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Research article

Dual effects of a dispersant and nutrient supplementation on weathered Endicott oil biodegradation in seawater

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Abstract: Laboratory-scale experiments were conducted to evaluate the biodegradation of physically (WAF) and chemically dispersed (CEWAF) Endicott oil in seawater (salinity: 29.1‰) from Prince William Sound, Alaska, under low nutrient (LN) (background seawater) and high nutrient (HN) (addition of 100 mg NO_3-N/L and 10 mg PO_4-P/L to background seawater) at 15 ± 0.5 °C for 42 days. The dispersant was Corexit 9500. The dispersed oil concentration of the WAF (0.019 g/L ± 0.002) was an order of magnitude lower than that in the CEWAF (0.363 g/L ± 0.038). While remaining negligible in the WAF, the total oil removal in the CEWAF was 26% and 44% in LN and HN treatments, respectively. Nutrient supplementation significantly accelerated the rate of oil biodegradation as confirmed by ANOVA coupled with Tukey's test at 95% confidence intervals ($\alpha = 0.05$). GC/MS analyses revealed that biodegradation affected mainly alkane compounds. In the CEWAF, O_2 consumption, CO_2 production and biomass were much larger in HN than in LN treatments, which suggests that chemical dispersion of oil coupled with high nutrient concentration could be very useful in terms of remediation strategies and effective responses to oil spill at sea.

Keywords: Biodegradation; bioremediation; Endicott oil; dispersants; nutrients; seawater

1. Introduction

Accidental marine oil spills (e.g., Exxon Valdez, Alaska, in 1989; BP Deepwater Horizon, Gulf of Mexico, in 2010) can lead to devastating environmental and socio-economic impacts [1,2]. These impacts can last for many years, and call for adequate mitigating response by decision makers. The primary response, which is commonly focused on the physical/mechanical removal of oil from the impacted environment, can be limited, particularly when large spills occur at sea. For instance, extensive mechanical operations from the Deepwater Horizon release have led to only ~3% of oil removal by skimming [3]. The breakdown of an oil slick into smaller droplets in the water column (a process known in the oil literature as "dispersion") causes dilution of the oil, thus reducing its potential impacts on the shorelines and coastal ecosystems. Dispersion can be enhanced by the addition of chemical dispersants. The mass fraction of oil entrained in the water column as discrete small droplets relative to the total mass of oil is referred to as dispersant effectiveness. Many factors can conceivably influence oil chemical dispersion including the type and quantity of dispersants, salinity and temperature of seawater, wave-mixing energy [4] and oil physicochemical properties, such as viscosity, gravity, interfacial tension with water [5].

An added benefit of oil chemical dispersion is the acceleration of oil biodegradation. Chemical dispersion can lead to the formation of tiny and stable oil droplets in the water column, thus increasing the surface area between oil and water and, subsequently, oil availability for microbial uptake. Biodegradation of dispersed oil droplets tends to occur preferentially at the oil-water interface since many oil components are poorly soluble in water. Enhanced hydrocarbon biodegradation appears to be correlated with a decrease in the droplet-size [6] and microbial attachment to oil droplets [7,8]. However, there are concerns that chemical dispersion could increase the oil concentration in the water column to toxic levels [9].

In addition to the potential effects of chemical dispersion on oil dilution and biodegradation, the presence of sufficient concentrations of nutrients (N and P) and/or oxygen could enhance the rate of oil biodegradation by indigenous hydrocarbon-degrading microorganisms [10-13]. The range of NO_3-N needed for maximum oil biodegradation has been estimated to be from ~2 to 10 mg/L [13-15]. Generally, the recommended N:P ratio, on a mass basis, is about 10:1 [16-19].

Water salinity also plays an important role on oil biodegradation in that it can affect the solubility of many compounds including dispersants and oil components, and the action of microorganisms on hydrocarbons. Research on the influence of salinity on petroleum accommodation by dispersants reported an increase in the effectiveness of two dispersants, Corexits 9527 and 9500, with increasing salinity in the range of 0 to 35 ppt [20]. A study reported that the action of microorganisms on the different fractions of Ashtart crude oil was dependent on salinity concentration [21]. The two worst spills in U.S. history (1) the Exxon Valdez occurred near island shorelines with low water salinity, and (2) the BP Deepwater Horizon occurred in deep sea with higher salinity. Thus, research to increase our understanding on oil biodegradation at different salinity conditions, resembling the salinity of coastal and far offshore waters, is critical for informed remediation and response strategies to limit the impacts of oil spills.

Recently, we reported our findings on oil biodegradation in brackish water [22]. This paper reports the results of lab-scale experiments on aerobic biodegradation of Endicott oil (Endicott Island, Alaska, USA) in seawater. The objectives were three: (1) Compare the effectiveness of physical and chemical dispersion on the rate of oil biodegradation in seawater, (2) Evaluate the influence of nutrient availability

on the rate of biodegradation of physically and chemically dispersed oil, and (3) Determine whether the use of chemical dispersants combined with exogenous nutrients enhances oil biodegradation in seawater.

2. Materials and Methods

Seawater samples (salinity: 29.1‰) were collected from Prince William Sound (PWS), Alaska (60.79109°N, 146.90607°W). The samples were filtered using a 10 μm Whatman paper, filtering flask and Buchner funnel (Figure S1 of the supplementary information-SI). A volume of 20 ml of weathered on Endicott oil was placed in 4 L bottle (i.e., oil to water ratio is 1/200 on a volume basis). The oil was obtained from Ohmsett (The National Oil Spill Response Research & Renewable Energy Test Facility), New Jersey, USA, and referred as weathered oil based on its history. The oil was then either physically or chemically dispersed (Corexit 9500, dispersant-to-oil ratio, 1:10 v/v) by mixing for 16 hours over a magnetic stirrer and allowing the suspension to settle for 30 minutes (Figure S2 of SI). For the chemical dispersion, the stirring speed was adjusted to create a small vortex (~1/4 of water depth), and the dispersant was applied gently to the oil slick with a syringe. Then, the mixing speed was adjusted to draw a vortex in the water column all the way to the bottom of the aspirator bottle. Packs of ice were placed around the bottle to keep the mixtures at low temperature and limit potential oil biodegradation during the 16 hour mixing time. The physically dispersed oil is referred herein as water accommodated fraction (WAF) and the chemically dispersed oil as chemically enhanced water accommodated fraction (CEWAF). After the settling time, 100 mL were collected from a spigot in the bottom of the bottle and were discarded. This resulted in the removal of undispersed oil from the spout at the bottom of the bottle. Then, a volume of 100 mL WAF or CEWAF was transferred to each microcosm.

The oil biodegradation was evaluated under low nutrient (LN) and high nutrient (HN) (Table S1 of the SI) for both WAF and CEWAF in triplicate sealed microcosms (Figure S3 of the SI). The LN included only background nutrient in seawater (0.013 ± 0.001 mg NO_3-N/L; 0.200 ± 0.022 mg PO_4-P/L) while the HN was supplemented with nutrient (background nutrient in seawater + 100 mg NO_3-N/L and 10 mg PO_4-P/L). Prior to the experiments, all microcosms were tested for potential gas leak by filling them with O_2 and submerging them under water for 5 minutes. None of the microcosms used in the experiment showed any leak. The microcosms were constructed using modified 250-mL wide-mouth, screw-cap Erlenmeyer flasks [23], and included (a) a CO_2 trap filled with a trapping solution (sodium hydroxide: NaOH), (b) a sample port in the cap of the trap tubes for removing or replacing the trapping solution periodically, and (c) a sidearm for controlled introduction of oxygen by attaching an oxygen-filled ground-glass syringe to the sidearm and allowing the pressure on the syringe barrel to equalize with the headspace gas pressure inside the microcosm. The microcosms were continuously agitated at 140 rpm by a shaker system and incubated at constant temperature (15 ± 0.5 ℃) for 42 days (Figure S4 of the SI). The background seawater nutrient concentrations (Table S1 of the SI) were measured using test kits (HACH 2668000, 2429800, 2672245, 2106069, and 2742645) and a spectrophotometer (Thermo Scientific Evolution 201 UV-Visible). Key parameters (oil removal, O_2 consumption, CO_2 production, biomass production and nutrient consumption) of aerobic Endicott oil biodegradation were determined for both WAF and CEWAF microcosms. In addition to the active microcosms, the experiments included control microcosms that were poisoned with sodium azide (0.5% w/v) to prevent microbial growth and oil biodegradation.

Temporal changes in the concentration of total petroleum hydrocarbon (TPH) and oil components including alkanes, polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs were

determined by gravimetric methods and gas chromatography/mass spectrometry (GC/MS), respectively. The most probable number (MPN) method was used to estimate biomass production. These measurements were performed at days 0 (i.e., after the mixing of oil with water), 14, 28 and 42 from three independent active microcosms. In the control microcosms, TPH concentration and biomass were measured at days 0 and 42. For TPH measurement, the weight of oil was measured after extraction with dichloromethane (DCM) as per EPA Method 3510C [24]. The extraction procedures are based on the separation of the oil content from the oil-water mixtures with DCM in a separatory funnel. The separated DCM-oil phase was collected in a clean pre-weighted glass beaker. Then the beaker was kept in the laboratory hood until complete DCM evaporation, and the weight was measured. The extracted oil was further analyzed for alkanes, PAH and alkylated PAH by GC/MS following the protocol reported in our recent paper [22]. The oil component values were normalized to hopane (17a (H), 21b (H)-hopane)**,** a conservative biomarker that is very resistant to biodegradation. Analysis of variance (ANOVA) coupled with Tukey's test at 95% confidence intervals ($\alpha = 0.05$) was performed on the oil data to determine whether the measured temporal changes were significant. The MPN assay was conducted using 96-well tests and selective growth substrates (Table S3 of the SI) to estimate biomass production of alkane degraders, PAH degraders, and heterotrophic bacteria [25]. The amount of O_2 introduced in the microcosms was recorded, and CO_2 produced was trapped into a solution of NaOH (Figure S3 of the SI). The CO_2-trapping solution was periodically removed and titrated using sulfuric acid to the phenolphthalein endpoint (pH = 8.3) to determine CO_2 production. Theoretical N and P consumption was calculated (see Equations used in the SI) based on biomass formula ($C_5H_7O_2NP_{0.1}$) [26].

Table 1. Summary of materials and methods.

Methods	Description
Seawater collection/ Preservation	Collected at Prince William Sound/Alaska (Salinity: 29.1‰) using Certified-clean amber glass bottles, kept at ~4 °C
Sample filtration	Vacuum filtration (10 μm Whatman paper filter)
Dispersed oil	Chemically (Corexit 9500) enhanced water accommodated fraction (CEWAF) Physically water accommodated fraction (WAF) Oil-to-water ratio (OWR) = 1:200 (v/v) Dispersant-to-oil ratio (DOR) = 1:10 (v/v)
Nutrient	Low (LN): CEWAF or WAF only (0.013 ± 0.001 mg NO_3-N/L; 0.200 ± 0.022 mg PO_4-P/L) High (HN): CEWAF or WAF +100 mg NO_3-N/L and 10 mg PO_4-P/L
Active/control microcosms	Active: No addition of bactericide Control: Addition of bactericide (0.5% w/v of sodium azide)
Oil measurements	Total Petroleum Hydrocarbon (TPH): Gravimetric methods Oil components (Normal and Methyl Alkanes, PAHs, Alkylated PAHs): GC/MS Statistical significance in oil data: ANOVA coupled with Tukey's test
Biomass estimation	Most probable number (MPN): alkanes, PAHs, and heterotrophic bacteria
O_2 Consumption	Measured of volume
CO_2 production	Titration of CO_2 trapping solution
Nutrient consumption	Theoretical calculations of nitrogen and phosphorus consumption based on biomass formula ($C_5H_7O_2NP_{0.1}$) [26]

A summary of this section is presented in Table 1 including sample collection and filtration, physical and chemical oil dispersion in seawater, determination of oil concentration, O_2 consumption, CO_2 production, biomass estimation and nutrient consumption estimation. The methods described here are further detailed in a recent article [22].

3. Results

3.1. Oil biodegradation as a function of time

The physically dispersed oil (WAF) microcosms showed comparable initial (day 0) total petroleum hydrocarbon (TPH) concentrations at 0.018 g/L ± 0.001 for the low nutrient (LN) and 0.021 g/L ± 0.001 for the high nutrient (HN) treatments The chemically dispersed oil (CEWAF) also showed comparable initial TPH: 0.347 g/L ± 0.033 for the LN and 0.379 g/L ± 0.042 for the HN, approximately an order of magnitude larger than the WAF. Figure 1A shows the temporal changes in TPH for both WAF and CEWAF microcosms during the 42 days of the experiments. The changes were negligible in the WAF for both treatments, providing no evidence of oil biodegradation. At day 42, the TPH concentration in the control WAF remained comparable with the TPH in the active microcosms (Figure 1A). However, in the CEWAF microcosms, the TPH removal reached 26% and 44% in LN and HN treatments, respectively. Statistical analyses (ANOVA and Tukey tests) revealed no difference in oil concentration in the LN between 0 and 14 days. However, the difference became significant at day 28. After day 28, no significant changes were observed. In the HN, the statistical analyses showed evidence of a significant oil biodegradation from day 0 to day 14. A summary of the statistical data results is reported in Table S2 of the SI. As expected, the control CEWAF microcosms showed no evidence of oil biodegradation with time as indicated by the measured TPH at day 42 (Figure 1A). Figure 1B and 1C showed the temporal changes in oil components (alkanes, alkylated PAHs and PAHs from GC/MS analyses) for LN and HN in the CEWAF microcosms, respectively. The GC/MS analyses revealed that alkanes and alkylated PAHs accounted for at least 97% of the compounds in both LN and HN at day 0: Alkanes (~71%), alkylated PAHs (~26%) and PAHs (only ~3%). The results showed (a) statistically significant changes in alkane biodegradation in the HN from day 0 to day 14 while remaining insignificant in the LN from day 0 to 42, (b) no significant changes in alkylated PAH from day 0 to day 42 for both LN and HN, and (c) significant changes in PAH biodegradation from day 0 to day 14 for both LN and HN.

Our results demonstrate that (1) chemical dispersion with Corexit 9500 has considerably increased dispersed Endicott oil in seawater, (2) nutrient supplementation to seawater significantly increased the rate of chemically dispersed oil biodegradation by a factor of ~2 compared with its biodegradation solely in background seawater, and (3) the oil biodegradation concerned mainly alkane compounds.

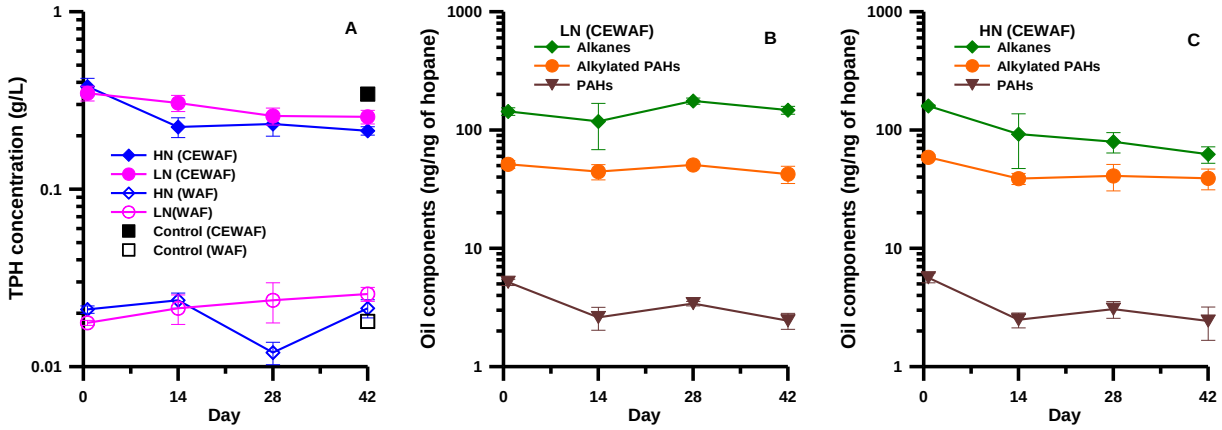

Figure 1. Temporal changes in oil concentrations. (A) Total petroleum hydrocarbon (TPH) for both low nutrient (LN) and high nutrient (HN). Hollow symbols represent TPH concentration in WAF microcosms (i.e., no addition of dispersant). Filled symbols are for CEWAF microcosms (i.e., addition of the dispersant Corexit 9500). Error bars represent one standard deviation based on independent triplicates. TPH concentrations at the end of the experiments in the control microcosms are shown for WAF (hollow square) and CEWAF (filled square). (B) Oil components (alkanes, alkylated PAHs and PAHs) for LN (CEWAF) from GC/MS analyses. C) Oil components for HN (CEWAF).

3.2. Oxygen consumption and carbon dioxide production

Figure 2 shows the cumulative amount of O_2 consumed and CO_2 produced for both WAF and CEWAF during the 42 days of the experiments. In the WAF microcosms, O_2 consumption remained relatively low and comparable in the LN ($2.18 < O_2 < 5.56$ mg) to the HN ($3.37 < O_2 < 6.25$). In the CEWAF microcosms, O_2 consumption was substantially lower in the LN ($1.19 < O_2 < 5.26$ mg) than in the HN ($6.65 < O_2 < 14.19$ mg). Overall, the cumulative O_2 consumption was at least two times higher in the HN of the CEWAF than the other observed values.

Carbon dioxide production remained relatively low and comparable in the WAF microcosms: $0.82 < CO_2\text{-}C < 1.17$ mg in the LN and $0.90 < CO_2\text{-}C < 1.16$ mg in the HN. In the CEWAF microcosms, CO_2 production was substantially lower in the LN ($1.06 < CO_2\text{-}C < 1.67$ mg) than in the HN ($3.62 < CO_2\text{-}C < 4.15$ mg). Overall, the cumulative CO_2 production was at least two times higher in the HN of the CEWAF than the other observed values.

3.3. Biomass production

The population of alkane degraders (AD) and heterotrophic bacteria (HB) varied temporally in both WAF and CEWAF microcosms (Figure 3). The microbial population increased during the first 14 days, but decreased thereafter. In the WAF, the AD population (Figure 3A) increased from ~1×10^0 count/mL at day 0 to 1.03×10^3 count/mL in the LN and 3.62×10^2 count/mL in the HN at day 14. Then, it decreased to ~0 count/mL at day 28 and remained the same at day 42 for both LN and HN treatments. In the CEWAF, the AD population (Figure 3A) increased from ~ 5.8×10^1 count/mL at day 0 to 1.03×10^3 count/mL in the LN and 7.2×10^4 count/mL in the HN at day 14. Then, the AD population decreased to ~0 count/mL in the LN at day 28 and remained the same at day 42. In the HN, it slightly decreased to 4.0×10^4 count/mL at day 28 and by an order of magnitude (1.4×10^3) count/mL at day 42.

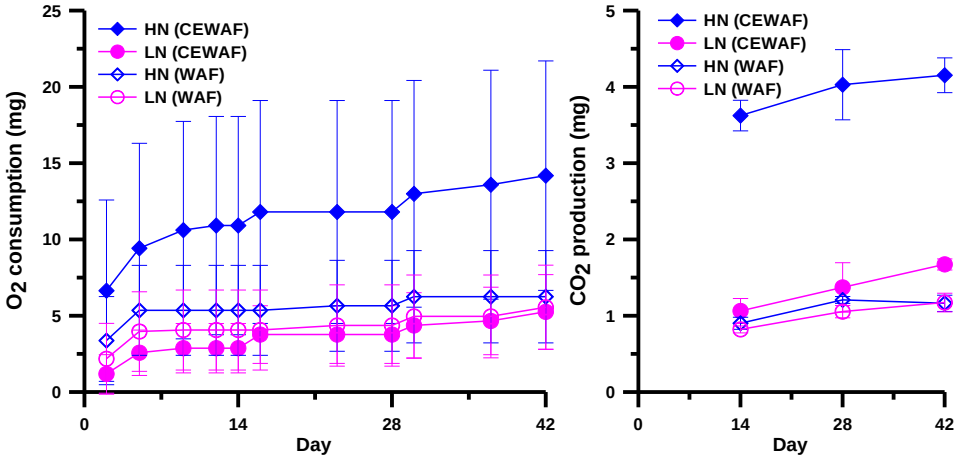

Figure 2. Oxygen (O_2) consumption (left panel) and carbon dioxide (CO_2) production (right panel) as a function of time for both low nutrients (LN) and high nutrients (HN). Hollow symbols represent measured values in the physically dispersed oil (WAF) microcosms (i.e., no addition of dispersants) while filled symbols are for chemically dispersed oil (CEWAF) microcosms (i.e., addition of a dispersant: Corexit 9500). Error bars represent one standard deviation based on triplicates.

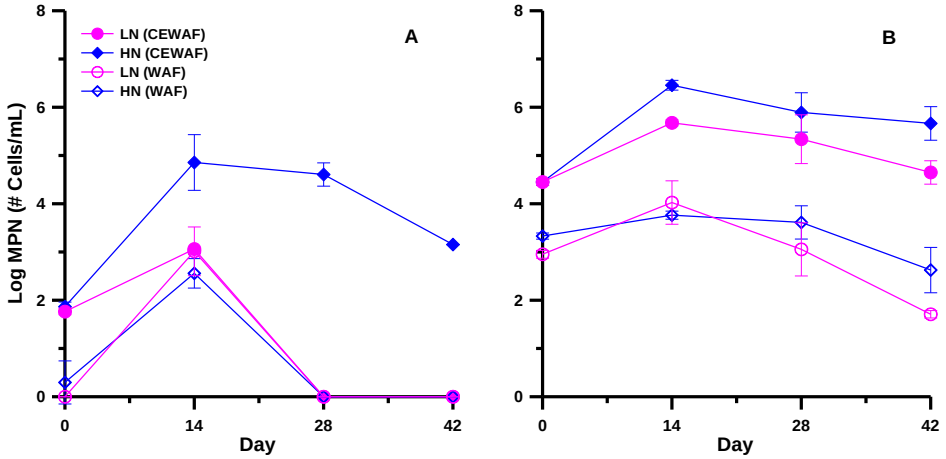

Figure 3. Variation in microbial population as a function of time for both low nutrient (LN) and high nutrients (HN): (A) Alkane degraders and (B) Heterotrophic bacteria. Hollow symbols represent microbial population in physically dispersed oil (WAF) microcosms (i.e., no addition of dispersants) while filled symbols are for chemically dispersed oil (CEWAF) microcosms (i.e., addition of a dispersant: Corexit 9500). Error bars represent one standard deviation based on triplicates.

In the WAF microcosms, the HB population (Figure 3B) increased from ~1.0×10^3 count/mL at day 0 to 1.0×10^4 count/mL in the LN and 5.8×10^3 count/mL in the HN at day 14. Then, it decreased in both LN and HN by at least an order of magnitude at day 42. In the CEWAF microcosms, the HB population (Figure 3B) increased from ~2.8×10^4 count/mL at day 0 to 4.6×10^5 count/mL in the LN and 2.9×10^6 count/mL in the HN at day 14. Then, it decreased in both LN and HN to approximately an order of magnitude at day 42. Unexpectedly, no PAH degraders (PAHD) were observed using the MPN assay. As

expected, no microbial population (AD, PAHD and HB) was observed in the control microcosms.

Based on the AD and HB population and assuming a cell dry weight of 2.8×10^{-10} mg [26], the net yield of biomass was calculated for the CEWAF microcosms and reported in Table 2. The net yield of biomass was lower in the LN (1.375×10^{-3} mg biomass/mg oil) than in the HN (4.923×10^{-3} mg biomass/mg oil).

Overall, the results indicated that the microbial population (1) increased during the first 14 days of the experiment and decreased thereafter, (2) was more important in the CEWAF than in the WAF microcosms, and (3) was lower in the LN than in the HN for the CEWAF microcosms. No microbial population (AD, PAHD, and HB) was observed in the control microcosms.

Table 2. Net yield of biomass (YX) for alkane-degraders, PAH degraders and heterotrophic bacteria in low nutrient (LN) and high nutrient (HN) of the chemically dispersed oil (CEWAF) microcosms.

	S (mg oil/L)		X (mg biomass/L)		Y_X (mg biomass/mg oil)	
	LN	HN	LN	HN	LN	HN
Alkane degraders			0.000	0.020	3.339×10^{-6}	1.210×10^{-4}
PAH degraders			0.000	0.000	-	-
Heterotrophic bacteria	91.667	165.333	0.125	0.794	1.364×10^{-3}	4.802×10^{-3}
Total biomass			0.126	0.814	1.375×10^{-3}	4.933×10^{-3}

S—substrate (Endicott Oil); X—biomass (alkane degraders, PAH degraders and heterotrophic bacteria); Y_X—net yield (mg of biomass per mg of oil). No substrate observed to be degraded in samples without dispersant; therefore, no yield of biomass was calculated.

3.4. Nutrient consumption

Table 3 shows the estimated N and P consumption based on biomass growth as per the equations described in the SI. The increase in biomass (<0.003 mg/L) and N and P consumption (0.000 mg/L) remained negligible in the WAF microcosms. In the CEWAF microcosms, the LN showed a substantially lower increase in biomass (0.126 mg/L) production, nitrogen (0.015 mg/L) and phosphorus (0.003 mg/L) consumption than the HN (biomass: 0.814 mg/L, nitrogen: 0.098 mg/L and phosphorus consumption: 0.022 mg/L).

Table 3. Estimated phosphorus and nitrogen consumption due to biomass growth for both low nutrient (LN) and high nutrient (HN) treatments of the physically dispersed oil (WAF) and chemically dispersed oil (CEWAF) microcosms.

	Biomass Increased (mg/L)	Estimated Nitrogen Consumption (mg/L)	Estimated Phosphorus Consumption (mg/L)
WAF-LN	0.003	0.000	0.000
WAF-HN	0.001	0.000	0.000
CEWAF-LN	0.126	0.015	0.003
CEWAF-HN	0.814	0.098	0.022

3.5. Comparison of oil biodegradation and O_2 consumption

Temporal changes in oil removal (%) and O_2 consumption (mg/L) in both LN and HN treatments of the CEWAF are compared in Figure 4. In the LN treatment, O_2 consumption slowly increased from 11.09 mg/L at day 2 to 28.77 mg/L at day 14, which corresponded to only 11.90% of oil removal (Figure 4A). After day 14, O_2 consumption continued to increase to a cumulative value of 52.58 mg/L at day 42. As a result, oil removal reached 26% at day 42. In the HN treatment, a rapid increase in O_2 consumption (from 66.47 mg/L at day 2 to a cumulative value of 109.13 mg/L at day 14) coincided with an important oil removal (41% at day 14) (Figure 4B). After day 14, changes in O_2 consumption varied only from 109.13 mg/L to a cumulative value of 141.86 mg/L at day 42. Similarly, the oil removal only slightly changed after day 14 to a cumulative value of 44% at day 42.

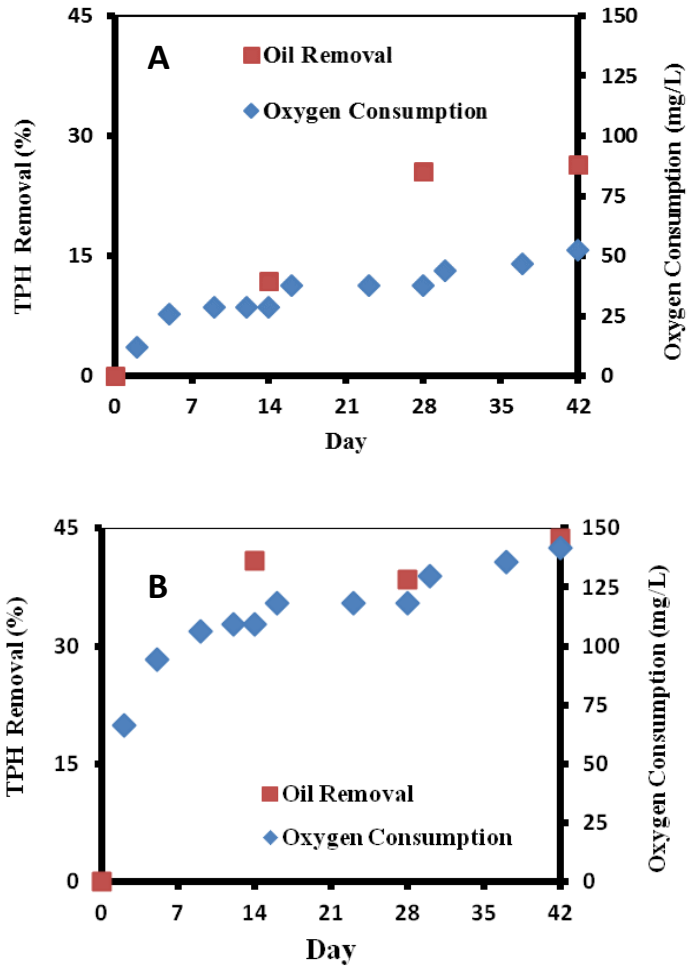

Figure 4. Comparison of total petroleum hydrocarbon (TPH) removal with oxygen consumption as a function of time for both low nutrients (LN) (Panel A) and high nutrients (HN) (Panel B) of the chemically dispersed oil (CEWAF) microcosms (i.e., addition of a dispersant: Corexit 9500).

4. Discussion

This experiment demonstrated that the application of a chemical dispersant (Corexit 9500) and nutrient supplementation (nitrogen and phosphorus) led to substantial aerobic biodegradation of

Endicott oil in seawater. The concentration of dispersed oil in seawater as result of physical processes (WAF) was an order of magnitude lower than that from the chemical dispersion (CEWAF) despite the same mixing conditions. Thus, the chemical dispersion increased the bioavailability of oil and its subsequent biodegradation as depicted by a removal of up to 44% of total petroleum hydrocarbon (TPH) (Cf. Figure 1A). In the WAF, TPH removal remained negligible during the entire experiment, which could probably be attributed to the formation of unstable oil droplets that coalesce rapidly; thereby decreasing the surface area between the oil and water, and the availability of oil droplets for microbial uptake. Our results are consistent with the findings of our recent research on oil biodegradation in brackish water [22] as well as previous studies that reported the effectiveness of Corexit 9500 in dispersing oil in seawater [27] and enhancing oil biodegradation rates [6]. They also agree with previous findings from research on the *Exxon Valdez* spill showing that the effectiveness of oil biodegradation was largely determined by the availability of a sufficient concentration of oil and nutrients [11]. In addition to the effects of chemical dispersion on oil bioavailability, nutrient supplementation significantly accelerated the rate of oil biodegradation as confirmed by ANOVA coupled with Tukey's test at 95% confidence intervals ($\alpha = 0.05$). The increase of microbial metabolic activity with availability of nutrients also appeared to be consistent with the rate of oil biodegradation as indicated by the amounts of O_2 consumed (109.13 mg/L) and oil removal (41%) during the first 14 days of the experiment (Cf. Figure 4B). When nutrients were not supplemented, only 28.77 mg/L of O_2 consumption and 11.90% of oil removal were observed during the same time period (Cf. Figure 4A). A higher production of CO_2 (Cf. Figure 2) and biomass (Cf. Figure 3) in the microcosms supplemented with nutrients also provided evidence for the effects of nutrient availability on accelerating the biodegradation rate of chemically dispersed oil. The MPN method most likely underestimated the biomass growth as sometimes only ~10% of indigenous bacteria in marine system would develop in culture [28]. Unexpectedly, no PAH degraders were observed. The fact that alkylated PAHs biodegradation was not observed and that the PAHs compounds formed only about 2.5% of the components could explain in part the non-detection of PAH degraders using the MNP methods. Another explanation could be associated with the limitations of the MPN method itself. An inhibition of PAH degrader growth by high salinity (29‰) was also possible. In similar experiments that we performed at low salinity (6.5‰) conditions, PAH degraders were observed at ~10^5 cells/mL [22]. A previous study also showed that an increase in salinity can cause an inhibition of PAH degradation activity [29]. These authors observed a suppression of the activity of *Sphingomonas paucinobilis* BA 2 (anthracene degraders) and strain BP 9 (pyrene degraders) as well as autochthonous soil bacteria, which they attributed to an increase in pore-water salinity in the soil to the range of marine environments.

After day 14, the biodegradation rate remained relatively steady in the LN until day 28 while it became very slow in the HN. In the LN, the slower biodegradation rate was presumably due to the lack of nutrients to stimulate maximum microbial activity. The estimated N and P consumption due to biomass growth was much lower in the LN than the HN (Cf. Table 3). The net yield of biomass per mass oil removal was ~4 times lower in the LN than the HN (Cf. Table 2). As a result, it took a longer time for the bacteria to remove the most easily degradable hydrocarbon compounds in the LN. In the HN, the most easily degradable alkane compounds have been presumably depleted within 14 days due to the abundance of nutrients favorable to maximum microbial activity as O_2 was not a limiting factor. This is consistent with the statistical analyses providing evidence of significant alkane biodegradation within the first 14 days of the experiments while no significant changes were

observed after day 14. Once these compounds were removed, it appeared that the biodegradation rate became no longer dependent on the availability of nutrients. The decrease in the biodegradation rate after day 28 in the LN and day 14 in the HN could have resulted from an increase in the mass fraction of recalcitrant compounds i.e., barely biodegradable compounds such as high-molecular-weight alkylated PAHs, resins and asphaltenes [30]. Consistently, there was no statistical evidence of alkylated PAHs biodegradation during the experiment. In addition, during the aerobic biodegradation processes, some hydrocarbons that have not fully mineralized could form recalcitrant oxy-hydrocarbons as reported in a recent study on oil weathering after the Deepwater Horizon disaster [31]. Our findings are concordant with previous studies showing an increase in oil biodegradation rate with adequate nutrient supplementation [13,32] and a decrease in the rate concomitant with the disappearance of low-molecular-weight hydrocarbons [13,30,33] even if nutrient supplementation was maintained [34].

Our findings have also some limitations. Although gas chromatographic–mass spectrometric (GC-MS) approaches are commonly used for the analyses of oil components [35-37], it has been recently recognized that many oil compounds are not chromatographically separated or amenable to GC-MS due to volatility. Thus, our findings on oil biodegraded components do not account for polar compounds that can't be analyzed by conventional techniques. The controlled conditions in the microcosms are also different from those expected in real oil spill scenarios. For instance, dispersed oil and applied nutrients at sea would likely spread and dilute in a large volume of water, thus affecting the overall biodegradation processes. Nonetheless, our microcosm tests indicate that dispersants combined with nutrient supplementation can potentially accelerate oil biodegradation. Although dispersants have been widely used on a large scale to mitigate oil spill like after the Deepwater Horizon wellhead failure in 2010 [2] and field studies have shown the effectiveness of nutrient supplementation in enhancing oil biodegradation [13], we recognize that our findings herein need to be verified on large scale or real oil spill scenarios.

5. Conclusions

The dual effects of chemical dispersion by Corexit 9500 and nutrient supplementation have led to a substantial enhancement of aerobic biodegradation of Endicott oil in seawater. The chemical dispersion (CEWAF) has resulted in a dispersed oil concentration that was an order of magnitude higher than that from the physical dispersion (WAF) under similar mixing conditions. Oil biodegradation remained negligible in the WAF while it was considerable in the CEWAF (up to 44% of TPH removal). Nutrient supplementation (N and P) has increased oil biodegradation by a factor of ~2 in comparison to its biodegradation with the background seawater nutrient conditions (i.e., no nutrients supplemented). The observed values of key biodegradation parameters including O_2 consumption, CO_2 production, biomass increase and estimation of nutrient consumption support our findings regarding the effect of nutrient supplementation on the biodegradation of chemically dispersed Endicott oil in seawater. The findings of this research suggest that the application of chemical dispersants in a high nutrient environment could help mitigate the impacts of oil spills at sea.

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made available upon request and placed in ResearchGate.

Conflict of Interest

Researchers working for oil companies, which might have a policy difference with respect to the use of dispersants.

References

1. McGenity TJ (2014) Hydrocarbon biodegradation in intertidal wetland sediments. *Curr Opin Biotechnol* 27: 46-54.
2. Atlas RM, Hazen TC (2011) Oil biodegradation and bioremediation: a tale of the two worst spills in U.S. history. *Environ Sci Technol* 45: 6709-6715.
3. NOAA (2010) Deepwater Horizon/BP Oil Budget: what happened to the oil. National Oceanic and Atmospheric Administration Report Silver Spring, MD Authors: Lubchenco, Jane; McNutt, Marcia; Lehr, Bill; Sogge, Mark; Miller, Mark; Hammond, Stephen; Conner, William.
4. Kaku VJ, Boufadel MC, Venosa AD (2006) Evaluation of mixing energy in laboratory flasks used for dispersant effectiveness testing. *J Environ Eng* 132: 93-101.
5. Zhao L, Torlapati J, Boufadel MC, et al. (2014) VDROP: a numerical model for the simulation of droplet formation from oils of various viscosities. *Chem Eng J* 253: 93-106.
6. Venosa AD, Holder EL (2007) Biodegradability of dispersed crude oil at two different temperatures. *Mar Pollut Bull* 54: 545-553.
7. Ron EZ, Rosenberg E (2002) Biosurfactants and oil bioremediation. *Curr Opin Biotechnol* 13: 249-252.
8. Rosenberg E, Ron E (1999) High-and low-molecular-mass microbial surfactants. *Appl Microbiol Biotechnol* 52: 154-162.
9. NRC (2005) Oil spill dispersants: efficacy and effects: National Academies Press.
10. Boufadel MC, Sharifi Y, Van Aken B, et al. (2010) Nutrient and oxygen concentrations within the sediments of an Alaskan beach polluted with the Exxon Valdez oil spill. *Environ Sci Technol* 44: 7418-7424.
11. Bragg JR, Prince RC, Harner EJ, et al. (1994) Effectiveness of bioremediation for the Exxon Valdez oil spill. *Nature* 368: 413-418.
12. Sharifi Y, Van Aken B, Boufadel MC (2011) The effect of pore water chemistry on the biodegradation of the Exxon Valdez oil spill. *Water Qual Expos Hea* 2: 157-168.
13. Venosa AD, Suidan MT, Wrenn BA, et al. (1996) Bioremediation of an experimental oil spill on the shoreline of Delaware Bay. *Environ Sci Technol* 30: 1764-1775.
14. Boufadel M, Reeser P, Suidan M, et al. (1999) Optimal nitrate concentration for the biodegradation of n-heptadecane in a variably-saturated sand column. *Environ Technol* 20: 191-199.
15. Geng X, Boufadel MC, Personna YR, et al. (2014) BIOB: a mathematical model for the biodegradation of low solubility hydrocarbons. *Mar Pollut Bull* 83: 138-147.
16. Atlas RM, Bartha R (1973) Stimulated biodegradation of oil slicks using oleophilic fertilizers. *Environ Sci Technol* 7: 538-541.
17. Oh YS, Sim DS, Kim SJ (2003) Effectiveness of bioremediation on oil-contaminated sand in intertidal zone. *J microbiol biotechnol* 13: 437-443.
18. Smith VH, Graham DW, Cleland DD (1998) Application of resource-ratio theory to hydrocarbon biodegradation. *Environ sci technol* 32: 3386-3395.

19. Zahed MA, Aziz HA, Isa MH, et al. (2010) Optimal conditions for bioremediation of oily seawater. *Bioresour technol* 101: 9455-9460.
20. Blondina GJ, Singer MM, Lee I, et al. (1999) Influence of salinity on petroleum accommodation by dispersants. *Spill Sci Technol B* 5: 127-134.
21 Mille GM, Almallah M, Bianchi M, et al. (1991) Effect of salinity on petroleum biodegradation. *Fresenius J anal chem* 339: 788-791.
22. Personna YR, King T, Boufadel MC, et al. (2014) Assessing weathered Endicott oil biodegradation in brackish water. *Mar Pollut Bull* 86: 102-110.
23 Fisher SD (2005) Enhanced biodegradation of highly weathered crude oil by chemical oxidation. . St. Louis, MO: Washington University.
24. EPA (1996) Method 3510C: Separatory funnel liquid-liquid extraction. Revision 3, pp. 1-8.
25. Wrenn BA, Venosa AD (1996) Selective enumeration of aromatic and aliphatic hydrocarbon degrading bacteria by a most-probable-number procedure. *Can J Microbiol* 42: 252-258.
26. Rittmann BE, McCarty PL (2001) Environmental biotechnology: McGraw-Hill New York.
27. Venosa AD, Holder EL (2013) Determining the dispersibility of South Louisiana crude oil by eight oil dispersant products listed on the NCP Product Schedule. *Mar Pollut Bull* 66: 73-77.
28. Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol rev* 59: 143-169.
29. Kästner M, Breuer-Jammali M, Mahro B (1998) Impact of inoculation protocols, salinity, and pH on the degradation of polycyclic aromatic hydrocarbons (PAHs) and survival of PAH-degrading bacteria introduced into soil. *Appl Environ Microbiol* 64: 359-362.
30. Atlas R, Bragg J (2009) Bioremediation of marine oil spills: when and when not–the Exxon Valdez experience. *Microb biotechnol* 2: 213-221.
31. Aeppli C, Carmichael CA, Nelson RK, et al. (2012) Oil weathering after the Deepwater Horizon disaster led to the formation of oxygenated residues. *Environ sci technol* 46: 8799-8807.
32. Venosa AD, Campo P, Suidan MT (2010) Biodegradability of lingering crude oil 19 years after the Exxon Valdez oil spill. *Environ sci technol* 44: 7613-7621.
33. Gallego JR, González-Rojas E, Peláez AI, et al. (2006) Natural attenuation and bioremediation of Prestige fuel oil along the Atlantic coast of Galicia (Spain). *Org Geochem* 37: 1869-1884.
34. Wrenn BA, Sarnecki KL, Kohar ES, et al. (2006) Effects of nutrient source and supply on crude oil biodegradation in continuous-flow beach microcosms. *J Environ Eng* 132: 75-84.
35. Douglas GS, McCarthy KJ, Dahlen DT, et al. (1992) The use of hydrocarbon analyses for environmental assessment and remediation. *Soil Sediment Contam* 1: 197-216.
36. Prince RC, McFarlin KM, Butler JD, et al. (2013) The primary biodegradation of dispersed crude oil in the sea. *Chemosphere* 90: 521-526.
37. McFarlin KM, Prince RC, Perkins R, et al. (2014) Biodegradation of dispersed oil in arctic seawater at-1 C. *PloS one* 9: e84297.

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