. The explanation for the improved TCE degradation rate is presumably the elimination of the competitive substrate, ammonia.

An attempt was made to isolate nitrogen-fixing methanotrophs from the existing consortia. This would allow elimination of the nitrate in the nutrient media, since the culture would obtain nitrogen from the air. In batch culture tests to date, only a pure culture of Methylosinus trichosporium strain OB3b has exhibited growth in the absence of a nitrogen source in the liquid media. One degradation test was conducted with this isolate in the absence of nitrate. No loss of TCE was observed, indicating no degradation.

Pathogenicity of Microbial Cultures

All the mixed cultures being evaluated were plated on seven types of media that encompassed both clinical and environmental protocols. All the mixed cultures typically contained six to twelve distinct types of microorganisms, some of which could be tentatively identified. No potential pathogens were identified in any of the cultures under consideration for use in the field demonstration.

CONCLUSIONS

Several of the cultures appeared to offer selected advantages, and no culture was obviously superior to the others in an overall sense. Thus it was necessary to rank the various performance criteria with respect to importance for achieving the goals of the technology demonstration.

The KCMC culture appeared to produce the lowest TCE concentrations after 24 hours in batch tests in the absence of ammonia. However, the other cultures appeared to provide adequate TCE reduction. The DT1 culture had somewhat better tolerance to high pH excursions (up to 7.2), and it grew better at 310°K than at ambient temperature. These advantages were judged to be of minimal value because the pilot plant is equipped with temperature and pH control. It is reasonable to assume that these control systems will operate properly.

The KCMC culture also was the fastest and most reliable grower for the batch studies. These characteristics were judged to be valuable to the project objectives; minimization of startup time (fast growth rate) is important to meet schedules and to allow the maximum flexibility for whatever circumstances may be encountered. Furthermore, should a culture failure occur for whatever reason, the restart time should be minimized with the fastest growing culture. The TCE-degrading characteristics of the KCMC culture were judged to be adequate -- comparable to the other cultures within the confidence level of the data.

The data obtained from operation of the continuous-flow biofilm bioreactors were consistent with the above arguments derived from the suspended-growth batch experiments. Although the DT1 culture may have slightly better TCE degradation characteristics than KCMC and DT2, the differences were not judged to be significant. The KCMC culture was observed to be one of the faster growing cultures in the formation of biofilms on the packing.

On the basis of these arguments, the KCMC culture was selected for initial inoculation of the bioreactors. The second choice would be DT2; it had comparable TCE degradation kinetics, but grew more slowly. DT1 grew poorly at ambient temperature, but grew quite well at 310°K.

STATUS OF THE FIELD DEMONSTRATION

The inoculation and start-up phase for the field unit was carried out at the relatively rich nutrient conditions used in the batch experiments. This condition appears to favor rapid formation of biofilms to minimize the start-up time, and heavy biomass loading appears to favor higher TCE degradation rates. The inoculation and start-up were carried out at total recycle, so the actual consumption of nutrients was minimal and no nutrients were discharged. After a heavy biofilm loading and stable operation are achieved, then it may be quite feasible to reduce the nutrient concentration by ten-fold or perhaps 100-fold. However, the change will be carried out carefully, with frequent monitoring of TCE degradation, to prevent potential loss of TCE degradation activity.

Acclimatization of the culture to the seep water has been initiated by periodic addition of small quantities of seep water while the system is maintained in total recycle. Sustained operation while feeding seep water has not commenced yet.

ACKNOWLEDGMENTS

The authors are pleased to acknowledge the excellent technical assistance from C. A. McGuire, J. M. Cosgrove, and S. L. Pye. Valuable advice and discussion were provided by T. J. Phelps, University of Tennessee. This work is sponsored by the Office of Technology Development, U.S. Department of Energy, under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

REFERENCES

1. T. L. DONALDSON, A. V. PALUMBO, P. A. BOERMAN, H. L. JENNINGS, A. J. LUCERO, and G. W. STRANDBERG, **Bench-Scale Process Development Studies for the Methanotrophic Technology. Co-Metabolic Bioreactor Demonstration at the Oak Ridge K-25 Site**, ORNL/TM-12024, Oak Ridge National Laboratory, Oak Ridge, Tennessee, March 1992.

2. C. D. LITTLE, A. V. PALUMBO, S. E. HERBES, M. E. LIDSTROM, R. L. TYNDALL, and P. J. GILMER, "Trichloroethylene Biodegradation by a Methane-Oxidizing Bacterium," **Appl. Environ. Microbiol. 54**, 951-956 (1988).

3. R. WHITTENBURY, K. C. PHILLIPS, and J. F. WILKINSON, "Enrichment, Isolation, and Some Properties of Methane-Utilizing Bacteria," **J. Gen. Microbiol. 61**, 205-218 (1970).

4. W. ENG, A. V. PALUMBO, S. SRIHARAN, and G. W. STRANDBERG, "Methanol Suppression of Trichloroethylene Degradation by Methylosinus trichosporium (OB3b) and Methane-Oxidizing Mixed Cultures," **Appl. Biochem. Biotech. 28/29**, 887-899 (1991).

5. G. W. STRANDBERG, T. L. DONALDSON, and L. L. FARR, "Degradation of Trichloroethylene and Trans-1,2-Dichloroethylene by a Methanotrophic Consortium in a Fixed-Film, Packed-Bed Bioreactor," **Environ. Sci. Technol. 23**, 1422-1425 (1989).