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POTENTIAL FOR INTRINSIC AND ENHANCED CRUDE OIL BIODEGRADATION IN LOUISIANA'S FRESHWATER MARSHES

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Abstract: This study determined the intrinsic rates of biodegradation of Louisiana "sweet" crude oil (LSCO) in a *Panicum hemitomon* freshwater marsh using kinetic microcosm studies and verified the results in a large intact core study. In addition, the potential to enhance biodegradation using inorganic nutrient additions was determined. These freshwater marsh soils have high intrinsic rates of degradation (2.0%/day) for the measured alkane fraction (C11–C66) and even higher rates (6.8%/day) for the measured polycyclic aromatic hydrocarbon (PAH) fraction (naphthalene, methylated naphthalenes, phenanthrene, and methylated phenanthrenes). However, there were compound-specific effects with intrinsic rates of degradation highest for the smaller alkanes (C<15) (8.5–2.1%/day), while rates for longer chain alkanes (C>15) were much lower (0.7–1.2%/day). Results from the intact core study indicate that these rates are similar to those experienced *in situ*, with the exception of the PAH fraction, whose rate constants will be substantially lower than those determined in the kinetic study. Nitrogen (ammonium) was primarily the limiting nutrient and increased degradation rate constants (2–3 fold). Few differences were seen between different classes of alkanes after fertilization. Critical nitrogen loading rates (amount needed to produce significant degradation increases) were similar for both the microcosm and core study (2.2–8.8 mg NH_4^+-N/g oil), while maximum rates of degradation were observed at higher loading rates (22–44 mg NH_4^+-N/g oil). While crude oil degradation can be enhanced by fertilization, the benefits need to be weighed against the presence of high intrinsic biodegradation rates in these systems.

Key Words: freshwater marsh, biodegradation, crude oil, nutrients

INTRODUCTION

Freshwater marshes are important ecosystems providing valuable hydrologic and ecological functions on the landscape (Mitsch and Gosselink 1993). Louisiana contains a large portion of the near-coast freshwater marshes in the contiguous United States. These marshes are at risk of a large crude oil release from a variety of sources. Freshwater marshes are a major site of oil and gas production in the region and are crisscrossed by thousands of kilometers of oil pipelines, in addition to trans-shipment of oil in nearby waterways. Unlike many other ecosystems, physical cleanup methods are not an option due to the sensitivity of these marshes.

Biodegradation is now one accepted tool for the remediation of crude oil released into the environment. Extensive research has been conducted on the ability of nutrient additions to stimulate biodegradation of crude oil by native microorganisms (for a review, see Leahy and Colwell 1990). Most of this research has been confined to beach or terrestrial systems, with much of the literature resulting from the Exxon Valdez spill in Alaska. Recently, two studies have shown statistically the ability of nutrients to stimulate crude oil degradation on contaminated beaches (Bragg et al. 1996, Venosa et al. 1996). The few crude oil bioremediation studies conducted in marshes have primarily focused on coastal salt marshes (Lee and Levy 1993, Jackson et al. 1996, Jackson and Pardue 1997). These systems were found to have high inherent crude oil degradation rates as compared to other coastal systems, and degradation rates could be enhanced by nutrient additions. However, overall rates of degradation are probably dependent on both nutrient and oxygen availability (Swannell and Head 1994, Jackson et al. 1996). Little information is available on the potential of freshwater marshes to degrade crude oil under intrinsic or fertilized conditions. A study investigating seasonal variations in crude oil degradation in coastal freshwater marshes found a high potential for intrinsic crude oil degradation, with rate enhancement after fertilization for certain seasons and compounds (Jackson and Pardue 1997).

Louisiana freshwater marshes are characterized by near constant flooded conditions, with long periods of inundation and less frequent periods of partial drying. The marsh soil is a highly organic peat (>80%) with a large surface area per gram soil, and nutrient conditions are eutrophic, although much of the nutrient content is unavailable (Jackson and Pardue 1997). The aerobic layer is quite small (1–5 cm) due to the high organic content, with most activity dominated by anaerobic processes. Once the oil penetrates into the anaerobic layer, further degradation of the majority of its components is much slower (Delaune et al. 1990, Lee and Levy 1993). Nitrogen fixation, internal recycling, and input of N-laden flood waters are the primary sources of nitrogen; in general, these marshes have low-levels of bioavailable N (Delaune et al. 1986, Feijtel et al. 1989). Addition of fertilizers to Louisiana freshwater marshes stimulated macrophyte primary production, suggesting N-limitation (DeLaune and Lindau 1990). In this study, very little ^{15}N was lost from the peat due to incorporation of NH_4^+-N into soil organic matter. Louisiana's freshwater marshes are less efficient at phosphorus retention than other wetlands. Flooded soils are phosphate-deficient, although the microbial community is capable of short-term storage of incoming phosphorous (Masscheleyn et al. 1992).

This study was conducted to investigate the potential for intrinsic and nutrient-enhanced crude oil biodegradation in freshwater marshes in Louisiana. The effect of nutrient amendments on the kinetics of crude oil degradation was also investigated. Crude oil degradation was investigated both in laboratory microcosms and in an intact-core study. Both studies used a "sweet" Louisiana crude oil (SLCO), which is a relatively non-toxic oil with high alkane, low polar, and moderate polycyclic aromatic hydrocarbon (PAH) concentrations. Information produced by this study is useful in developing remediation strategies and highlighting the potential role of intrinsic degradation or natural attenuation of crude oil biodegradation.

METHODS AND MATERIALS

Site Description and Sampling

The site location was near Lake Salvador at the northern end of the Barataria Basin in Louisiana. The site is an intermittently flooded freshwater marsh, with a seasonal water-level flux of approximately −5 to +70 cm, and is dominated by *Panicum hemitomon Schult.* Samples for microcosm studies were taken with a thin-walled aluminum core (15-cm diameter) and transported to the laboratory in an upright position. The upper 5 cm of the core was removed and used to construct slurries for biodegradation experiments. Samples for intact core studies were removed using a machete to cut out sections of the marsh (900 cm^2 and 20 cm in depth). Samples were immediately transferred to glass aquaria in the field and brought to the greenhouse. The sides of the aquaria were darkened by aluminum foil to prevent algal growth.

Laboratory Microcosm Kinetic Studies

Crude oil biodegradation kinetics experiments were conducted in completely mixed microcosms (Masscheleyn et al. 1992). The microcosms contained 1.8 liters of a 40:1 (water/soil) (w/w) slurry produced from surface soils (0–5 cm) obtained from the study site. Microcosms were amended with unweathered SLCO at a concentration of 0.7 g oil/g soil. This is approximately equal to a moderate oil spill loading rate. Microcosms were operated in a completely aerated mode with air flow greater than 15 ml/min. Three treatments were monitored: microbially-inhibited (2 g NaN_3/flask), unamended (no fertilizer amendment), and ammonium and phosphate amended (16 mg-NH_4^+-N and 5.0 mg-KH_2PO_4-P/g soil). All treatments were conducted in duplicate. Late in the experiment (at 40 days), both unamended treatments were fertilized. Unamended 1 and 2 treatments received 16 mg NH_4^+-N and 5.0 mg KH_2PO_4-P/g soil and 1.6 mg NH_4^+-N and 0.5 mg KH_2PO_4-P/g soil, respectively. Temperatures in the microcosms were 25±2°C.

The microcosms were sampled for oil components by removing 5 ml of slurry, which was extracted with 1:1 (V/V) hexane:acetone (Jackson et al. 1996). The phases were separated using centrifugation, and the extracts were passed through anhydrous Na_2SO_4 to remove residual water. Finally, the samples were concentrated under a stream of dry nitrogen to a suitable volume for analysis. The oil extracts were analyzed by GC-MS using 17α,21β-hopane as a normalizing compound (Prince et al. 1994). Using hopane as a normalizing compound (i.e., expressing the results as the ratio of the measured compound to hopane concentration) allows biodegradation to be separated from other loss processes. Alkanes C11–C66, pristane, and parent C1 and C2 naphthalenes and phenanthrenes were monitored. In addition to the transformation of oil, ammonium concentration was monitored throughout the experiment using an ion-selective electrode.

Biodegradation data were fitted to a first-order degradation equation:

$$C/C_o = A + B(e^{-kt})$$

where C = hopane ratio at time t (days), C_o = initial hopane ratio, A = fraction of hopane ratio that does

not degrade (i.e., the asymptote), B = the fraction of hopane ratio that degrades ultimately, and k = first order degradation rate (day^{-1}). First-order rate constants were expressed as %/day after multiplying (k) by (B*100). The equation was fit using non-linear regression using the Marquardt algorithm.

Intact Core Studies

Additional studies used large box cores removed intact from the field and transferred to glass aquaria, which were subsequently placed in the greenhouse. Water levels in the aquaria were adjusted to approximately the level of the soil surface using water from the site. The cores were contaminated with 113 mg oil/cm^2. This concentration is indicative of a light-to-moderate oil spill. Five cores were used as an unamended control, and five cores each received phosphate (0.5 mg P-K_2HPO_4/cm^2) and a range of ammonium nitrogen (0.1, 0.5, 1.0, 5.0, and 10.0 mg NH_4^+-N/cm^2). A beaker with a thin layer of oil (5 cm) was placed alongside the aquaria and allowed to weather in the greenhouse under the same conditions. At the end of 4 weeks, the mesocosms were cored using 15.0-cm-diameter, thin-walled aluminum cores. Approximately 20% of the surface area of each treatment was sampled by removing the top 5 cm of soil and homogenizing. Subsamples were taken and mixed with $MgSO_4$ to reduce the water content. The oil was extracted using supercritical fluid extraction with unmodified CO_2 (flow rate = 11 ml/min; extraction time = thirty minutes; oven temperature = 100°C; restrictor temperature = 175°C; and a collection solvent (dichloromethane) held at 4°C). This extraction procedure had a recovery of 85–95% depending on the compound (Jackson et al. 1997). Oil analysis on the extracts was conducted as in the microcosm study.

RESULTS AND DISCUSSION

Kinetic Study

The freshwater marsh demonstrated a substantial capacity to degrade the alkane fraction of crude oil. Microcosm studies showed rapid and nearly complete (greater than 90%) degradation of parent alkanes (C11–C66) in the fertilized (phosphate and ammonium) treatments (Figure 1A). Unamended microcosms also showed substantial (60%) degradation of the alkane fraction (Figure 1B). Microbially inhibited controls did not demonstrate large reductions in hopane ratios (Figure 1C). First order rates of degradation of the summed alkane fraction (C11–C66) for the nutrient enhanced treatments were more than double those of the unamended, 5.8% day^{-1} for the ammonium and phosphate fertilized versus 2.0% day^{-1} for the unamended treatment (Table 1), which is significantly different ($p < 0.05$).

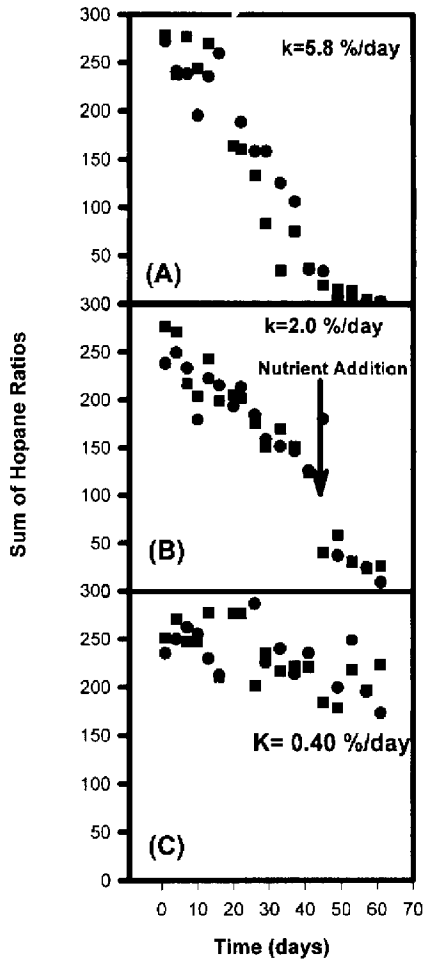

Figure 1. Total alkane degradation in freshwater marsh soil (aerobic slurries) under ammonium- and phophate-amended (A), unamended (B), and microbially inhibited (C) conditions. Nutrients were added to unamended treatments on day 41.

The cumulative rate of PAH degradation in the marsh microcosms was higher than the alkane rate (Figure 2A). High PAH degradation rates have also been observed in other studies involving both freshwater and salt marshes (Jackson et al. 1996, Jackson and Pardue 1997). Significant decreases in the hopane ratios of the microbially inhibited treatment were observed and are attributable to the unsubstituted, parent naphthalene compound, which is volatile (Figure 2B). Additions of phosphate and ammonium enhanced the rates of degradation (Figure 2C). The first order rate constant of the fertilized treatment (13.3 day^{-1}) was statistically higher ($p<0.05$) than the unamended treatment (6.8 day^{-1}) using a paired t-test (Table 1).

To further determine the possibility of stimulating

Table 1. Pseudo-first order degradation rate constants (%/day) of crude oil in microcosm.

Treatment	Alkane Chain Length												Sum	
	11	12	13	14	15	16	17	18	20	30	40	44	66	Alkane	PAH
Killed	3.1	1.3	0.42	0.37	0.64	0.15	0.30	0.30	0.15	0.09	0.20	0.05	0.01	0.40	3.2
(StdV.)	(0.2)	(0.10)	(0.03)	(0.10)	(0.60)	(0.08)	(0.02)	(0.01)	(0.06)	(0.09)	(0.15)	(0.01)	(0.02)	(0.10)	(0.4)
Control	8.5	3.9	1.80	2.10	0.84	0.72	0.87	0.70	1.6	1.2	1.1	1.40	N/A	2.0	6.8
(StdV.)	(0.08)	(1.20)	(0.30)	(0.20)	(0.06)	(0.01)	(0.30)	(0.01)	(0.50)	(0.50)	(0.50)	(1.90)		(0.30)	(0.60)
Ammonium and Phosphate	10.1	6.4	7.5	6.6	6.2	6.5	7.5	4.9	7.1	6.6	7.3	6.1	6.00	5.8	13.3
(StdV.)	(0.90)	(1.30)	(0.30)	(1.10)	(1.20)	(0.40)	(0.80)	(0.30)	(0.30)	(1.40)	(1.20)	(2.30)	(0.80)	(0.80)	(4.70)

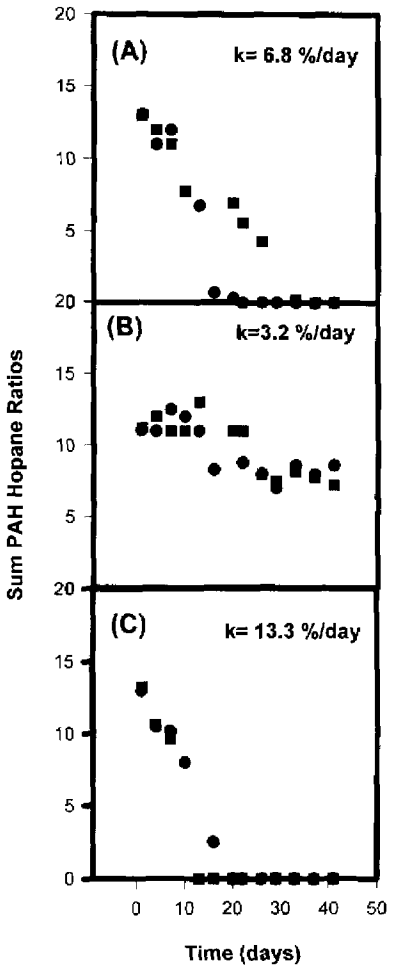

Figure 2. Total PAH degradation in freshwater marsh soil (aerobic slurries) under unamended (A), microbially inhibited (B), and ammonium- and phosphate-amended (C) conditions. Nutrients were added to unamended treatments on day 41.

crude oil degradation by nutrient addition, both phosphate and ammonium were added to the unamended treatments on day 41. Both concentrations were approximately equally effective at increasing the degradation rates of the alkane fraction (first order degradation rates of unamended treatment 1 = 8.2%/day and unamended treatment 2 = 6.2%/day), providing supporting evidence of the ability of nutrients to enhance the alkane degradation in a freshwater marsh after a spill. The PAH fraction was already nearly completely degraded, so no data were available.

The two- to three-fold increase in degradation rate achieved by nutrient addition is substantial and is supported by previous seasonal studies (Jackson and Pardue 1997). However, the effectiveness of nutrient additions is more clearly shown by the examination of the degradation rates of individual components of the alkane fraction. The unamended microcosms had high

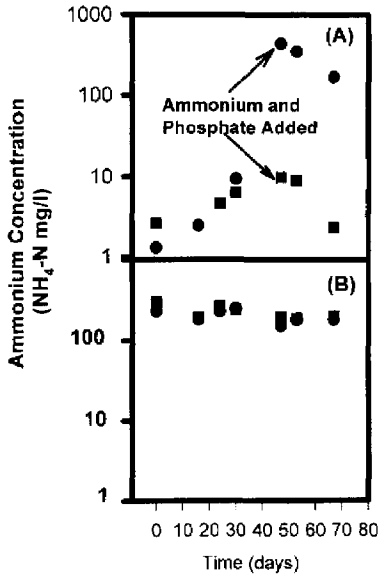

Figure 3. Ammonium concentrations in porewater of aerobic slurries under unamended (A) and ammonium- and phosphate-amended (B) conditions. Ammonium and phosphate added to unamended treatments on day 41.

rates of degradation (ranging from 2–8 %/day) of the smaller-chain alkane fraction C11–C14. Higher chain length alkanes had comparatively lower rates of degradation (0.7–1.6 %/day) in unamended microcosms. After fertilization, however, there was little difference in rate constants between the various alkanes. For alkanes C15 and greater, the increase in degradation rate constants from fertilization was 5–7 fold. Clearly, a greater benefit for fertilization is seen from the more persistent, longer chain alkanes. The persistence of this fraction of crude oil in the absence of nutrient additions for salt marsh systems has been previously documented for salt marshes (Lee and Levy 1993).

The level of fertilization necessary for stimulation of crude oil degradation in freshwater marshes is of interest. Figure 3 presents the measured porewater concentrations of ammonium throughout the experiment. Porewater ammonia concentrations have been hypothesized to be a critical variable in stimulating biodegradation after oil spills (Bragg et al. 1994). Addition of nutrients increased porewater ammonium concentrations, as expected (Figure 3). The increasing ammonium concentrations in the unamended treatments over time (Figure 3A) is probably due to mineralization of organic matter in these closed microcosms.

Previous studies have shown that while these near-coast freshwater marshes contain large amounts of total nitrogen, most is unavailable (DeLaune et al. 1986). Nitrogen limitation of macrophyte primary production in these systems has been documented (DeLaune and Lindau 1990). A seasonal build-up of ammonia in the winter has been seen in several studies (Sasser et al. 1991, Jackson and Pardue 1997). Ammonia concentrations remain low during the plant growing season (spring and early summer), as it is rapidly taken up by plants. In a previous study, fertilization enhanced the mineralization of hexadecane (C16) and phenanthrene throughout the year but much less than in a salt marsh within the same basin (Jackson and Pardue 1997).

Intact Core Study

In order to study how freshwater marsh systems respond to crude oil degradation in a more realistic manner, an intact core study was initiated, which was designed to minimally disturb the marsh soil. In particular, the applicability of rate constants determined in microcosm studies to this more realistic study were of interest.

Substantial degradation for all compounds except pristane occurred in all treatments compared to a weathered control oil sample (Figure 4). The unamended cores showed fairly uniform degradation (60–70% remaining after 4 weeks). In addition, the standard deviations were fairly small for this type of data (~20%) and are indicative of limited, small scale variation of crude oil degradation in the marsh. The percent reduction in crude oil hopane ratios was consistent with the calculated unamended rates (1–2%/day) from the microcosm study. In addition, similar class-specific effects were observed (i.e., higher rates of degradation with lower alkane length). Interestingly, the degradation of the PAH fraction in the unamended cores was approximately equal to the alkane degradation, indicating that the high intrinsic PAH degradation rates found in the kinetic study may not be observed in the field. One potential explanation is the lower mixing energies that may reduce the availability of these compounds.

Fertilization had a significant impact on crude oil degradation in the intact cores. Nitrogen additions of 1 mg/cm^2 and higher greatly increased the total degradation that occurred for all compounds (alkanes and PAHs). Even pristane, which was not significantly degraded in the unamended treatments or low fertilizer treatments (0.1–0.5 mg/cm^2) was substantially degraded in the higher ammonium loading rates (1–10 mg/cm^2) treatments. In general, over 80% degradation occurred in the monitored compounds for the two highest loading rates. This is consistent with the calculated rates from the microcosm kinetic experiment in this nutrient range over this time scale. The optimum nitrogen fertilization rate seems to be approximately 5 mg/cm^2. This loading rate produced an equal degradation rate as achieved by the 10 mg/cm^2 loading rate

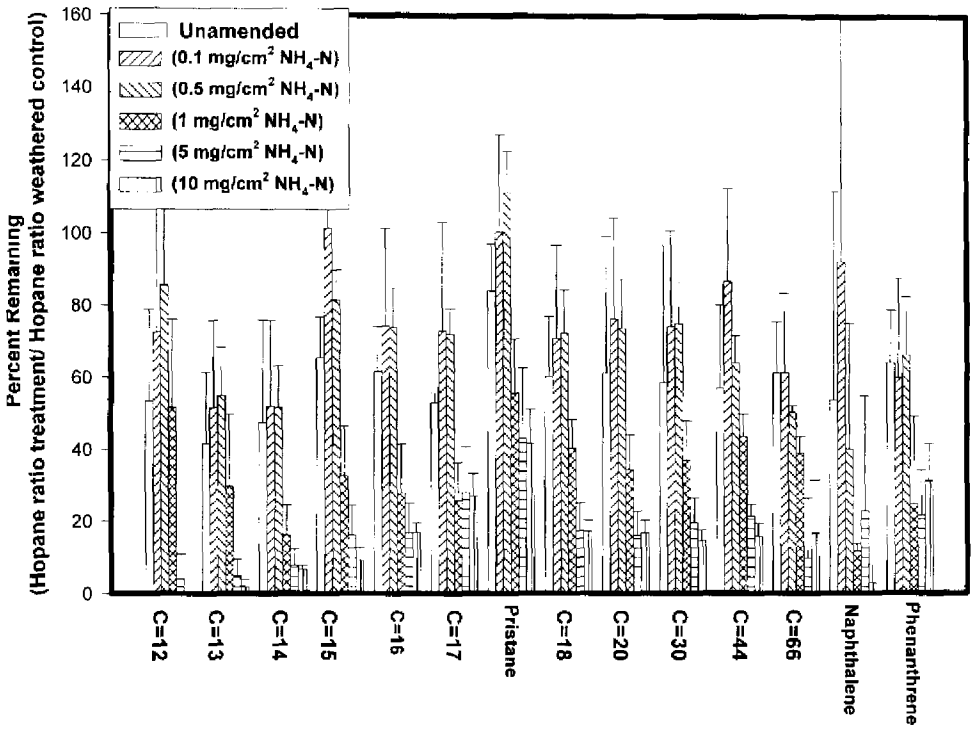

Figure 4. Percent remaining (as compared to a weathered control) of crude oil components after 4 weeks in intact box cores from a freshwater marsh.

but was substantially higher than the 1 mg/cm^2 loading rate, the lowest loading rate in which any enhancement was observed.

If it is decided that nutrient enhancement is desirable, then remediation efforts will need to be tailored for this marsh. Nitrogen in some form (ammonium) is a necessity. Nitrate or other oxidized nitrogen forms would be unlikely suitable fertilizers, given the low sorption rate (high wash out potential) and their large potential loss through dentrification. Oleophilic formulations are probably unnecessary given the high sorption rates of ammonium on organic matter and the potential for the formulation to cause further oxygen depletion. Researchers have also shown that a critical concentration of nitrogen is required for biostimulation to be successful (Bragg et al. 1994). Loading rates below this concentration will be a waste of resources, while overfertilization may cause secondary problems such as algal blooms. Actual optimum loading rates will depend on a number of factors, such as the height of water on the marsh, time of year, and exact sorption characteristics of the amendment. The kinetic study suggests that a critical concentration of 2.2 mg N/g oil is needed to see a significant biostimulation effect. This is of the same order of magnitude as the minimum loading rate that produced a significant effect in the core study (8.8 mg N/g oil). Maximizing degradation rates would require 2 to10-fold greater ammonium loading rates, although more studies would be needed to compare the benefit-to-cost ratio of high dose single application versus repeated low dose applications.

CONCLUSIONS

Freshwater marshes have a high inherent degradation potential for many crude oil components and seem to respond well to nutrient enhancements. A two- to three- fold increase in degradation is achievable by the addition of the proper nutrients: nitrogen and, less importantly, phosphate. However, the benefits of degrading the oil at 2 to 3 times the intrinsic rate will need to be weighed against the cost. Near-coast freshwater marshes accrete at a high rate, and the oxidized layer is small (1–5 cm). If the oil is buried or moved to an anaerobic zone, it is likely that the degradation rate of crude oil would slow considerably (Hambrick et al. 1980, Delaune et al. 1990, Lee and Levy 1993). Degradation of many crude oil components has been demonstrated under anaerobic conditions, although long lag times are involved (Coates et al. 1996). In addition, while this study has shown the potential for freshwater marshes to degrade the alkane and the measured PAH fraction, some of the more recalcitrant and toxic compounds (e.g., the 4-ring PAHs) may be stable under intrinsic conditions. A risk assessment would determine if these components are present at high enough levels to pose a threat to system biota.

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LITERATURE CITED

Bragg, J. R., R. C. Prince, E. J. Harner, and R. M. Atlas. 1994. Effectiveness of bioremediation for the Exxon Valdez spill. Nature 368:413–418.

Coates, J. D., R. T. Anderson, and D. R. Lovley. 1996. Oxidation of polycyclic aromatic hydrocarbons under sulfate reducing conditions. Applied Environmental Microbiology 62:1099–1101.

DeLaune, R. D., R. P. Gambrell, J. H. Pardue, and W. H. Patrick, Jr. 1990. Fate of petroleum hydrocarbons and toxic organics in Louisiana coastal environments. Estuaries 13:72–80.

Delaune, R. D., C. J. Smith, and M. N. Sarafyan. 1986. Nitrogen cycling in a freshwater marsh of *Panicum hemitomon* on the deltaic plain of the Mississippi River. Journal of Ecology 74:249–256.

DeLaune, R. D. and C. W. Lindau. 1990. Fate of added ^{15}N labelled nitrogen in a *Sagittaria lancifolia L.* Gulf Coast marsh. Journal of Freshwater Ecology 5:429–431.

Feijtel, T. C., R. D. DeLaune, and W. H. Patrick, Jr. 1989. Carbon, nitrogen and micronutrient dynamics in Gulf Coast marshes. p. 47–60. *In* R. R. Sharitz and J. W. Gibbons (eds.) Freshwater Wetlands and Wildlife. Proceedings of the International Symposium on Ecology and Management of Wetlands. PUBLISHER, Charleston, SC, USA.

Hambrick, G. A. III, R. D. DeLaune, and W. H. Patrick, Jr. 1980. Effect of estuarine sediment pH and oxidation-reduction potential on microbial hydrocarbon degradation. Applied Environmental Microbiology 40:365–369.

Jackson W. A. and J. H. Pardue. 1997. Seasonal variability of crude oil respiration potential in salt and fresh marshes. Journal of Environmental Quality 26:1140–1146.

Jackson W. A., J. H. Pardue, and R. Araujo. 1996. Monitoring crude oil mineralization in salt marshes: Use of stable carbon isotope ratios. Environmental Science and Technology 30:1139–1144.

Leahy, J. G. and R. R. Colwell. 1990. Microbial degradation of hydrocarbons in the environment. Microbiological Reviews 54: 305–315.

Lee, K. and E. R. Levy. 1993. Bioremediation: waxy crude oils stranded on low energy shorelines. p. 541–547. *In* Proceedings 1993 International Oil Spill Conference. Washington, DC, USA.

Masscheleyn, P. H., J. H. Pardue, R. D. Delaune, and W. H. Patrick, Jr. 1992. Phosphorous release and assimilatory capacity of two lower Mississippi valley freshwater wetland soils. Water Resources Bulletin 28:763–773.

Mitsch, W. J. and J. G. Gosselink. 1993. Wetlands. 2nd edition. Van Nostrand Reinhold Company, New York, NY, USA.

Prince, R. C., E. L. Elmendorf, J. R. Lute, S. H. Chang, E. H. Copper, J. D. Senius, G. J. Dechert, G. S. Douglas, and E. L. Butler. 1994. Hopane as a conserved internal marker for estimating the biodegradation of crude oil. Environmental Science and Technology 28:142–145.

Sasser, C. E., J. G. Gosselink, and G. P. Shaffer. 1991. Distribution of nitrogen and phosphorus in a Louisiana freshwater floating marsh. Aquatic Biology 41:317–331.

Swannell, R. J. and I. M. Head. 1994. Bioremediation comes of age. Nature 368:396–397.

Venosa, A. D., M. T. Suidan, B. A. Wrenn, K. L. Strohmeier, J. R. Haines, B. L. Eberhart, D. King, and E. Holder. 1996. Bioremediation of an experimental oil spill on the shoreline of Delaware Bay. Environmental Science and Technology 30:1764–1775.

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