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Journal of Hazardous Materials 92 (2002) 89–102

Journal of Hazardous Materials

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Effects of *n*-hexadecane and PM-100 clay on trichloroethylene degradation by *Burkholderia cepacia*

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Abstract

Trichloroethylene (TCE) is a non-flammable, volatile organochlorine compound which was a widely used degreasing agent, anesthetic, and coolant prior to 1960, but has since been placed on the Environmental Protection Agency's (EPA) list of priority pollutants. The inadequate disposal practices for TCE have created numerous TCE-contaminated superfund sites. The most commonly employed practice for remediating TCE-contaminated sites is to purge the contaminant from the source and trap it onto an adsorbent which is disposed of in a landfill or by incineration. This investigation was undertaken to evaluate the effectiveness of *Burkholderia cepacia* strain G4 (G4) to regenerate used sorbents by degrading TCE from the sorbent directly or indirectly. The results of this investigation showed that G4 was capable of reducing TCE attached to PM-100 clay but at significantly reduced rate due to the slow desorption rate. Conversely, it was shown that G4 was capable of degrading TCE dissolved in *n*-hexadecane at the same rate as systems without *n*-hexadecane present. The reduction in TCE degradation when the TCE is attached to the PM-100 clay could be overcome by solvent rinsing the TCE from the clay with subsequent removal of the TCE from the *n*-hexadecane by G4.

Keywords: Trichloroethylene; *Burkholderia cepacia* strain G4; PM-100 clay

1. Introduction

Windholz et al. [1] describes trichloroethylene (TCE) as a non-flammable, volatile organochlorine compound with a density of 1.4649 at 20 °C, practically insoluble in water,

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0304-3894/02/$ – see front matter
PII: S0304-3894(01)00376-4

but soluble in fixed and volatile oils and most alcohols. In 1985, 90,000 t of TCE were used by industry in the US alone [2]. Folsom et al. [3] stated that prior to 1960, TCE was a widely used degreasing agent, anesthetic, and coolant but has since been placed on the Environmental Protection Agency's (EPA) list of priority pollutants. Shannon [4] reported that the inadequate disposal practices for TCE have created numerous TCE-contaminated superfund sites. In fact, 246 of the 1035 superfund sites have TCE contamination [4].

The most commonly employed practice for remediating TCE-contaminated sites is to purge the contaminant from the source and trap it onto an adsorbent which is disposed of in a landfill or by incineration. Both methods are costly and disposal in a landfill transposes the problem with this potential carcinogen from the TCE-contaminated site to the landfill. While incineration converts the TCE to innocuous carbon dioxide, it is expensive.

Burkholderia cepacia strain G4 (G4) has been shown to effectively degrade TCE [3,5–7]. G4 was isolated from an industrial waste treatment facility at the Naval Air Station (NAS) in Pensacola, FL [6]. Initially, TCE mineralization only occurred when G4 was grown using the NAS water. G4 growth on acetate, succinate, lactate, glucose and ethanol inhibited TCE degradation [6]. The component in the NAS water that induced TCE degradation was phenol [8]. Furthermore, TCE-degradative ability of G4 was also induced by toluene, *o*-cresol, and *m*-cresol [8]. Lun et al. [7] reported that the enzyme that initiates TCE mineralization is the toluene *o*-monooxygenase (TOM).

Folsom et al. [3] reported G4 mineralization rates of TCE to be 1.05 μg/min/mg of protein. Folsom and Chapman [9] reported that G4 mineralized TCE at a rate of 0.39 μg/min/mg of protein in a model recirculating bioreactor. These rates of mineralization were determined in systems where the TCE was readily available. No literature on the effects of an adsorbent on the rate at which G4 mineralizes TCE was found.

This investigation was undertaken to evaluate methods by which absorbent used to remove TCE from contaminated water can be recycled. Two options were investigated. The first option was to determine if G4 could remove the TCE from the clay directly (by degrading the TCE in the aqueous phase). The second option was to determine if TCE, which had been removed from the clay by a solvent (*n*-hexadecane), could be removed from the solvent by G4.

2. Materials and methods

2.1. Mineral salts broth

The mineral salts broth (MSB) employed in this study consisted of

Sodium phosphate, dibasic	1.42 g
Potassium phosphate, monobasic	1.36 g
Ammonium sulfate	0.75 g
Magnesium sulfate·$7H_2O$	0.07 g
Nitrilotriacetic acid, disodium salt	0.06 g
Trace mineral solution	10 ml
DI water	990 ml

The water used in this study had been deionized (DI) and purified by a Milli-Q UV plus system equipped with a Q-Pak deionizing cartridges. The pH of the MSB was adjusted to 7.0 + 0.02 by adding 1 N potassium hydroxide once the solution was clear.

The trace mineral solution contained the following ingredients/l of DI water

Ethylenediamine tetraacetic acid, disodium salt	5.00 mg
Zinc sulfate·$7H_2O$	109.50 mg
Ferric sulfate·$7H_2O$	50.00 mg
Manganese sulfate·H_2O	15.40 mg
Cupric sulfate·$5H_2O$	3.92 mg
Cobalt nitrate·$5H_2O$	2.48 mg
Sodium borate·$10H_2O$	1.77 mg

2.2. Sterilization

All media and glassware were sterilized by autoclaving at 121 °C under 15 lbs/in.^{2} pressure. The duration of the exposure to autoclaving was dependent on the volume of the media; but never less than 15 min. The sterilizer used in this study was a castle steam sterilizer.

2.3. Microbial cultures

G4 was obtained from the EPA laboratory, Gulf Breeze, FL. This organism was maintained on MSB with 0.05% (w/v) phenol. The organism was subcultured using a Wheaton 500 ml sidearm flask containing 200 ml of sterile MSB with 0.05% phenol. A 100 ml of the culture was used to inoculate the subculture. The flask was incubated under shake conditions (100 rpm) using a New Brunswick G53 tier shaker at 30 °C. The cells were harvested by centrifugation. A 30 ml of the culture was placed into a 30 ml centrifuge tube and into a Beckman type 28 rotary centrifuge head. The tubes were centrifuged at 15,000 rpm for 10 min, removed and the medium decanted. A 1 ml of MSB was added and the tubes vortexed until the cells were resuspended.

A hexadecane-degrading microorganism (HDM) was isolated using classical enrichment techniques. The HDM was maintained in MSB with 0.5% hexadecane as the sole carbon and energy source. The organism was subcultured using a Wheaton 500 ml sidearm flask containing 200 ml of sterile MSB with 0.5% hexadecane. A 100 ml of the culture was used to inoculate the subculture. The flask was incubated under shake conditions (100 rpm) using a New Brunswick G53 tier shaker at 30 °C. The cells were harvested by centrifugation. A 30 ml of the culture was placed into a 30 ml centrifuge tube and into a Beckman type 28 rotary centrifuge head. The tubes were centrifuged at 15,000 rpm for 10 min, removed, and the medium decanted. A 1 ml of MSB was added and the tubes vortexed until the cells were resuspended.

2.4. Analysis of dissolved TCE

Aqueous TCE levels were determined using an OI analytical 16 port MPM purge unit. The volume of sample (10–5000 μl) placed in the sample tube was dependent upon the expected

TCE concentration in the aqueous phase. This system purges the volatiles from the tube with helium and traps them with an OI analytical 4560 sample concentrator equipped with a OI analytical #7 trap maintained at 25 °C. Subsequently, the trap was heated to 180 °C for 5 min. The organics volatilized from the trap were introduced into a gas chomatograph (GC) via 1/4 in. stainless steel tubing. TCE was quantitated using a Hewlett Packard 5890 Series II GC equipped with an OI analytical photon ionization detector (PID). The detector temperature was maintained at 180 °C. The injector temperature was 275 °C. The GC was equipped with a 30 m Restek RTX-5 0.53 diameter capillary column. The carrier gas, helium, had a flow rate of approximately 1.2 ml/min. Helium also was employed as the makeup gas for the detector using a flow rate of 40 ml/min. The oven temperature was set initially at 35 °C and was increased 8 °C/min to a final temperature of 120 °C where it remained for 2 min.

2.5. Experimental conditions

For all experiments the vials and ampules were incubated under shake conditions (100 rpm) using a New Brunswick G53 tier shaker at 30 °C unless otherwise stated. All tests were conducted in triplicate.

2.6. TCE equilibrium: Henry's Law constant

In order to mass balance TCE in the test systems, the mass of TCE in the gas phase had to be calculated from the mass of TCE in the aqueous phase. Using Henry's constant along with the concentration of TCE in the aqueous phase, the TCE mass in the gas phase can be calculated. This study was conducted to determine Henry's constant for the test system.

This experiment was conducted in 10 ml ampules containing 2.5 ml of MSM with 10 ml of headspace. Three concentrations of TCE (5, 30 and 1000 ppm) in triplicate were evaluated. The TCE was added as a TCE–methanol solution. The ampules were flame-sealed and incubated. The medium in the aqueous phase was analyzed for TCE at 48 h.

2.7. Distribution of TCE between MSB and hexadecane

The analytical method employed for analysis of TCE was limited to the aqueous phase. Therefore, any determination of the amount of TCE dissolved in hexadecane would require that a relationship between the aqueous TCE concentration and the hexadecane TCE concentration be established. The test vessel used in this experiment was a 40 ml VOA vial with Teflon-lined cap. The treatments contained 273 μl of hexadecane. The TCE concentrations tested were 0.5, 2.0, 5.0, 10.0 and 20.0 ppm. The vials were capped immediately after the TCE was added, inverted and incubated. Duplicate controls for these experiments did receive hexadecane. The controls provided the means of determining losses of TCE due to volatilization.

2.8. PM-100 organophilic clay adsorption/desorption experiment

Studies were conducted to determine what effect TCE adsorbed to clay would have on the TCE-degradation rate by G4. Therefore, it was necessary to know the equilibrium

concentrations between the aqueous phase and the clay at a concentration of 30 ppm TCE.

A 40 ml VOA vials with Teflon-lined caps were employed as the test vessels. The treatment vials contained 1 g of clay added prior to the addition of the MSB. To the VOA vials, 41.5 g of MSB was added, vials were capped, inverted, and incubated under stationary conditions for 24 h at room temperature to hydrate the clay. After the hydration period, the caps were removed and TCE–methanol solution added to obtain a concentration of 30 ppm TCE in the aqueous phase. Immediately after the addition of TCE, the vials were recapped, inverted, and incubated. A 100 μl sample of MSB was taken every hour for 10 h and analyzed for TCE using the GC. A final 100 μl sample was taken at 24 h and analyzed for TCE. The wet weight of the clay was determined. The TCE mass in the remaining MSB was subtracted from the mass of TCE that was adsorbed. To determine the TCE-desorption rate, 41.5 g of clean MSB was added to the VOA vials which then were capped and incubated. A 100 μl sample was taken every hour for 10 h and then daily for 5 days and analyzed for TCE. Controls for this study did not receive clay.

2.9. TCE-degradation studies

TCE-degradation studies were conducted in 10 ml glass Kimble ampules which contained 2.5 l of MSB. The ampules were sterilized and phenol was added from a 5% stock solution. A 25 μl of the 5% phenol solution yielded 500 ppm phenol in the ampule. A 100 μl of the resuspended cells containing 0.021 g dry weight of cells was added to the ampules. A 5 μl of a TCE–methanol solution was added to the ampules below the surface of the MSB and the ampules sealed. After incubation, the MSB in the ampules was analyzed for TCE.

2.10. The effects of initial TCE concentration on TCE degradation by G4

In order to determine the effect of the initial concentration of TCE on the TCE-degrading ability of G4, three concentrations of TCE were employed, 10, 20 and 60 ppm. A 10 ml ampules containing 2.5 ml of MSB were inoculated with 100 μl of a 3-day-old culture of G4 and TCE added in the form of a TCE–methanol solution. The ampules were immediately sealed, incubated under shake conditions and the MSB analyzed for TCE after 24 h. Ampules without the G4 inoculum served as controls.

2.11. The effects of initial phenol concentrations on TCE degradation

Since G4 requires induction of the TCE-degrading enzyme by phenol, an experiment was conducted to determine the effect of initial phenol concentration on the TCE-degrading ability of G4. A 10 ml ampules containing 2.5 ml of MSB served as the test system. A 100 μl of a 3-day-old culture was used to inoculate the ampules. The phenol concentrations used in this study were 0, 60, 120, 250, 600 and 1200 ppm. TCE was added to the ampules in the form of a TCE–methanol solution to yield an aqueous phase TCE concentration of 60 ppm. The ampules were immediately sealed and incubated for 48 h. The MSB in the ampules was sampled and analyzed for TCE. Ampules containing no inoculum served as controls.

2.12. Determination of the TCE-degradation ability of the hexadecane-degrading microorganism

Prior to the start of experiments to determine the ability of the HDM to increase the bioavailability of TCE by degrading hexadecane, information was needed about its ability to degrade TCE. This experiment was conducted in 10 ml ampules containing 2.5 ml of MSB, hexadecane (100 μl) and 5 ppm TCE. A 100 μl of the resuspended cells was used as an inoculum. The TCE was added in the form of a TCE–methanol solution. The ampules were immediately sealed and incubated for 48 h on an orbital shaker. The MSB was sampled and analyzed for TCE. TCE degradation was confirmed by increases in chloride content in the system. Controls for this experiment did not contain the HDM.

Chloride analyses were conducted using a Dionex DX500 ion chomatograph equipped with a 4 mm anion IonPac column. The chloride analytical method uses two mobile phases. Eluent A is a 200 mM sodium hydroxide solution and eluent B is distilled deionized water (DDI water) degassed with helium. The flow rate is 1 ml/min with a total run time of 15 min. The elution time is 2.5 min. The chloride standard curve was prepared using five concentrations of chloride in triplicate. The standards were prepared in distilled DI water.

2.13. The effects of a HDM on the equilibrium between TCE concentrations in the aqueous phase and the hexadecane phase

An experiment was conducted to determine if degradation of hexadecane by a HDM would release TCE into the aqueous phase resulting in an increase in the bioavailability of TCE. The 10 ml ampules containing 2.5 ml of MSB served as the test system. A 50 μl of hexadecane was added to each ampule. The TCE was added in the form of a TCE–methanol solution so that an aqueous phase TCE concentration of 5 ppm was obtained. Based on the isotherm data, 37.5 μg of TCE yielded 5 ppm in the aqueous phase. The ampules were immediately sealed without the addition of the HDM and incubated for 24 h to allow TCE time to reach equilibrium between the three phases before the start of degradation. A 100 μl of 3-day-old HDM culture was added through a small opening in the ampule and then immediately resealed. The controls were treated similarly but the HDM was not added. The MSB in the ampules was sampled at 2 h, 1, 3 and 4 days and analyzed for TCE.

2.14. The effects of 0.5% hexadecane on TCE degradation by G4

This experiment was conducted to determine the effect of TCE dissolved in hexadecane on the TCE-degrading ability of G4 using 10 ml ampules containing 2.5 ml of MSB and 50 μl of hexadecane. The TCE was added in the form of a TCE–methanol solution to yield an aqueous phase TCE concentration of 5 ppm (57.5 μg of TCE). The ampules were immediately sealed without the addition of G4 and incubated for 24 h to allow TCE time to reach equilibrium between the three phases before the start of degradation. A 100 μl of 3-day-old culture of G4 was added through an opening in the ampule and then immediately resealed. The controls were treated similarly but the G4 was not added. The ampules were immediately sealed for 48 h. The MSB in the ampules was sampled and analyzed for TCE.

2.15. The effects of PM-100 clay on TCE degradation

In order to determine the effect of TCE adsorption to PM-100 on the TCE-degrading ability by G4, three concentrations of TCE (10, 20 and 30 ppm) were employed. A 1 g of the clay was added to each 10 ml ampule using a spatula. Sterile MSB (2.5 ml) was added to the ampules and TCE was added in the form of a TCE–methanol solution. All ampules were immediately sealed without the addition of G4 and incubated for 24 h on an orbital shaker to allow TCE time to reach equilibrium between the three phases before the start of degradation. A 100 μl aliquot of a 3-day-old G4 culture was added to the ampules through an opening in the ampules and then immediately resealed. The controls were treated similarly but the G4 was not added. The ampules were incubated for 36 h under shake conditions. The neck of the ampules was broken off and the medium decanted. A 1 ml of methanol was added to each ampule and the ampules sonicated for 30 min. The methanol was removed from the ampules using a Pasteur pipette and 100 μl of the methanol extract was analyzed for TCE.

2.16. Statistical analysis

The statistical analysis used to determine significance for all data obtained was the two-sample t test for small-sample test described by Miller et al. [10]. The formula used was

$$t = \frac{(x_1 - x_2) - \delta}{((n_1 - 1)(s_1^2) + (n_2 - 1)(s_2^2))^{1/2}} (n_1 + n_2)^{1/2}$$

where x_1 is the mean for one set of data and the x_2 the mean for the second set of data, s_1 and s_2 are the standard deviation for the means, and n_1 and n_2 the degrees of freedom for the number of samples in the sets.

3. Results

3.1. Henry's Law constant

The mean of Henry's constant plus or minus the standard deviation for all three concentrations of TCE was 0.40 ± 0.05. The Henry's constant found in this study agreed with the 0.4 coefficient observed by Folsom et al. [3]. Therefore, the concentration of TCE in the headspace could be calculated with a high degree of confidence using Henry's Law.

3.2. Distribution of TCE between water and hexadecane

The effects of initial TCE concentration (0.0, 0.5, 2.0, 5.0 and 10.0 ppm) on the equilibrium of TCE between hexadecane and the aqueous phases in the VOA vials are shown in Fig. 1. A linear regression of the results resulted in a R^2-value of 0.9863. Therefore, the linear curve formula was used to calculate the concentration of TCE in one phase based on the TCE concentration in the other phase.

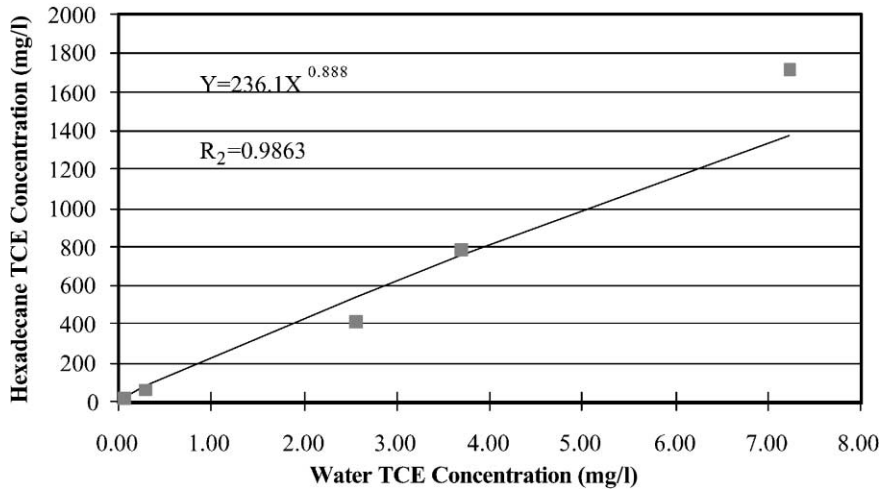

Fig. 1. TCE equilibrium isotherm using MSB and hexadecane.

3.3. PM-100 clay and TCE adsorption and desorption results

The adsorption and desorption of TCE to and from PM-100 clay suspended in MSB over time is presented in Fig. 2. Adsorption was rapid during the first 10 h after which time it remained constant at 47.5 ppm for the next 14 h. Desorption of TCE from the PM-100 clay was rapid for the first 6 h and then remained constant for the remaining 42 h of the experiment and 84% of the TCE remained adsorbed to the PM-100 clay.

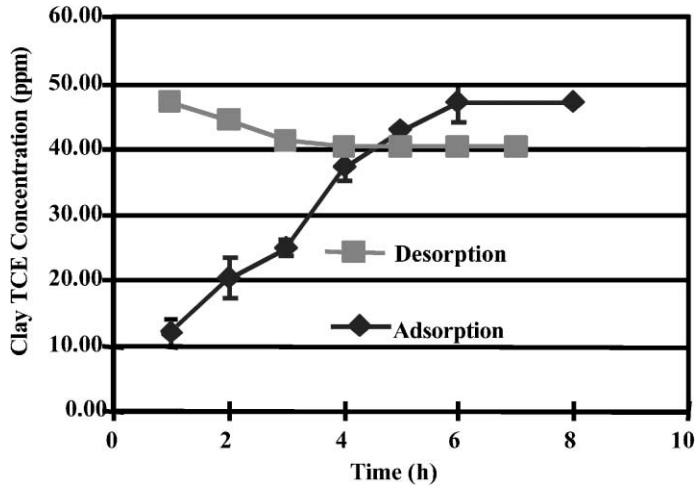

Fig. 2. PM-100 clay batch adsorption and desorption kinetics.

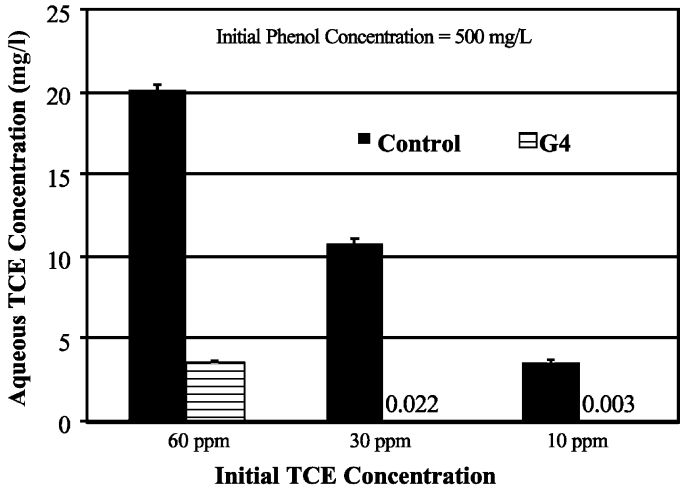

Fig. 3. TCE degradation by G4 with different initial TCE concentrations and incubated for 48 h.

3.4. The effects of initial TCE concentration on TCE degradation by G4

The effects of TCE concentration on the utilization of TCE by G4 are shown in Fig. 3. In this experiment, TCE was added in amounts calculated to yield aqueous TCE concentrations of 10, 30 and 60 ppm if all of it remained in the aqueous phase. Actual analyses revealed aqueous concentrations of 3.552, 10.739 and 20.102 ppm, respectively. Nearly 65% of the added TCE was in the headspace and would partition into the aqueous phase as the aqueous TCE was utilized by G4. Thus, the amounts of TCE actually consumed were far greater than indicated in Fig. 3. The mass of TCE consumed was corrected for the mass of TCE in the headspace using Henry's constant and were calculated to be 0.0075, 0.055 and 8.9425 ppm. Statistical analyses of the data in Fig. 3 revealed that there were highly significant differences between the initial aqueous TCE concentrations and final aqueous concentrations for all concentrations of TCE tested.

3.5. The effects of initial phenol concentration on TCE degradation by G4

The effects of initial phenol concentration on TCE utilization by G4 are shown in Fig. 4. Phenol was added to the aqueous phase in the ampule in concentrations of 0, 60, 120, 240, 480, 600 and 1200 ppm. G4 utilized nearly all of the TCE for samples treated initially with 60, 120, 240, 480 and 600 ppm phenol. Statistical analyses of these data proved that the reduction in TCE by G4 was highly significant. Even with no initial phenol, G4 utilized 60% of the total TCE (corrected using Henry's constant), thus indicating that G4 remained induced for TOM for a substantial period of time. There was no significant reduction in TCE concentration when the initial phenol concentration was 1200 ppm indicating that this phenol concentration was inhibitory.

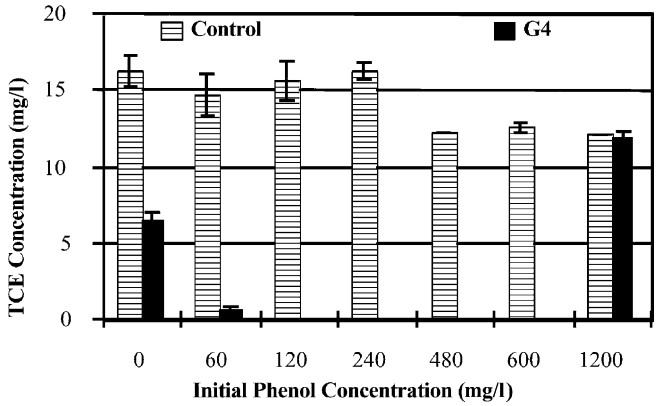

Fig. 4. TCE degradation by G4 with different initial phenol concentrations.

3.6. The effects of PM-100 clay on TCE degradation by G4

The effects of PM-100 clay on TCE utilization by G4 conducted in the ampules are shown in Fig. 5. The statistical analysis of the data showed a highly significant difference between the controls and the treatment with G4 for initial TCE concentrations of 10, 20 and 30 ppm. A possible explanation is that G4 significantly lowered the aqueous TCE concentration so that the equilibrium driving force was in favor of the MSB.

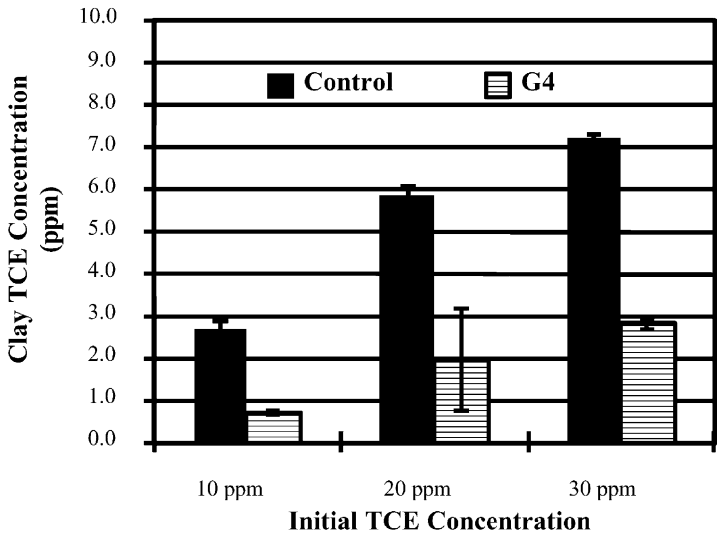

Fig. 5. TCE-degradation studies with PM-100 clay and G4 for 36 h of incubation.

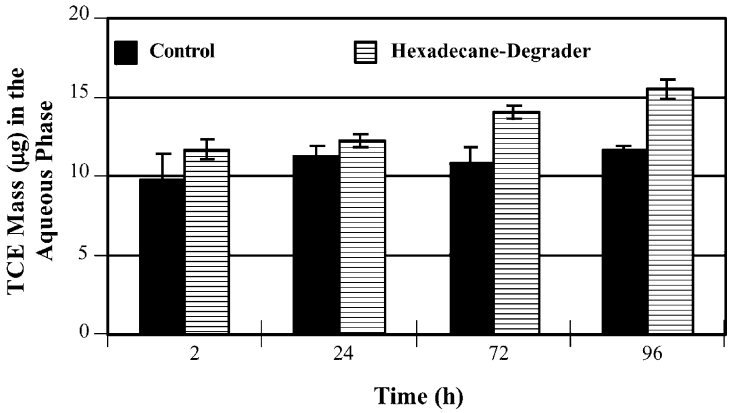

Fig. 6. TCE released into the headspace and MSB due to hexadecane utilization by the hexadecane-degrader.

3.7. Determination of the TCE-degrading ability of the HDM

TCE degradation by the HDM was assessed by measuring the loss of TCE and an increase in the chloride ion concentration. The results of the TCE and chloride ion analyses did not show a significant difference at the 0.05 level of significance between the treatments with the HDM and the control without the HDM. Therefore, it was concluded that the HDM did not degrade the TCE in the ampules within the 48 h period of incubation.

3.8. The effects of a HDM on the equilibrium between TCE concentrations in the aqueous phase and the hexadecane phase

The data in Fig. 6 show that the HDM caused the concentration of TCE in the aqueous phase to increase with increased time of incubation due to hexadecane utilization by the HDM. Although not statistically significant after 24 h, the increase in TCE concentration in the MSB was highly significant after 72 h and continued to increase after another 24 h of incubation when compared to systems that did not contain the HDM. Importantly, the concentration of TCE in the aqueous phase after 72 and 96 h of incubation is significantly greater than the amount of TCE in the aqueous phase after 2 h of incubation. Statistical analyses of the data found the differences to be highly significant. Correspondingly, there is no statistically significant increase in the concentration of TCE in the controls over the same period of time.

3.9. The effects of 0.5% hexadecane on TCE degradation by G4

The impact of 0.5% hexadecane on TCE utilization by G4 is shown in Figs. 7 and 8. In the first 24 h of incubation, no significant difference at the 0.05 level of significance was observed between treatments with hexadecane and those without hexadecane for TCE degradation by G4. With an additional 24 h of incubation, the amount of TCE in the aqueous

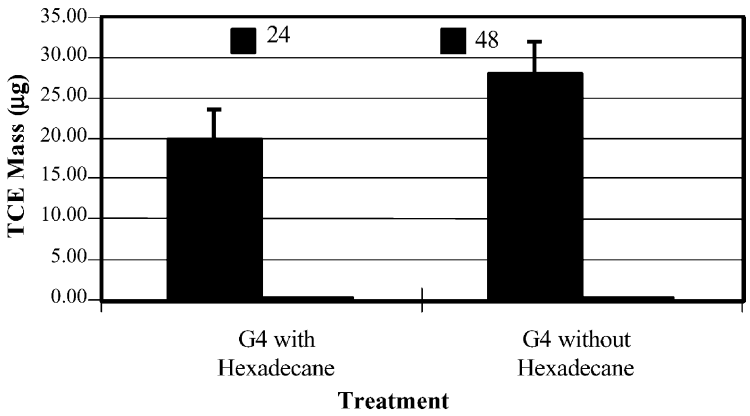

Fig. 7. TCE mass left in the system by G4 for systems treated with hexadecane and systems without hexadecane.

phase was undetectable by gas chomatography. Statistical analyses of the data revealed no statistically significant impact at the 0.05 level of significance made by hexadecane on the utilization of TCE by G4 for the 24 and 48 h sampling times. Chloride analyses (Fig. 8) were performed on these samples to confirm TCE utilization. Statistical analyses of the data for the 24 and 48 h sampling times showed no statistically significant difference between the chloride ion concentration in samples containing hexadecane and those without hexadecane. This suggest that desorption of TCE from the hexadecane is equal to or greater than the TCE-degradation rate of G4.

The rate of TCE degradation by G4 with no adsorbent present was calculated using the data presented in Fig. 3. The rates for degradation when the initial TCE concentrations were 60, 30 and 10 ppm were 57.38, 37.21 and 12.33 μg TCE/h/g of dry cells, respectively. The rate of TCE degradation by G4 when hexadecane was present at an initial TCE concentration of 30 ppm was 34.69 μg TCE/h/g of dry cells. This suggests that the rate of dissolution of

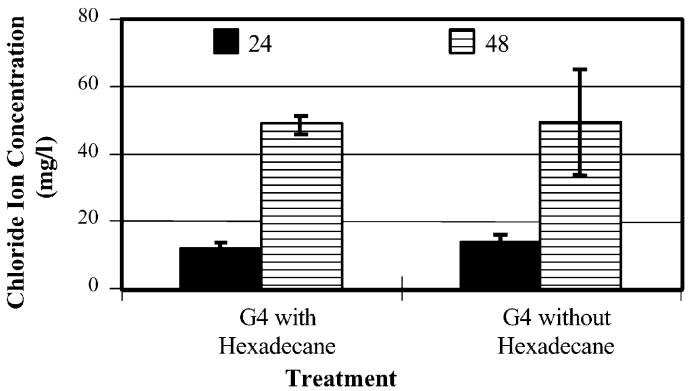

Fig. 8. Chloride ion production by G4 for systems treated with hexadecane and systems without hexadecane.

TCE from hexadecane is approximately the same or greater than the degradation of TCE by G4.

4. Discussion

G4 degraded 12.33, 37.21 and 57.38 μg of TCE/h/g of cells from initial TCE concentrations of 10, 30 and 60 ppm, respectively

The effects of the initial phenol concentration on TCE degradation were determined for aqueous concentrations of 0, 60, 120, 240, 480, 600 and 1200 ppm. The greatest mass of TCE degraded per mass of phenol was observed with an initial phenol concentration of 60 ppm which yielded a TCE-to-phenol ratio of 0.234 μg of TCE/μg of phenol. It was found that the inoculum, preinduced with phenol, did not require further induction to be able to effectively degrade all of the available TCE which amounted to 0.078 mg of cells/mg of TCE. These data indicate that for a field-scale TCE-cleanup reactor, phenol would not be required in a reactor if operated in a batch mode, but would require additional phenol if the reactor is operated in a continuous mode. In a report not published, Teeter et al. [11] found that G4 was capable of degrading phenol to below 5 ppb in 5 days.

The question arises as to whether G4 would be able to effectively degrade the TCE attached to the clay or dissolved in hexadecane. The desorption rate of TCE from the clay was determined to be 1.115 μg of TCE/h/g of clay. The dissolution rate of TCE from hexadecane was not determined because the volume of hexadecane (50 μl) required to remove the same mass of TCE from the same volume of MSB (40 ml) as the 1 g of clay was so minute that all of the hexadecane could not have been removed and would have created a large standard deviation.The results of the studies conducted using hexadecane and PM-100 clay showed that the hexadecane–TCE concentration was below detectable limits in 48 h and TCE could still be detected on the clay at 36 h. The rate of degradation for G4 without any hexadecane or PM-100 clay present was calculated to be 37.21 μg TCE/h/g of dry cells when the initial TCE concentration was 30 ppm and a similar degradation rate, 34.69 μg TCE/h/g of dry cells was observed when 0.5% hexadecane (w/w) was present. This suggests that the rate of dissolution of TCE from hexadecane is approximately the same or greater than the degradation of TCE by G4. Using the data from the TCE adsorption and desorption study, the desorption rate of the TCE was calculated to be 1.115 μg of TCE/h/g of clay for the first 6 h of incubation, the time at which equilibrium was reached, which is far less than the degradation rate of G4; thereby, severely limiting the bioavailability of the TCE. When studies were conducted using the PM-100 clay, TCE was still present after 36 h of incubation with G4, but it was capable of reducing the TCE concentration on the PM-100 clay significantly (0.05 level of significance). However, this rate of TCE degradation was significantly less than the rate observed when no PM-100 clay was present. Alternatively, the desorption rate of the TCE from the PM-100 clay may be enhanced by the addition of a synthetic surfactant but the impact of the surfactant on the G4 and its degradative ability is unknown.

In summary, this investigation showed that G4 could effectively remove TCE from PM-100 clay and in effect rejuvenate the absorbent, however, the desorption rate of TCE from the clay into the aqueous phase was rate limiting. If TCE were removed from the

PM-100 clay using hexadecane, G4 was shown to remove the TCE from the hexdecane at a rate significantly faster than TCE removal from the clay itself.

Acknowledgements

The authors would like to thank Brad Rogers for his help with conducting and analyzing experiments in this study. We would like to also thank the Strategic Environmental Research and Development program for supplying part of the funding.

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