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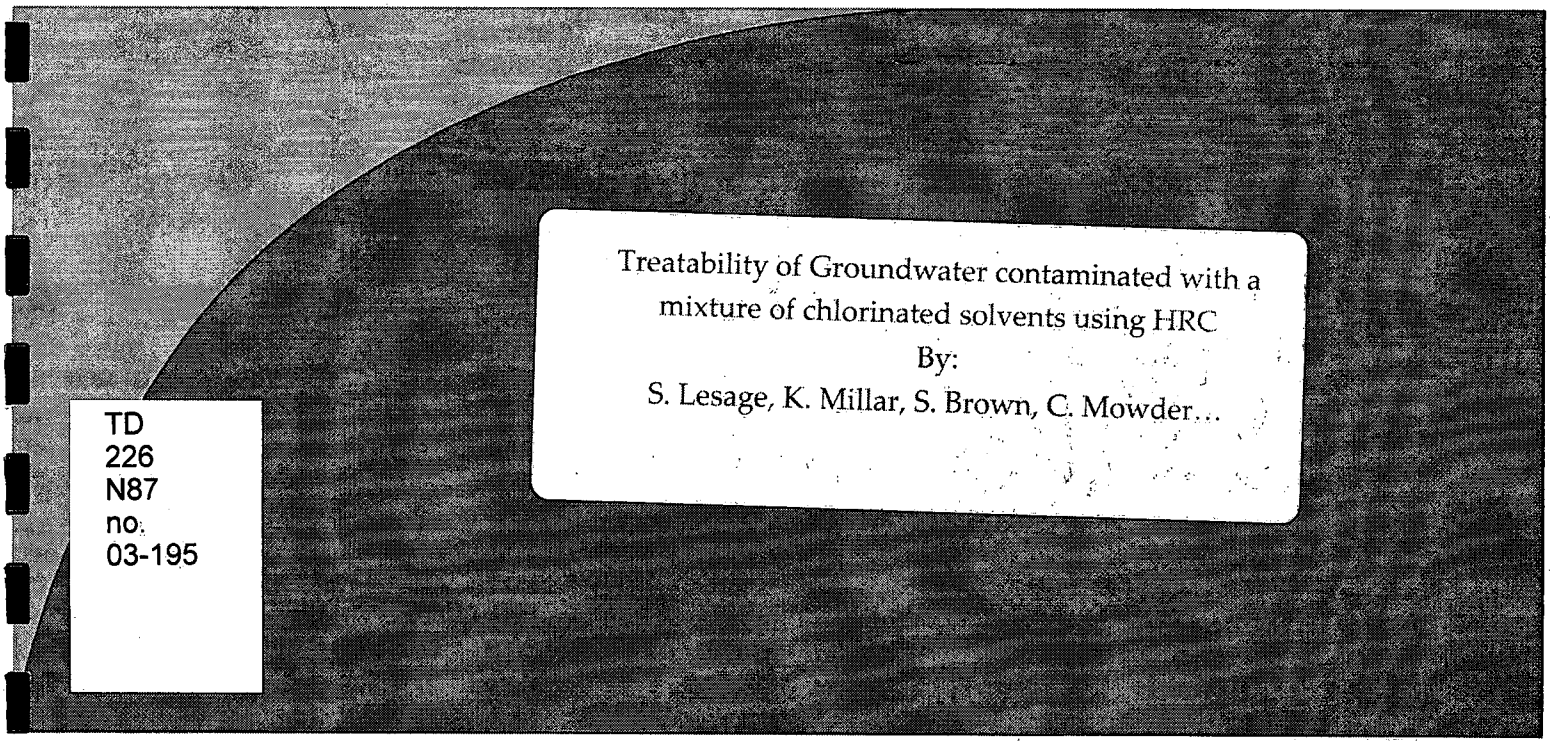

Treatability of Groundwater contaminated with a mixture of chlorinated solvents using HRC

By:

S. Lesage, K. Millar, S. Brown, C. Mowder...

03-195

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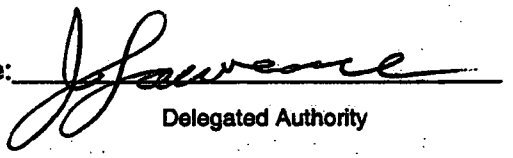
Signature: 

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Treatability of Groundwater Contaminated with a Mixture of Chlorinated Solvents Using HRC®.

Suzanne Lesage, Kelly Millar, Susan Brown, Carol Mowder, Don Green

Passive *in-situ* remediation techniques can be a very cost effective means of site remediation. Hydrogen Releasing Compound (HRC®) has been used successfully at many sites. However the contaminant at the vast majority of these sites was trichloroethene (TCE). Groundwater at Graces Quarters at Aberdeen Proving Ground was contaminated predominantly with a mixture of 1,1,2,2-tetrachloroethane (TeCA; 1,349 μg/L), carbon tetrachloride (CT; 221 μg/L), and TCE (171 μg/L) . Previous attempts at inducing reductive dechlorination with water and aquifer solids from the site and the addition of various carbon sources had achieved very limited success. A series of microcosms with aquifer solids and groundwater from the site were used to assess whether the addition of HRC® could effectively support the biodegradation of the mixture of chlorinated compounds. CT was degraded to chloroform (CF), which accumulated. There was no evidence of biological degradation of TeCA and a minimal amount of TCE degradation in these microcosms. There is evidence in the literature of the inhibition by CT and CF of the reductive dechlorination of tetrachloroethene. It is possible that this inhibition also affects the degradation of TeCA and TCE.

Keywords: Hydrogen release compound, HRC, 1,1,2,2-tetrachloroethane, carbon tetrachloride, trichloroethene, reductive dechlorination, lactic acid, inhibition

Traitabilité d'eaux souterraines contaminées par un mélange de solvants chlorés au moyen du HRC ®.

Suzanne Lesage, Kelly Millar, Susan Brown, Carol Mowder et Don Green

Les techniques d'assainissement passif *in situ* peuvent s'avérer un moyen très rentable pour décontaminer un site. Un composé libérant de l'hydrogène (HRC®) a été utilisé avec succès à de nombreux sites. Toutefois le contaminant présent, dans la grande majorité de ces sites, était le trichloroéthène (TCE). Les eaux souterraines au polygone d'essai d'Aberdeen, à Graces Quarters, étaient contaminées principalement par un mélange de 1,1,2,2-tétrachloréthane (TeCA; 1 349 µg/L), de tétrachlorure de carbone (CT; 221 µg/L), et de TCE (171 µg/L) . Des essais antérieurs, visant à induire une déchloration réductrice de l'eau et de la phase solide de l'aquifère du site par l'ajout de sources variées de carbone, ont connu un succès très limité. Une série de microcosmes composés d'eau souterraine et de la phase solide de l'aquifère du site ont été utilisés pour évaluer si l'addition de HRC® peut effectivement soutenir la biodégradation du mélange de composés chlorés. Le CT a été dégradé en chloroforme (CF), qui s'est accumulé. Nous avons détecté la dégradation d'une quantité minime de TCE, mais aucun signe de dégradation biologique du TeCA. Des études ont rapporté les propriétés inhibitrices du CT et du CF dans la déchloration réductrice du tétrachloroéthène. Il se peut que cette inhibition influe également sur la dégradation du TeCA et du TCE.

Mots clés: Composé libérant de l'hydrogène, HRC, 1,1,2,2-tétrachloroéthane, tétrachlorure de carbone, trichloroéthène, déchloration réductrice, acide lactique, inhibition

NWRI RESEARCH SUMMARY

Plain language title
Testing a polymer to promote biodegradation of chlorinated solvents in groundwater

What is the problem and what do scientists already know about it?
Adding a lactate polyester to groundwater provides a continuous release of lactic acid that can be used as a food source for bacteria in groundwater and promote the biodegradation of chlorinated solvents. The system has been widely promoted by the manufacturer, but only really tested with perchloroethylene and trichloroethylene.

Why did NWRI do this study?
We were asked by the US Army at Aberdeen Proving Grounds to run an independent test with soil and contaminated water from their site. The contaminant was a mixture of 1,1,2,2-tetrachloroethane, carbon tetrachloride and trichloroethene.

What were the results?
The only transformation was that of carbon tetrachloride to chloroform. Nothing else happened

How will these results be used?
These negative results were used for the Army in deciding upon the best remedial strategy for their site. It is important to disseminate the information to other potential users so they will be aware of the limitation of the product.

Who were our main partners in the study?
Arcadis Inc. and the US Army at Aberdeen Proving Grounds.

Sommaire des recherches de l'INRE

Titre en langage clair
Mise à l'essai d'un polymère favorisant la biodégradation des solvants chlorés dans les eaux souterraines.

Quel est le problème et que savent les chercheurs à ce sujet?
L'ajout d'un polyester d'acide lactique dans les eaux souterraines fournit un apport continu de nourriture (acide lactique) pour les bactéries vivant dans les eaux souterraines et favorise la biodégradation des solvants chlorés. Ce système a fait l'objet d'une promotion à grande échelle de la part du fabricant, mais n'a été testé seulement qu'avec le perchloréthylène et le trichloroéthylène.

Pourquoi l'INRE a-t-il effectué cette étude?
L'armée américaine au polygone d'essai d'Aberdeen nous a demandé d'effectuer un test indépendant avec le sol et l'eau contaminée de leur site. Le contaminant consistait en un mélange de 1,1,2,2-tétrachloroéthane, de tétrachlorure de carbone et de trichloroéthène.

Quels sont les résultats?
La seule transformation détectée a été celle du tétrachlorure de carbone en chloroforme. Aucune autre transformation chimique ne s'est produite.

Comment ces résultats seront-ils utilisés?
L'armée s'est servie de ces résultats négatifs dans le choix de la meilleure stratégie d'assainissement pour leur site. Il est important de transmettre ces renseignements à d'autres utilisateurs potentiels de façon à ce qu'ils soient conscients des limitations du produit.

Quels étaient nos principaux partenaires dans cette étude?
Arcadis Inc. et l'armée américaine au polygone d'essai d'Aberdeen.

Treatability of Groundwater Contaminated with a Mixture of Chlorinated Solvents Using HRC®.

Suzanne Lesage, Kelly Millar, Susan Brown – Environment Canada, Ontario, Canada

Carol Mowder, Arcadis, Millersville,MD, USA

Don Green – US Army, Aberdeen Proving Ground, MD, USA

1.0 Abstract: Passive *in-situ* remediation techniques can be a very cost effective means of site remediation. Hydrogen Releasing Compound (HRC®) has been used successfully at many sites. However the contaminant at the vast majority of these sites was trichloroethene (TCE). Groundwater at Graces Quarters at Aberdeen Proving Ground was contaminated predominantly with a mixture of 1,1,2,2-tetrachloroethane (TeCA; 1,349 μg/L), carbon tetrachloride (CT; 221 μg/L), and TCE (171 μg/L) . Previous attempts at inducing reductive dechlorination with water and aquifer solids from the site and the addition of various carbon sources had achieved very limited success. A series of microcosms with aquifer solids and groundwater from the site were used to assess whether the addition of HRC® could effectively support the biodegradation of the mixture of chlorinated compounds. CT was degraded to chloroform (CF), which accumulated. There was no evidence of biological degradation of TeCA and a minimal amount of TCE degradation in these microcosms. There is evidence in the literature of the inhibition by CT and CF of the reductive dechlorination of tetrachloroethene. It is possible that this inhibition also affects the degradation of TeCA and TCE.

Keywords: Hydrogen release compound, HRC, 1,1,2,2-tetrachloroethane, carbon tetrachloride, trichloroethene, reductive dechlorination, lactic acid, inhibition

2.0 Introduction

Passive *in-situ* remediation techniques can be a very cost effective means of site remediation. Hydrogen Releasing Compound HRC®, a polylactic acid ester formulated for the slow release of lactic acid produced by Regenesis Inc. (Koenisgberg and Farone, 1999) has been used successfully at many sites contaminated with chlorinated solvents (Koenisgberg, 2000). However the contaminant at the vast majority of these sites was trichloroethene (TCE). Graces Quarters at Aberdeen Proving Ground (APG) is contaminated predominantly with a mixture of 1,1,2,2-tetrachloroethane (TeCA), carbon tetrachloride (CT), and TCE. Previous attempts at inducing reductive dechlorination with water and soil from the site and the addition of various carbon sources, including lactic acid, have achieved very limited success in three-month experiments (Dames and Moore, 1999). In the previously conducted bioremediation treatability tests, CT was the only compound degraded, and the degradation sequence stopped at chloroform (CF).

There is relatively little information on the biodegradation of TeCA in the literature. Chen et al. (1996) studied the transformation of TeCA under methanogenic conditions. They identified all the potential products of biotic and abiotic processes. They showed that TCE is formed by abiotic elimination of HCl. Two main biotic routes are available: sequential reductive dechlorination to 1,1,2-trichloroethane, 1,2-dichloroethane, chloroethane, and ethane, and dichloroelimination to *cis*- and *trans*-1,2-dichloroethene (DCE), followed by reductive dechlorination to vinyl chloride (VC) and ethene. Two studies were conducted in conjunction with other areas of APG that are contaminated with TeCA and TCE but not CT (Lorah and Olsen, 1999; Jackson et al., 2000). Both of these studies showed significant amounts of *cis*- and *trans*- DCE and VC as the degradation products. Jackson et al. (2000) found that the addition of butyrate, yeast extract, and vitamin B_{12} was the most effective in inducing degradation, but after 14 weeks of treatment. After six weeks, there was no significant difference in contaminant

concentrations. The authors also found that the activity varied within the site. In some instances, a reduction in the concentrations of TeCA was not accompanied by the appearance of daughter products.

CT and CF have been found to inhibit dechlorination of tetrachloroethene (PCE) in microcosms (Bagley et al., 2000) and in a column study (Kaseros et al., 2000). While Kaseros et al. (2000) were able to partially acclimate a column already degrading PCE to increasing amounts of CT and CF, they were unable to achieve significant removal of CF in their system. In batch studies with trichloroethane (TCA), inhibition by CF was constant and concentration-dependent. (Hughes and Parkin, 1996).

The study discussed in this document was designed to find out whether the addition of HRC® could induce bacterial dechlorination of the mixture of contaminants present at Graces Quarters. Historic site data indicate that the groundwater pH in the contaminant plume is low (4.5 – 5.0), a pH that has been found unsuitable to most bacteria expressing dechlorinating activity. In addition, the buffering of groundwater that occurred in conjunction with the use of vitamin B_{12} at Graces Quarters helped bacterial growth in the area (Lesage et al. 2001, 2002; Mowder et al. 2000). Therefore, the HRC treatability test included two groups of microcosms, buffered and unbuffered. A mixture of calcium and sodium carbonate was used for buffering. Because the addition of the carbonate buffer was expected to cause contaminant losses by volatilization (due to carbon dioxide formation), half of the microcosms were spiked with 4 mg/L each TeCA and CT and 1 mg/L TCE. An unamended control was also included. Each microcosm condition was prepared in triplicate to confirm reproducibility of results. Each microcosm was monitored initially, and after 2, 8, 21, 49, 77, and 175 days for volatile chlorinated compounds (VOCs) and their expected daughter products as well as volatile fatty acids (VFAs).

3.0 Materials and Methods

3.1 Microcosms

Microcosms were established in 160-mL serum vials consisting of 100 grams (g) of soil and 125 mL of groundwater from the site (near former sample location DMGP04), for a total microcosm volume of 140 mL. To minimize aeration of the soil, soil was added through the narrow neck of the microcosm vials in an anaerobic chamber under an atmosphere of purified nitrogen. The addition of site water and other components was conducted outside the chamber for consistency with a parallel study conducted by Regenesis/Applied Power Concepts, California.

Five microcosm conditions were established with site soil and groundwater in triplicate as follows:

- Treatment 1 –unbuffered, HRC;
- Treatment 2 –buffered, HRC;
- Treatment 3 –unbuffered, HRC, spiked with TeCA, CT, and TCE;
- Treatment 4 –buffered, HRC, spiked with TeCA, CT, and TCE;
- Control – Site soil and groundwater only, no buffer.

HRC® (1.2 g) was added to microcosm treatments 1 through 4 directly, as supplied and in the amount recommended by the manufacturer. Buffering of microcosms 2 and 4 was conducted 48 hours after the initial setup of the microcosms to not interfere with the initial dissolution of HRC®. Calcium carbonate (1g) added to each microcosm was allowed to dissolve for 2 hours before adding sodium carbonate to a final pH of 7.5.

Stock solutions of TeCA, CT, and TCE were prepared in water using neat standards, at concentrations approximately half of their maximum aqueous solubilities for spiking additional chlorinated compounds into microcosms sets 3 and 4. A total of 2 mL was added to each sealed microcosm via a glass syringe,

after first removing an equivalent volume of site water. Treatment 3 microcosms were spiked on day 0 of the experiment, while treatment 4 microcosms were spiked on day 2, after buffering.

3.2 Analyses

Sample analyses were conducted at time 0, 2 days, 1, 3, and 7 weeks.

3.2.1 VOCs. VOCs were analyzed by a modified EPA method 8260 using a 200 μL sample of liquid from a microcosm and injecting the sample into a 40 mL VOC vial filled with milli-Q water. A Dynatech PTA-30 autosampler transferred 10 mL of sample to a Tekmar 3100 purge and trap equipped with a Vocarb 3000 trap. The purge and trap was connected to a Agilent 6890 GC equipped with a 0.32 DB-624 30m, 1.8μm film thickness column. The detector used was an Agilent 5973 MSD.

3.2.2 VFAs. Organic acids were analyzed by ion exclusion chromatography using an IC-Pak column (Waters) and a Waters 430 conductivity detector. Eluent was 1 mM HCl at 1 mL/min. pH was monitored by removing a drop of sample and using multirange pH paper. It is recognized that this is not very accurate, but, it was not possible to use an electrode on such a small sample. Electrodes were used whenever possible.

3.2.3. Hydrocarbon Gases. Methane, ethane, and ethene were monitored by analysis of 100 μL of headspace using a SRI 8610A gas chromatograph equipped with a GS-Q column (J&W) and FID detector.

3.2.4. Hydrogen. Headspace hydrogen was quantified using a RGD detector (Trace Analytical). Headspace analysis of microcosms were conducted by diluting 500 μL samples into empty 160-mL serum vials for a dilution of 320X.

4.0 Results and Discussion

4.1. VOCs

The results are presented by parent compound, followed by their respective daughter products. The graphs show the average of three replicates for each microcosm condition, with error bars for the relative standard deviation..

4.1.1 Carbon tetrachloride. Results for all treatments are shown in Figure 1. The apparent increase in the buffered spiked treatment (#3) is because spiking was done after a two-day equilibration period with the HRC®, after the buffer was added. Similarly the large decrease between Day 0 and Day 2 for treatment 2 can be attributed to volatilization due to carbon dioxide evolution from the carbonate buffer. From Day 2 onwards, there was a slow but definite decrease in CT amounts in all treatments containing HRC®. This reduction averaged 84% for treatment 1, 3, and 4, 90% for treatment 2, and 48% for the control. Some reduction of amount is expected because of sampling, since each time a 1 mL portion of water was removed and 600 μL headspace for the gas analyses. Other losses could be due to sorption or degradation.

4.1.2 Chloroform. The results for CF are shown in Figure 2. There was an average of 27 μg/L chloroform in the site water as received. There was a significant formation of CF in both spiked samples. While initially there was more than double the amount formed in the unbuffered spiked treatment (#3) compared to the buffered one (#4), after 175 days, there was virtually no difference between the two treatments with an average of 2,362 and 2,590 nmoles of CF formed respectively. In the unspiked samples, there was also no difference between the buffered compared to the unbuffered treatment after 175 days. The amount of CF formed accounted for approximately 2/3 of the CT losses in all treatments except #4, where the amount CF formed accounted for 80% of the CT losses.

4.1.3 Dichloromethane. The results for dichloromethane (DCM) are shown in Figure 3. The amount of DCM formed peaked at Day 49. There was some formed in all treatments, but most in

treatment 3, then 4, in keeping with the relative amount of CF in both treatments. The formation of DCM arises from the reductive dechlorination of CF. It is not entirely clear as to why it the amount decreased after day 49, but a similar pattern was seen in the formation of methane (see below). Chloromethane (CM) only appeared in the last day of sampling, in treatment 3, in two of the replicates, at 11 and 14 nmoles respectively. Therefore the decrease in DCM after day 49 cannot be attributed to further reductive dechlorination.

The variation between replicates was very high in the spiked samples; therefore no conclusion can be drawn as to the effect of buffering. There was no difference in the amount of DCM found in the unspiked samples and the control. The amount of DCM formed at 49 days for the spiked samples accounted for a quarter of the decrease in the amount of CT over the period.

4.1.4 1,1,2,2-Tetrachloroethane. The results for 1,1,2,2-Tetrachloroethane (TeCA) are shown in Figure 4. While during the first 21 days of the study there was an apparent decrease in the concentration of TeCA, over the whole study period there were no losses. The conclusion is that there was no attenuation of TeCA during the study. Early decreases may be attributed to sorption to the HRC.

TeCA can be dehydrochlorinated to form TCE. However, this is a base-catalyzed reaction and was not expected to be a major pathway in these microcosms, even in the buffered ones, because the pH was below 7 for most of the study (Table 2).

As outlined in the introduction, the presence of CT and CF has been found to inhibit the reductive dechlorination of PCE and TCA. This inhibition was concentration-dependent. Therefore a similar phenomenon could be happening here. This was one of the reasons for conducting the study with both spiked and unspiked microcosms, the hypothesis being that the concentration of CT and CF in the unspiked microcosms would drop below the toxicity threshold. These results show that this was not achieved during the 175 days of the study.

4.1.5 Trichloroethene. TCE was present in the site water and also spiked. The average amounts are shown in Figure 5. While there was an apparent decrease in amounts between Day 2 and Day 21, the amount did not decrease subsequently. In the unspiked microcosms, there was no difference between the samples and the control.

4.1.6 1,1,2-Trichloroethane. TeCA can be reductively dechlorinated to 1,1,2-TCA. As shown in Figure 6, there were minimal amounts of 1,1,2-TCA in most of the microcosms, with slightly higher concentrations in microcosms 3 and 4 after spiking. The amounts did not change significantly after Day 2.

4.1.7 Cis-Dichloroethene . The results for *cis*-DCE are shown in Figure 7. There was no *cis*-DCE formed until Day 20 in any of the microcosms. After that period, the highest amount of *cis*-DCE was formed in treatment 3, (spiked, unbuffered). At the last sampling, all microcosms, including the control, contained some, with treatment 3, the most. There was no further increase past Day 75.

4.1.8 Trans-Dichloroethene. The results for *trans*-DCE are shown in Figure 8. Up to and including Day 21, they parallel the results for *cis*-DCE. After that, unlike what was observed for *cis*-DCE, the concentration continued to increase, with the most found in treatment 3. The highest amount found was in the unbuffered microcosm. Its formation from abiotic dichloroelimination from TeCA is highly unlikely at pH 3. There was an average of 113 nmoles formed, which may have appeared from the reductive dechlorination of TCE. This represents less than 10% of the spiked TCE.

4.1.9 Vinyl chloride. There was no measurable amount of vinyl chloride in any of the microcosms.

4.1.10 Tetrachloroethene. The results for PCE are shown in Figure 9. PCE was present in the site water at an average amount of 15 nmoles. While there were fluctuations in the concentration of PCE in the microcosms, the differences between treatments and control cannot be considered significant.

4.2 Gases

4.2.1 Ethene and Ethane. The results for ethene and ethane are shown in Figures 10 a and b. There were small amounts of ethene and ethane formed in all samples, including controls, throughout the experiment. They are most probably biogenic, but their presence cannot be linked to the degradation of *cis*- and *trans*-DCE, because they appeared before either of these compounds. The concentrations also peaked at Day 49, similar to that of DCM and methane.

4.2.2 Methane. There were two potential sources for methane: reductive dechlorination of CT and CF and methanogenic activity. Although methanogens cannot use lactic acid, degradation of lactic acid by other bacteria releases hydrogen, which can be converted to methane by methanogenic bacteria. Methane production via both pathways is possible and cannot be distinguished in experiments such as these. The results are shown in Figure 11. The rate of methane generation was relatively low and could have arisen from the reductive dechlorination process. However there was no correlation between the amount of CT and CF degraded and the methane formed. While treatment 3 had the highest amount of methane, it was not significantly different than the amount generated from treatment 1, and only marginally higher than treatment 2. There was some formed in the control, which could be associated with the reductive dechlorination process of CT and CF in those samples, because they contained neither lactic acid nor hydrogen. The addition of a buffer seemed to have a negative effect on methane generation. It is

interesting to note that the trend in methane concentration followed those of DCM formation, with the highest amount formed at 49 days followed by a leveling off.

4.3 Biochemical Indicators

4.3.1 VFA. The results for lactic acid are shown on Figure 12. Because HRC® is a source of lactic acid, it was expected that lactic acid concentrations would increase rapidly. After two days of equilibration, buffers were added to treatments 2 and 4. There was no apparent effect on the lactic acid concentrations. The amount of lactic acid continued to increase in all treated microcosms throughout the experiment. This suggests that the precautionary 2-day equilibration period may not have been required.

Traces of VFAs started to appear by day 8. Pyruvic acid appeared first and by Day 48 was present in small amounts in all replicates of both unbuffered treatments. Acetic acid appeared on Day 48 and was present in both buffered groups, but not the unbuffered microcosms. This may suggest the stimulation of different bacterial consortia depending on the pH. Traces of formic acid appeared in two of the controls.

4.3.2 Hydrogen. The results for hydrogen are shown in Table 1. Hydrogen was measured from Day 8 onward. Initially, the unbuffered microcosms contained on average three times as much hydrogen as the buffered ones. However, by Day 21 there was no significant difference between treatments, except for the controls, where there was no measurable hydrogen. Therefore, any lack of dechlorination could not be linked to a shortage of hydrogen. Although the amount of lactic acid continued to increase throughout the experiment, the amount of H_2 present did not change. The amount of variability between replicates was relatively high and the difference could not be correlated with the amount of methane formed.

4.3.3 pH. The pH measurements are listed in Table 2. The site water as received had a neutral pH, unlike what had been observed previously (Dames and Moore, 1999). The addition of HRC® caused an immediate drop of about 1 pH unit, and by Day 2, the pH had dropped to 3-4. It is important to note that once the microcosms were sealed, the pH measurements were done using pH paper using a small drop of water from the microcosms. This method was sufficient to distinguish between an average pH of 3 for the unbuffered treatments and an average of 6 for the buffered treatments. These results show that the calcium carbonate added was sufficient to buffer for the duration of the study, even as more lactic acid was being released.

One of the interesting results of the study is that HRC® can support reductive dechlorination of CT, even at a low pH. After 175 days, there was no significant difference in the amount of CF formed between buffered and unbuffered microcosms.

5.0 Conclusion

The results of this study indicate that the addition of HRC® does provide a large concentration of lactic acid, which in turn supports bacterial activity that leads to the formation of hydrogen. These results were not significantly affected by the pH of the environment.

Most reductive dechlorination was observed in microcosms to which additional amounts of contaminants had been spiked. The only significant transformation was that of CT to CF. Although some DCM was formed, its production stopped and CF seemed to be accumulating. As was discussed in the introduction, there is documented evidence in the literature of the inhibition by CT and CF of the reductive dechlorination of PCE.

After 175 days no TeCA and very little TCE was degraded. This paper shows that the degradation of TeCA and TCE can also be inhibited by CT and CF. This implies that whenever CT and CF are present at a site, biostimulation through the addition of a carbon source such as HRC® may not be sufficient as a bioremediation strategy.

6.0 Acknowledgements

The authors wish to thank Regenesis Inc. for providing the polylactate, and William Farone of Applied Power Concept Inc. for advice on product utilization and experimental design. The study was funded by the US. Army at Aberdeen Proving Grounds.

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Table 1. H_2 produced in microcosms, average of three replicates ± standard deviation in μmoles per serum bottle.

	Day 8	Day 21	Day 52	Day 72	Day 148
HRC	15 ±7	19 ±9	21 ±11	16 ±12	21 ±12
HRC/buffered	4 ±3	30 ±3	23 ±10	16 ±15	19 ±13
HRC/spiked	13 ±5	21 ±5	28 ±7	22 ±11	32 ±12
HRC/spiked/buffered	1 ±1	13 ±1	20 ±8	22 ±9	17 ±7
control	0 ±0	0 ±0	0 ±0	0 ±0	0 ±0

Table 2. pH Measurements were done using pH paper except where indicated by an asterisk.

		Day 0	Day 2	Day 2 after $CaCO_3$	Day 2 after $NaCO_3$	Day 8	Day 21	Day 48	Day 78	Day 148
HRC	1A	6	3-4			3	3	3	3	3
	1B	6	3-4			3	3	3	3	3
	1C	6	3-4			3	3	3	3	3
HRC + Buffer	2A	5-6	3.2*	5.3*	7.3*	6-7	6	6	6	6
	2B	5-6	3.2*	5.4*	7.6*	6-7	6	6	6	6
	2C	5-6	3.2*	5.3*	7.5*	6-7	6	6	6	6
HRC Spiked	3A	5-6	3-4			3	3	3	3	3
	3B	5-6	3-4			3	3	3	3	3
	3C	5-6	3-4			3	3	3	3	3
HRC + Buffer Spiked	4A	5-6	3.2*	5.6*	7.3*	6	6	6	6	6
	4B	5-6	3.3*	5.7*	7.4*	6	6	6	6	6
	4C	5-6	3.2*	5.7*	7.4*	6	6	6	6	6
Control	5A	7	7			6-7	6-7	6-7	6-7	6-7
	5B	7	7			6-7	6-7	6-7	6-7	6-7
	5C	7	7			6-7	6-7	6-7	6-7	6-7

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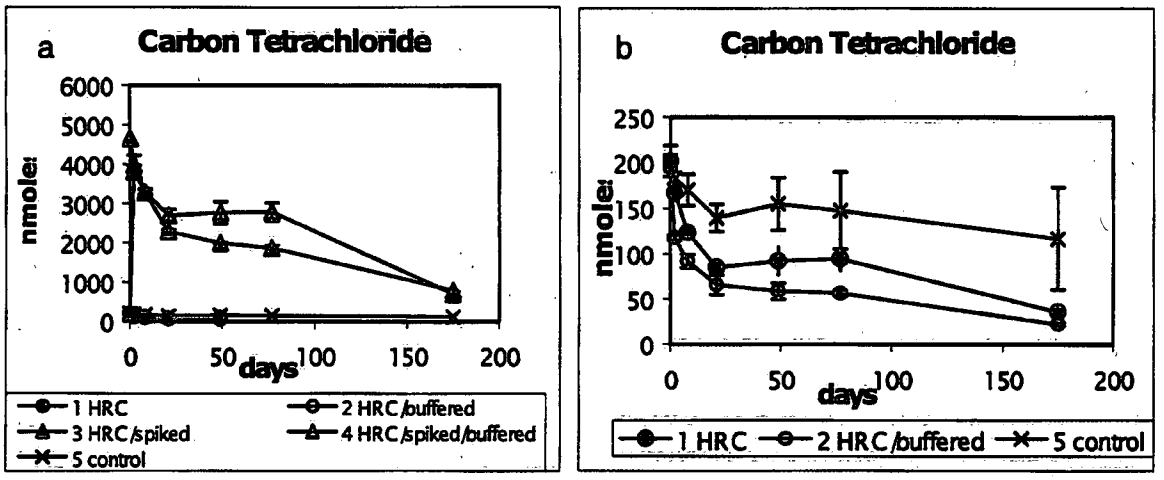

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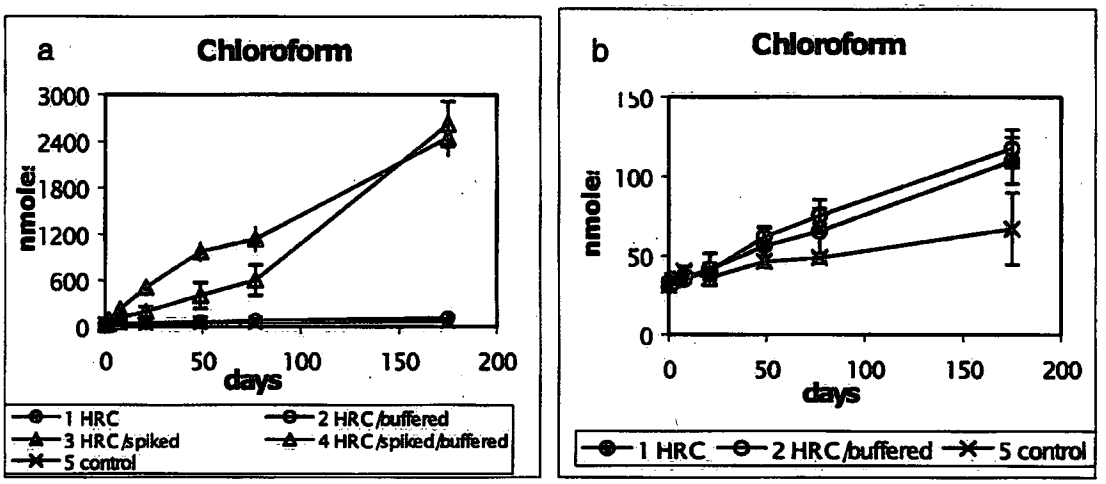

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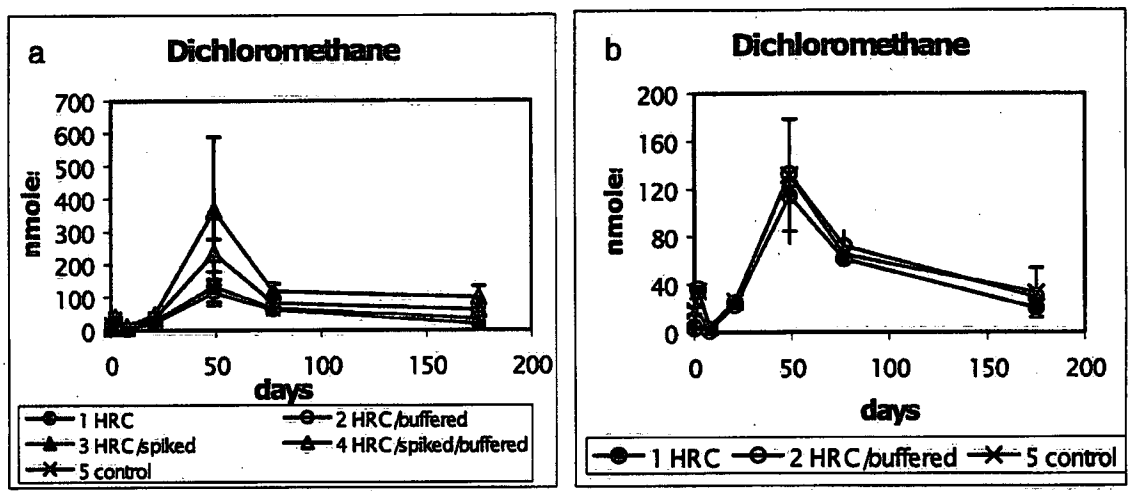

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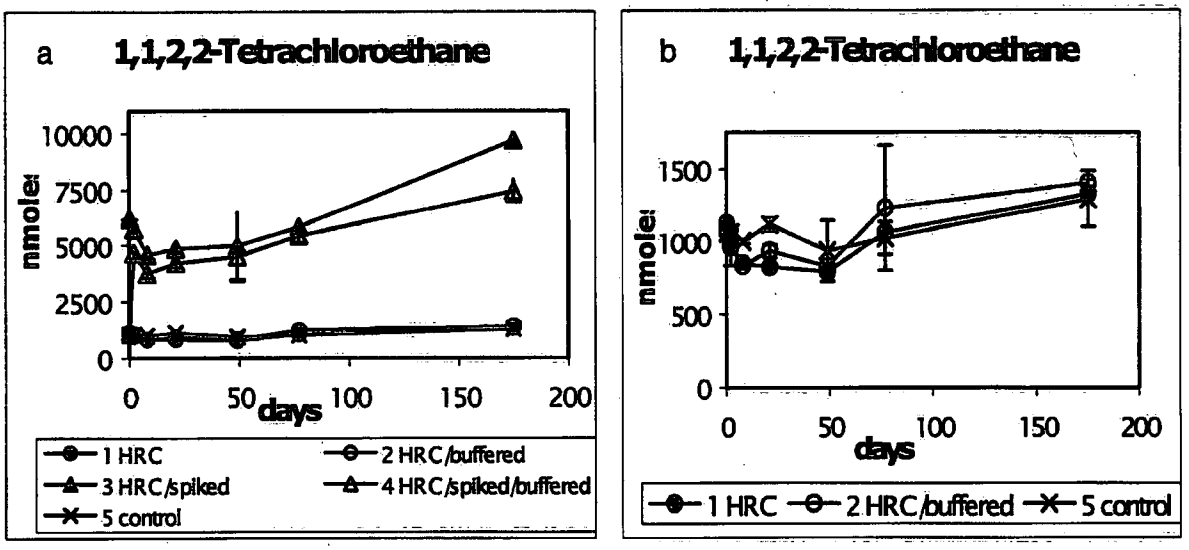

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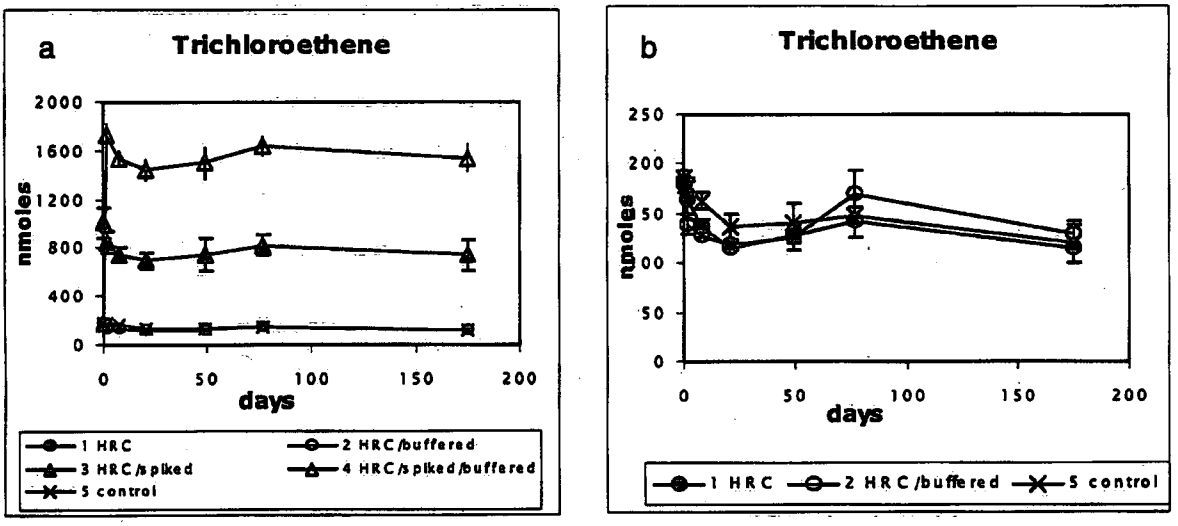

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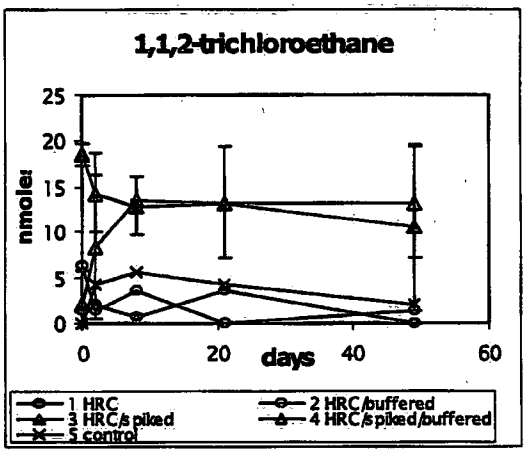

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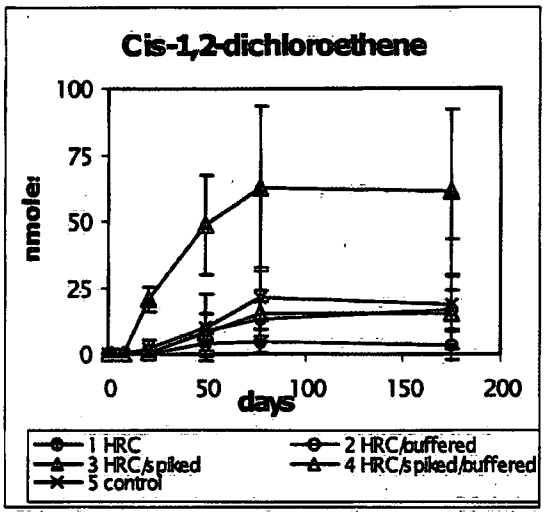

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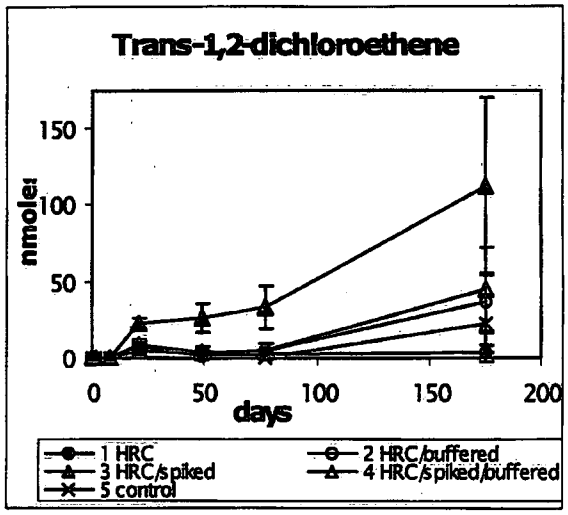

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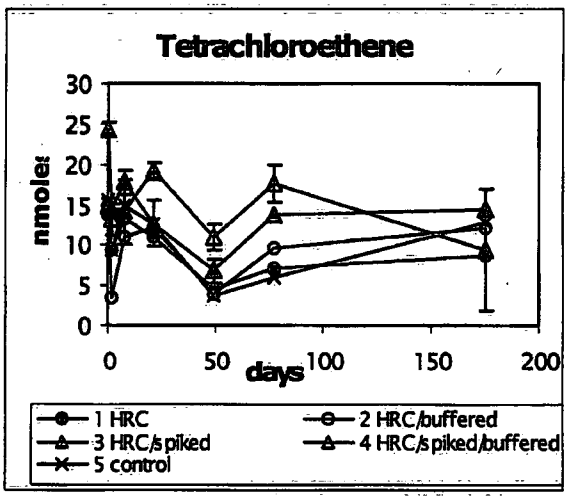

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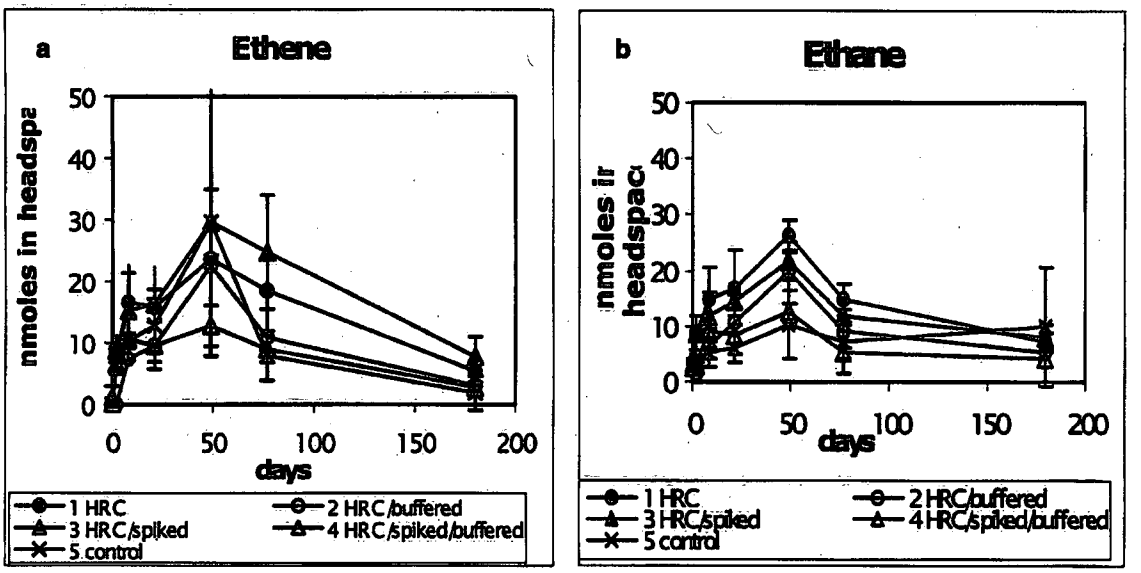
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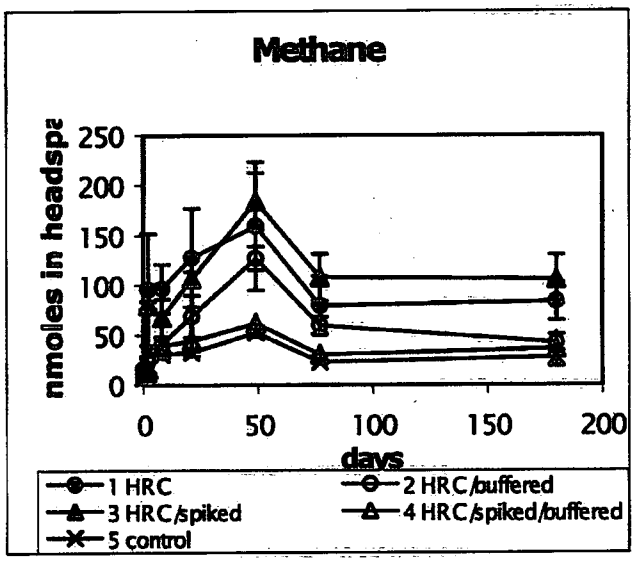
Figure 10.



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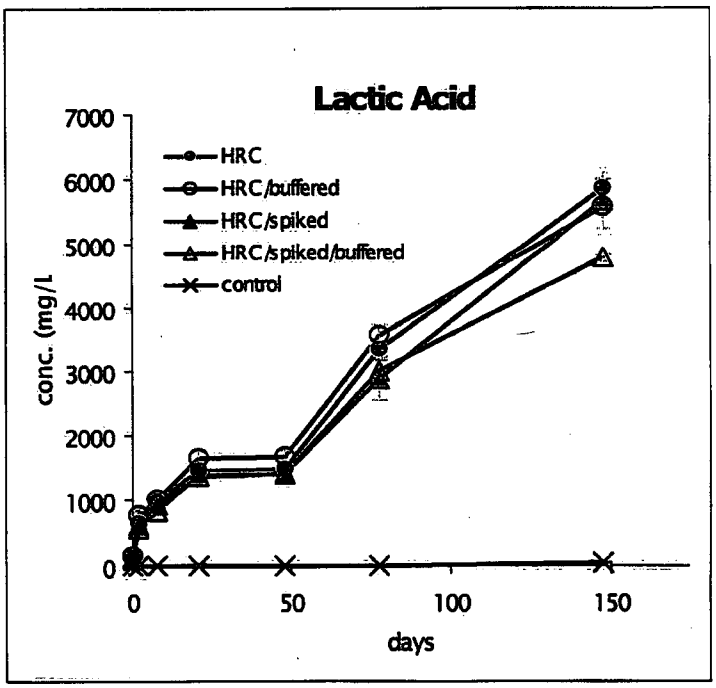

Figure 12..

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