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Nutrient and Oxygen Concentrations within the Sediments of an Alaskan Beach Polluted with the *Exxon Valdez* Oil Spill

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Measurements of the background concentrations of nutrients, dissolved oxygen (DO), and salinity were obtained from a beach that has oil from the *Exxon Valdez* oil spill in 1989. Two transects were set across the beach, one passed through an oil patch while the other transect was clean. Three pits were dug in each transect, and they ranged in depth from 0.9 to 1.5 m. The DO was around 1.0 mg L^{-1} at oiled pits and larger than 5 mg L^{-1} at clean pits. The average nutrient concentrations in the beach were 0.39 mg-N L^{-1} and 0.020 mg-P L^{-1}. Both concentrations are lower than optimal values for oil biodegradation (2 to 10 mg-N L^{-1} and 0.40 to 2.0 mg-P L^{-1}), which suggests that they are both limiting factors for biodegradation. The lowest nitrate and DO values were found in the oiled pits, leading to the conclusion that microbial oil consumption was probably occurring under anoxic conditions and was associated to denitrification. We present evidence that the oxygen level may be a major factor limiting oil biodegradation in the beaches.

Introduction

Residues of spilled oil from the tanker vessel *Exxon Valdez* in 1989 are still found within the beaches of Prince William Sound (PWS). The subsurface oil is observed in the middle and lower intertidal zones, which are habitat for foragers. Studies of subsurface oiling conditions (*1*–*3*) showed that, more than 20 years later, the oil is only slightly weathered and contains high concentrations of polycyclic aromatic hydrocarbon (PAHs) known to be potentially harmful to the marine and coastal ecosystem (*4, 5*). The oil is typically located under a layer of gravel, pebbles, and cobbles in a low porosity layer 5 to 50 cm below the surface. The average thickness of the oil layer is about 0.1 m, but it could reach up to 0.22 m (*6*).

One remediation strategy attempted after the spill was bioremediation (*7*), which relies on enhancing the biodegradation of oil. Biodegradation of hydrocarbons has been extensively studied and is known to occur fast under aerobic conditions. Potential limitation to the process includes low concentrations of oxygen (O_2, electron acceptor) and nutrients, such as nitrogen (N) and phosphorus (P). In an attempt to overcome the low levels of N and P in Prince William Sound beaches, large amounts of fertilizers (approximately 50,000 kg of N and 5000 kg of P) were added to 300 miles of contaminated shorelines over the summers of 1989 to 1992 (*7*–*9*). However, large amounts of oil (60 to 100 tons) still persist in the beaches (*1, 10*).

Atlas and Bragg (*11, 12*) conducted measurements in PWS beaches and reported for the beach studied in this paper, EL056C on Eleanor Island (Figure 1), an average nitrate concentration of 0.36 mg-N L^{-1} and DO values larger than 5.0 mg L^{-1}. Li and Boufadel (*13*) found that the beach consists of two layers, an upper layer with high permeability, overlaying a low permeability layer, where the oil could be trapped with limited exchange with upper layer. They also found that the low permeability zone is anoxic. This was a new finding as it was traditionally assumed that the levels of oxygen in beach sediments are large enough for bioremediation (*14*).

The objective of the present study is to provide detailed measurements of nutrient and DO concentration deep into the beach and to evaluate the impact of these values on oil biodegradation. We present the first evidence that the oxygen level may be a major factor limiting oil biodegradation in the beaches.

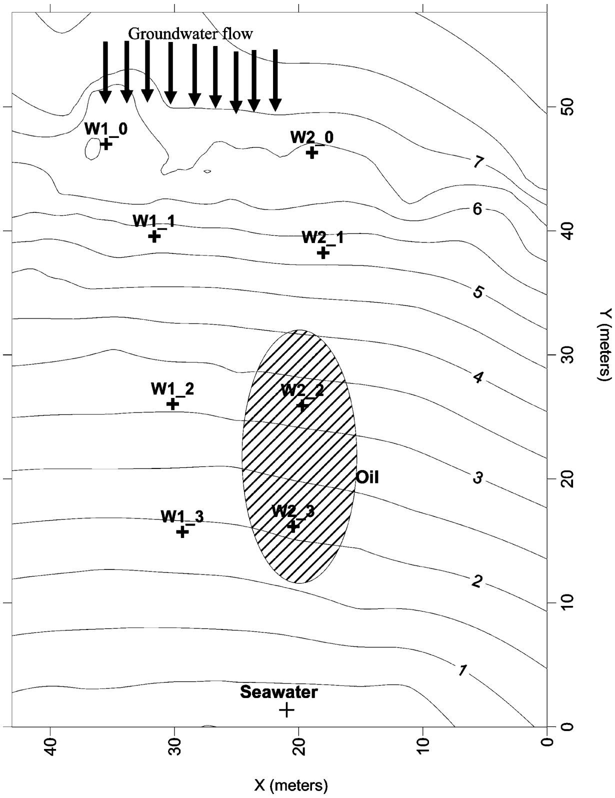

FIGURE 1. Topographic map of EL056C, showing the location of the sampling wells in each transect. The oil was observed at W2_2 and W2_3. The tidal variation was between −0.1 and 5.7 m, and the high tide line was roughly 1 m landward of W1_1 and W2_1.

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FIGURE 2. A photo of the beach El056C, showing the two transects, each well is labeled.

Experimental Section

Site Description. Our investigation was conducted on beach EL056C on Eleanor Island. This beach is a single pocket beach with along-shore width of about 40 m and an across-shore length of about 50 m. The coordinates of the beach are 147° 34′ 17.42″ W and 60° 33′ 45.57″ N. The sediments of the beach are coarse ranging from gravel (a few millimeters diameter) to pebbles and cobbles (10 to 20 cm diameter), and the beach has a low exposure to the wave energy. The beach was heavily oiled after the *Exxon Valdez* oil spill in 1989, but latter studies found that one side (right) of the beach had heavy oil residue (HOR), according to the ASTM classification (*1, 15*), while the other side (left) was completely clean. The oil was located at lower to middle intertidal zone on the right of the beach (*1, 3, 11*−*13*). The persistence of oil on the right side was explained by Li and Boufadel (*13*) as due to a water table that drops into the lower layer at low tide. The upper (high permeability) layer on the left side of the beach was much deeper and high freshwater recharge sustained the water table in it (i.e., a low freshwater recharge would have resulted in water table dropping into the lower layer). Li and Boufadel (*13*) analyzed the water motion within this beach and found that the beach fills with seawater through the beach surface near the high tide line (landward of W1_2 and W2_2 in Figure 1) during high tide and fills from the landward aquifer during the rest of the tidal cycle. They also found that the beach drains to sea through the beach surface in the seaward portion of the intertidal zone (at and seaward of W1_3 and W2_3), especially at low tide.

Site Preparation. Due to the coarse sediments of the beach and the presence of cobbles and boulders, driving sensors into the beach is extremely difficult, and it is essentially impossible to drive in PVC tubings. Hence we excavated pits and placed the sensors in them and then refilled the pits. Because the concentration in the lower layer was sought, we waited nine weeks for the sediments to return to their natural setting before we took any samples. Otherwise, the sensor measurements from the lower layer would be "contaminated" by water from the upper layer. The nine weeks wait period was chosen based on long-term monitoring of sensors about 1.0 m deep in the beach that occurred from July 2008 through June 2009. The sensors provided measurements of water pressure, salinity, and temperature. We noted that temperature and salinity fluctuations with tide decreased sharply after six weeks, which indicates that the beach returned to its two-layer configuration. In this work, we waited three additional weeks as a precautionary measure. This required two field trips. The first was conducted on June 16−28 and the second on August 18−29, during which the samples were collected.

In total, six pits were dug for the purpose of evaluating beach chemistry, three on the left transect which was clean and three on the right transect which crossed the oil zone (Figures 1 and 2). The surface elevation of the wells, on both transects, was selected in a way that there would be one well in the upper tidal zone, one in the intermediate zone, and one in the lower intertidal zone. The tidal range on this beach varied from −0.1 to 5.7 m, and the high tide line was 1 m landward of the wells W1_1 and W2_1.

The overall approach was to dig the pits to about 1.5 m deep and place a polyvinyl chloride (PVC) pipe, a sampling well that contains multiple sampling ports (SP), and two sampling boxes (SB) in the pits. The PVC pipe had an inner diameter of one inch and was slotted across over the whole length to allow water passage. A pressure transducer (Mini-Diver DataLogger, Schlumberger, Sugar Land, TX) was placed at the bottom of each PVC pipe to record the water pressure at 10 min interval. The barometric pressure, monitored by an air-pressure sensor (DL-500 baroLogger, Schlumberger, Sugar Land, TX), was subtracted from the readings of the pressure

TABLE 1. Depth of Sampling Ports (SP) and Sampling Boxes (SB) Used for Taking Samples along with the Number of Different Samples Collected from Each Well

location	W1_1	W2_1	W1_2	W2_2	W1_3	W2_3
Port A depth (m)	0.79	0.68	0.29	0.58	0.63	0.48
Port B depth (m)	0.6	0.49	0.1	0.39	0.44	0.29
SB depth (m)	0.89	0.85	0.47	0.68	0.82	0.55
DO (numbers of samples)	4	6	2	4	4	4
nutrients (numbers of samples)	6	6	3	5	12	12

transducers to obtain the water level. No rainfall occurred during the field measurement period in August 2009.

The multiport sampling wells were made of stainless steel and contained sampling ports (SP) at various levels. The ports were spaced at the interval of 0.19 m and were labeled A, B, C, and D from the bottom up. Each port was connected via a steel tube that extended to the top of the pipe. A 1/8 in. (3.2 mm) inner diameter Tygon tube (Cole-Parmer, Vernon Hills, IL) was placed on each of the steel tubes, and it was connected to a Luer-Lok three-way valve (Cole-Parmer, Vernon Hills, IL). To prevent blockage by fine sediments and to guarantee good hydraulic connection between the beach pore-water and the water inside the well, the ports were wrapped with fine stainless-steel screen.

The SB consisted of two perforated concentric cylinders made of PVC Schedule 40 (see Supporting Information Figure S1). The chamber between them was filled with sand #16 from Alaska Sand and Gravel Co. (Anchorage, AK). The diameter of the sand grains ranged from 0.21 to 1.41 mm with an average size of 0.88 mm. The uniformity coefficient was 1.68, a low value that indicates uniform particle-size sand. Both cylinders were covered with mesh size 100 (opening diameter 150 μm) steel screen. The diameter of the inner cylinder was 0.05 m (2 in.) and the length was 0.15 m (6 in.), which results in a volume of 200 mL. The inner diameter of the outer cylinder was 0.1 m (4 in.), and considering the thickness of the inner cylinder wall, the spacing between the cylinders was around 15 mm. Twelve SBs were designed and built for the dual purpose of measuring dissolved oxygen (DO) of pore-water and as a backup for taking water samples in case the SPs were clogged. The depth of SBs and SPs at each well is given in Table 1.

Sampling Methods. DO, nutrients, and salinity of the pore-water samples were measured in this study. The DO samples were measured in the field, while the nutrient concentration and salinity were determined in the lab.

DO was measured in the field using a Thermo Scientific, RDO optical probe and an ORION 4 Plus hand-held meter (Thermo Scientific, Beverly, MA). The water was pumped from the SBs into a measuring chamber using a peristaltic pump (Masterflex, Cole-Parmer, Vernon Hills, IL), allowed to overflow from the chamber. The measuring chamber was a 60-mm long, 50-mm (2-in.) wide (ID) PVC pipe, Schedule 40. The volume of the chamber was about 120 mL. Half-inch thick fiberglass plates were glued to the top and bottom of the pipe. Silicon glue was used to ensure that no water or air leaked at the contact of the pipe and the fiberglass plates. One hole (5/8-in. (15.9 mm) diameter) was drilled and threaded in each of the top and bottom plates for insertion of brass pipe and fitting to allow the water to flow in and out of the chamber. Inflow to the chamber was from the bottom plate and the outflow was from top. All connections were sealed with Teflon tape. In addition, another hole (3/4-in. (19.05 mm) diameter) was drilled in the middle of the top plate and threaded so that the DO probe would be screwed into the measuring chamber. A Teflon tape was rolled around the DO probe to seal the probe in the place.

For sampling, one end of the Masterflex tubing (peristaltic pump tubing, Masterflex, Cole-Parmer, Vernon Hills, IL) was lowered to the bottom of the sampling box and the other end was connected to the inflow of the measuring chamber (Supporting Information Figure S2). Then the peristaltic pump was started. The initial flow rate was 0.33 L min^{-1}, and it was adjusted to have a steady flow with no air bubbles. It was assumed that the steady state was achieved 10 min after running the pump, the first reading was taken and considered the representative value for the pore water.

Nutrient samples were collected from different ports of the multiport sampling wells (SP) at different times, depending on accessibility (due to tide level) and resources. The water samples were collected using presterilized 60-mL syringes (BD Luer-Lok, Fisher Scientific, Pittsburgh, PA). The sampling started from the port closest to the surface and moved to the deeper ports. The samples were collected and stored in 30-mL high density polypropylene bottles (Nalgene, Pittsburgh, PA). All the bottles were acid washed (in 10%-HCl acid bath for 18 h) and rinsed 3 times with DI water prior to the field sampling. The first volume (60 mL in the syringe) taken from each port was wasted. An additional volume of 25 mL was used to rinse the bottles. Once the rinsing was finished, the remaining water in the syringe was disposed, and a new water aliquot was taken from the port using the same syringe and emptied into the rinsed bottle. All bottles were labeled indicating the beach/pit/depth, time, and date of sampling. The samples were kept cooled in an ice-filled cooler on the beach, and they were immediately frozen in a -5 °C freezer when they arrived on board (*16*). The samples were air shipped to Temple University in dry ice to keep the samples frozen all the time during the shipping. Once in the lab, the samples were kept in a -20 °C freezer until analysis (*17*). Six measurements were made in the lab: ammonia (NH_3), nitrite (NO_2^-), nitrite/nitrate (NO_2^-/NO_3^-), phosphate (PO_4^{3-}), silicate (SiO_2), and salinity.

Nutrient and Salinity Analysis of the Pore-Water Samples. The nutrient compounds were measured using an AutoAnalyzer3 (Seal Analytical, Mequon, WI). The frozen samples were defrosted and kept in the fridge (below 4 °C) in batches of 76 samples. At the time of analysis, the samples were taken out of the fridge, hand shaken for 15 s, and filtered through 0.45 μm PTFE membrane filters (Puradisc, Whatman, Florham, NJ) into the AutoAnalyzer3 cups. The segmented flow method was used, and the concentrations were detected by colorimetric analysis. Ammonia in seawater was measured using the Berthelot reaction where a blue-green colored complex was formed that was measured at 660 nm wavelength. Nitrate in the solution was reduced to nitrite by a copper–cadmium reactor column (*18, 19*). The nitrite reacted with sulfanilamide under acid condition to form a purple azo dye that was analyzed at 550 nm wavelength (*18, 19*). Phosphate was measured following the Murphy and Riley (*18, 19*) method based on the formation of a blue complex by reaction of orthophosphate, molybdate ion, and antimony ion followed by reduction with ascorbic acid at $\text{pH} < 1$. The blue complex was analyzed at 880 nm wavelength (*18, 19*). The soluble silicate was determined based on the reduction of silicomolybdate in acidic solution to molybdenum blue by ascorbic acid. The complex was analyzed at 820 nm wavelength (*18, 19*).

The salinity of the same pore-water samples was measured using a digital refractometer (Salinity-300035, Sper Scientific, Scottsdale, AZ). The samples were filtered, about 1.5 mL of sample was poured into the measuring cup of the instrument, and the salinity was determined based on the refraction index of the sample. The refractive index of the samples is related to the density, which in turn relates to the salinity.

TABLE 2. Average of the Values Obtained from the Two Deepest Ports for DO, Nitrate-N, Ammonia-N, Phosphate-P, Silicate, and Salinity Shown Throughout the Beach for Each Well[a]

location	W1_1	W1_2	W1_3	seawater
DO (mg L^{-1})	7.06 ± 0.70	7.50 ± 0.24	4.76 ± 0.48 (*)	----
NO_3^--N (mg-N L^{-1})	0.115 ± 0.043	0.139 ± 0.030	0.132 ± 0.147	0.048 ± 0.001
NH_3^+-N (mg-N L^{-1})	0.255 ± 0.289	0.115 ± 0.050	0.234 ± 0.133	0.169 ± 0.005
PO_4^{3-}-P (mg-P L^{-1})	0.010 ± 0.002	0.011 ± 0.009	0.023 ± 0.026	0.014 ± 0.001
SiO_2 (mg L^{-1})	1.63 ± 0.88	1.47 ± 0.05	2.32 ± 0.83	0.55 ± 0.14
salinity (g L^{-1})	7.7 ± 7.3	15.0 ± 9.8	11.4 ± 3.9	25.0 ± 11.3

location	W1_1	W1_2	W1_3
DO (mg L^{-1})	5.43 ± 1.26 (*)	0.80 ± 0.14 (*)	1.25 ± 0.57 (*)
NO_3^--N (mg-N L^{-1})	0.503 ± 0.362(*)	0.051 ± 0.026	0.057 ± 0.024
NH_3^+-N (mg-N L^{-1})	0.200 ± 0.215	0.323 ± 0.145	0.227 ± 0.145
PO_4^{3-}-P (mg-P L^{-1})	0.014 ± 0.008	0.034 ± 0.022	0.024 ± 0.014
SiO_2 (mg L^{-1})	2.68 ± 0.67	2.05 ± 0.54	1.48 ± 0.65 (*)
salinity (g L^{-1})	9.2 ± 4.7	18.4 ± 3.0 (*)	8.9 ± 6.2

[a] The samples used for seawater measurements were taken from the boat about 100 m offshore. Values that are statistically different from others based on the ANOVA test were marked in Table 2 by an "*".

The statistical analysis of the experimental results was performed as follows. In order to determine the statistical significance of the differences recorded between the mean concentrations across all the wells, one way analysis of variance (ANOVA) was used. Where a significant difference between the values obtained for the wells in the beach was observed, the Tukey's honesty significant difference (HSD) test was performed to determine the well(s) that showed significantly different values by comparison to other wells. Paired student *t*-tests were used for the pairwise comparison between the values obtained for two single wells as well as to compare the pond values with the ones of each well. The level of significance used was 95% ($\alpha = 0.05$).

Results

As described earlier, the beach recharges with seawater near the tide line at high tide and with fresh aquifer water during the rest of the tidal cycle. Therefore, water moves seaward within the beach and exits near the low tide line. This has consequences on the biotransformation of nutrients and depletion of DO as they move within the beach. The number of samples for DO and nutrient analyses taken at each well is given in Table 1. The average of the values obtained from the two deepest ports for DO, nitrate-N, ammonia-N, phosphate-P, silicate, and salinity are presented in Table 2.

The wells on the left (clean) side of the beach, W1_1 and W1_2, showed the highest DO concentrations (around 7 mg L^{-1}), which could be explained by landward recharge and high exchange with seawater. The DO concentration decreased moving seaward to W1_3 (around 5 mg L^{-1}), which could be a sign of oxygen depletion within the beach due to microbial activity. Although the DO concentration decreased, it did not cause the system to become anoxic (<1 mg L^{-1}).

On the right transect, the DO concentration at W2_1 (landward) was around 5 mg L^{-1} which could be an indication of groundwater recharge and seawater mixing. Moving seaward to the oily wells (W2_2 and W2_3), the DO concentration decreased drastically, reaching 0.8 mg L^{-1} at W2_2 and 1.25 mg L^{-1} at W2_3. The paired two tailed student's *t*-test was used to compare the means of the observed values in the beach. The level of significance was 95% ($\alpha = 0.05$). The statistical analysis showed that the DO level in the oily wells can be considered as significantly lower than the DO level in the rest of the wells in the beach, ($P(t) = 0.0003 < P(t) = 0.05$). These results show a positive correlation between oil degradation and oxygen level, suggesting that either oil degradation was slower under anaerobic conditions that under aerobic conditions or that oil consumption by aerobic microbial activity resulted in anaerobic conditions in the sediments.

The NO_2^-/NO_3^--N concentrations on the left transect (clean) were about 0.11−0.14 mg-N L^{-1} with a slight increase moving seaward. However, the differences between the mean values of the three wells were not statistically significant (5% level of significance). On the contrary, the concentration of nitrate-N on the right (oily) transect was the highest at W2_1 (0.5 mg-N L^{-1}) and then decreased significantly (an order of magnitude) moving seaward to W2_2 and W2_3 to about 0.05−0.06 mg-N L^{-1} ($P(t) = 0.03 < P(t) = 0.05$). These two values were the lowest in the beach. The seawater samples were taken from the boat away from the beach to minimize contamination from shore runoff and fish spawning. The nitrate-N concentration of these samples (0.048 mg-N L^{-1}) was lower than the concentrations in the beach but close to the seawater values found in other studies in PWS (*20*) and in the Delaware Bay (*21*). The high average concentration of nitrate detected on the beach (0.139 mg-N L^{-1}) revealed that either the beach received high levels of nitrate from inland or that nitrification occurred in the beach, which is consistent with the aerobic conditions observed. Potential sources of reduced nitrogen include decomposition of organic matter and microbial fixation of atmospheric nitrogen. The latter could be due to nitrogen fixation in the beach, either through bacteria in the beach sediments (e.g., cyanobacteria) (*22*) or through groundwater recharge to the beach enriched with nitrogen-fixing *Frankia* sp. from the vegetation (mainly alder trees) covering the inland of the upper intertidal zone of the beach (*12, 23*). Nitrogen inputs to groundwater can be a consequence of leaching from soil organic matter or biological nitrogen fixation (*22*). Lower nitrate concentrations observed in the oily region of the beach could be explained by anaerobic degradation of oil components using nitrate as electron acceptor, i.e., denitrification, or by a lower rate of nitrification of ammonia under low oxygen conditions. However, the latter explanation would result in a higher level of ammonia in oily spots, which was not observed (the higher value of ammonia observed in well W2_2 is not statistically significant), leading to one to conclude that oil degradation occurred mainly through denitrification.

The ammonia concentrations on the left transect decreased from 0.255 mg-N L^{-1} at W1_1 to 0.115 mg-N L^{-1} at W1_2 and then increased again to 0.236 mg-N L^{-1} W1_3. On the oily transect, the concentration of the ammonia increased from 0.200 mg-N L^{-1} at W2_1 to 0.32 mg-N L^{-1} at W2_2 and then decreased to 0.227 mg-N L^{-1} at W2_3. The highest concentration of ammonia was observed at the well with the highest oil concentration. However, the differences between mean ammonia concentrations throughout the beach were not significant ($P(t) = 0.05 < P(t) = 0.3$). The ammonia concentration of the seawater (0.169 mg-N L^{-1}) was lower than most of the concentrations in the beach. This suggests a net input of reduced nitrogen to the beach, either through atmospheric nitrogen fixation in the beach or through recharge from inland, as discussed regarding nitrate. In addition, the aerobic conditions and high nitrate concentrations observed in the beach suggest a significant loss of ammonia through nitrification. The higher level of ammonia in the oily well (i.e., 0.32 mg-N L^{-1} at W2_2), even though not statistically significant, is consistent with the detection of near-anoxic conditions, as high concentrations of groundwater−ammonia are commonly observed under low oxygen concentration (*21, 24*).

The concentration of phosphate-P was essentially the same for the two left transect wells, W1_1 and W1_2 (0.01 mg-P L^{-1}), but almost doubled when moving seaward to W1_3. On the right transect, the phosphate concentration at W2_1 was relatively close to the value observed for W1_1 and W1_2 but increased at W2_2 (0.034 mg-P L^{-1}) and W2_3 (0.024 mg-P L^{-1}). In general, the phosphate concentration of the pore-water samples was approximately 1 order of magnitude lower than the average reported in the literature (*21*). The phosphate concentration in seawater was 0.014 mg-P L^{-1}, which is comparable to average values reported in the literature on PWS (*20*) and the Delaware Bay, 0.035 mg-P L^{-1} (*21*).

Higher concentrations of phosphate and ammonia observed at W2_2 (and to a lesser extent at W2_3) and low DO and nitrate values at these wells are consistent with the existence of anoxic conditions (*21, 24*). As phosphorus in groundwater is mostly present as inorganic dissolved phosphate, it precipitates rapidly by reaction with ferric iron, forming iron phosphate under oxic conditions (*24*). However, under anoxic conditions, phosphate removal by precipitation is less efficient, resulting in higher concentrations (*24*). The concentration of phosphate at the two most seaward wells (W1_3, 0.023 mg-P L^{-1} and W2_3, 0.024 mg-P L^{-1}) were higher than the level observed in the rest of the beach. This might be explained by the decomposition of algae residues or spawning fishes at the lower intertidal zone of the beach (note that our seawater measurements were taken around a mile offshore). It is worth noting that the statistical analysis did not show a significant difference between the mean phosphate concentrations throughout the beach ($P(t) = 0.05 < P(t) = 0.1$).

Silicate is a constituent of the sediments and the seawater, allowing one to trace the origin of the nutrients in a beach (*21*). On the left transect, the silicate concentration decreased slightly from W1_1 (1.63 mg L^{-1}) to W1_2 (1.47 mg L^{-1}) and then increased moving seaward to W1_3 (2.32 mg L^{-1}). On the right transect, the highest concentration was at W2_1 (2.68 mg L^{-1}) and decreased moving seaward. In a typical beach, the upper and lower intertidal zones should have the highest silicate concentration (*21*) which could be an indication of mixing of seawater and groundwater in different zones of the beach. This phenomena was observed on the left transect where the concentration decreased from W1_1 to W1_2 and then increased to W1_3. On the right transect, this trend was not observed. It started with a high concentration and then decreased at W2_2 and W2_3. These low values indicate a lack of water exchange between the sea and the beach at W2_3, which is also supported by the low DO observed at that well.

The seawater salinity was found to be about 25 g L^{-1}. In both transects, the salinity was highest in the midintertidal zone and decreased going landward and seaward. This configuration is due to tidal hydraulics (*25−27*), and it was demonstrated in detail for this beach by Li and Boufadel (*13*). In essence, freshwater tends to be highest near the high tide line (see Figure 1), due to proximity to the source (the landward aquifer or runoff). As the freshwater propagates into the beach, the rising tide pushes it downward causing the freshwater to propagate further seaward and to pinch out near the low tide (*27*). Li and Boufadel (*13*) found that a large freshwater flow enters the beach through the left transect in comparison with the right transect. Therefore, the average salinity on the left transect was expected to be lower than on the right transect. However, due to the high variability of the salinity with tide level, the statistical analysis did not show significant differences throughout the beach, ($P(t) = 0.05 < P(t) = 0.3$). As our focus in this paper is on nutrients, we elect to investigate this issue in a future work using detailed modeling as conducted by Li and Boufadel (*13*).

Discussion

Atlas and Bragg (*12*) conducted measurements on this beach by driving piezotubes into the beach until reaching the top of the oil layer. They reported an average nitrogen concentration of 0.36 mg-N L^{-1} and DO concentrations larger than 5.0 mg-N L^{-1}. The average nutrient values they reported are comparable to ours, 0.392 mg-N L^{-1} (based on the nitrate and ammonia). The largest discrepancy resides in the DO measurements and could be due to two nonexclusive reasons: 1) in our investigation, we placed the sensors in the beach nine weeks prior to taking samples to provide sufficient time for the beach to return to its natural state. When piezotubes are driven into this beach, as done by Atlas and Bragg (*12*), they would invariably hit cobbles in their way which would disturb the path, and we believe that one should allow a sufficient time for the sediments to return to their natural state, otherwise, the sampling at depth might be contaminated by water from shallower depths. Unfortunately, neither the time between driving the piezotubes and sampling nor the diameter of the piezotubes were provided by Atlas and Bragg (*12*). 2) Atlas and Bragg (*12*) measured the nutrient and oxygen concentrations above the oil layer, while our measurements were made from below the oil layer, which, we argue, is more relevant for oil biodegradation considering the lithology and the hydraulics in this beach; Li and Boufadel (*13*) found that this beach can be viewed as consisting of two layers, an upper layer with a high hydraulic conductivity (around 1.0 cm s^{-1}) underlain by a layer whose hydraulic conductivity is 100 to 1000 times smaller. Based on comparison between the oily and clean transects, they concluded that oil resides a few centimeters below the layers' interface. This is illustrated in Figure 3. Therefore, sampling above the oil zone would, most likely, provide high oxygen concentration due to the high conductivity in the upper layer. In addition, due to tidal hydraulics, seawater (rich in DO) does not enter the beach near W2_3; on the contrary, it leaves the beach through that location (*13*) and it opposes the molecular diffusion of oxygen from the sea. Tidal hydraulics therefore explains the findings by Atlas and Bragg (*12*) that the lower part of the oil layer is not weathered, while the top part exhibits gradual weathering increasing upward. The lower part of the oil layer is not degraded because the water that contacts it is depleted of oxygen, as our measurements at W2_2 and W2_3 showed (Table 2).

The nitrogen concentrations were about an order of magnitude lower than the concentrations (2 to 10 mg-N L^{-1}) required for optimal biodegradation of hydrocarbons (*28−31*).

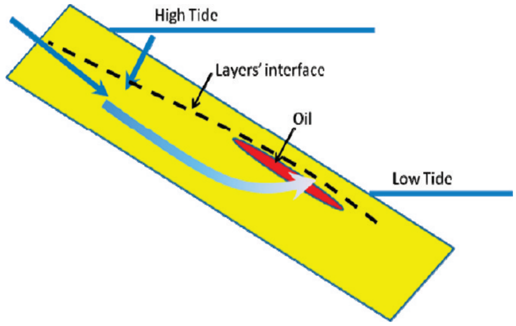

FIGURE 3. Schematic of water flow within the oiled transect (right transect, see Figure 1). The beach fills from the landward side and from the sea near the high tide line (large blue arrows). As water moves seaward within the beach (curved arrow), it loses its dissolved oxygen and nitrate. Measurements above the oil layer in the upper layer would invariably provide high dissolved oxygen values due to the large permeability of the upper layer.

Bragg et al. (*7*) and Atlas and Bragg (*11, 12*) used the ratio of nitrogen/nonpolar hydrocarbons to assess the required nutrient concentration. Although this may be the correct approach based on the stoichiometry, it may not take into account the kinetics, since the microorganisms need to be subjected to the high nutrient concentration for them to thrive. In addition, the fact that the N:P ratios in this study are greater than the optimum ratio for biodegradation (around N:P = 10) (*32*) suggests that both nitrogen and phosphorus are limiting factors.

Based on our observation that the presence of oil was negatively correlated with DO, we hypothesize that oxygen may be a limiting factor for oil biodegradation. Indeed, previous studies have provided extended evidence that the beach was heavily contaminated shortly after the spill and that the observed oil reduction is mainly the result of biodegradation, even though some weathering would occurred (*12*).

We have found that the concentrations of nutrients and DO are limiting for the biodegradation of oil when compared to the values reported in the literature. It has been argued in some studies on the *Exxon Valdez* spill that the lack of oil biodegradation could be due to a low bioavailability, or mass transfer limitation, due to sequestration of oil (*3, 11, 12, 14*). We believe this to be a valid consideration that could be only addressed by a field remediation study where the nutrients and DO concentrations are increased through an appropriate delivery technique and the oil concentrations are monitored as a function of time. Nevertheless, the near anoxic conditions along with denitrification at oiled wells indicate that the beach geochemistry at oiled areas is significantly different from the rest of the beach.

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Supporting Information Available

The quality assurance of the analytical, the methods detection limits (Table S1), and the details of sampling box (SB) and dissolved oxygen (DO) measuring set up (Figures S1 and S2, respectively). This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Short, J. W.; Lindeberg, M. R.; Harris, P. M.; Maselko, J. M.; Pella, J. J.; Rice, S. D. Estimate of oil persisting on the beaches of Prince William Sound 12 years after the Exxon Valdez oil spill. *Environ. Sci. Technol.* **2004**, *38*, 19–25.

(2) Owens, E. H.; Taylor, E.; Humphrey, B. The persistence and character of stranded oil on coarse-sediment beaches. *Mar. Pollut. Bull.* **2008**, *56*, 14–26.

(3) Taylor, E.; Reimer, D. Oil persistence on beaches in Prince William Sound - A review of SCAT surveys conducted from 1989 to 2002. *Mar. Pollut. Bull.* **2008**, *56*, 458–474.

(4) Carls, M. G.; Babcock, M. M.; Harris, P. M.; Irvine, G. V.; Cusick, J. A.; Rice, S. D. Persistence of oiling in mussel beds after the Exxon Valdez oil spill. *Mar. Environ. Res.* **2001**, *51*, 167–190.

(5) Peterson, C. H.; Rice, S. D.; Short, J. W.; Esler, D.; Bodkin, J. L.; Ballachey, B. E.; Irons, D. B. Long-term ecosystem response to the Exxon Valdez oil spill. *Science* **2003**, *302*, 2082–6.

(6) Michel, J.; Nixon, Z.; Cotsapas, L. Exxon Valdez Oil Spill Restoration Project Report, Evaluation of Oil Remediation Technologies for Lingering Oil from the Exxon Valdez Oil Spill in Prince William Sound, Alaska Restoration Project 050778. 2006; pp 1−61.

(7) Bragg, J. R.; Prince, R. C.; Harner, E. J.; Atlas, R. M. Effectiveness of bioremediation for the *Exxon Valdez* oil spill. *Nature* **1994**, *368*, 413–418.

(8) Pritchard, P. H.; Costa, C. F. EPA's Alaska oil spill bioremediation project. *Environ. Sci. Technol.* **1991**, *25*, 372–379.

(9) Claxton, L. D.; Houk, V. S.; Williams, R.; Kremer, F. Effect of Bioremediation on the Mutagenicity of Oil Spilled in Prince-William-Sound, Alaska. *Chemosphere* **1991**, *23*, 643–650.

(10) Short, J. W.; Maselko, J. M.; Lindeberg, M. R.; Harris, P. M.; Rice, S. D. Vertical distribution and probability of encountering intertidal Exxon Valdez oil on shorelines of three embayments within Prince William Sound, Alaska. *Environ. Sci. Technol.* **2006**, *40*, 3723–3729.

(11) Atlas, R.; Bragg, J. R. Bioremediation of marine oil spills: when and when not - the *Exxon Valdez* experience. *Microbial Biotechnol.* **2009**, *2*, 213–221.

(12) Atlas, R. Bragg, J. R. Evaluation of PAH depletion of subsurface *Exxon Valdez* oil residues remaining in Prince William Sound in 2007−2008 and their likely bioremediation potential. *Proc. 32nd Arctic and Marine Oil Spill Program (AMOP) Technical Seminar*; Environment Canada: Ottawa, ON, 2009; Vol. 2, pp 723−747.

(13) Li, H.; Boufadel, M. C. Long-term persistence of oil from the Exxon Valdez spill in two-layer beaches. *Nat. Geosci.* **2010**, *3*, 96–99.

(14) Page, D. S.; Boehm, P. D.; Neff, J. M. Shoreline type and subsurface oil persistence in the *Exxon Valdez* spill zone of Prince William Sound, Alaska. *Proc. 31st Arctic and Marine Oil Spill Program (AMOP) Technical Seminar*; Environment Canada: Ottawa, ON, 2008; Vol. 2, pp 545−563.

(15) ASTM F1687-97. Standard guides for terminology and indices to describe oiling condition on shoreline. 2003.

(16) U.S. Environmental Protection Agency. Sample Preservation. In *Methods for Chemical Analysis of Water and Wastes*; U.S.E.P.A.: Cincinnati, Ohio, USA, 1983; pp xv−xx.

(17) Dore, J. E.; Houlihan, T.; Hebel, D. V.; Tien, G.; Tupas, L.; Karl, D. M. Freezing as a method of sample preservation for the analysis of dissolved inorganic nutrients in seawater. *Mar. Chem.* **1996**, *53*, 173–185.

(18) Grasshoff, K.; Kremling, K.; Ehrhadt, M. *Methods of Seawater Analysis*; Wiley-VCH: Germany, 1999

(19) Seal Analytical. *AutoAnalyzer3 user guide;* SEAL Analytical: Mequon, WI, 2008.

(20) Eslinger, D. L.; Cooney, R. T.; McRoy, C. P.; Ward, A.; Kline, T. C.; Simpson, E. P.; Wang, J.; Allen, J. R. Plankton dynamics: observed and modelled responses to physical conditions in Prince William Sound, Alaska. *Fish. Oceanogr.* **2001**, *10*, 81–96.

(21) Ullman, W. J.; Chang, B.; Miller, D. C.; Madsen, J. A. Groundwater mixing, nutrient diagenesis, and discharges across a sandy beachface, Cape Henlopen, Delaware (USA). *Estuarine, Coastal Shelf Sci.* **2003**, *57*, 539–552.

(22) Postgate, J. R. *Nitrogen Fixation*; Cambridge University Press: United Kingdom, 1998; Vol. 1, p 112.

(23) Cooper, W. S. Vegetation of the Prince William Sound Region, Alaska; With a Brief Excursion into Post-Pleistocene Climatic History. *Ecol. Monogr.* **1942**, *12*, 1–22.

(24) Slomp, C.; Van Capellen, P. Nutrient inputs to the coastal ocean through submarine groundwater discharge: controls and potential impact. *J. Hydrol.* **2004**, *295*, 64–86.

(25) Wrenn, B. A.; Suidan, M. T.; Strohmeier, K. L.; Eberhart, B. L.; Wilson, G. J.; Venosa, A. D. Nutrient transport during bioremediation of contaminated beaches: Evaluation with lithium as a conservative tracer. *Water Res.* **1997**, *31*, 515–524.

(26) Ataie-Ashtiani, B.; Volker, R. E.; Lockington, D. A. Tidal effects on sea water intrusion in unconfined aquifers. *J. Hydrol.* **1999**, *216*, 17–31.

(27) Boufadel, M. C. A Mechanistic Study of Nonlinear Solute Transport in a Groundwater-Surface Water System under Steady State and Transient Hydraulic Conditions. *Water Resour. Res.* **2000**, *36*, 2549–2565.

(28) Venosa, A. D.; Suidan, M. T.; Wrenn, B. A.; Strohmeier, K. L.; Haines, J.; Eberhart, B. L.; King, D.; Holder, E. Bioremediation of an experimental oil spill on the shoreline of Delaware Bay. *Environ. Sci. Technol.* **1996**, *30*, 1764–1775.

(29) Boufadel, M. C.; Reeser, P.; Suidan, M. T.; Wrenn, B. A.; Cheng, J.; Du, X.; Venosa, A. D. Optimal nitrate concentration for the biodegradation of n-heptadecane in a variably-saturated sand column. *Environ. Technol.* **1999**, *20*, 191–199.

(30) Du, X.; Reeser, P.; Suidan, M. T.; Huang, T. L.; Moteleb, M.; Boufadel, M. C.; Venosa, A. Optimal nitrate concentration supporting maximum crude oil biodegradation in microcosms. In *Proceedings of 1999 International Oil Spill Conference*; Anonymous; American Petroleum Institute: Washington, DC, 1999.

(31) Zhu, X.; Venosa, A.; Suidman, M.; Lee, K. *Guidelines for the bioremediation of marine shorelines and freshwater wetlands*; U.S. Environmental Protection Agency, Office of Research and Development National Risk Management Laboratory, Land Remediation and Pollution Control Division: Cincinnati, 2001.

(32) Liebeg, E. W.; Cutright, T. J. The investigation of enhanced bioremediation through the addition of macro and micro nutrients in a PAH contaminated soil. *Int. Biodeterior. Biodegrad.* **1999**, *44*, 55–64.

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