Effect of temperature and dispersant (COREXIT® EC 9500A) on aerobic biodegradation of benzene in a coastal salt marsh sediment

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HIGHLIGHTS

- Benzene degradation was 6 time faster under aerobic than anaerobic condition.
- Benzene degradation was in an order of $20\,°C > 10\,°C > 30\,°C$ in a saline environment.
- Dispersant shows an inhibitory effect on benzene degradation.

ARTICLE INFO

ABSTRACT

The coastal ecosystem in the northern Gulf of Mexico (GoM) has been seriously impacted by the 2010 BP oil spill. Two experiments were conducted to study the effect of temperature and addition of the dispersant on biodegradation of benzene, as a representative of petroleum hydrocarbon, in a coastal salt marsh sediment under aerobic conditions. The results show that benzene biodegradation was approximately 6 time faster under aerobic conditions (Eh > +300 mV) than under anaerobic iron-reduction conditions (+14 mV < Eh < +162 mV). Benzene biodegradation in response to temperature was in an order of $20\,°C > 10\,°C > 30\,°C$ as expected in a saline environment. Application of the dispersant caused initial fluctuations of benzene vapor pressure during the incubation due to its hydrophobic and hydrophilic nature of the molecules. Presence of the dispersant shows an inhibitory effect on benzene biodegradation, and the inhibition increased with concentration of the dispersant. The Gulf coast sediment seems in a favorable scenario to recover from the BP oil spill with an average temperature around $20\,°C$ in spring and fall season. Application of the dispersant may be necessary for the oil spill rescue operation, but its side effects may deserve further investigations.

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1. Introduction

The 2010 BP (British Petroleum) oil spill in the Gulf of Mexico (GoM) was unprecedented with approximately 4.9 million barrels of petroleum hydrocarbons released into the ocean (BP, 2010; NOAA, 2010). This is equivalent to spilling 0.5 mL crude oil on every square meter of the GoM (1.6 million km^2) that is larger than all five Gulf States combined (1.3 million km^2). In addition, approximately 7 million L of dispersants (mainly COREXIT EC9500A and some COREXIT EC9527A) were applied in response to the oil spill, among which 3 million L were allocated to the oil wellhead at the sea floor and 4 million L on the Gulf surface (National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2010). Initial survey shows that 1773 km of GoM shoreline was significantly oiled, of which 45% was coastal marsh (Michel et al., 2013). Louisiana alone accounts for 40% of the total wetlands in mainland US (Richardson and Pahl, 2006), and generates 30% of the nation's seafood production (Day et al., 2005). As a transition zone from terrestrial to marine ecosystems, coastal wetlands play a critical role in maintaining the ecosystem integrity of the Gulf of Mexico. However, presence of oil and dispersant and their degradation products provides additional stresses that affect the survival and ecological functions of the coastal wetlands. The environmental and human health impacts from this oil spill may take decades to unveil.

Recovery of ecological services from the coastal wetlands largely relies on a significant removal of oil toxicity. Bioremediation is the

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most effective treatment for removing oil pollutants, a major conclusion from a joint Exxon-USEPA-State of Alaska monitoring effort (Atlas, 1991). Both aerobic (Atlas, 1981) and anaerobic (Chakraborty and Coates, 2004; Phelps and Young, 2001) mechanisms have been studied for the biodegradation of petroleum hydrocarbons. During this process, complex petroleum compounds are degraded to volatile components, and further to gaseous end products of carbon dioxide (CO_2) and possibly methane (CH_4) (Masumoto et al., 2012). Aerobic oil degradation has been deemed more effective due to the presence of oxygen (O_2). Documented recovery of petroleum contaminated marsh is more rapid in the warmer Gulf coast than the boreal regions (Cross et al., 2003; Van Stempvoort et al., 2004). Even in the same region, seasonal temperature changes will have significant impacts on the efficiencies of oil biodegradation.

Using benzene as a representative petroleum hydrocarbon, we conducted two experiments to explore the potential of stimulating oil biodegradation in the Gulf coast marsh sediments under anaerobic conditions. The first study explored if a pre-exposure of benzene to a salt marsh sediment could stimulate biodegradation of benzene in a later-exposure (Yu et al., 2012). The second study explored the possibility of nitrate amendment (as a nutrient and an electron acceptor) to stimulate benzene degradation in coastal marsh sediments with a salinity gradient (Tao and Yu, 2013). Both attempts showed little improvement in benzene biodegradation in the marsh sediments. To complement existing knowledge, the objectives of this study were to study benzene biodegradation in a salt marsh sediment under aerobic conditions, and (1) the effect of temperature, and (2) the effect of oil dispersant.

2. Materials and methods

2.1. Sediment and oil dispersant sample

A salt marsh sediment sample (top 30 cm and composite from 5 locations) was taken in Louisiana coast near Lake Pontchartrain (N30° 08.78′, W89° 44.67'). Major plant species at the sampling site were *Spartina alterniflora* and *Spartina patens*. The composite sediment sample was put in an ice cooler and immediately shipped to the laboratory. The sample was stored in a refrigerator (4 °C) less than a month before the experiment. One-liter dispersant sample, COREXIT® EC 9500A, was obtained from Nalco Environmental Solution LLC (Sugar Land, TX) with agreement to use for this study. Brief physical and chemical characteristics of the sediment and

Table 1
Basic characteristics of the salt marsh sediment and dispersant.

Sample	Characteristics	Values
Salt marsh sediment		
	Texture	Silty Clay Loam
	Sand	11%
	Silt	57%
	Clay	32%
	Total carbon	7.2%
	Total nitrogen	0.43%
	Iron	19.91 g kg^{-1} dry sediment
	Manganese	0.12 g kg^{-1} dry sediment
	Sulfur	5.30 g kg^{-1} dry sediment
	Salinity	12‰ (ppt)
	pH	7.0
Oil dispersant		
	Distillates, hydro-treated light	10−30%
	Organic sulfonic acid salt	10−30%
	Propylene glycol	1−5%
	Density	0.95 kg L^{-1} at 15.6 °C.
	pH	6.2

dispersant are summarized in Table 1. More detailed characteristics of the sediment are available in previous publications (Yu et al., 2012; Tao and Yu, 2013).

2.2. Experimental setup

Two batches of experiment were conducted, one for studying the effect of temperature and the other for studying the effect of dispersant on benzene biodegradation in the sediment. For all experiments, a sediment slurry was made by mixing 40 g (wet weight) sediment and 80 mL water of the same salinity as at the sampling site. Saturated saline water (36 ppt, ‰) was made 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 experiments. The experimental unit was a 237-mL wide-mouth glass bottle with a screw cap sealed with Teflon tape to prevent gas leakage. A hole was drilled in the middle of the cap, and then a rubber stopper was installed for introducing benzene/dispersant to the sediment slurries and later for gas sampling from headspace of the bottle during incubation. All experimental treatments were prepared without replacing the headspace air in the bottles (note: headspace air can be replaced with pure nitrogen gas for an anaerobic incubation) to ensure that benzene biodegradation was under aerobic conditions. Approximately half of the incubation bottle as headspace volume provided sufficient O_2 (ambient concentration 21%) to support microbial aerobic respiration in a 4-week incubation.

For the experimental batch studying the temperature effect, 9 sediment slurry bottles were prepared for three temperature treatments (Temp - 10, Temp - 20, and Temp - 30) incubated in three separate water baths, and three replicates were applied for each treatment. The selected temperatures represent the mean air temperature in winter (10 °C), spring/fall (20 °C) and summer (30 °C) at the sampling location, respectively (Yu et al., 2008). After all bottles were capped, 1-mL benzene (>99.8% purity, analytical grade, Sigma-Aldrich Chemical Company) was injected to each sediment slurry using a micro-syringe through the rubber stopper of the bottle.

For the experimental batch studying the dispersant effect, 12 sediment slurry bottles were prepared. Four treatments with three replicates were applied, including Control (no dispersant), B:D = 10:1 (benzene: dispersant = 10:1), B:D = 20:1 (benzene: dispersant = 20:1), and B:D = 40:1 (benzene: dispersant = 40:1). For all treatments (including the batch for studying the temperature effect), the quantity of benzene applied was the same (1 mL). The sediment had 72% water content before the experiment. With 1 mL benzene (density 0.88 g mL^{-1}) addition, it is equivalent to 78.6 mg benzene per g dry sediment. Solubility of benzene in water varies slightly (Arnold et al., 1958) with temperature (about 1.8 g L^{-1} in a range of 10−30 °C). Approximately 20% of the added benzene was dissolved in the water phase of the sediment slurry, with the remaining 80% subject to be dispersed by the dispersant. A mixture of benzene and dispersant (10:1) was serially diluted to 20:1 and 40:1 and injected to each corresponding treatment using a micro-syringe. To monitor potential fluctuations of pH and redox potential (Eh) during the incubation and effect of the dispersant addition, another 12 bottles with the same four treatments were prepared. This is because uncovering the bottles for the pH and Eh measurements would unavoidably cause loss of benzene from the system. This experimental batch was conducted only under room temperature (20 °C).

All sediment slurries with different treatments were thoroughly mixed on a rotary shaker for 2 h to allow the system to reach equilibrium, and were incubated for approximately a month in the dark except for gas sampling and for pH/Eh measurement. Benzene
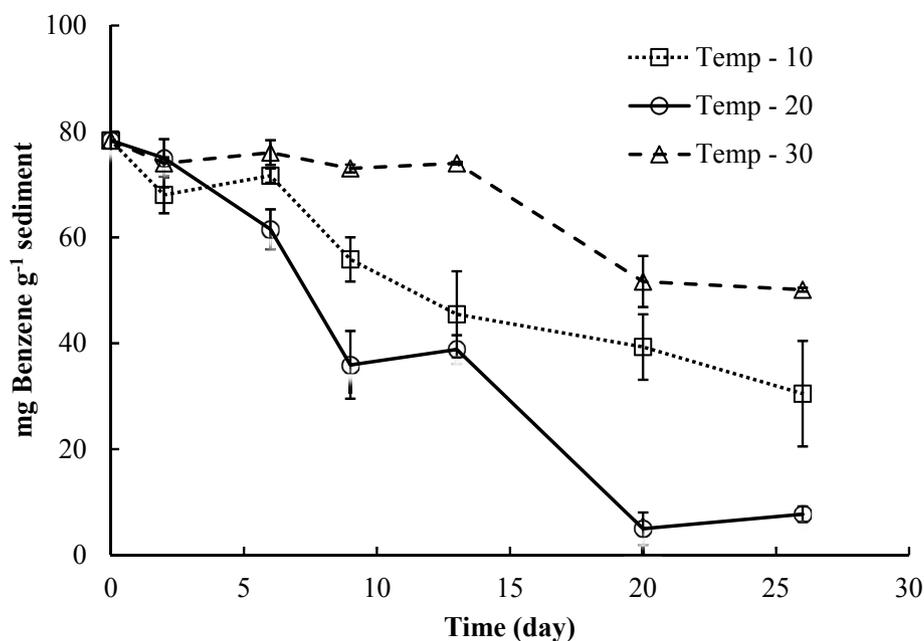
Fig. 1. Effect of temperature on benzene biodegradation in the sediment. Error bars represent standard deviations of the replicates (n = 3).

concentrations in the headspace of the bottles were monitored by taking 0.1 mL gas samples using a Luer-lok glass syringe through the rubber stopper. For pH and Eh measurement, the bottles were uncovered briefly and then were covered again and maintained at the room temperature (20 °C).

2.3. Sample analysis

Frozen sub-samples of the sediment were shipped to the Central Analytical Instruments Research Laboratory at Louisiana State University (LSU) for determination of major chemical and physical characteristics according to standard protocols with appropriate QA/QC (Yu et al., 2012; Tao and Yu, 2013). Benzene vapor pressure in the headspace was assumed to be in equilibrium with its liquid phase in the sediment slurry. Benzene concentration in the sample was quantified by using a Shimadzu GC-2014 gas chromatograph (GC) equipped with a Supel-Q PLOT fused silica capillary column (30 m × 0.32 mm) and a flame ionization detector (FID). The retention time for benzene in the GC was approximately 2.70 min with a total analysis time of 5 min for each sample. Helium (ultra high purity grade) was used as a 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 for the GC were 160, 150 and 200 °C, respectively. The pH and Eh measurements were made 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, after the readings stabilized in about 2 min.

2.4. Calculations and statistical analysis

All data are reported based on dry weight of the sediment. The redox potentials were calibrated to the standard H$_2$ electrode by adding the correction factor (+247 mV at 20 °C) for the calomel reference to the observed instrument reading (DeLaune and Reddy, 2005). Loss of benzene through sampling (0.1 mL each time) is negligible comparing to the headspace volume of the incubation bottle (approximately 120 mL). 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 t-test (significance level α chosen at 0.05).

3. Results and discussion

During the 4-week incubation, substantial benzene biodegradation occurred in the salt marsh sediment (Fig. 1). In this study, the sediment slurries were saturated with the added benzene (1 mL), making the benzene biodegradation at the maximum capacity. In a previous study using the same sediment and under the same temperature (20 °C), anaerobic (+14 mV < Eh < 162 mV for iron reduction) benzene biodegradation was estimated at the maximum rate of 0.24 mg g^{-1} d^{-1} (Yu et al., 2012). Under the aerobic conditions (for O$_2$ reduction) of this study, maximum benzene
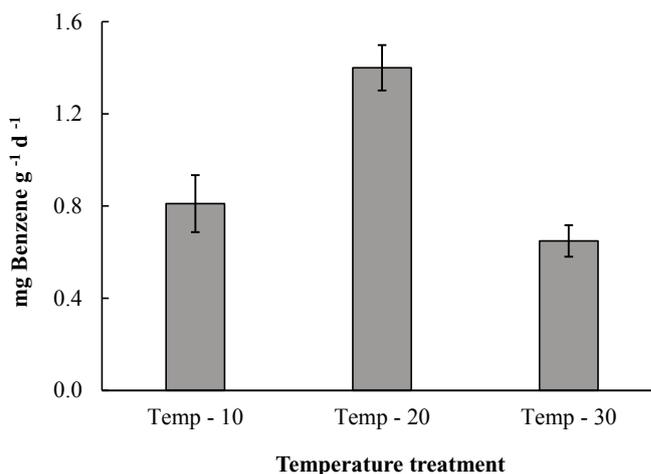

Fig. 2. Benzene biodegradation rate in the sediment under different temperatures. Error bars represent standard deviations of the replicates (n = 3). There was a significant difference ($p < 0.05$) between the Temp − 20 and the other two treatments, but no significant difference ($p > 0.05$) was found between the Temp − 10 and Temp − 30 treatments.
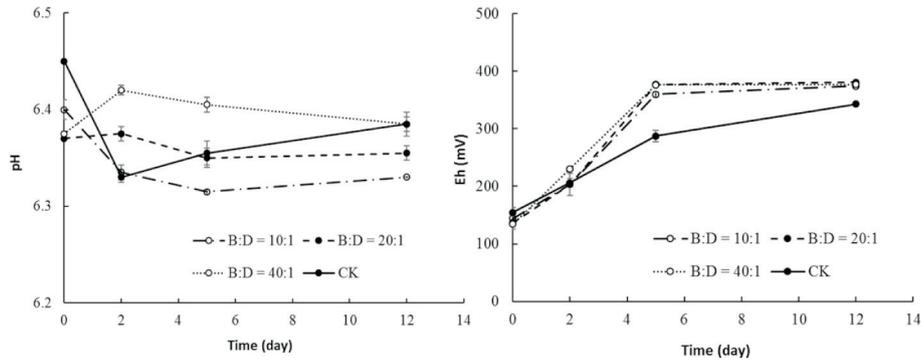
Fig. 3. Changes of pH and redox potential (Eh) in the sediment during the incubation (20 °C) under dispersant treatments. Error bars represent standard deviations of the replicates (n = 3 for pH, and n = 6 for Eh). For both pH and Eh, no significant difference was found among the four treatments ($p > 0.05$).

biodegradation rate reached 1.40 mg g^{-1} d^{-1} (Fig. 2), which was about 6 times higher than that under the anaerobic conditions. The results support the general conclusion that biodegradation of petroleum hydrocarbons is more efficient under aerobic conditions than under anaerobic conditions (Atlas, 1981).

The results clearly demonstrate that temperature plays an important role in benzene biodegradation (Figs. 1 and 2). Statistical analysis indicates that the benzene biodegradation rates were significantly higher ($p < 0.05$) at 20 °C than the other two temperature treatments, and were in an order of 20 °C > 10 °C > 30 °C (no significant difference between 10 °C and 30 °C). A commonly used Q_{10} value (Gibbs et al., 1975) cannot be obtained from this study, because the benzene biodegradation rates were not proportionally correlated with the temperature changes. The optimum temperature for hydrocarbon biodegradation depends on salinity in the environments. As reviewed by Das and Chandran (2011), maximum hydrocarbon degradation was found at 30−40 °C for soil environments, 20−30 °C for freshwater environments, and 15−20 °C for marine environments. The salt marsh sediment used in this study represents a resemblance to a marine environment. The reason is more likely due to the difference in microbial communities and

activities under different salinity conditions. The difference in solubility of hydrocarbons under different temperatures cannot interpret the temperature effects in this study, since the sediment was saturated with added benzene. The results show that the Gulf coastal marsh ecosystem is in a favorable scenario to combat with the BP oil spill with average temperature around 20 °C for half of the year (spring and fall), in which hydrocarbon biodegradation reaches the maximum in the salt marsh sediment.

Application of dispersant in the BP oil spill treatment is very controversial, and its effectiveness and environmental consequences remain uncertain. The dispersant used in this study was slightly acidic in nature (Table 1). A small fluctuation of pH and Eh was found during the first week of the incubation (Fig. 3), and there is no significant ($p > 0.05$) difference among the treatments (with and without the dispersant). Mixing of the sediment slurries facilitated O_2 in the headspace of incubation bottle dissolving in the water phase, as seen the Eh increase in early phase of the incubation. These fluctuations of pH and Eh tended to stabilize within two weeks, thus the monitoring was ended. Redox potential measurements verified that the sediment slurries were under aerobic conditions with Eh > +300 mV (Patrick and Jugsujinda, 1992), and
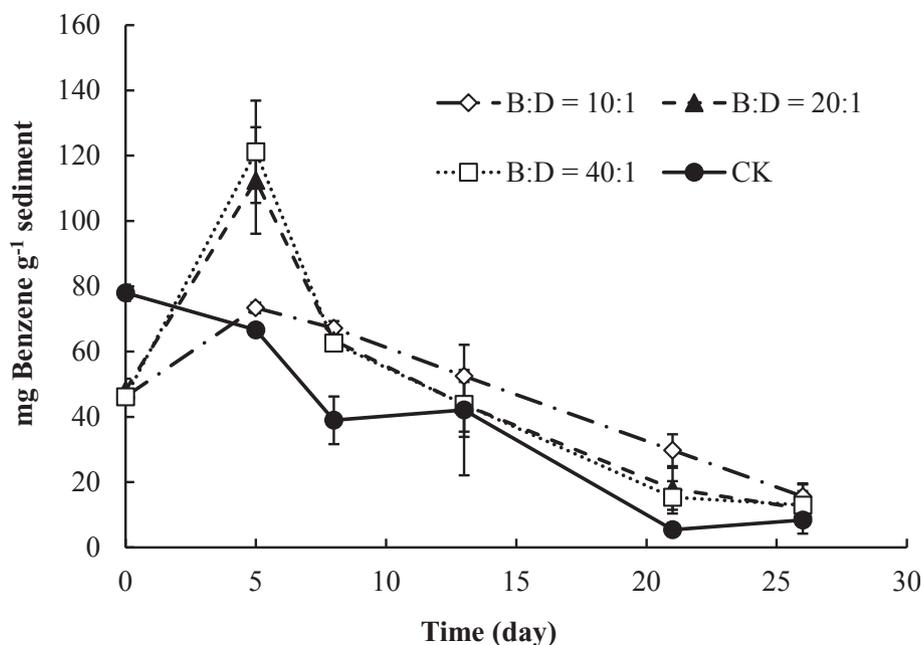

Fig. 4. Effect of dispersant on benzene biodegradation (20 °C) in the sediment. Error bars represent standard deviations of the replicates (n = 3).
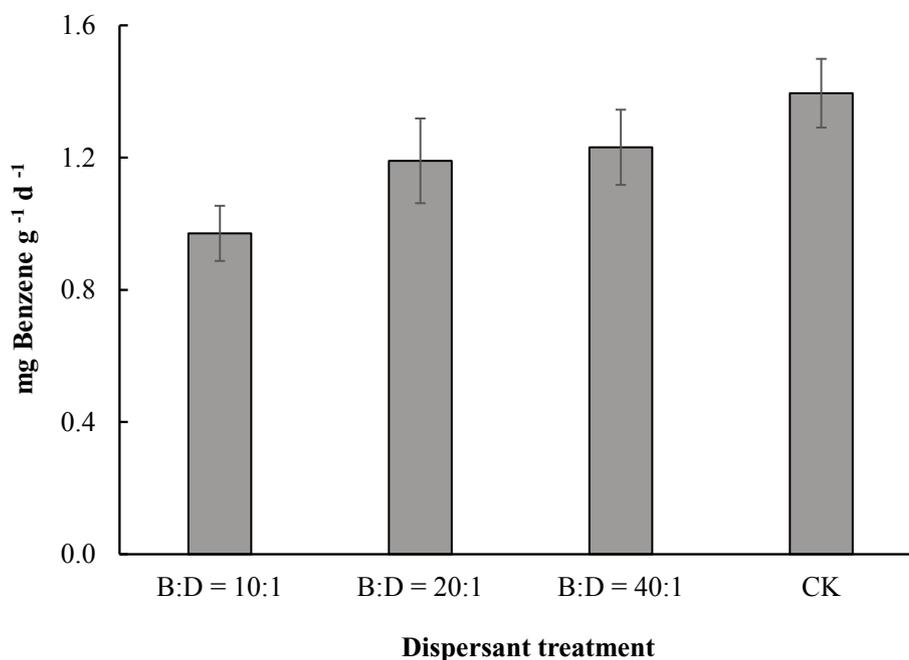
Fig. 5. Effect of dispersant on benzene biodegradation rate in the sediment. Error bars represent standard deviations of the replicates (n = 3). There was no significant difference among the four treatments ($p > 0.05$).

corresponding $O_2 > 10\%$, estimated by the correlation between Eh status and O_2 concentration (Yu et al., 2006).

Even the same amount (1 mL) of benzene was added to all the treatments, presence of the dispersant caused a large initial fluctuation of benzene vapor pressure in the headspace of incubation bottles (Fig. 4). In comparison to the control treatment without the dispersant (CK), addition of the dispersant caused a decrease of benzene vapor pressure at beginning of the incubation, and a spike a week later. The treatments without the dispersant showed a continuous decline of benzene vapor pressure in the headspace due to its biodegradation in the sediment (Figs. 1 and 4). The experiment was designed intentionally to add extra benzene with approximately 80% of the benzene not dissolved in the water phase of the sediment slurry. It seems that the dispersant interacted with the undissolved benzene in a dynamic hydrophobic and hydrophilic mechanism due to its amphiphilic molecular structure. At beginning of the dispersion process, the dispersant emulsified the undissolved benzene into smaller droplets that sunk into water column (John et al., 2016). This process increased the total benzene surface area, but actually resulted in less benzene vapor pressure in the headspace of the bottles, because the smaller benzene droplets were wrapped by the dispersant molecules by hydrophobic forces. On the other hand, due to the hydrophilic nature of the molecule, the dispersant eventually dissolved into the water phase of the sediment slurry, exposing the smaller benzene droplets to the surface environment that could cause a spike of benzene vapor pressure due to its larger surface area after dispersion. It seems that this dynamic dispersing process took place within a week or so, and would not repeat again in the rest of the incubation. Therefore, the benzene biodegradation rate in presence of the dispersant was estimated by using the data after a week to avoid the interference of large fluctuation of benzene vapor pressure in the headspace of the incubation bottles.

In a closed incubation system of this study, the dispersant remained in the system after the dynamic dispersing processes (Fig. 4), and its effect on benzene biodegradation rate in the sediment is summarized in Fig. 5. The results show that addition of the dispersant actually slightly inhibited the benzene biodegradation in this study with saturated substrate, in which dispersing the substrate would not contribute to the biodegradation by increasing the contact surface area with microbes. This inhibition effect of biodegradation increased with the dispersant concentration in benzene (Fig. 5), and shows a clear negative linear relationship:

Biodegradation rate $= -4.03 \times$ dispersant concentration in benzene $+1.37$ ($R^2 = 0.97$)

where benzene biodegradation rate is in mg g^{-1} d^{-1}, and the dispersant concentration in benzene is in percentage. No significant difference ($p > 0.05$) in benzene biodegradation rate was found among the four treatments (Fig. 5). From this regression equation, it can be estimated that the benzene biodegradation would be completely terminated when the dispersant:benzene ratio reached approximately 1:3 (34%), comparing to the control treatment with no dispersant. Similar inhibition effects of dispersant on oil biodegradation were reported in other recent studies on the BP oil spill (Rahsepar et al., 2016). The mechanism of inhibitory effect of the dispersant on benzene degradation is unknown and beyond the scope of this study.

4. Conclusions

Recovery of the services and functions in the Gulf coast ecosystem from the BP oil spill largely depends on effective biodegradation of petroleum hydrocarbons. In comparison with previous studies under anaerobic conditions (Yu et al., 2012), this study provides evidence to support that oil biodegradation is more efficient under aerobic conditions (Eh $> +300$ mV) than under anaerobic iron-reduction conditions ($+14$ mV $<$ Eh $< +162$ mV). The results show an encouraging finding that the oil biodegradation rate in the Gulf salt marsh sediment was significantly ($p < 0.05$) higher at 20 °C (average temperature for spring and fall of this region) than at 10 °C (winter) and 30 °C (summer). This response of oil biodegradation to temperature is favorable for a speedy removal

of the petroleum hydrocarbons from this oil spill event.

Application of oil dispersant may be necessary during the oil spill in order to make various rescue operations possible to stop the blow-out wells (National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2010). However, if the dispersant remains in the coastal marsh sediment with the petroleum hydrocarbons, it may not help the coastal ecosystem to recover from this oil spill event. The study clearly demonstrates an inhibitory effect of dispersant addition on biodegradation of benzene, as a model petroleum hydrocarbon. The Gulf coast environment seems quite adaptable to oil spill due to historical natural seepage in the region (Etkin, 2009; Mahmoudi et al., 2013), but is not used to exposure of the oil dispersant. Other harmful effects of the dispersant application may deserve further investigations (Almeda et al., 2014; Shi and Yu, 2014; Toyota et al., 2016).

Acknowledgements

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