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Low aqueous solubility electron donors for the reduction of nitroaromatics in anaerobic sediments

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Abstract

Studies are presented investigating the ability to enhance aryl nitro-reduction processes in sediments through electron donor addition. In particular, high molecular weight (starch and guar gum) and/or low aqueous solubility electron donors (oleic acid) were studied, since they should be less prone to diffusive loss to the water column after addition to contaminated areas. For comparison, complimentary studies were conducted with water-soluble electron donors (acetate and dextrose). The ability to enhance activity was measured by methane production and reduction of either nitrobenzene or 1,3,5-trinitrobenzene to aniline or dinitroaniline. The results demonstrate that all electron donors resulted in increased methane production after a lag phase. The highest level of methane production and the shortest lag phase in uncontaminated sediment microcosms was observed in acetate-fed systems. Sorption studies of all electron donors showed that starch was partitioning the least into the water phase. In microcosms containing nitrobenzene, trinitrobenzene and acetate, methane production did not occur and nitro-reduction was not observed. Conversely, the addition of dextrose or starch yielded methane production and aryl nitro-reduction with each contaminant tested. Neither nitrobenzene nor trinitrobenzene was significantly reduced in $HgCl_2$-killed controls. From these studies, it appears that starch may be well suited for applications of in-place, anaerobic sediment bioremediation.

Keywords: Sediments; Bioremediation; Nitroaromatics; Anaerobic

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1. Introduction

Due to their recalcitrant and hydrophobic nature, the widespread use of nitroaromatic compounds has resulted in their accumulation in bottom sediments. Many nitroaromatics represent both acute and chronic toxic effects, and in sediments they pose a concern for ecosystem health (Field et al., 1995; Haderlein and Schwarzenbach, 1995). An important process influencing the fate of nitroaromatics in anaerobic sediments is aryl nitro-reduction (McCormick et al., 1976; Hallas and Alexander, 1983; Kaplan, 1990; Boopathy and Kulpa, 1992). This process results in the formation of corresponding aminoaromatic compounds via nitroso- and hydroxylamino-intermediates. The reducing equivalents for this process often originate from microbial activity (directly or indirectly) and the reduction rate has been correlated to overall methanogenic activity (Mohn and Tiedje, 1992; Boopathy and Kulpa, 1994).

In natural anaerobic systems including sediments, the flow of reducing equivalents to aryl nitro-reduction is often limited by the availability of readily biodegradable electron donors. The addition of electron donor(s) to support reduction processes is, therefore, a potential method for in-place treatment of contaminated sediments. Studies have demonstrated the enhancement of nitro-reduction rates following the addition of organic electron donors such as glucose, pyruvate, formate, starch, etc. (Preuss et al., 1993; Boopathy, 1994; Roberts et al., 1995); however, most of the electron donors that have been investigated are highly water soluble. For in-place sediment bioremediation systems, this is a practical concern since non-sorbing, hydrophilic electron donor introduced into the sediment could be lost to the overlying water column—reducing the efficiency of the process. Kaake et al. (1992) and Roberts et al. (1996) showed an enhancement of nitroaromatics degradation by the addition of starch. However, Roberts et al. (1996) also noted that the use of insoluble starch did not lead to the complete disappearance of trinitrotoluene (TNT).

The purpose of the present study focuses on the ability to enhance aryl nitro-reduction processes in sediments through the addition of various electron donors with low aqueous solubility. In particular high molecular weight (starch and guar gum) and/or hydrophobic electron donors (oleic acid) were studied that should be less prone to diffusive loss to the water column. For comparison, complimentary studies were conducted with water-soluble electron donors (acetate and dextrose). The ability to enhance activity was measured by methane production and reduction of nitrobenzene and 1,3,5-trinitrobenzene.

2. Materials and methods

2.1. Chemicals

Nitrobenzene and 1,3,5-trinitrobenzene were selected as the model nitroaromatic contaminants in this study, since nitrobenzene is a common solvent and 1,3,5-trinitrobenzene is a stable end product of TNT photolysis (Burlison, 1980). Nitrobenzene (purity 99%) and 1,3,5-trinitrobenzene (99%) were obtained from Chem Service (West

Chester, PA) and used without further purification. Aniline and dinitroaniline (metabolites of nitrobenzene and 1,3,5-trinitrobenzene, respectively) were also obtained from Chem Service (West Chester, PA). Electron donors were obtained from Fisher, Fair Lawn, NJ (sodium acetate, reagent grade), EM Science, Gibbstown, NJ (dextrose, reagent grade), Aldrich, Milwaukee, WI (oleic acid and potato starch) and Sigma, St. Louis, MO (guar gum). Physical and chemical properties of the electron donors used in this study are presented in Table 1. Dichloromethane (chromatography grade, Fisher Fair Lawn, NJ) was used for extraction. Sodium azide (MCB, Cincinnati, OH) and mercuric chloride (Malinckrodt, Paris, KY) were used in controls for sorption experiments and nitroaromatic transformation experiments, respectively.

2.2. Sediments

Sediment samples were taken from a freshwater pond at a former TNT manufacturing area of the Alabama Army Ammunition Plant, Childersburg, AL. The uppermost section of unconsolidated sediments was collected and samples were transferred to an anaerobic glovebox. Sediments were then sieved (1 cm) to remove large debris such as leaves and wood, and stored in sealed glass bottles. Sieving and storage was conducted in an anaerobic glovebox (100% N_2). The sediments contained $\sim$10% of organic matter as measured by the burning loss of organic carbon.

2.3. Analytical

Nitroaromatics and reduction products were analyzed by gas chromatography (model 5890, Hewlett Packard, Palo Alto, CA) equipped with a mass-selective detector. Samples (1 μl) were injected in the splitless mode onto an HP Ultra 2 column (length 25 m, 0.2 mm inner diameter, 0.11 μm film thickness), and data collected with HP ChemStation software (Version C.01.05). Helium (ultra-high-purity grade) served as the carrier gas at a flow rate of 0.9 ml/min, the injector temperature was 300°C. The temperature program was: 40°C for 4 min, then ramped at 5°C per min to 250°C, followed by a 10 min hold. The interface temperature was 280°C, and the electron impact detector operated at 70 eV (scanning rate of 1.7/s over an m/z range of 40–400).

Table 1
Physical and chemical properties of selected electron donors

Electron donors	Synonyms	Formula	Aqueous solubility
Sodium acetate	–	$C_2H_3O_2Na$	82 g/l (20°C)
Dextrose	D-Glucose	$C_6H_{12}O_6$	soluble in water
Oleic acid	Red oil	$C_{18}H_{34}O_2$	insoluble in water
Potato starch	Amylodextrin	$C_6H_{10}O_5N$	1 ~ 10 g/l (19°C)
Guar gum	*Cyamopsis tetragonolobus*	NA[a]	$<$1 g/l (20°C)

[a]Guar gum is a mixture of many individual substances and cannot be adequately represented by a single molecular structure.

Methane was quantified with a GC-FID (model 5890, Hewlett Packard) equipped with a 10 ft $\times$ 0.125 in. 100/120 HayeSep D column (Supelco, Bellefonte, PA). The GC was operated isothermally at 100°C with a helium carrier flow of 12 ml/min. Total gas production was determined by displacement of a calibrated syringe against atmospheric pressure. The produced gas volume was released following measurements. Dissolved organic carbon (DOC) concentrations were determined with a Shimadzu TOC-500 analyzer (Shimadzu, Kyoto, Japan).

2.4. Stimulation of anaerobic activity

The ability to enhance sediment microbial activity through the addition of various electron donors was tested in sediment–water microcosms using methane production as a gross indicator of anaerobic microbial activity. Oxygen-free tap water (60 ± 1 ml) was added to serum bottles (120 ml) containing sieved sediment (3 g dry basis) under a nitrogen purge. Electron donors were added to bottles in neat form (starch, guar gum, and oleic acid) or from oxygen-free aqueous stock solution (dextrose and acetate) and the bottles were then sealed with gray butyl rubber stoppers and aluminum crimp seals. Bottles were purged with a gas mixture of N_2/CO_2 (90:10), with pressure released through a syringe puncturing the septa and shaken for 1 h after setup.

For each electron donor, six microcosms were established with three mixing regimes —allowing for duplicate systems in all cases. The different mixing conditions included: static, intermittent mixing (i.e., mixed each time before measuring methane), and continuously mixing (using a planar shaker rotating at 150 rpm). The amount of electron donor added to individual bottles was equal on an electron equivalent basis, thus the theoretical methane production potential (1.41×10^{-3} mmol) was the same for all systems. All experiments were conducted at 20°C.

2.5. Sorption of electron donors

The sorption of electron donors was evaluated in sediment water systems identical to those described previously, except that a 2% sodium azide (NaN_3) solution was used to minimize microbial activity. DOC was used to determine the extent of sorption. Controls without electron donors were used to determine the background DOC concentration of the sediments and the NaN_3 solution.

Duplicate systems for each electron donor were constructed as described previously and shaken on a planar shaker rotating at 150 rpm (20°C) until in equilibrium. Samples (2 ml) were centrifuged at $2000 \times g$ for 4 min, the supernatant recovered and filtered through a pre-rinsed 0.45 μm nylon filter (Fisher, Fair Lawn, NJ), then diluted in acidified Millipore water ($pH < 1$). The fraction of electron donor sorbed to the sediment was calculated from the resulting DOC concentrations in the water phase and compared to electron donor free controls.

2.6. Biotransformation of nitrobenzene and trinitrobenzene

The degradation experiments were performed in sediment microcosms as described previously. The addition of trinitrobenzene and nitrobenzene to microcosms was suffi-

cient to produce aqueous phase concentrations (neglecting sorption) of 0.47 and 0.97 mmol/l, respectively. Trinitrobenzene was added as a solid prior to sediment addition. Neat liquid nitrobenzene was added with a 10-μl syringe after purging bottles. Bottles were maintained on a shaker for approximately 1 h to facilitate homogenous distribution of nitroaromatics and electron donors. Electron donors were added in a tenfold excess to the necessary amount calculated for the complete reduction of the added nitrobenzene and trinitrobenzene to their corresponding amines.

Samples (2 ml) taken from microcosms for analysis of nitroaromatics and transformation products were centrifuged at 13,000 × *g* for 5 min, the supernatant extracted with 1 ml of DCM under basic conditions (pH > 11.5), and analyzed by GC/MS. No sediment extractions were performed. Methane production was determined by GC/FID.

3. Results and discussion

3.1. Stimulation of anaerobic activity

Microcosms amended with electron donors under various mixing regimes were monitored for methane production over a 65-day period. Results of methane production from static systems are shown in Fig. 1. Methane production was observed in all systems augmented with electron donors. In all but the acetate-amended systems, the extent of methane production remained slightly below the theoretical amount that could

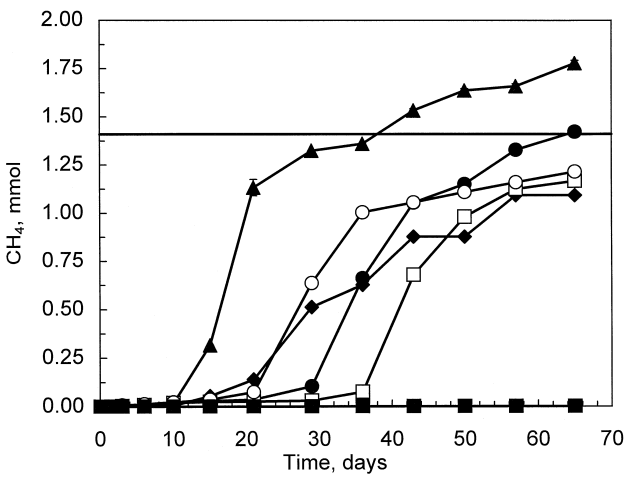

Fig. 1. Methane production in static systems. Electron donor additions: not supplemented control (■), supplemented with dextrose (●), acetate (▲), oleic acid (♦), starch (□), and guar gum (○). The horizontal line represents the theoretical methane production for 100% conversion of the electron donor added with no cell growth assumed. Values are averages of duplicates. To ensure the readability of the graph, error bars, representing the standard error of the mean averaged over all the systems (excluding the not-supplemented systems), were only included for the acetate-supplemented setup. However, since the error bars are very small, they are in most cases hidden behind the markers.

have been produced based on complete conversion of the substrate to methane. This may be due to assimilation of carbon for cell growth. In the systems fed acetate, however, the amount of methane produced actually exceeded the theoretical level. Presumably, the excess methane in these systems resulted from the decay of sediment organic matter in addition to the acetate added. Methane production was observed first in the acetate-amended microcosms after 10 days of incubation while methane production was not observed in other systems until 15 to 28 days after electron donor addition. The rate of methane production following the lag period was similar for all systems.

The extent of methane production was not influenced by mixing conditions, although the lag phase prior to rapid methane production was longer in systems that were not mixed continuously. This is shown in Fig. 2 for systems fed acetate and starch. Methane production from acetate occurred with a shorter lag period than from starch, and yielded a greater extent of methane production over the period of study.

3.2. Sorption of electron donors

The potential sorption of electron donors to the sediment is shown in Fig. 3. As expected for dextrose and acetate nearly all added organic carbon was recovered as DOC in the supernatant. For starch, oleic acid and guar gum, low fractions of organic carbon were expected to be recovered since these compounds show a fairly low aqueous solubility and/or high hydrophobicity (Table 1, Perry et al., 1984). Lowest DOC levels were observed in the starch systems where less than 10% was recovered in the

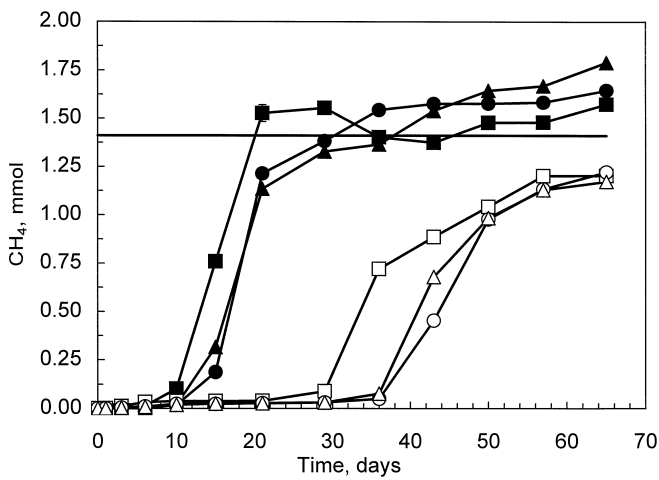

Fig. 2. Methane production in sediment–water systems supplemented with starch (empty symbols) or acetate (solid symbols) under various mixing regimes: continuously shaken (□, ■), intermittent shaking (○, ●) and static (△, ▲). The horizontal line represents the theoretical methane production for 100% conversion of the electron donor added (no cell growth assumed). Values are averages of duplicates. To ensure the readability of the graph, error bars, representing the standard error of the mean averaged over all the systems (excluding the not-supplemented systems), were only included for the continuously shaken, acetate-supplemented setup. However, since the error bars are very small, they are in most cases hidden behind the markers.

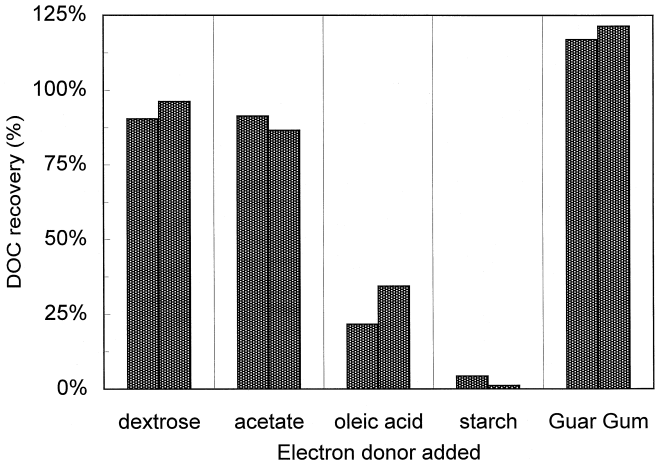

Fig. 3. Percent recovery of electron donors as DOC in the supernatant of soil microcosms.

supernatant. Since the starch used was slightly soluble, these results indicate that starch is not significantly solubilized in these systems and may have therefore been more highly associated with the sediments than the other electron donors. The oleic acid systems had low DOC fractions in the supernatant; however, an oily layer was observed at the water surface and presumably accounted for a large fraction of the oleic acid added. Interpretation of results from the guar gum systems was complicated due to the plugging of the nylon filters prior to DOC analysis. High pressure was required for samples to pass through the filter and the integrity of the filters may have been affected. This may have been the reason for DOC concentrations in excess of the maximum calculated on the basis of the organic carbon added. A loss of the added organic carbon due to microbial activity can presumably be neglected since the addition of NaN_3 and the relatively short experimental time of less than 24 h should have prevented methane production in these systems (in uninhibited controls methane production was not observed until the fifth day of the experiment, see Figs. 1 and 2).

3.3. Biotransformation of nitrobenzene and trinitrobenzene

These experiments evaluated the ability of acetate, dextrose, and starch to enhance the reduction of trinitrobenzene and nitrobenzene. Starch and dextrose were used since they both are capable of stimulating a range of anaerobic organisms, but vary in their tendency to associate with sediment. Systems with oleic acid and guar gum were not set up since the sorption experiments did not clearly indicate that oleic acid or guar gum associated with the sediments after addition. Acetate addition targeted the stimulation of acetoclastic methanogens only. Trinitrobenzene, a stable end product of TNT photolysis (Burlison, 1980), is readily reduced via amino-dinitrobenzene (dinitroaniline) and di-amino-nitrobenzene to triaminobenzene. It has been observed that the reduction of the

first two nitro groups occurs readily, while more reducing power is required for the reduction of the third nitro group (McCormick et al., 1976; Preuss et al., 1993). The single nitro group of nitrobenzene is more difficult to reduce than the first nitro group of trinitrobenzene (Boopathy, 1994).

In all systems, the concentrations of the nitroaromatics, measured immediately after addition, were below the levels predicted if the compounds were 100% in the water phase. Rapid sorption to sediments would explain these findings, although chemical reduction may also have been a contributing factor. Gorontzy et al. (1993), Preuss et al. (1993), and Haderlein and Schwarzenbach (1995) have observed the rapid chemical reduction of polynitroaromatics by chemical reductants such as sulfides, reduced iron or hydroquinone. According to their findings, the first and second nitro group of polynitroaromatic compounds can be chemically reduced whereas the reduction of the third nitro group is usually mediated by microbial activity. The sediments utilized were strongly anaerobic, produced noticeable levels of H_2S, and the chemical reduction of nitroaromatics may have occurred quickly after their addition—making it impossible to take the first sample undisturbed.

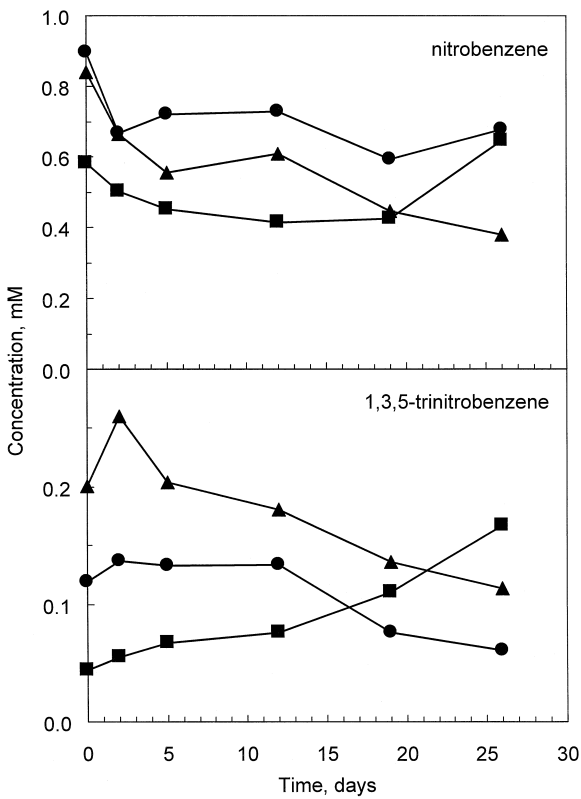

Fig. 4. Nitrobenzene (top) and trinitrobenzene (bottom) concentrations in $HgCl_2$-killed controls over time. Electron-donor additions: not supplemented (■), dextrose-supplemented (●) and starch-supplemented (▲).

In $HgCl_2$-killed controls, regardless of whether electron donors where added or not (Fig. 4), the remaining trinitrobenzene and nitrobenzene indicated that the sediment's potential for chemical reduction was not high enough to reduce them completely. Although the trinitrobenzene concentrations in the dextrose or starch amended killed controls slowly decreased throughout the experiment, indicating that the addition of $HgCl_2$ may not have been sufficient to inhibit microbial activity completely, the decrease in nitrobenzene and trinitrobenzene concentrations was significantly less than in the non-inhibited microcosms. The initial decrease of the nitroaromatic concentrations is most likely due to sorption and not to chemical reduction since the concentrations of the more readily reducible trinitrobenzene did not decrease between the first and the 5th day. The sediments contained a relatively high amount of organic matter (ca. 10%), which may explain the relatively high sorption tendency of nitrobenzene.

In biotic systems not supplemented with electron donor (Fig. 5), trinitrobenzene concentrations decreased to levels below detection limits within 5 days, with a commensurate production of dinitroaniline (aminodinitrobenzene). This reduction in trinitrobenzene was not observed in abiotic controls, meaning that either the sterilization agent affected abiotic redox active agents in the sediments, or the sediment microorganisms were capable of reducing trinitobenzene without electron donor addition. On the other hand, aqueous phase concentrations of nitrobenzene decreased in the first 5 to 10 days and then remained relatively constant at approximately 50% of the added concentration over the rest of the experiments. No aniline was detected in these systems.

In biotic systems supplemented with dextrose (Fig. 6) or starch (Fig. 7), trinitrobenzene and nitrobenzene disappeared completely. Dinitroaniline accumulated between the second and the twelfth day and then disappeared. Further reduction to trinitrobenzene metabolites were not detected. The detection of diamino-nitrobenzene and triaminoben-

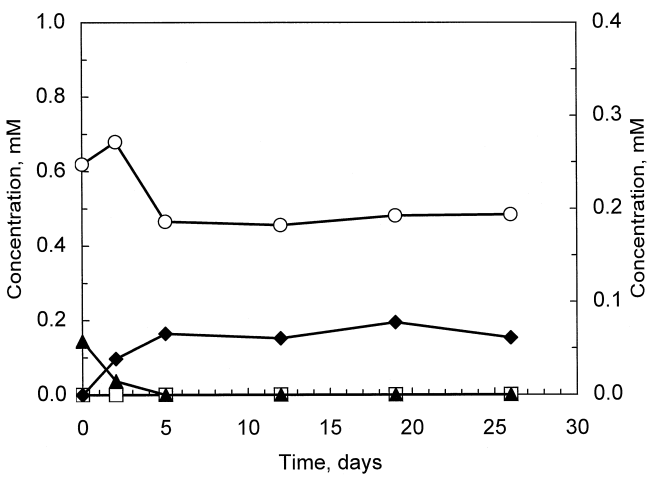

Fig. 5. Transformation of nitrobenzene and trinitrobenzene in non-supplemented anaerobic sediments. Nitrobenzene (○) and aniline (□) are plotted against the left axis. Trinitrobenzene (▲) and dinitroaniline (◆) are plotted against the right axis.

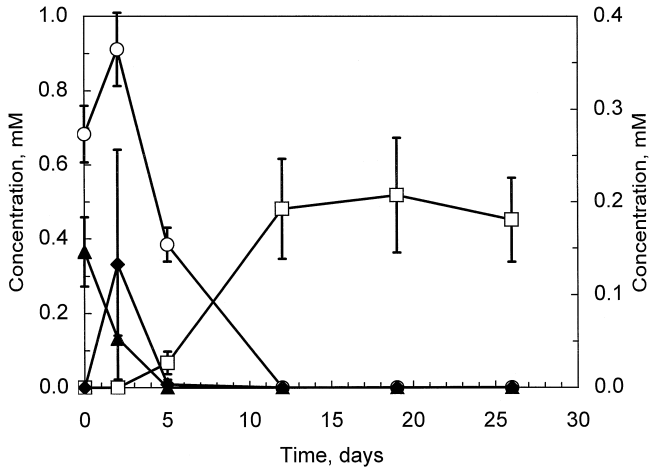

Fig. 6. Transformation of nitrobenzene and trinitrobenzene in dextrose-supplemented anaerobic sediments. Nitrobenzene (○) and aniline (□) are plotted against the left axis. Trinitrobenzene (▲) and dinitroaniline (♦) are plotted against the right axis. The error bars indicate one standard deviation.

zene is generally difficult in soil–water or sediment–water systems since they tend to strongly associate with the organic fraction and clay layers (Rieger and Knackmuss, 1995). Nitrobenzene concentrations decreased rapidly in all systems supplemented with dextrose or starch, and it was not detected after 18 days. Instead, aniline accumulated and persisted throughout the experiment. The addition of these substrates greatly enhanced reduction processes for nitrobenzene and facilitated a more rapid reduction of

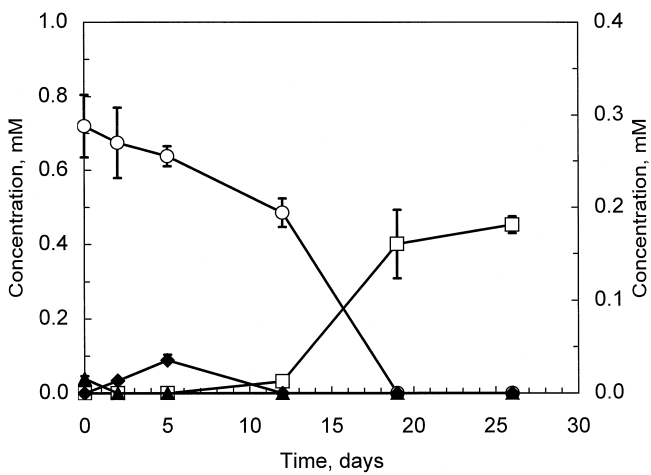

Fig. 7. Transformation of nitrobenzene and trinitrobenzene in starch-supplemented anaerobic sediments. Nitrobenzene (○) and aniline (□) are plotted against the left axis. Trinitrobenzene (▲) and dinitroaniline (♦) are plotted against the right axis. The error bars indicate one standard deviation.

amino-derivatives of trinitrobenzene through direct metabolism or indirect processes. Higher rates were observed in systems fed dextrose. This may be explained by the lower degree of dextrose partitioning to the sediment compared to starch, or by a longer lag phase for starch utilization. In studies of methane production, a longer lag period was required for methanogenesis from starch, followed by high activity, generally supporting the latter possibility.

The addition of acetate did not appear to have any impact on the reduction of nitrobenzene or trinitrobenzene (Fig. 8). Trinitrobenzene was partially reduced to dinitroaniline and concentrations were decreasing near the end of the study, but similar activity was observed in unfed systems. In systems fed nitrobenzene, activity was very similar to unfed controls (e.g., slow decrease in concentration with no aniline production). Acetate, despite most effective in stimulating the overall methanogenic activity in uncontaminated systems, did not stimulate the reduction of nitroaromatics. From the data obtained in this study, it is not possible to exactly determine why acetate did not stimulate aryl–nitro reduction, but the results obtained are consistent with previous observations made by Boopathy (1994), Boopathy and Kulpa (1994), and Roberts et al. (1996) who reported that the addition of acetate did not successfully stimulate the transformation of nitroaromatics. Since the acetate supplemented systems did not show any methane production throughout the experiment (Fig. 9), it is likely that the nitroaromatics, at the concentrations added, were toxic to the acetate utilizing organisms in the sediments. Wang et al. (1991), Donlon et al. (1995), and Haghighi Podeh et al. (1995) described the toxicity of nitoaromatics to anaerobic bacteria and noted that acetoclastic methanogens were extremely sensitive to toxicants such as polynitroaromatics. This explanation is further supported by the fact that in both dextrose and starch supplemented systems, methane production was delayed only as long as nitrobenzene,

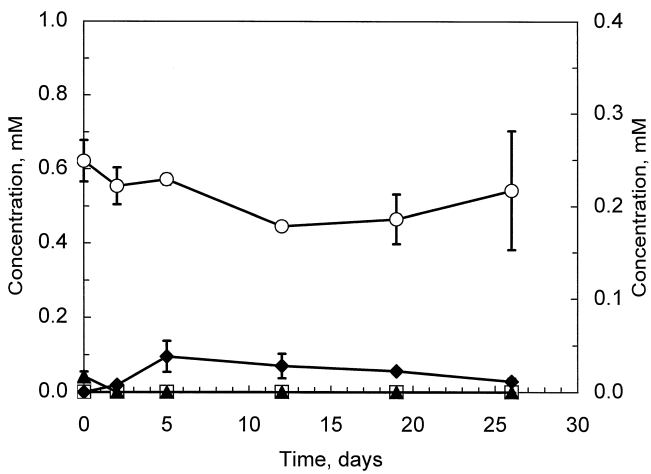

Fig. 8. Transformation of nitrobenzene and trinitrobenzene in acetate-supplemented anaerobic sediments. Nitrobenzene (○) and aniline (□) are plotted against the left axis. Trinitrobenzene (▲) and dinitroaniline (◆) are plotted against the right axis. The error bars indicate one standard deviation.

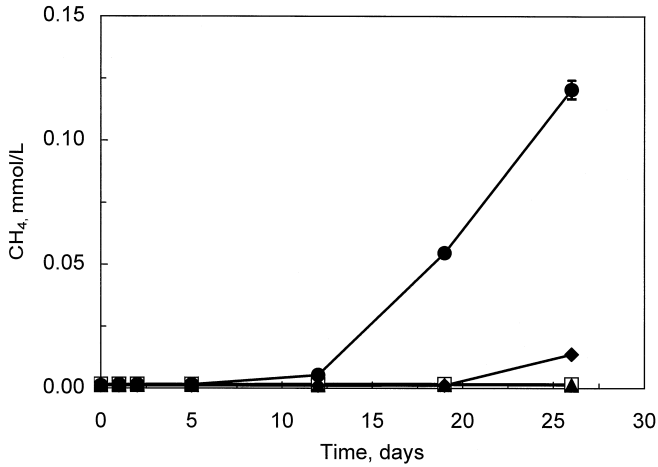

Fig. 9. Methane production in anaerobic sediments, spiked with nitrobenzene and trinitrobenzene. Electron donor additions: not supplemented (□), supplemented with dextrose (●), acetate (▲), starch (♦). The error bars indicate one standard deviation.

trinitrobenzene, or trinitrobenzene reduction products were detectable. This observation is supported by Fedorak et al. (1990) who reported that aniline, the degradation product of nitrobenzene, did not inhibit methanogenesis.

4. Summary and conclusions

The results presented in this paper show that certain electron donors such as starch can be used to enhance the reduction of nitroaromatics in anaerobic sediments. The experimental setup did not allow distinction between direct reduction of the nitroaromatics by microorganisms and microbially mediated chemical reduction (e.g., via metals or hydroquinone structures). Supplementation with dextrose or starch led to the complete disappearance of nitrobenzene and trinitrobenzene. Acetate, which rapidly stimulated methanogenesis in nitroaromatic-free systems, did not significantly enhance the contaminant reduction. This may have been due to the toxicity of nitroaromatics to acetoclastic methanogens.

Among the five electron donors tested, starch was the most appropriate for an in situ treatment of contaminated sediments. Starch associated to the highest degree with sediments (as opposed to the overlying water column), remained bioavailable, and promoted nitro-reduction. Determining the extent to which other low aqueous solubility electron donors (e.g., oleic acid and guar gum) sorbed to sediments was problematic with the methods used.

The observed reduction of nitroaromatics to its corresponding amino-compounds is in many cases required before ring-fission and mineralization of these contaminants can occur. While the nitro-group reduction generally requires anaerobic conditions, the

ring-fission and subsequent mineralization is favorable under aerobic conditions. During both anaerobic and aerobic phases, nitroaromatics and their degradation products may also be removed from the system due to polymerization or the formation of bound residues (Funk et al., 1993; Gorontzy et al., 1994).

In view of a possible field application, a system would have to be developed that allows the change of redox conditions throughout the process. One technique offering this feature is proposed by Anid et al. (1991). In this study, a barge with injection equipment was used for the injection of electron donors into the sediment and the subsequent oxygenation of sediments. Thus, after degrading the nitroaromatics to their (partially) amino-counterparts, the aeration could lead to the fission of the aromatic system. Another technique proposed by Abramovic (1994) describes the use of in situ treatment cells that can be lowered into the sediment, encapsulating the sediment and the overlying water. The cells can be equipped with mixing devices, reducing mass transfer limitations and also enabling the aeration. After achieving the degradation goal in a cell, the cell could be moved to another place. In view of a field or pilot-scale application, further research should be conducted to assess the potential for a range of other hydrophobic electron donors that are best suited for the contaminants of interest and the costs of various materials.

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