RPI'S CAT 100 SUCCESSFULLY TREATS 1,4-DIOXANE AND CVOCS

ABSTRACT

Many sites exist that are contaminated with a mix of compounds that do not lend themselves to a single technique for remediation. CVOC impacted sites that are also impacted by 1,4-dioxane are notoriously problematic, and the best approaches mandate multiple remedies and exacerbated costs. RPI investigated the potential of CAT 100 as a single-step remedy for such sites. Step 1 involved screening the CAT consortia to verify it contained microorganisms capable of using 1,4-dioxane (1,4-D) as a sole source of carbon. The second phase was bench testing with groundwater from a candidate site, and the last step is proving the efficacy in a pilot test at a site in New Jersey. CAT 100 is granular activated carbon that is impregnated with metallic iron combined with nutrients, substrate, and a proprietary consortium.

PRELIMINARY WORK TO SCREEN RPI BACTERIA FOR ABILITY TO GROW ON 1,4-DIOXANE

RPI engaged an outside biological laboratory to screen the individual strains in our Trap and Treat CAT 100 blend of microorganisms to look for organisms with the ability to use 1,4-D as the sole food source supporting growth. The typical protocol is to streak the individual strains onto petri dishes containing non-nutrient agar (NNA). These NNA "only" plates contain no organic material which can be easily used to sustain growth. Tiny amounts of macro- and micro-nutrients were added to the agar to provide mineral nutrients essential for metabolism.

These NNA "only" plates were incubated in a desiccator jar, containing 2ml of 1,4-D which evaporates and contaminates the plates, as shown in Figure 1. After one week an additional 2ml of 1,4 D was added. Growth on the surface of these plates demonstrates the ability of those microbes to use the compound as the only carbon and energy source for their growth. These "vapor incubation" plates were used to indicate which strains were presumptive degraders.

The laboratory observed a total of twelve strains that displayed growth on the media using the protocol described. Based on this activity, RPI is confident that its consortia are capable of degrading 1,4-D under typical site conditions. Although RPI's CAT 100 has been used at numerous CVOC sites, 1,4-D has not been a contaminant of concern so data collection on this compound has been sparse.

PROTOCOL FOR THE BENCH TESTING

The objective of this study is quite simple. It is designed to answer the question, "Can CAT 100 successfully degrade 1,4-D"? In this case, it is presumed that degradation is occurring if the dissolved 1,4-D concentration trends down over time, taking into consideration the initial effects of adsorption by activated carbon added in the form of BOS 100®. Additional testing is planned to identify degradation by products to verify assimilation of 1,4-D.

TREATMENTS PROPOSED

The study includes two control samples along with various treatment compositions that are variations of the CAT 100 technology.

- F 400 Carbon Control – This is the granular activated carbon used to manufacture BOS 100 and will gauge losses due to sample handling and provide data relevant to adsorption of the target contaminants.
- BOS 100 Control – This is F 400 granular activated carbon impregnated with metallic iron. The material is roughly 6.7% (wt.) metallic iron. This product was first introduced by RPI in 2003 and was designed to rapidly degrade halogenated organic compounds through abiotic dehalogenation to non-toxic hydrocarbons.
- CAT 100 treatment takes advantage of the synergy between BOS 100 and biological enhanced reductive dechlorination (ERD). Food-grade starch is the substrate added to support ERD related biological activity.
- CAT 100 + FAC treatment is identical to regular CAT 100 with 200 mg of ferric ammonium citrate added as a supplemental nutrient.
- CAT 100-PF treatment is identical to regular CAT 100 except the starch has been replaced with food-grade pea fiber.
- CAT 100-PS treatment is identical to regular CAT 100 except now the substrate is 50% pea fiber and 50% starch.

All test vials were prepared in 150 ml serum vials with crimp septa seals. Examples are shown in pictures 1 and 2. Trace metals and nutrients were added to all vials, including controls. Substrate, yeast extract, and standard blend of CAT 100 microbes were added to all treatment vials. In general, 1-gm of substrate, 0.5-gm yeast extract, 0.5-gm BOS 100 (or F 400 carbon), and 2 ml of bacterial concentrate were added to test vials.

SAMPLING FREQUENCY

A set of seven vials were prepared for each control or treatment. Because BOS 100 will rapidly reduce COC concentrations due to adsorption, and there will also be measurable degradation of chlorinated ethanes/ethenes, a sample must be taken early on to establish a pseudo-baseline post-adsorption condition. Consequently, the first sample was taken after four hours of contact.
The next proposed sampling events were at: 3 days, 1 week, 2 weeks, 1 month, 1.5 months, and 2 months.

ANALYTICAL TESTING

The study will be conducted with groundwater collected from one of the "hot" wells from a site in NJ. RPI plans to analyze study samples for the following parameters.

1. VOCs using method 8260B
2. Headspace gases – RSK 175
3. Anions – 300.1 Ion-Chromatography
4. Ammonia – Ion-Chromatography (cationic) ASTM D6919-17
5. pH - SM 4500

In addition to tracking changes in concentration of the CVOCs present, RPI will also be looking at degradation by products of the CVOCs and indications of abiotic and biotic degradation pathways. Detection of low molecular weight hydrocarbon gases including methane, ethane, ethene, and carbon dioxide will assist with identification of these pathways.

Because all other VOCs were readily determined by method 8260B, it was desirable to develop the method to also measure 1,4-D at as low a concentration as possible. 1,4-D is infinitely soluble in water and does not perform well using purge and trap techniques. A modification of the method was developed that combined purging a 20 ml sample and adding 5 to 6 grams sodium sulfate to saturate the sample, increasing its ionic strength considerably. This improved method performance and enabled a method detection limit of 5 ppb.

Monitoring of standard inorganic anions such as nitrate and sulfate will be included along with a suite of volatile fatty acids. These organic anions are windows into biological activity and indicators of the CAT effect.

GROUNDWATER COMPOSITION (PPB)

1,1,1-TCA – 25,960
1,1-DCE – 13,020
1,1-DCA – 200
TCE – 102
1,4-D – 120

Groundwater was received in 1-liter bottles and kept under refrigeration. The day prior to assembling controls and treatment vials, ten bottles of GW were composited into a 10-liter Cali-Bond sample bag. Samples were withdrawn for analysis to establish baseline conditions. After analysis, sufficient 1,4-D was spiked into the bag to raise the baseline concentration by 1 ppm.

SUBSTRATE AND YEAST EXTRACT

Preliminary development of CAT 100 was performed using food-grade starch. This material was chosen as it is available everywhere and very inexpensive. Further, starch is not very soluble in water and tends to ferment very slowly, providing a time-release source of sugars. The downside of starch is that it is simply a polymer of sugar; it has no nutritive value whatsoever. Yeast extract is rich in protein, containing essential amino acids and vitamins such as cobalamin. Pea fiber consists of usable carbohydrate, protein, and dietary fiber.

Pea fiber has substantial nutritive value and ferments even more slowly than starch because of its fiber content. It is for these reasons that RPI included this material in the study, as it may provide the time-release source of small organic compounds that can be beneficially utilized to degrade site COCs and provide essential nutrients supporting cell growth.

DISCUSSION OF RESULTS

Sets of vials were assembled, two at a time so that the fourteen vials could be placed onto a rotary mixer and continuously mixed for two to three hours. This is a short enough time that no significant biological activity had begun and reductions in compound concentrations are due to adsorption by the activated carbon or BOS 100. After this first phase is over, vials were placed in an incubator maintained at a temperature of 30 degrees C. The vials are managed in trays as shown in Picture 2 and can be manually inverted to mix the contents a couple of times each day.

Controls 1,4-D

Figure 2 details the change in concentration of 1.4-D in controls and CAT 100 treatment over a period of 124 to 207 days. As expected, there is a rapid drop in concentration due to adsorption by the carbon. Concentration then trends down slowly, approaching an equilibrium that appears to be relatively stable.
There are no known pathways for degradation of 1.4-D available by granular activated carbon or the BOS 100, and the same activated carbon makes up both materials. The BOS 100 is far more interesting as in this case we know that the CVOCs are not only adsorbed but are rapidly being degraded as well. This is evident in the data as CVOC concentrations continue to fall with clear differences from the F-400 control even within just three weeks. The more important result is that BOS 100 appears to be doing more than simply adsorbing the 1,4-D.
The BOS 100 used in the bench test was prepared from F-400 carbon in RPI's lab and contains approximately 6.5% (wt.) metallic iron residing in the micro-pores of the carbon. This means BOS 100 has less active carbon surface area and would perform slightly less effectively than the F-400 when it comes to simple adsorption. We would expect the 1,4-D concentration persisting in the BOS 100 only vials to be roughly equal to that in the F-400 or a bit higher, perhaps close to 300 ppb. Inspection of the data demonstrates that within the first three weeks of contact, there has been over a 92% reduction in the 1,4-D concentration.
This strongly suggests 1,4-dioxane is interacting with the metallic iron in addition to simple sorption stemming from the carbon of BOS 100.

FIG 1.

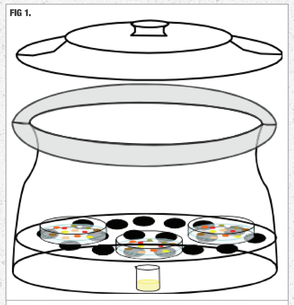

FIG 2. 1,4-DIOXANE PLOTS CONTROLS VS CAT 100 TREATMENT

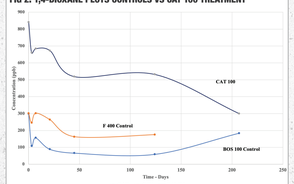

FIG 3. 1,4-DIOXANE PLOTS CONTROLS VS CAT 100 + FAC TREATMENT

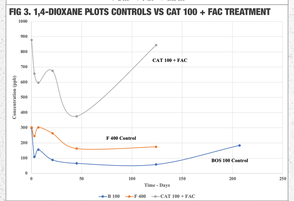

FIG 4. 1,4-DIOXANE PLOTS CONTROLS -VS- CAT 100-PS TREATMENT

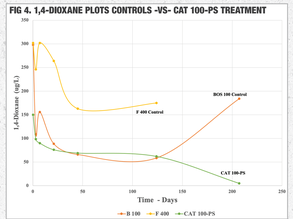

FIG 5. CAT 100-PS VS BOS 100 CONTROL

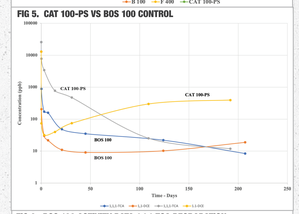

FIG 6. BOS 100 ACTIVITY DATA 1,1,1-TCA DEGRADATION

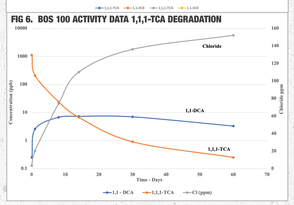

FIG 7. CARBON DIOXIDE (PPM)

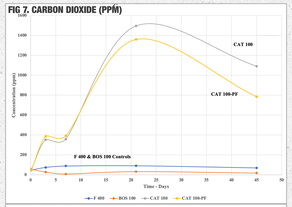

FIG 8. PLOT OF ACETATE ACROSS FOUR TREATMENTS CAT 100, CAT 100-PF, CAT 100+FAC, CAT 100-PS

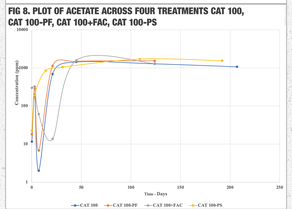

FIG 9. PLOT OF SULFATE ACROSS FOUR TREATMENTS CAT 100, CAT 100-PF, CAT 100+FAC, CAT 100-PS

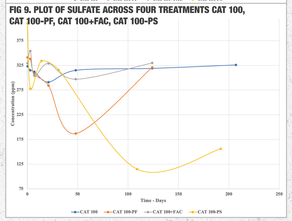

IMAGE 1.

IMAGE 2.

IMAGE 3.

DISCUSSION OF RESULTS CONT.

Treatments 1,4-D

Recalling that at time zero the concentration of 1,4-D is 1.12 ppm, there is a substantial difference between the rates of adsorption between CAT 100 and Controls. The CAT 100 is lagging far behind. Adsorption is a thermodynamic process and is exothermic for all organic compounds, so energy is released through adsorption. It is similar to a change in state.

The obvious difference between CAT 100 and the controls is the addition of food-grade starch, yeast extract, and a complex consortium of microorganisms. Even though solubility of the starch and yeast extract is limited, enough dissolves from the 1.5-gram addition to easily dwarf the 30 ppm of VOCs present in GW. A complex mixture results and there is competition for adsorption sites. This results in retardation of VOCs adsorption as observed. After just over two hundred days of contact, a 73% drop in 1,4-D was measured in the dissolved phase.

The heat of adsorption for 1,4-D is not high so it does not take much energy to displace this compound from sorption sites. In many of the treatment systems, there were initial drops in concentration followed by an increase. The most likely reason for this type of response is competitive desorption of 1,4-D resulting from the onset of vigorous biological activity and generation of substantial amounts of small molecules like acetic acid, butyric acid, and other fermentation byproducts including alcohols and various esters. A slight change in the treatment mixture can result in this type of behavior and is illustrated by data plotted on Figure 3 involving CAT 100 to which a small amount of ferric ammonium citrate had been added. This compound provides ammonia, iron, and citric acid which is a common type of nutrient for stimulating cell growth. However, in this case, the added citrate, combined with the on-going degradation of CVOCs and fermentation of the starch and yeast extract results in a system where 1,4-D is thermodynamically displaced from sorption sites within the CAT 100 carbon.

CAT 100 based on complete replacement of the starch with pea fiber results in a similar 1,4-D response. However, when the CAT is prepared from a 50:50 mixture of starch and pea fiber (CAT 100-PS), a quite different response is observed as shown in Figure 4. Initially, it tracks closely to that of the BOS 100 control. Instead of remaining flat or increasing, the 1,4-D concentration continues to fall to below detection limit.

Based on calculations using an isotherm provided by Calgon Carbon for 1,4-D and F-400 carbon, at equilibrium, 0.5 grams of carbon added to 150 ml of water containing 1.12 ppm 1.4-D, the concentration will be around 200 ppb. This is right in line with the concentration observed in the F-400 and BOS 100 controls but is clearly inconsistent with the 1,4-D falling below detection limit. The data strongly supports the presumption that 1,4-D is being degraded.

Treatment CVOCs

Principal CVOC contaminants in groundwater are 1,1,1-TCA and 1,1-DCE. Inspection of Figure 5 shows performance of CAT 100-PS with respect to these contaminants. Again, the decline in concentration of TCA lags the BOS 100 performance initially but catches up after about three months and TCA is far below the MCL of 200 ppb. The more interesting data is that related to 1,1-DCE.

Biological degradation of 1,1,1-TCA produces 1,1-DCA, CA, and ethane as daughter products while abiotic degradation can produce lesser amounts of 1,1-DCE, although this is a minor pathway. The only reasonable explanation for the rise in 1,1-DCE that began after about one week is that it is desorbing from the CAT 100. There is no known mechanism for significant generation of this compound through degradation of 1,1,1-TCA. Reaction of BOS 100 with 1,1,1-TCA is discussed in the next section.

BOS 100 Activity Test

Activity testing is a routine practice at RPI. The idea of activity testing is for rapid screening for reactivity of BOS 100 with specific compounds. Serum vials are assembled in a glove box with a measured dose of BOS 100 and 150 ml of reagent water and sealed. Removal from the glove box allows a measured dose of a specific compound like 1,1,1-TCA to be directly injected into the vial through the rubber septa while the vial is shaken. After specified periods of time, roughly 1 ml samples of the water can be withdrawn from the vial with a syringe for analysis of chloride. Generation of chloride is direct evidence of the ability of BOS 100 to degrade the chlorinated compound.

Figure 6 provides data from an activity test on 1,1,1-TCA. Five grams of BOS 100 was added to the serum vial along with 150 ml of reagent water. Then 30 mg of reagent grade TCA was injected into the vial. As expected, there is a rapid drop in concentration after 1-hour (over 99% drop). Generation of chloride begins quickly, and after sixty days of contact, 96% of the theoretical chloride has been generated, which is equivalent to 28.65 mg out of the original 30 mg being degraded. During that time, no 1,1-DCE was detected above 5 ppb and no significant 1,1-DCA was produced.

Based on this data, we know that 1,1,1-TCA is rapidly being degraded within the CAT 100-PS system and that no 1,1-DCE is being formed so it must be desorbing from the carbon. As degradation continues, this trend will reverse and 1,1-DCE will also be degraded.

EVIDENCE OF BIOLOGICAL ACTIVITY

Within the first week, internal pressure had built up in many of the serum vials to the point that venting was necessary. Regular mixing was required as biofilm formation tended to encase the BOS 100 and starch or pea fiber so contact with CVOCs were impeded. This is clearly shown in Picture #3 where the vial had been inverted and whitish slime has encased grains of BOS100. Shaking the vial easily broke this up so that effective contact with the aqueous solution and the grains of BOS 100 was restored, and microbes had more effective access to substrate and nutrient.

Carbon dioxide was the principal gas generated, and a typical plot of CO2 generation over time is shown on Figure 7. Controls (F-400 and BOS 100) remained flat over the course of the test, and this is not surprising as no microbes were added to these vials. However, gas generation in the CAT 100 and CAT 100-PF was substantial. At the temperature in the incubator (30 degrees C), solubility of CO2 is 2500 ppm. Although saturation was not observed, concentrations approaching saturation were common.

Fermentation of starch will produce alcohols, esters, and low molecular weight fatty acids like acetic, propionic, and butyric. Figure 8 displays acetate data over time on four of the treatment chemistries: CAT 100, CAT 100-PF, CAT 100+FAC, and CAT 100-PS. The pattern of generation is similar for all treatments. Initial generation followed by a rapid drop and then rising to level out at a concentration of between 1,000 and 2,000 ppm. The initial drop is likely due to microbial recognition of readily available food and exponential growth. The rate of acetate production outpaces the rate of assimilation, and, in the end, an equilibrium is attained where the rate of production is roughly equal to the rate of assimilation. This same pattern was observed with respect to the production of propionate and butyrate.

Plots of sulfate (Figure 9) over time across the same four treatments as above shows a wide range in response. In many of the treatments, sulfate remained flat with slight variation. Pathways involving oxidation using the sulfate oxygen are not active in these treatment chemistries as evidenced in the CAT 100 and CAT 100+FAC vials. Pea fiber elicited a somewhat different response. The CAT 100-PS treatment exhibited the strongest evidence of sulfate utilization as a terminal electron acceptor.

SUMMARY

This was a preliminary study to evaluate the potential of CAT 100 chemistries to effectively degrade both chlorinated solvents and the 1,4-dioxane often present in chloroethane impacts in a single treatment scenario. Several chemistries were evaluated, however, one in particular (CAT 100-PS) emerged as a potential candidate. Preliminary screening of RPI's blend of microbes demonstrated the presence of twelve strains capable of degrading 1,4-D. Exposing this blend to site groundwater with elevated CVOC concentrations along with over 100 ppb of 1,4-D with a specific blend of substrate and nutrient produced a result strongly suggesting degradation of 1,4-D and the CVOCs in a single treatment is possible.

NEXT STEPS

It is apparent that excessive biological activity interfered with normal functionality of activated carbon and BOS 100. This is supported by the lack of methane generation and excessive production of CO^2. Also, the lag in adsorption of CVOCs observed in all the treatment vials and excessive production of volatile fatty acids.

A follow up study is planned, capitalizing on what was learned in this initial effort. Dosing of substrate and yeast extract will be scaled back. Various ratios of starch, pea fiber, and yeast extract will be investigated to maximize rates of COC degradation. Once these variables are better defined, a pilot demonstration will be completed.