SUPPORTING INFORMATION

Detailed Hydrocarbon Analysis Methodology

Extraction and analysis of hydrocarbon compounds was performed according to a modified version of EPA method 3510C with accompanying QA/QC protocols. Bacterial and control treatments were extracted for quantification of total petroleum hydrocarbons (TPHs) as well as the specific hydrocarbon compound classes aliphatics (*n*-alkanes C_{12}-C_{40}, and isoprenoids pristine and phytane) and polycyclic aromatic hydrocarbons (PAHs). Samples (25 ml) were transferred to 250 ml separatory funnels, and 15 ml of dichloromethane was added. Sample glassware containers were rinsed with an additional 15 mL of dichloromethane to collect all residual oil. Separatory funnels were shaken for 2 minutes, and the organic layer was collected in a 60-ml vial. The extraction step was repeated 3x, and 2-4 g of anhydrous sodium sulfate was added to the resulting organic extracts. Hydrocarbon fractions were separated using solid-phase extraction (SPE) with silica/cyanopropyl glass columns (SiO2/C3-CN, 1 g/0.5 g, 6 mL) made at the University of South Florida. Fractions were collected by sequentially eluting the extracts with hexane (100%) to collect aliphatic hydrocarbons, and hexane/dichloromethylene mixture (3:1, v:v) to collect aromatic hydrocarbons. Both fractions were concentrated and spiked with d14-terphenyl. All solvents used were at the highest purity available. All solvents used were at the highest purity available and without further purification. All glassware used was previously combusted at 450°C for 4 hours, and rinsed with dichloromethane prior to extraction. An extraction blank was included with each set of samples (10-12 samples) to ensure no contamination from chemicals, glassware and/or laboratory equipment. Extracts were stored in glass amber vials at 4 °C until GC analysis.

Aliphatics and aromatics were quantified in a gas chromatograph/mass spectrometric

detector (GC/MS) in full scan mode (*m/z* 50-550). Splitless injections of 1µL of the sample were conducted, and a RXi®5sil column (30 m x 0.25 mm x 0.25 µm) was used. Quantitative analysis of aliphatics and PAHs were conducted using the IS (internal standard) method. Samples were spiked before extraction with perdeuterated *n*-alkane (d_{50}-Tetracosane, Ultra Scientific) and PAHs (Deuterated PAH Mixture: d_{10}-acenaphthene, d_{10}-phenanthrene, d_{10}-fluoranthene, d_{12}-benz(a)anthracene, d_{12}-benzo(a)pyrene, d_{14}-dibenz(ah)anthracene, d_{14}-benzo(ai)perylene; Ultra Scientific). For *n*-alkanes analysis, GC oven temperature was programmed as 80°C held for 0.5 min, then increased to 320°C at a rate of 10°C min^{-1} and held for 5.5 min. Injector temperature was set to 280°C. Identification and quantification of *n*-alkanes (nC_{12}-nC_{40}) and isoprenoids pristine (Pr) and phytane (Phy) were conducted by comparing with a reference standard (Fuel Oil Degradation mixture, Ultra Scientific; C8-C40 Alkane Certification Standard; Supelco) and the spike standard (d_{50}-Tetracosane). Recoveries were high within QA/QC criteria of 90-120%. Concentrations are expressed as volume sample (L) concentrations. For PAHs, a GC oven temperature was programmed as 60°C held for 8 min, then increased to 290°C at a rate of 6°C/min and held for 4 min, then increased to 340°C at a rate of 14°C/min, and held at the upper temperature for 5 min. The temperature of the MS detector was 250°C. Concentrations of PAHs were calculated using response factors by comparison with a known standard mixture (16-unsubstituited EPA Priority Pollutants and selected congeners: Ultrascientific US-106N PAH mix, NIST 1491a) and the spike standard. When no commercial reference standard was available, compounds were quantitated using the response factor for an isomer. Therefore, the concentrations determined for many of the alkylated PAHs were semiquantitative. Recoveries were generally within QA/QC criteria of 70-120%. Aromatic compounds are expressed as volume sample (L) concentrations.

Outlier in Acinetobacter Oil Degradation

There was an outlier in the dispersed oil treatments inoculated with *Acinetobacter* which produced a higher total peak area than any of the control treatments, after correction for extraction efficiency and total mass of oil added to each replicate. In the main text this outlier was removed (n=2), and the results are displayed in Figure 2B. If we include this outlier (n=3), no significant degradation was observed in the *Acinetobacter*-treated dispersed oil treatments relative to the un-inoculated control ($t = -1.08$, $p = 0.38$, Figure S3).

Table S1. Welch's t-test results comparing the mean total protein concentration at the final timepoint for each treatment listed compared to the respective un-inoculated controls (n=3 in all cases).

Treatment	Mean (µg/ml)	t	p-value
Alcanivorax			
Crude Oil	563.8	17.17	0.004
Dispersed Oil	618.1	9.4	0.011
COREXIT	58.3	4.05	0.015
Acinetobacter			
Crude Oil	1988	36.8	0.0007
Dispersed Oil	1310	7.79	0.016
COREXIT	183.2	20.99	0.002

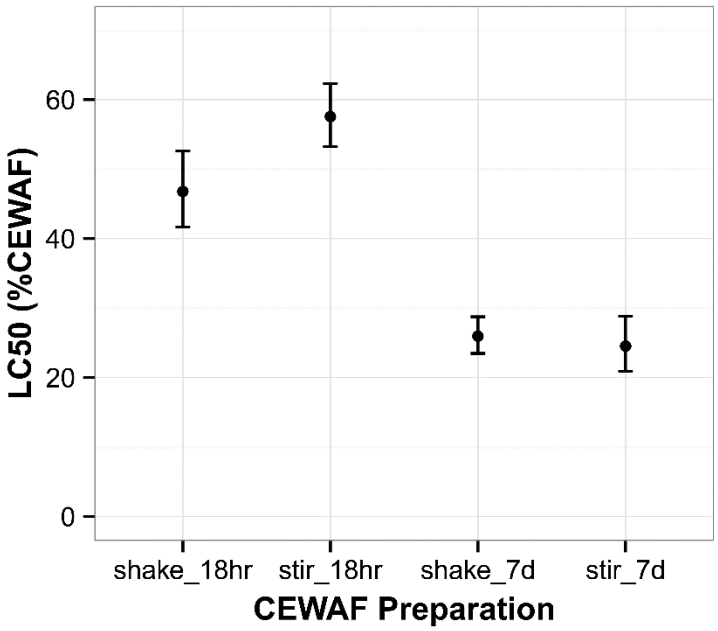


Figure S1. Comparing toxicity results from our modified method for generating WAF compared to Singer et al. (Mar Pollut Bull 40:1007-1016, 2000). Results only include toxicity of the CEWAFs since WAFs were not toxic to *Brachionus plicatilis*.

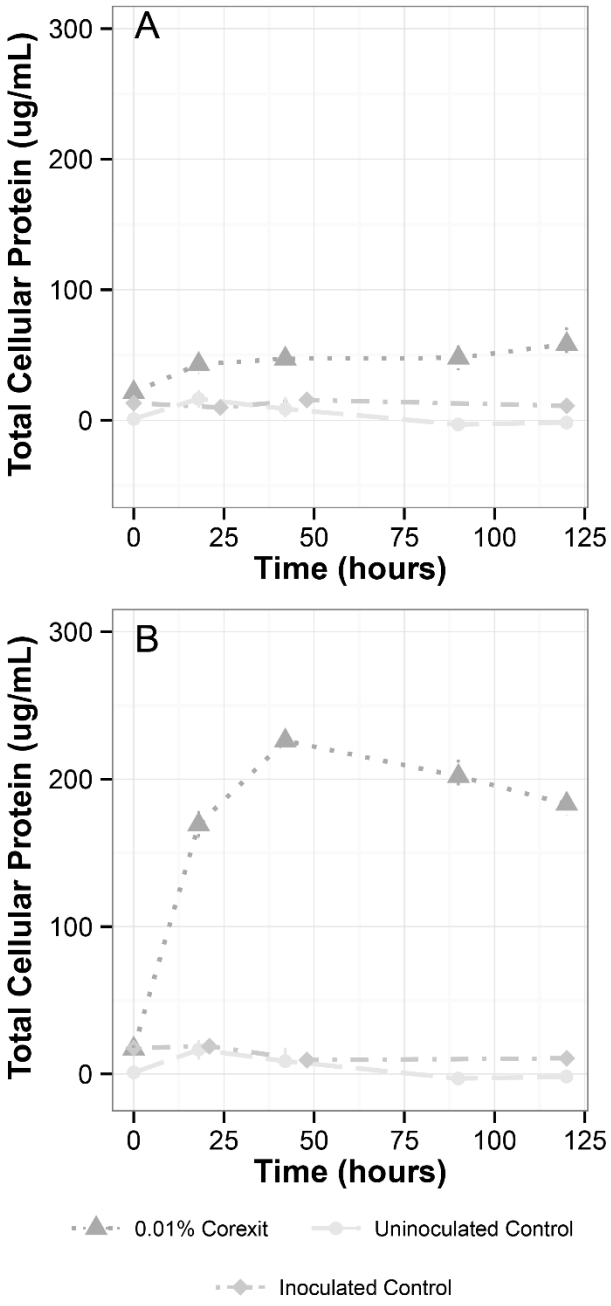


Figure S2. Modified version of Figure 1 demonstrating growth of (A) *Alcanivorax* and (B) *Acinetobacter* on COREXIT alone.

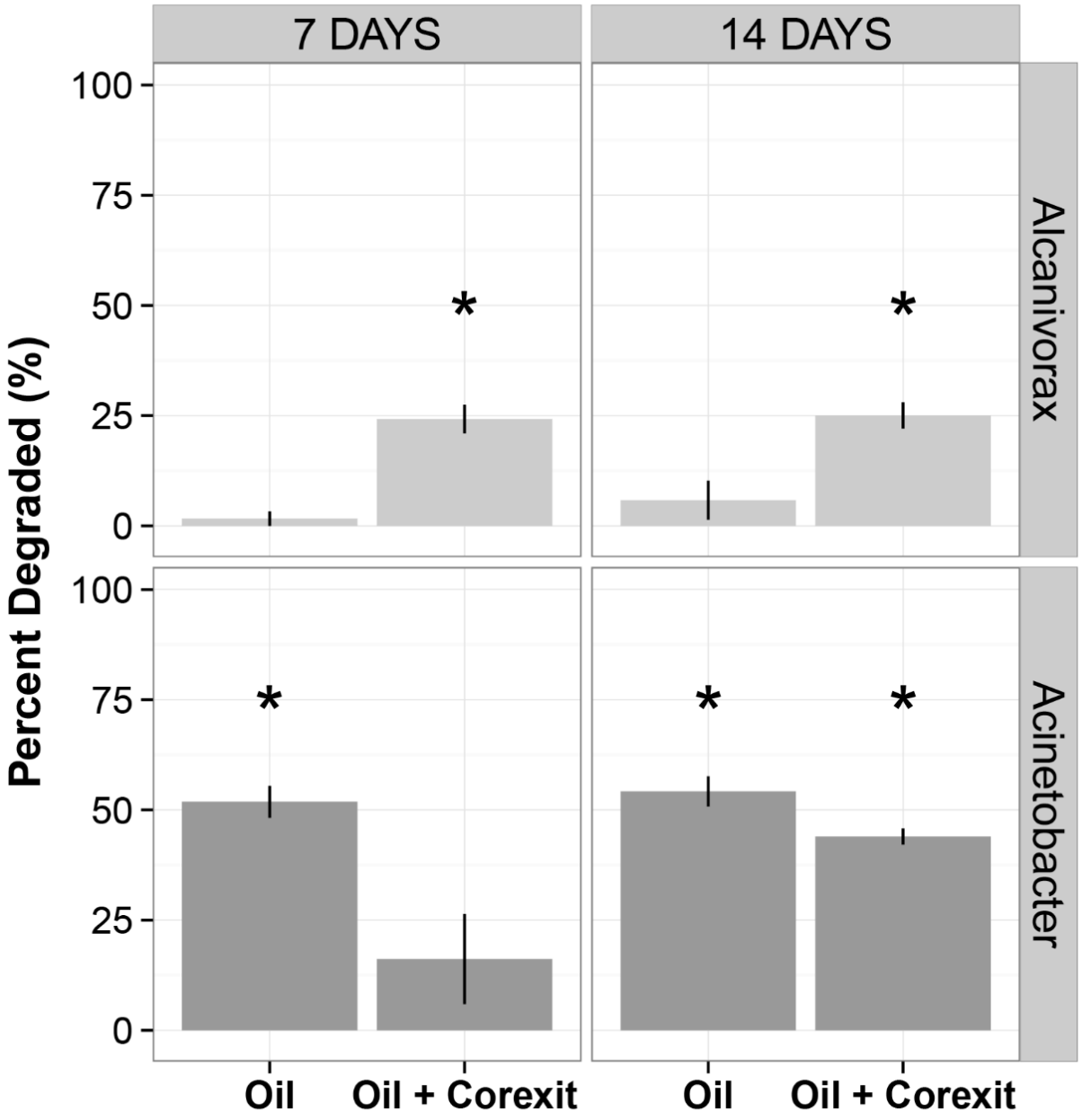


Figure S3. Biodegradation potential of *Alcanivorax* and *Acinetobacter* assessed after extending incubation times to 14 days.

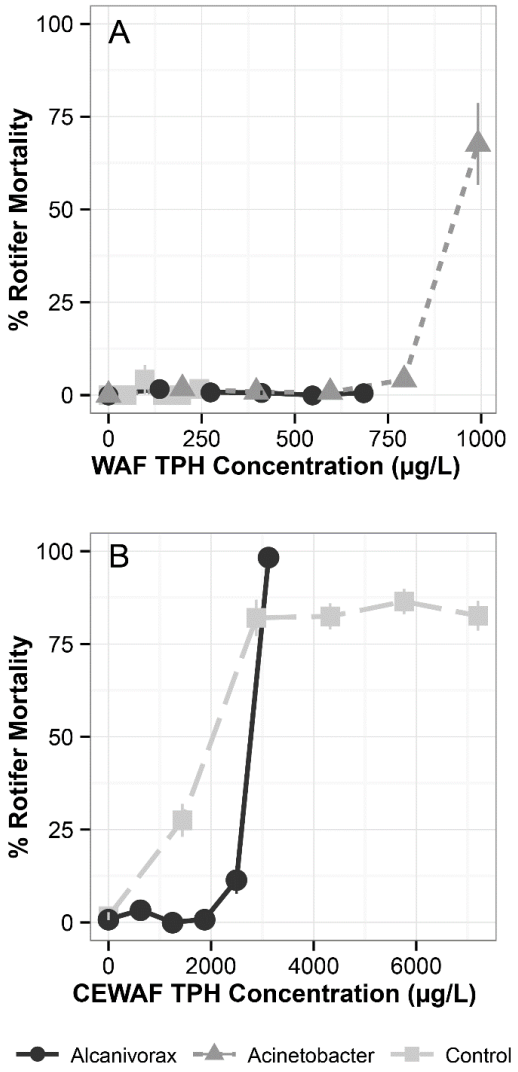


Figure S4. Ecotoxicity of bacterial treated crude oil and dispered crude oil normalized to TPH concentrations using GC/MS values. *Acinetobacter* treated dispersed oil samples were excluded from this figure due to extraction difficulties in separating EPS from hydrocarbons. All toxicity measurements were determined using the marine rotifer *Brachionus plicatilis*. (A) Toxicity associated with 0.5% (v/v) Macondo surrogate crude oil. (B) Toxicity associated with 1:50 COREXIT 9500A dispersed Macondo crude oil.

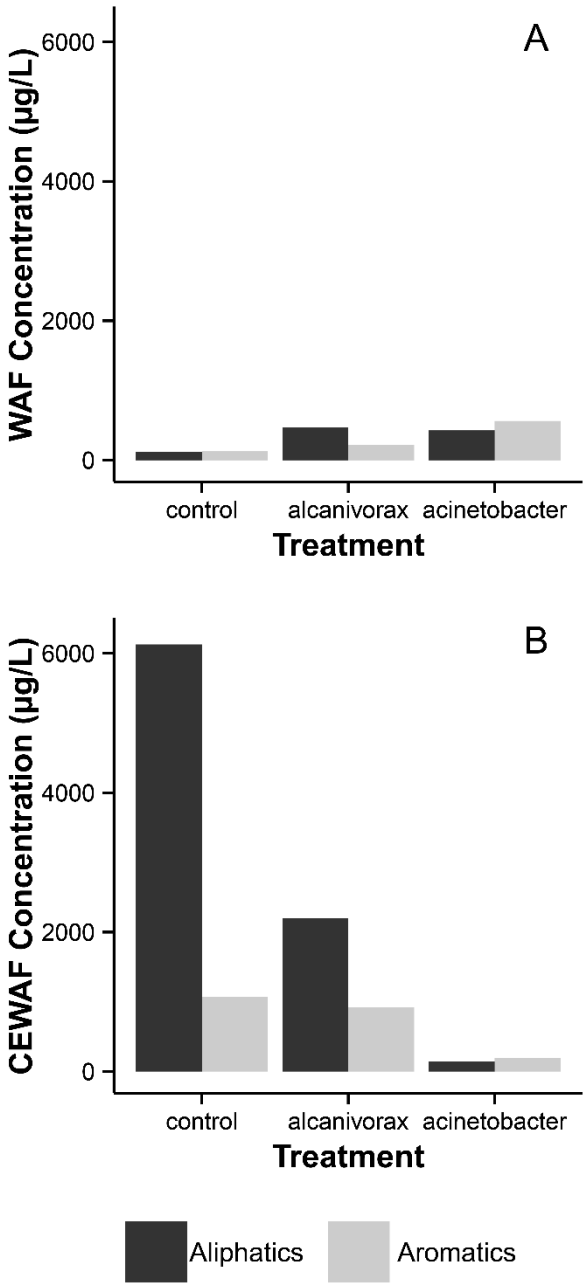
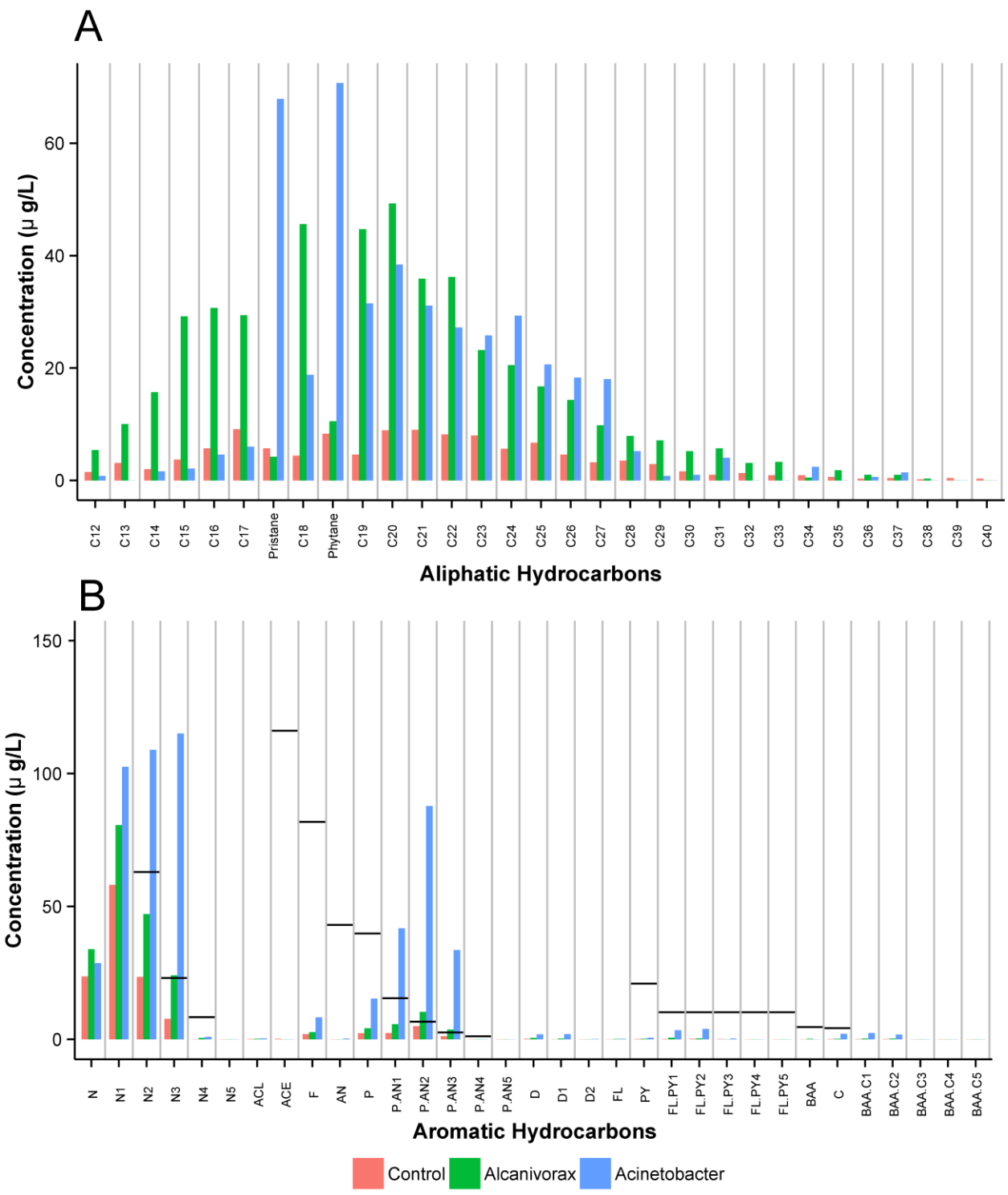


Figure S5. Total aliphatic and aromatic hydrocarbon concentrations in the (A) WAF (crude oil) or (B) CEWAF (dispersed crude oil).


A
Concentration (µ g/L)
0
20
40
60
C12
C13
C14
C15
C16
C17
Pristane
C18
Phytane
C19
C20
C21
C22
C23
C24
C25
C26
C27
C28
C29
C30
C31
C32
C33
C34
C35
C36
C37
C38
C39
C40
Aliphatic Hydrocarbons
B
Concentration (µ g/L)
0
50
100
150
N
N1
N2
N3
N4
N5
ACL
ACE
F
AN
P
P.AN1
P.AN2
P.AN3
P.AN4
P.AN5
D
D1
D2
FL
PY
FL.PY1
FL.PY2
FL.PY3
FL.PY4
FL.PY5
BAA
C
BAA.C1
BAA.C2
BAA.C3
BAA.C4
BAA.C5
Aromatic Hydrocarbons
Control
Alcanivorax
Acinetobacter

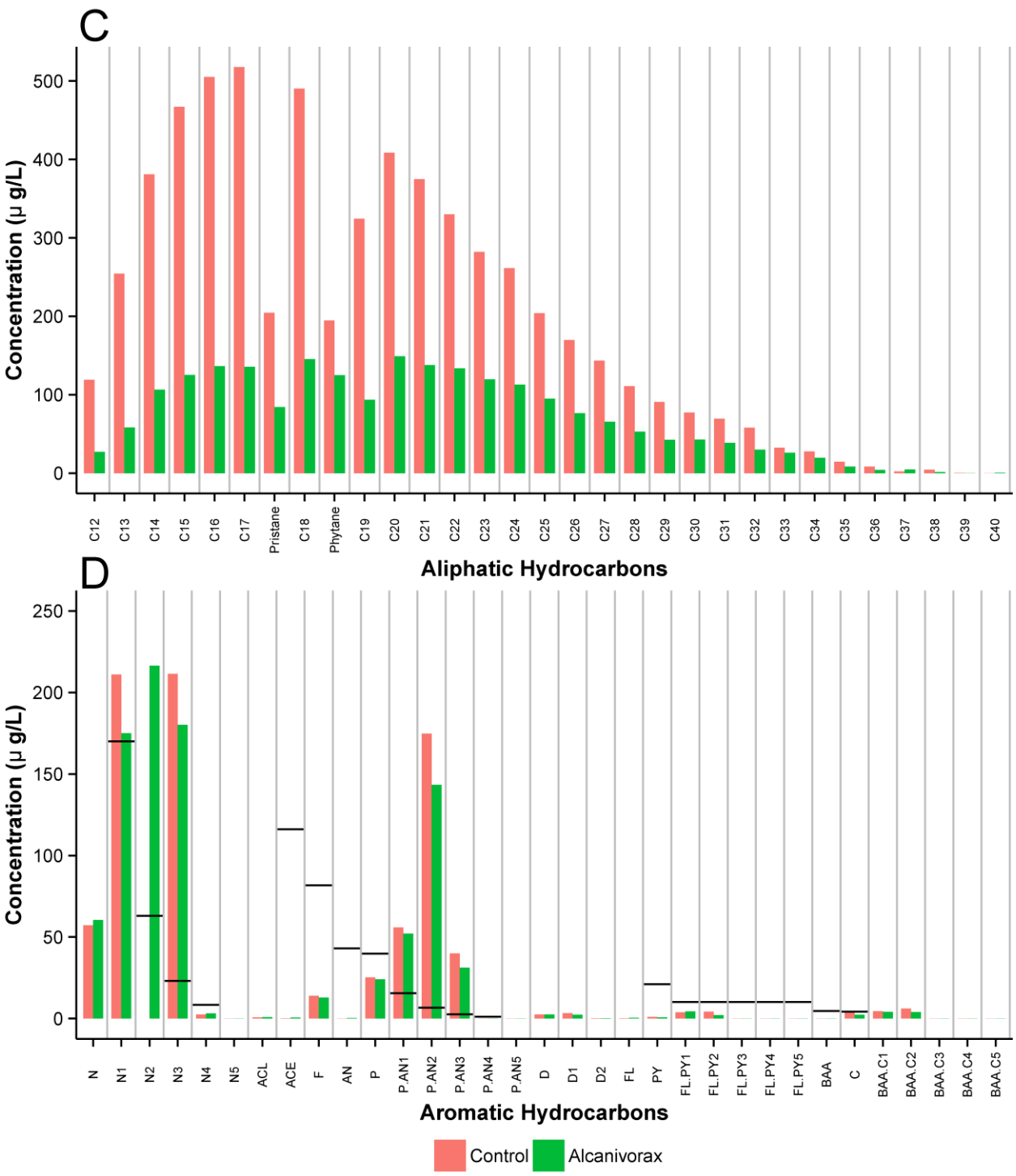


Figure S6. Expanded Figure 4: Detailed analysis of aliphatic (A, C) and aromatic (B, D) hydrocarbons present in the water accommodated fraction from the crude oil treatment (A, B) or the chemically enhanced water accommodated fraction from the dispersed oil treatment (C, D). Controls are indicated by red bars, *Alcanivorax* treated samples by green bars, and *Acinetobacter* treated samples by blue bars. *Acinetobacter* treated dispersed oil samples were excluded from this figure due to extraction difficulties in separating EPS from hydrocarbons. EPA acute

potency divisor values for aromatic hydrocarbons are indicated on plots B, D by black bars. Target PAHs are: Naphthalene (N) and alkylated homologues (N_{C1-C4}), Acenaphthylene (ACL), Acenaphthene (ACE), Fluorene (F), Dibenzothiophene (D) and alkylated homologues (D_{C1-C2}), Phenanthrene (P), Anthracene (AN), and their alkylated homologues (P/AN_{C1-C4}), Fluoranthene (FL), Pyrene (PY), and their alkylated homologues (FL/PY_{C1-C4}), Benz[a]anthracene (BAA), Chrysene (C), and their alkylated homologues (BAA/C_{C1-C4}), Benzo[b]fluoranthene (BBF), Benzo[k]fluoranthene (BKF), Benzo[a]pyrene (BAP), Dibenz[a,h]anthracene (DA), and alkylated homologues (BP/PER_{C1-C4}), Indeno[1,2,3-cd]pyrene (ID), and Benzo[ghi]perylene (BGP).