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EFFECTS OF AQUIFER ENVIRONMENTAL FACTORS ON BIODEGRADATION OF ORGANIC CONTAMINANTS

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EFFECTS OF AQUIFER ENVIRONMENTAL FACTORS ON BIODEGRADATION OF ORGANIC CONTAMINANTS

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ABSTRACT

Aquifers in New Mexico are contaminated with a wide variety of organic compounds. Benzene and 1,1,1-trichloroethane (TCA) are frequently detected and represent two major structural contaminant classes, aromatic and chlorinated aliphatic organic compounds, respectively. Important environmental effects on the biodegradation of these two compounds were examined in laboratory batch incubations of regional aquifer material with ^{14}C-radiolabelled benzene and TCA. Redox status, changes in redox conditions, and long and short pre-exposure periods were investigated for their effects on complete biodegradation as indicated by $^{14}CO_2$ generation over time. Additional variables which were quantified include residual organics in aquifer material, residual volatile organics, and $^{14}CO_2$ incorporated into microbial biomass.

Results showed that significant biodegradation of each contaminant occurred within 40 and 120 days for benzene and TCA, respectively. Starting levels of benzene present in batch aquifer material were reduced by approximately 90% during the incubation period, and TCA levels decreased approximately 80%. Disappearance of both contaminants occurred under aerobic and anaerobic conditions, but the specific biochemical fate of each compound was influenced by the prevailing redox conditions and the length of contaminant pre-exposure.

These results may be useful in establishing boundary conditions to predict biodegradation rates for benzene and TCA by native microbial communities in comprehensive ground water hydrologic models which track the fate of contaminants in New Mexico's aquifers. Biodegradation

is a factor requiring consideration when evaluating the fate of organic contaminants in New Mexico's aquifers.

Keywords: benzene, 1,1,1-trichloroethane, TCA, biodegradation, aquifer contamination, redox effects, pre-exposure

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JUSTIFICATION

Many New Mexico aquifers are contaminated with a wide variety of organic pollutants and this problem is one of the most critical environmental problem facing New Mexico. In New Mexico leaking underground storage tanks (LUST) contribute significantly to this problem. At least 10 to 30% of the 14,000 registered tanks are known to leak; many more are expected to leak in the near future. Contaminants range from petroleum products, including gasoline, diesel and kerosene, to chemical solvents and waste products. Over 48 LUST sites in Albuquerque alone are known to have contaminated groundwater supplies. The reorganization of the Environmental Improvement Division into the Environment Department under Governor Bruce King's current administration was effected in large part to deal with this particular issue.

However, very few data exist regarding the fate of contaminants within the subsurface environments of the Southwest. Information is particularly scarce with respect to biological transformations of contaminants and how regional environmental factors may affect these transformations. The majority of studies addressing biodegradation of organic contaminants have been conducted in the eastern regions of the United States, and it is doubtful that results obtained in these studies

are directly applicable to areas of the Southwest. In order to design effective remediation strategies for contaminated aquifers in this region, the data base regarding biological fate of pollutants within these systems must be expanded.

Traditional remediation of LUST contaminated aquifers begins by pumping off any floating product from the water table. Many contaminants remain dissolved in groundwater and adsorbed onto soil particles where they remain an environmental hazard. Therefore, further aquifer remediation is usually required before the site can be closed. Technologies currently being implemented for this purpose, such as subsurface removal and disposal, soil venting, and air stripping, have serious limitations. These treatments are often prohibitively expensive, and contamination is not actually eliminated, but merely transferred elsewhere. Land Ban and Clean Air legislation can also prevent these techniques from being viable options at particular sites. Given these enormous problems associated with current remediation technologies, bioremediation appears to offer a viable alternative at a number of sites. The U.S. Environmental Protection Agency (EPA) has specifically called for the development, refinement and implementation of innovative and effective remediation technologies (Office of Underground Storage Tanks 1988). Bioremediation has been declared by EPA to represent an important remediation tool, and that agency, along with the U.S. Department of Energy (DOE) and a variety of regional funding groups, has urged the scientific community to conduct extensive basic research required to make bioremediation a viable site-specific alternative.

Development of successful bioremediation techniques depends on a thorough characterization of the biological aspects of contaminant behavior and fate in the subsurface. Therefore, research must focus on providing a better understanding of the mechanisms and consequences of interactions between environmental factors and biodegradation. It is critical, for example, to determine if active microbial communities capable of contaminant degradation are present within the local subsurface environment. Knowledge of the extent to which these populations can potentially degrade a particular contaminant is prerequisite to attempting to maximize this capacity. Microbial degradation rates of organic contaminants vary significantly depending on contaminant structure (Walton and Anderson 1988). Aromatic petroleum constituents and chlorinated aliphatic solvents are two major structural groups detected routinely in New Mexico's aquifers, and are ideal candidates for this research.

Of the numerous environmental factors that may significantly affect microbial degradation of organic contaminants, local redox conditions are particularly relevant. Manipulation of redox status, which may be described in terms of the tendency of an environment to receive or supply electrons, has been proposed as a potentially powerful bioremediation tool. Contaminant structure largely determines which redox conditions will favor degradation. For example, many petroleum constituents occur in highly reduced forms with net negative charges associated with their carbon atoms. Oxidizing redox conditions tend to promote the degradation of these molecules, and microbially mediated

oxidation reactions can degrade these compounds quite effectively. However, the relationships between contaminant structure, redox conditions and microbial communities are not necessarily straightforward. There may be any number of complicating factors in the subsurface, especially when local redox conditions shift significantly during the biodegradation process.

Recently, changes in redox status from anaerobic to aerobic have been identified as a potentially crucial step in the complete degradation of a variety of contaminants (McKinney 1986, Richards and Shieh 1986). Southwestern aquifers undergo natural shifts in redox status as the water table rises and falls and concentrations of inorganic and organic constituents change. With respect to bioremediation, changes in subsurface redox status can be affected in a variety of ways. Aerobic conditions can be induced by processes such as air sparging (Raymond 1974), and hydrogen peroxide pumping (Raymond et al. 1986), whereas anaerobiosis can often be achieved in the subsurface by the introduction of simple non-hazardous organic compounds (Major et al. 1988). The current study was designed to characterize significant variables affecting biodegradation of pollutants in regional aquifer material. To successfully utilize these strategies, the dynamics of contaminant biodegradation must be well characterized with respect to redox conditions and shifts.

Another important environmental variable related to contaminant biodegradation is the pre-exposure history of a site to a particular

pollutant. Subsurface systems which have had previous contact with contaminants may contain microbial populations which are "adapted" to these compounds, and different rate kinetics of biodegradation may therefore be observed in response to a current contamination event. With a few notable exceptions (Aelion et al. 1987, Aelion et al. 1989), this phenomenon has not been well described for a variety of subsurface materials and contaminant types. Pre-exposure effects are likely to be highly compounded and to some degree site specific. Results generated from regional studies with relevant contaminant types can, however, provide a first approximation for remediation planners dealing with specific sites within comparable hydrologic, geologic, and climatic regimes.

METHODS

Small scale laboratory batch incubations were used to evaluate the effects of four environmental factors on the biodegradation of organic compounds in a southwestern aquifer system. The experimental factorial design included 1) indigenous microbial activity (biotic vs. abiotic [irradiated] samples); 2) contaminant structure type (aromatic vs. chlorinated aliphatic); 3) pre-exposure conditions (none, short, and long pre-exposure periods); and 4) redox status (aerobic throughout, anaerobic throughout, and anaerobic to aerobic shift).

Bulk aquifer sediment samples were collected at approximately 2 meters depth from the shallow aquifer of the Santa Fe Formation with the assistance of Mr. John Shomaker and Mr. Bob Newcomer, local professional hydrologists. The sampling site was just north of the Old Town part of Albuquerque, New Mexico. The samples were collected from within the historic floodplain of the Rio Grande in the riparian zone. The samples were collected with a hollow stem auger which was rinsed with ethanol and air dried before sampling. Samples were immediately transferred to sterilized plastic bags, placed in an ice chest, and transported to the laboratory. This aquifer is typical of the relatively permeable large aquifers in New Mexico (personal communication with Mr. Newcomer). Samples were stored at 5^0C prior to analysis. Samples were not amended with additional nutrients.

Benzene and 1,1,1-trichloroethane (TCA) were chosen for this

study based on their prevalence in regional contaminated aquifer systems, their listing as EPA priority pollutants, and their significant difference in structure, representing aromatics and chlorinated aliphatic organic compounds, respectively. To quantify the complete decomposition of these compounds, mineralization studies using uniformly ^{14}C-radiolabelled benzene and TCA were conducted, and the ^{14}C-CO_2 produced by mineralization was quantified using scintillation counting techniques.

Methodologies developed by our research group (Watwood et al. 1991) which facilitate the examination of mineralization in regional unsaturated, calcareous soils and saturated sediments were utilized. Each ^{14}C-labelled compound was added in environmentally relevant concentrations (approximately 1 ppm) to 20 g aquifer samples in standard 160 mL dilution flasks. Abiotic controls were provided by cobalt source irradiation at Sandia National Laboratories with exposure to 300 kilorads/hr at 43^0C for at least 48 hours. Incubations were conducted for up to 50 and 120 days for benzene and TCA, respectively. Samples were incubated under aerobic and anaerobic conditions. Anaerobic conditions were established within a Forma Scientific anaerobic system. The samples were flushed repeatedly with an anaerobic gas mixture within the transfer compartment of the system and then moved into the anaerobic glove box where sample manipulations were carried out. The anaerobic stoppered flasks were then incubated at 25^0C in an incubator located inside the anaerobic system. An oxygen sensor monitored the atmosphere within the anaerobic system and confirmed

that anaerobic conditions were established and maintained throughout the experiments. A set of samples incubated with benzene and TCA, respectively, were initially subjected to anaerobic incubation conditions and then purged with oxygen after 20 and 30 days for benzene and TCA respectively. These samples were then incubated aerobically for the remainder of the incubation period.

Prior to adding the ^{14}C-labelled compounds, two sets of samples were pre-exposed to non-labelled benzene and TCA. One sample set was pre-exposed to the contaminant for a relatively short period (1 and 3 months for benzene and TCA, respectively), while the second set was pre-exposed for a longer period (2 and 4 months, benzene and TCA, respectively).

At each sampling time, triplicate samples were acidified to release all the CO_2. The acidification process released all of the inorganic carbon and mineralized organic matter into the flask headspace. Using a specially adapted shunt apparatus developed in our laboratory (Watwood et al. 1991), the CO_2 was transferred into a trapping solution of 4 N NaOH. The flasks were purged with nitrogen gas to ensure total transfer of the gas into the trapping solution. The shunt contained activated charcoal to prevent any volatile radiolabelled organic compounds from reaching the CO_2 trap. Five hundred uL aliquots of the trapping solution were then counted for $^{14}CO_2$ radioactivity in 20 mL of Scintiverse II scintillation cocktail (Fisher Scientific) enriched with 10% methanol. A Packard 1500 liquid scintillation counter was used to

generate the total amount of radioactivity, which was then extrapolated to determine the total amount of $^{14}CO_2$ resulting from the mineralization of each labelled organic contaminant, and the percentage of each radiolabelled compound that was biodegraded to yield CO_2. Prior studies have confirmed that this technique results in $^{14}CO_2$ recovery efficiencies of approximately 90%, and total recoveries in the current study typically ranged from 70 to 95% (Watwood et al. 1991).

In addition to quantifying $^{14}CO_2$ produced by contaminant mineralization, several other biodegradation parameters were measured. Incorporation of contaminant-derived ^{14}C into microbial biomass was determined according to chloroform fumigation methods of Jenkinson and Powlson (1976). Briefly, at each sampling time, a subset of samples was subjected to chloroform fumigation. The samples were first opened and purged to remove the $^{14}CO_2$. The samples were then restoppered with a small container attached to the bottom of the stopper into which approximately 1 ml of chloroform was added. The samples were next vacuumed and released repeatedly to permeate chloroform vapors throughout the sediment. Chloroform fumigation was carried out for 12 hours. The chloroform was then removed, 0.5 g of fresh sediment added, and the samples incubated for 10 additional days. The samples were then acidified and processed as described above to determine the percentage of each ^{14}C-radiolabelled compound which had become incorporated into microbial biomass. This amount of radiolabelled microbial biomass is calculated from the amount of $^{14}CO_2$ released from decomposing microbial biomass during the 10 day incubation (Jenkinson

and Powlson 1976).

A subset of samples was sacrificed at each sampling time to determine the amount of residual ^{14}C-labelled organic material in the aquifer material. These samples were exposed to ether for 3 days, after which time a 500 uL aliquot of the ether solution was subjected to scintillation counting. Volatile organics were also assayed by extracting charcoal removed from the $^{14}CO_2$ transfer shunts with ether for 3 days, then subjecting 500 uL aliquots to scintillation counting. These determinations provided additional information regarding the biological fate of benzene and TCA in the aquifer material.

Microbial analyses were conducted to provide a preliminary characterization of the populations potentially responsible for the observed biodegradation results. Pristine aquifer material was analyzed for numbers of total heterotrophic bacteria (organic carbon degraders), specific contaminant degraders, gram positive / gram negative ratios, and rod / cocci ratios using culturing techniques. Cultures were grown using nutrient agar, trypticase soy agar, and dilute (1:10) nutrient agar. Tests for specific contaminant degraders were done with mineral salts media with the sole carbon source being benzene or TCA. The effect of long-term contaminant exposure on the numbers of contaminant degraders was also examined with mineral salts media and the specific contaminant.

RESULTS AND SIGNIFICANCE

Study results are illustrated in the series of figures which follow in the text. For all figures, error bars represent one standard error, and lines containing the cross symbols indicate results of killed (cobalt irradiated) control experiments.

Benzene

Levels of benzene present within the aquifer material dropped to approximately 10% of the original amount added within a 40-day incubation period (Figure 1). Decreases of approximately the same amount were observed for samples incubated aerobically or anaerobically. However, the specific fate of benzene during the incubations was substantially different, depending on the redox conditions of incubation.

For samples incubated aerobically (Figure 2), levels of $^{14}CO_2$ generated by mineralization showed several trends. First, production of $^{14}CO_2$ occurred rapidly with significant levels (over 10%) produced within 3 days. Maximum levels of $^{14}CO_2$ were characteristically observed after approximately 10 days, with levels generally declining as the incubations proceeded. Because these assays determined the amount of $^{14}CO_2$ present within closed incubation flasks, decreases in $^{14}CO_2$ levels observed over time mean that some of the $^{14}CO_2$ produced by microbial mineralization earlier in the incubation period was being removed from

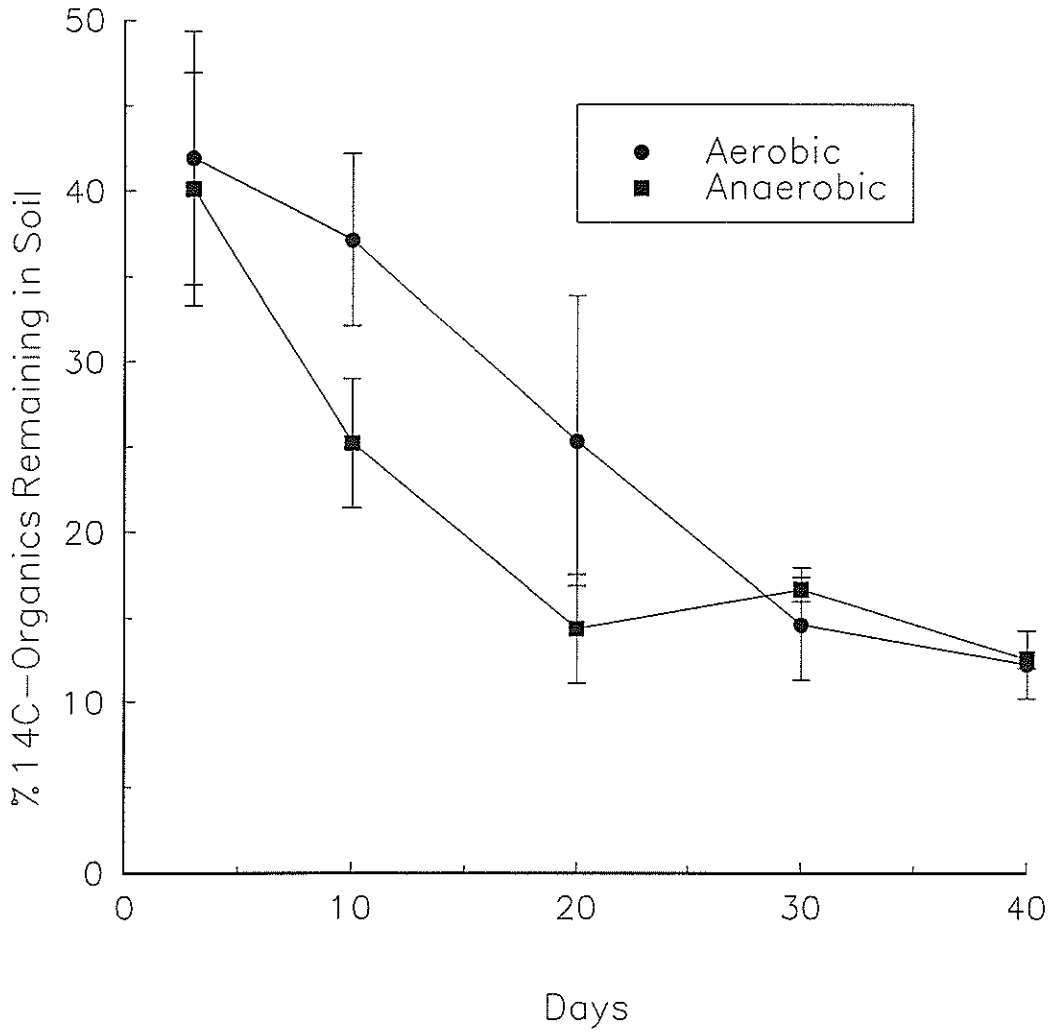

FIGURE 1. Residual benzene recovered in aquifer material following aerobic and anaerobic incubations.

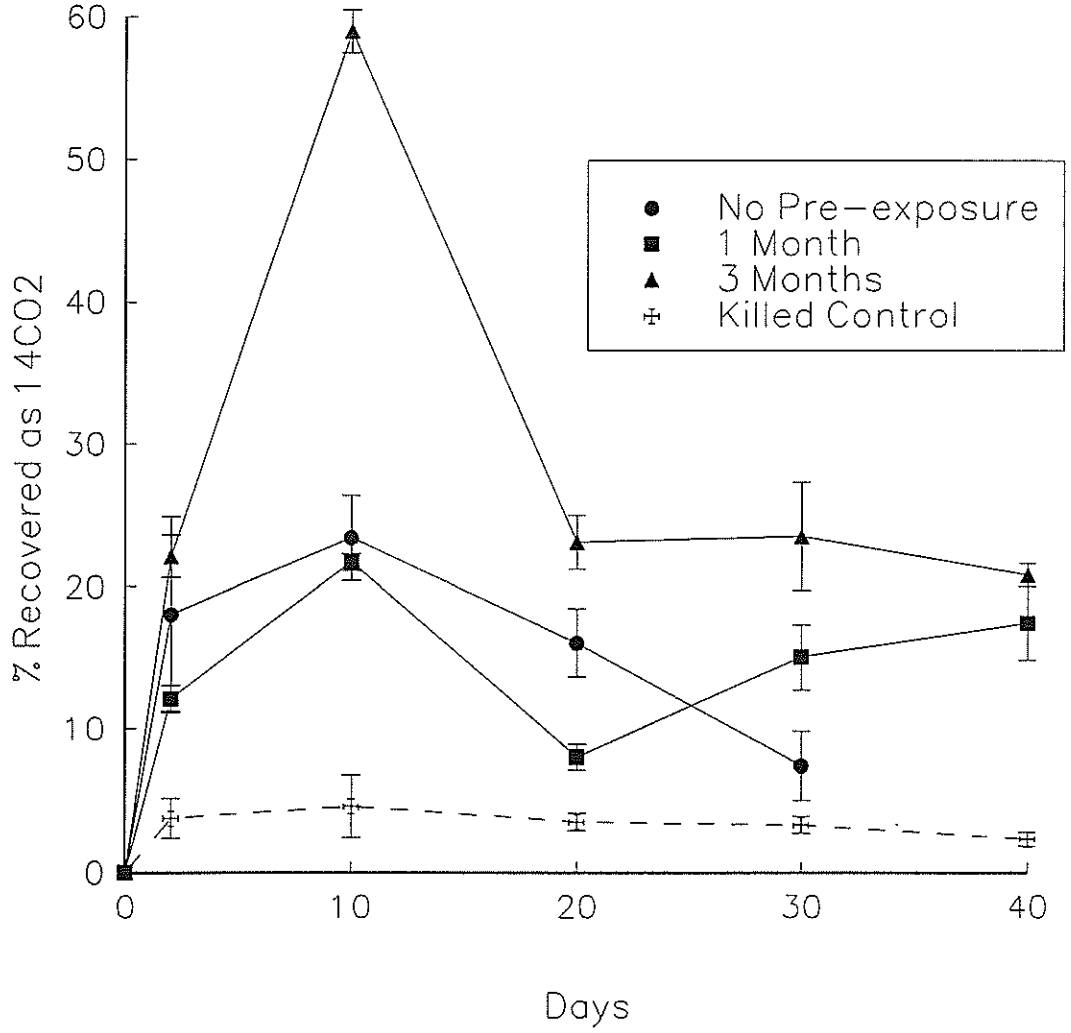

FIGURE 2. $^{14}CO_2$ production in aerobic incubations of benzene with aquifer material subjected to 0-, 1- and 3-month pre-exposure.

the inorganic carbon pool. The most likely fate for this $^{14}CO_2$ is uptake by bacterial autotrophs. Levels of $^{14}CO_2$ shown in these figures really represent net mineralization; more $^{14}CO_2$ was actually generated during the incubations but a proportion of this $^{14}CO_2$ was later incorporated into microbial biomass via autotrophic uptake.

With respect to pre-exposure effects on aerobic biodegradation of benzene, there was not a significantly higher level of $^{14}CO_2$ production associated with the shorter 1-month pre-exposure samples than with the non-exposed samples. However, the longer 3-month pre-exposure period resulted in significantly higher levels of $^{14}CO_2$ production, with almost 60% of the original benzene being converted to $^{14}CO_2$ within 10 days. Less than 5% of the benzene was converted to $^{14}CO_2$ in samples which had been irradiated to eliminate microbial activity.

Figure 3 shows the results obtained during anaerobic incubations of the aquifer material with benzene. Although somewhat lower levels of $^{14}CO_2$ were typically produced as compared to the aerobic incubations, fairly substantial amounts were generated, and significant fluctuations in $^{14}CO_2$ levels were observed, regardless of pre-exposure history. For anaerobic incubations, pre-exposure apparently affected the timing of $^{14}CO_2$ generation and cycling. Samples pre-exposed to benzene for short or long periods produced maximum net amounts of $^{14}CO_2$ after approximately 10 days, whereas maximum $^{14}CO_2$ generation was delayed somewhat (until 20 days) in those samples which received no pre-exposure. The pattern of $^{14}CO_2$ generation and cycling is extremely

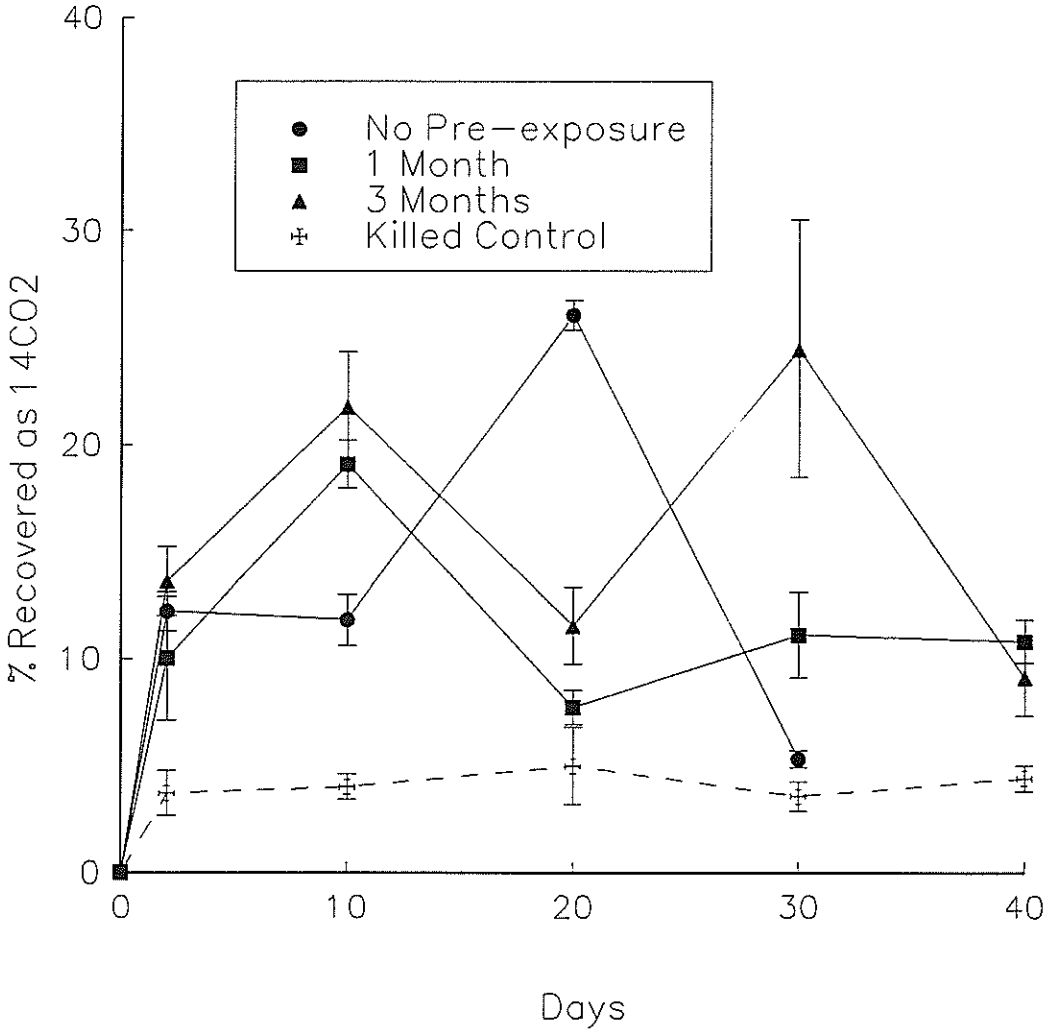

FIGURE 3. $^{14}CO_2$ production in anaerobic incubations of benzene with aquifer material subjected to 0-, 1-, and 3-month pre-exposure.

apparent in these results. For example, with respect to the 3-month pre-exposure samples, maximum $^{14}CO_2$ levels were observed after 10 days. Subsequently, $^{14}CO_2$ was removed from the system and the resulting lowered levels were measured at 20 days. This pattern was then repeated, with $^{14}CO_2$ apparently being re-released and again removed. Similar observations were made for samples with different pre-exposure histories. Much stronger patterns of $^{14}CO_2$ cycling were evident with anaerobic as compared to aerobic incubations.

Shifts in redox conditions from anaerobic to aerobic were imposed on a subset of samples after 20 days of anaerobic incubation. The results of this shift for short and long pre-exposure samples are shown in Figure 4 in comparison with samples which were allowed to incubate anaerobically for the experiment's duration. Steady increases in $^{14}CO_2$ production were observed after the redox shift for both 1- and 3-month pre-exposed samples. Levels of $^{14}CO_2$ produced in samples pre-exposed to benzene for the longer period were somewhat higher. For both groups of samples the shift to aerobic conditions appeared to reduce the $^{14}CO_2$ cycling phenomenon.

Aerobic incorporation of benzene-derived ^{14}C into microbial biomass is shown in Figure 5. Levels of incorporation increased over time, although there is some evidence (1 month pre-exposure, 30 days) of a release of $^{14}CO_2$ resulting in decreased incorporation levels. Samples were not replicated for these experiments, so it is possible only to comment on, not to statistically verify, trends. Irradiated samples

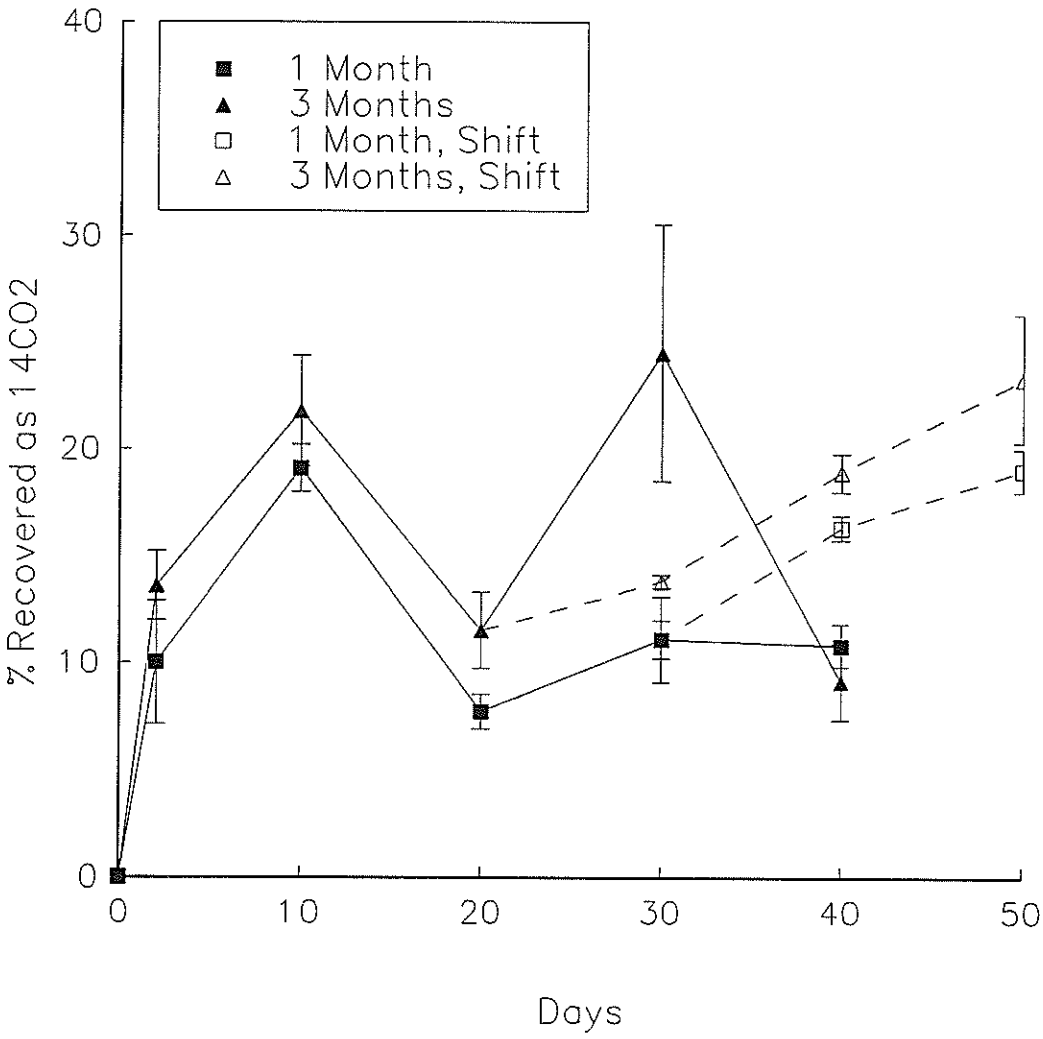

FIGURE 4. Influence of redox shift from anaerobic to aerobic conditions on the production of $^{14}CO_2$ in incubations of benzene with aquifer material subjected to 1- and 3-month pre-exposure.

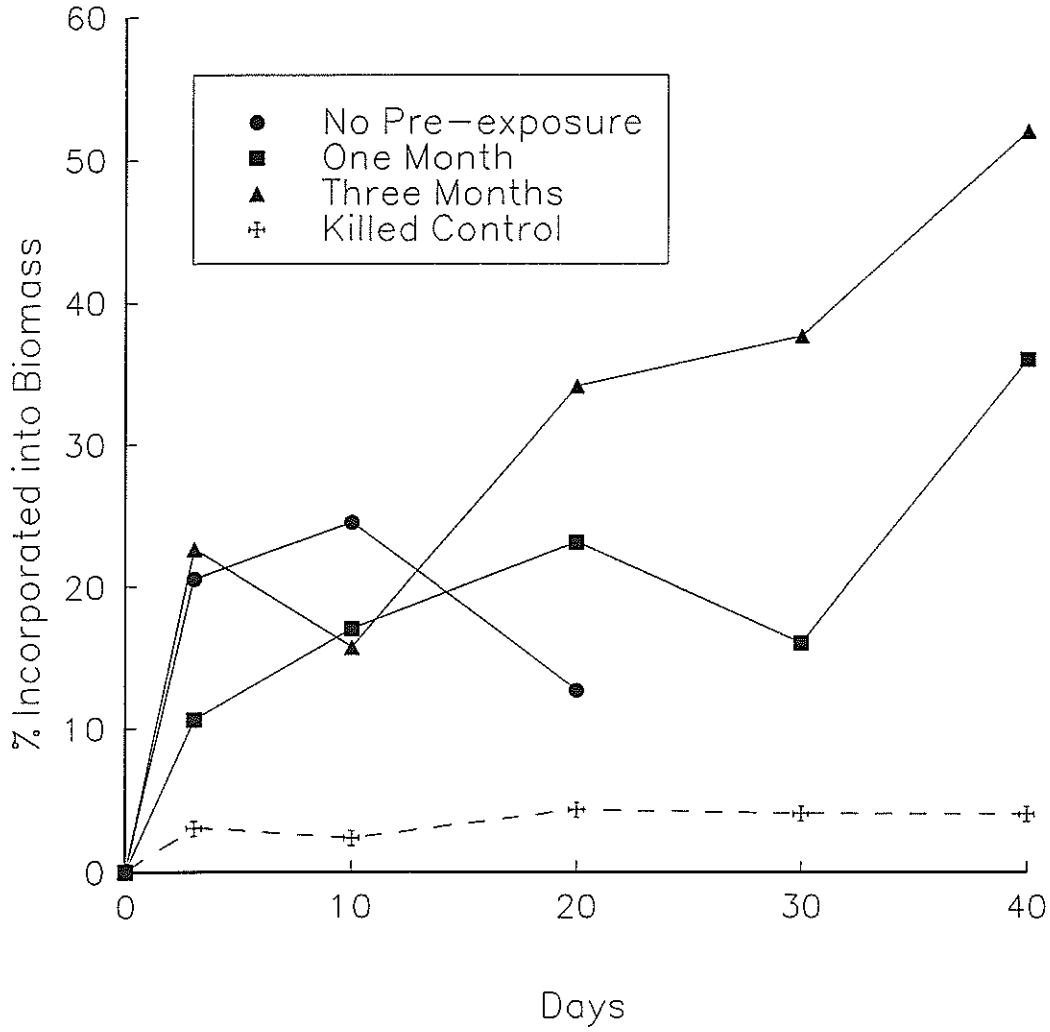

FIGURE 5. Incorporation of benzene-derived ^{14}C into microbial biomass for samples receiving 0-, 1-, and 3-month pre-exposure.

showed very low levels of $^{14}CO_2$ incorporation, which follows as a result of having microbial activity (including incorporation) curtailed by irradiation. These results also indicate that the longer pre-exposure period resulted in elevated levels of $^{14}CO_2$ incorporation into biomass. After 40 days, over 50% of the carbon in the added benzene was recovered as microbial biomass carbon.

In addition to calculating the amount of residual benzene present in aquifer material (Figure 1) the amount trapped by the charcoal shunt filters as volatile organics (theoretically benzene and biodegradation intermediates combined) was determined and is shown in Figure 6. Volatile organics increased during the course of the study, although this fraction never exceeded 10% of the original amount of contaminant added to the flasks. Levels of volatile organics also increased over time in the irradiated sample flasks, which is not unexpected since benzene volatilization is primarily controlled by physical-chemical, not biological, parameters.

1,1,1-Trichloroethane

Several similarities were observed between the biodegradation of TCA and that of benzene, but there were also some significant differences. One of the main points to keep in mind when interpreting results of incubations with TCA is that the time frame during which biodegradation proceeded was substantially longer (120 days) than that provided for the benzene incubations (40 in most cases). In this

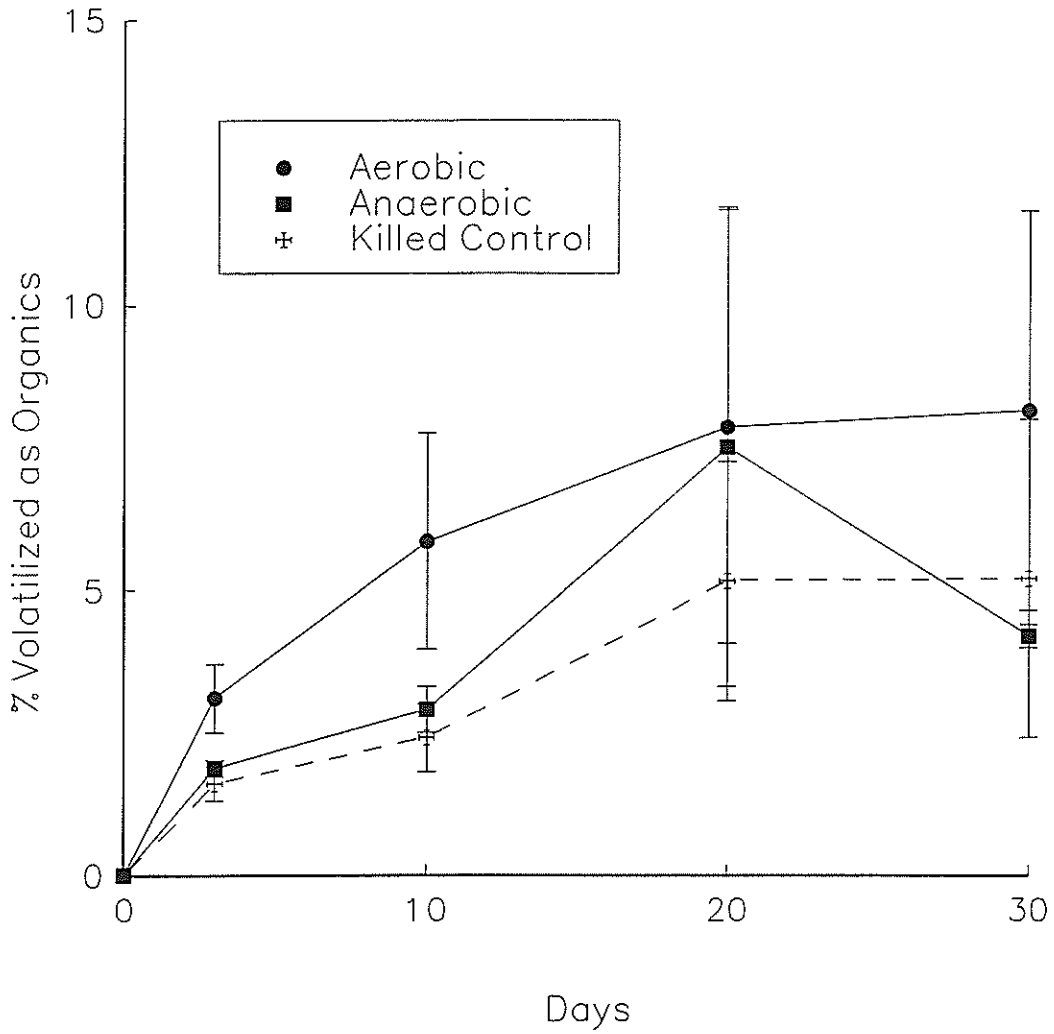

FIGURE 6. Volatilized organics trapped by charcoal filters during aerobic and anaerobic incubations of benzene with aquifer material.

expanded time frame levels of TCA present in the aquifer material dropped significantly during the experiments, approaching 20% of original concentrations after approximately 60 days (Figure 7).

Both aerobic (Figure 8) and anaerobic (Figure 9) incubations with TCA resulted in substantial levels of $^{14}CO_2$ production, although higher total amounts of CO_2 were produced under anaerobic conditions. This was not unexpected in view of the enhanced rate of dehalogenation normally measured under anaerobic conditions and the slightly oxidized structure of TCA which would tend to favor degradation under reducing (anaerobic) conditions.

In the benzene incubations, pre-exposure history influenced the timing of maximum net CO_2 production in the anaerobic incubations only. However, with respect to TCA incubations, this was true for both aerobic and anaerobic incubations. In both cases samples pre-exposed to the contaminant generated maximum amounts of $^{14}CO_2$ very quickly, whereas non-exposed samples did not generate maximum net levels of $^{14}CO_2$ until after approximately 90 days. Again, as with the benzene samples, it is apparent that $^{14}CO_2$, once generated by TCA mineralization, is incorporated into microbial biomass within the incubation system. However, the degree of cycling apparent for benzene samples incubated anaerobically is not evident with respect to TCA (Figure 9). Levels of $^{14}CO_2$ production in irradiated controls were characteristically low under both aerobic and anaerobic conditions.

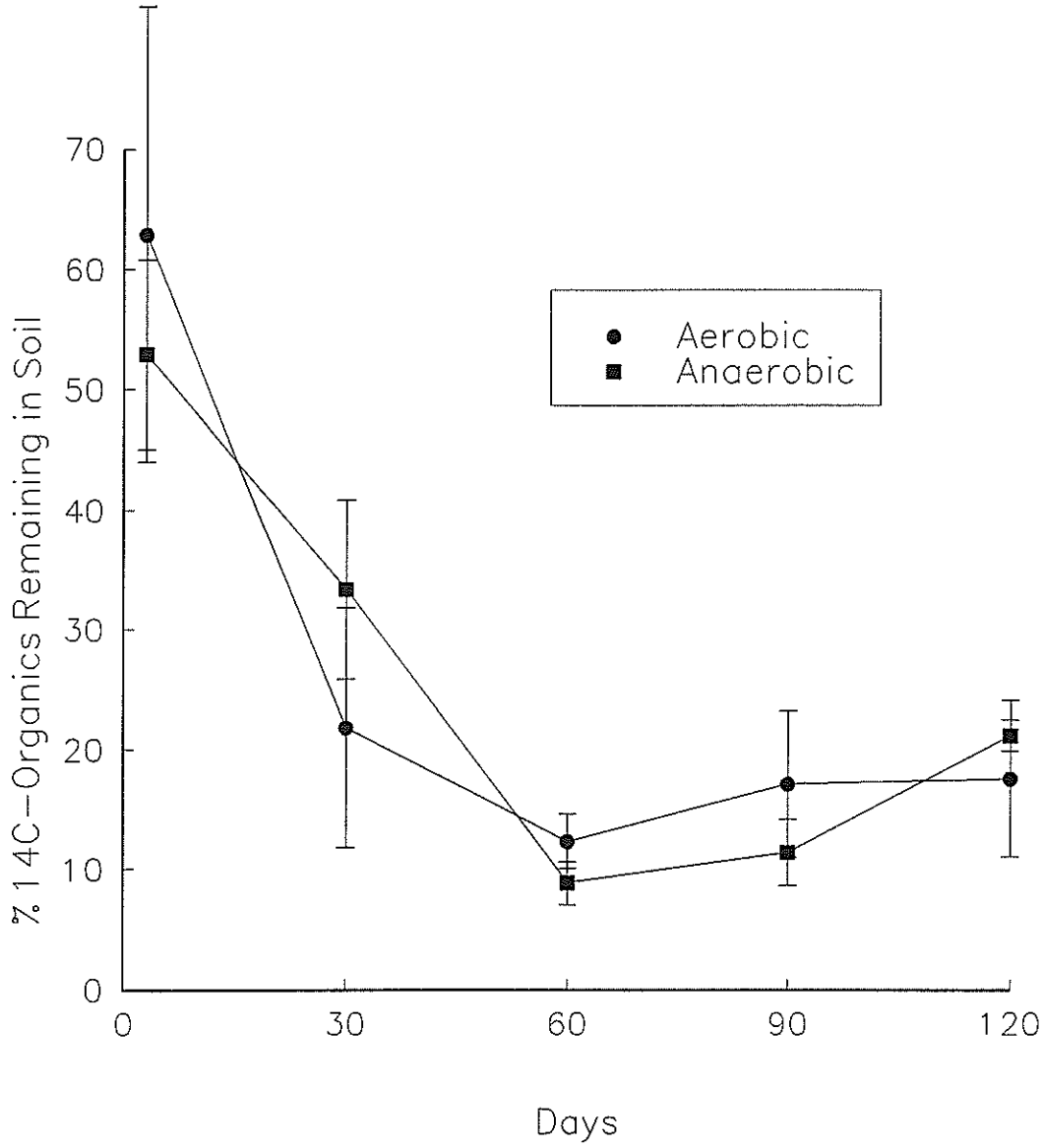

FIGURE 7. Residual TCA recovered in aquifer material following aerobic and anaerobic incubations.

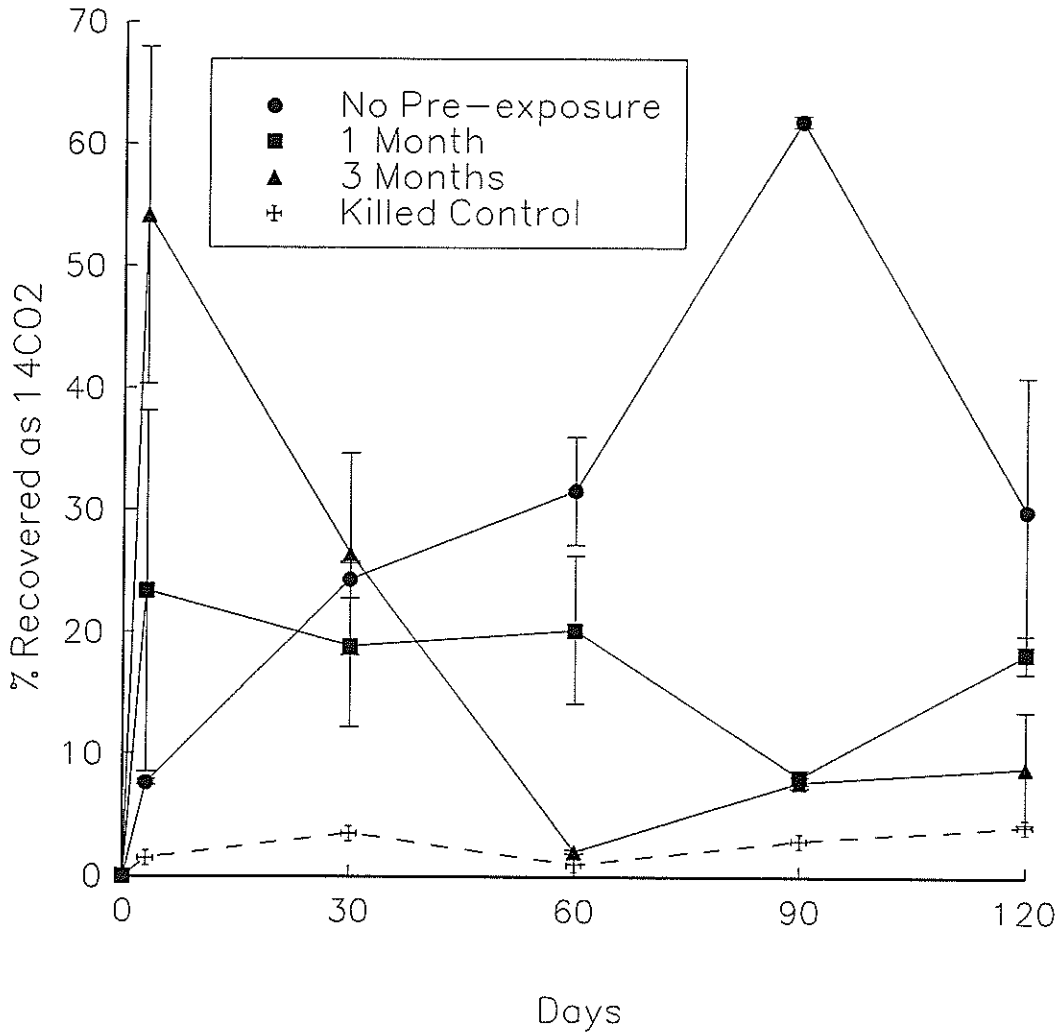

FIGURE 8. $^{14}CO_2$ production in aerobic incubations of TCA with aquifer material subjected to 0-, 2- and 4-month pre-exposure.

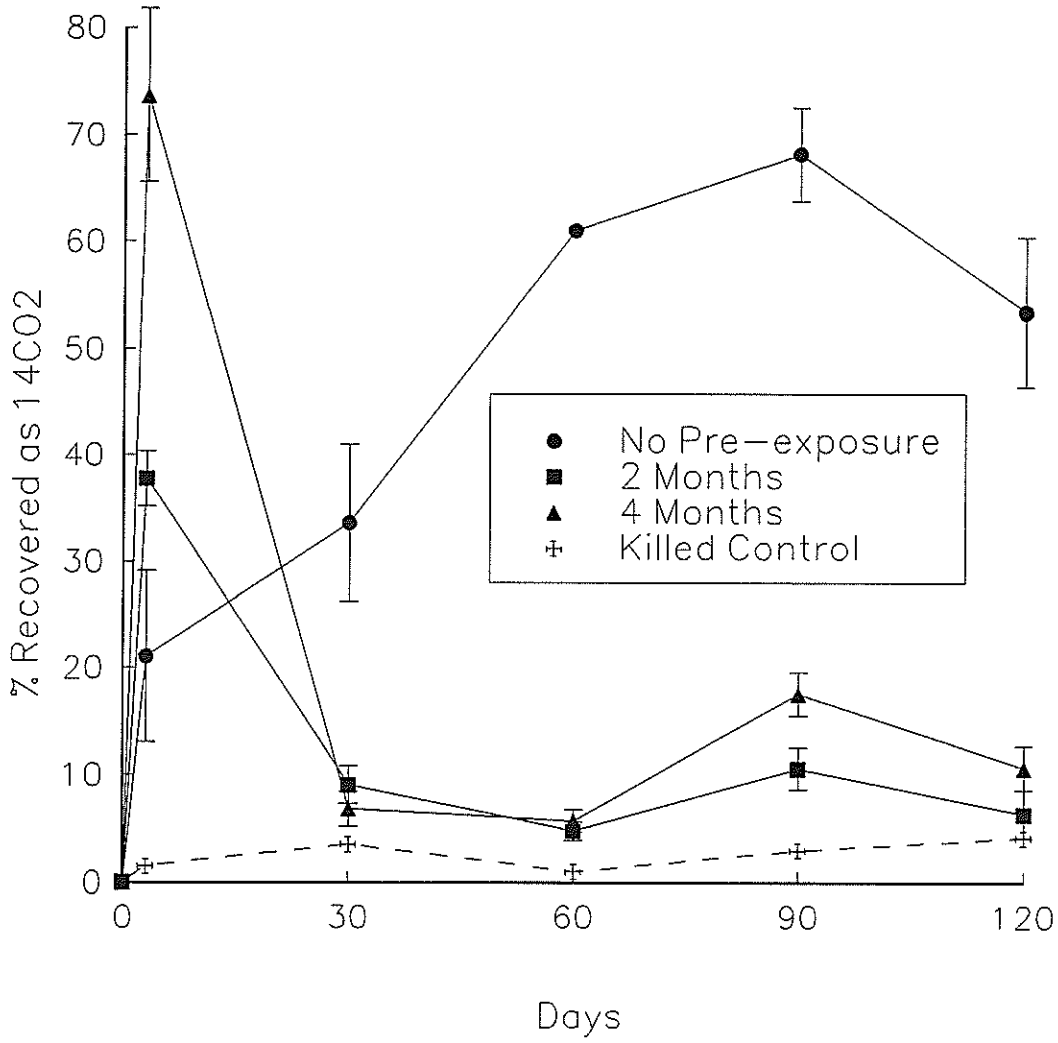

FIGURE 9. $^{14}CO_2$ production in anaerobic incubations of TCA with aquifer material subjected to 0-, 2-, and 4-month pre-exposure.

Results of shifting redox conditions from anaerobic to aerobic are shown in Figure 10. In the case of TCA, samples with no pre-exposure were also subjected to a redox shift, but it did not have a significant effect on $^{14}CO_2$ production. With respect to both long and short pre-exposure samples, however, the shift from anaerobic to aerobic conditions resulted in sustained elevated levels of net $^{14}CO_2$ generation. These results indicate that shifting redox conditions in the incubation flasks had significant effects either on the biodegradation of TCA, the biological cycling of $^{14}CO_2$, or both.

Figure 11 shows the incorporation of TCA-derived ^{14}C into microbial biomass under aerobic conditions. While substantial levels of incorporation (approaching 70% recovered) occurred for both the non-pre-exposed and the 2-month pre-exposure samples, relatively low amounts (less than 20%) were incorporated into microbial biomass in the 4-month pre-exposure samples. This result is very different than that obtained for benzene, and the explanation for why this would occur is not immediately apparent. It is also interesting that levels of $^{14}CO_2$ incorporation remained fairly constant in the non-pre-exposure samples, whereas substantial amounts of $^{14}CO_2$ appeared to be released from biomass subsequent to incorporation in the 2-month pre-exposure samples, resulting in the lower levels shown in the figure at 90 and 120 days.

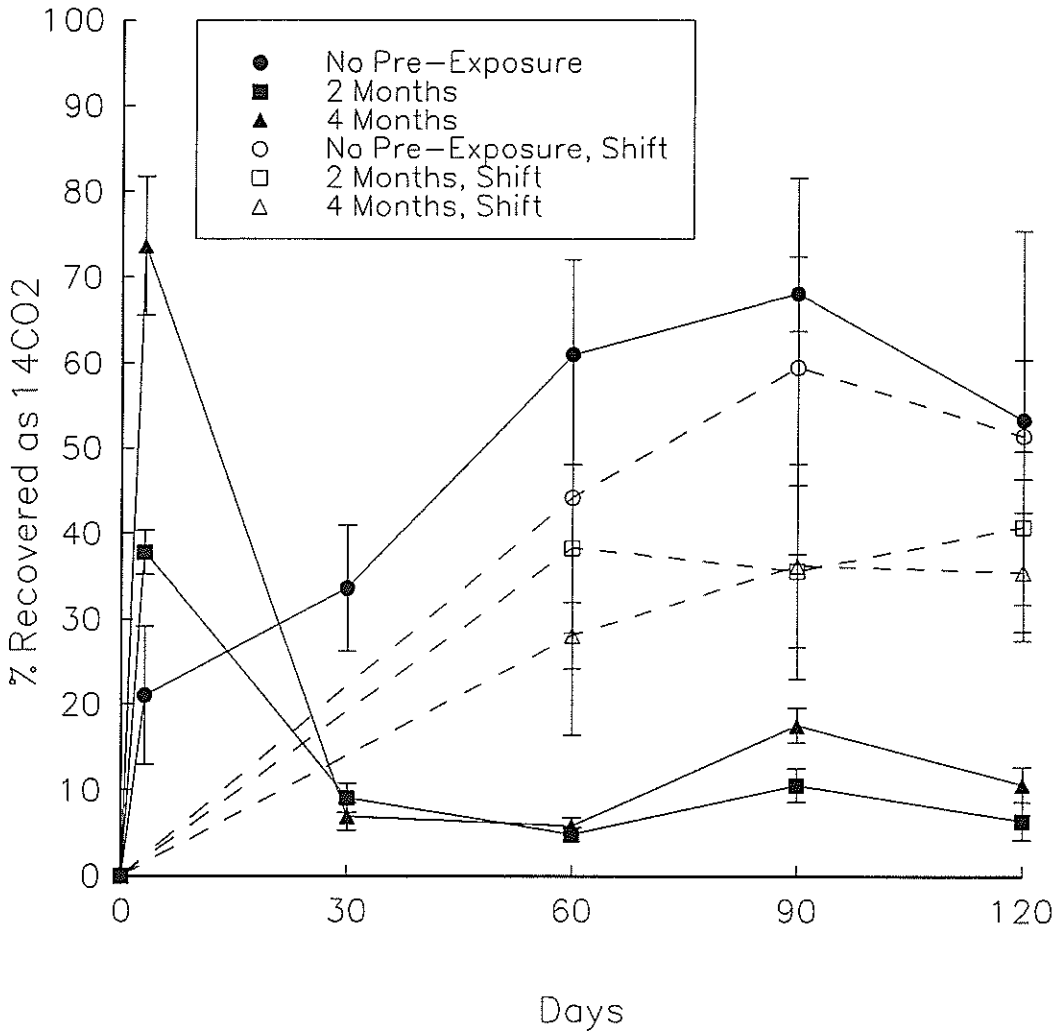

FIGURE 10. Influence of redox shift from anaerobic to aerobic conditions on the production of $^{14}CO_2$ in incubations of TCA with aquifer material subjected to 0-, 2- and 4-month pre-exposure.

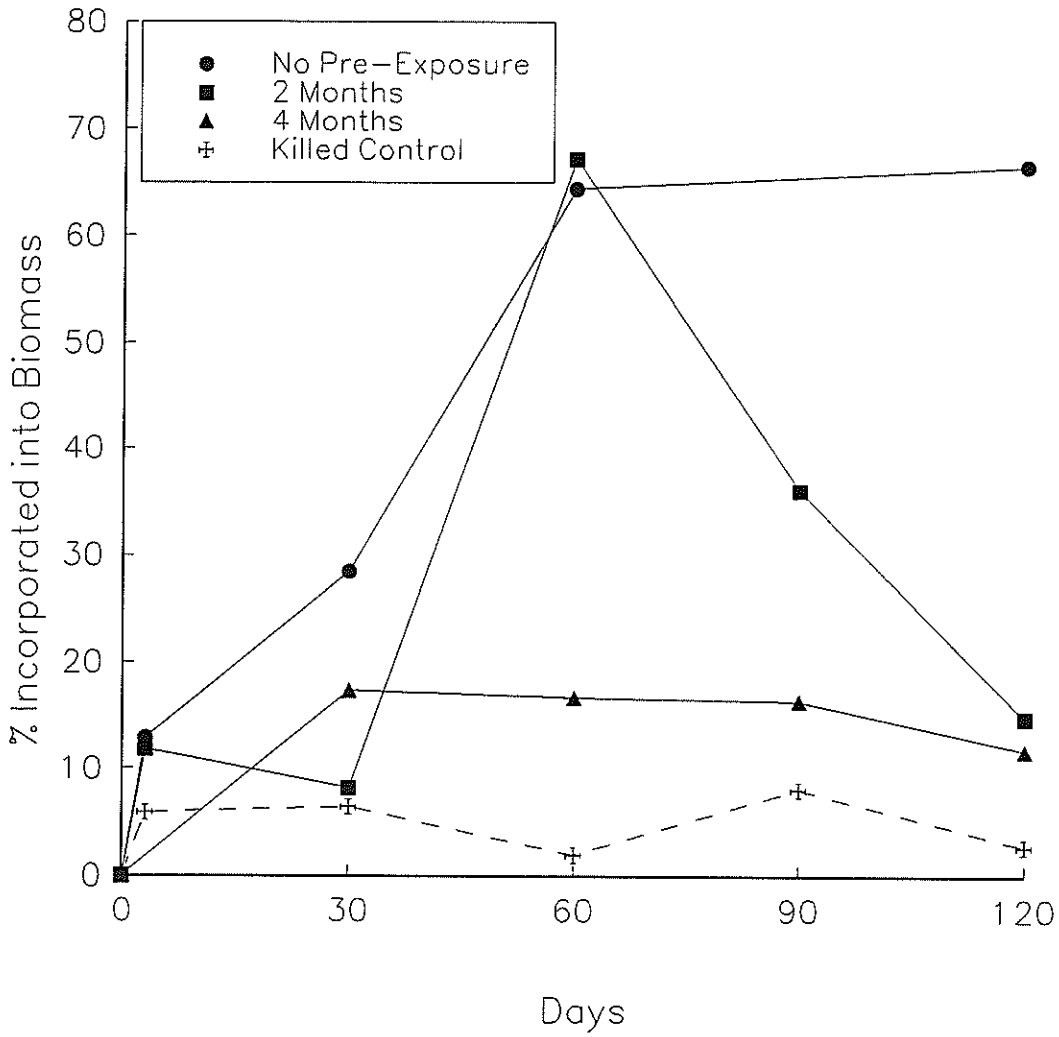

FIGURE 11. Incorporation of TCA-derived ^{14}C into microbial biomass for samples receiving 0-, 2-, and 4-month pre-exposure.

The amount of TCA (and intermediates) recovered as volatiles trapped in the charcoal shunt filters is shown in Figure 12 for aerobic and anaerobic incubations. Levels of volatiles reached a maximum at approximately 30 days for all samples, then declined. Volatile organic levels never exceeded 20% of the original amount of TCA added to the aquifer samples. It is interesting to observe that the patterns of volatile organics trapped by the filters were extremely similar for aerobic and anaerobic incubations, as well as for irradiated killed control samples.

Microbial Populations

Microbial analyses revealed that approximately 8.5 x 10^8 cells/ gram wet weight of total heterotrophic microorganisms were present in the aquifer material. Of these numbers approximately 86% of the cultured cells were gram negative, 7% gram positive, and 7% gram variable. Of the heterotrophs cultured on nutrient agar, rods and cocci were present in approximate equal numbers, although rods outnumbered cocci 75 to 25% when grown on trypticase soy agar.

We were unable to isolate TCA degraders from pristine aquifer material, and benzene degraders were isolated from these samples only with extensive organic enrichment in the laboratory. Subsequent to 7 days of enrichment in nutrient broth approximately 6.6 x 10^4 cells / mL enrichment medium were isolated from the pristine aquifer sediment. These organisms were confirmed as benzene degraders by their ability

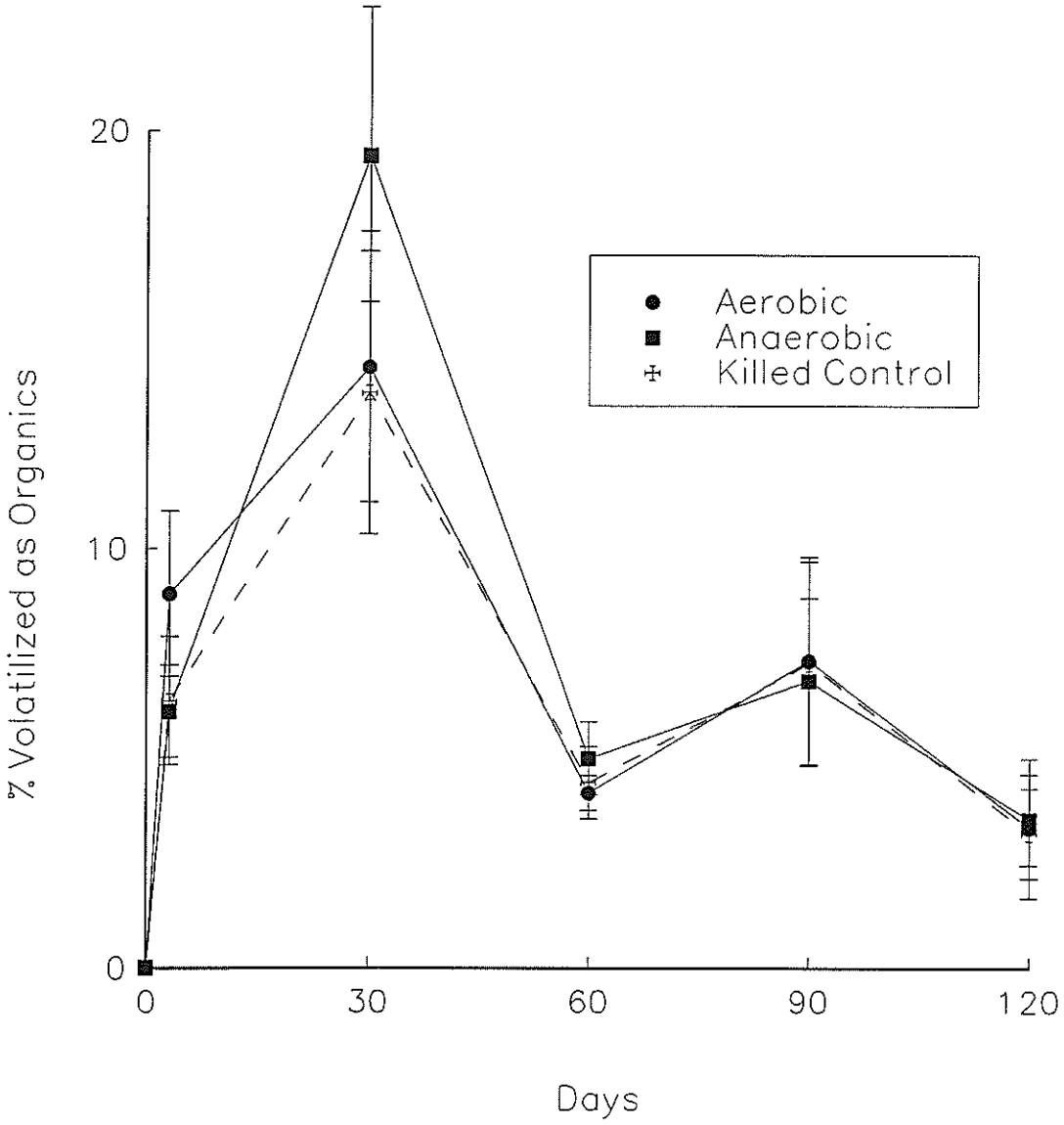

FIGURE 12. Volatilized organics trapped by charcoal filters during aerobic and anaerobic incubations of TCA with aquifer material.

to grow heterotrophically with benzene as the sole source of organic carbon. Exposure of the aquifer sediment to 100 ppm levels of benzene or TCA for 6 months selected for bacteria capable of degrading the specific contaminant. Approximately 5.0×10^6 and 2.3×10^7 cells / gram wet weight aquifer material, benzene and TCA degraders, respectively, were isolated subsequently from the exposed sediment.

PRINCIPAL FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

Principal Findings

* Both benzene and TCA were effectively removed from pristine aquifer material within a relatively short time. Benzene levels decreased more rapidly than TCA, and benzene removal was more complete.

* With respect to benzene biodegradation, $^{14}CO_2$ production was slightly higher during aerobic incubations, although significant amounts of $^{14}CO_2$ were also produced under anaerobic conditions. Uptake of $^{14}CO_2$ subsequent to mineralization was observed in most of the samples, although $^{14}CO_2$ recycling patterns was stronger for the anaerobic incubations.

* TCA incubations revealed similar patterns of $^{14}CO_2$ generation and uptake, with higher total net amounts of $^{14}CO_2$ being generated anaerobically.

* In several of the incubations, contaminant pre-exposure (particularly the longer pre-exposure) resulted in higher net levels of $^{14}CO_2$ being produced, but this was not always the case. The most significant result of the pre-exposure periods appears to be that pre-exposure resulted in more rapid release of $^{14}CO_2$ as compared with non-pre-exposed samples. This pattern was true for anaerobic benzene incubations and for both aerobic and anaerobic TCA incubations.

* Shifts in redox conditions from anaerobic to aerobic appeared to mitigate the $^{14}CO_2$ recycling phenomenon for both benzene and TCA. Relatively constant increases in $^{14}CO_2$ levels were observed following the shift, although for TCA, samples which had not been pre-exposed to the contaminant did not exhibit such increases.

* Incorporation of ^{14}C into microbial biomass played an important role in incubations of benzene and TCA. With respect to benzene, samples receiving the longer pre-exposure exhibited the highest level of ^{14}C incorporation, whereas the reverse was true for samples incubated with TCA.

* Recovery of volatile organic compounds increased with time for the benzene incubations and increased and then declined for the TCA samples. In both cases, irradiated killed controls followed very similar patterns.

* Gram negative rods and cocci predominated in the microbial populations in this aquifer. Benzene and TCA degraders were difficult to grow under laboratory conditions, but benzene degrading populations were successfully isolated from the pristine material following enrichment. Exposure to contaminants for a lengthy period facilitated the isolation of both benzene and TCA degraders.

Conclusions

* Pristine aquifer material from the riparian zone of the Rio Grande in New Mexico contains microbial populations able to degrade benzene and TCA.

* Benzene was more rapidly degraded than TCA. No difference between aerobic and anaerobic release of $^{14}CO_2$ was measured with benzene; anaerobic conditions favored TCA degradation. Benzene and TCA were degraded to some extent both aerobically and anaerobically.

* Once $^{14}CO_2$ was generated by the mineralization of contaminant compounds, strong patterns of uptake and release were noted within these closed systems. This dynamic system of $^{14}CO_2$ release and uptake was likely associated with bacterial autotrophy.

* Contaminant pre-exposure increased levels of $^{14}CO_2$ production in some cases, but was most strongly associated with lessening the time required for maximum $^{14}CO_2$ generation.

* Redox shifts from anaerobic to aerobic affected either the generation of $^{14}CO_2$, the recycling of this compound to microbial biomass, or both.

* Incorporation of ^{14}C generated by the mineralization of contaminants played an important role in ^{14}C cycling; this process represented an important biological fate of contaminants in our batch incubations.

* Large populations of microorganisms occur in this aquifer, and the contaminant degraders make up a significant proportion. However, the contaminant degrading populations were extremely difficult to culture under laboratory conditions without prior exposure to the contaminant of interest. Prior exposure increased the ease with which these microorganisms were isolated; this may reflect a microbial adaptation to the contaminant which rendered the populations less fastidious.

Recommendations

* Based on these results and conclusions we recommend that bioremediation of contaminated aquifers in New Mexico receive careful attention. Site-specific analysis should be conducted to ensure the feasibility of remediation treatment, but this study indicates that microorganisms capable of degrading very different types of contaminants are present within large regional aquifers.

* In estimating the time required for successful bioremediation, the site history of contaminant exposure should be considered. Recent releases will probably require longer for complete biodegradation if there has been no previous exposure of the site to the contaminant of interest.

* Redox status at a remediation site should be monitored and/or controlled to provide the optimum environment based on the contaminant structure and the local microbial response. Shifting redox status from anaerobic to aerobic may increase biodegradation, particularly of chlorinated aliphatics, but more research needs to be conducted to thoroughly address this question.

* Finally, these results can provide a first approximation for the importance of biotic processes in the fate of contaminants in ground water models. Non-conservative microbial biodegradation of organic contaminants needs to be considered in ground water transport of contaminants. These results can serve as initial boundary conditions for

introducing these terms into such models.

SUMMARY

Effects of several important environmental factors on the biodegradation of benzene and 1,1,1-trichloroethane (TCA) were examined in laboratory batch experiments. Regional uncontaminated aquifer material was incubated with ^{14}C-radiolabelled benzene and TCA. Mineralization was quantified by measuring amounts of $^{14}CO_2$ produced over time. Redox conditions (aerobic, oxygen rich vs. anaerobic, oxygen depleted), changes in redox conditions, and short and long pre-exposure periods were analyzed for their effects on biodegradation. Additional variables that were quantified include residual organics in aquifer material, residual volatile organics, and ^{14}C incorporated into microbial biomass. We also conducted preliminary analysis of the microbial populations present in the aquifer material.

Significant levels of biodegradation occurred for both contaminant types, with degradation occurring under aerobic and anaerobic conditions. The biochemical fate of each compound was influenced by the prevailing redox conditions as well as by the length of pre-exposure to the contaminant. Aerobic and anaerobic degradation of benzene resulted in comparable levels of complete mineralization, whereas anaerobic conditions favored TCA degradation. Pre-exposure resulted in higher levels of mineralization in some cases, but the major effect was to decrease the time required for maximum levels of mineralization to occur. After $^{14}CO_2$ was generated by contaminant mineralization, the compound was subject to uptake and recycling through autotrophic pathways, although shifts in redox conditions from anaerobic to aerobic

appeared to lessen the recycling phenomenon.

Incorporation of ^{14}C into microbial biomass occurred during incubations with both contaminants, and this process was found to represent an important biological fate of organic contaminants in the subsurface.

Microbial populations were largely gram negative rods and cocci. Although substantial numbers of benzene and TCA degraders were present, they were very difficult to grow under laboratory conditions. Lengthy exposure to the contaminants resulted in microbial adaptation to the compounds, rendering the populations less fastidious and easier to isolate in the laboratory.

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