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Biodegradation of RDX within soil-water slurries using a combination of differing redox incubation conditions

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Abstract

Biodegradation of ^{14}C-tagged hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) was studied in aerobic, anaerobic, and anaerobic/aerobic slurries to identify the conditions maximizing RDX-mineralization in Cornhusker Army Ammunition Plant (CAAP, NE) groundwater. Supplementation with phosphate and adequate quantities of acetate caused 25% mineralization of RDX in 3 weeks by microorganisms native to CAAP. Under anaerobic conditions, the same supplementation resulted in 20% mineralization in 3 weeks and 30% mineralization in 6 weeks. The highest degree of mineralization (50%) was obtained under aerobic conditions when the contaminated groundwater was augmented with a consortium of three microbes isolated from another RDX contaminated soil (Hastings, NE) in addition to supplemented with phosphate and acetic acid. Use of complex organic sources (potato or corn starch) slowed down the rates of mineralization under anaerobic conditions, but rapid mineralization ensued as soon as the aerobic conditions were created. Final RDX concentrations in aqueous phase were below detection limit under most conditions. Assimilation of RDX by the cells was negligible.

Keywords: RDX mineralization; Co-metabolism; Aerobic biodegradation; Radiometry; Column studies

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1. Introduction

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and its formulations with 2,4,6-trinitrotoluene (TNT) have widely been used by militaries around the world since the Second World War [1,2]. The production, processing, and usage of these explosives have resulted in the vast contamination of soil and water at military installations worldwide [3]. The solubility of RDX in water is 43.2 mg/l at 25 °C [2] and it is highly mobile in groundwater [4]. RDX is a suspected mutagen and a type C carcinogen [5]. It causes nausea, irritability, convulsions, unconsciousness, and amnesia in humans [6,7,5,8]. As a result, soil and groundwater contamination with RDX is cause for major concern. EPA drinking water-quality criterion for RDX is 0.3 μg/l [9]. For RDX-contaminated groundwater, the target treatment level is typically 2 μg/l [5].

Several researchers have discussed environmental fate of RDX, including volatilization [10], hydrolysis [11,12], sorption [13], photodegradation [11], and biodegradation [14–17]. Volatilization and hydrolysis are not major factors in determining the fate of RDX in groundwater under natural conditions even though RDX degrades rapidly and completely in the pH range of 10–12 at elevated temperatures (50–80 °C) [18]. Similarly, photodegradation is also not a viable fate for RDX in subsurface and groundwater in spite of its rapid photolysis in aqueous solution [11] and the resulting reduction in toxicity for daphnia [19]. Adsorption of RDX to soil particles occurs reversibly [20,21]. Biodegradation of RDX has been reported by a number of authors [22–25,9,26–29,14,30,31]. The general consensus of these studies was that RDX is biodegraded co-metabolically under aerobic as well as anaerobic conditions. It is metabolized by a number of indigenous microorganisms and its metabolic products include hexahydro-1-nitroso-2,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), dimethylhydrazine, hydrazine, nitrite, and formaldehyde [14,32]. Several of the reported metabolites in biodegradation of RDX are considered hazardous, although [33] reported accumulation of no potentially carcinogenic byproducts under aerobic conditions. The rates of biodegradation under aerobic conditions were not as high as those under anaerobic conditions [23].

In view of the fact that RDX and several of its metabolites are potentially carcinogenic, it is necessary to maximize the extent of RDX-mineralization (conversion to carbon dioxide) in any process. The objective of this study was to establish the conditions under which the extent of mineralization of RDX in the contaminated CAAP-groundwater and aquifer material can be maximized. This was achieved by examining the fate of ^{14}C-labled RDX in shake-flasks under different conditions. The variables investigated were the carbon and energy sources, redox conditions, and effect of native (to CAAP) and non-native RDX-degrading microorganisms.

2. Materials and methods

Aquifer soil and groundwater were obtained from the Cornhusker Army Ammunition Plant (CAAP), Nebraska, USA. A composite soil sample was collected from a depth of 30–40 ft below ground level. Groundwater was collected from a monitoring well that had high levels of RDX contamination. Soil as well as groundwater were transported to

Table 1
Concentration of different contaminants in CAAP groundwater and soil

Analyte	Water (mg/l)	Soil[a] (μg/kg)
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	4.53	<20
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	16.40	<20
1,3,5-Trinitrobenzene (TNB)	93.60	<20
1,3-Dinitrobenzene (DNB)	<0.20	<20
TETRYL	<0.50	<20
2,4,6-Trinitrotoluene (TNT)	330.00	<20
4-Amino-2,6-dinitrotoluene (4A-DNT)	51.40	<20
2-Amino-4,6-dinitrotoluene (2A-DNT)	54.90	<20
2,6-Dinitrotoluene (2,6-DNT)	<0.20	<20
2,4-Dinitrotoluene (2,4-DNT)	0.56	<20

[a] Detection level of the different contaminants in soils was 20 μg/kg soil. In water, the detection limit for the different contaminants was 0.20 μg/l.

Vicksburg, MS under ice and stored in a cooler at 4 °C pending experiments. Prior to its analysis and use, the soil was passed through a #10 sieve and manually homogenized. Extensive homogenization, however, was avoided for the fear of causing extensive disturbance in the microbial ecology of the subsurface soil. According to the Unified-Soil-Classification System analysis, the soil contained between 73 and 95% sand, with the rest being silts and clays. Soil pH was 7.3. Total organic content was 362 mg/kg and the cation exchange capacity was 0.370 meq./100 g. Microbial count in the soil was estimated by fluorescence direct count screening at 440,000 cells/g dry soil. Chemical analytical data for the samples are presented in Table 1. The concentrations of most contaminants in soil were below the detection limit. The groundwater contained RDX, trinitrobenzene, trinitrotoluene, aminonitrotoluenes, and HMX in significant quantities, and small amounts of dinitrotoluenes. The presence of aminonitrotoluenes in groundwater suggests some TNT biodegradation activity in the soil. Of the compounds present, only the dinitrotoluenes have been reported to be able to support cell growth as carbon and energy sources [34].

Uniformly ring-labeled [^{14}C]RDX with a radiochemical purity of >99% and stock solution concentration of 9.8 mg/l was obtained from New England Nuclear Life Science Products (Boston, MA). In some experiments, [^{14}C]RDX of 93% radiochemical purity and stock solution concentration of 6.3 mg/l was used. Non-radioactive RDX was obtained from Hallston Army Ammunition Plant (Kingston, TN) and used in the form of a stock solution containing 10 mg RDX/l. Reagents (chromatography grade) used in Bligh–Dyer extractions were purchased from Burdick and Jackson (Muskegon, MI).

The radiometric experiments were conducted in 250 ml biometric flasks (Ace Glass). KOH solution placed in the center well was used to capture any carbon dioxide evolved. The side arm of the flasks was fitted with a helium grade balloon to accommodate changes in pressure during addition and recovery of solutions. Before each experiment, the flasks were thoroughly cleaned, checked for radioactivity, heat sterilized at 121 °C for 15 min, and then assembled inside sterile hoods. The assembly consisted of placing 2.5 ml of 1 N KOH solution in the center well, followed by introducing 20 g dry weight soil, 80 ml CAAP groundwater, 5 ml sterile additive-solution, 1–2 ml RDX stock solution (10 mg/l), and 1 ml

^{14}C-RDX solution in the flask itself. The additive solution contained KH_2PO_4, candidate carbon source, and surfactant (in some cases). Four candidate carbon sources (sodium acetate, ethanol, potato starch, and corn starch) were evaluated. Only one candidate carbon source was added to one flask and its level was either 92 or 7000 mg/l aqueous phase. All the candidate carbon additives are easily metabolized by most soil microorganisms and can serve as carbon and energy sources. Acetate is a commonly used co-metabolite for stimulating soil microbes and serving as an electron donor. Ethanol is commonly used in solvent flushing technologies and is an environmentally acceptable electron donor. Selection of starches was based on their widespread availability, biodegradability, and complex nature of the molecules. Earlier experience in our laboratory had shown that Tween 80 (polyoxyethylene (20) Sorbitan monooleate, CAS# 9005-65-6) enhances biodegradation of RDX in slurry reactors [35]. Hence, Tween 80 was also used as an additive either alone or with other carbon sources at a level of either 6908 mg/l or at 7000 mg/l, depending on whether it was present with carbon source or alone. The concentration of KH_2PO_4 in the slurry was 5.73 mg/l. No nitrogen source was added as the presence of nitrates has been shown to suppress biodegradation of RDX [30]. Measurements of biochemical oxygen demand of the additives using an electrolytic respirometer suggested that soil microorganisms did in fact metabolize all the additives (including Tween 80).

A consortium of three microorganisms (internally referred to as Hastings Triplet), *Pseudomonas nitroreducens, Alcaligenes denitrificans, and Pseudomonas fluorescens*, isolated from explosives-contaminated soil from the Hastings Army Ammunition Plant (Nebraska, USA) was also used in some aerobic experiments. *Morganella morganii* B2 cells obtained from American Type Culture Collection (ATCC 51596) were used to inoculate some flasks maintained under anaerobic conditions. In flasks augmented with non-native microorganisms, the CAAP soil and groundwater were double autoclaved at 121 °C for 20 min to kill the native microorganisms. In the flasks with native microorganisms, CAAP soil/groundwater used was not autoclaved and no additional microorganisms were added. Sterile controls were prepared by adding 0.3 g mercuric chloride to the slurry in the flasks.

The flasks were sealed and incubated in orbital shakers set at 100 rpm and 25 ± 2 °C. KOH solutions from the center well were withdrawn and replaced with fresh KOH solution every 2–3 days. Samples were also collected from the flask for analyses. pH and oxidation–reduction potential (ORP) in the samples were measured immediately upon sampling. In the aerobic experiments, gas in space above the flask-fluids was allowed to exchange with outside air for 5–10 min during KOH withdrawal and refilling. Anaerobic experiments were conducted in Coy$^{@}$ glove bags [heavy duty, optically clear, radio frequency welded, one piece flexible 20–40 mil PVC bag mounted on a rigid base] purged with oxygen-free gas mixture containing 96% nitrogen and 4% hydrogen. In this case, KOH solutions were exchanged using syringes with precautions to avoid introduction of air. At the end of the experiment, KOH solution in the center well was exchanged with fresh KOH solution and the flasks were sealed again. A total of 1 ml concentrated phosphoric acid was then added to the flask contents to convert any carbonates in solution to carbon dioxide that was then captured in the KOH traps for measurement.

The samples collected at the end of each experiment were centrifuged to separate the solids from liquid. Radioactivity in the supernatant was measured directly, while the soil was extracted using a modified Bligh–Dyer organic solvent extraction procedure [36] to

quantitatively recover the extractable polar and non-polar radioactivity from soil. The extracted soil was oxidized in a Packard Model 307 Sample Oxidizer to measure the amount of non-extractable radioactivity in it. All the radioactivity measurements were conducted in presence of Ultima Gold (Packard Instruments) scintillation cocktail in a Packard 2500TR Liquid Scintillation Analyzer. Thus, the radioactivity data provided for a carbon-balance in the form of carbonate species (mineralization to carbon dioxide evolved as gas and as dissolved carbonate in solution), metabolites and RDX in the aqueous phase, and adsorbed metabolites as extractable non-polar, extractable polar (likely associated with or actually within cell lipids), and non-extractable from the soil.

Aqueous samples were analyzed for RDX using a HPLC with UV-detector, generally following USEPA protocol 8330. Prior to injection, the samples were pre-concentrated by passing each sample through a Porapak@ RDX Sep-Pak@ Vac Cartridge (consisting of a divinylbenzene/vinylpyrrolidone copolymer, packed in syringe barrels). The adsorbed nitramines and nitroaromatics were eluted from the cartridges by acetonitrile and injected into HPLC. The HPLC protocol has been described by [37]. The detection limit of RDX in aqueous phase was $\leq$45 μg/l. No attempt was made to analyze the samples for metabolites of RDX. All the experiments were conducted in replicates of five.

The respiration studies were conducted in a BI-1000 electrolytic respirometer from Biosciences Inc. This allowed for screening for biological activity by measurements of oxygen uptake.

3. Results

The effect of several different carbon additives (co-metabolites) on biodegradation and mineralization of RDX in aerobic, anaerobic, and aerobic/anaerobic conditions was studied in this research.

3.1. Aerobic experiments

Preliminary aerobic experiments were conducted with 92 mg/l candidate carbon source in liquid phase in the flasks. Although the data are not shown, the results are summarized as follows. Initial RDX concentration in each flask was 202 μg/l (mass ratio of supplemental carbon source to RDX 455:1) and the flasks contained only the native CAAP microorganisms. The aqueous-phase residual concentration of RDX after 3 weeks of incubation was around 50% of initial value in all these flasks. However, the mineralization of RDX to carbonates was insignificant. The recovery of radioactivity during the experiments was $100 \pm 8\%$ (one standard deviation) of the amount initially introduced into the flasks. Most of the radioactivity was measured as RDX and its soluble metabolites in the aqueous phase; only 3% of [^{14}C] was recovered as extractable non-polar fraction (methylene chloride fraction of the Bligh–Dyer extraction) and only 1–2% as the extractable polar portion. This suggests that little RDX was assimilated by the cells. In one set of flasks, Tween 80 was also added (at a level of 6908 mg/l aqueous solution) along with acetate (92 mg/l) bringing the mass ratio of supplemental carbon source to RDX to 34.7 mg/μg RDX. In these flasks, the concentration of RDX after 3 weeks of incubation was below detection limit, 43.3%

RDX was mineralized (i.e. recovered as total carbonates in the KOH and the aqueous phase), and 44.1% radioactivity was recovered as soluble intermediates in the aqueous phase. Thus, the addition of Tween 80 to the medium along with acetate significantly enhanced the mineralization of RDX under aerobic conditions. This supports the observations of [35] that indicated significant enhancements to TNT biodegradation by addition of Tween 80 to the soil systems.

Since Tween 80 is a surface-active agent as well as a carbon source, aerobic experiments were conducted with high level of other carbon additives as well to elucidate the role of surface-active agent vis-à-vis the total amount of metabolizable organic carbon additives added to the flasks. In these experiments, the total amount of carbon additives (Tween 80 alone, or acetic acid alone, or a mixture of Tween 80 and acetic acid) in each flask was 7000 mg/l. The initial RDX concentration in the flasks was 328 μg/l (ratio of supplemental carbon source to RDX = 21.3 mg/μg). Each set of flasks was divided into two subsets—one contained native CAAP microbes and the other contained Hastings triplet. The results of these experiments are presented in Figs. 1–3.

In all the cases, the sterile controls showed little change in the concentration of RDX over 3 weeks (Fig. 1). Over the same time, RDX concentration in the flasks supplemented with acetate-only was below the detection limit, regardless of whether these were inoculated with Hastings triplet or with native CAAP microbes. Final residual RDX concentration in flasks supplemented with Tween 80 (either alone or with acetate) was dependent on whether these had native microorganisms or Hastings Triplet. In flasks inoculated with Hastings Triplet, the final residual aqueous phase RDX concentration was below detection levels regardless of any candidate carbon addition; the corresponding RDX concentration in flasks inoculated with the native CAAP microorganisms was around 20% of initial value (Fig. 1).

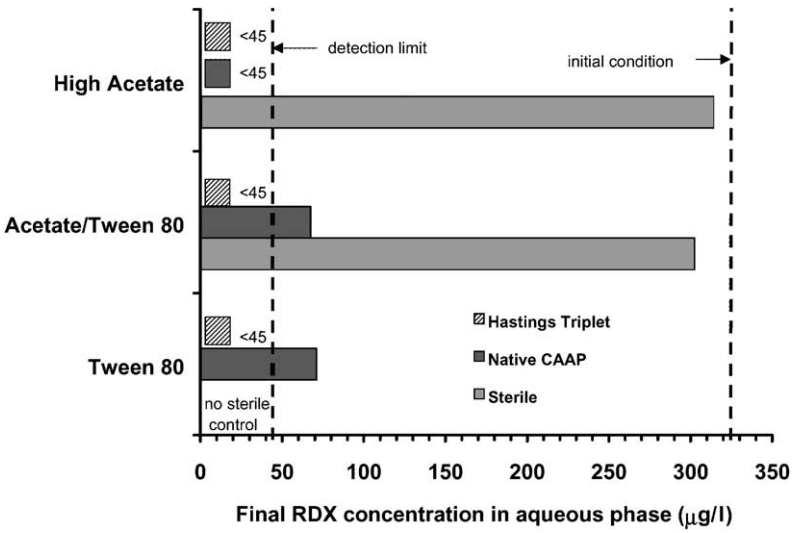

Fig. 1. Final RDX concentration in aerobic flasks after 3 weeks of incubation.

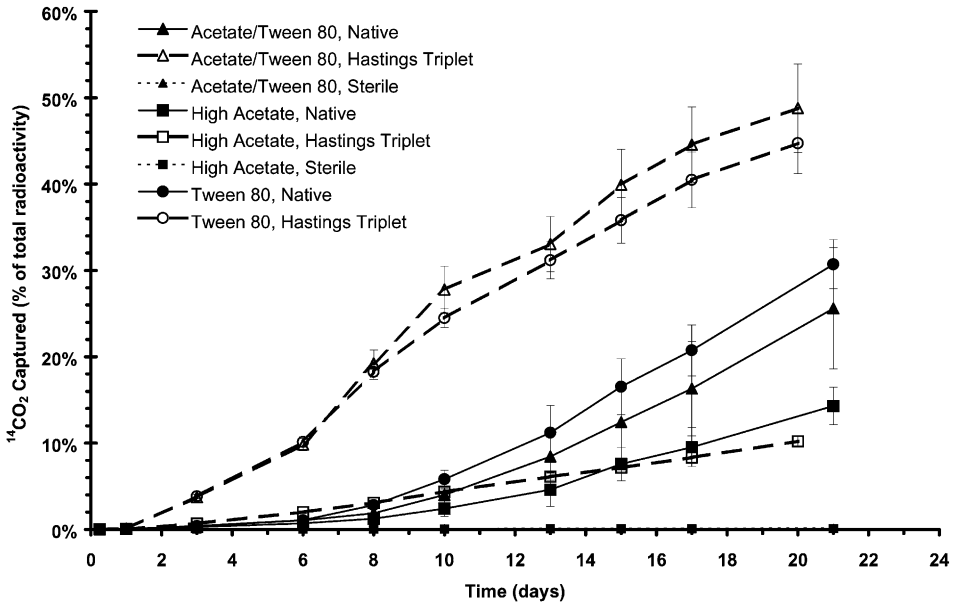

Fig. 2. Evolution of $^{14}CO_2$ vs. time in aerobic experiments.

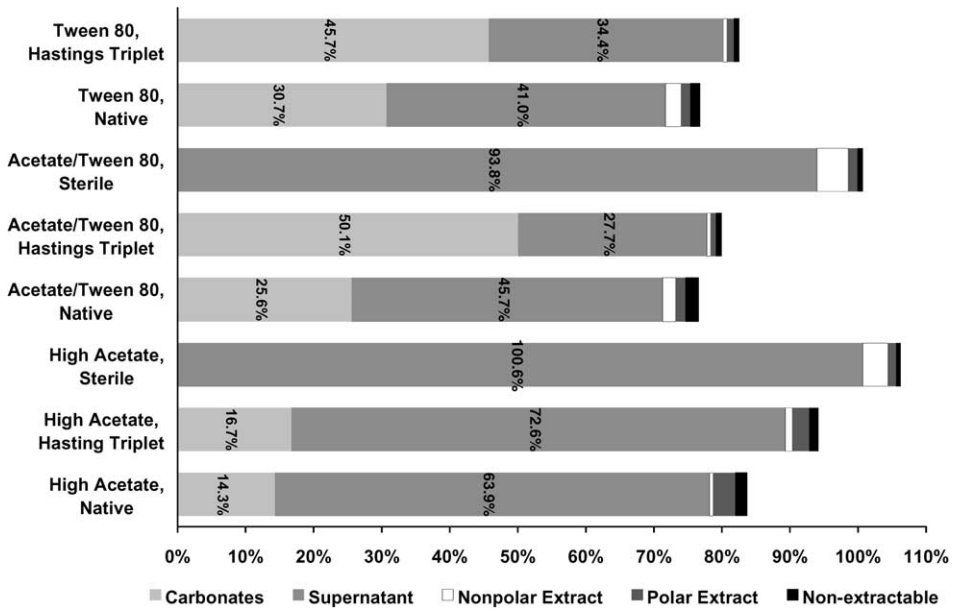

Fig. 3. Distribution of ^{14}C among different phases in aerobic flasks after 3 weeks of incubation.

The evolution of radioactive carbon dioxide (captured in the KOH solution) in the different flasks is presented in Fig. 2. Those flasks where acetate was added, with or without seeding with the Hastings consortia, indicated much lower ^{14}C evolution, both in rate and extent. The addition of Tween 80, without benefit of Hastings consortia and with or without acetate, performed well as compared to the acetate addition. However, when Tween 80 and the Hastings consortium were both added, by far the greatest extent and rate of ^{14}C evolution was observed. These systems yielded percent recoveries of ^{14}C approaching 50%. Since the experiments were halted after 3 weeks, the difference between native microorganisms and Hastings Triplet could not be established in terms of extent of mineralization. Flasks containing native microorganisms showed a long lag phase, but seemed to achieve the same rate of CO_2 production as Hastings triplet in the third week (Fig. 2). Hastings Triplet was isolated for their ability to biodegrade explosives, but the inoculated flasks could simply have higher concentrations of more active degraders at the start of experiments.

The mass balance data of ^{14}C recovered from the different flasks are presented in Fig. 3. Total recovery of ^{14}C in the sterile flasks was approximately 100%, with most of radioactivity recoverable from CO_2 evolved and the aqueous supernatant after the contents of the microcosms were centrifuged. The 'active' flasks had ^{14}C balances ranging from 75 to 95%, with most at about 80%. This indicates an additional fraction not accounted for as $^{14}CO_2$ or ^{14}C-metabolites in slurry. Only small amounts of ^{14}C material were present with the solvent extractable or soil-bound fractions.

pH in all the flasks remained between 7 and 9.5. Reducing conditions did not develop in any of the aerobic flasks. The measured values of ORP were lowest (+150 to +170 mV) in the flasks containing Hastings Triplet. The ORP values in all the other flasks were above +200 mV. These results showed that high extent of RDX mineralization and biodegradation is possible without strict anaerobic conditions. The results suggest that native microorganisms rapidly transformed RDX in presence of easily metabolizable carbon supplements but mineralization to carbon dioxide was assisted by presence of Tween 80.

3.2. Anaerobic experiments

Anaerobic experiments were conducted for a period of 6 weeks and the experimental data are presented in Figs. 4 and 5. Initial RDX concentration in the flasks was 202 μg/l. The carbon additives in the flasks were acetate, starches, or ethanol at levels of 92 mg/l. One set of flasks was provided no carbon-additive, serving as an experimental control. A killed control was also maintained. Native microorganisms were the only biological activity present in unsterile flasks. The ORP values in all the sterile flasks at the end of 6 weeks were around +450 mV while those in the flasks with biological activity ranged from −300 to −480 mV suggesting development of highly anaerobic conditions in the 'active' flasks.

In all the active flasks, the residual RDX concentrations after 6 weeks ranged from non-detect to barely detect (Fig. 4). This reduction to barely-detect levels in the concentration of RDX was observed even in the set to which no carbon supplement was added. Apparently, the amount of carbon present in soil/groundwater matrix was sufficient to stimulate the transformation of RDX when supplemented with phosphate under anaerobic conditions.

The ^{14}C-recovery (Fig. 5) within the flasks ranged from 100 ± 10% in all the flasks. The extent of mineralization in the flasks ranged from 8 to 14%. Considering that ^{14}C-RDX

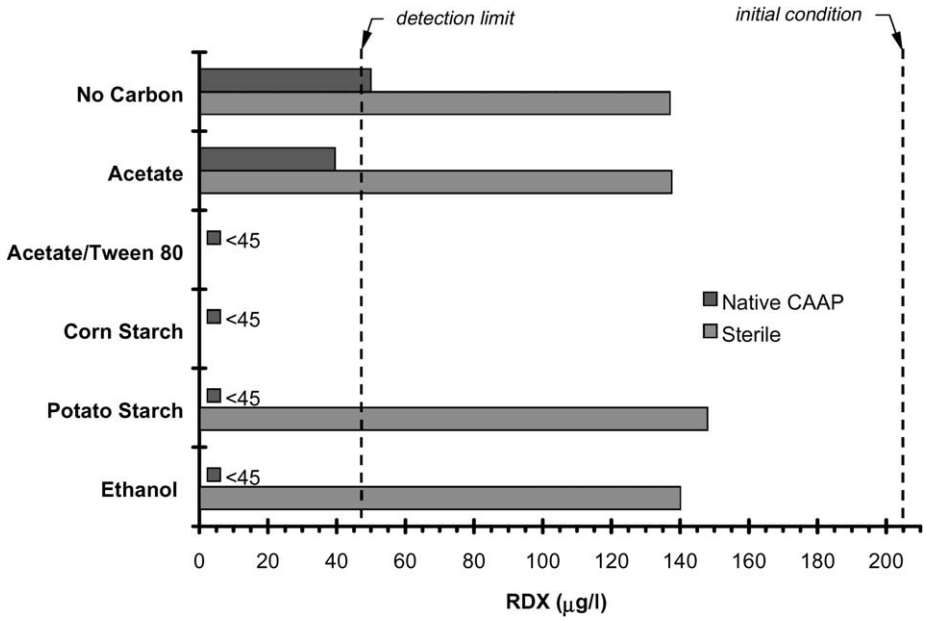

Fig. 4. Final RDX concentration in anaerobic flasks after 6 weeks of incubation.

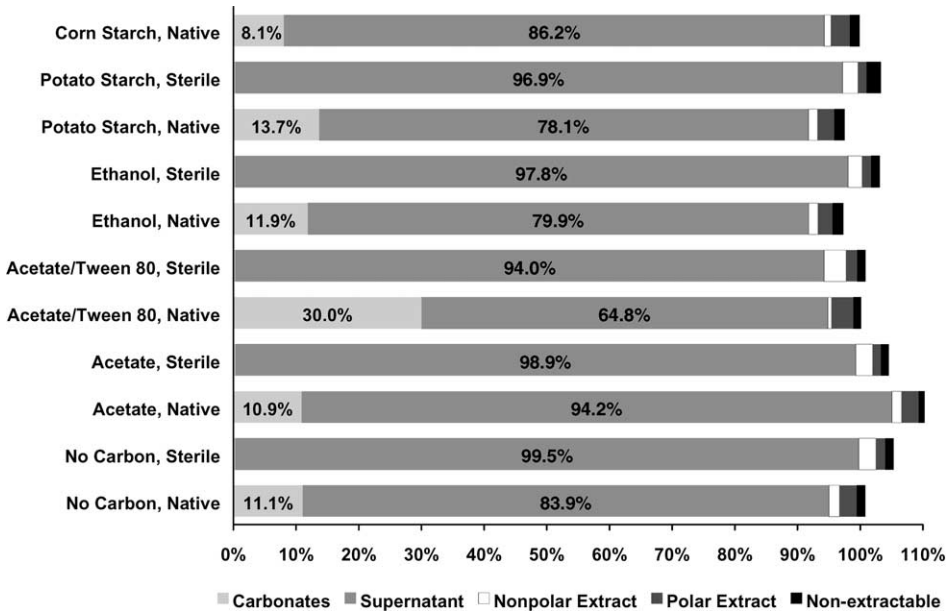

Fig. 5. Distribution of ^{14}C among different phases in anaerobic flasks after 6 weeks of incubation.

Table 2
Distribution of ^{14}C after 6 weeks of anaerobic incubation in the form of total carbonate and dissolved components in supernatant in flasks supplemented with 7000 mg/l carbon sources

Additives	Carbonate (%)	Supernatant (%)	Total recovery (%)
Acetate, Tween 80, native[a]	30	64.8	100
Tween 80, native	2.3	94.2	101
Tween 80, *M. Morganii*	14.8	77.3	102
Potato starch, *M. Morganii*	23	66.9	95
Potato starch, native	22.6	67	94
Potato starch, sterile	0	98.7	102.5
Potato starch, Tween 80, *M. Morganii*	13.9	76.1	94
Potato starch, Tween 80, native	5.3	87	97.5
Potato starch, Tween 80, sterile	0.5	93.8	98

[a] Ninety-three percent purity ^{14}C-RDX used in this flask. Hence, the % carbonates are estimated to be 23%, purity of ^{14}C-RDX in all the other flasks was >99%.

used in these experiments had a purity of 93%, the extent of RDX mineralization in these flasks may be considered to be 7% or less. In the sterile flasks, 30% loss of initial aqueous-phase RDX concentration was observed (see Fig. 4). However, no mineralization of RDX occurred in sterile flasks because the entire radioactivity was recovered from aqueous phase of slurry. In the set of the anaerobic flasks where Tween 80 (6908 mg/l) was added along with acetate (92 mg/l), 23% mineralization (30% minus 7% for radioactive impurities in RDX) was observed after 6 days. This is comparable to mineralization (25.6 and 30.7%) observed with similar supplementation of native microbes with Tween 80 and acetic acid under aerobic conditions (Fig. 3). Earlier reports [38] have suggested that the degree of mineralization under anaerobic conditions is much higher than under aerobic conditions. This conclusion was not supported by the experimental results of this work. Clearly, Tween 80 was also effective with the removal of RDX under anaerobic conditions.

Anaerobic experiments were also conducted using much higher concentrations of carbon supplements (7000 versus 92 mg/l) to evaluate carbon loadings similar to those in Tween 80-supplemented flasks. The mineralization results after 6 weeks of these incubations are presented in Table 2. Potato starch, Tween 80, and acetate were used as carbon supplements and the RDX used was 99% pure. Some flasks were inoculated with cells of *Morganella morganii* B2 (ATCC 51596) that has been shown to be able to degrade RDX under anaerobic conditions [39]. The base case considered was that of supplementation with acetate + Tween 80 in native CAAP microorganisms (23% mineralization, Fig. 5).

Addition of Tween 80 alone resulted in very little mineralization of RDX by native microbes. When Tween 80 was present with potato starch (92 mg/l) in native microbes, RDX mineralization under anaerobic conditions was same as if only low concentration of potato starch were present (Fig. 5). Augmentation with the ATCC culture did not show any improvement over the native CAAP microorganisms. The supplementation with potato-starch (7000 mg/l) resulted in 23% mineralization of RDX both with native microbes as well as with ATCC 51596 cells. It appears that Tween 80 is ineffective in enhancing RDX mineralization by native CAAP microorganisms under anaerobic conditions when present with

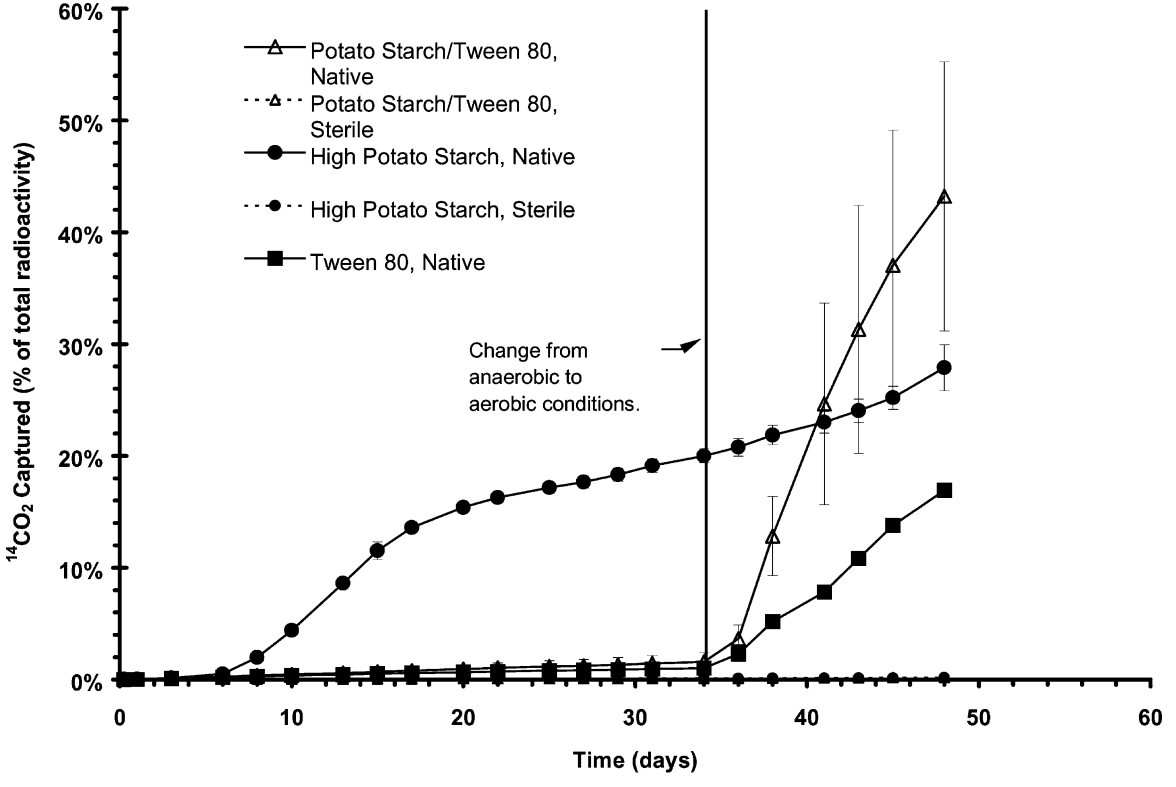

Fig. 6. Evolution of $^{14}CO_2$ vs. time in anaerobic/aerobic flasks.

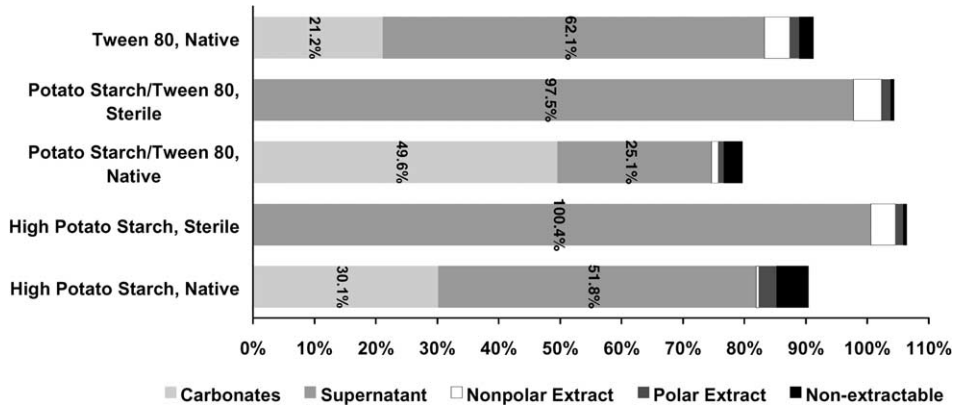

Fig. 7. Distribution of ^{14}C among different phases in anaerobic/aerobic flasks after 7 weeks of incubation.

complex carbohydrates. Later experiments involving sequential anaerobic/aerobic conditions confirm this observation regarding Tween 80.

3.3. Anaerobic/aerobic experiments

Experiments were also conducted in which anaerobic phase treatment was followed with aerobic treatment. [40] have eloquently suggested such a strategy for biotreatment of polynitroaromatic compounds. Here, the carbon additives used were potato starch, Tween 80, and potato starch with Tween 80. Total carbon source supplementation to the flasks was at a level of 7000 mg/l. Only native microbes present in the CAAP soil and groundwater were allowed in the flasks. The anaerobic phase was maintained for 5 weeks. Then the aerobic conditions were restored and allowed to incubate for 2 weeks. The results are shown in Figs. 6–8.

The production of $^{14}CO_2$ during the anaerobic/aerobic experiment is shown in Fig. 6. During the anaerobic phase, significant ^{14}C carbon dioxide production (amounting to 20%

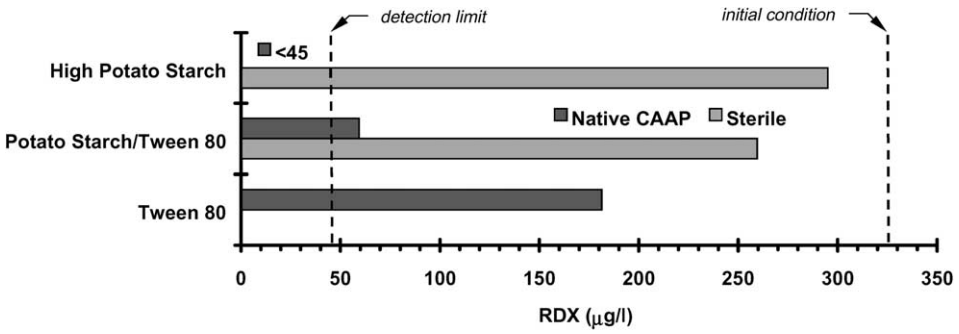

Fig. 8. Final RDX concentration in aerobic/anaerobic flasks after 7 weeks of incubation.

in 5 weeks took place only in the flasks supplied with potato starch. This observation compared well with the mineralization obtained under anaerobic conditions after 6 weeks (see Table 2). When the conditions were shifted to aerobic conditions (weeks 6 and 7), the evolution of carbon dioxide continued at the same pace and reached a final value of 28%. Flasks supplied with Tween 80 alone or Tween 80 and potato starch showed little $^{14}CO_2$ production during the anaerobic phase, but the CO_2 production increased rapidly as soon as aerobic conditions were restored. This confirmed that Tween 80 alone or Tween 80 in presence of complex carbohydrate (potato starch) does not cause significant mineralization of RDX under anaerobic conditions. When the redox conditions were shifted to aerobic, $^{14}CO_2$ production increased rapidly. With Tween 80 alone, mineralization after 2 weeks of aerobic operation was 20%, an observation comparable to that from aerobic flasks (Fig. 3) in 2 weeks. In flasks supplemented with Tween 80 + acetate, the $^{14}CO_2$ production increased even more rapidly and reached $>$ 40% in 2 days after shifting to aerobic conditions.

Distribution of radioactivity among the different phases at the end of anaerobic/aerobic operation is presented in Fig. 7. Again, small amount (5%) of radioactivity was observed in solids as non-polar, polar, and non-extractable matter, suggesting little if any assimilation of RDX in cell mass. Again, as observed with the aerobic systems, the recovery of ^{14}C was only 80%. This appeared to be a common observation for the cases with high RDX transformation (see Fig. 3).

Data concerning the final residual RDX concentration in aqueous phase are shown in Fig. 8. Final RDX concentrations in the aqueous phase show little change in the sterile flasks. RDX transformation was complete in the flask with potato starch and it was progressively smaller in those containing Tween 80 and potato starch or Tween 80 alone (Fig. 8).

4. Conclusions

The results of our study showed that RDX could not only be transformed, but also significantly mineralized in all treatment systems maintained under various redox conditions (aerobic, anaerobic, and sequential anaerobic/aerobic). Under aerobic conditions, efficient transformation of RDX required supply of large excesses of Tween 80. Supplying Tween 80 resulted in a slight reduction in the extent of RDX transformed, but an enhancement in the degree of mineralization observed. Under anaerobic conditions, transformation of RDX to below detection level took place even when little carbon was added to the slurry.

Percent mineralization under anaerobic conditions was at best comparable to those achieved under aerobic conditions. During the anaerobic phase of the anaerobic/aerobic experiments, the transformation patterns were identical to those seen in the anaerobic experiments, but quickly changed to those observed in aerobic conditions when air was introduced into the flasks.

All the results presented here suggest that microorganisms require external carbon source to biodegrade RDX, and a significant quantity of ^{14}C was respired as $^{14}CO_2$ while only little ^{14}C was incorporated into biomass. The published literature also suggests that RDX biodegradation takes place by a co-metabolic process. The carbon sources used in this study belong to those rapidly metabolized by the soil microbes. Hence, it is not surprising that a high degree of RDX transformation was observed when sufficient amounts of supplemental

carbon were supplied. Addition of Tween 80 to the native cultures resulted in higher degrees of RDX-mineralization but reduced rates of RDX-biodegradation.

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