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ORIGINAL PAPER

Effect of biostimulation on the microbial community in PCB-contaminated sediments through periodic amendment of sediment with iron

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Abstract Reductive dehalogenation of polychlorinated biphenyls (PCBs) by indigenous dehalorespiring microorganisms in contaminated sediments may be enhanced via biostimulation by supplying hydrogen generated through the anaerobic corrosion of elemental iron added to the sediment. In this study, the effect of periodic amendment of sediment with various dosages of iron on the microbial community present in sediment was investigated using phospholipid fatty acid analysis (PLFA) over a period of 18 months. Three PCB-contaminated sediments (two freshwater lake sediments and one marine sediment) were used. Signature biomarker analysis of the microbial community present in all three sediments revealed the enrichment of *Dehalococcoides* species, the population of which was sustained for a longer period of time when the sediment microcosms were amended with the lower dosage of iron (0.01 g iron per g dry sediment) every 6 months as compared to the blank system (without iron). Lower microbial stress levels were reported for the system periodically amended with 0.01 g of iron per g dry sediment every 6 months, thus reducing the competition from other hydrogen-utilizing microorganisms like methanogens, iron reducers, and sulfate reducers. The concentration of hydrogen in the system was found to be an important factor influencing the shift in microbial communities in all sediments with time. Periodic amendment of sediment with larger dosages of iron every 3 months resulted in the early prevalence of *Geobacteraceae* and sulfate-reducing bacteria followed by methanogens. An average pH of 8.4 (range of 8.2–8.6) and an average hydrogen concentration of 0.75% (range of 0.3–1.2%) observed between 6 and 15 months of the study were found to be conducive to sustaining the population of *Dehalococcoides* species in the three sediments amended with 0.01 g iron per g dry sediment. Biostimulation of indigenous PCB dechlorinators by the periodic amendment of contaminated sediments with low dosages of iron metal may therefore be an effective technology for remediation of PCB-contaminated sediments.

Keywords Biostimulation · Sediment · Iron · Phospholipid fatty acid analysis · *Dehalococcoides*

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Introduction

Sediments contaminated with polychlorinated biphenyls (PCBs) are a major concern due to the toxicity associated with PCB exposure and their biomagnification in the food chain. PCBs are subjected to physical, chemical, and biological degradation to reduce their toxicity. Biodegradation, that is, the degradation of compounds by bacteria or other microorganisms, is a slow yet possible method for destroying PCBs in both aerobic and anaerobic environments. Because PCBs are more persistent with increasing chlorination of the congener, aerobic biodegradation involving biphenyl ring cleavage is restricted to the lightly chlorinated congeners. Reductive dehalogenation is an important process in the biodegradation of various halogenated aliphatic and aromatic compounds such as PCBs and is the only known biodegradation process for the higher chlorinated PCB congeners. Research literature

shows that microbial dechlorination of PCBs is prevalent in lake sediments [19] and estuarine systems [5] but the rates, extent, and mechanism of dechlorination vary greatly from one site to another. Reductive dehalogenation depends at least in part on the electron donor requirements and the efficiency with which electrons can be used. Reductive dechlorination is a two-electron transfer process in which hydrogen is assumed to be directly or indirectly the electron donor [23]. Hydrogen (H_2) can select a consortium of different competence or it might serve as an alternate electron donor to the same consortium which changes the pathway, products, and possibly the rate of PCB dechlorination [28]. Furthermore, competition from other microorganisms such as sulfate reducers and methanogens for hydrogen is an important factor affecting the rate of dechlorination of PCBs in weathered sediments. Addition of elemental iron results in anaerobic corrosion thus generating hydrogen which may stimulate the microbial reductive dechlorination of PCBs in sediments [37]. Elemental iron oxidizes to ferrous ion and produces hydrogen gas with a standard redox potential of 0.44 V making the reaction thermodynamically feasible:

$$Fe^0 + 2H_2O \rightarrow Fe^{2+} + 2OH^- + H_2(g)$$

The corrosion of iron generates hydrogen, which serves as an electron donor for the growth of microbes like dehalorespirers, methanogens, sulfate reducers, and iron-reducing bacteria, which then can grow by gaining energy from inorganic molecules like hydrogen [25]. Addition of iron provides two mutually beneficial effects. First, it provides hydrogen in situ to enhance microbial activity. Second, ferrous iron removes sulfide formed during sulfate reduction by forming the insoluble precipitate ferrous sulfide, thus decreasing the availability and toxicity of sulfide [40].

Laboratory studies have observed anaerobic dechlorination of PCBs spiked into uncontaminated sediments [1]. Studies also show that the rates of dechlorination of PCBs are higher in contaminated sediments than in uncontaminated sediments spiked with PCBs. For example, a higher rate of dechlorination (46–1,646 μmol l^{-1} day^{-1}) and lesser lag times were observed for the degradation of tetrachlorobiphenyl 2346-CB and trichlorobiphenyl 246-CB in contaminated Woods Pond, MA, USA sediment samples compared to spiked sediment from an uncontaminated site from Sandy Creek Nature Park, Athens, GA, USA [36]. This supports the observation that microbial communities present in PCB-contaminated sites are better adapted for PCB dechlorination than those in uncontaminated sites [22]. Most of the bacteria that reductively dechlorinate halogenated industrial pollutants are members of the genus *Dehalococcoides*. Another such obligate anaerobe capable of aryl reductive dehalogenation, *Desulfomonile tiedjei*, has been identified and isolated from the Hudson River, N.Y. sediments [26].

There have been many studies on reductive dechlorination of chlorinated compounds under methanogenic conditions [29]. Dechlorination of PCBs in sediments has been observed with an accompanying generation of methane [20]. Methanogenic archaea are known to depend on fermentative bacteria for substrates which are provided by the dechlorination of PCBs. Extensive studies carried out to establish the role of methanogenic archaea in PCB degradation revealed that pasteurized microbial cultures failed to generate methane but continued to exhibit dechlorination of Aroclor 1242 at a lesser rate as compared to untreated sediment samples from the Hudson River, N.Y. [38]. Further research in the field of enrichment cultures for sediments has shown the presence of highly diverse 2,3,5,6-CB dechlorinating microbial communities containing the deeply branching *Dehalococcoides* species as well as other species with sequence similarity to *Methanomicrobiales* and *Methanosarcinales* archaea and the *Thermotogales* subgroups [12]. Evaluation of the role of methanogenic archaea demonstrated that methanogens were not involved in the *ortho*-dechlorination of PCBs [38].

It has been shown that discrete microbial populations are responsible for different dechlorination mechanisms. *Dehalococcoides* fall within the *Chloroflexi* phylum and are known to dechlorinate chlorinated ethenes. Most of the *Dehalococcoides* species are strictly hydrogenotrophic and dechlorinate perchloroethene (PCE) via trichloroethylene (TCE) and finally to ethene [18]. *Dehalococcoides ethenogenes* strain 195 and *Dehalococcoides* strain CBDB1 were shown to be able to dechlorinate Aroclor 1260 in a sediment-free culture by the N process of dehalorespiration [3]. This also suggests that *Dehalococcoides* populations may play an important role in the natural attenuation and bioremediation of commercial PCB mixtures in sediments.

Even though there are other species of bacteria such as *Dehalobacter*, *Desulfitobacterium*, *Desulforomonas*, *Sulfurospirillum*, *Anaeromyxobacter*, and SZ-type *Geobacteraceae* deriving energy from dehalorespiration, their role in utilizing PCBs as electron acceptors is not well documented. Furthermore, competition from other microorganisms such as sulfate reducers and methanogens for hydrogen is an important factor affecting the rate of dechlorination of PCBs in weathered sediments. In sulfate-rich environments like marine or estuarine sediments, sulfate is usually the predominant electron acceptor for anaerobic metabolic processes. A number of studies have shown that sulfates partially inhibit microbial dehalogenation [11]. Dechlorination of Aroclor 1242 was found to be inhibited completely at high sulfate concentrations [6, 24]. In contrast, PCB dechlorination by the addition of ferrous sulfate ($FeSO_4$) following a lag in the onset of the

dechlorination process has also been reported [39]. Those authors concluded that sulfates stimulate the growth of *para*-dechlorinating sulfate reducers like *Desulfomonile tiedjei*, and the presence of iron results in the formation of insoluble FeS which reduces the toxicity associated with sulfides. Sulfate reducers were shown to be able to outcompete CO_2-reducing methanogenic Archaea owing to their higher affinity for hydrogen and higher growth yield [16]. Threshold concentration of hydrogen rather than the kinetic parameters was shown to be responsible for the outcome of the competition for trace hydrogen concentrations [14]. Thus the process of dechlorination in a mixed consortium might be associated with a delicate balance in the availability of electron acceptors and the concentration of electron donor which is hydrogen in the current study.

Phospholipid fatty acid analysis

The study of microbial diversity and community structure in sediment slurries has grown rapidly with the advent of new and improved techniques for analysis. The limitations in obtaining pure enrichment cultures of a certain microbial species (especially anaerobes) from a mixed consortium have resulted in the development of various culture-independent molecular techniques for the analysis of microorganisms. Microbial biomarkers (chemical compounds) are a useful way to qualitatively and quantitatively estimate the microbial community present in samples obtained from different environments. Membrane lipids and related fatty acids are essential components of all living cells and their structural and functional diversity makes them useful biomarkers for the characterization of microorganisms. Phospholipids consist of a single molecule of glycerol in which two hydroxy (OH) groups are attached to the fatty acid chains and another OH group is attached to the phosphate group; thus phospholipids have hydrophobic and hydrophilic ends.

Phospholipid fatty acids (PLFA) can be classified into ester-linked and ether-linked fatty acids. The ester-linked fatty acids further comprise saturated, hydroxy, monounsaturated, and polyunsaturated fatty acids. Furthermore, hydroxy-substituted fatty acids present in the lipopolysaccharide (LPS) portion of the cell wall in gram-negative bacteria fall under a separate category. Ether-linked fatty acids are prevalent in the membranes of Archaea. PLFAs were reported to be hydrolyzed into diglycerides by cellular enzymes within minutes to hours of cell death [32]. The polar fraction of the lipids present in soils and sediments consists of PLFA of viable microorganisms [33].

For complex matrices such as soils, PLFA analysis has been proven to be a valuable tool in detecting changes in microbial communities in response to various sources of pollution [21, 27]. Thus the measurement of lipid biomarkers, specifically PLFA profiles of microbes provides an insight into important characteristics like community structure, biomass, and nutritional status and stress responses of gram-negative bacteria [35]. Stressed gram-negative bacteria show increases in the ratios of saturated to unsaturated fatty acids, *trans* to *cis* monoenoic fatty acids and cyclopropyl acids, and cy17:0 and cy19:0 to their monoenoic precursors 16:1ω7c and 18:1ω7c respectively [10]. Fatty acids 15:0 and 17:0 are generally indicative of bacteria, *iso* and *anteiso* isomers of 15:0 for gram-positive bacteria [9], β-hydroxy fatty acids from the LPS position of the cell are representative of gram-negative bacterial populations, 10Me18:0 for Actinomycetes, and 18:2ω6 for a fungal biomarker [9]. Although PLFAs possess great structural diversity and are also highly specific in nature, they can be applied to other groups of microorganisms with certain fatty acids more dominant in a particular group than others. For example, *Geobacteraceae* species contain 16:1ω7c, i15:0, and 16:0 with 16:1ω7c as the dominant fatty acid [17]. Fatty acids i15:0, ai15:0, 16:0, and i17:0 were reported as the major fatty acids for *Desulfovibrio* species [8]. The presence of phospholipid furan fatty acids (Fu17:2ω6, Fu17:2ω5, Fu18:2ω6, and Fu18:2ω7) was reported for the first time in bacterial phospholipids namely, *Dehalococcoides* species [34]. The presence of high proportions of fatty acids 14:0 and 16:0 was also reported, apart from Fu17:2ω6, Fu17:2ω5, Fu18:2ω6, and Fu18:2ω7 in various strains of *Dehalococcoides* species [34]. Specific patterns of PLFA can also be considered to be indicative of the physiological or nutritional status of the microbial community. Starvation and stationary growth phase result in the conversion of monoenoic acids (16:1ω7c, 18:1ω7c) to cyclopropyl fatty acids (cy17:0, cy19:0) respectively. Even though variations are observed from one organism to another the ratio of cyclopropyl fatty acids to their respective monoenoic precursors is usually in the range 0.1 (for log phase) to 5 or greater (in stationary-growth phase) [10]. An increase in cyclopropyl PLFA formation with an increase in anaerobic activity of facultative heterotrophic bacteria in monoculture studies was also reported [31]. The isomerization of *cis*-monoenoic fatty acids (16:1ω7c, 18:1ω7c) to *trans*-monoenoic PLFAs (16:1ω7t, 18:1ω7t) as an adaptation mechanism has also been reported to be associated with starvation and stress in microbial communities [10]. Reports in the literature have shown that stress responses could be as result of exposure to high temperature, presence of toxic compounds, low pH and starvation.

The following nomenclature is used to represent the fatty acids: the total number of carbon atoms, followed by the number of double bonds, followed by the position of the double bond from the methyl end of the molecule (ω). Suffixes c and t indicate *cis* and *trans* geometry. For

example 18:1ω9t is a fatty acid with 18 carbon atoms, 1 double bond located between positions 9 and 10 from the methyl end of the chain in a *trans* configuration. The prefixes a and ai refer to *iso* and *anteiso* branching, 10Me indicates a methyl group on the 10th carbon from the carboxyl group, a number before the OH symbol indicates the position of the hydroxyl group (e.g., 2-OH 16:0), cy represents cyclopropane fatty acids (e.g., cy17:0) and the presence of a furan ring in the chain is indicated by an Fu prefix (e.g., Fu18:2ω7).

Research objectives

Reductive dehalogenation of PCBs in the sediment microcosms occurs as a result of microbial activity. The microbes present in the sediment niches establish a synergistic relationship with other microbial communities present in the system depending on the availability of electron donor. For this purpose the following three different sites historically contaminated with PCBs were selected to study the microbial composition and diversity with varying sediment characteristics and PCB contamination levels: Lake Hartwell, South Carolina (freshwater sediment); Roxana Marsh, Indiana (freshwater sediment); and New Bedford Harbor (marine sediment). The major source of PCB contamination in the Lake Hartwell, SC site was the disposal of PCB-laden dielectric fluid used in capacitors from the Sangamo-Weston plant in the 1950s and 1960s. Sediment from New Bedford Harbor, MA was contaminated by the discharge of PCB-containing wastewaters directly into the harbor by two industrial electronic manufacturers, Aerovox and Cornell-Dubilier. Roxana Marsh, IN in the Grand Calumet River Basin was contaminated with PCBs by both point sources and non-point sources including leachate runoff, industrial wastewater discharge, and combined sewer overflows. Establishing the diversity of the microbial consortium as well as its relative quantification between the different systems in the current study should be a function of the amount of degradation observed in that particular system. The research objectives thus focus on the analysis of the phospholipid fatty acids obtained from viable microbial cells in the sediment and use signature biomarker analysis to distinguish the type of microbial communities in the sediments. Further focus is directed at studying the variation or shift in the pattern of the microbial communities with respect to time, and to the frequency and dosage of iron amendment. The concentration of hydrogen and the rate of cathodic hydrogen production in the microcosms are vital for the enrichment of dechlorinating populations and also induce competition from sulfate reducers. The addition of iron as a source of cathodic hydrogen may also result in variation in the populations of Fe(III)-reducing bacteria like *Geobacteraceae,* sulfate reducers, methanogens, and dehalorespirers. This study discusses the variation and comparison of microbial consortia present in Lake Hartwell, New Bedford Harbor, and Roxana Marsh sediment microcosms with different modes of enhancement over a period of 18 months.

Materials and methods

Contaminated sediments

Surface sediments were collected from a marine site and two freshwater lake sites. The sediments were collected from the top 10-cm layer of the sediment. Overlaying natural water was collected from the same location as the sediment. The characterization of the sediments is presented in Table 1 for the Lake Hartwell, New Bedford Harbor, and Roxana Marsh sediments.

Biodegradation experiments

The natural water from a site was purged with nitrogen gas overnight to decrease the dissolved oxygen level to less than 1 mg/l. The contaminated sediment and the corresponding site natural water were mixed together in 125-ml borosilicate serum bottles using a sediment/water ratio of 1:4 (25 g of wet sediment/100 ml of natural water), where serum bottles served as microcosm. A controlled atmosphere chamber was used to fill the headspace of bottles with nitrogen gas under strict anaerobic conditions. The chamber was purged with an anaerobic gas mixture (10% H_2/5% CO_2/85% N_2) to remove the residual oxygen (O_2) from the system via reaction with hydrogen in the presence of a palladium catalyst. The chamber was then flushed with nitrogen to fill the bottle headspace with nitrogen gas to provide for anaerobic microcosms. The serum bottles were then capped with Teflon-faced butyl rubber septa, crimped with aluminum seals, removed from the chamber, and placed inside a rotating tumbler at 12 rpm for mixing in the dark at 25°C. All biodegradation experiments were carried out in triplicate.

(a) Blank (no iron)

Sediment and natural overlaying water were mixed in serum bottles, and the bottle headspace was filled with nitrogen gas. The blank system was prepared without iron to simulate natural attenuation and to study the state of the microbial community without any biostimulation.

(b) Initial amendment of iron (no recharge)

The generation of H_2 by the corrosion of elemental iron stimulated the microbial reductive dechlorination of PCBs in sediment microcosms when added at the appropriate

Table 1 Sediment characteristics

Sediment	pH	Moisture (%)	Particle size distribution	Organic carbon (w/w)	Aroclor	Aroclor contamination (mg/kg dry weight)	Heavy metal	Metal contamination (mg/kg dry weight)
Lake Hartwell	6.2	54.5	39% sand	2.83	1221	2.73	Zinc	100
			36% silt		1232	3.66	Copper	20
			25% clay		1242	3.26	Nickel	12
					1248	2.88	Lead	16
					1254	0.90	Chromium	33
					1260	0.69	Mercury	0.06
New Bedford Harbor	7.3	56	57% sand	7.5	1221	886	Zinc	1,132
			32% silt		1232	363	Copper	452
			11% clay		1242	301	Nickel	32
					1248	174	Lead	358
					1254	92	Chromium	156
					1260	45	Mercury	1.2
					1268	7.74		
Roxana Marsh	7.0	72	37% sand	20.5	1221	5.01	Zinc	4,528
			38% silt		1232	3.45	Copper	510
			25% clay		1242	3.33	Nickel	179
					1248	2.98	Lead	3,260
					1254	1.75	Chromium	309
					1260	1.06	Mercury	2.9

rate. The sediments are mixed with a given quantity of iron. Iron corrosion followed by the production of H_2 will aid the bacteria to degrade the PCBs. Iron powder (99% purity and 325 mesh) was obtained from Fisher Chemicals (Fairlawn, NJ), and was used without modification. Several dosages of iron (0.003–0.1 g iron/g dry weight of sediment) were employed to evaluate the effect of hydrogen concentration on the microbial community.

(c) Periodic amendment of iron (iron recharge)

Because of weathering of iron in sediment systems, the passivation of iron corrosion and the consequent subsidence in generation of hydrogen may occur with time. Periodic amendments of the microcosm with fixed amounts iron may aid the hydrogen generation and sustain the rate of dechlorination. This will prolong the growth phase of the microorganisms by providing a continuous supply of hydrogen as the electron donor. Several dosages of iron (0.003–0.1 g iron/g dry weight of sediment) were added to two systems, with periodic amendment of the same iron dosages every 3 months and every 6 months. The effect of the periodic addition of iron on the microbial consortium was evaluated.

Sediment extraction and PCB analysis

After the biodegradation and the desorption experiments, the concentration of PCBs remaining in the sediments was determined by solvent extraction. Batch solvent extraction involved the extraction of 2.5 g of sediment with 25 ml of acetone (ACS certified, Fisher Chemicals) using a solid/liquid extraction ratio of 1:10 (g/ml) and shaking at 16 rpm for 24 h. After batch solvent extraction, the sediment extract was separated from the sediment using centrifugation. Liquid–liquid extraction was performed on sediment extract samples to prepare the samples in hexane (Optima, Fisher Chemicals) for injection into the gas chromatograph for PCB analysis. Five representative PCB congeners were analyzed: 2,2′,5-trichlorobiphenyl (PCB 18), 2,2′,3,5′-tetrachlorobiphenyl (PCB 44), 2,2′,3,4,5′-pentachlorobiphenyl (PCB 87), 2,2′,4,4′,5,5′-hexachlorobiphenyl (PCB 153), and 2,2′,3,4,4′,5,5′-heptachlorobiphenyl (PCB 180). The solvent extracts were analyzed for PCBs according to EPA method 8082 [30] using gas chromatography (GC) with an Agilent (Newark, DE) Model 6890 GC system equipped with a micro electron capture detector (μECD). A PCB congener mixture obtained from AccuStandards (New Haven, CT) was used for standard calibration. Decachlorobiphenyl (Supelco, Bellefonte, PA) was used as the internal standard and tetrachloro-*m*-xylene (Supelco) was used as the recovery standard. A DB-5 (J&W, Folsom, CA) capillary GC column with dimensions of 30 m × 530 μm × 1.5 μm was employed. The GC carrier and the ECD makeup gases were helium and argon-methane, respectively.

Hydrogen, methane, and sulfate analysis

Before the microcosm bottles were opened for sediment sample withdrawal, gas samples from the bottle headspace were withdrawn using a gas-tight locking syringe. The headspace gas samples were analyzed for hydrogen and methane on a GC system equipped with a thermal conductivity detector (TCD) using molecular sieve and silica gel columns, respectively. The concentration of sulfate in solution was determined using ion chromatography (IC) with a Dionex IC25 IC calibrated with sulfate standards.

Microbial community analysis

The extraction of lipids was carried out using a hybrid method to extract PLFA and fatty acid methyl esters (FAME) [13]. The whole procedure relies on the extraction of 'signature' lipids from the cell wall and membranes of microorganisms which serve as biomarkers for different microbial communities [35]. Lipids from the sediment samples were extracted using the modified Bligh and Dyer [4] procedure in triplicates to account for variation between samples.

Sediment samples frozen at −15°C were thawed, and extracted with phosphate buffer prepared using potassium hydrogen phosphate (ACS certified, Midwest Scientific, St. Louis, MO) and potassium dihydrogen phosphate, chloroform, and methanol (all ACS certified, Fisher Chemicals) in the ratio 0.8:1:2. Methyl esters of the fatty acids were prepared using a shorter and simpler procedure described by Microbial ID (Hayward, CA, USA). Preparation of FAMEs prior to analysis was carried out in four steps: saponification, methylation, extraction, and base washing.

FAME analysis

The dried lipids were then resuspended in a 1:1 mixture of hexane and methyl *tert*-butyl ether (HPLC grade, Fisher Chemicals) for analysis on an Agilent Model 6890 GC system equipped with a flame ionization detector (FID). Direct 1.0-µl injections at an inlet temperature of 250°C were used. The detector temperature was maintained at 300°C. A DB-5 capillary GC column (30 m × 320 µm × 0.25 µm) was used to separate the FAMEs. The temperature program was initial temperature = 100°C for 2 min, 5°C/min to 150°C, 1°C/min to 165°C, and 5°C/min to 250°C. Individual FAMEs detected in the samples were identified by comparison with the retention times obtained using the bacterial acid methyl ester (BAME) mix (Supelco) and methyl 8-(5-hexyl-2-furyl)octanoate standard (Matreya LLC, Pleasant Gap, PA).

Results and discussion

Lake Hartwell sediment

Among the recharge systems tested the 0.01 g iron/g dry sediment 6-month recharge set contains the least amount of iron and the 0.1 g iron/g dry sediment 3-month recharge set contains the maximum quantity of iron. Because the ratio of fatty acids cy17:0 to 16:1ω7c is indicative of the physiological condition or state of stress for the anaerobic microbial consortium, Fig. 1 shows that the stress at 6 months is lowest for the 0.1 g iron/g dry sediment 3-month recharge set followed by the 0.01 g iron/g dry sediment 6-month recharge set as the next best case. The microbial consortium exhibits log-phase growth between the 6-month to the 12-month period in the 0.01 g iron/g dry sediment 6-month recharge system. With an increase in the iron dosage, an increase in stress is observed at 12 and 18 months respectively, with the highest stress being exhibited by the system which contains the most iron. Therefore, after the 12-month period the physiological condition of the microbial consortium present in the sediment is in an intermediate phase between the log phase and stationary phase. As shown in Table 2, the recharge sets generally exhibited lower stress levels compared to the no-recharge systems and the blank system (without iron). Overall higher stress levels were observed for the higher dosages of iron (0.03 and 0.01 g iron/g dry sediment).

As is evident from Fig. 1, at the 18-month period, the recharge systems involving 0.01 g iron/g dry sediment exhibit maximum concentration of Fu18:2ω7 which translates into a proportional increase in the population of *Dehalococcoides* species in the sediment. As shown in Fig. 2, after 6 months a drastic increase in the amount of signature fatty acid Fu18:2ω7 was observed in the blank, 0.01 g iron/g dry sediment 6-month recharge and in the 0.1 g iron/g dry sediment 3-month recharge systems as compared to the control (0 month). Compared to the blank set (190 nmol/g dry) the recharge systems perform better, with the 0.01 g iron/g dry sediment 6-month recharge set (300 nmol/g dry) containing the highest concentration of Fu18:2ω7. This clearly indicates that amendment of low dosages of iron to the system enriched the population of *Dehalococcoides* owing to the continuous availability of hydrogen following anaerobic corrosion of iron which is evident from Fig. 3. Conversely, a decrease in the concentration of Fu18:2ω7 is observed in the 0.1 g iron/g dry sediment 3-month recharge system at 12 months, whereas an increase in the concentration of Fu18:2ω7 is observed in the 0.01 g iron/g dry sediment 6-month recharge system at 12 months. This could be as a result of the large amounts of iron being present in the 3-month recharge set and the resultant competition by sulfate reducers, iron-reducing bacteria like *Geobacteraceae*, and

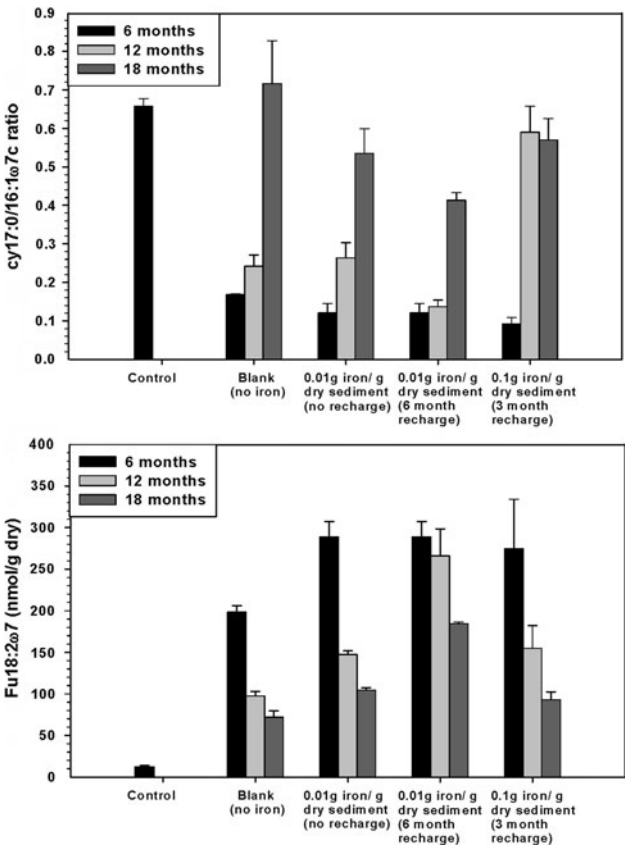

Fig. 1 Ratio of fatty acids cy17:0/16:1ω7c and concentration of *Dehalococcoides* biomarker (Fu18:2ω7) in Lake Hartwell sediment as function of time and iron recharge

methanogens for the generated hydrogen. This is consistent with high concentrations of H_2 observed in the 0.1 g iron/g dry sediment 3-month recharge systems. A decrease in Fu18:2ω7 concentration is also observed in the blank system at 12 months (Fig. 2) probably because of the unavailability of sufficient levels of electron donors like hydrogen in the system. At the 18-month period (Fig. 2), all the sets exhibit a gradual decline in the concentration of Fu18:2ω7, with the recharge systems exhibiting significantly higher concentrations compared to the blank (without iron) set. The rate of decrease in Fu18:2ω7 concentration in the 0.01 g iron/g dry sediment 6-month recharge set (300–280 nmol/g dry) is significantly lower than the other sets which signifies its effectiveness compared to the other systems. At the 18-month period, owing to the periodic amendment of iron, the recharge systems have a high concentration of iron resulting in high levels of hydrogen which might subsequently result in competition with sulfate reducers, *Geobacteraceae* species, and methanogens which have affinity to high levels of hydrogen. This is evident from the rate of increase in methane generation in the 0.1 g iron/g dry sediment 3-month recharge system as shown in Fig. 3. It is also interesting to note that despite the relatively higher levels of iron present in the 0.01 g iron/g dry sediment 6-month recharge systems, the concentration of Fu18:2ω7 is higher compared to the blank (without iron) and the 0.1 g iron/g dry sediment 3-month recharge sets. Results from Table 2 show that the 0.01 g iron/g dry sediment 6-month recharge system is most effective in sustaining the population of *Dehalococcoides*. This again indicates that periodic addition of small quantities of iron (0.01 g iron/g dry sediment) resulted in the continuous generation of lower levels of hydrogen compared to the 0.1 g iron/g dry sediment iron set and therefore sustained the *Dehalococcoides* population present in the sediment. The increase in stress with an increase in the amount of iron can be attributed to an increase in the activity of methanogenic Archaea which outcompete sulfate reducers for the terminal electron acceptor because of the presence of very low levels of sulfate in the sediment (Fig. 2). This is consistent with reports in the literature which show that in a coculture, nitrate or fumarate > sulfate > CO_2/CH_4 > sulfur or CO_2/acetate were the preferred electron acceptors and a greater percentage of hydrogen was transferred to the microbial consortia able to utilize the preferred terminal electron acceptors [7]. In the blank (without iron) set, even though no hydrogen gas was detected (Fig. 2), a gradual increase in methane (CH_4) production was also observed, which might explain the gradual increase in stress between 12 and 18 months. High pH conditions are also not conducive to the survival of microbial consortia in the sediment because they thrive at optimal pH values in the range of 7.0–8.0. As shown in Fig. 2, conditions of high pH (>8.6) are prevalent in the 0.1 g iron/g dry sediment 3-month recharge system which also exhibits high stress levels after the 12-month period. Therefore the recharge system using the lower dosage of iron (0.01 g iron/g dry sediment) was more effective in preserving optimal conditions representative of the nutritional and physiological status of the microbial consortium present in the sediment.

New Bedford Harbor sediment

As shown in Fig. 3, the 0.01 g iron/g dry sediment 6-month recharge set exhibits significantly lower stress levels compared to all the other systems. In general, the recharge systems exhibit lower stress levels when compared to the corresponding no-recharge systems (Table 3). After 6 months (Fig. 3), the lowest stress was observed in the 0.1 g iron/g dry sediment 3-month recharge set which contains the maximum quantity of iron, possibly owing to the presence of optimal quantities of hydrogen following the amendment of iron. As is evident from Fig. 3, the 0.01 g iron/g dry sediment 6-month recharge system exhibits relatively lower stress at the 9- and 12-month time periods compared to the 6-month set, whereas the blank (without iron) and the 0.1 g iron/g dry sediment 3-month

Table 2 Ratio of fatty acids cy17:0/16:1ω7c and concentration of *Dehalococcoides* biomarker (Fu18:2ω7) in Lake Hartwell sediment at the 18 month period

	Control	Blank (no iron)	0.01 g iron/g dry sediment			0.03 g iron/g dry sediment			0.1 g iron/g dry sediment		
			No recharge	Recharge @ 3 month	Recharge @ 6 month	No recharge	Recharge @ 3 month	Recharge @ 6 month	No recharge	Recharge @ 3 month	Recharge @ 6 month
cy17:0/16:1ω7c ratio	1.18 ± 0.06	0.57 ± 0.20	0.40 ± 0.06	0.42 ± 0.13	0.32 ± 0.03	0.40 ± 0.14	0.44 ± 0.05	0.47 ± 0.11	0.38 ± 0.04	0.40 ± 0.05	0.42 ± 0.04
Fu18:2ω7 (nmol/g dry)	9.04 ± 0.82	15.20 ± 0.73	31.82 ± 1.11	59.29 ± 2.91	60.06 ± 1.66	24.62 ± 2.39	37.30 ± 1.03	37.95 ± 1.19	28.18 ± 1.24	46.96 ± 1.47	48.73 ± 1.91

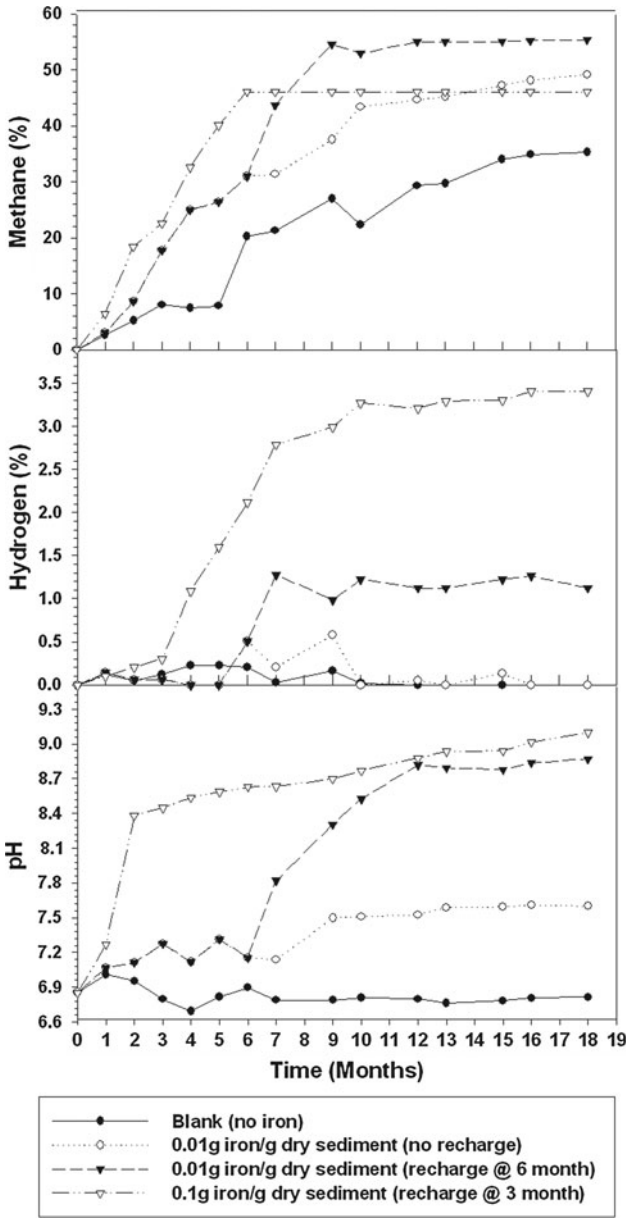

Fig. 2 pH, hydrogen concentration, and methane production in Lake Hartwell sediment as function of time and iron recharge

recharge sets exhibit higher stress levels. The 0.01 g iron/g dry sediment 6-month recharge set exhibits log-phase growth pattern up to the 15-month period after which it enters an intermediate phase between log and stationary phase. This suggests that optimal conditions were prevalent in the sediment up to the 15-month period using the 0.01 g iron/g dry sediment 6-month recharge system. The increase in stress levels in all the sets between months 15 and 18, (Fig. 3) can be attributed to the high levels of hydrogen present in the recharge sets resulting in competition between sulfate reducers, *Geobacteraceae*, and methanogenic Archaea. Hydrogen was detected in

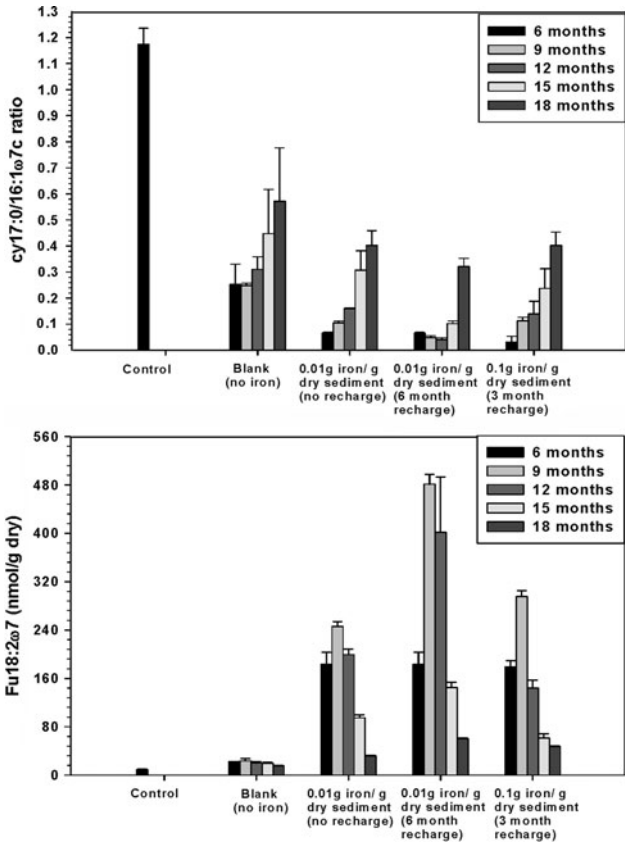

Fig. 3 Ratio of fatty acids cy17:0/16:1ω7c and concentration of *Dehalococcoides* biomarker (Fu18:2ω7) in New Bedford Harbor sediment as function of time and iron recharge

exceptionally high concentrations in the 0.1 g iron/g dry sediment 3-month recharge system when compared to the 0.01 g iron/g dry sediment 6-month recharge system as shown in Fig. 6. High concentrations of sulfate are found in marine sediments like New Bedford Harbor sediment which also exhibit high sulfate-reduction activity. The 0.1 g iron/g dry sediment 3-month recharge system exhibits the highest rate of sulfate reduction among the systems being studied. As shown in Fig. 6, sulfate gets depleted in the 0.1 g iron/g dry sediment 3-month recharge system at the 6-month period, which is followed by a community shift where methanogenesis becomes the dominant electron-accepting process. The prevalence of high pH conditions ($\sim$9.1) in the 0.1 g iron/g dry sediment 3-month recharge system could also explain the increasing stress levels over the 18-month period. The increase in stress levels in the blank set can be explained by the absence of sufficient quantities of a readily available electron donor like hydrogen (Fig. 6) in the presence of large quantities of an electron acceptor like sulfate.

As shown in Fig. 3, at 18 months the highest concentration of Fu18:2ω7 is exhibited by the 0.01 g iron/g dry sediment 6-month recharge system. There is a tenfold increase in the concentration of Fu18:2ω7 in the 0.01 g iron/g dry sediment 6-month recharge set (470 nmol/g dry) when compared to the blank set (40 nmol/g dry). Results from Table 3 indicate that the recharge systems generally exhibit higher concentrations of Fu18:2ω7 compared to the no-recharge, blank sets. As shown in Fig. 3, at 9 months a relatively drastic increase in the amount of the signature biomarker Fu18:2ω7 was observed in the 0.01 g iron/g dry sediment 6-month recharge (470 nmol/g dry) when compared to the 0.1 g iron/g dry sediment 3-month recharge system (300 nmol/g dry). Between the 9- and 12-month period for the 0.01 g iron/g dry sediment 6-month recharge set, the population of *Dehalococcoides* remains constant (470 nmol/g dry) compared to an approximately 50% decline in the 0.1 g iron/g dry sediment 3-month recharge system in the same time period from 300 to 160 nmol/gdry. This clearly indicates that amendment of low dosages of iron to the system created conditions conducive to the proliferation of *Dehalococcoides* species owing to the continuous availability of hydrogen generated through anaerobic corrosion of iron. Conversely, a decrease in the concentration of Fu18:2ω7 in the 0.1 g iron/g dry sediment 3-month recharge system between 9 and 12 months was observed despite the continuous amendment of iron to ensure hydrogen generation. This could be attributed to the increasing rate of hydrogen generation in the system for the 0.1 g iron/g dry sediment 3-month recharge set followed by the onset of methanogenesis and a corresponding depletion of sulfate between the 6-, 9-, and 12 -month periods (Fig. 6). The blank system exhibits low concentrations of Fu18:2ω7 which remain relatively constant for the entire duration of the study. At the 18-month time period, the entire set exhibits a gradual decline in the concentration of Fu18:2ω7 with the recharge systems exhibiting significantly higher concentrations compared to the blank set. At the 18-month period, owing to the periodic amendment of iron, the recharge systems have a high concentration of iron resulting in high levels of hydrogen (0.1 g iron/g dry sediment 3-month recharge set) which might subsequently result in competition with sulfate reducers (0.01 g iron/g dry sediment 6-month recharge system) and methanogens (0.1 g iron/g dry sediment 3-month recharge system) which have greater affinity towards high levels of hydrogen. It is also interesting to note that despite the relatively higher levels of iron present in the 0.01 g iron/g dry sediment 6-month recharge systems at 18 months, the concentration of Fu18:2ω7 is higher when compared to the blank and the 0.1 g iron/g dry sediment 3-month recharge set. This again indicates that periodic addition of small amounts of iron (0.01 g iron/g dry sediment) resulting in the continuous generation of low levels of hydrogen sustained the *Dehalococcoides* population present in the sediment to a greater extent than the 0.1 g iron/g dry sediment iron set.

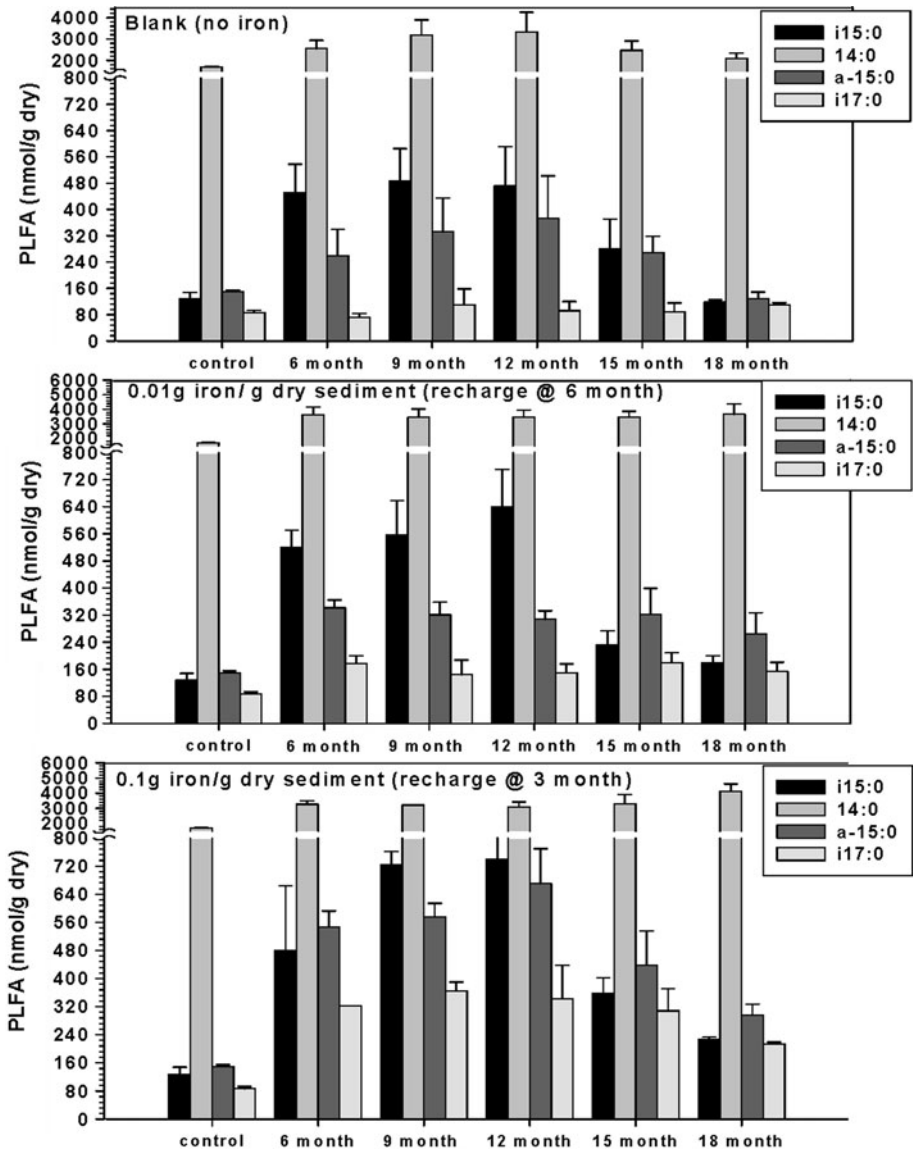

Fig. 4 Fatty acids i15:0, 14:0, a-15:0, and i17:0 in New Bedford Harbor sediment as function of time and iron recharge

At 6 months, an increase in the PLFA concentration for sulfate reducers is observed, with the most significant increase in the 0.1 g iron/g dry sediment 3-month recharge set followed by the 0.01 g iron/g dry sediment 6-month recharge set. In the case of the 0.01 g iron/g dry sediment 6-month recharge and 0.1 g iron/g dry sediment 3-month recharge set at 9 and 12 months a further increase in sulfate reducers was observed owing to a high concentration of sulfate present in the sediment. Beginning from the 12-month period, a gradual decline in the concentration of the PLFAs characteristic of sulfate reducers was observed for all the sets till the 18 month period. The relative decrease in the population of sulfate reducers was highest for the 0.1 g iron/g dry sediment 3-month recharge set followed by the 0.01 g iron/g dry sediment 6-month recharge set and the lowest for the blank system.

Even though methanogens and sulfate reducers co-exist in sediments, when sulfate is not limiting sulfate-reducing bacteria inhibited methane production by lowering the hydrogen partial pressure below the threshold level for methanogenic Archaea [16]. In the 0.1 g iron/g dry sediment 3-month recharge set, no sulfate was detected in New Bedford Harbor sediment at 15 months and relatively low concentrations of sulfate were present in the 0.01 g iron/g dry sediment 6-month recharge set also as indicated in Fig. 6. A corresponding increase in methanogenesis is also observed following the depletion of sulfate in the sediment (Fig. 6). Consistent with the findings of Lovley et al. [16], the decrease in the population of sulfate reducers between months 15 and 18 can be explained by the gradual depletion of sulfate and a subsequent microbial community shift with the methanogens becoming the dominant hydrogen-utilizing microbial species in the sediment.

Table 3 Ratio of fatty acids cy17:0/16:1ω7c and concentration of *Dehalococcoides* biomarker (Fu18:2ω7) in New Bedford Harbor sediment at the 18-month period

	Control	Blank (no iron)	0.01 g iron/g dry sediment			0.03 g iron/g dry sediment			0.1 g iron/g dry sediment		
			No recharge	Recharge @ 3 month	Recharge @ 6 month	No recharge	Recharge @ 3 month	Recharge @ 6 month	No recharge	Recharge @ 3 month	Recharge @ 6 month
cy17:0/16:1ω7c ratio	0.66 ± 0.02	0.72 ± 0.11	0.54 ± 0.06	0.35 ± 0.04	0.41 ± 0.02	0.51 ± 0.10	0.56 ± 0.05	0.54 ± 0.04	0.64 ± 0.01	0.57 ± 0.06	0.49 ± 0.01
Fu18:2ω7 (nmol/g dry)	12.39 ± 1.56	72.04 ± 7.46	104.54 ± 3.17	193.57 ± 8.62	184.33 ± 2.02	95.10 ± 3.49	122.32 ± 11.34	108.74 ± 7.25	60.91 ± 2.41	93.01 ± 9.30	71.15 ± 1.74

From Figs. 4 and 5 it is clearly evident that the collective concentration for the representative fatty acids for *Geobacteraceae* is highest for the 0.1 g iron/g dry sediment 3-month recharge set followed by the 0.01 g iron/g dry sediment 6-month recharge set. At the 9- and 12-month periods, the concentration of i15:0, 16:1ω7c, and 16:0 are higher compared to the 6-month set and between 15 and 18 months, a significant decrease in the population of *Geobacteraceae* is observed in all the systems. On the basis of previous research [15], the particle size and the type of Fe(III) oxides formed might be a factor in sustaining the population of *Geobacteraceae*. Gradual accumulation of sulfide from the reduction of sulfate and a simultaneous decline in the population of *Geobacter* was reported, with a continuous supply of acetate to a uranium-contaminated aquifer [2]. Those authors concluded that the microbial community shift was observed owing to the depletion of Fe(III) over time and the preference for sulfate as the terminal electron acceptor over other electron-accepting species. Because New Bedford Harbor sediment is associated with high levels of sulfate, the microbial reduction of sulfate (Fig. 6) and the onset of methanogenesis (Fig. 6) might be the major factors effecting a decline in the population of *Geobacteraceae* at 15 and 18 months

Roxana Marsh sediment

Both the 3-month and 6-month recharge sets for the 0.01 and 0.03 g iron/g dry sediment dosages exhibit significantly lower stress levels compared to all the other systems (Fig. 7). The recharge systems exhibit generally lower stress levels when compared to the corresponding no-recharge systems (Table 4). After 6 months, marginally lower stress levels were observed in the 0.03 g iron/g dry sediment 3-month recharge set when compared to the 0.01 g iron/g dry sediment 6-month recharge set. This could possibly be due to the presence of optimal quantities of hydrogen following the initial amendment of higher dosages of iron. The 0.01 g iron/g dry sediment 6-month recharge and the 0.03 g iron/g dry sediment 3-month recharge sets exhibit log-phase growth pattern throughout the duration of the study. This suggests that optimal conditions are prevalent in the sediment throughout the study. In the 0.01 g iron/g dry sediment 6-month recharge and 0.03 g iron/g dry sediment 3-month recharge sets, the stress levels were found to remain relatively constant throughout the duration of study. Hydrogen was detected in the 0.03 g iron/g dry sediment 3-month recharge system in concentrations approximately 10 times that in the 0.01 g iron/g dry sediment 6-month recharge system as shown in Fig. 8. As shown in Fig. 8, the highest sulfate-reduction activity was observed in the 0.03 g iron/g dry sediment

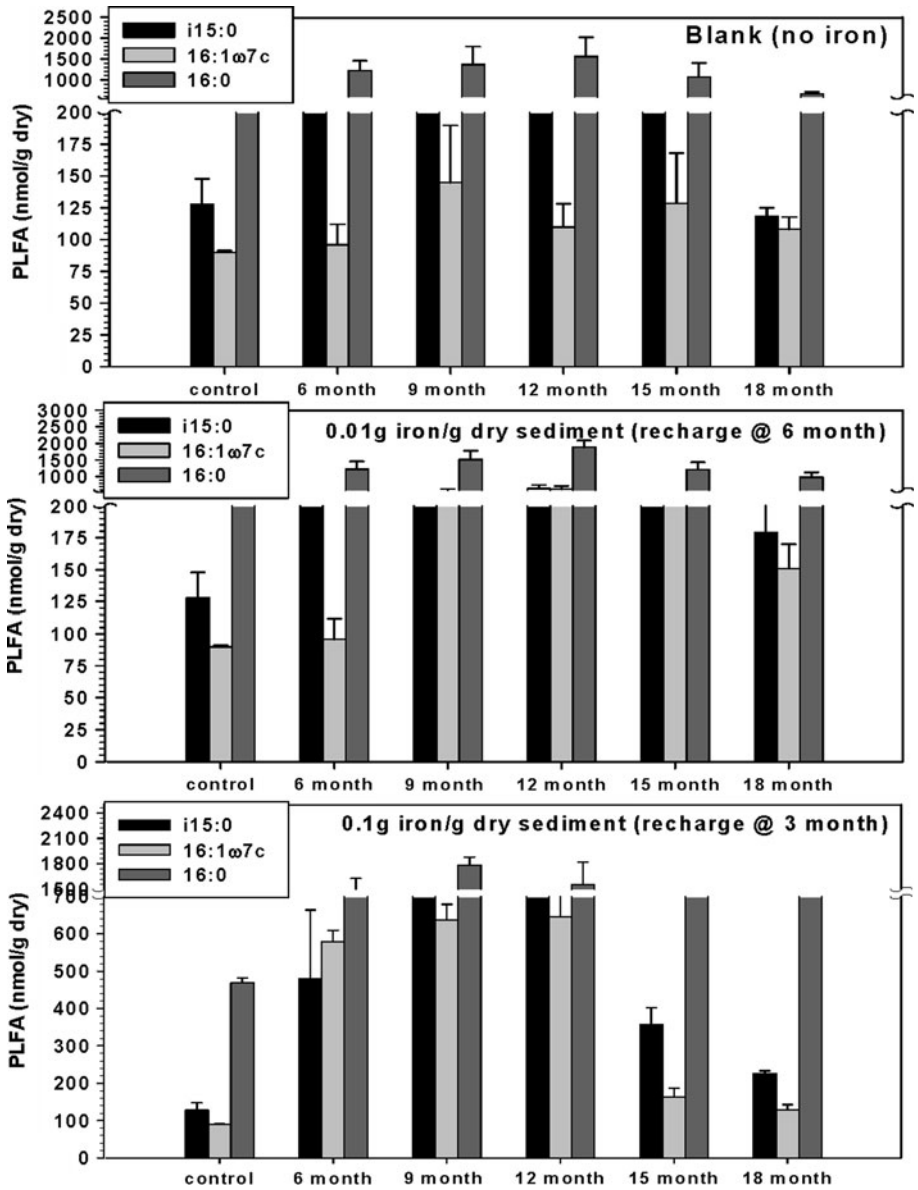

Fig. 5 Fatty acids i15:0, 16:1ω7c, and 16:0 in New Bedford Harbor sediment as function of time and iron recharge

3-month recharge system when compared to the 0.01 g iron/g dry sediment 6-month recharge system. This can be attributed to the presence of sulfate-reducing bacteria which are known to possess affinity for high levels of hydrogen or other electron donors in the system to reduce sulfate to sulfides. Figure 8 shows that detectable levels of hydrogen in the recharge systems coincide with the generation of methane. The generation of methane is found to be proportional to the quantity of iron (thus hydrogen) supplied to the system. The increase in stress levels in the blank set can be explained by the absence of sufficient quantities of a readily available electron donor like hydrogen (Fig. 8) in the presence of large quantities of an electron acceptor like sulfate. Therefore, the trend observed in the stress levels in Roxana Marsh sediment indicate that irrespective of the high pH (Fig. 8) and high concentrations of H_2 detected in the systems with periodic amendment of iron, optimal conditions for the indigenous microbial consortium were prevalent in the recharge systems.

As shown in Fig. 7, at 18 months the highest concentration of Fu18:2ω7 is exhibited by the 0.01 g iron/g dry sediment 3- and 6-month recharge systems. The concentration of Fu18:2ω7 in the 0.01 g iron/g dry sediment 6-month recharge set (300 nmol/g dry) is found to be 8 times higher when compared to the blank set (40 nmol/g dry). In general, the recharge systems exhibit significantly higher concentrations of Fu18:2ω7 compared to the no-recharge, blank sets (Table 4). At 6 months, a drastic increase in the concentration of the signature biomarker Fu18:2ω7 was observed in the 0.01 g iron/g dry sediment

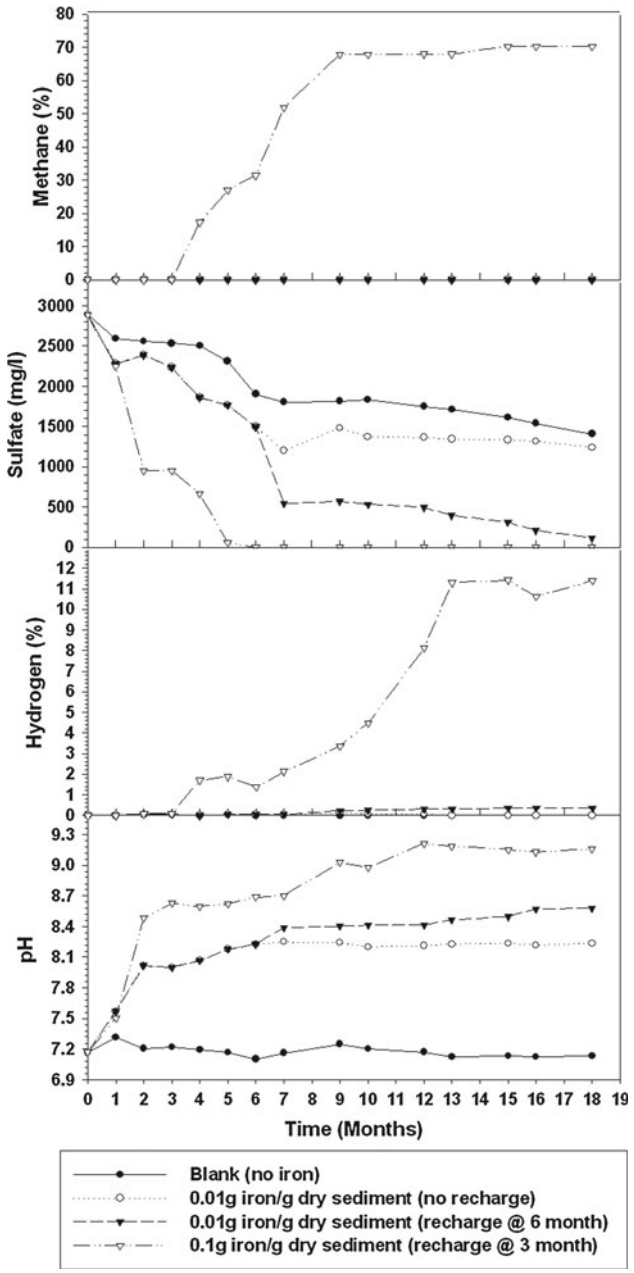

Fig. 6 pH, hydrogen concentration, sulfate concentration, and methane production in New Bedford Harbor sediment as function of time and iron recharge

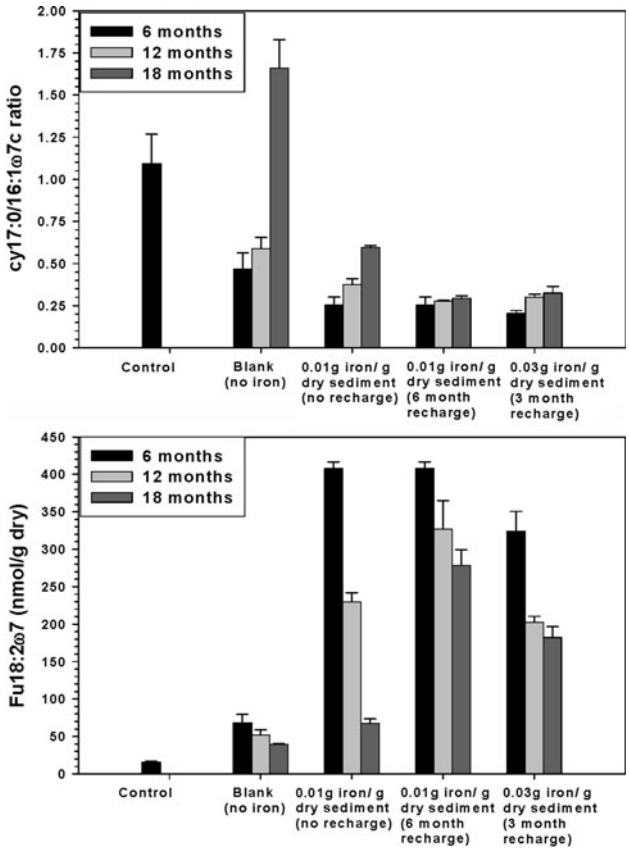

Fig. 7 Ratio of fatty acids cy17:0/16:1ω7c and concentration of *Dehalococcoides* biomarker (Fu18:2ω7) in Roxana Marsh sediment as function of time and iron recharge

6-month recharge system (420 nmol/g dry) and the 0.03 g iron/g dry sediment 3-month recharge system (340 nmol/g dry) compared to the blank (75 nmol/g dry). This indicates the presence of an indigenous population of *Dehalococcoides* in the sediment that got enriched on the addition of iron. Between the 6- and 12-month period for the 0.01 g iron/g dry sediment 6-month recharge set, the population of *Dehalococcoides* shows a 25% decline compared to an approximately 42% decline in the 0.03 g iron/g dry sediment 3-month recharge system in the same time period from 370 to 200 nmol/gdry. This clearly indicates that amendment of low dosages of iron to the system created conditions conducive to the proliferation of *Dehalococcoides* species owing to the continuous availability of hydrogen following anaerobic corrosion of iron. This could be attributed to the increasing rate of hydrogen generation in the 0.03 g iron/g dry sediment 3-month recharge set followed by the onset of methanogenesis between the 6- and 12-month periods (Fig. 8). The blank system exhibits low concentrations of Fu18:2ω7 which remain relatively constant for the entire duration of the study. At the 18-month time period, the entire set exhibits a gradual decline in the concentration of Fu18:2ω7, with the recharge systems exhibiting significantly higher concentrations compared to the blank set. Owing to the periodic amendment of iron, the recharge systems have a high concentration of iron at the 18-month period, thus resulting in high levels of hydrogen (0.03 g iron/g dry sediment 3-month recharge set) which might subsequently result in competition with sulfate reducers and methanogens which have greater affinity towards high levels of hydrogen (Fig. 8). This again indicates that periodic addition of small

Table 4 Ratio of fatty acids cy17:0/16:1ω7c and concentration of *Dehalococcoides* biomarker (Fu18:2ω7) in Roxana Marsh sediment at the 18-month period

	Control	Blank (no iron)	0.003 g iron/g dry sediment			0.01 g iron/g dry sediment			0.03 g iron/g dry sediment		
			No recharge	Recharge @ 3 month	Recharge @ 6 month	No recharge	Recharge @ 3 month	Recharge @ 6 month	No recharge	Recharge @ 3 month	Recharge @ 6 month
cy17:0/16:1ω7c ratio	1.09 ± 0.17	1.66 ± 0.17	0.88 ± 0.12	0.76 ± 0.03	0.59 ± 0.05	0.59 ± 0.01	0.38 ± 0.07	0.29 ± 0.02	0.66 ± 0.04	0.32 ± 0.04	0.33 ± 0.02
Fu18:2ω7 (nmol/g dry)	15.44 ± 1.72	39.50 ± 0.91	44.25 ± 0.64	117.64 ± 1.07	164.63 ± 10.47	67.23 ± 6.25	261.87 ± 8.67	278.24 ± 21.13	39.93 ± 1.12	182.18 ± 14.33	193.96 ± 16.25

amounts of iron (0.01 g iron/g dry sediment) resulting in the continuous generation of low levels of hydrogen sustained the *Dehalococcoides* population present in the sediment to a greater extent than the 0.03 g iron/g dry sediment iron set.

Biodegradation of PCBs in sediment

The biodegradation experiments were carried out for all the three sediments for a period of 18 months where five PCB congeners were monitored. Periodic amendment of sediment with iron significantly enhanced the extent of degradation of the selected congeners when compared to the blank system as shown in Table 5. In addition, the systems amended with lower levels of iron were found to produce greater biodegradation as compared to higher dosages of iron. In other words, the microbial consortia ultimately prefer low concentration of hydrogen as enhancements for reductively dechlorinating the PCB molecule which is also evident in the proliferation of *Dehalococcoides* species and lower stress levels exhibited by the corresponding systems. The quality and quantity of hydrogen produced from the lower dosage iron systems was found to be easily accessible for the dehalorespiring microbes which justifies their dominance over the systems with higher levels of hydrogen. For freshwater sediments like Lake Hartwell and Roxana Marsh the addition of very high concentrations of iron to the pre-enriched dechlorinating microbes inhibited dechlorination of the PCB congeners studied. In the New Bedford Harbor estuarine sediment, the higher dosages of iron were equally effective in dechlorinating PCBs possibly enhanced by the precipitation of toxic sulfides from solution. Therefore, in contaminated sediments where dechlorinators are only a small part of a consortium that includes hydrogen-utilizing sulfate reducers, metal reducers, and methanogens, the addition of high concentrations of iron is not an effective way to stimulate their growth and activity because other populations are likely to grow more rapidly at high electron donor (H_2) concentrations and change the local environment. Therefore the reductive dechlorination of the selected PCB congeners was enhanced by periodic amendment of iron to the system.

Conclusions

Generally, lower stress levels were observed in systems with the lower dosage of iron for Lake Hartwell, New Bedford Harbor, and Roxana Marsh sediments compared to systems with higher dosage of iron. In the case of Lake Hartwell sediment, the amendment of the lower dosage of iron proved to be more useful in enhancing the population

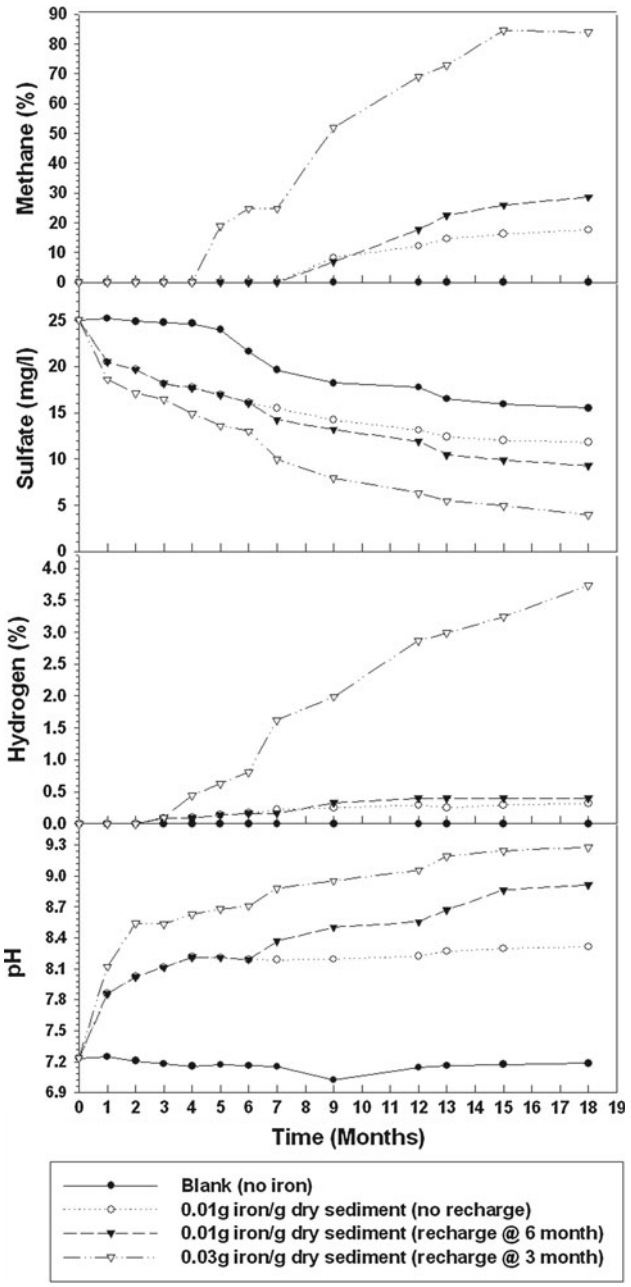

Fig. 8 pH, hydrogen concentration, sulfate concentration, and methane production in Roxana Marsh sediment as function of time and iron recharge

of *Dehalococcoides* species in the sediment, and a similar trend was observed in the New Bedford Harbor and Roxana Marsh sediment where the increase in *Dehalococcoides* population in the recharge systems relative to the blank set was more pronounced and significant than that observed in Lake Hartwell sediment. Both Lake Hartwell and New Bedford Harbor sediments exhibited lower stress levels in the amendment system with the lower iron dosage, showing a gradual increase in stress levels with the

Table 5 Percent biodegradation of PCB congeners in contaminated sediments after 18 months

PCB	Lake Hartwell sediment			New Bedford Harbor sediment			Roxana Marsh sediment		
	Blank (no iron)	0.01 g iron/g dry recharge @ 6 month	0.1 g iron/g dry recharge @ 3 month	Blank (no iron)	0.01 g iron/g dry recharge @ 6 month	0.1 g iron/g dry recharge @ 3 month	Blank (no iron)	0.01 g iron/g dry recharge @ 6 month	0.03 g iron/g dry recharge @ 3 month
18	43.84	72.28	57.07	14.39	39.95	45.77	5.25	26.33	15.41
44	33.07	63.90	50.43	5.26	34.07	29.61	28.81	52.40	38.57
87	57.05	75.42	60.61	55.77	79.69	72.17	41.32	61.51	70.04
153	56.18	76.15	54.82	44.76	70.17	66.58	18.02	41.56	41.06
180	50.38	74.06	65.60	49.35	75.84	73.68	32.17	72.45	41.53

introduction of increasing quantities of iron. Conversely, in the Roxana Marsh sediment a minimum and maximum threshold concentration of iron was observed to exhibit lower stress levels. High levels of an electron donor like H_2 in the sediment induces competition between various microbial communities utilizing the same electron donor which leads to higher stress levels exhibited by the less dominant microbial species in the consortium. Maximum increase in populations of *Dehalococcoides* species, *Geobacteraceae*, and sulfate reducers was observed between 6 and 12 months for both Lake Hartwell and New Bedford Harbor sediments and up to 6 months in Roxana Marsh sediment. Between 15 and 18 months, high levels of hydrogen in the system resulted in establishment of methanogens as the dominant microbial species in the sediment accompanied by a gradual decline in populations of *Dehalococcoides* species, *Geobacteraceae*, and sulfate reducers in Lake Hartwell, New Bedford Harbor, and at 6 months in Roxana Marsh sediments.

In Lake Hartwell, New Bedford Harbor, and Roxana Marsh sediments, *Dehalococcoides* species proliferated in the early stages of the study, owing to the presence of low quantities of H_2 in the system to be eventually outcompeted by *Geobacteracea*, methanogens, and/or sulfate reducers with continuous amendment of higher dosages of iron, depending on the abundance and preference of the terminal electron acceptor (either Fe(III) or sulfate) present in the sediment. Therefore, periodic amendments of the sediment with the lower iron dosage (0.01 g iron/g dry sediment) every 6 months were found to be most effective in stimulating *Dehalococcoides* species by providing low concentrations of H_2 and eliminating competition by *Geobacteraceae*, methanogens, and sulfate reducers which have affinity for high levels of H_2. The capability to stimulate anaerobic PCB dehalogenation is in the nascent phase and has the potential to materialize as an efficient ex situ remediation technology for contaminated sediments. Biostimulation of indigenous PCB dechlorinators by the periodic amendment of contaminated sediments with low dosages of iron metal may therefore be an effective technology for remediating PCB-contaminated sediments.

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