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Utilization of Toxic Gases and Vapors as Alternate Electron Acceptors in Biofilters

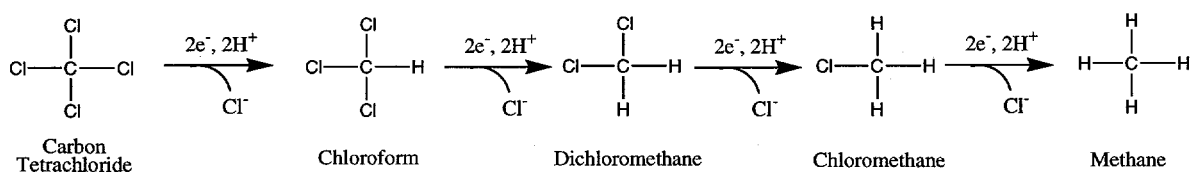**Brady D. Lee, William A. Apel and Michelle R. Walton**

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Introduction

Conceptually, biofilters are vapor phase bioreactors that rely on microorganisms in the bed medium to oxidize contaminants in off-gases flowing through the bed to less hazardous compounds. In the most studied and utilized systems reduced compounds such as fuel hydrocarbons are enzymatically oxidized to compounds such as carbon dioxide and water. In these types of reactions the microorganisms in the bed oxidize the contaminant and transfer the electrons to oxygen which is the terminal electron acceptor in the process. In essence the contaminant is the carbon and energy source for the microorganisms in the bed medium and through this catabolic process oxygen is reduced to water. An example of this oxidation process can be seen during the degradation of benzene and similar aromatic compounds (Figure 1). Aromatics are initially attacked by a dioxygenase enzyme which oxidizes the compounds to a labile dihydrodiole which is spontaneously converted to a catechol. The dihydroxylated aromatic ring is then opened by oxidative "ortho" or "meta" cleavage yielding *cis, cis*-muconic acid or 2-hydroxy-*cis, cis*-muconic semialdehyde, respectively (1). These organic compounds are further oxidized to carbon dioxide or are assimilated for cellular material.

Operation of industrial waste treatment and environmental remediation processes often produce highly chlorinated compounds that are highly oxidized and are not susceptible to further oxidation by microorganisms. Examples of such compounds are perchloroethylene (PCE) and carbon tetrachloride which have not been shown to biodegrade under aerobic conditions. Reduction of these compounds to less toxic products has been demonstrated to occur in many instances when an adequate supply of reducing equivalents is supplied. Carbon tetrachloride and PCE are not degraded under aerobic conditions, but can be transformed by microbially mediated reductive dehalogenation under anaerobic conditions (2). These reductive reactions involve the transfer of electrons to the chlorinated compound, resulting in the replacement of a chloride ion with an atom of hydrogen (3). Carbon tetrachloride is reduced by the following pathway:

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Factors affecting the dechlorination process include the type of electron donor, the presence of specific electron acceptors, temperature and substrate availability to the microbe. The most effective electron donor appears to be dependent on the microbial population involved. Reductive dechlorination has been shown to use hydrogen as the direct electron donor (4). Low molecular weight compounds such as methanol, ethanol, lactic acid, acetic acid, formic acid, butyric acid or more complex carbon sources such as glucose, all of which can be fermented to hydrogen, have been shown to cause microbial dechlorination of PCE (5). In most studies looking at the anaerobic transformation of chlorinated solvents only 0.005 to 1.6% of the electrons from the primary electron donor are used for dechlorination. The addition of electron acceptors affects the population composition of microbial communities by establishing the redox conditions and chemical speciation in the vacinity of the cell and has been correlated to reductive dehalogenation activity (6). Specifically, microorganisms capable of utilizing sulfate, nitrate, or carbon dioxide as terminal electron acceptors have been shown to degrade these contaminants (7). The transfer of electrons between the electron donor and acceptor provides energy for synthesis of cellular material from carbon produced during the oxidation of the electron donor or carbon source.

Anaerobic biofiltration has been previously demonstrated for nitrogen oxide (NO_x) compounds (8,9). Compost based biofilters operated at empty bed residence times between 12 seconds and three minutes and nitric oxide (NO) concentrations between 0 and 500 ppmv were shown to effectively remove NO (>90%). At inlet NO concentrations above 250 ppmv, the rate and extent of NO removal was enhanced by adding supplemental carbon to the bed medium. Glucose, lactate, molasses and acetate were tested and all were shown to provide adequate carbon and energy for biological reduction of NO. In the absence of oxygen NO_x compounds, specifically nitrate as an example, can act as terminal electron acceptors in a process known as nitrate respiration or dissimilatory nitrate reduction. In the process a carbon source such as a sugar, alcohol or organic acid is oxidized yielding two protons (hydrogen) and two electrons. The hydrogen atoms and electrons are passed through a series of oxidation-reduction systems, and the energy inherent in the substrate is released as biologically useful energy in the form of adenosine triphosphate or an equivalent. Reductase enzymes in the cytoplasmic membrane of these microorganisms use electrons from the process to drive the reduction of the nitrogen oxygen bond while the protons are translocated to oxygen liberated from the nitrogenous oxide to form water. Enzymes and electron carriers in the chain are oxidized and reduced leading to the formation of N_2, the final product of denitrification.

The purpose of this paper is to further demonstrate the utility of biofilters operating under anaerobic conditions as a method of destroying contaminants by using them as electron acceptors, in essence destruction by reduction rather than oxidation. This paper will discuss experiments and results of anaerobic biofilters using methanol as the primary carbon and energy source for the subsequent transformation of carbon tetrachloride.

Experimental Methods

Biofiltration of Carbon Tetrachloride

A general schematic of the laboratory-scale, compost based biofilters used for the biofiltration of carbon tetrachloride can be seen in Figure 2. Biofilters were constructed using 12" x 3" I. D. Kimax Beaded Process Pipe coupled bead to bead to 3" x 2" Kimax straight reducers. The ends of the biofilters were capped with Teflon plugs ported for sampling as well as gas and liquid delivery. Biofilter sampling was

done at the column inlet and outlet. The biofilters were run at ambient room temperature (20 to 25°C) in a fume hood. The biofilters were packed with a municipal yard waste compost (Schenectady County Soil and Water Conservation District, Scotia, NY), yielding a bed volume of 1.4 L and an empty bed residence time of 28 minutes. The bed medium consisted of the municipal yard waste compost amended with 100 g of calcite as a method of controlling the pH in the biofilter during operation, and 10g of Max Bac Customblen a fertilizer formulated for use during bioremediation operations.

Methanol was used as a carbon and electron source during this phase of testing, with helium as the makeup gas yielding a total flow of 50 ml·min^{-1}. Target methanol concentrations tested were 500, 1,000, and 1,500 ppmv (parts per million by volume). Methanol vapors were supplied by sparging helium through liquid methanol held in a stainless steel vessel.

Previous experiments indicating biofilters could be operated using denitrifying conditions led to an initial attempt to use denitrifying conditions for reductive dechlorination. During the first portion of the feasibility testing for biofilters receiving 500 and 1,000 ppmv methanol as the carbon source, NO gas was supplied at a target inlet concentration of 1,000 ppmv. All of the biofilters were acclimated to the anoxic conditions for at least one month prior to carbon tetrachloride exposure in order to build up an anaerobic microbial population. A target inlet carbon tetrachloride concentration of 50 ppmv was used during testing. Moisture was added to the bed medium by injecting 40 ml of water into the column inlet, optimally, every two days. Methods of sampling and analysis are presented below.

Concentrations of carbon tetrachloride and methanol in the biofilters were measured via gas chromatographic analysis using a Hewlett-Packard 5890 Series II gas chromatograph (GC). Methanol concentrations were determined using a flame ionization detector (FID) held at 250°C, with an injection temperature of 250°C. All runs were isothermal at an oven temperature of 40°C. Biofilter inlets and outlets were sampled manually in triplicate. Sample sizes of 200 µl and 500 µl were taken from the inlet and outlet, respectively. An electron capture detector (ECD) held at 275°C with nitrogen as the make up gas and an injector temperature of 225°C was used to analyze carbon tetrachloride. Runs were isothermal at 50°C. Carbon tetrachloride inlet and outlet samples were injected into the GC via automated gas sampling valves, a volume of 250 µl was used for sampling carbon tetrachloride. Column inlet and outlet were sampled in quadruplicate and the values were averaged.

Results and Discussion

Following are results from biofiltration experiments that were performed to determine the feasibility of operating biofilters in which carbon tetrachloride was used as an alternate electron acceptor rather than the primary carbon and energy source.

Three laboratory scale biofilters were set up in order to determine the feasibility of removing carbon tetrachloride from contaminated vapor streams. As an initial starting point for these feasibility tests, three inlet methanol concentrations were tested. The inlet carbon concentration was varied, in theory, to increase the amount of protons and electrons that would be available for the reductive dechlorination process. Two biofilters were initially operated to enrich for denitrifying microorganisms by supplying nitric oxide to the bed medium microorganisms. Denitrifying conditions were selected in these two biofilters because denitrifying microorganisms had previously been demonstrated in compost based biofilters (8,9). These two biofilters were supplied with 500 and 1,000 ppmv methanol as the carbon and energy source. Both biofilters removed all of the NO which was supplied to them, but the determination

was never made whether the bed medium microorganisms were using the NO as an electron acceptor or as a nitrogen source during the growth of sulfate reducing bacteria or methanogens. At the end of the experiments run at inlet methanol concentrations of 500 and 1,000 ppmv methanol a third biofilter was set up which received 1,500 ppmv methanol as the primary carbon and energy source. During analysis of methanol, methane peaks were noted on the chromatograms for outlet samples of all three biofilters, but these peaks were not quantified. The presence of methane in samples from the biofilter outlet indicated that there was an apparent succession of redox conditions from denitrifying to methanogenic conditions in some portion of the bed.

Carbon tetrachloride removal for a biofilter that received 500 ppmv methanol as the primary carbon source can be seen in Figure 3. At a target inlet carbon tetrachloride concentration of 50 ppmv the removal efficiency of the biofilter remained at an average value of 25% for approximately 71 days. At a loading rate of approximately 7.36×10^5 µg of carbon tetrachloride•m^{-3} of bed•hr^{-1} the elimination capacity was 1.67×10^5 µg of carbon tetrachloride•m^{-3} of bed•hr^{-1}. The sudden drop in removal efficiency was attributed to the inlet line being inadvertently disconnected for an extended period of time following sampling. As stated in the "Methods" section, NO was supplied to the biofilter for the initial portion of the experiment. On day 60 the NO was turned off, while all other parameters for biofilter operation were held constant. Figure 4 shows the inlet and outlet methanol concentrations during the acclimation and carbon tetrachloride dechlorination portions of the experiment. The methanol removal efficiency ranged from 80 to 90% on average depending on the time frame of the experiment. Carbon tetrachloride was supplied to the column after 39 days of exposure to methanol under anoxic conditions. The data indicates no change in the methanol removal efficiency at three important points during the experiment at day 39 when carbon tetrachloride flow was initiated to the biofilter; on day 100 when the NO was turned off; or on day 115 when the inlet line was left disconnected.

A biofilter that received 1,000 ppmv methanol as the carbon and energy source demonstrated carbon tetrachloride removal efficiencies averaging 40% during the first 50 days of operation (Figure 5). At an average loading rate of 7.36×10^5 µg of carbon tetrachloride•m^{-3} of bed•hr^{-1} the elimination capacity was 2.83×10^5 µg of carbon tetrachloride•m^{-3} of bed•hr^{-1}. The biofilter received NO for the first 40 days of operation with carbon tetrachloride in the inlet, the NO was turned off after day 40. The cause of the decrease in carbon tetrachloride removal that started approximately 10 days after the NO was turned off is not known. One possible explanation is that the bed medium microorganisms were using the NO as a nutrient rather than an electron acceptor and when the NO supply was shut off the microorganisms growth was limited by the lack of nitrogen. Nitrogen was also supplied to the microorganisms by the MaxBac fertilizer that had been added to the bed. This particular blend of time release fertilizer has a release period of 6 to 7 months which means the fertilizer was probably still being released into the bed medium. A second explanation is that the bed medium microbes were growing under nitrate reducing conditions and when the NO was turned off the population did not have ample electron acceptor to facilitate respiration. Methanol removal during the experiment varied between 70 and 80% for the duration of the experiment, with the exception of almost complete removal at the beginning of the experiment, eventually tailing off to the above 70 to 80% (Figure 6). Carbon tetrachloride flow was initiated to the biofilter after approximately 55 days of exposure to methanol. As demonstrated by the biofilter receiving 500 ppmv methanol, removal of the primary carbon source was not affected by the addition of carbon tetrachloride.

A third biofilter that never received NO demonstrated carbon tetrachloride removal at a level midway between the two previously mentioned biofilters (Figure 7). This biofilter, that received 1,500 ppmv methanol as the carbon source, demonstrated an average removal efficiency of 30%. Carbon tetrachloride removal remained at this level for over 50 days, at the target inlet concentration of 50 ppmv. At an average carbon tetrachloride loading rate of 7.36×10^5 $\mu g \cdot m^{-3}$ of bed$\cdot hr^{-1}$ the elimination capacity was 2.04×10^5 μg of carbon tetrachloride$\cdot m^{-3}$ of bed$\cdot hr^{-1}$. Methanol removal demonstrated by the biofilter was nearly complete (> 98%) upon initial exposure and decreased to near 85 percent after 10 days of operation, remaining at that level until approximately day 76 (Figure 8). Carbon tetrachloride flow was initiated to the biofilter on day 76, at that time there appeared to be a slight drop in methanol removal. Although NO was never supplied to the biofilter nitrogen limitation was not thought to be a problem due in part to both the short time of the run and the fact that MaxBac fertilizer had been added to the biofilter.

Based on removal efficiency, the biofilter operated using 1,000 ppmv methanol in the inlet appears to be out performing the biofilters operated at 500 and 1,500 ppmv methanol as the carbon and electron source. A comparison of the mass of methanol used to the mass of carbon tetrachloride transformed per cubic meter of air treated indicates that the biofilters operating at 500 (Biofilter A) and 1,000 (Biofilter B) ppmv methanol in the inlet were performing equally well, and both were performing better than the biofilter operated with 1,500 (Biofilter C) ppmv methanol in the inlet (Figure 9). The biofilters operated at the lower inlet methanol concentrations used approximately 10 mg of methanol for every mg of carbon tetrachloride converted while the biofilter operated at 1,500 ppmv methanol used nearly 18 mg of methanol per mg of carbon tetrachloride converted. Carbon source to chlorinated removal ratios were better than those demonstrated for methanogenic bacteria transforming perchloroethylene, chloroform or 1,1,1-trichloroethane in liquid cultures using acetate as the carbon source. The ratio of acetate mass used per mass of chlorinated compound transformed in these tests varied between 100:1 and 1,000:1 (10). Methanol mass used to carbon tetrachloride transformed ratios were closer to those demonstrated by a methanogenic system that also used methanol as the primary electron donor. In this system nearly one-third of the electrons from methanol oxidation went to dechlorination of PCE (11). The only feasible explanation for the differences in carbon tetrachloride removal between Biofilters A and B compared to Biofilter C was the fact that NO was not supplied to the latter biofilter any time during operation. Related to the NO limitation there are two potential explanations; first, the lack of NO may have lead to enrichment of a different microbial population with lower activity in Biofilter C than the biofilters operated at lower methanol concentrations. The second explanation is related to the same microbial population was present in all three biofilters, but in Biofilter C the microorganisms were growing under nitrogen limitation, thereby decreasing carbon tetrachloride removal.

The liquid effluent from the bottom of the biofilters was periodically checked for pH while carbon tetrachloride was supplied to the system. Over the greater than 100 day test period the pH of this liquid remained between pH 6 and 8, values that would not be expected to be inhibitory to the anaerobic microbes present. The pH in the bed medium could potentially drop due to the liberation of chloride ion during dechlorination. Since there was loss of carbon tetrachloride for the duration of the experiment it has been determined that the calcite added to the bed was an adequate method for pH control.

Conclusions

Results from the research presented demonstrate the feasibility of using biofiltration as a treatment system for off-gases containing highly chlorinated compounds, such as carbon tetrachloride. Methanol was shown to be an adequate carbon and energy source for the anaerobic transformation of carbon tetrachloride in biofilters. The use of methanol as a carbon source in biofilters is advantageous because it can be delivered in the vapor phase and the use of the carbon and energy source by the bed medium microbes can be easily monitored. Inlet methanol concentrations of near 1,000 ppmv or less appeared to facilitate greater carbon tetrachloride removal on a mass of methanol used per mass of carbon tetrachloride transformed. A biofilter operated at an inlet methanol concentration of 1,500 ppmv required nearly twice as much methanol to remove the same amount of carbon tetrachloride as biofilters operated at 1,000 ppmv methanol or below. Further testing needs to be done to determine whether other carbon sources would yield better carbon tetrachloride removal results. Results from batch testing done with liquid systems has indicated that organic acids may yield better dechlorination than alcohols and sugars (5).

For the feasibility testing the empty bed residence time of the biofilters was set at approximately 28 minutes. This initial value was chosen due to the recalcitrance of carbon tetrachloride to biological dechlorination. When studying reductive dechlorination in liquid systems the typical time frame for batch testing is on the order of days or weeks for complete removal of the contaminant. Related to biofiltration, 28 minutes for the removal of the contaminant would lead to increased cost due to the overly large biofilter that would be required for complete removal of carbon tetrachloride. Now that the feasibility of vapor phase carbon tetrachloride removal has been shown, experiments are in progress looking at biofilters in which the empty bed residence time has been decreased an order of magnitude.

The effect of NO addition to the biofilter inlet has not been completely elucidated. As stated previously the NO may have allowed for selection of a denitrifying microbial population in the biofilters, but as the experiment proceeded a succession to a methanogenic population in at least a portion of the biofilter occurred as indicated by the presence of methane in the biofilter effluent. Since dechlorination proceeded following the removal of NO from the biofilter inlet it is thought that the bed medium microorganisms were probably using the NO as a source of nitrogen.

Over the 3 to 4 month time frame of the experiment pH did not appear to be a problem. The calcite that had been added to the bed appeared to buffer the pH of the bed medium during the operation period of the biofilter. Further research using higher loads of carbon tetrachloride as well as extended operation need to be performed to determine how long this buffering capacity lasts.
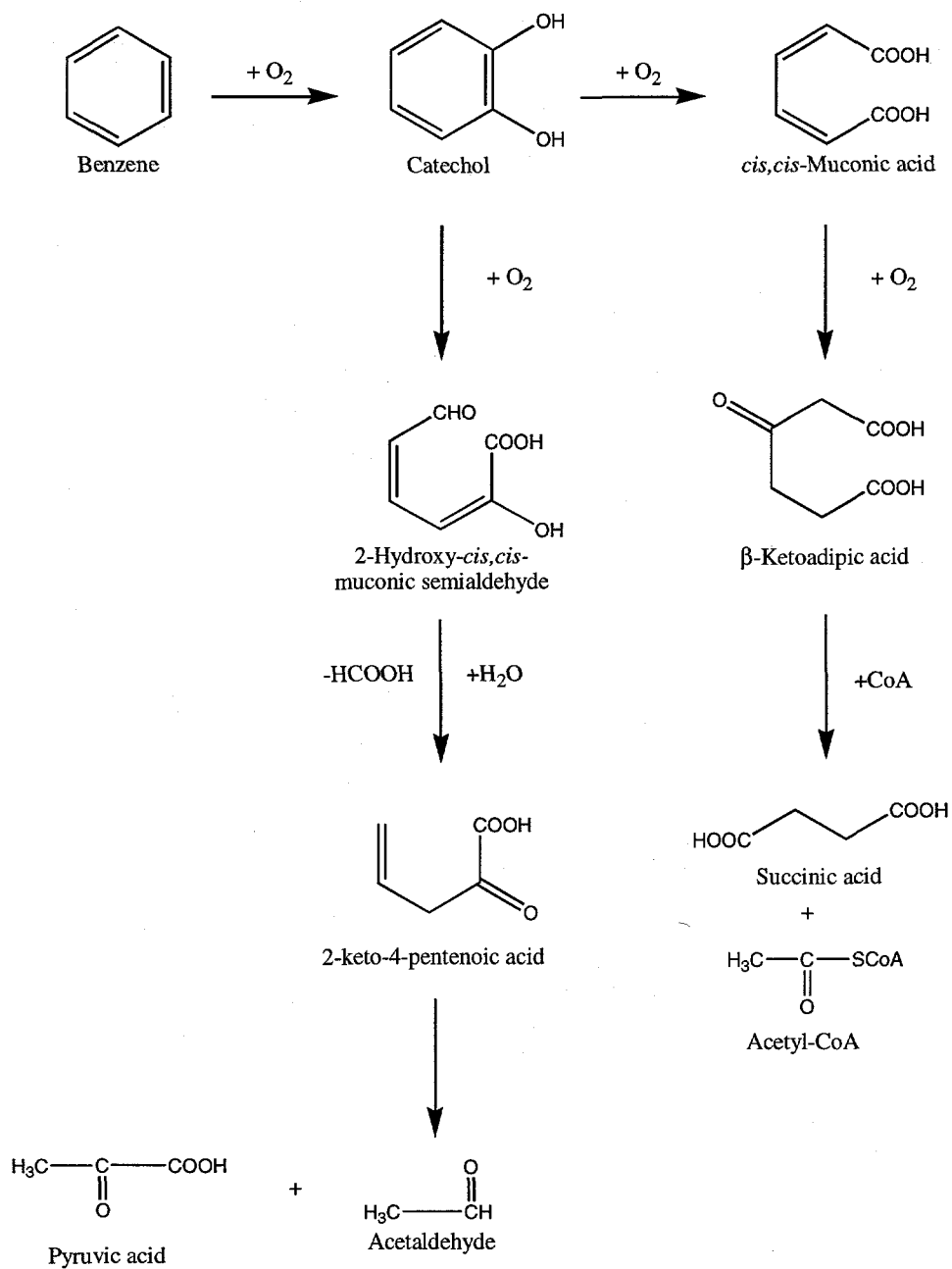
Acknowledgment

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Figure 1. Microbial metabolism of the aromatic ring of benzene by *meta* or *ortho* cleavage (1).
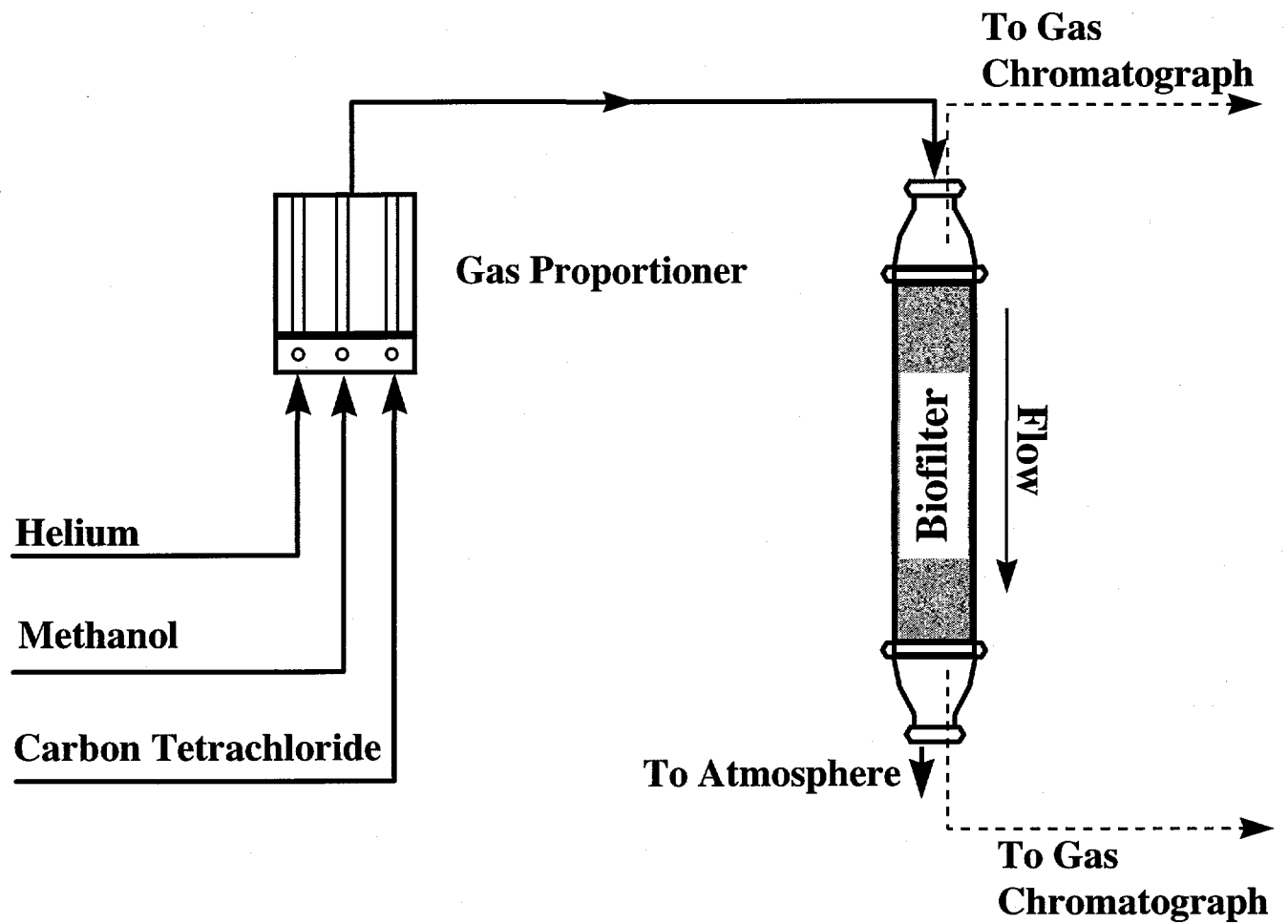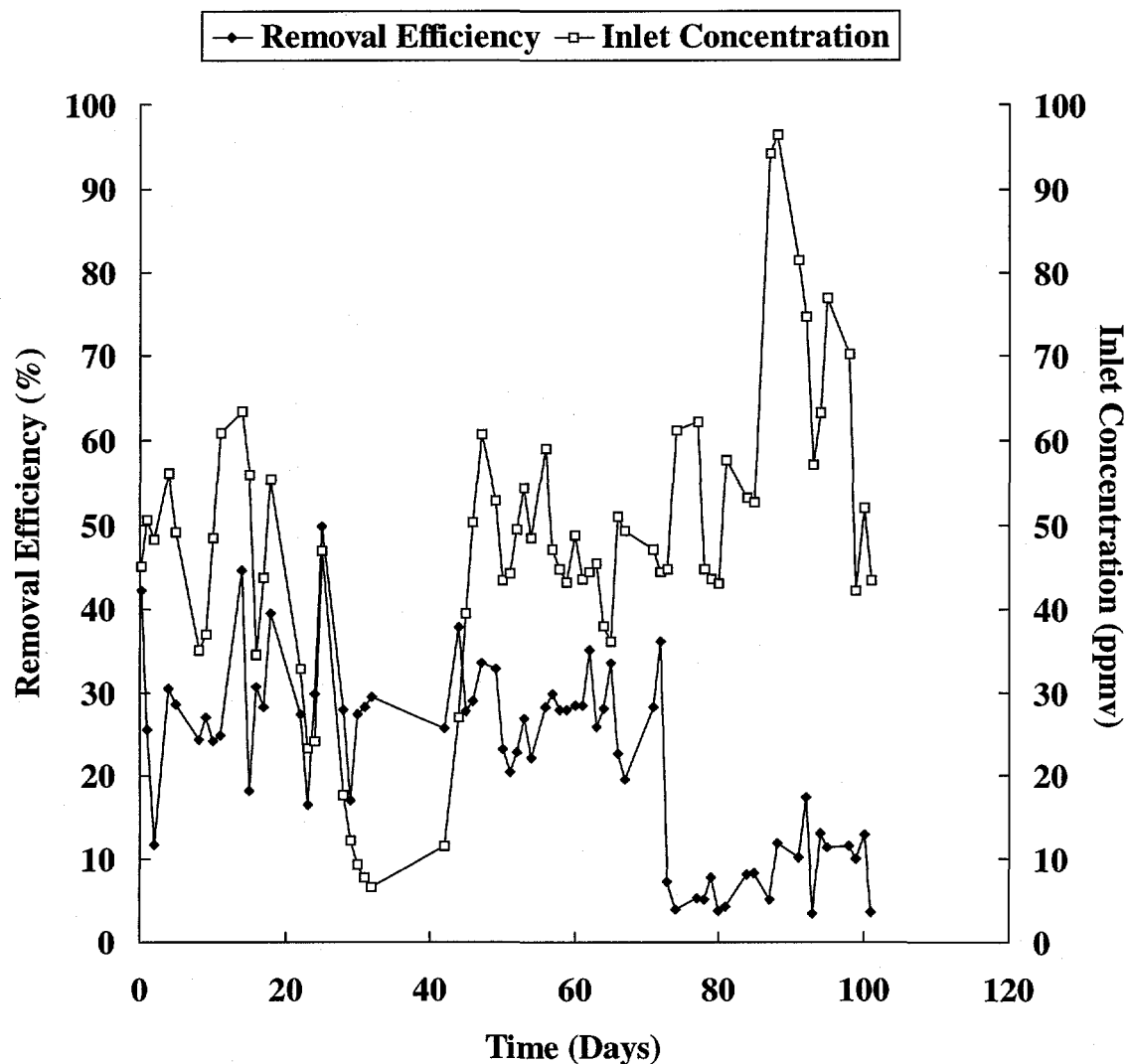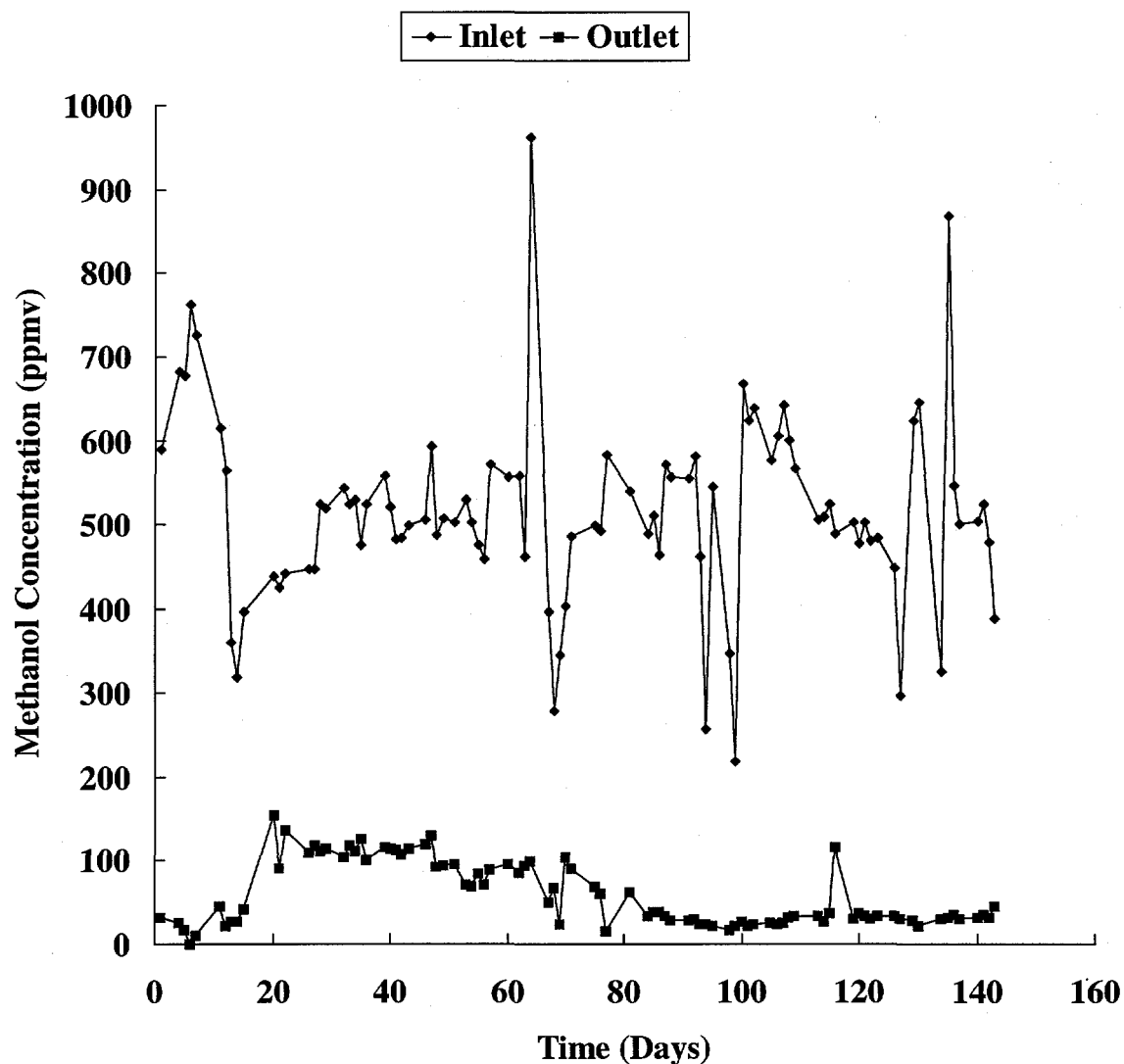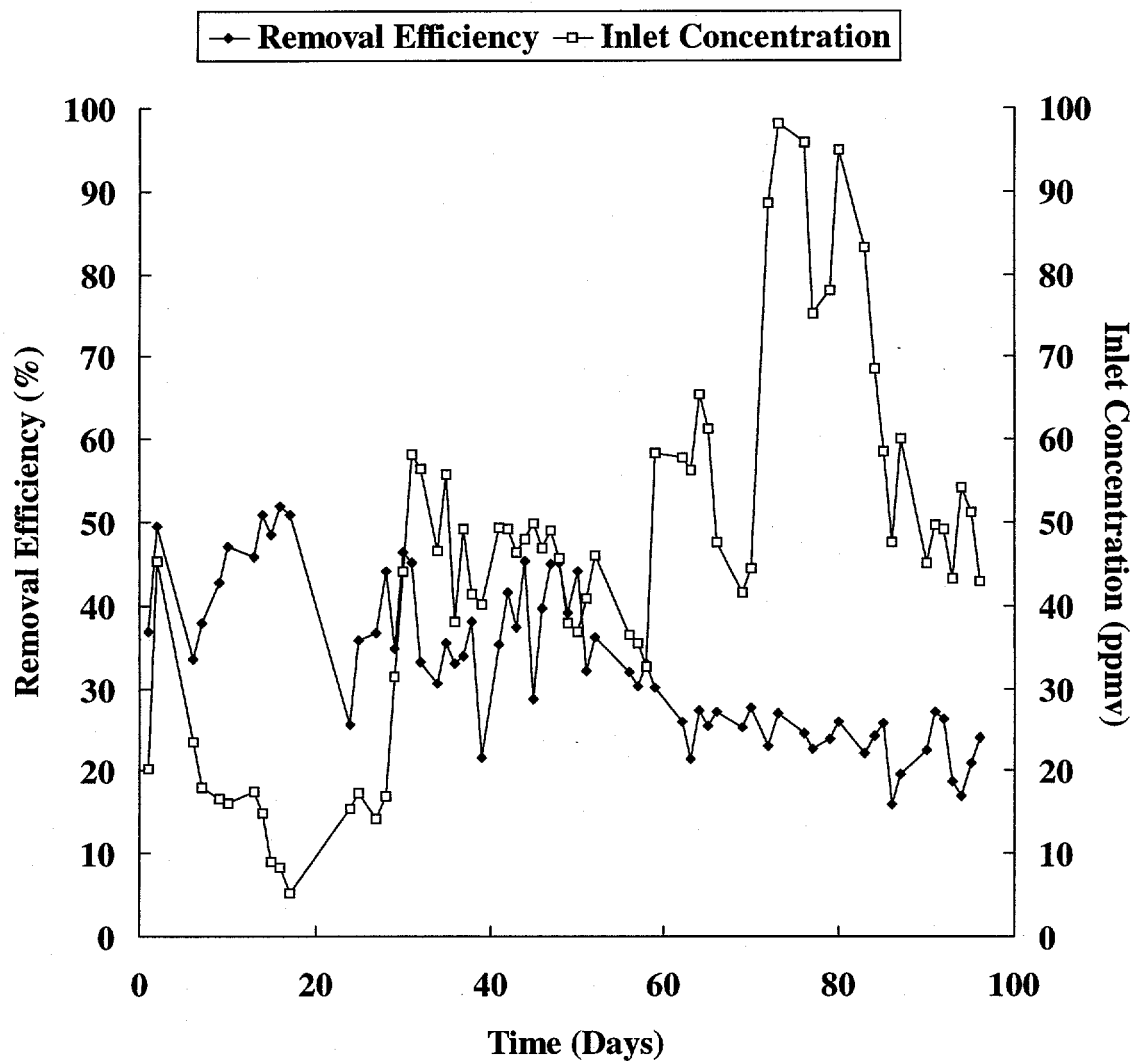
Figure 2. **General schematic of continuous-flow, compost based biofiltration system for carbon tetrachloride.**
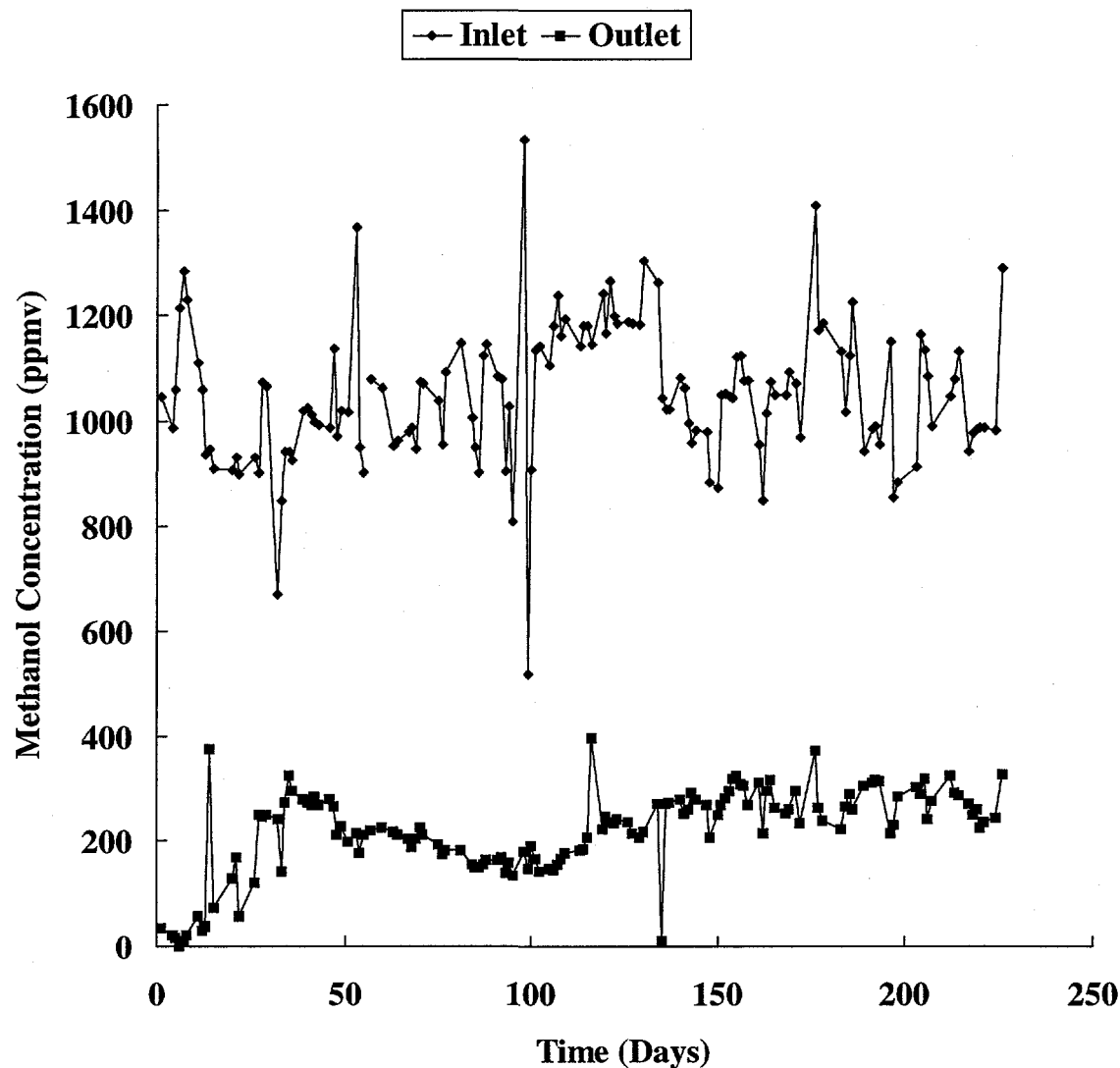
Figure 3. Inlet carbon tetrachloride concentration and carbon tetrachloride removal efficiency for a biofilter receiving 500 ppmv methanol as the primary carbon and energy source. Biofilters were operated at an empty bed residence time of 28 minutes at room temperature.

Figure 4. Methanol removal from a biofilter receiving a target inlet methanol concentration of 500 ppmv and carbon tetrachloride at a target inlet concentration of 50 ppmv. Biofilters were operated at an empty bed residence time of 28 minutes at room temperature.

Figure 5. Inlet carbon tetrachloride concentration and carbon tetrachloride removal efficiency for a biofilter receiving 1,000 ppmv methanol as the primary carbon and energy source. Biofilters were operated at an empty bed residence time of 28 minutes at room temperature.

Figure 6. Methanol removal from a biofilter receiving a target inlet methanol concentration of 1,000 ppmv and carbon tetrachloride at a target inlet concentration of 50 ppmv. Biofilters were operated at an empty bed residence time of 28 minutes at room temperature.
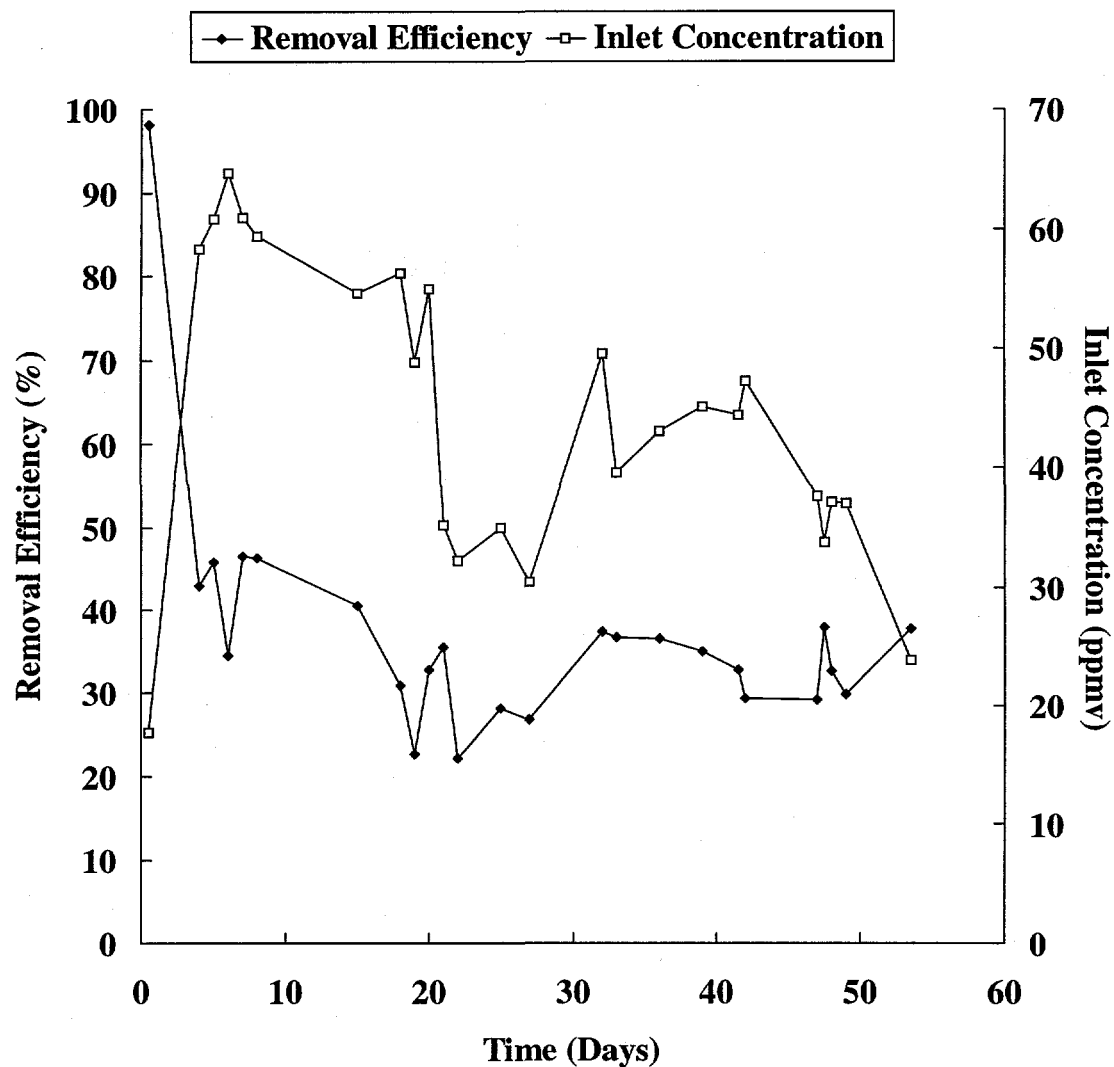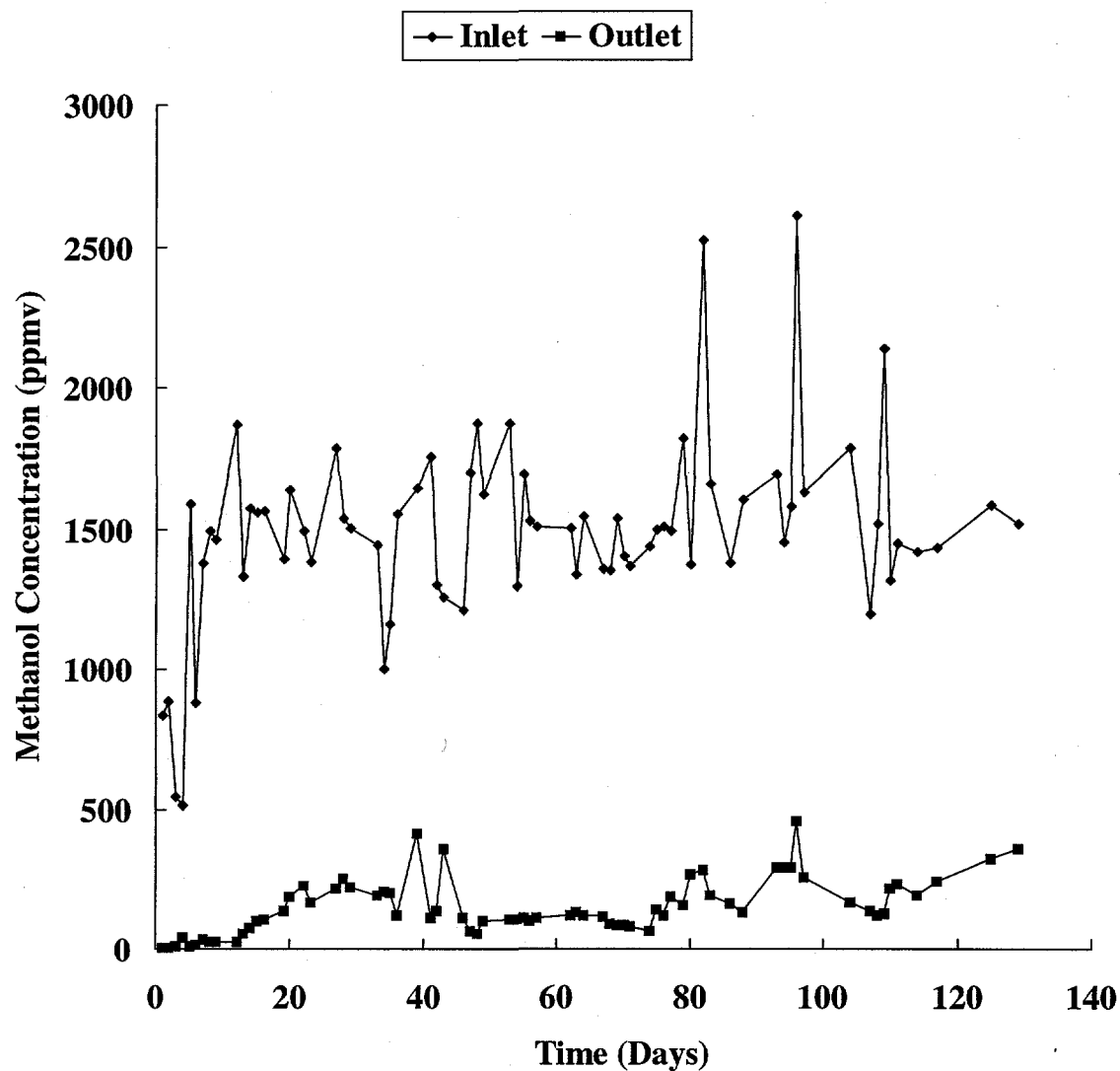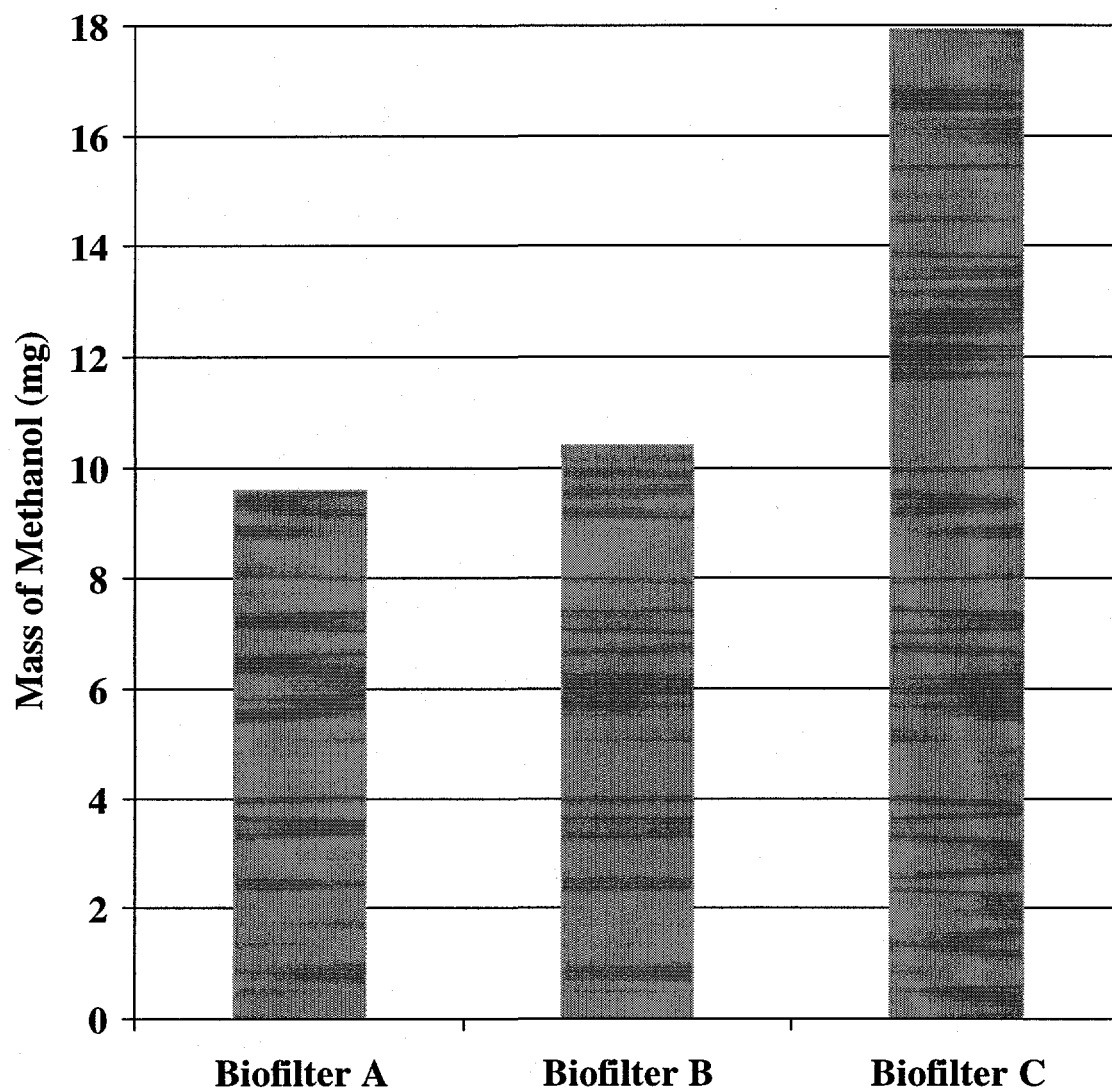
Figure 7. Inlet carbon tetrachloride concentration and carbon tetrachloride removal efficiency for a biofilter receiving 1,500 ppmv methanol as the primary carbon and energy source. Biofilters were operated at an empty bed residence time of 28 minutes at room temperature.

Figure 8. Methanol removal from a biofilter receiving a target inlet methanol concentration of 1,500 ppmv and carbon tetrachloride at a target inlet concentration of 50 ppmv. Biofilters were operated at an empty bed residence time of 28 minutes at room temperature.

Figure 9. Mass of methanol required to transform 1 mg of carbon tetrachloride for every cubic meter of air treated. Biofilter A is biofilter receiving 500 ppmv methanol. Biofilter B is biofilter receiving 1,000 ppmv methanol. Biofilter C is biofilter receiving 1,500 ppmv methanol. Biofilters were operated at an empty bed residence time of 28 minutes at room temperature.