Proposed Remedial Action Plan - GE Main Plant - Supporting Information

SUMMARY of MICROCOSM & BIOREMEDIATION STUDIES

Introduction

Two microcosm studies were performed to determine the feasibility of enhanced bioremediation as a remedial alternative to treat two suspected source areas (former Building 109 Wire Mill (Wire Mill) and Wastewater Treatment Plant (WWTP) areas) of chlorinated solvents in groundwater at the GE Main Plant Site in Schenectady, NY. The studies were performed between September 2002 and April 2003 at GE Global Research (GEGR) in Niskayuna, NY, in accordance with standard practices developed through GE's partnership in the Remedial Technology Development Forum (RTDF) Bioremediation of Chlorinated Solvents Consortium. The purpose of these microcosm studies was to determine whether the complete reductive dechlorination of TCE could be stimulated in soil and groundwater taken from these two suspected source areas of chlorinated solvents. The studies were designed to determine if an optimal carbon source (e.g. sodium lactate, molasses, chitin, or soybean oil) exists for stimulating this dechlorination and whether other nutrients (e.g. nitrogen, phosphate, or trace minerals) must also be added to promote optimal biodegradation. Finally, the studies were used to determine whether bioaugmentation (e.g. the addition of non-native dechlorinating bacteria) is necessary or beneficial to the active biodegradation process naturally occurring at the site.

The overall conclusions, based upon statistical analysis of the data summarized in this appendix, are as follows:

Enhanced bioremediation is capable of reducing high levels (40-50 mg/L) of TCE to ethene in Wire Mill soil and is a feasible remedial option for this area of the site. Lactate and chitin were more effective than molasses or soybean oil at promoting the complete dechlorination of TCE. The addition of supplemental nutrients and bioaugmentation each increased the rate at which the biodegradation occurred but did not have significant impact on the ultimate extent of degradation achieved.

Enhanced bioremediation is also capable of rapidly reducing moderate (5 mg/L) and high (40 mg/L) levels of TCE to ethene in WWTP soil and is a feasible remedial option at this area of the site as well. Both lactate and various forms of chitin tested were equally effective at promoting the complete dechlorination of TCE. In this case, the addition of supplemental nutrients and bioaugmentation did not have a noticeable effect on either the rate or extent of biodegradation beyond that which was already observed. The complete dechlorination of TCE to ethene was also observed in the unamended control in this study, confirming that natural attenuation is quite prevalent in this area. Because this study took place at room temperature, actual degradation rates observed in the field are expected to be at least fifty percent slower due to temperature effects alone.

Anaerobic Biodegradation Background and Process

Accelerated anaerobic biodegradation (the natural breakdown of chemical constituents in an low oxygen environment by active in-situ microorganisms) of chlorinated solvent source areas represent a preferable remedial option, where natural attenuation processes are already naturally occurring, to mitigate risk to human health and the environment at a contaminated site. Accelerated biodegradation involves the addition of simple and safe sources

of carbon and nutrients to the subsurface in order to stimulate anaerobic bacteria capable of reductively dechlorinating chlorinated solvents like tetrachloroethene (PCE) and trichloroethene (TCE) to innocuous byproducts like ethene and ethane. Reductive dechlorination involves the step-wise replacement of individual chlorine atoms with hydrogen atoms, such that

PCE ® TCE ® c-DCE ® VC ® Ethene (® Ethane)

where c-DCE and VC are cis-dichloroethene and vinyl chloride, respectively.

In the last decade, the biological conversion of PCE and TCE to ethene or ethane by anaerobic reductive dechlorination has been demonstrated both in the laboratory and in the field (Freedman & Gossett, 1989; Major, et al., 1991; de Bruin, et al., 1992). In this process, the chlorinated ethene acts as an electron acceptor, while an electron donor is required to provide energy for this process (McCarty, 1994). Hydrogen is generally considered to be the direct electron donor for reductive dechlorination, but is typically produced from the anaerobic oxidation of other carbon substrates, such as sugars, organic acids, or alcohols (Maymo-Gatell, et al., 1995).

Complete dechlorination of PCE or TCE to ethene or ethane has been demonstrated to occur at many chlorinated solvent sites. However, this extensive dechlorination does not occur at all sites (Ellis, 1997). In rare instances, no dechlorination is observed on a site. More commonly, partial dechlorination is observed, where the process stops at an intermediate product like c-DCE, rather than proceeding all the way to ethene or ethane. This may occur for one or more of the following reasons.

- Carbon sources (electron donors), which provide energy for the dechlorinating microorganisms, are not present or not present in sufficient quantity.
- Other important nutrients, such a nitrogen or phosphate, are lacking.
- The proper dechlorinating microorganisms are not present.

There are many carbon sources suitable for promoting reductive dechlorination of chlorinated solvents by anaerobic bacteria. Among these, sugars (e.g. molasses), organic acids (e.g. lactic acid or sodium lactate), and alcohols (e.g. methanol) have most widely used in enhanced biodegradation applications to date. Because these substrates are soluble in water and highly biodegradable, they must be added periodically in order to maintain effectiveness. Substrate addition and maintenance of the injection system is typically the most expensive aspect of this remedial approach (Harkness, 2000).

More recently, water insoluble carbon sources have seen increasing application in enhanced biodegradation. These carbon sources biodegrade slowly over time and include substances like lactic acid polymers, soybean oil, chitin (derived from crustacean shells), and wood chips. Unlike water soluble substrates, these materials do not require continuous or batch additions in order to maintain their effectiveness and therefore can be cheaper to apply due to reduced substrate addition and system maintenance costs. However, in some cases the cost of these materials can be prohibitive (Harkness, 2000).

Bacteria also require basic nutrients like nitrogen and phosphorus in order to support their growth. These nutrients are often present in sufficient quantities in soil and groundwater, but can be limiting in some cases. In the same way, dechlorinating bacteria are generally present in the environment, particularly those capable of dechlorinating

PCE and TCE to c-DCE. However, the bacteria responsible for dechlorinating c-DCE to ethene and ethane are more sensitive to environmental conditions, and are not present at all sites. In this case, they can be added via bioaugmentation and will grow and proliferate in the subsurface under favorable conditions (Harkness, et al., 1999, Ellis, et al., 2000).

Materials and Methods

The soil and groundwater used in the Main Plant microcosm study was obtained from the former Wire Mill and Wastewater Treatment Plant areas of the site. Two to three kilograms of soil were collected from subsurface soil borings using a Geoprobe® drill rig proximal to each source area site using 4-inch diameter steel Shelby tubes. The tubes were capped and sealed with wax immediately upon retrieval, then packed in coolers and sent to GEGR in Schenectady. Approximately one gallon of groundwater was also obtained from a well in close proximity to the location of each soil sample. The groundwater samples were collected in one-liter glass containers, which were completely filled with groundwater, capped, and shipped to GEGR in coolers. The soil and groundwater samples were stored at $4^{o}C$ prior to use.

The microcosm study was performed in sterile 120 ml serum bottles. Fifty (50) grams of soil were weighed out and dispensed into each bottle in an anaerobic glove box containing an atmosphere of ~5% hydrogen in nitrogen. Each serum bottle was then filled with 75 mL of non-sterile, filtered ground water that was sparged with nitrogen to remove any preexisting solvents.

Electron donors (sodium lactate, molasses, chitin, and soybean oil), yeast extract, supplemental nutrients, in the form of reduced anaerobic mineral media (RAMM), and dechlorinating microorganisms were added to the microcosms alone and in combination to determine the optimum conditions for carrying out the dechlorination. The design consisted of one set of unamended microcosms, and multiple sets of microcosms where the individual electron donors and yeast extract were added alone and in combination with supplemental nutrients. This design allowed the efficacy of different electron donors to be assessed and the necessity of supplemental nutrients to be determined. In addition, one set of microcosms was amended with sodium lactate, yeast extract, supplemental nutrients and was bioaugmented with an active PCE-dechlorinating culture to act as a positive control. This treatment ensured that there were dechlorinating bacteria available to biodegrade the solvent. Finally, there was also a killed control to monitor non-biological losses from the microcosm bottles. The killed control consisted of removing the existing microorganism population in the sample through the addition of toxins.

All the microcosms were set-up and analyzed in triplicate (Wilson, et al., 1997). The microcosm bottles were sealed with Teflon™-coated septa and aluminum crimp seals and incubated upside-down in the dark at room temperature.

Sodium lactate and molasses are water-soluble substrates and were added to the microcosms each week using a gas-tight syringe. Chitin and soybean oil are non-water-soluble substrates and were added only at the beginning of the study. In each case 0.5 grams of the substrate were added to each bottle in a glovebox under anaerobic conditions. Yeast extract (30 mg/L) was added once to all the bottles with the exception of the killed control at the beginning of the study, using a gas tight syringe. RAMM, consisting of a phosphate buffer, potassium and ammonium salts, and trace metals (Shelton & Tiedje, 1984) was added to designated bottles at the beginning of the study. Resazurin, a redox-sensitive color indicator for anaerobic conditions, was added to all the bottles at the

beginning of the study. No reducing agents were used in this experiment.

The positive control was bioaugmented with the Pinellas consortium, which contains bacteria able to completely dechlorinate PCE to ethane (Flanagan et al., 1995). The Pinellas consortium was recently used to bioaugment a field test at Dover AFB, resulting in first successful use of bioaugmentation to promote the complete dechlorination of TCE to ethene in the field (Harkness, et al., 1999, Ellis et al., 2000). The killed controls were created by autoclaving the bottles or by adding mercuric chloride using a gas-tight syringe. The unamended controls did not receive any electron donor or nutrient amendments.

TCE was spiked into the microcosms at the beginning of the study using a gas-tight syringe from a saturated stock solution. The purpose of adding TCE was to establish an initial source of nutrients for the microorganisms to grow and feed. The microcosms were sampled at the start and then at two to three-week intervals throughout the study. PCE, TCE, c-DCE, VC and ethene/ethane were measured using a gas chromatograph (GC) equipped with a flame ionization detector (FID). Under the most highly reduced conditions, carbon dioxide is reduced to methane gas by methanogenic bacteria. This activity is significant because reductive dechlorination of c-DCE and VC to ethene and ethane typically occurs under these most highly reduced conditions. Therefore, any gas produced in the microcosm bottles was collected and measured on a weekly basis during the study.

Results - Former Wire Mill Area Study

The electron donors and electron donor/RAMM combinations tested in this study are summarized in Table 1. The soil used for the study was collected from the glaciolacustrine deposits located 15 to 17 feet below ground surface, in the vicinity of monitoring well DM-421G which is proximal to the former Building 109 Wire Mill suspected source area. This soil was made up of silts and clays and contained high residual concentrations of solvents. Due to these high concentrations in the soil, the TCE concentrations present in the bottles ranged from 14 to 179 mg/L. The mean starting TCE concentration in the study was 40 to 50 mg/L, which is indicative of a source zone. The study ran for 210 days (30 weeks).

Set	Amendment
1	Unamended Control
2	Lactate only
3	Molasses only
4	Chitin Only
5	Soybean Oil only
6	Lactate + RAMM
7	Molasses + RAMM
8	Chitin + RAMM
9	Soybean Oil + RAMM
10	Lactate + Pinellas + RAMM
11	Killed Control

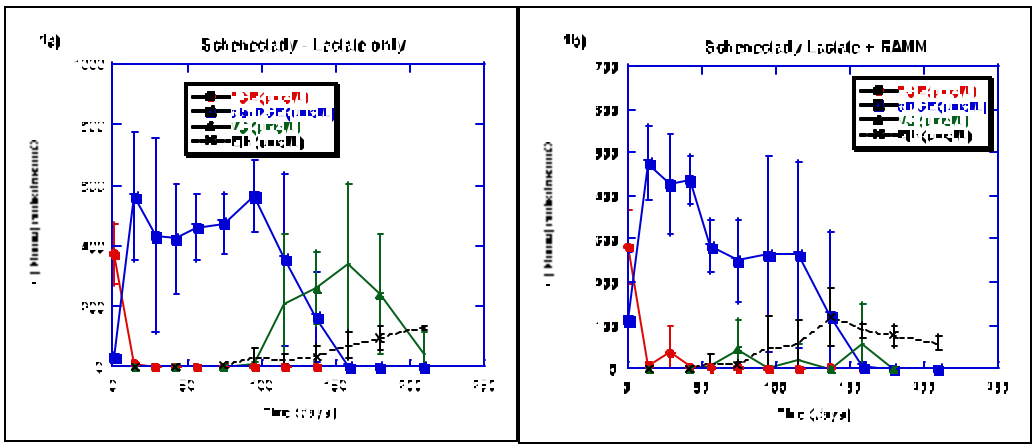
Table 1. Treatments used during the former Wire Mill Microcosm Study

With the exception of the killed control, the microcosm bottles were biologically active from the beginning of the experiment, indicative of the naturally occurring biodegradation of chlorinated solvents in the suspected source area samples. The redox indicator (resazurin) turned from blue to clear in about a day in each of the microcosm bottles, indicating that these systems had become anaerobic as a result of biological processes. Methane gas was later produced in the bottles, indicting that methogenesis was occurring.

In this study, both lactate and chitin stimulated the complete reductive dechlorination of TCE to ethene, while molasses and soybean oil did not (Figures 1-4). Complete conversion of TCE to ethene was observed in 116 days in the bioaugmented bottles containing lactate+RAMM (Figure 1c), in 180 days in the bottles containing lactate+RAMM (Figure 1b), and in 210 days in the bottles containing lactate alone (Figure 1a). In the microcosm sample bottles containing lactate only, two of three bottles went to complete conversion. Complete conversion of TCE to ethene was observed in 137 days in the bottles containing chitin+RAMM (Figure 3b) and in 210 days in the bottles containing chitin alone (Figure 3a).

The soybean oil and molasses amended bottles did not achieve complete dechlorination of cDCE and VC, with little ethene production observed. The molasses supported dechlorination of TCE to cis-DCE, but dechlorination stopped there (Figure 2a&b). Soybean oil was marginally more successful than molasses, as both cis-DCE and VC were formed in these bottles (Figure 4a&b). However, significant amounts of these contaminants persisted in the bottles at the end of the study. Very limited dechlorination of TCE to cis-DCE was observed in the unamended and killed controls (data not shown).

When statistical analyses using ANOVA and t-tests were applied to the data set, the analysis found that the choice of electron donor impacted the extent of dechlorination observed at a 95% confidence level. In addition, the analysis indicated that both RAMM and bioaugmentation had a positive effect on dechlorination rate at a 95% confidence level. In total, these results indicate that lactate and chitin were superior to molasses and soybean oil in promoting complete reductive dechlorination of TCE to ethene at the site and that complete dechlorination occurred more rapidly in the presence of RAMM and the Pinellas bacteria.



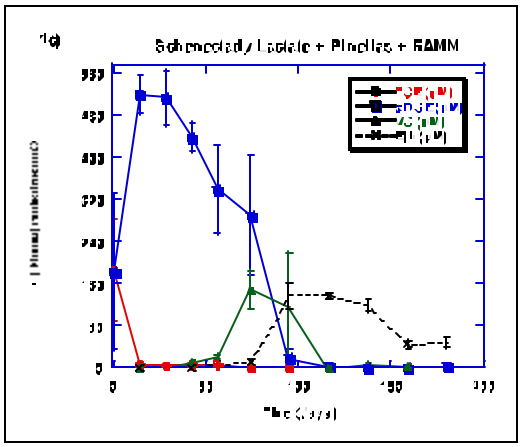
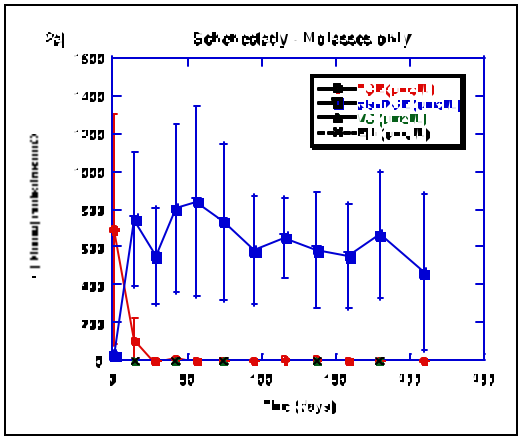


Figure 1a. Lactate Only **b.** Lactate + RAMM **c.** Lactate + Pinellas + RAMM



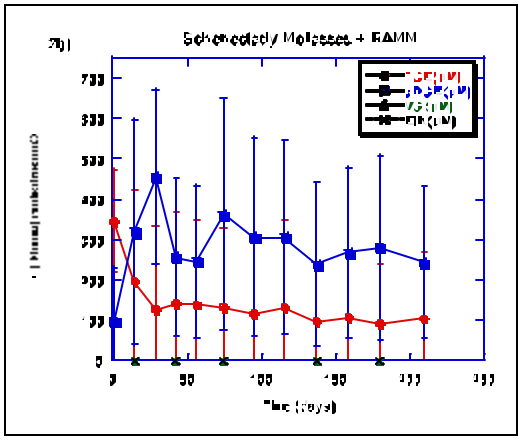


Figure 2a. Molasses Only **b.** Molasses + RAMM

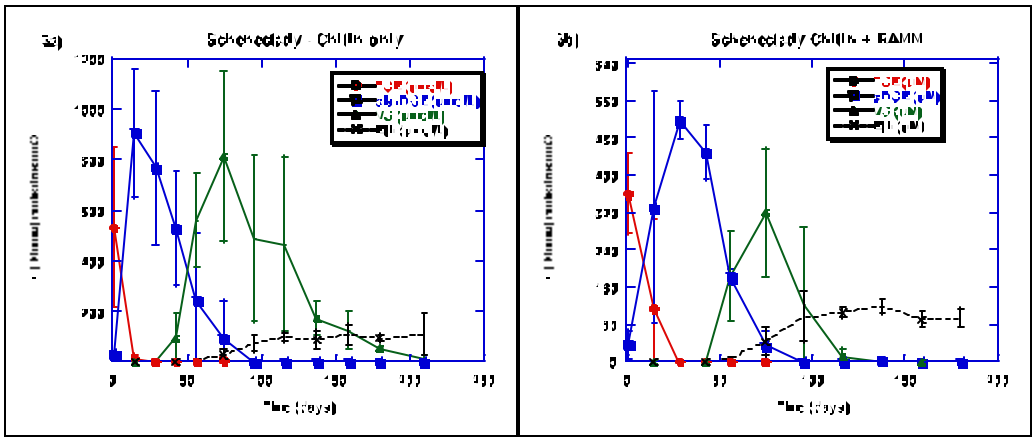


Figure 3a. Chitin Only **b.** Chitin + RAMM

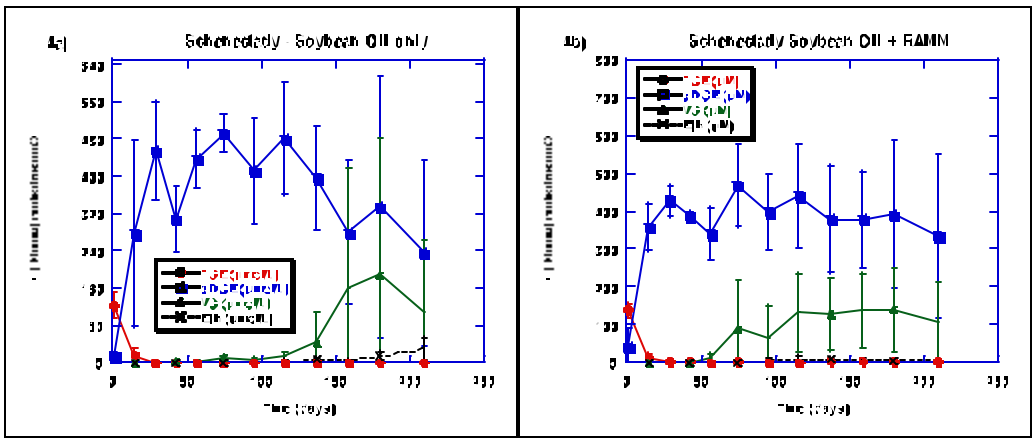


Figure 4a. Soybean Oil Only **b.** Soybean Oil + RAMM

Results - WWTP Area Study

The electron donors and electron donor/RAMM combinations tested in this study are summarized in Table 2. In this case, two commercial grades of chitin were used, chitin fines and SC-40. The chitin fines consist of approximately 80% chitin and 20% inert material including water. The SC-40 grade consists of approximately 40% chitin and 60% carbonates obtained from the remaining shells. In addition, two different particle sizes (coarse and

ground) of SC-40 were tested. The soil used for the study was collected from floodplain deposits located 18 to 20 feet below ground surface in the vicinity of well GW-WWTP-2, which is proximal to the suspected source area south of the Site's WWTP. This soil was made up of silts, clays, and fine sands. High residual concentrations of solvents were not detected. The bottles were initially spiked with 5 mg/L TCE. The TCE was rapidly degraded in all the donor-amended bottles, so these bottles were re-spiked to 40 mg/L TCE at 75 days into the study to assess biodegradation performance at higher TCE concentrations. The study ran for 193 days (28 weeks).

Set	Amendment
1	Killed Control
2	Unamended Control
3	Lactate Only
4	40% coarse Chitin Only
5	40% ground Chitin Only
6	Chitin fines Only
7	Lactate + RAMM
8	40% coarse Chitin + RAMM
9	40% ground Chitin + RAMM
10	Chitin fines + RAMM
11	Lactate + Pinellas + RAMM

Table 2. Treatments used during the WWTP microcosm study

As previously observed in the Wire Mill study, the microcosm bottles were biologically active from the beginning of the experiment, which is an indication of how strong the natural biodegradation of chlorinated solvents is occurring in this suspected source area. The redox indicator turned from blue to clear in less than a day in each of the bottles and methane gas was later produced, indicting that methanogenesis was occurring.

Both lactate and the various forms of chitin rapidly dechlorinated the initial spike of TCE to ethene (Figures 5-8). In all cases, dechlorination was complete in less than 39 days. With the exception of the bottles amended with chitin fines (Figure 8a), all the donors also rapidly dechlorinated the second TCE spike as well. In this case, dechlorination to ethene was generally complete in 25-75 days after the second TCE addition, despite the fact that the second TCE spike was eight fold higher than the original amount. This reflects a significant increase in the biodegradation rates from the first TCE spike to the second, likely caused by adaptation and growth of dechlorinating bacteria over time in the bottles.

TCE levels in the killed control remained roughly constant throughout the study, indicating that abiotic loss of TCE is not occurring to a significant extent at this site (Figure 9a). However, the initial TCE spike was completely dechlorinated to cis-DCE, VC, and ultimately to ethene in the unamended control by the end of the study (Figure 9b). This again confirms that natural attenuation is very strong in this portion of the site. The corresponding half-lives for these rates are 22.4 days for TCE, 8.5 days for cis-DCE, and 36.5 days for VC. Because the microcosm bottles were kept at room temperature (20-22˚C) and groundwater temperatures typically range from 10-15˚C, rates in the field would be expected to be about half the values observed in the laboratory.

When statistical analyses using ANOVA and t-tests were applied to this data set, the analysis found that lactate and the various forms of chitin were all equivalent in both the rate and extent to which they promoted the complete dechlorination of TCE to ethene. Although all donors increased the degradation rate above the unamended level, there was no performance difference between donors in either spike study. Similarly, the addition of RAMM and the Pinellas bacteria also did not increase the rate or promote the extent of dechlorination to a statistically significant degree (95% confidence level). This is understandable given the strong natural attenuation capacity of the native soil in this area.

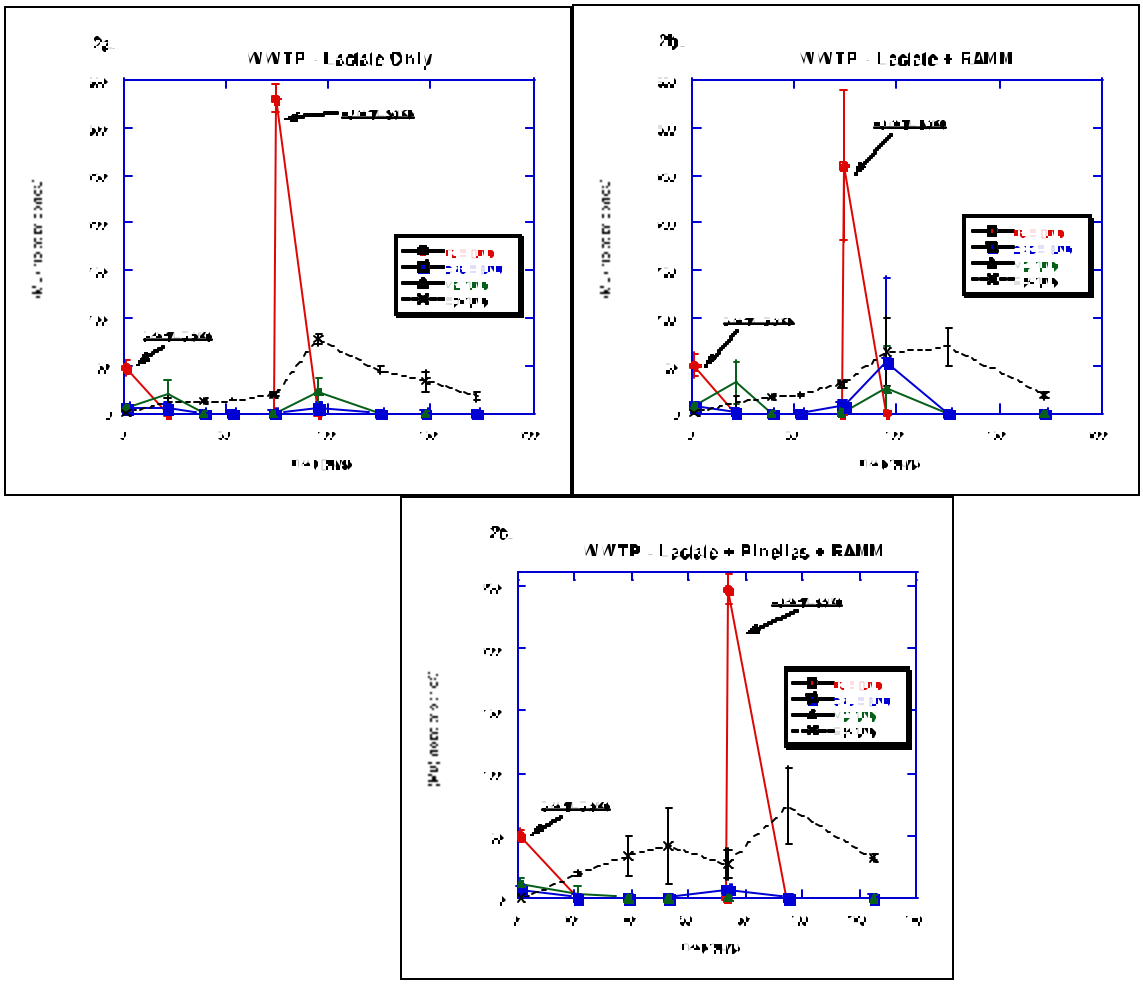


Figure 5a. Lactate Only **b.** Lactate + RAMM **c.** Lactate + Pinellas + RAMM

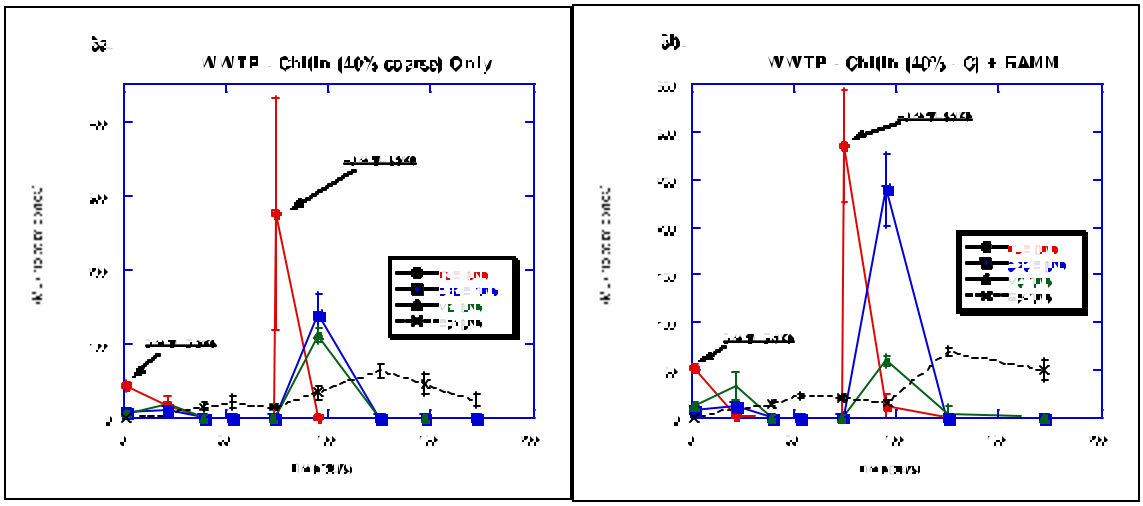


Figure 6a. Chitin (40% Coarse) Only **b.** Chitin (40% Coarse) + RAMM

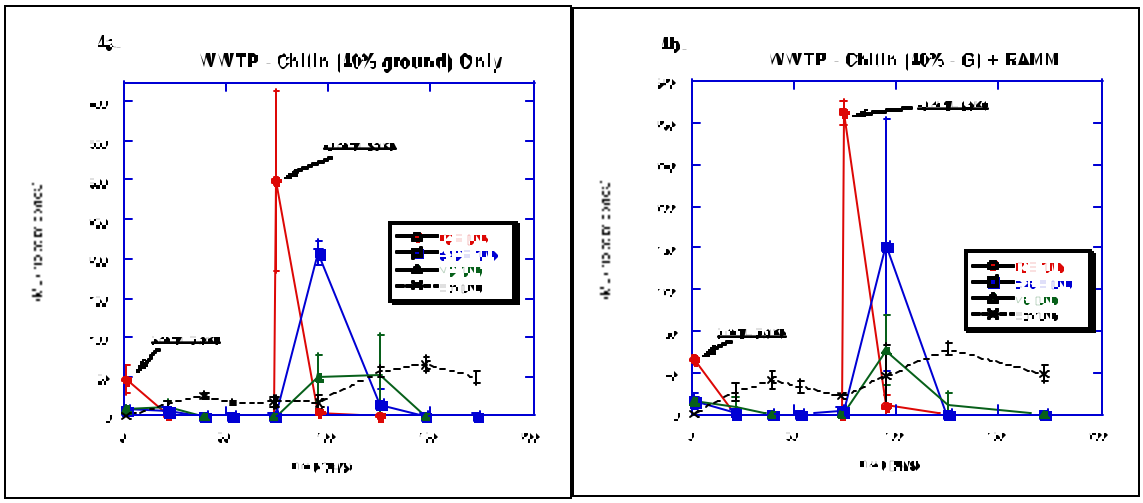


Figure 7a. Chitin (40% ground) Only **b.** Chitin (40% ground) + RAMM

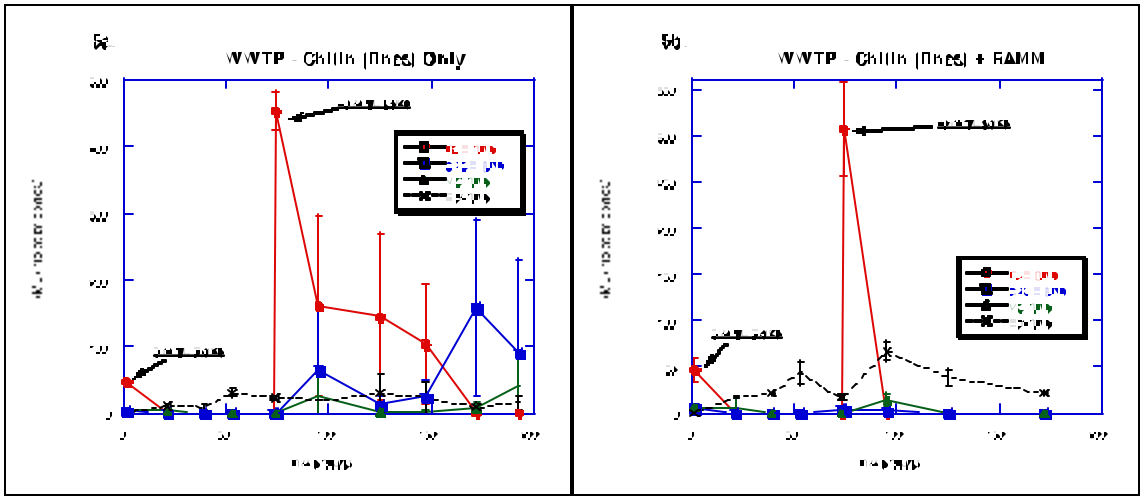


Figure 8a. Chitin (fines) only **b.** Chitin (fines) + RAMM

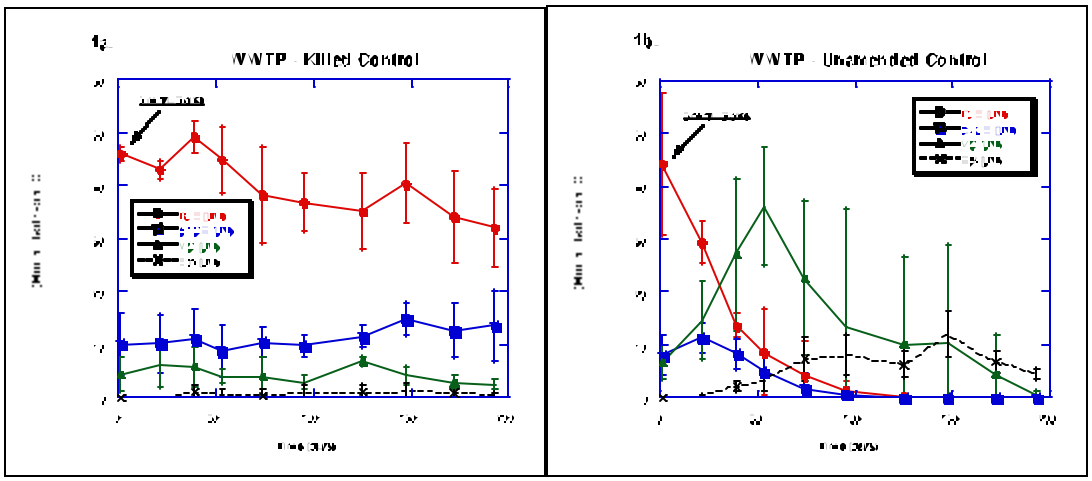


Figure 9a. Killed Control **b.** Unamended Control

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