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ORIGINAL RESEARCH

Measuring Groundwater and Contaminant Flux: Passive Flux Meter Field Applications and Issues with Alcohol Degradability

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Abstract: The passive flux meter (PFM) developed at the University of Florida is an innovative device that is inserted into a well in order to measure groundwater and contaminant flux. The in-situ device consists of an activated carbon matrix impregnated with known amounts of alcohols that are desorbed at rates proportional to the groundwater flux through the device. After exposure the sorbent is extracted to quantify the contaminant mass intercepted and the resident alcohol mass remaining. Since the alcohols employed in bioactive sites are degradable, studies were conducted to investigate biodegradation issues and microbial acclimation times in field application. Also, silver-impregnated activated carbon was compared to unamended activated carbon in batch and column studies to determine silver ion effects on degradation. The studies confirm degradation and microbial acclimation occurrence, and demonstrate that silver impregnated activated carbon does inhibit degradation. Issues remain with biofilm/biofouling observed in the field as well as column studies.

Keywords: passive flux meter, alcohol degradability, groundwater, contaminant flux

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Introduction

Groundwater hydrologists estimate water and contaminant mass fluxes to define boundary conditions and source terms that are then used to predict risk, compliance, and contaminant attenuation.[1–4] Estimation of subsurface contaminant mass flows is difficult using field data since spatial variations in both concentrations and groundwater flows induce mass flow variations of several orders of magnitude. As a result, hydrologists typically approximate contaminant mass flows using calculated (i.e. not measured) groundwater fluxes and depth-averaged concentrations gathered from wells.

The PFM technique[5–7] involves the deployment of a permeable unit, which is a matrix of hydrophobic permeable sorbents, into a well or boring such that intercepts groundwater flow but does not retain it. The sorbent is selected such that it retains dissolved contaminants present in the intercepted groundwater. The sorbent matrix is also impregnated with known amounts of water-soluble alcohols that are desorbed at rates proportional to the groundwater flux through the device.

Through previous column studies, the desorption rates of the alcohols from activated carbon have been studied and reported.[6] However, since the PFM is applied to assess the effects of remediation strategies that include enhanced microbial degradation via biostimulation or bioaugmentation, there is a need to assess bacterial degradation of the alcohols apart from the desorption rates. The batch and column studies presented in this paper are based on the use of the PFM technology at a bioactive site contaminated with trichloroethylene: the National Aeronautics and Space Administation (NASA) Launch Complex 34 site (LC34) at Cape Canaveral, Florida.[8] This site was chosen as a demonstration of the use of microbial bioaugmentation for remediation of trichloroethylene. (Fig. 1). Prior to addition of the dechlorinating culture, the site was flushed with an ethanol solution for several months. To demonstrate the performance of the PFM method, groundwater and contaminant flux was evaluated during 4 deployments. The first phase and application of the PFM study involved assessing conditions under steady water flow. Flux was then monitored during the next three phases of the study, which involved ethanol biostimulation and

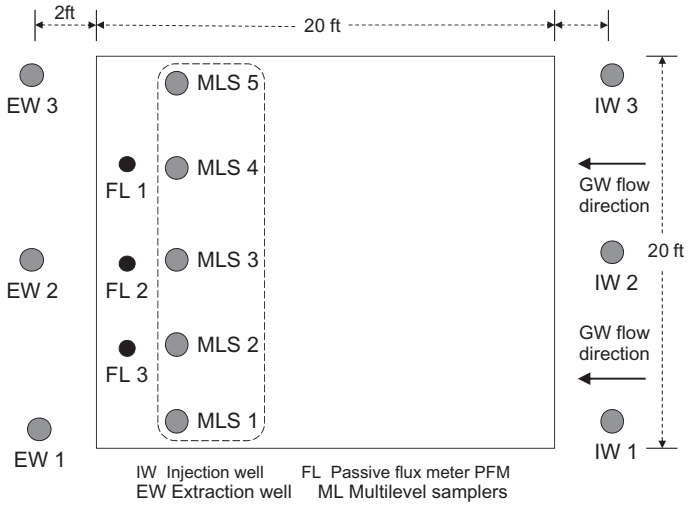

Figure 1. Well layout at NASA LC34.

bioaugmentation microbial treatment. For these three phases, due to ethanol presence for biostimulation in the aquifer, ethanol was no longer included in the suite of "tracer alcohols" to evaluate groundwater flux. Note that additional in-situ field testing has been conducted by the University of Florida group at several dense non-aqueous phase (DNAPL) contaminated sites including the Canadian Forces Base Borden, Ontario, Canada; Hill Air Force Base, Utah; Ft Lewis, Washington; and Patrick Air Force Base in Florida. Documentations of these field tests are in various stages.

Since site LC34 is bioactive, it was essential to evaluate the biodegradability of alcohol tracers used in the flux device as degradation could reduce the remaining alcohol mass on the activated carbon matrix and alter resulting groundwater flux estimates. This alcohol degradability is influenced by the length and branching of the alkyl chain, the number of hydroxyl groups and the presence of other substituent types. Additionally, degradability initiation depends on the required microbial acclimation time as well as concentration effects.[9] For the aliphatic alcohols evaluated in the laboratory studies, methanol and ethanol are easily degraded in both aerobic and anaerobic environments[10,11] Isopropanol is less easily degraded while tetra-butyl alcohol and 2,4-dimethyl-3-pentanol (2,4-DMP) are resistant due to alkyl branching[12–15] Specific alcohol biodegradability rates were reported in the published Handbook of Environmental Degradation Rates.[16] This handbook compilation resulted

from work by the Syracuse Research Corporation under the Environmental Protection Agency (EPA) auspices. Rates as reported in Table 1 demonstrate that ethanol is the most readily degraded alcohol; an ethanol aerobic degradation rate of 100 mg/L in 7 days and an anaerobic degradation rate of 100 mg/L in 3–25 days have been reported depending on conditions.[17] The structural characteristics of ethanol favor rapid biodegradation; it is expected to rapidly biodegrade in essentially all environmental conditions (i.e. temperature, pH, and pressure). Microorganisms capable of metabolizing ethanol are ubiquitous in the environment.[18,19]

Given the length of time the PFM devices were placed in the wells and then removed for extraction at NASA LC34, the batch studies were designed to determine specific alcohol microbial acclimation times as well as alcohol degradation rates. When natural microbial communities are exposed to xenobiotic alcohols, a change in metabolic activity may be observed after a lag phase of varying length. During this phase, the microbes are acclimating to new conditions. Suggested mechanisms for this acclimation include selection of specialized organisms, genetic adaptation or induction of enzyme expression.[20–23]

Another complication to accurate flux measurements was observed in field testing of the device. Biofouling was observed in the wells at LC34; therefore, there was an additional need to investigate potential biofouling and the presence of a biofilm on calculated mass measurements. Biofouling was also observed at another another field site, Patrick Air Force Base in Florida. At both sites, initial PFM measurements prior to site biotreatment were less variable than subsequent deployments.

Table 1. Half-lives of alcohols in groundwater based on acclimated aerobic aqueous biodegradation half-lives.

Tracer alcohol	Aerobic half-life/ high hours	Aerobic half-life/ low, hours
Ethanol	52	13
Methanol	168	24
Isopropanol	336	48
Tert-Butyl Alcohol	8640	1334
2,4-dimethyl-3-pentanol	Not available-recalcitrant	Not available-recalcitrant

Hypothesized explanations for this include bacterial adhesion mechanisms that are essential to biofilm development and can affect biodegradation availability of alcohols on the media/sorbent surface.[24–26] Additionally, the chemical composition of bacterial cell surface polymers can be influenced by changes in environmental conditions and time.[27] Often, after bacteria have been attached to a surface for hours or days, hydrated amorphous polymers accumulate along with increasing numbers of attached cells. An intercellular slimy matrix composed of polysaccharides[28–32] can be formed that constitutes a major portion of the biofilm. Laboratory studies of model systems have been developed to examine bacterial interaction with surfaces by manipulating surface charge, hydrophobicity, and solution chemistry.[33–35]

After an investigation of types of activated carbon available and in an effort to reduce in field alcohol degradation, a silver-impregnated activated carbon was selected for use in the PFM. It was hypothesized that this media would inhibit microbial growth and delay the biofilm development that is a component of the acclimation process. The use of silver-impregnated activated carbon as a sorbent (silver AC) derives from the historical application of silver for bacterial inhibition.[36] It was reported in 1000 BC that water was kept in silver or copper vessels; Aristotle advised Alexander the Great (335 BC) to store water in silver vessels and boil before use when transporting for field campaigns, inferring that the antimicrobial activity of silver was observed and exploited in these times.[37]

The actual mechanisms of antimicrobial action were first explored in the last decade of the nineteenth century. Research showed that silver was an extremely active biocide at silver ion concentrations of 10^{-9} to 10^{-6}.[38] There is experimental evidence that this was in fact due to the silver ion.[39] Additional research showed that the silver has a bactericidal effect in water.[40–43] It was further shown that the silver ion reacts strongly with the sulphydryl (S-H) groups in the bacterial cell found in both the bacterial structure and functional enzymes; the ion also interacts with the nucleic acids.[44–46] Medical studies on silver-impregnated devices for intravenous catheters showed that silver ion decreases bacterial adhesion and biofilm formation.[47–49] Other effects of silver effects on activity, reported in the literature include

structural changes in the cell envelope, cell membrane detachment and formation of silver deposits inside the bacterial cell.[50,51,52]

An additional consideration in evaluating silver bactericidal efficacy in field testing is the development of bacterial resistance to silver. This was documented in medical environments where silver toxicity selects for resistance. Mechanisms of resistance include the efflux pumping of toxic Ag^+ ions from the bacteria and enzymatic detoxification, which decreases the Ag^+ ion concentration (reduction to Ag^o for example). Bioaccumulation or bio-sequestration, either intracellularly or within the cell surface, is another mechanism of silver resistance.[37,53,54]

Since the PFM devices are placed in-situ for a period of days or weeks as a function of groundwater velocity, degradation, microbial acclimation and biofilm issues could alter the flux results. In order to evaluate these effects, 1) batch studies and 2) column studies were performed. Additionally, the inhibitory effect of the silver ion on degradation was addressed. Laboratory experiments were designed to mimic environmental conditions during PFM field deployment at LC34. In both batch studies and column studies, the effects of silver on degradation were evaluated. The column experiments were conducted to study porous media flow and nutrient/electron donor amendment on alcohol degradation as well as the potential development of a biofilm.[55,13]

Methods and Materials

Batch studies

To evaluate tracer degradation along with controlling environmental factors, batch microcosms were constructed with site specific groundwater and soil. A preliminary batch study (Series A) was performed to evaluate alcohol degradation and microbial acclimation. A second batch study was then conducted under confirmed oxygen-limited (Series B) conditions. Both alcohol-equilibrated activated carbon and silver-impregnated carbons were used in the second set of batch experiments in order to assess antimicrobial activity of the silver ion. Site groundwater and soil from LC34 were used as the source of microbes in the batch tests.

Preliminary Series A batch tests for assessment of alcohol degradation employed four separate batch systems in a sealed environment. The alcohols used were methanol, ethanol, isopropanol (IPA), tert-butyl alcohol (TBA) and 2, 4-dimethyl-3-pentanol (2, 4-DMP); these are the same alcohols equilibrated with the activated carbon sorbent used in the field PFM tests. All alcohol tracers used for the study were reagent grade and purchased from Fisher Scientific Company. A solution of these alcohols was prepared with resulting alcohol concentrations as shown in Table 2.

Approximately 200 g Fisher Brand 6–14 mesh activated carbon were added to 1.5 liters of alcohol solution and shaken for 24 hours. After equilibration with the alcohol solution, 50 g wet activated carbon and 300 g deionized (HPLC) water were placed into a 500 mL jar which was tightly capped and designated System 1. System 2 was designed to show system response to spiking with NASA site groundwater (GW); both site groundwater and soil were added to System 3. System 4 was set up as a control as shown in Table 3.

The lids on all systems were sealed; the solutions were then sampled daily. Systems 1, 2 and 3 were placed on magnetic stirrers at approximately 200 rpm during the course of the experiment. Three hours after initial set up, a 5 mL sample was pipetted from each system. This was allowed to settle at least one hour before subsampling into two duplicate 2 mL GC vials, which were then tightly sealed with Teflon caps and refrigerated for analysis. The alcohols were then

Table 2. Series A alcohol solution preparation, solution concentrations.

	Tracer solution preparation	Concentration mg/L
Series A	1.5 mL methanol 1.5 mL ethanol 3 mL isopropyl alcohol 3 mL tertiary butyl alcohol 3 mL 2,4-dimethyl-3 pentanol 3 L deionized water	395 MeOH 395 EtOH 785 IPA 786 TBA 829 DMP
Series A spike solution (Day 24)	60 µL methanol 60 µLethanol 90 µL isopropyl alcohol 100 mL NASA Groundwater	790 MeOH 790 EtOH 1800 IPA

Table 3. Series A system set-up.

Series A	System 1	System 2	System 3	System 4
	50 g wet AC* 300 g HPLC water 22–23 °C	50 g wet AC* 300 g site groundwater 22–23 °C	50 g wet AC* 300 g site groundwater 2 g site soil 22–23 °C	Control no AC 300 g HPLC water 22–23 °C

analyzed using a Perkin-Elmer Gas Chromatograph (GC) equipped with automated liquid injection and a flame ionization detector (FID). The experiments for Systems 1, 2 and 4 were conducted for 23 days while System 3 which included the soil and groundwater was run for 40 days. On Day 24, System 3 was spiked with a methanol, ethanol and isopropanol groundwater solution (Table 2). Tert-butyl alcohol and 2, 4-dimethyl-3-pentanol were not included in the spike alcohol solution since degradation of these alcohols was not observed for these alcohols in the previous 23 days; the purpose of the spike was to compare degradation rates for the alcohols before and after spiking and demonstrate that microbial acclimation in the first days accounted for prompt degradation at similar rates after the spike. From Day 23 to Day 40, samples were taken every 24 hours and prepared for GC analysis.

Series B (O2 limited) batch equilibrium tests employed 8 separate batch reactors. The same suite of alcohols was used for both Series A and B. Approximately 7.5 g Fisher AC was added to 100 g NASA groundwater and then spiked with the alcohol solution. Table 5 shows the preparation for spiking the activated carbon and water solution. To minimize dissolved oxygen, the system was then degassed with helium before alcohol tracer addition. Dissolved oxygen measurements ranged from 1.8 to 2.1 mg/L. Each of the 8 systems (Table 6) was spiked with 300 µL alcohol solution, then septa sealed and shaken for several hours. After the systems settled for 24 hours, initial samples were taken for analysis. System 6 was prepared with silver-impregnated AC (Barnesbey Sutcliffe Type 989 12 × 30 0.026% metallic silver). A dose of 25 mg lactate was added to System 3 as a nutrient (approximately 100 mg/L as carbon). Lactate was added since it had been originally chosen to biostimulate the microbes at LC34 prior to PFM field test deployment; ethanol was the ultimate choice for field site biostimulation.

A 1 mL Teflon syringe was used to sample through septa sealed systems into 0.4 mL sample vials, which were then allowed to settle several hours. To avoid possible carbon contamination of the GC equipment, the settled 0.4 mL samples were then subsampled again into 0.2 mL GC vials for chromatographic analysis. System 2 was maintained at 15 ºC to simulate a representative cool aquifer environment.

Column studies

Sand column preparation. Two columns (2.5 cm × 14 cm Kontes chromatography column) were incrementally packed with NASA sand/soil and NASA site groundwater. The caps were screened on both ends with fine mesh and a coarse mesh anodized steel screen. Vibration of the soil increments was

Table 4. Series A acclimation times and degradation rates.

	Alcohol	Acclimation time, days	Est. 0 order K mg-L^{-1} day^{-1}	Est. 1st order K day^{-1}
Series A System 3	Methanol ethanol IPA TBA	16 8–9 9 –	25 25 4 –	1.0 0.8 0.1 –
Series A System 3 **Day 24 spiked** with alcohol tracers	Methanol ethanol IPA TBA	0 0 0 –	25 25 4 –	1.0 0.8 0.2 –

Table 5. Series B alcohol solution preparation, solution concentrations.

	Tracer solution preparation	Concentration mg/L
Series B	40 µL methanol	350
	40 µL ethanol	350
	80 µL isopropyl alcohol	700
	80 µL tertiary butyl alcohol	700
	80 µL 2,4-dimethyl-3-pentanol	700
	100 mL NASA Groundwater	

performed to improve packing characteristics. NASA groundwater was filtered with 0.45 µm mesh paper and used for influent to the control column. For the sand column influent, the filtered groundwater was spiked with ethanol at 280 mg/L to mimic field site application of ethanol (the field site had an induced pump gradient). This alcohol/groundwater solution was then pumped through the column at approximately 0.1 mL/min to simulate NASA field site ethanol flushing of the bioaugmentation site. The second column, the control column, was flushed with ethanol-free groundwater at the same rate. (Two set-ups/2 pumps were run simultaneously.) These columns were flushed for 9 days to provide adequate time for microbe acclimation, based on the previous batch experiments. The effluent from the experimental soil column was monitored daily by gas chromatographic analysis to assess ethanol concentration level. At day 9, none was detected confirming the hypothesis that bacteria were now acclimated and degrading ethanol within the sand column. The temperature for all components was approximately 25 °C.

Activated carbon column preparation. An alcohol solution was prepared with 45 mL methanol, 45 mL ethanol, 90 mL isopropanol, 90 mL tert-butyl alcohol and 45 mL 2, 4-dimethyl-3-pentanol. After mixing the alcohols, 2.09 mL of this solution was added to 400 mL tap water in an 800 mL glass teflon-capped bottle. This was done to match field PFM activated carbon preparation and resulting alcohol concentrations. A quantity of 100 g Fisher activated carbon (Columbus, Ohio) was added to this water/alcohol solution and rotated for 24 hours. The equilibrated carbon was then drained and incrementally packed and vibrated into two smaller-scale glass columns (2.5 cm × 5 cm Kontes chromatography column). To most closely match field condition inflow to the PFM, these columns were then connected to the effluent of the soil columns; the pumping rate of 0.1 mL/min was maintained through both set-ups. Table 7 outlines the experimental design, and Figure 2 shows a schematic of the prepared AC column.

Calculations of water flux were made according to column volumes and pumping rates. The respective volumes of the NASA soil column and smaller AC column were 275 mL (5 cm diameter, 14 cm length) and 25 mL (2.5 cm diameter, 5 cm length). Given a pumping rate of 0.1 mL/min or 144 mL/day and an effective porosity of 0.4 for the soil/sand column, the pore volume rate was 1.2 pore volumes/day. The AC column volume and porosity resulted in a rate of over 10 pore volumes/day. Darcy flow through the smaller column was 28.8 cm/day. The LC34 site Darcy flow was 7.62 cm/day.

Table 6. Series B system set up.

System 1	System 2	System 3	System 4
7.5 g "dry AC" 100 g NASA GW 0.6 g NASA soil Alcohol spiked 22–23 °C	7.5 g "dry AC" 100 g NASA GW 0.6 g NASA soil Alcohol spiked 15 °C	7.5 g "dry AC" 100 g NASA GW 0.6 g NASA soil 500 µL Lactate soln. Alcohol spiked 22–23 °C	7.5 g "dry AC" 100 g NASA GW 0.6 g NASA soil Alcohol spiked 22–23 °C
System 5	**System 6**	**System7**	**System 8**
100 g NASA GW Alcohol spiked 22–23 °C	7.5 g "dry silver AC" 100 g NASA GW 0.6 g NASA soil 500 µL lactate. Alcohol spiked 22–23 °C	100 g NASA GW 0.6 g NASA soil Alcohol spiked 22–23 °C	Control 100 g deionized H_2O Alcohol spiked 22–23 °C

Table 7. Experimental design of column studies.

Step 1	Flush sand column (5 cm × 14 cm) packed with NASA soil (sand) with ethanol/NASA groundwater solution. Flush for 9–10 days to allow microbial acclimation time. Monitor effluent to confirm tracer degradation. At same time, flush second column packed with NASA sand with NASA groundwater only, no ethanol. Also flush for 9–10 days. Monitor effluent.
Step 2	Attach small AC column (2.5 cm × 5 cm) packed with tracer equilibrated Fisher activated carbon to each large NASA sand column (one is flushed with ethanol solution). Monitor effluent daily to assess tracer mass loss. Continue using ethanol/groundwater solution to flush columns. Use groundwater only to flush second set of columns.
Step 3	At conclusion of each run, sample AC column at "front, middle, end" (10 mg samples). Extract alcohol mass using isobutyl alcohol.
Step 4	Repeat step 2 substituting silver-impregnated AC for Fisher AC.

Immediately after the smaller AC columns were attached to the sand columns, samples of effluent from the AC columns were collected every 12 hours for several days in order to assess alcohol concentrations. The pumping rate was lowered to 0.02 mL/min to more accurately match LC34 groundwater velocity. The samples were collected in 0.5 mL inserts for 2 ml GC vials. All samples were analyzed using a Perkin-Elmer Gas Chromatograph (GC) equipped with automated liquid injection and FID.

Prior to construction of each AC column, the activated carbon was sampled to establish initial concentrations of the sorbed resident alcohols. These concentrations were used in subsequent calculations to ascertain the relative mass of each alcohol remaining on the activated carbon following a period of exposure to flow in the column. Sampling of the carbon at the conclusion of each run involved extracting the activated carbon with isobutyl alcohol (IBA). From the extract, all alcohols, including 2, 4-DMP were analyzed using a Perkin-Elmer Gas Chromatograph (GC) equipped with automated liquid injection and FID. The sand column was then eluted with water at a flow rate of 0.5 mL/min. Frequent volumetric measurements were taken to develop plots of cumulative elution volume versus time. Whenever the eluent volume was measured, a sample was collected and analyzed to assess transient changes in dissolved concentrations of all resident alcohols.

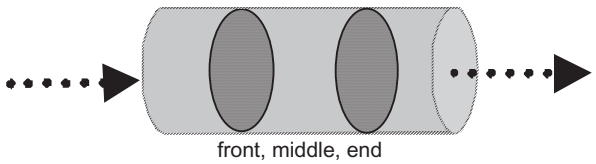

Figure 2. Schematic of smaller activated carbon column, showing three sampling sections for mass extraction.

At the end of the experimental run, mass was sampled from front/entry, middle and end/exit sections of the AC column (Fig. 2). This was done to determine spatial differences in alcohol mass remaining on the Fisher activated carbon as a result of flow from the front to the end of the column. The experiment was run for a 9.8 pore volume flush at 0.02 mL/min.

Given the actual site groundwater velocity, another experiment was prepared with the lowered pumping rate of 0.02 mL/min. This experiment then compared the effect of silver AC versus Fisher AC on alcohol degradation. Silver-impregnated carbon was substituted for the Fisher carbon in the second column; the subsequent tests ran at a pumping rate of 0.02 mL/min for approximately 9.8 pore volumes and 17.5 pore volumes for the Fisher AC column and 20.5 pore volumes for the silver-impregnated AC column. Initial concentrations of sorbed alcohols were again measured; samples of effluent were collected for GC analysis. At the end of the each experimental run (9.8, 17.5 pore volumes for Fisher AC column and 20.5 pore volume flush for Silver AC column), alcohol mass was again sampled from front/entry, middle and end/exit sections of the Fisher AC column and the silver AC carbon.

Results and Discussion

Preliminary Series A. Systems 1, 2 and 4 showed no degradation activity whereas System 3 with added soil exhibited alcohol degradation (Fig. 3). Figure 4 shows results of spiking the System 3 on day 24. Table 4 shows estimated zero order and first order degradation rates obtained from linear and log scale plots along with estimated acclimation time for microbial growth. In System 3, alcohol degradation initiated in the following order: 1) ethanol, 2) methanol

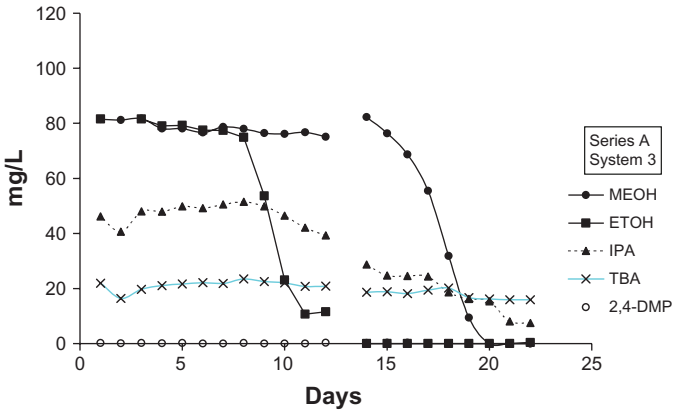

Figure 3. System 3 alcohol concentration as function of time. 50 g wet AC 300 g site ground water 2 g site soil.

and then 3) isopropanol. This order was expected as the ethanol has preferential degradation over methanol. As ethanol becomes less available, the bacteria then utilizes methanol. Researchers[56,57] confirmed in laboratory studies that ethanol is considerably more readily available as a carbon source than methanol. Moreover, a shorter adaptation time was needed. Experiments conducted showed that sludge once adapted to ethanol[58] shows an increased activity toward other organic materials such as methanol and acetic acid. System 3, after the alcohol spike on day 24, yielded identical degradation rates for ethanol and methanol with no acclimation time required.

Series B. The Series B batch experiment employed 7 separate batch reactors using Fisher activated carbon. System 6 was prepared with silver-impregnated carbon. The degradation activity shown for System 1 with AC and soil (Fig. 5) followed expected results with ethanol and methanol degrading initially followed by isopropanol within a 50 day time period. Acclimation

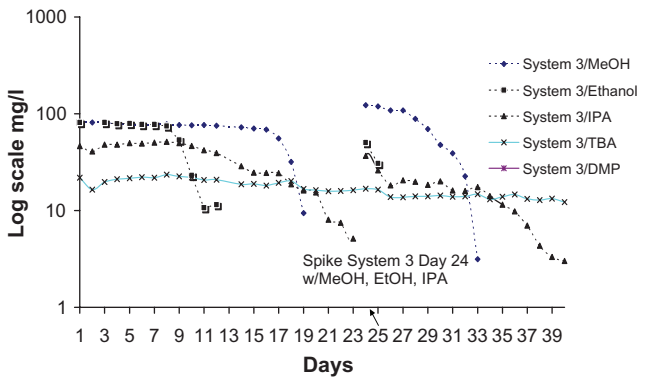

Figure 4. Series A System 3 spiked Day 24, alcohol concentration as function of time.

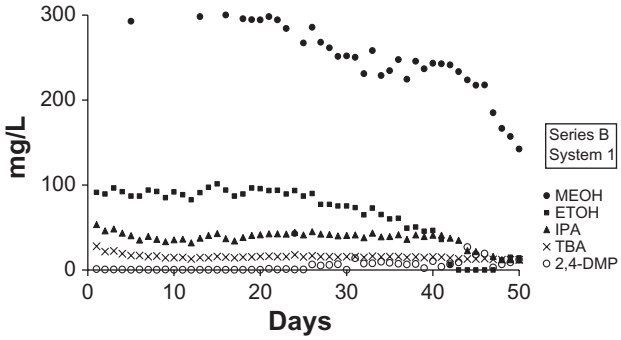

Figure 5. Series B System 1 alcohol concentration with time. 7.5 g dry AC,100 g NASA GW, 0.6 g NASA soil.

times were significant and ranged from 25 to 42 days. Tert-butyl alcohol did not degrade. System 2 (Fig. 6) which was identical to System 1 but held at 15 °C showed no degradation activity. This was interesting since this was the average groundwater temperature at Cape Canaveral at the time of our PFM deployment and is evidence that colder temperature groundwater would inhibit degradation. System 3 (Fig. 7) which was identical to Systems 1 and 2, but included an additional 25 mg lactate in 500 µL solution as nutrient addition, followed expected results with ethanol and methanol degrading initially followed by isopropanol within a 50 day time period. Tert-butyl alcohol did not degrade (this lactate addition was included in the experiment since the LC34 bioaugmentation site design originally called for the lactate amendment). It is interesting that with the lactate addition the acclimation times for ethanol and iso-propyl alcohol were 30%–40% shorter (23 and 30 days respectively). Highly sorbed 2,4-DMP remained at very low concentrations. This was confirmed by extraction of the alcohols remaining on the AC after the experiment was concluded. System

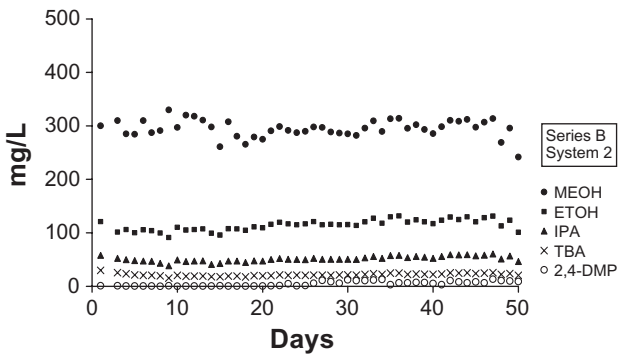

Figure 6. Series B System 2: alcohol concentration with time 15 °C. 7.5 g dry AC, 100 g NASA GW, 0.6 g NASA soil Alcohol spiked.

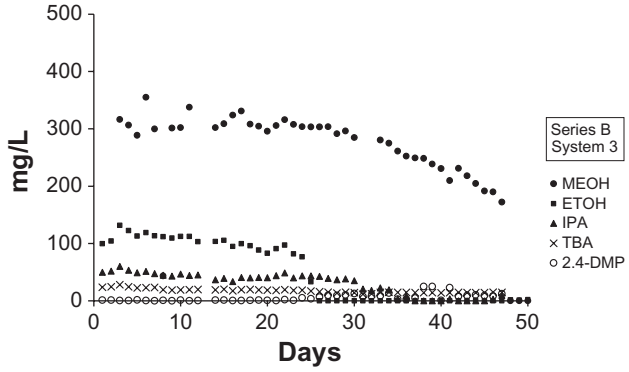

Figure 7 Series B System 3 alcohol concentration with time. 7.5 g dry AC,100 g NASA GW, 0.6 g NASA soil, 500 µl Lactate/alcohol spike.

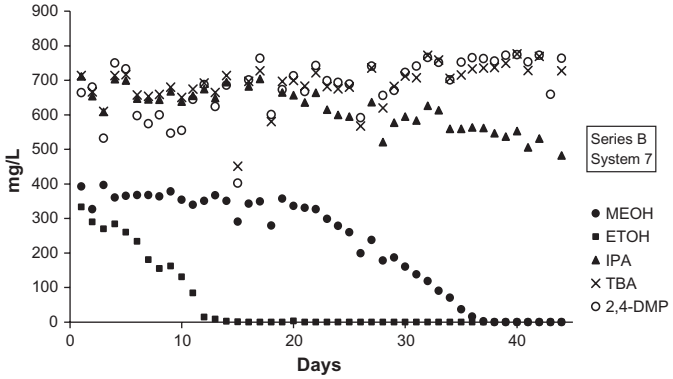

Figure 9. Series B System 7 alcohol concentration with time. 100 g NASA GW, 0.6 g NASA soil, alcohol spike.

4 included AC but no soil and showed no alcohol degradation. System 5 included neither AC nor soil, but did exhibit degradation of ethanol with a much shorter 1–2 day acclimation time. For a 50 day period, System 6 (Fig. 8), prepared with silver-treated carbon, soil and amended with lactate (identical to System 3 but prepared with silver AC), exhibited no degradation, an apparent inhibition of microbial activity due to the silver ion. Alcohol degradation occurred in System 7 (Fig. 9); this system was prepared with soil and an alcohol spike (no "dry" AC included). It is interesting that this system showed a much shortened 1–2 day acclimation time for ethanol degradation. More study is needed to determine how the presence of AC affects microbial acclimation. System 8 (control) showed no activity.

Table 8 compiles the estimated zero order, first order degradation rates obtained from linear and log scale plots with estimated acclimation times for microbial growth.

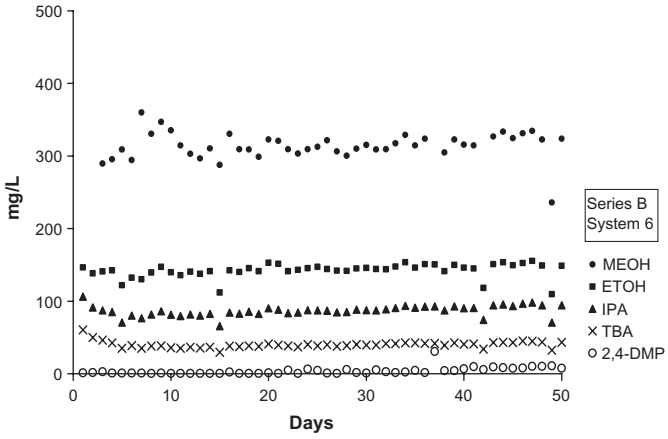

Figure 8. Series B System 6 alcohol concentration with time. 7.5 g silver AC, 100 g NASA GW, 0.6 g NASA soil, 500 µl lactate., alcohol spike.

Column Studies. Several trial runs were necessary to determine the appropriate length of run (number of pore volumes) and pumping rate. Several set-ups compared effluent concentrations and extracted alcohol mass remaining on the AC column after disassembly. Initial pumping rates ranged from 0.02 mL/min to 0.5 mL/min. The rate of 0.02 mL/min was selected in order to compare with LC34 site groundwater velocity. Calculated pore volume flushes ranged from about 10 to 43 pore volumes. This assured a measurable alcohol removal for comparison to typical aquifer groundwater flux rates at PFM field sites.

The alcohol mass remaining on the activated carbon, after a recorded number of pore volumes was pumped, was expressed as a concentration per unit AC mass and then calculated as a ratio of the original mass concentration. The front, middle, and end section alcohol mass ratios for the AC column through which only groundwater flowed were compared to the AC column sections through which groundwater and ethanol flowed (GW/ethanol). For the specific 9.85 pore volumes flush at 0.02 mL/min, Figure 10 compares the tracer alcohol mass remaining on both the experimental GW/ethanol influent Fisher AC column and the groundwater only influent (control) Fisher AC column. This mass ratio, concentration/original concentration (C/C_o), was calculated for each activated carbon column by section: 1) front/entry, 2) middle, and 3) end/exit. For the isopropanol and tert-butyl fractions, there is no significant difference for mass measured in front/entry, middle and end/exit sections. However, there is a significant difference in ethanol mass remaining on the groundwater/ethanol influent AC column compared to the groundwater

Table 8. Series B acclimation times and degradation rates.

Series B	Description	Alcohol	Acclimation time, days	Est. 0 order K mg-L^{-1} day^{-1}	Est. 1st order K day^{-1}
1	AC NASA GW/soil alcohol spiked 22–23 °C	Methanol ethanol IPA TBA	25 40 42	4 20 5	0.01 1.8 0.15
2	AC NASA GW/soil alcohol spiked 15 °C	Methanol ethanol IPA TBA	No activity		
3	AC NASA GW/soil alcohol spiked lactate 22–23 °C	Methanol ethanol IPA TBA	25 23 30	5 25 5	0.02 1.0 0.01
4	AC NASA GW alcohol spiked 22–23 °C	Methanol ethanol IPA TBA	No activity		
5	No AC NASA GW/ alcohol spiked 22–23 °C	Methanol ethanol IPA TBA	35	12	1.0
6	Silver AC NASA GW/soil alcohol spiked lactate 22–23 °C	Methanol ethanol IPA TBA	No activity		
7	No AC NASA GW/soil alcohol spiked 22–23 °C	Methanol ethanol IPA TBA	20 1–2 35	20 20 7	0.8 1.0 0.01
8	No AC Deionized water alcohol spiked 22–23 °C	Methanol ethanol IPA TBA	No activity		

only column. The loss on the groundwater/ethanol influent column is about 10 times greater than the groundwater only influent column.

To assess the effect of silver-impregnated AC on alcohol degradation, Figure 11 shows the results of ethanol tracer mass remaining on the silver-impregnated AC column extracted after 20.5 pore volumes. This is plotted and compared to that on Fisher activated carbon, extracted at 9.8 and 17.8 pore volumes (another experiment was run with silver-impregnated AC at 43 pore volumes; no ethanol remained on AC after flushing). The GW/ethanol influent column results, for the two runs at 9.8 and 17.8 pore volumes, both using Fisher activated carbon, show large decreases in tracer ethanol mass. However, the GW/ethanol influent silver-impregnated AC middle and end sections show significantly less reduction compared to the groundwater only influent, silver-impregnated AC sections.

Figure 12 shows laboratory photos of the experimental set up. The leftmost photo shows a black substance appearing at the entry to the sand column receiving the ethanol influent; the control sand column maintained its original color throughout the experiment. This color change suggested significant bacterial-growth and strong reducing conditions. Indeed, the results for silver-impregnated AC show that the ethanol mass remaining on the extracted (GW/ethanol influent) AC column is significantly higher when compared to the Fisher activated carbon used as the sorbent. However, there is still a significant reduction in ethanol mass in the front and middle experimental sections (94% and 51% respectively) of the silver-impregnated AC column; only in

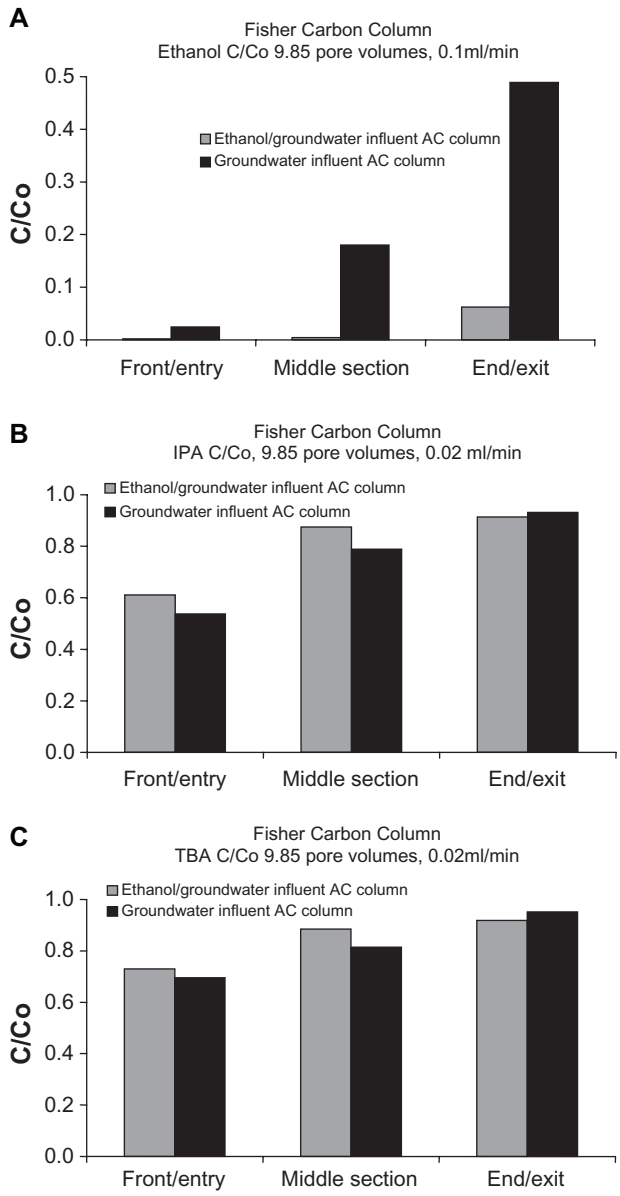

Figure 10. C/C_o comparison of alcohol fractions according to column section, 9.85 pore volumes, 0.02 mL/min. A) Ethanol. B) Isopropanol. C) Tert-butyl alcohol.

the end/exit section does all mass remain (compared to the groundwater only, control column). It is interesting that the front entry section shows a 94% reduction in mass for ethanol and apparently no protection from the silver. This may be due to the silver ion flushing out, resulting in silver ion concentrations too low to be bactericidal. However, the middle section had only about a 50% reduction in ethanol mass whereas the end section showed a slight increase in mass compared to the control. Thus, the end/exit section of the silver-impregnated AC column appears to have the most protection from bacterial degradation.

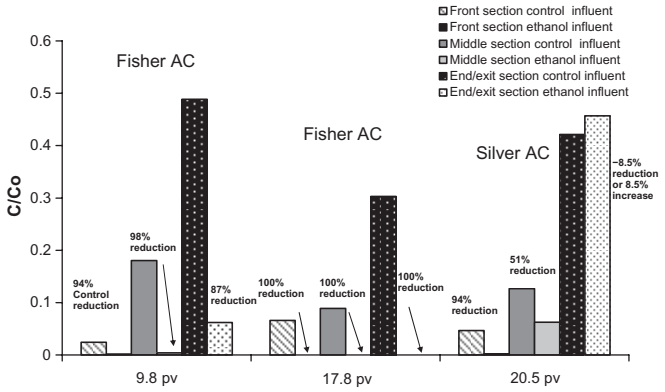

Figure 11. C/C_o comparison of ethanol mass remaining on Fisher AC and silver-impregnated AC after specified number of pore volumes. Control signifies flushed with groundwater only.

A white substance was observed on the entry part of the AC column upon unpacking of the column sections. This biofilm-type substance may protect the bacteria from inhibitory activity of the silver ion. Also, this biofilm may lower the silver ion concentration that is accessible to the bacterial membrane. In the middle section, there appeared to be a smaller amount of this substance; and therefore it is hypothesized that silver ion was better able to transport to the bacterial membrane. Measured effluent concentrations and mass remaining on extracted AC confirm that the GW/ethanol influent to the AC column enhances degradation when compared to an influent of only groundwater. Observations suggest that that the GW/ethanol influent biostimulates the microbes so that they are growing and

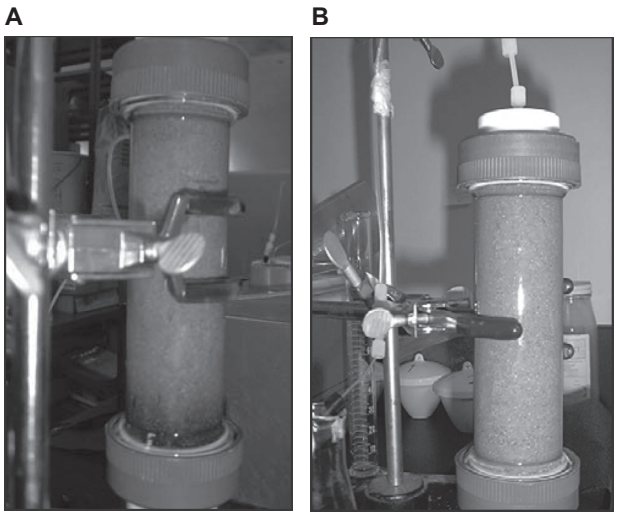

Figure 12. Photos of sand columns: A) LC34 sand column, GW/ethanol influent, black substance at the entry to the column. B) LC34 sand column, groundwater only influent.

well-acclimated for ethanol degradation within the activated carbon column. Results show that this additional growth may compromise the integrity of the silver ion that is impregnated on the activated carbon by the development of a biofilm. Further research is needed to evaluate biofilm impacts which may also include short circuiting of flow through the column.

Conclusions

The laboratory batch studies confirmed field site potential for alcohol degradation in the PFM. The batch studies also confirmed that microbial acclimation lag time is required for initiation of alcohol degradation; this could affect PFM groundwater flux estimates depending on the length of time the PFM is in place prior to removal and extraction of alcohols. In an effort to reduce or inhibit microbial activity and alcohol biodegradation, the properties of silver-impregnated activated carbon were evaluated in both batch and column studies. The silver-impregnated carbon batch system with limited oxygen exhibited no alcohol degradation.

Results from column studies comparing silver-impregnated carbon to Fisher carbon, as a sorbent for alcohol "tracers" exposed to a water flux, revealed that a biofilm could develop that could protect the degrading bacteria from the silver's inhibitory effect. The water flux through the column could be impacted by this microbial colonization and biofilm polysaccharide substance. The GW/ethanol influent to the sand column appeared to enhance bacterial activity and resultant tracer alcohol degradation in the connected AC column. Additionally, although silver appears protective to tracer integrity, this inhibitory activity may be either depleted in a highly bioactive environment or silver ion may be flushed from the AC surface. Further research is needed to understand this phenomenon; the silver ion may be in lower concentrations, or in a reduced form in certain environments or may develop resistance to the silver ion.

Disclosures

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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