Journal of Hazardous Substance Research

Volume 2 Article 2

1-1-1999

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**Cover Page Footnote**
Funding was provided by the U.S. Department of Energy through a subcontract between Florida A&M University and the University of Georgia - Grant/Contract #C-9594/10-21-RR176-234.

# BIOSORPTION AND TRANSFORMATION OF TETRA-CHLOROETHYLENE AND TRICHLOROETHYLENE USING MIXED-SPECIES MICROBIAL MATS

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## ABSTRACT

Microbial mats are naturally occurring assemblages of aquatic organisms capable of sorbing and degrading various organic compounds. Kinetic transformation and sorption experiments were conducted in sealed batch vials, using microbial mats dosed with unlabeled tetrachloroethylene (PCE) and trichloroethylene (TCE) and radiolabeled forms of these compounds ([$^{14}$C]PCE and [$^{14}$C]TCE). Results indicated that sorption equilibrium was attained for both chemicals within a few hours. The sorption isotherm was linear, demonstrating that the sorption mechanism was partitioning. PCE was completely degraded in periods as short as 50 days under favorable growth conditions. Samples were also tested for degradation products, with analyses indicating that PCE and TCE are transformed through both aerobic and anaerobic pathways. The ability of the microbial mats to sorb and degrade PCE and TCE suggests a potential for their application to the bioremediation of media contaminated by these compounds.

***Key words:*** *tetrachloroethylene, trichloroethylene, partitioning, biosorption, microbial mats*

## INTRODUCTION

Microbial mats are self-sustaining, photoautotrophic assemblages of mixed-aquatic microbial species, primarily consisting of cyanobacteria. Although cyanobacteria (formerly termed blue-green algae) are neither a true algae or plant, there is significant promise for the application of microbial mats to contamination problems in a manner similar to conventional phytoremediation techniques. In recent years, microbial mats have been used to successfully treat wastewater contaminated by metals and metalloids including lead, cadmium, copper, zinc, cobalt, iron, uranium, and manganese (Bender et al., 1995a). The mats have also been shown to degrade many organic compounds including trinitrotoluene, chrysene, naphthalene, hexadecane, phenanthrene, polychlorinated biphenyl, trichloroethylene, and the pesticides chlordane, carbofuran, and paraquat (Bender et al., 1995b; Kuritz and Wolk, 1994). Recent experiments have demonstrated a significant potential for microbial mats to assimilate and transform the organic compound tetrachloroethylene (PCE) and trichloroethylene (TCE) in aqueous media (O'Niell et al., 1997 and 1998).

PCE and TCE are common xenobiotic contaminants that are recalcitrant to degradation. Widespread and prolonged use of these volatile solvents in industrial applications has led to extensive contamination of soils, groundwater, and surface waters (Riley and Zachara, 1992; Westrick et al., 1984). *In situ* remediation technologies (e.g., natural attenuation, phytoremediation, biodegradation, reactive subsurface barriers, etc.) presently used for the removal of PCE and TCE from contaminated sites may require many decades to achieve acceptable treatment goals (Newman et al., 1997). Thus, there is a need to develop more efficient, cost-effective, and low maintenance

methods for remediation. Our investigations have focused on the use of microbial mats as a possible PCE/TCE remediation alternative that would meet these requirements.

The objectives of this study were to investigate the sorption and transformation of PCE and TCE by both living and dried microbial mats. Kinetic batch experiments were conducted to determine when sorption equilibrium was attained, and to evaluate the capacity of the mats to degrade PCE and TCE. Sorption batch experiments were conducted to calculate PCE and TCE sorption coefficients for both living and dried mats. Results of the experiments were confirmed in replicate experiments using $^{14}C$-labeled PCE and TCE. The TCE sorption results were compared to TCE sorption data available for other media. Samples of the mat and/or liquid phases were analyzed for various daughter products to determine possible PCE and TCE degradation products and pathways.

## MATERIALS AND METHODS

### *Microbial mats*

The mats used in the sorption and transformation experiments were grown in-house from inocula provided by Dr. Judith Bender and Dr. Peter Phillips of Clark-Atlanta University, using a patented technique developed in their laboratory (U.S. Patent Nos. 5,522,985 and 5,614,097). Microbial mats can be readily constructed in a laboratory environment to produce a unique symbiotic community that mimics those found in natural ecosystems. The constructed mats were grown on a substrate of ensilaged grass clippings in Allen-Arnon liquid growth media and are held together by slimy secretions from the various microbial components. The mats have few requirements for growth and maintenance and are thus capable of surviving and flourishing in even very harsh environments (Bender et al., 1995a).

Like mats in natural habitats, constructed microbial mats stratify into aerobic and anaerobic zones. The aerobic upper layer of the constructed mat is composed primarily of cyanobacteria (e.g., *Oscillatoria*, *Nostoc*, and *Anabaena* spp.), which overlies an anaerobic lower substrate of silage and various bacteria (e.g., *Pseudomonas* spp., *Flavobacterium* spp., sulfur-reducing bacteria, purple autotrophic bacteria) (Bender et al., 1995a). The near-surface layers of the mat are photosynthetic and can degrade halogenated compounds through aerobic pathways, while bacteria in the lower layers can biodegrade these compounds through reductive dehalogenation (Bender et al., 1995b; Nzengung et al., 1998).

### *Reagents/chemicals*

PCE and TCE were obtained from Aldrich Chemical Company, Milwaukee, Wis. Analytical grade methanol from the Fisher Scientific Co., Pittsburgh, Penn., was the solvent used to prepare stock solutions of PCE and TCE. HPLC-grade hexane, also from the Fisher Scientific Co., and methyl-tert butyl ether (MTBE) from Aldrich Chemical Co., were used for extraction of PCE, TCE, and their metabolites from both liquid and solid phases of samples. All chemicals were greater than



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DOI: 10.4148/1090-7025.1012

99% purity, as confirmed by analysis using a gas chromatograph/ mass spectrometer (GC/MS). Chemicals were used as obtained without further purification. A diazomethane/MTBE mixture used to derivatize polar-chlorinated products (chloroethanols and chloroacetic acids) was prepared in-house using a diazomethane-generation apparatus from Aldrich. The diazomethane/MTBE mixture was stored in sealed vials at -4°C until use, or for a maximum of 14 days. The Allen/Arnon growth media providing nutrients to the mats were prepared in-house using deionized water and other commercially available chemicals.

***Transformation experiments***

Kinetic experiments were conducted to evaluate transformation of PCE and TCE by the microbial mats and to determine when sorption equilibrium was attained. Batch samples of living mat (2, 8, or 12 g wet weight) and dried mat (1 g) were placed in 20-ml glass vials filled with approximately 12 to 21 ml of deionized water. The mat mass/liquid volume ratio was kept constant for each mat mass tested. At least five replicates and controls were prepared for each experiment. The headspace in the vials was minimized, and each vial was dosed with PCE or TCE to obtain initial solution concentrations of 2, 4, or 8 mg/l. The vials were sealed with Teflon-faced screw-top septa. The first sample was sacrificed for analysis within 15 minutes of dosing. All other samples were maintained in either a 12/12 hour light/dark environment at 25 ± 2°C and analyzed at intervals of one to seven days, depending on the solution concentration of the most recently analyzed sample. Several artificial light intensities (15 to 120 μmols $s^{-1}$ $m^{-2}$) were tested during these experiments.

Hexane was used to extract PCE, TCE, and their metabolites from the liquid and solid (mat) phases of each sample. Prior to extraction, centrifuging the sample at 2500 rpm for 10 minutes separated the liquid and solid phases of the samples. An aliquot (1 ml to 6 ml) of the liquid phase was removed from each sample vial, added to a second vial containing hexane (6 ml to 12 ml), and agitated for one minute. The liquid in the second vial was then centrifuged at 2500 rpm for five minutes to enhance separation of the hexane and aqueous phases. Aliquots of the hexane were collected and analyzed for PCE and TCE using a gas chromatograph with an electron capture detector (GC/ECD).

After decanting the remaining liquid from the sample vials, PCE, TCE, and their metabolites were extracted from the solid phase. To enhance extraction, 1 ml of methanol and 6 or 12 ml of hexane were added to each vial, and the vials were sonicated for 30 minutes. The vials were then centrifuged at 2500 rpm for five minutes to separate the hexane phase from the mat. Aliquots of hexane were removed from the vials using a gas-tight syringe and analyzed using a GC/ECD. The mat in the sample vials was then subjected to a second extraction procedure using the technique described above. During the second mat-phase extraction, samples were sonicated for 60 minutes and allowed to equilibrate for 24 hours before the hexane was removed from the vials for analysis.

Two extractions of the mat phase were sufficient to remove 90+ % of recoverable PCE or TCE from the mats. All experiments were replicated.

### *Sorption experiments*

Batch sorption experiments were conducted using both unlabeled and radiolabeled PCE and TCE to dose living and dried microbial mats. The living mats were placed on absorbent tissues to remove excess moisture immediately prior to weighing. The water content of the living mats was calculated at approximately 90%. Dried mat samples were prepared for sorption experiments by air drying the living mat at 30°C for 48 hours and manually grinding the dried material into coarse granules.

Samples of the living mat (8 g wet weight) and dried mat (0.5, 1, 1.5, and 2 g) were weighed into 20-ml glass vials. Five vials were prepared for each mat mass, filled with deionized water to minimize headspace as much as possible and dosed with PCE or TCE at liquid-phase concentrations of 1, 2, 4, 7, and 10 mg/l. Five control samples were prepared using 20-ml glass vials filled with deionized water and dosed with PCE or TCE at the specified solution concentrations. Vials were sealed using Teflon-faced septa, placed on a mechanical shaker, and allowed to reach equilibrium at an ambient temperature 25°C ± 2°C. Samples dosed with PCE were analyzed after six hours, and those dosed with TCE were analyzed after 12 hours. PCE and TCE were extracted from the liquid and mat phases using the procedure described above. The concentrations of these compounds were analyzed using a GC/ECD. All experiments were replicated. The resulting data were used to prepare sorption isotherms for the various masses of living and dried mat.

Four sorption experiments were conducted using $^{14}C$-labeled PCE and TCE from Sigma Chemical Co., St. Louis, Mo., to replicate and confirm previous results. The [$^{14}C$]PCE and [$^{14}C$]TCE experiments generally provide better sensitivity and accuracy than GC analysis of unlabeled PCE and TCE (Nzengung et al., 1996 and 1997). Sorption batch experiments were set up using techniques similar to those previously described. Each sample vial was dosed with a PCE or TCE tracer having an initial specific activity of approximately 20,000 disintegrations/minute (dpm) in 1 ml of liquid.

After a 24-hour equilibration period, the vials were centrifuged for 10 minutes at 2500 rpm. One ml of liquid was removed from each vial and added to a separate vial containing 14 ml of ScintiSafe 30% scintillation cocktail from Fisher Scientific Co. The sample and cocktail mixture was agitated for one minute and analyzed on a Beckman 5801 liquid scintillation (LS) counter. The LS data were used to quantify the PCE concentrations in the liquid phases and to prepare sorption isotherms. Similar PCE and TCE sorption kinetic experiments were conducted using 0.25 g of silage.

### *Analysis of transformation products*

Headspace was created in the sample vials after removal of 6-ml of liquid for PCE and TCE analyses. Vapor-phase samples were collected from this headspace and analyzed for transformation products such as dichloroethylene, vinyl chloride, ethene, and ethane. One-ml samples were



http://newprairiepress.org/jhsr/vol2/iss1/2
DOI: 10.4148/1090-7025.1012

withdrawn from the headspace using a gas-tight syringe and were analyzed without further preparation on the GC/FID.

Liquid-phase samples were tested for polar ionizable transformation products using GC/ECD. These samples were derivitized using diazomethane (in MTBE) prior to injection into the GC/ECD. Derivitization was accomplished by first placing 6 ml of liquid phase sample in a vial. Three drops of sulfuric acid (98.08%) were added, and the vial was slowly agitated for one minute. Six ml of MTBE were then added to the vial. The acidified liquid and MTBE were manually agitated for one minute and then centrifuged at 2500 rpm for five minutes to enhance separation of the MTBE and aqueous phases. Five ml of the MTBE phase were removed from the vial and placed in a second vial. The second vial was placed in an ice-water bath, and the MTBE mixture was derivitized by adding 0.5 ml of diazomethane (in MTBE). The mixture was chilled for a minimum of 20 minutes after the addition of the diazomethane and was then analyzed using a GC/ECD. Chloroacetic acid standards and blanks of neat MTBE were treated using the derivitization procedure and analyzed by GC/ECD.

***Gas chromatography***

A Shimadzu GC-14A gas chromatograph equipped with an electron-capture detector and a Shimadzu CR501 Chromatopac integrator were used for quantitative and qualitative analysis of the PCE, TCE, and chloroacetic acids in liquid- and solid-phase samples. All analyses were by direct splitless injection of 0.5 to 1 µl of hexane or MTBE sample extract using a Shimadzu AOC 17 auto injector and AOC 1400 automatic sampler. The separation column used in the analyses was a DB-5 megabore * 0.53 mm * 1.5 µm (methylpolysiloxane phase) manufactured by J.W. Scientific, Folsom, Calif. Column lengths of 30 m and 15 m were used during the analyses. For the 30-m length, the column temperature was programmed at 35°C for two minutes, followed by a temperature increase of 5°C/min to 90°C, and a subsequent temperature increase of 35°C/min to 160°C for two minutes. Nitrogen was used as the carrier gas with a combined flow rate of 40 ml/min. For the 15-m length, the column temperature was programmed at 35°C for six minutes, followed by a temperature increase of 5°C/min to 90°C, and a subsequent temperature increase at 35°C/min. to 160°C for two minutes. Nitrogen was used as the carrier gas with an in-column flow rate of 10 ml/min. For both columns, the injector and detector temperatures were 200°C and 340°C, respectively. Methanol solutions containing 1 mg/l dichlorobenzene were analyzed as external standards. PCE and TCE standards prepared in-house were used to construct calibration curves each week to ensure accurate quantitative results.

A Hewlett-Packard 5890 gas chromatograph with a flame-ionization detector (GC/FID) was used to analyze vapor-phase samples for dichloroethylene, vinyl chloride, ethene, and ethane. All analyses were by direct splitless injection of 1 ml of vapor sample collected from the headspace of the batch vials following removal of the liquid phase. A 30-m DB-VRX narrowbore column * 0.25

mm * 1.4 µm was used for these analyses. The column temperature was programmed at 30°C for five minutes, followed by a temperature increase of 5°C/min to 60°C for one minute, and a subsequent temperature increase at 25°C/min to 200°C for 10 minutes. Helium was used as the carrier gas with a flow rate of 2 ml/min, and air and hydrogen were used to produce the flame in the detector. The injector and detector temperatures were 200°C and 300°C, respectively.

### *Data analysis*

The direct and difference methods were used to calculate the concentrations of PCE or TCE that were sorbed to the mat. Sd (mg/Kg) is defined as the sorbed concentration of a compound that was calculated using the direct method, and Se (mg/Kg) is the sorbed concentration calculated using the difference method. For the direct method, the actual concentration measured by extracting either PCE or TCE from the mat phase was used to calculate the concentration of solute per gram of mat. The difference method data were calculated by subtracting the final liquid-phase concentrations (Ce in mg/l) from the initial concentrations ($C_0$ in mg/l) and attributing this difference to sorption. Both Sd and Se were calculated for the unlabeled PCE and TCE experiments, but only Se was calculated for the [$^{14}$C]PCE and [$^{14}$C]TCE sorption experiments. Regression analyses of the final liquid-phase concentrations (Ce) versus the sorbed concentrations (Sd and Se) were used to determine the partitioning coefficient (Kd in ml/g) between the mats and aqueous media.

The statistical analysis software package SAS was used to conduct an analysis of variance for the PCE and TCE sorption data. The level of significance (p-value) was calculated and used to determine the probability that mat mass affected the values of the sorption coefficients (Kd). The interpretation of the statistical results was based on the assumption that the null hypothesis—that mat mass did not affect sorption response for the concentrations ranges tested—could be rejected if the p-values were less than 0.05. A regression analysis was also conducted to determine upper and lower 95 percent prediction bands for the PCE and TCE sorption isotherms (Ott, 1993).

## RESULTS AND DISCUSSION

### *Sorption and transformation kinetics*

The kinetic experiments indicated very rapid removal of PCE and TCE from the liquid phase within 15 minutes of dosing. The rapid decrease was attributed to sorption and did not change significantly within a 12-hour period (Figure 1). These data are consistent with previous experiments, which have shown that organic compounds partition to algal material in a few minutes and partition to bacteria in a few hours (Johnson and Kennedy, 1973; Grimes and Morrison, 1975; Matter-Müller et al., 1980; Harding and Phillips, 1978; Baughman and Paris, 1981; McRae, 1985; Smets and Rittmann, 1990).

Degradation of the parent compounds was also considered when choosing the equilibration periods because longer periods might result in losses through transformation. In samples dosed with



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DOI: 10.4148/1090-7025.1012

PCE, TCE typically appeared in the liquid- and mat-phase of the samples, generally within 12 hours to one week (Figure 2). TCE is more soluble and less susceptible to biodegradation and was allowed to equilibrate for a longer period. Therefore, equilibration periods were limited to six hours for PCE and 12 hours for TCE.

The time required to completely remove PCE from the liquid phase varied from 50 to 628 days (Table 1). The mat-phase PCE was also completely removed within this period (Figure 2). The wide variability in time is due in part to the natural heterogeneity of the mats, but can also be attributed to the varying light conditions to which the vials were exposed. The general trend observed was that increase in light resulted in a healthier mat (no putrefaction of the mat) along with faster PCE and TCE degradation.

Mat mass also affected the degradation rate of PCE and TCE within the concentration ranges used in these experiments. Because of the vial size chosen for the experiments, smaller masses of mat (e.g. 4 g) had more surface area exposed to light than larger mat masses (8 and 12 g). Consequently, mat in the 12 g samples began to die and putrefy as the experiment proceeded and degradation rates slowed. The 4 g samples remained healthy throughout the experiment and degraded the compounds fastest. Degradation rates calculated for the 8 g samples were between those of the 4 g samples and 12 g samples. Analysis of the control samples indicated that photodegradation did not affect the PCE and TCE concentrations in the vials. These results were replicated using [$^{14}$C]PCE and [$^{14}$C]TCE as tracer compounds, and similar results were observed.

***Transformation products studies***

The appearance of TCE in the sample dosed with PCE verifies that the PCE was being dehalogenated and is indicative of reductive dehalogenation, an anaerobic transformation pathway. Aerobic transformation pathways were identified as well. Dichloroacetic and trichloroacetic acid, transformation products indicative of oxidative transformation, were identified in batch samples exposed to higher levels of artificial light. Aerobic degradation products were not identified in samples maintained in low-light conditions (less than 120 mmols $s^{-1}$ $m^{-2}$). The radiolabeled studies indicated that a significant fraction (7%) of the parent compound or metabolites was irreversibly bound to the mat as indicated by the identification of the radio-labeled compounds in dried samples of previously extracted mat.

Degradation of PCE through a strictly anaerobic pathway may produce more hazardous compounds such as TCE, dichloroethylene (DCE), and vinyl chloride (VC). Degradation through multiple pathways may result in production of more innocuous metabolites such as acetic acid and carbon dioxide, while DCE and VC are either not produced or are rapidly aerobically degraded. A detailed investigation of the metabolic pathways for PCE and TCE by mats grown under different light intensities is presented elsewhere (O'Niell and Nzengung, 1999).

***Sorption isotherms***

All sorption isotherms were linear within the specified concentrations, as demonstrated by the correlation coefficients ($R^2$ values) ranging from 0.95 to 0.99 (Tables 2 and 3). The Linear Isotherm Models described the sorption data presented in Tables 2 and 3:

$$Kd = \frac{Se}{Ce} \text{(difference method) or } Kd = \frac{Sd}{Cd} \text{(direct method)}$$

The linearity of the data indicates that sorption of PCE and TCE from the liquid phase to the mat phase in the batch samples is a partitioning phenomenon. The values of Kd were normalized to the organic carbon content of the mat using the following formula:

$$Koc = \frac{Kd}{foc}$$

Where: Koc = Sorption coefficient normalized to organic carbon content, foc = Fraction organic carbon content of the mat.

For the living mat, an average partition coefficient of 6.63 ± 0.30 (SE) ml/g (wet weight mass) was determined for PCE while the average partition coefficient for TCE was calculated at 2.34 ± 0.59 (SE) ml/g. Because mat is composed of approximately 90% water, a more direct comparison of living and dry sorption responses is accomplished by comparing the sorption coefficients of living mat (5g wet weight) to the dry mat mass of 0.5 g. The PCE partition coefficient for 0.5 g of dry mat varied from 52.6 ± 5.9 to 73.1 ± 4.6 (SE) ml/g, and the TCE partition coefficient for 0.5 g of dry mat varied from 12.8 ± 1.4 to 23.9 ± 3.3 (SE) ml/g. The average partition coefficient of PCE to dried mat was 58.2 ± 3.29 ml/g and 16.2 ± 1.50 (SE) ml/g for TCE (Tables 2 and 3). The difference between the partitioning coefficients of PCE and TCE is attributed to the greater aqueous solubility of TCE. The partition coefficients for dried mat are an order of magnitude greater than living mat (Figures 3 and 4), which is consistent with the trends observed for sorption of organic compounds to wet and dry soils (Chiou, 1990). The dry mat was ground into smaller particles before each experiment. As a result, for the same dry weight, the ground dried mat provided a greater surface area for adsorption than the wet living mat. Also, the unhydrated mat presented a more hydrophobic surface for partitioning of PCE, TCE, and their hydrophobic metabolites.

A linear regression analysis was used to calculate a 95% prediction interval for the sorption coefficients of dried mat. The prediction interval is constructed about variables rather than parameters, and is used to predict the value of random variables with a 95% certainty (Ott, 1993). The dried mat sorption data fits within these prediction bands for both PCE and TCE (Figures 5 and 6).

An analysis of variance was conducted to analyze if the population means of the PCE and TCE partition coefficients remained equal as mat mass varied. The p-values calculated for the PCE data (0.15 for the direct method and 0.44 for the difference method) are greater than 0.05, indicating that the mean values of Kd calculated for the four masses are not statistically different. Similar



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DOI: 10.4148/1090-7025.1012

results were obtained for TCE, with p-values of 0.14 for the direct method and 0.67 for the difference method. Therefore, the partitioning of PCE and TCE between the mat phase and water is not dependent on mat mass for the concentration ranges and mat masses specified.

The [$^{14}$C]PCE and [$^{14}$C]TCE sorption experiments yielded similar results. Average sorption coefficients of 57.3 ± 4.66 ml/g and 13.8 ± 0.99 ml/g were calculated for sorption of PCE and TCE to dried mat, respectively (Tables 2 and 3). The sorption isotherms were also linear for the concentration range of 1 to 10 mg/l. PCE and TCE also sorbed to the silage with sorption coefficients of 41.3 ml/g and 12.9 ml/g, respectively. Because the partition coefficients for silage were similar to those for dried mats, and silage only accounted for approximately 1% or less of total mat mass, the sorption of PCE and TCE to the mats was not significantly affected by the silage substrate.

Because published values of partitioning data for PCE are scarce, the sorption data calculated for the mats were compared to published TCE sorption data for other media including soils, humins, peat moss, granulated activated carbon, algae, and other substances (Table 4). Dried microbial mat had a lower value for Koc than the other materials listed in the literature, but this comparison ignores several advantages of the mat. Mat can be dried for easy transport and will begin growing upon rehydration; it is a self-replenishing resource that is easy to produce and can withstand very harsh environmental conditions which may facilitate cost-effective application of this material as a remedial biosorbent. The primary advantage of the microbial mats is the ability to degrade PCE and TCE through multiple pathways, resulting in the mineralization of the parent compound rather than a simple phase transfer. Commercial production of the mats would utilize natural nutrients and solar energy to minimize production costs.

Desorption of PCE and TCE from the mats was not studied extensively. Two treatments with stringent solvents and sonication for 30 to 60 minutes are required to extract the sorbed PCE and TCE from the mats. This fact, along with evidence of continued degradation of PCE and TCE, suggests that desorption of these compounds may not be significant in a natural environment.

## CONCLUSIONS

PCE and TCE partitioned rapidly to the microbial mats, with equilibration occurring in less than 24 hours. A slower transformation and degradation of both compounds followed this rapid partitioning to the mat phase. Metabolites of PCE detected in the samples included TCE, dichloroacetic acid, and trichloroacetic acid. Despite repeated analyses, dichloroethylene and vinyl chloride were not detected in any of the samples. These results indicate that the compounds were being degraded through two different pathways, reductive dehalogenation and oxidative transformation. The close proximity of aerobic and anaerobic zones within the mat may enhance degradation through these alternate routes.

Light intensity was observed to affect mat health and influence the degradation mechanism. Mat mass also affected the degradation rates of these compounds. As mat mass increased, degra-

dation rates decreased. This trend was attributed to mass transfer limitations in the vials and declining mat health as mass increased due to the lower availability of light at higher masses.

The ability to degrade these organic compounds through aerobic and anaerobic pathways in as little as 50 days under favorable growth conditions, in addition to a capability for sequestering metals in the waste stream, provides a unique advantage over other media that are able to sorb PCE and TCE from water. The minimal growth requirements and the ability of the microbial mats to survive and grow in harsh aquatic environments are additional features that enhance the potential for their use as a cost-effective means of remediating surface water contaminated by PCE and TCE, or in a bioreactor for the treatment of waste effluents.

## ACKNOWLEDGEMENTS

Funding was provided by the U.S. Department of Energy through a subcontract between Florida A&M University and the University of Georgia - Grant/Contract #C-9594/10-21-RR176-234.

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**Table 1.** Time needed for the complete removal of PCE from liquid phase by microbial mats grown under different light intensities.

| Concentration | Mass (wet) 4 g | Mass (wet) 8 g | Mass (wet) 12 g |
|---|---|---|---|
| 2 mg/L | 68.7 days (LL)<br>144.5 days (VLL) | 176.7 days (LL)<br>179.7 days (VLL) | 173.5 days (LL)<br>628.1 days (VLL) |
| 4 mg/L | | 65.8 days (ML)<br>254.4days (VLL) | |
| 8 mg/L | | 49.8 days (ML)<br>230.7 days (VLL) | |

VLL — very low intensity artificial light (15.2 µmols $s^{-1}$ $m^{-2}$), LL — low intensity artificial light (71.7 µmols $s^{-1}$ $m^{-2}$), ML—medium intensity artificial light (120 µmols $s^{-1}$ $m^{-2}$)

**Table 2.** Summary results for batch sorption experiments – microbial mat dosed with PCE.

| Material | Mass (g) | Kd (ml/g) Difference Method | $R^2$ | Kd (ml/g) Direct Method | $R^2$ | Kd (ml/g) Average |
|---|---|---|---|---|---|---|
| Living Mat | 5.0 | 6.33 ± 0.30 | 0.97 | 6.93 ± 0.30 | 0.99 | 6.63 ± 0.30 |
| Dried Mat | 0.5<br>1.0<br>1.5<br>2.0 | 73.08 ± 4.61<br>49.57 ± 0.85<br>46.10 ± 4.22<br>62.84 ± 5.31 | 0.97<br>0.99<br>0.95<br>0.96 | 67.40 ± 3.88<br>58.64 ± 1.47<br>50.47 ± 8.26<br>57.58 ± 0.01 | 0.99<br>0.99<br>0.99<br>0.97 | 58.2 ± 3.29 |
| Dried Mat using[$^{14}$C]PCE | 0.5<br>2.0 | 52.64 ± 5.91<br>61.96 ± 3.40 | 0.99<br>0.98 | -<br>- | -<br>- | 57.3 ± 4.66 |
| Silage [$^{14}$C]PCE | 0.25 | 41.3 | 0.99 | - | - | 41.3 |

± Standard error



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DOI: 10.4148/1090-7025.1012

**Table 3.** Summary results for batch sorption experiments – microbial mat dosed with TCE.

| Material | Mass (g) | Kd (ml/g) Difference Method | $R^2$ | Kd (ml/g) Direct Method | $R^2$ | Kd (ml/g) Average |
|---|---|---|---|---|---|---|
| Living Mat | 5.0 | 2.93 ± 0.60 | 0.99 | 1.75 ± 0.60 | 0.98 | 2.34 ± 0.59 |
| Dried Mat | 0.5<br>1.0<br>1.5<br>2.0 | 15.57 ± 1.40<br>11.44 ± 0.53<br>18.22 ± 3.68<br>13.97 ± 0.22 | 0.99<br>0.99<br>0.99<br>0.99 | 23.86 ± 3.33<br>18.63 ± 1.36<br>10.86 ± 3.68<br>17.32 ± 0.92 | 0.99<br>0.99<br>0.97<br>0.99 | 16.2 ± 1.50 |
| Dried Mat using [$^{14C}$C] TCE | 0.5<br>2.0 | 12.78 ± 1.40<br>14.77 ± 1.01 | 0.99<br>0.99 | -<br>- | -<br>- | 13.8 ± 0.99 |
| Silage [$^{14}$C]TCE | 0.25 | 12.9 | 0.99 | - | - | 12.9 |

± Standard error

**Table 4.** Comparison of partition coefficients of TCE for different media (sorbents).

| Medium | Kd (ml/g) | foc | Koc (ml/g) | Concentration Range (mg/l) |
|---|---|---|---|---|
| Wet Microbial Mat | 2.34 ± 34 | 1 | 2.34 | 1.0 to 10 |
| Dry Microbial Mat | 16.2 ± 1.50 | 1 | 16.2 | 1.0 to 10 |
| Humin [a] | 1.28 | 0.0088 | 145 | ≈ 2 |
| Oxidized Humin [a] | 0.4 | 0.0014 | 287 | ≈ 2 |
| Fats, Waxes, Resins [a] | 348 | 0.833 | 460 | ≈ 2 |
| Algae [b] | 1400-5370 | 1 | 1440-5370 | 0.04-4.4 |
| Humic Coated Alumina [c] | 0.308 | 0.0045 | 68.4 | - |
| Sediment (< 1 mm) [c] | 0.12 (0.11-0.13) | 0.0019 | 84.2 | - |
| Sandy Loam Soil [d] | 0.5 | 0.001 | 50 | 175 - 875 |
| Organic Topsoil [d] | 13.5 | 0.117 | 115 | 175 - 875 |
| Peat Moss [d] | 93.4 | 0.494 | 189 | 175 - 875 |
| Granular Activated Carbon [d] | 81076 | 1 | 81076 | 175 - 875 |

[a] Garbarini and Lion (1986); [b]Smets and Rittmann (1990), [c]Allen-King et al. (1996); [d] Zytner (1992)

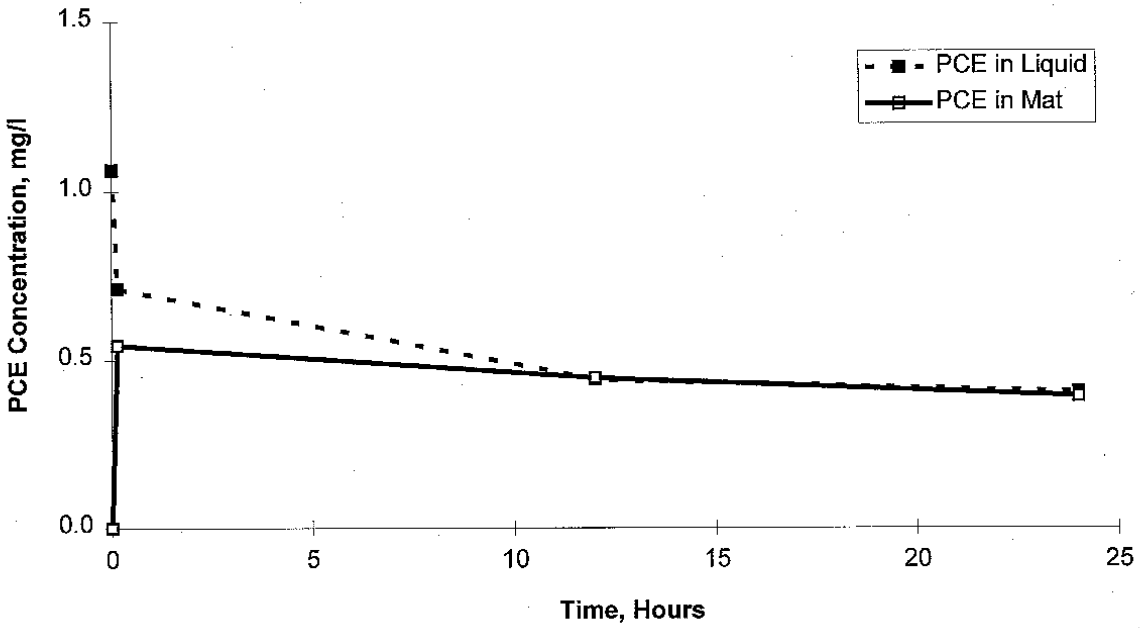


**Figure 1.** Liquid and solid (mat) phase concentrations of PCE versus time. Sorption equilibrium was attained in about 12 hours.

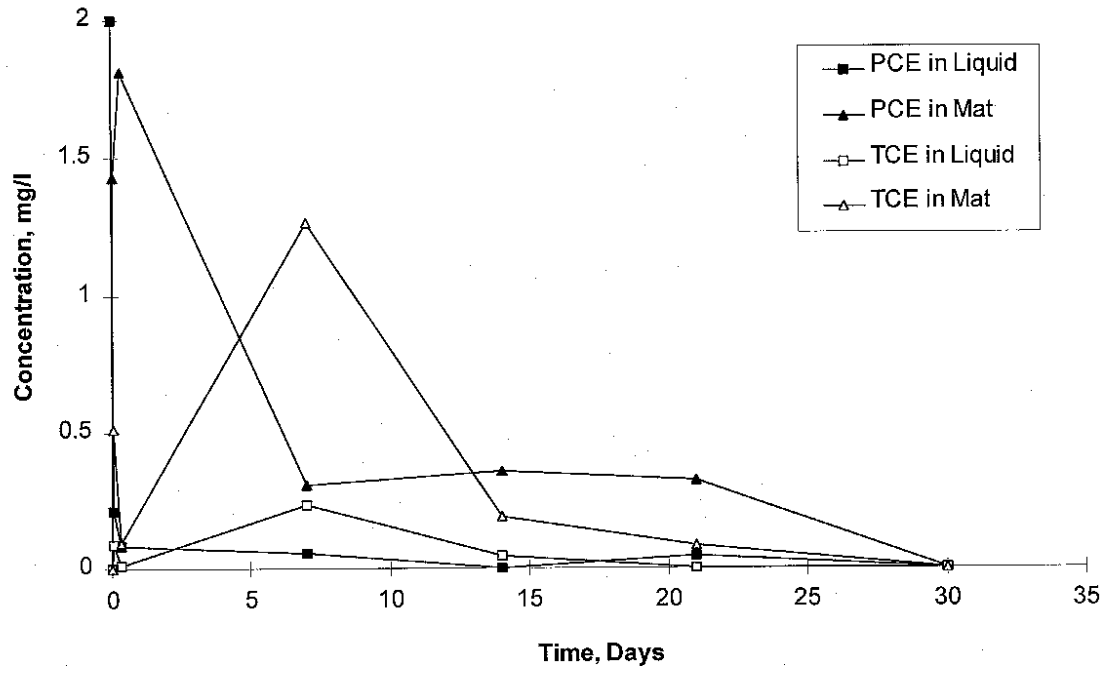


**Figure 2.** Sorption accompanied by transformation of PCE and TCE for batch studies. The parent compound (PCE) and reductive transformation product (TCE) were both transformed to non-detectable levels in the liquid and mat phases.



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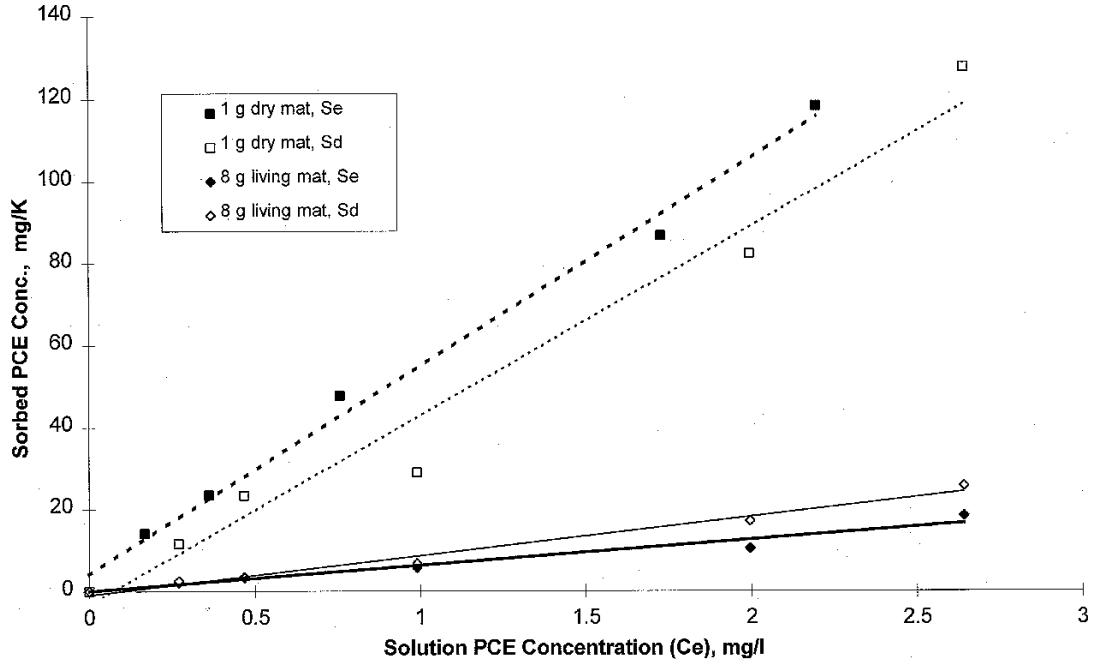


**Figure 3.** Comparison of the PCE sorption effectiveness using equivalent masses of living and dried mat.

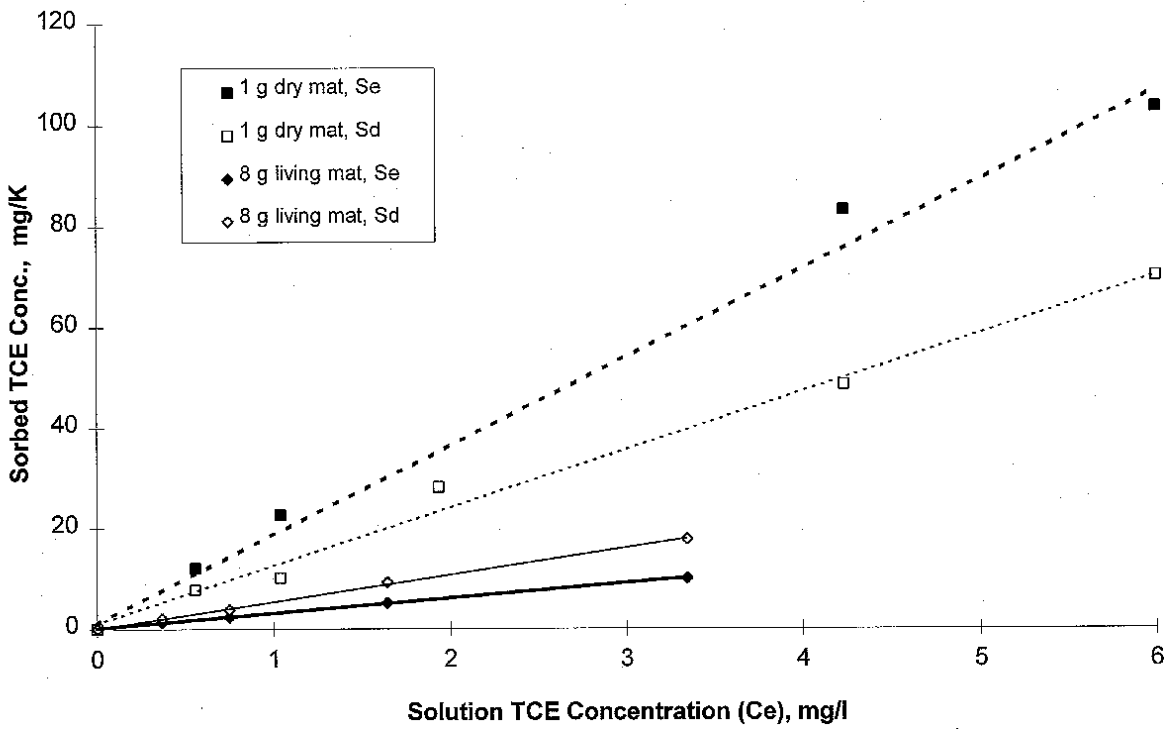


**Figure 4.** Comparison of the TCE sorption effectiveness using equivalent masses of living and dried mat.

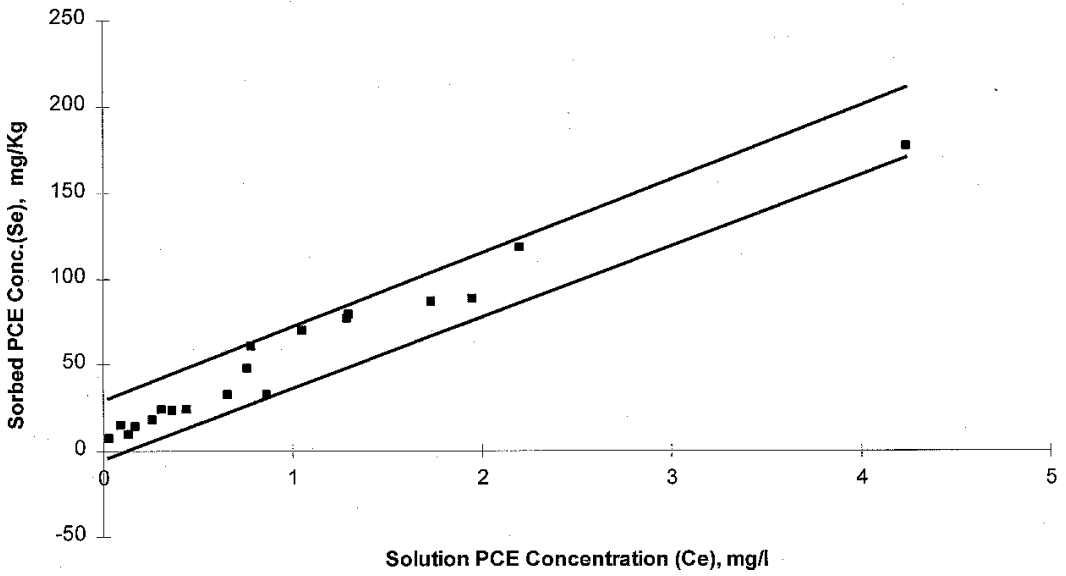


**Figure 5.** Pooled, dried-mat sorption data for PCE with upper and lower 95% prediction bands. A linear sorption model described all data from the replicate experiments.

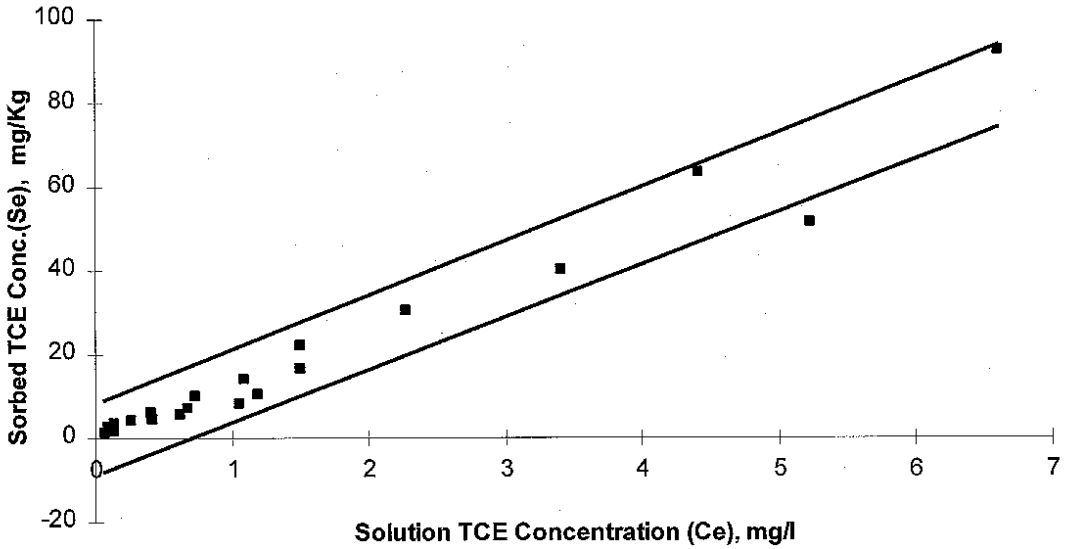


**Figure 6.** Pooled, dried-mat sorption data for TCE with upper and lower 95% prediction bands. A linear sorption model described all data from the replicate experiments.



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DOI: 10.4148/1090-7025.1012