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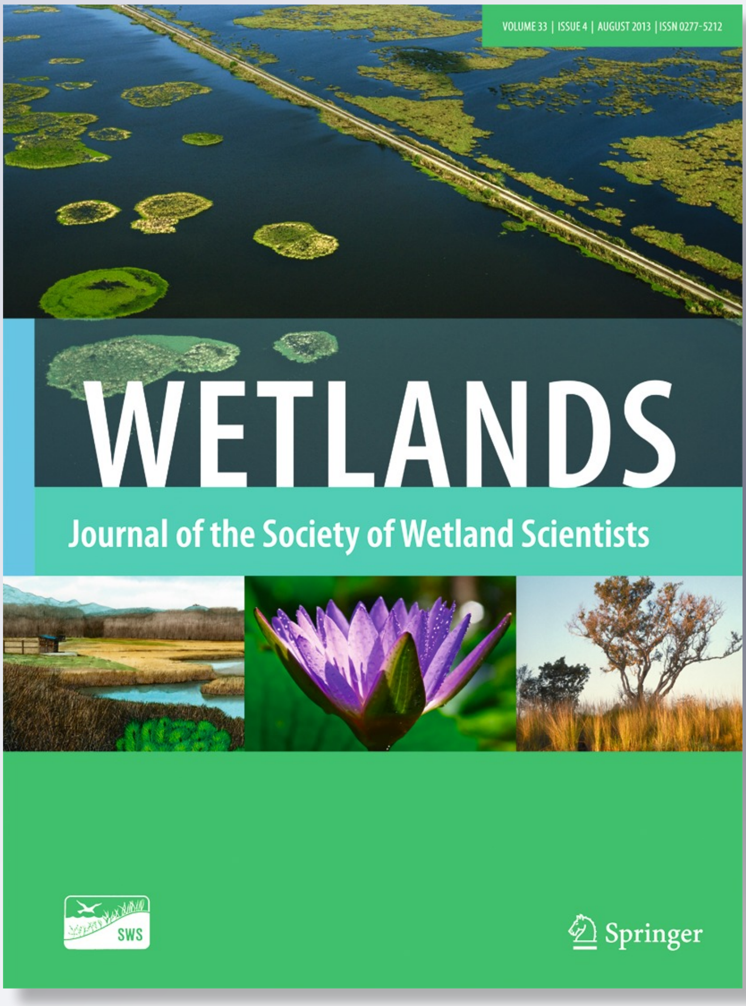

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ARTICLE

Nitrate Addition Has Minimal Effect on Anaerobic Biodegradation of Benzene in Coastal Saline (salt), Brackish and Freshwater Marsh Sediments

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Abstract In response to the 2010 British Petroleum (BP) Deepwater Horizon Macondo well oil spill, saline (salt), brackish and freshwater marsh sediments were used to study whether nitrate addition could stimulate petroleum biodegradation. Benzene was selected as a model petroleum hydrocarbon, and its biodegradation was assumed to follow first-order kinetics. The results show that the intrinsic benzene degradation activities in the marsh sediments were inversely correlated (R^2=0.83, n=9) with the sediment salinity, with following order of benzene degradation rate regardless of nitrate treatments, freshwater > brackish > salt ($P<0.05$). Addition of nitrate shows a minimal potential to stimulate the benzene degradation. Increase of redox potentials by addition of nitrate even shows a slight inhibition effect on benzene degradation in these coastal marsh sediments without a clear mechanism. Excessive loading of nitrate has been causing frequent occurrence of coastal eutrophication and hypoxia in the Gulf of Mexico. Therefore, application of additional nitrate to the coastal marsh sediments for the remediation of the 2010 BP oil spill is not recommended.

Keywords BP oil spill · Deepwater Horizon · Petroleum hydrocarbon · Biodegradation · Marsh sediment · Salinity · Nitrate · Redox potential

Introduction

The 2010 BP oil spill became the worst environmental incident in US history with approximately 5 million barrels of petroleum hydrocarbons released into the Gulf of Mexico (BP 2010; NOAA 2010). Application of large amount of dispersant makes its environmental impacts even more complicated. Natural forces can push large amounts of the spilled oils into the fragile coastal wetlands that have been experiencing substantial losses due to subsidence, drainage, eustatic global sea level rise and tropical storms (Conner and Day 1988; Bourne 2000). As a transition zone from terrestrial to marine ecosystems, coastal wetlands play a critical role to maintain the ecosystem integrity of the Gulf of Mexico. However, presence of oil/dispersant and their degradation products provides additional stresses that affect the survival and ecological functions of coastal wetlands.

Biodegradation of petroleum hydrocarbons is an important mechanism to remove the oils from both natural seepage and human sources. It has an advantage over various mechanical remediation practices due to less destruction and low cost (Venosa and Zhu 2003; Mitsch 2010). It is commonly believed that aerobic biodegradation of petroleum hydrocarbons is more effective than anaerobic biodegradation because of the presence of oxygen. The aeration status of wetland sediment can be easily determined by its oxidation-reduction (redox) potential using a redox electrode (Bohn 1971). In wetland sediments, a series of redox reactions can be initiated with organic matters, including petroleum hydrocarbons, as electron donors and various electron acceptors, in their thermodynamic and redox potential order including oxygen (high redox), nitrate/nitrite, manganese (IV), iron (III), sulfate, acetate and carbon dioxide (CO_2) (low redox) (Cross et al. 2003; Van Stempvoort et al. 2004; Fan et al. 2006). During this process, complex petroleum compounds are degraded to volatile components, and further to gaseous end products of CO_2 and possibly methane (CH_4) (Masumoto et al. 2012).

Application of nitrate for petroleum contaminated sites has been used to stimulate biodegradation of petroleum hydrocarbons due to nitrate's high redox potential and high mobility (Jin et al. 2010). Addition of nitrate may stimulate

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the growth of microbes that are responsible for the biodegradation activities. However, excessive amount of nitrate introduced may pose a potential consequence of stimulating coastal eutrophication and hypoxia (water O_2 level less than 2 mgL^{-1}) as documented over the last several decades (Turner and Rabalais 1994; Rabalais et al. 1994). It has been concluded that the Mississippi River discharge containing elevated levels of nitrate from various non-point sources in the watershed is the major cause of the expanding area of hypoxia in the Gulf of Mexico each year. This is because the Mississippi River levee built for flood control has prevented nutrients from entering the coastal wetlands, which historically serves as an important N sink to reduce excessive nitrate loading into the Gulf (Addison 1999).

As an immediate response to the 2010 BP oil spill, this experiment aims to study whether addition of nitrate could stimulate the biodegradation of petroleum hydrocarbons in the Gulf coastal wetlands, and how much nitrate addition would be appropriate. Saline (salt), brackish and freshwater marsh sediments are used to cover the full-range salinity gradient of the coastal environment. The observed toxicity of petroleum mainly comes from its lower-molecular-weight components (Fuller et al. 2004). Degradation of complex aliphatic and aromatic petroleum hydrocarbons generates a steady stream of such highly toxic compounds, most of which belong to a class of carcinogenic polycyclic aromatic hydrocarbons (PAHs), and then a family of mono-aromatic compounds (BTEX, including benzene, toluene, ethlybenzene, and xylene). The stable aromatic chemical structure in PAHs and BTEX makes them very persistent in nature. Benzene, as a common constituent of petroleum hydrocarbons and a derivative of petroleum degradation, has been intensively studied. For the same reason and to simplify the experimental design, benzene was selected in this study as a model petroleum hydrocarbon. It is hypothesized that benzene degradation is greater as the sediment salinity increases since more saline sediment tends to have previous oil exposure from historical oil spills or natural seepage. The study anticipates that nitrate would stimulate benzene degradation by providing N as a nutrient and by providing an electron acceptor for the microbial metabolism.

Materials and Methods

Sediment Samples

Marsh sediments on the Louisiana Gulf coast were sampled in May 2011, 1 year after the 2010 BP oil spill (Fig. 1). Both the saline and brackish sites were dominantly covered by marsh plant species of *Spartina alterniflora* and *Spartina patens*, while the freshwater site by a mixture of *Sagittaria lancifolia*, *Typha angustifolia*, *Potamogeton nodosus* and *Scirpus olneyi*. After removing the surface vegetation, top 30-cm of the sediment was taken from each site and stored in ice before being transported to the laboratory. The samples were then stored in a refrigerator (4 °C) before this experiment in August 2011. Major physical and chemical characteristics of the sediments were analyzed before the experiment and are summarized in Table 1. Water content of the sediment was determined to be 76 %, 63 %, and 93 % for the salt, brackish and freshwater marsh sediment, respectively.

Experimental Procedure

Each sediment sample was incubated under its original salinity condition by mixing the sediment with water of designated salinity. Artificial ocean water (36 ppt, ‰) was prepared by dissolving sea salt (Instant Ocean, Spectrum Brands Inc.) into D.I. water, and was later diluted to a final salinity of 12 ppt (for the salt marsh sediment), 6 ppt (for the brackish marsh sediment). For the freshwater marsh sediment, only D.I. water was used since the salinity in the freshwater marsh was commonly <1 ppt. Sediment slurry was made by using a 237-mL wide-mouth glass bottle with 40 g (wet weight) sediment and 80 mL water of designated salinity. Thus, the sediments were incubated at the salinity conditions similar to the sampling sites with final salinity of 12.6, 6.5 and 0.1 ppt. The bottle was covered with a screw cap lined with Teflon tape to prevent gas leakage. A hole was drilled in the center of the cap, and then a rubber stopper was inserted for future gas sampling.

For each of the three sediments, the experiment was conducted in two separate batches. In the first batch, same amount of benzene (0.1 mL, 88 mg, benzene density 0.88 g mL^{-1}) was added to all four nitrate addition treatments, including Control (no nitrate), 1×Nitrate (0.1 mL, 1.4 g N), 2×Nitrate (0.2 mL, 2.8 g N), and 4×Nitrate (0.4 mL, 5.6 g N). In the second batch, same amount of nitrate (0.2 mL, 2.8 g N) was added to all four benzene addition treatments, including Control (no benzene), 1×Benzene (0.05 mL, 44 mg), 2×Benzene (0.1 mL, 88 mg), and 4×Benzene (0.2 mL, 176 mg). In fact, the treatment of 2×Nitrate and the treatment of 2×Benzene had the same quantity of benzene and nitrate combination, serving as an internal reference between the two batches of the experiments. For every treatment, three replicates were applied with a total of 36 bottles (4 treatments×3 replicates×3 sediments) needed for each batch of the experiment. For the nitrate addition treatments, 1 M KNO_3 solution was added using a pipette into the sediment slurries. After that, all bottles were capped and then flushed with pressurized nitrogen gas (ultra high purity grade) for 5 min to displace the headspace air and create an oxygen-free incubation environment. Then, benzene (> 99.8 % purity, analytical grade, Sigma-Aldrich

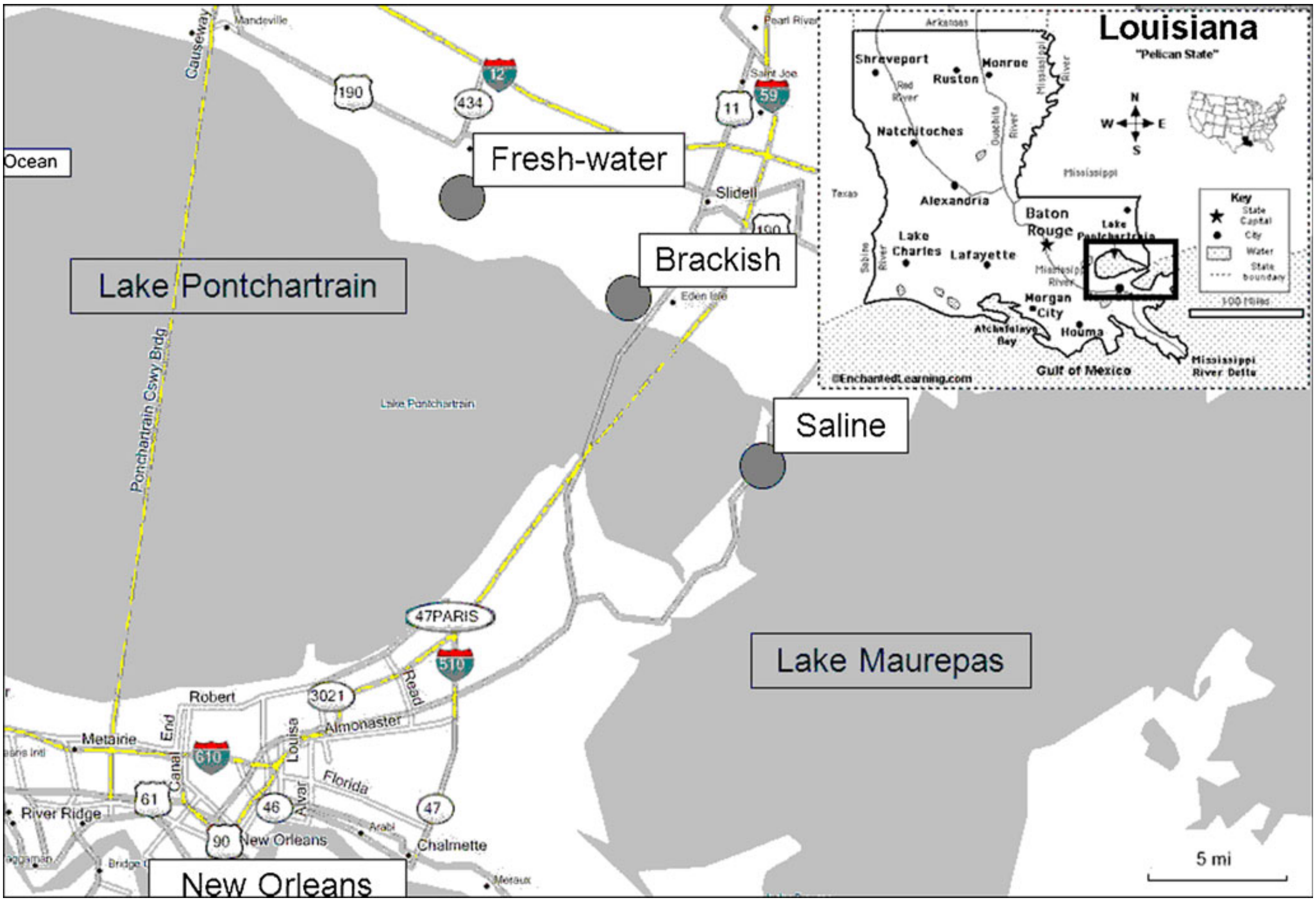

Fig. 1 Sampling locations for the marsh sediments across a salinity gradient on the Louisiana Gulf coast

Chemical Company) was added to each benzene treatment using a micro-syringe through the rubber stopper of the bottle.

Before initial measurements were taken, all sediment slurries were placed on a rotary shaker for 2 hours to allow the system to reach equilibrium. Then, 0.1 mL gas samples were taken using a Luer-lok glass syringe to determine the benzene concentrations in the headspace of the bottles. This initial measurement was considered as the starting time of experiment. Then, the benzene concentrations were monitored three times a week for 2 weeks. All experimental procedures were conducted under room temperature (23 °C), and all incubation bottles were kept in the dark except during the period of gas sampling. At the end of experiments, all bottles were uncovered for pH and Eh measurements.

Analytical Methods

Texture of the sediments was determined by hydrometer method according to USDA particle size classification (sand 2–0.05 mm, silt 0.05–0.002 mm, and clay <0.002 mm). EPA methods were used for analysis of anions (method 300.0), metals (method 200.7), and total phosphorus (method 365.3), respectively. Sediment pore water was obtained through centrifugation of the wet sediment. Salinity (chlorinity) was calculated by multiplying chloride content with a constant of 0.0018066 (Table 1). Benzene concentration was analyzed by using a Shimadzu GC-2014 gas chromatograph equipped with a flame ionization detector (FID). A Supel-Q PLOT fused silica capillary column (30 m × 0.32 mm) was used. The retention time for benzene was approximately 2.70 min with a total analysis time of 5 min. Helium (ultra high purity grade) was used as the carrier gas with a pressure of 132.2 kPa and a total flow rate of 4.0 mL min^{-1}. The oven, injector and detector temperatures were 160, 150 and 200 °C, respectively. The pH and Eh values of the sediment slurries were measured by using a pH/mV meter (Accumet AP62, Fisher Scientific) with a combination pH electrode (Fisher Scientific) and with an oxidation-reduction potential (ORP) electrode (SPER Scientific, Inc), respectively. The pH and Eh measurements were made at room temperature after the readings stabilized in about 5 min.

Table 1 Physical and chemical characteristics of the three marsh sediments

Analysis	Freshwater marsh	Brackish marsh	Salt marsh
GPS coordinates	N30° 17.073’	N30° 13.58’	N30° 08.78’
	W89° 55.17’	W89° 49.28’	W89° 44.67’
Total carbon %	18.4	2.8	7.2
Total nitrogen %	1.25	0.16	0.43
pH	7.9	7.6	7.3
Eh (mV) at pH 7	+333	+108	+173
Texture	Silt Loam	Loam	Silty Clay Loam
Sand %	38	33	11
Silt %	54	41	57
Clay %	8	25	32
Dry sediment analysis in g $\mathrm{kg^{-1}}$			
Iron	7.99	12.65	19.91
Potassium	0.56	0.993	2.36
Magnesium	3.23	2.46	5.56
Manganese	0.03	0.05	0.12
Phosphorus	0.42	0.27	0.61
Sulfur	5.43	4.25	5.30
Sediment pore water analysis in mg $\mathrm{L^{-1}}$			
Nitrate	1.1	14.9	11.4
Nitrite	3.1	20.00	21.7
Chloride	165	4558	7947
Sulfate	73	220	109
Phosphate	ND	0.10	0.03
Total Phosphorus	0.2	1.4	2.4

ND Not Detected. The values of pH and Eh were determined before the experiment

Calculations and Statistical Analysis

Benzene degradation was assumed to follow first-order kinetics as following:

$$C = C_0 \times e^{-kt}$$

where C represents the benzene concentration at time t, C_0 the initial benzene concentration (t=0), and k the first-order kinetics constant, which is independent of concentration. Higher k values represent greater benzene degradation rates. The k values were directly determined by exponential regressions of the benzene concentrations during the incubation. All data are reported based on the dry weight of the sediments. The redox potentials were calibrated to the standard H_2 electrode by adding the correction factor (+245 mV at 23 °C) for the calomel reference to the observed instrument reading. The effect of pH on Eh was calculated according to the inverse relationship of Eh and pH as described by the Nernst equation (Bohn 1971). All statistical analysis was conducted using SAS 9.3 (SAS institute Inc, Cary, NC USA). Difference among the means of different treatments was determined by a two-way ANOVA (significance level α=0.05).

Results

The redox potential measurements at end of the experiments verified that the benzene degradation process in this study was under anaerobic conditions (Figs. 2 and 3). All sediments under all treatments generally remained under nitrate/nitrite and iron (III) reduction conditions (Patrick and Jugsujinda 1992; Yu et al. 2007). Generally pH of the sediment slurries slightly lowered (< 1 pH unit) after 2-week incubation, and remained close to neutral conditions for all treatments (data not shown). After the incubation, redox potentials in the salt marsh and freshwater marsh sediments were lower than the initial values (Table 1). Surprisingly, the brackish marsh sediment slurries showed about 100 mV increase in redox potential from its initial value, making the mean Eh values in the brackish marsh sediment significantly higher ($P<0.05$) than the other two sediments. For the salt marsh and brackish marsh sediments, there was no significant difference in Eh values ($P>0.05$) among different treatments. The freshwater marsh sediment showed no significant difference in Eh values among the treatments in the first batch of the experiment (Fig. 2), but showed a significant difference ($P<0.05$) among the treatments in the second batch of the experiment (Fig. 3).

Benzene biodegradation dynamics during the incubation were illustrated in Figs. 4 and 5. It seems the coastal marsh sediments are ready for benzene degradation, resulting in most of the added benzene was consumed in two weeks. According

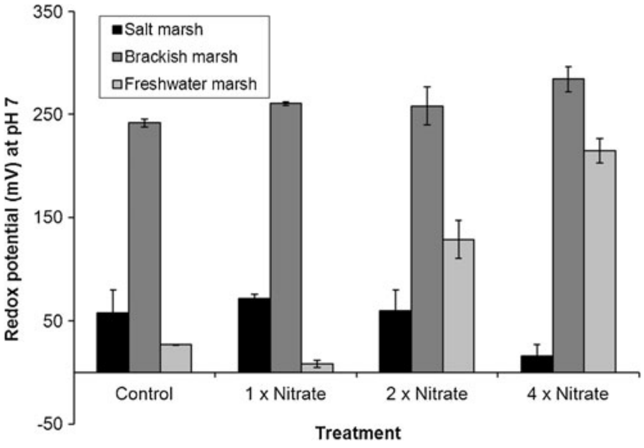

Fig. 2 Redox potential measurements at end of the incubation with different nitrate additions. Same amount of benzene (0.1 mL, 88 mg) was added to all treatments. For each sediment, additions of nitrate (1 M KNO_3) included Control (no nitrate), 1×Nitrate (0.1 mL, 1.4 mg N), 2× Nitrate (0.2 mL, 2.8 mg N), and 4×Nitrate (0.4 mL, 5.6 mg N). *Error bars* represents standard deviations of the replicates (n=3)

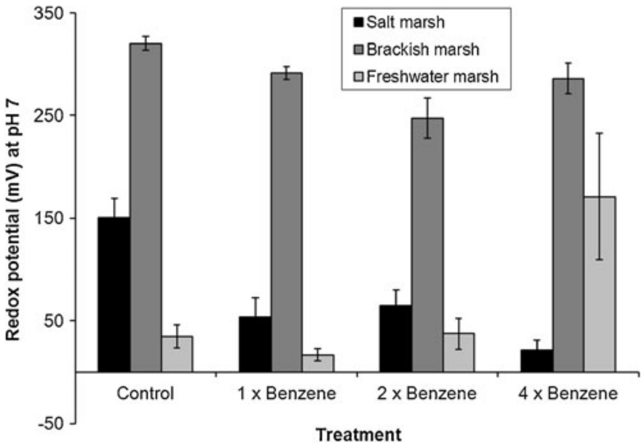

Fig. 3 Redox potential measurements at end of the incubation with different benzene additions. Same amount of nitrate (0.2 mL 1 M KNO_3, 2.8 mg N) was added to all treatments. For each sediment, benzene additions included 1×Benzene (0.05 mL, 44 mg), 2×Benzene (0.1 mL, 88 mg), and 4×Benzene (0.2 mL, 176 mg). *Error bars* represents standard deviations of the replicates (n=3)

to the patterns of benzene concentration decrease, it seems the assumption of first-order kinetics degradation is appropriate for this study. The results show distinctly different benzene degradation rates among different marsh sediments, but not among different treatments of the individual sediment.

Due to the difference in sediment water content, addition of the same amount of benzene and nitrate represents different quantities applied to the three marsh sediments in dry weight basis. Therefore, the benzene degradation rates among different sediments, which depend on the benzene concentrations in dry weight of the sediments, are not comparable. Thus it is an advantage of comparing the intrinsic first-order kinetics constants of benzene degradation among different sediments, because the constants are independent of benzene concentrations. Overall, benzene degradation in the marsh sediments showed the following order of activity with statistical significance ($P<0.05$), freshwater > brackish > salt, regardless of nitrate treatments (Fig. 6). This order of activity was not apparent ($P>0.05$) in the second batch of the experiment where different amounts of benzene were applied (Fig. 7). The results showed no statistical difference ($P>0.05$) in the benzene degradation under different nitrate additions in the salt and brackish marsh sediments. In the freshwater marsh sediment, however, higher dose of nitrate even showed some inhibition effect on benzene degradation activity (Fig. 6). Addition of different amount of benzene showed little effect on the first-order kinetics constants of benzene degradation in all sediments, except abnormally high degradation activity was found at the lowest benzene addition treatment (1×Benzene) in the brackish marsh sediment (Fig. 7).

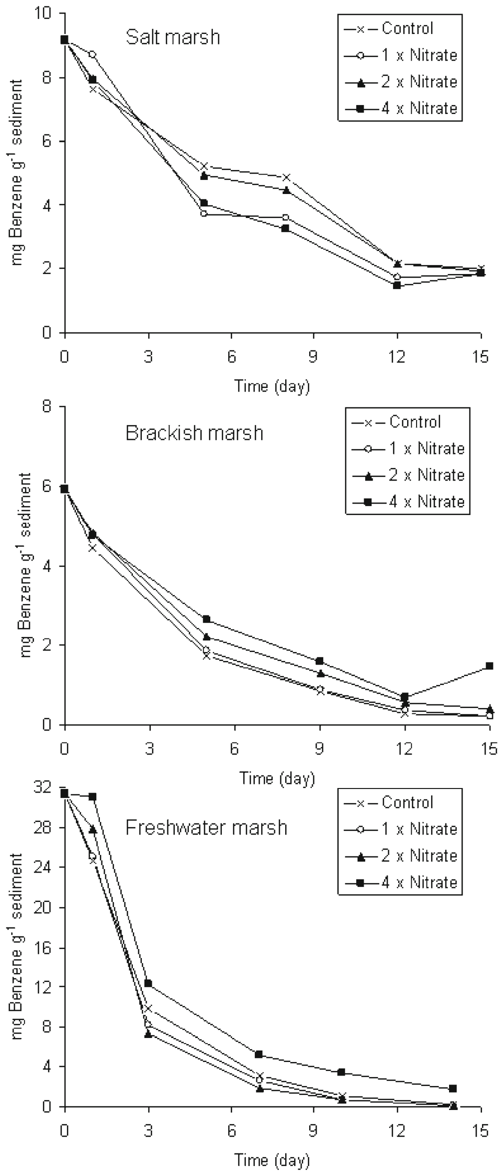

Fig. 4 Anaerobic degradation of benzene under different nitrate treatments. Same amount of benzene (0.1 mL, 88 mg) was added to all treatments. For each sediment, additions of nitrate (1 M KNO_3) included Control (no nitrate), 1×Nitrate (0.1 mL, 1.4 mg N), 2×Nitrate (0.2 mL, 2.8 mg N), and 4×Nitrate (0.4 mL, 5.6 mg N). Standard deviations of the replicates (typically<30 % of the means, n=3) are not denoted for clarity purpose

Discussion

Recovery of petroleum contaminated ecosystems through biodegradation can take weeks to decades depending primarily on geographical locations (NRC 2005). For a specific location, petroleum degradation rate is critical for the remediation process. Due to lack of oxygen, anaerobic degradation of petroleum is relatively slow and the highly toxic byproducts generated through degradation processes reside in the system for longer periods of time. This is particularly serious in water-

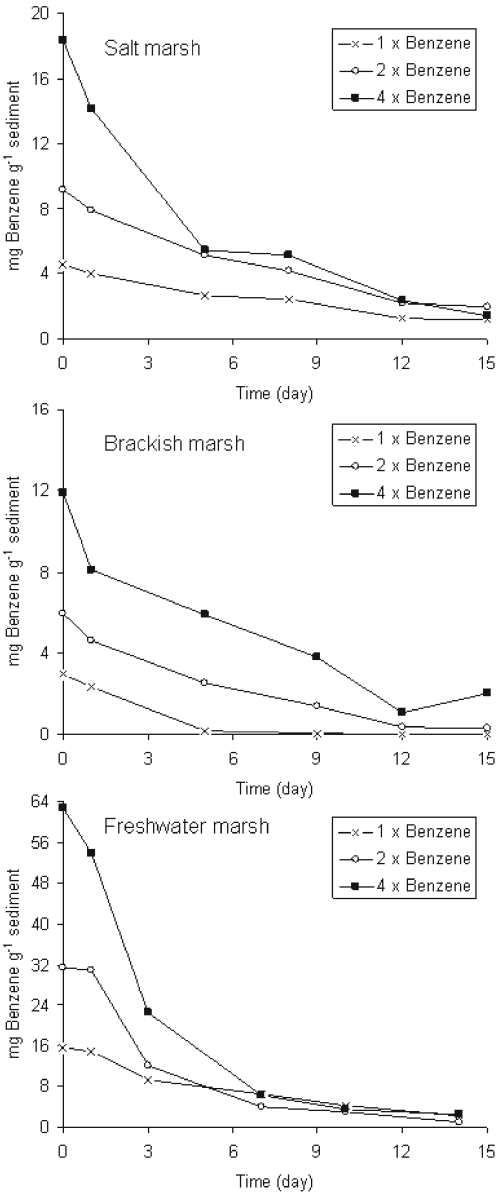

Fig. 5 Anaerobic degradation of benzene under the same nitrate treatment (0.2 mL 1 M KNO_3, 2.8 mg N). For each sediment, benzene additions included 1×Benzene (0.05 mL, 44 mg), 2×Benzene (0.1 mL, 88 mg), and 4×Benzene (0.2 mL, 176 mg). Standard deviations of the replicates (typically<30 % of the means, *n*=3) are not denoted for clarity purpose

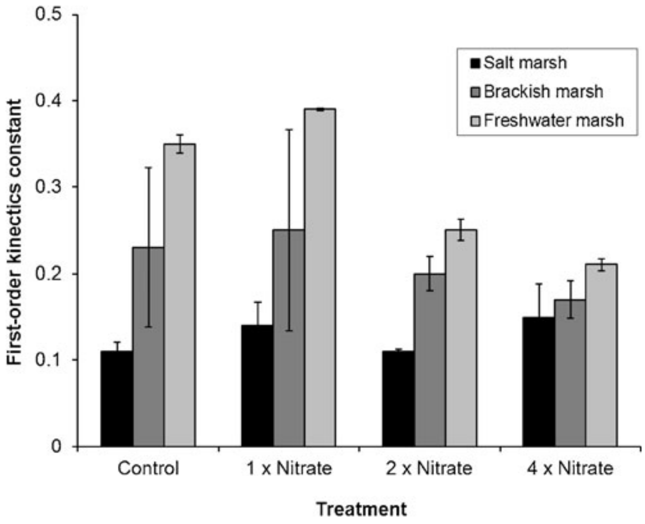

Fig. 6 Effect of nitrate addition on the first-order kinetics constant (day $^{-1}$) of anaerobic degradation of benzene. Same amount of benzene (0.1 mL, 88 mg) was added to all treatments. For each sediment, additions of nitrate (1 M KNO_3) included Control (no nitrate), 1× Nitrate (0.1 mL, 1.4 mg N), 2×Nitrate (0.2 mL, 2.8 mg N), and 4× Nitrate (0.4 mL, 5.6 mg N). Error bars represents standard deviations of the replicates (*n*=3)

saturated wetland ecosystems where toxic compounds can be transported by the movement of water within the system but are prevented from evaporating to the atmosphere due to the standing water layer. In the studied coastal marsh sediments, contents of redox active iron were two orders of magnitude higher than that of manganese. In moderately reducing conditions as in this study, most of the redox reactions occurred in nitrate/nitrite and iron (III) reduction range of the redox scale (Patrick and Jugsujinda 1992; Yu et al. 2007). A complete incubation of the salt marsh sediment verified that redox potential could be maintained in the iron reduction range for a month or so, after that it rapidly decreased to sulfate reduction range (data not shown). Due to the similarity in the characteristics of the three sample sediments, it is believed that iron reduction (moderately reducing as in this study) and sulfate reduction (strictly reducing in a prolonged incubation) are mainly responsible for the benzene degradation (as an electron donor). Marsh sediment profiles can be well stratified

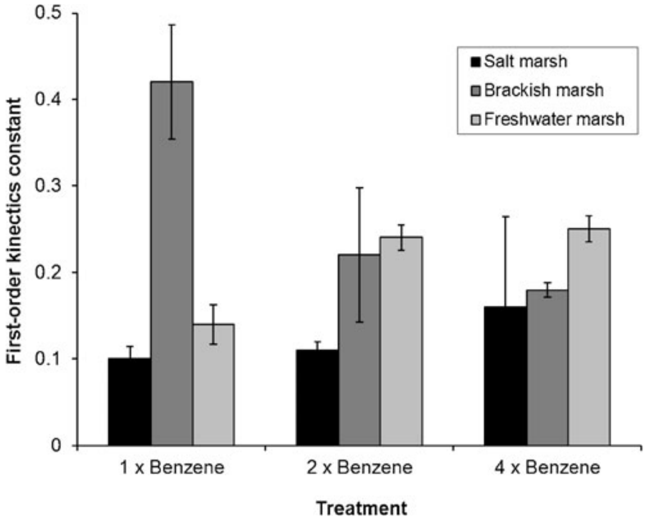

Fig. 7 First-order kinetics constant (day $^{-1}$) of anaerobic degradation of benzene under the same nitrate treatment (0.2 mL 1 M KNO_3, 2.8 mg N). For each sediment, benzene additions included 1× Benzene (0.05 mL, 44 mg), 2×Benzene (0.1 mL, 88 mg), and 4× Benzene (0.2 mL, 176 mg). Error bars represents standard deviations of the replicates (*n*=3)

in the field with moderately reducing conditions existing at upper layers of the sediments, while strictly reducing conditions at lower layers of the sediments (Pezeshki et al. 1991). Anaerobic biodegradation of benzene has been previously observed under iron reduction conditions (Coates et al. 1996; Caldwell et al. 1999), and sulfate reduction conditions (Weiner and Lovley 1998). Despite the high organic matter content in the studied sediments, benzene was still readily available as the electron donor for microbial metabolisms (Figs. 4 and 5). In all sediments, noticeable amounts of acetylene (C_2H_2), likely one of the byproducts of benzene degradation, were found during the incubation especially in treatments with higher benzene addition. Presence of C_2H_2 was not observed in the treatments without benzene addition, and at the initial measurement (starting time) after benzene addition. The water in the sediment slurries might somewhat prevent the C_2H_2 from contacting with the microbes in the sediments for further degradation. Some intermediate products of petroleum hydrocarbon biodegradation, such as quinones and phenols, are normally more toxic to organisms and more persistent in the environment (Teal and Howarth 1984).

Without introducing additional nitrate in the incubation, less redox potential decrease found in the salt marsh sediment (Eh from +173 mV to +58 mV) than in the freshwater marsh sediment (Eh from +333 mV to +27 mV) can be partially interpreted by the higher nitrate/nitrite and iron contents in the salt marsh sediment (Table 1; Fig. 2). It is unclear what caused the redox potential increase in the brackish marsh sediment during the incubation, but the highest nitrate/nitrite content is likely an important factor. This interpretation is supported by the higher redox potentials found at end of the incubation in the freshwater marsh sediment with higher addition of nitrate (Fig. 2). Due to the difference in water content of the sediments, same treatment of nitrate addition represents different dose in dry weight sediment. For example, the treatment of 1×Nitrate in the freshwater marsh sediment was about equivalent to the treatment of 4×Nitrate in the salt marsh sediment in dry weight basis.

It is worth to emphasize that addition of benzene in this study not only provided more organic matter (as an electron donor), but also potentially introduced additional toxicity (as a stressor) to the microbes that were responsible for the redox reactions in the sediments (Mueller et al. 2003). The coastal salt marsh sediment likely had a historic exposure to petroleum, since there are more than 1000 natural oil seepage locations reported in the Gulf of Mexico (Etkin 2009; Yu et al. 2012), while the brackish and freshwater marsh had less chance for previous petroleum exposure. The lack of previous exposure to petroleum may help to understand some unique behavior in the brackish and freshwater marsh sediment, especially when excessive benzene was added (Fig. 7). Since the results of 2×Nitrate and the treatment of 2×Benzene, as an internal reference, were fairly consistent ($P>0.05$), it is believe that the two batches of the experiments were comparable (Figs. 6 and 7).

When three replicates were calculated individually, the intrinsic benzene degradation activities (control treatment) in the studied sediments showed a clear inverse relationship ($R^2=0.83$, $n=9$) with the salinity as indicated by the chloride content (Table 1; Fig. 6). Inhibition of benzene degradation by high salinity was found in a study showing that the degradation rate was nearly 4 times lower when 5 M NaCl was added to non-saline sediments (Sei and Fathepure 2009). Increase of redox potentials by addition of nitrate even shows an inhibition effect on benzene degradation in the sediments. Intermediate products of denitrification (not monitored in this study), nitric oxide (NO) and nitrous oxide (N_2O), may contribute to the observed increase of redox potential (Lide, 1991). Abundance of NO and N_2O is very low in natural sediment, but could accumulate in this closed study system. Potential toxic effects from these intermediate products of denitrification may partially explain the negative correlations ($n=3$) between the end redox potentials in the sediments and the average benzene degradation kinetics constants in the salt ($R^2=0.28$), brackish ($R^2=0.51$) and freshwater ($R^2=0.96$) marsh sediments (Figs. 2 and 6). Unlike previous reported results (Cunningham et al. 2001; Xia et al. 2007), this study shows a minimal potential to stimulate the benzene degradation by addition of nitrate in these coastal marsh sediments. It is not clear whether the three marsh sediments were limited by N nutrient (Table 1). In fact, all the studied sediments are ready to release N during their organic matter mineralization processes as indicated by their low C/N ratio of 17 (salt and brackish marsh) and 15 (freshwater marsh). The effect of nitrite on benzene degradation is case dependent and concentration dependent (Burland and Edwards 1999; Wu et al. 2007; Dou et al. 2008). In most cases, nitrite accumulation is minor and temporal. In the studied marsh sediments, both nitrate and iron (III) likely play a dominant role in the anaerobic benzene degradation (Lovley 2000). In absence of oxygen, nitrate reduction occurs prior to iron (III) reduction according to their thermodynamic sequence (Patrick and Jugsujinda 1992). The reverse process is also valid that addition of nitrate would oxide ferrous iron (II) into ferric iron (III) (Straub et al. 1996). Thus, a mechanism of nitrate enhanced benzene degradation is likely due to regeneration of ferric iron (III) in anaerobic environment. In this experiment, iron (III) seems far from being depleted as indicted by the redox measurement (Figs. 2 and 3), which partially interprets the minimal effect of nitrate addition on benzene degradation.

Conclusions

Prompt recovery from petroleum contamination is crucial to maintain the ecosystem's integrity and to protect the public health. This study shows that sediment salinity is a good indicator (even without a clear mechanism) of the intrinsic benzene degradation potential under anaerobic conditions, which can be calculated as followings:

$$\text{First-order kinetics constant}\left(\text{day}^{-1}\right)$$
$$= -0.022 \times \text{salinity(ppt)} + 0.368\left(R^2 = 0.83,\ \text{n} = 9\right)$$

This study serves as an initial screening of the limiting factor that controls anaerobic biodegradation of petroleum hydrocarbons in the Gulf coast marsh sediments. In addition to salinity, total phosphorus (R^2=0.83), iron (R^2=0.82), Potassium (R^2=0.76), and manganese (R^2=0.76) all show strong correlation (n=9) with the benzene degradation constants (Table 1). It is not clear whether phosphorus is a limiting nutrient for the microbes responsible for benzene degradation (Table 1). Stimulation of hydrocarbons was found in a salt marsh by supplement of both phosphorus and nitrate, while nitrate alone did not stimulate the degradation (Wright et al. 1999). It deserves further studies to explore possible limiting factor(s) (such as phosphorus) and their interactions for biodegradation of BP oils in the Gulf coast environment. Despite the difference in the characteristics of the studied sediments, this study show little or minimal stimulation on benzene degradation by addition of nitrate in all the sediments. Excessive nitrate has already been causing near coast eutrophication in the Gulf of Mexico each year. Therefore, application of additional N to the coastal sediments is not recommended for the remediation of the 2010 BP oil spill.

Study of microbial community profiles and structures may unveil some insights of petroleum degradation processes. Interaction of petroleum with dispersant even further make the situation complicated. The degradation pathway of petroleum hydrocarbons/dispersant deserves careful studies to minimize the production of even more harmful byproducts during microbial metabolisms.

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