I, Yu Zhang, hereby submit this original work as part of the requirements for the degree of Master of Science in Environmental Engineering.

It is entitled:
Biodegradability of Dispersant and Dispersed Oil at 5 and 25 °C

Student's name: Yu Zhang

This work and its defense approved by:

Committee chair: Pablo Campo, Ph.D.

Committee member: Robyn N. Conmy, Ph.D.

Committee member: George Sorial, Ph.D.
Biodegradability of Dispersant and Dispersed Oil at 5 and 25 °C

A Thesis submitted to the
Division of Research and Advanced Studies
of the University of Cincinnati

In Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE

In the Department of Biomedical, Chemical and Environmental Engineering of the College of Engineering and Applied Sciences
2016
By

Yu Zhang
B.E., Environmental Engineering, University of Science and Technology Beijing, China, 2014

Committee

Dr. Pablo Campo-Moreno (Chair)

Dr. Robyn N. Conmy

Dr. George A. Sorial
Abstract

Increasing the surface area to volume ratio of oil droplets, chemical dispersant agents reduce the interfacial tension between oil and water and thus facilitate the biodegradation of spilled oil. Prior to field application of dispersants, several factors should be taken into account such as the product effectiveness, environmental conditions (e.g., temperature, wind and pressure), toxicity to the marine organisms, and the fate and transport of the dispersed hydrocarbons and dispersants themselves.

In this study, experiments were conducted with two crude oils (Alaskan North Slope, ANS and Endicott) and two dispersant products (Corexit 9500 and Finaosol OSR 52) at 5 and 25 °C to determine the biodegradation of dispersants and dispersed oil at environmentally-relevant concentrations. Two oil degrading cultures, isolated from the surface (meso) and deep sea (cryo) waters of the Gulf of Mexico (GOM), were enriched on crude oil at 25 and 5 °C and were used as the inocula. The biodegradation experiments were performed at 5 °C for 56 d and at 25 °C for 48 (or 45) d in sterile GP2 artificial seawater as medium. The time series concentration of dioctyl sulfosuccinate (DOSS), the anionic surfactant in the dispersants, was monitored by liquid chromatography with tandem mass spectrometry (LC-MS/MS), in addition to alkanes and polycyclic aromatic hydrocarbons (PAHs), which were measured by gas chromatography-tandem mass spectrometry (GC-MS/MS).

Although the initial concentration of DOSS in Finaosol OSR 52 was 20% higher than in Corexit 9500, over 90% of the anionic surfactant fraction was metabolized for both types of dispersant products by the end of the experiment at 25 °C, while DOSS persisted at 5 °C for both products. Around 90% of the total alkanes degraded regardless of the temperature or treatment. PAH removal was favored in the presence of dispersant at 5 °C, while there was
no significant degradation at 25 °C. The microbial community structures of the oil samples were also analyzed by polymerase chain reaction (PCR) analysis. Results indicated that the abundance of *alcanivorax*, *pseudidiomarina* and *thallassospira* correlated well with hydrocarbon degradation trends. This study significantly expands on our understanding of biodegradation process of dispersants and oil components at environmentally-relevant conditions and also provides information regarding the bacterial community composition that is important to hydrocarbon degradation.
Acknowledgements

First and foremost, I would like to own my most sincere gratitude to my supervisor Dr. Pablo Campo-Moreno for providing this precious research opportunity. His patience, support and encouragement greatly supported me to overcome many crisis situations in all the time of working on the experiment and writing this thesis.

My deepest thanks also goes to Dr. George Sorial and Dr. Robyn Conmy for devoting their effort and time by serving on my thesis committee. I am grateful to them for the all the discussions with them that helped me to address the technical problems of my work.

I would like to single out Dr. Jorge SantoDomingo for his endless support on PCR analysis. His guidance and encouragement enlightened me the first glance of microscopic world. I would also like to express my appreciation to Dr. Raghuraman Venkatapathy and Ms. Edith Holder for their assistance on experiment running.

I give my additional thanks to all of my fellow colleagues in my research group: Dr. Mobing Zhuang for her endless help and excellent guidance on all the research and sharing her experiment results to make this thesis complete; Miss Ruta Deshpande for her hard work, stimulating discussions and all the unforgettable companionship; and Dr. Devi Sundaravadivelu for her enthusiastic assistance and reminding to help me grow as an independent researcher. I greatly appreciate all of the group members’ contribution, kindness and friendship during my two-year study.

Last but not the least, I am deeply indebted to family, this accomplishment would not have been possible without their love and support.

Thanks you to all the people who have supported and helped me throughout this period!
# Table of Contents

Abstract ........................................................................................................................................... I  

Acknowledgements ........................................................................................................................ IV  

Table of Contents ................................................................................................................................ VI  

List of Figures .................................................................................................................................... VIII  

List of Tables ....................................................................................................................................... X  

Chapter 1 Introduction ......................................................................................................................... 1  
  1.1 Background ................................................................................................................................... 1  
  1.2 Dispersant use during the DWH spill ............................................................................................... 2  
    1.2.1 Dispersant and Dispersion .......................................................................................................... 2  
    1.2.2 Controversy of Dispersant .......................................................................................................... 3  
  1.3 Biodegradability of Dispersant and Dispersed Oil ......................................................................... 4  
    1.3.1 Biodegradability of dispersant ..................................................................................................... 5  
    1.3.2 Biodegradability of dispersed oil ................................................................................................. 5  
  1.4 Microbial Community of Degrading bacteria ................................................................................. 6  
  1.5 Research Objectives ....................................................................................................................... 6  
  1.6 Thesis Structure ............................................................................................................................ 7  

Chapter 2 Material and Method ........................................................................................................... 8  
  2.1 Material ......................................................................................................................................... 8  
    2.1.1 Microbial Cultures .................................................................................................................. 8  
    2.1.2 Chemicals and Reagents ......................................................................................................... 8  
    2.1.3 Sterile Artificial Seawater ...................................................................................................... 8  
  2.2 Treatments ...................................................................................................................................... 9  
    2.2.1 Dispersed oil treatment ............................................................................................................ 9  
    2.2.2 Non-dispersed oil treatment ..................................................................................................... 9  
    2.2.3 Dispersant alone treatment .................................................................................................... 10
2.3 Experimental setup and sampling.............................................................................. 11

2.4 Oil Components and Dispersants Analysis ................................................................. 12

2.4.1 Sampling Procedures ............................................................................................ 12
2.4.2 Hydrocarbon Analysis .......................................................................................... 13
2.4.3 DOSS analysis ...................................................................................................... 15

2.5 PCR Analysis .............................................................................................................. 16

Chapter 3 Biodegradability of Alaska North Slope Crude Oil Dispersed with Corexit 9500 and Finasol OSR 52 ............................................................................................................. 17

3.1 Introduction .................................................................................................................. 17

3.2 Results and Discussion .............................................................................................. 18

3.2.1 DOSS degradation ............................................................................................... 18
3.2.2 Hydrocarbon Degradation .................................................................................... 20
3.2.3 Bacterial Community Effect (PCR analysis) ......................................................... 28

3.3 Effect of dispersant comparison ................................................................................. 32

3.3.1 Biodegradability of Dispersants .......................................................................... 32
3.3.2 Dispersant effect on hydrocarbon degradation ....................................................... 33

Chapter 4 Biodegradability of Alaska North Slope and Endicott Crude Oil Dispersed with Corexit 9500 ............................................................................................................. 36

4.1 Introduction .................................................................................................................. 36

4.2 Results and Discussion .............................................................................................. 37

4.2.1 DOSS degradation ............................................................................................... 37
4.2.2 Hydrocarbon degradation ..................................................................................... 38

4.3 Effect of oil properties comparison ........................................................................... 44

Chapter 5 Summary and Future Research ....................................................................... 49

References ......................................................................................................................... 52
List of Figures

Figure 2.1 Dispersed Oil Preparation (A, B and C) and EU loading (D) ........................................ 11

Figure 3.1 Disappearance of DOSS in the absence (A, C) and presence (B, D) of ANS at 5 and 25 °C. Live treatment are in panels A and B, whereas KCs are in panels C and D ........................................ 19

Figure 3.2 Time series concentration of hopane ................................................................. 21

Figure 3.3 Biodegradation of hopane-normalized total alkanes (A, B) and PAHs (C, D) at 5 and 25 °C without (A, C) and with (B, D) Finasol dispersant. ........................................ 24

Figure 3.4 Removal extent of individual alkane by the end of experiment in the absence (A) and presence (B) of Finasol at two temperatures .............................................................. 25

Figure 3.5 Biodegradation of hopane-normalized total alkanes (A, B) and PAHs (C, D) at 5 and 25 °C without (A, C) and with (B, D) Finasol dispersant in KCs samples ........................................ 26

Figure 3.6 Loss rates of individual PAH by the end of experiment in the absence (A) and presence (B) of Finasol at two temperatures .............................................................. 27

Figure 3.7 Changes in Microbial community structures of meso cultures on DNA genus level in ANS alone treatment of Phase I (A) and Phase II (B) experiments ........................................ 29

Figure 3.8 Changes in Microbial community structures of meso cultures on RNA genus level in ANS alone treatment of Phase I (A) and Phase II (B) experiments ........................................ 29

Figure 3.9 DNA and RNA Changes in individual species on genus level in ANS alone treatment of Phase I (A, C, E) and Phase II (B, D, F) experiments ........................................ 31

Figure 3.10 Biodegradation of n-alkanes (A-H) and branched alkanes (I, J) with two dispersants at 5 (left panels) and 25 °C (right panels). .............................................................. 34

Figure 3.11 Loss rates of indvivial PAH for two dispersed oils after 56 days at 5 °C ...................... 35

Figure 4.1 Disappearance of DOSS in the absence (A, C) and presence (B, D) of Endicott at 5 and 25 °C. Live treatments are in panels A and B, whereas KCs are in panels C and D ........................................ 38

Figure 4.2 Time series concentration of hopane ................................................................. 39
Figure 4.3 Biodegradation of hopane-normalized total alkanes (A, B) and PAHs (C, D) at 5 and 25 °C without (A, C) and with (B, D) C9500 dispersant…………………………………………..41

Figure 4.4 Removal extent of individual alkanes by the end of experiment in the absence (A) and presence (B) of C9500 at two temperatures…………………………………………………………..41

Figure 4.5 Biodegradation of hopane-normalized total alkanes (A, B) and PAHs (C, D) at 5 and 25 °C without (A, C) and with (B, D) C9500 dispersant in KCs samples……………………………………..42

Figure 4.6 Removal extent of individual PAHs by the end of experiment in the absence (A) and presence (B) of C9500 at two temperatures…………………………………………………………..43

Figure 4.7 Alkanes distribution for ANS and Endicott crude oils ……………………………..45

Figure 4.8 PAHs distribution for ANS and Endicott crude oils ……………………………..45

Figure 4.9 Biodegradation of n-alkanes (A-H) and branched alkanes (I, J) with two oils dispersed by C9500 at 5 (left panels) and 25 °C (right panels)…………………………………………………………..47

Figure 4.10 Loss rates of individual PAHs for two dispersed oils after 56 days at 5 °C…………..48
List of Tables

Table 2.1 Summary of Experimental Layout for Phase II Experiment (ANS and Finasol).................12
Table 2.2 Instrumental Conditions for Crude Oil Analysis.........................................................14
Table 2.3 Gradient Conditions for DOSS Liquid Chromatography ........................................16
Table 2.4 Mass Spectrometer Parameters .............................................................................16
Table 3.1 Oil Residual in Cultures (phase II).................................................................22
Table 3.2 Initial Concentration of individual alkanes in dispersed oil at 25 °C..........................26
Table 3.3 Summary of Biodegradability of two Dispersants at 25 °C ..................................32
Table 4.1 Oil Residual in Cultures (phase III).................................................................39
Table 4.2 Physical Properties of ANS and Endicott.........................................................44
Chapter 1 Introduction

1.1 Background

Pollution caused by oil spills can be devastating for the environment and there is a continuing need to remediate accidental and operational releases of oil during production, transportation, and storage. Approximately 5.74 million tons of oil entered aquatic systems as a result of oil spill incidents from 1970 to 2014 worldwide (Oil Tanker Spill Statistics, 2015). Spilled oil forms slicks on the surface of water, which can prevent the photosynthesis and respiration of marine organisms, and subsequently disperses into the water column with potential impacts on shoreline biota (Nagarajan, 2008). Furthermore, spilled oil can directly affect human health through inhalation, ingestion, dermal irritation, or bio-accumulation (Solomon and Janssen, 2010). Additionally, spills may depress the local economy, since activities like fishing, shipping, and tourism are disrupted.

To minimize the effects of spilled oil on ecosystems and humans, various countermeasure strategies have been implemented, including physical and mechanical methods, thermal treatments, chemical and biological methods (USEPA, 1999). Containment and recovery are the primary response methods. The former consists in the application of booms and barriers to isolate the spill within a specific zone, while the latter includes skimming to remove oil slicks from the water surface. Nevertheless, these technologies are less effective under high winds or other harsh environmental conditions (Etkin, 2000). In-situ burning is another remediation method that involves the ignition and combustion of oil on the surface of the water at the location of spill (USEPA, 1999). The advantages of this approach are simple logistics, high elimination rates and burn efficiencies, versatility, and cost. The
disadvantages are the uncontrollability of the fires and potential environmental and human health threats owing to combustion (Mullin and Champ, 2003). In aquatic environments, chemical dispersants act on spilled oil by breaking down slicks into micron-sized buoyant droplets with high surface area, which facilitates evaporation and biodegradation (Prince et al., 2013; National Research Council of the National Academies, 2005). Although dispersants have been applied on a large scale in many responses, their use is still highly debated.

1.2 Dispersant use during the DWH spill

During the GOM incident in 2010, the oil spill lasted for almost three months, and approximately 4.9 million barrels of crude oil were released into GOM (Kostka et al. 2011; Reddy et al. 2012). As one of the remediation strategies, 2.1 Mgal of dispersant, mainly consisting of Corexit 9500 and 9527, were delivered to the surface waters (1.4 Mgal) and deep-sea wellhead (0.77 Mgal) in order to accelerate dispersion (Kujawinski et al., 2011; National Commission on the BP GOM Oil Spill and Offshore Drilling, 2011). The unprecedented nature of the spill resulted in the authorization to inject the dispersant directly into the subsea at the wellhead, which had never been tried before (Deepwater Horizon Incident Joint Information Center, 2010). However, several environmental scientists expressed concern about the toxicity, fate, and transport of the dispersant and the dispersed oil (Gong et al. 2014; National Research Council, 2005)

1.2.1 Dispersant and Dispersion

Dispersants are blends of solvents, surfactants, and additives (Gretchen et al., 2013; Kujawinski et al. 2011). Surfactants, as the active ingredient, are the amphiphile with lipophilic (‘oil-compatible’) tail and hydrophilic (‘water-compatible’) head (Abdelrahim,
2012; Salager, 2002; National Research Council, 2005). Hence, once the dispersant is added, the surfactants greatly reduce the oil-water interfacial tension and form a bridge between the water and oil phase by aligning hydrophilic groups towards the water and connecting the hydrophobic groups to oil (International Association of Oil & Gas Producers, 2015). Given that the effectiveness of dispersant is closely related to the solubility, hydrophilic-lipophilic balance (HLB) is a parameter often used to characterize the solubility of surfactant. This parameter ranges from 0 to 20 and varies with the affinity for oil and water: substances with lower values have a tendency to dissolve in water, whereas higher values indicate a greater affinity for oil. Most commercial oil dispersants have an overall HLB number in the range of 9 to 11 (Abdelrahim, 2012). Solvents reduce the viscosity and promote the transfer and distribution of surfactants to the oil-water interface. Together with sufficient mixing energy, surfactants enhance formation of tiny oil droplets which are small enough to be dispersed into the water column (Sorial et al. 2004). The mixing energy could be waves and wind at the surface area and turbulence and currents at the wellhead, respectively (International Tanker Owners Pollution Federation Limited, 2011).

1.2.2 Controversy of Dispersant

As one of the most effective oil spill response strategies, chemical dispersants continue to be widely studied. Dispersants can be used in harsh weather conditions even when other physical and chemical countermeasures may not be well suited (Lessard and Demarco, 2000). Additionally, the presence of surfactant around the oil droplets prevents minimizes the possibility of the recoalescence and resurfacing. At the same time, dispersion accelerates the biodegradation and alleviates the environmental impact of the spilled oil by increasing surface area exerted to hydrocarbon degrading bacteria (Chapman et al. 2007).
However, the effectiveness of dispersants is limited by numerous factors, like the properties of the oil, slick thickness, dispersant-to-oil ratio, surfactant loss at the water surface, mixing energy, emulsion formation, and water salinity (Sorial et al., 2004). Furthermore, toxicity is also concern for dispersants. In terms of acute toxicity, the two dispersants in this study, C9500 (96 h LC$_{50}$ = 25.2 mg/L) is less toxic than Finasol (96 h LC$_{50}$ = 11.66 mg/L) to *Menidia beryllina* (National Product Schedule, 2016). Many studies indicated that dispersants were generally less toxic than oil itself after evaluating the aquatic toxicity of crude oils, dispersants, and dispersed oil (USEPA, 2010; National Product Schedule, 2016; Hemmer et al., 2014). However, some tests indicated that the mixture of dispersant and oil increased the toxicity of crude oil to some organisms (Gulec and Holdway, 1997; Barron et al., 2014). Thus environmental scientists and toxicologists continue to heavily debate the use of dispersants for oil spill response.

### 1.3 Biodegradability of Dispersant and Dispersed Oil

As physical and, in some cases, chemical methods are rarely completely successful, the ubiquitous hydrocarbon-degrading bacteria are considered to be the predominant agents for actual removal of contaminants from the environment (Leahy and Colwell, 1990; Prince, 1997). In the aftermath of DWH, lingering concentration of DOSS, the anionic surfactant of Corexit, was detected both in deep waters. Campo. et al. (2013) and Zhuang et al. (2015) assessed the biodegradation of dispersant and dispersed oil at 5 and 25 °C to simulate the deep sea and surface conditions and concluded that the process was highly dependent on temperature.

As most of the previous laboratory studies were conducted at significantly high initial oil concentrations (Brakstad et al., 2015; Venosa and Holder, 2007), one of the challenges
was to test the biodegradability at a lower environmentally-relevant concentration (Lee et al., 2013). Zahed et al. (2010) studied the effect of initial oil concentration (100 to 2000 mg/L) on biodegradation, with and without the addition of dispersants and concluded that the highest hydrocarbon removal occurred at lower concentrations of crude oil. Thus, the objective of this test is to examine the biodegradability of dispersants and oils at a more environmentally-relevant concentration.

1.3.1 Biodegradability of dispersant

Recently, much research has aimed at the biodegradation of dispersant surfactants with and without oil. The degradation rates of C9500 and JD-2000 were compared in the absence and presence of South Louisiana crude oil (Campo. et al. 2013; Zhuang et al., 2015), as well as the ANS crude oil (Abulikemu, 2015). Furthermore, the fate of the surfactants was evaluated at 5 and 25 °C in all these three studies. JD-2000 presented a higher degradation rate at 5 °C than at 25 °C. Finasol is more toxic compared to the well characterized and studied C9500, and its effectiveness and biodegradability are still unknown. Additionally, all the available research related to the anionic surfactants degradation had been conducted at high concentration. Thus, the degradation at lower concentration was investigated in this study.

1.3.2 Biodegradability of dispersed oil

The effect of chemical dispersion on the biodegradation of hydrocarbons has been investigated for several decades. Nevertheless, several studies have contrary conclusions on whether the presence of dispersants enhanced the biodegradability of dispersed oil. While some researchers concluded that the addition of dispersant had a positive influence on oil degradation (Kleindienst et al., 2015; Venosa and Holder, 2007; Prince et al., 2013), other
studies seem to suggest otherwise (Kleindienst et al. 2015). Moreover, Rahsepar et al. (2016) found that the inhibition of biodegradation depends on the dispersant to oil ratios.

Hence, prior research suggests that the fate of dispersant surfactants and dispersed oil are dependent on the initial concentration of substrates, chemical properties of the surfactants, oil composition, temperature and community of microorganisms.

1.4 Microbial Community of Degrading bacteria

Several studies on bacterial enrichments have enabled identification of bacterial communities and active genes that are functionally important in the biodegradation of hydrocarbons (Gallego et al., 2014). Previous studies focused on the characterization of the dominant hydrocarbon-degrading bacteria among the bacterial consortia by 16S rRNA gene sequencing (Baelum et al., 2012; Cui et al., 2008).

To date, few studies have examined the effect of dispersants on both biodegradation and microbial community structures. Zolfaghari et al. (2012) explored the effects of dispersants on biodegradation of dispersed oil by analyzing the growth of specific bacteria, rather than the whole community. In addition, Coulon et al., (2007) focused on optimization of the most efficient temperature for biostimulation of the oil-degrading bacterial communities. Moreover, the difference between microbial community structures resulted in opposite effect of dispersant on hydrocarbon degradation by evaluating MC-252 oil and C9500 (Chakraborty et al., 2012; Baelum et al., 2012). The bacterial community investigation of the enriched cultures provided a more complete understanding of their roles in the fate of oil (Gutierrez et al., 2013).

1.5 Research Objectives

There were three phases of a longer project. Phase I studied the biodegradation of ANS
dispersed with C9500 which was conducted by Mobing Zhuang in 2013 (Zhuang, 2016). Phase II and III are the biodegradation studies herein reported of ANS dispersed with Finaol and Endicott dispersed with C9500, respectively. The effect of C9500 and Finaol on ANS was evaluated through comparative study of Phase I and II. Meanwhile, the impact of C9500 on ANS and Endicott crude oils was tested by comparing Phase I and III. The specific objectives of this thesis are:

1) to evaluate the biodegradation of two dispersants (Finaol OSR 52 and C9500) and two crude oils (ANS and Endicott) and two dispersed oils (ANS dispersed by Finaol and Endicott dispersed by C9500) at environmentally-relevant concentrations.

2) to measure biodegradation rates at 5 °C, corresponding to average temperature at depths closed to the wellhead, and at 25 °C, corresponding to average temperature in the top 5 m.

3) to study the time-dependent changes in the microbial communities and identifying active microbial populations for meso cultures in oil alone treatment.

1.6 Thesis Structure

This thesis includes four chapters. In Chapter 1, the background of this thesis is introduced followed by proposed research objectives. Chapter 2 focuses on experiment setup, treatment preparation and sample analysis methods. In Chapter 3, the biodegradability of Finaol and ANS dispersed with Finaol was investigated. Furthermore, the effect of the two dispersants (C9500 and Finaol) on ANS was compared. Chapter 4 discusses the effect of C9500 on Endicott and ANS by comparing the degradation of aliphatic and aromatic compound. Chapter 5 provides a brief summary of the results and the main finding of this research.
Chapter 2 Material and Method

2.1 Material

2.1.1 Microbial Cultures

Two frozen mixed cultures of oil degrading bacteria were provided by the U.S. Environmental Protection Agency (EPA). These cultures were previously enriched, grown, and frozen in 5-mL vials containing glycerol by EPA’s Gulf Ecology Division at Gulf Breeze, FL. The first mixed culture (denoted as cryo) was isolated from the water at a depth of 1240 m of the GOM and enriched on South Louisiana crude oil at 5 °C. The second culture (denoted as meso) was isolated from the top 5 m of the GOM and enriched at 25 °C.

2.1.2 Chemicals and Reagents

The Agency provided the ANS and Endicott crude oils. Two types of dispersant products were used in this study, Finasol OSR 52 and C9500, both of which were obtained from the EPA as well. Standards for DOSS were purchased from Aldrich (St. Louis, MO) and its deuterated surrogate (D_{17}-DOSS) was acquired from Isotec (Miamisburg, OH). Acetonitrile (ACN), deionized ultra-filtered water, mineral salts, and dichloromethane (DCM) were obtained from Fisher Scientific (Pittsburgh, PA). Sylon CT for deactivating the glassware was purchased from Sigma-Aldrich (St. Louis, MO).

2.1.3 Sterile Artificial Seawater

Sterile artificial seawater, GP2, was prepared by dissolving the required amounts of mineral salts in distilled water. This solution was autoclaved at 121 °C and 15 psi for 30 min in batches of 1 L. The concentration of each salt (expressed in g/L) were NaCl (21.03), MgCl_2.6H_2O (9.5), Na_2SO_4 (3.52), CaCl_2.2H_2O (1.32), KCl (0.61), KBr (0.088),
NaB₄O₇.10H₂O (0.034), SrCl₂.6H₂O (0.02), NaHCO₃ (0.17) FeCl₃.6H₂O (0.05), Na₅P₃O₁₀ (0.297) KNO₃ (2.89) (Bidwell and Spotte, 1985).

2.2 Treatments

2.2.1 Dispersed oil treatment

The procedure of preparing dispersed oil involved making three batches of dispersed oil in baffled flasks and compositing them in a glass carboy. The composite was continuously mixed to keep the suspension from settling. A single dispersed oil batch was prepared by spiking 240 µL of ANS onto the surface of 1.5 L GP2 artificial seawater in a 2-L baffled flask, followed by 10 µL of Finasol onto the oil slick to achieve a volumetric dispersant-to-oil ratio of 1:24 (Panels A and B of Fig. 2.1). The mixture was shaken at 200 rpm for 20 min to provide necessary turbulence for dispersion (Panel C of Fig. 2.1). The mixing was followed by a 10-min quiescent period to allow undispersed oil to float to the surface. Then, the dispersion was drained into the carboy with 12 L of sterile GP2 through the bottom stopcock without disturbing the top layer of undispersed oil. Meanwhile, the carboy was mixed continuously until 3 L of the dispersed oil was diluted. Subsequently, a series of 250-mL silanized shaker flasks were filled with 100 mL aliquots of the dispersed oil for both dispersed oil treatment and their killed controls (KCs). The same procedure was repeated for Endicott dispersed with C9500.

2.2.2 Non-dispersed oil treatment

In this treatment, only ANS or Endicott were tested. The final concentration of oil in the samples was approximately 28 µg/L, which was almost the same as the dispersed oil treatment. Hence, approximately 4 µL ANS (or Endicott) was directly pipetted into each shake flask containing 100 mL sterile GP2 media. The inoculum volume was 0.5 mL per
flask with the cryo and meso cultures used in the 5 and 25 °C experiments, respectively.

2.2.3  Dispersant alone treatment

In order to evaluate the biodegradation of Finasol (C9500) alone, tests were conducted where the only substrate was the dispersant. For this, a single batch of sterile GP2 (14 L) was spiked with 20 µL of Finasol or C9500 under continuous mixing, to yield a concentration similar to the dispersed oil treatment. From this stock, the experiment units (EUs) for both temperatures were prepared by transferring 100 mL of the solution into each flask. The cryo and meso cultures were added to the 5 and 25 °C EUs respectively. Additionally, the killed controls (KCs) were sterilized by adding NaN₃ in a final concentration of 500 mg/L per flask. After preparation, all the shaker flasks were placed on the orbital shakers in corresponding constant temperature rooms (5 or 25 °C) for the duration of the experiment.

Figure 2.1  Dispersed Oil Preparation (A, B and C) and EU loading (D)
2.3 Experimental setup and sampling

The experimental setup outlined in Table 2.1 was used to determine the biodegradation rates for ANS crude oil alone, ANS dispersed by Finasol, and Finasol dispersant alone. Each of the treatments were set up at 5 and 25 °C. At 5 °C, 11 sampling events were taken place at 0, 2, 4, 8, 12, 16, 24, 32, 40, 48, and 56 days, while at 25 °C, there were 10 sampling events on day 0, 2, 4, 8, 12, 16, 24, 32, 40, and 48. For both temperatures, three different treatments were set up, namely, Finasol alone, ANS dispersed by Finasol, and ANS alone. Additionally, Killed Controls (KCs) were run in triplicates for each treatment to account for abiotic losses, if any. These KCs include all ingredients as the live microcosms (i.e., oil, dispersant, nutrients, and culture) but they were sterilized with sodium azide (NaN₃) at a concentration of 500 mg/L per flask. In all the cases, 100 mL of autoclaved GP2 synthetic seawater were used as medium for microbial activity and the orbital shakers were rotated at 200 rpm (Panel D of Fig. 2.1). All the EUs at 5 and 25 °C were spiked with 0.5 mL of mixed cultures named cryo and meso, respectively. At time zero, cultures alone treatment were also prepared by spiking 0.5 mL of corresponding cultures into 100 mL GP2 for examining the residual oil. At a given sampling event, triplicate flasks of each treatment were sacrificed along with those KCs having dispersed oil and dispersant. As for the oil alone treatments, triplicates of the KCs were sacrificed only at the final event.

The experiment with Endicott dispersed by C9500 (Phase III) was set up in the same manner.
Table 2.1 Summary of Experimental Layout for Phase II Experiment (ANS and Finasol)

<table>
<thead>
<tr>
<th>Test</th>
<th>Temperature °C</th>
<th>Treatment</th>
<th>Sampling Events</th>
<th>Sample Replicates</th>
<th>Total Experimental Units (EU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>Finasol alone</td>
<td>11</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>ANS dispersed by Finasol</td>
<td>11</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>ANS alone</td>
<td>11</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>Killed ANS control</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>Killed Finasol control</td>
<td>10</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>Killed ANS + Finasol control</td>
<td>10</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>Biomass Alone</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Subtotal EU’s</strong></td>
<td><strong>165</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>Finasol alone</td>
<td>10</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>ANS dispersed by Finasol</td>
<td>10</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>ANS alone</td>
<td>10</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>11</td>
<td>25</td>
<td>Killed ANS control</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>25</td>
<td>Killed Finasol control</td>
<td>9</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>13</td>
<td>25</td>
<td>Killed ANS + Finasol control</td>
<td>9</td>
<td>3</td>
<td>27</td>
</tr>
<tr>
<td>14</td>
<td>25</td>
<td>Biomass Alone</td>
<td>1</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Subtotal EU’s</strong></td>
<td><strong>150</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Total EU’s for Finasol</strong></td>
<td><strong>315</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*For Endicott dispersed with C9500 (Phase III), all the experimental units are the same as ANS dispersed with Finasol (Phase II).

2.4 Oil Components and Dispersants Analysis

2.4.1 Sampling Procedures

At time 0 and for any given test, three flasks were sacrificed per treatment. Before samples were sterilized, a 2-mL subsample was withdrawn for microbial characterization, which included PCR assays and sequencing. Subsequently, bacterial activity was stopped by adding NaN₃ to every flask in a final concentration of 500 mg/L. KCs (triplicates) were also be removed for the dispersant alone and dispersed oil treatments. For the dispersant alone treatment, a 20-mL subsample was taken for the analysis of its anionic surfactant DOSS by LC-MS/MS. In the flasks containing oil alone, the 100 mL of seawater was extracted with
DCM (See Section 2.4.2) and analyzed for alkanes and aromatics by GC-MS/MS. Finally, in the dispersed oil treatments, after collecting the 20-mL subsample for DOSS analysis, the remaining 80-mL were extracted with DCM and analyzed for oil components by GC-MS/MS. Alkanes included normal aliphatics ranging in carbon number from 10 to 35 and branch alkanes (pristane and phytane). Aromatics included 2-, 3-, and 4-ring PAH compounds and their alkylated homologs (i.e. C\textsubscript{0-4}-naphthalenes, C\textsubscript{0-3}-fluorenes, C\textsubscript{0-3}-dibenzothiophenes, C\textsubscript{0-4}-phenanthrenes, C\textsubscript{0-4}-napthbenzothiophenes, C\textsubscript{0-1}-pyrenes, C\textsubscript{0-3}-chrysenes). Hopane was also detected as a conservative biomarker.

2.4.2 Hydrocarbon Analysis

Liquid-liquid extraction was used to separate the oil components from GP2 with DCM based on SW-846 Method 3500C. After pouring the GP2 into the 250 mL separatory funnels, 1mL of surrogate standard spiking solution (mixture of D\textsubscript{36}-heptadecane, D\textsubscript{50}-tetracosane, D\textsubscript{66}-dotriacontane, D\textsubscript{10}-1-methylnaphthalene, D\textsubscript{10}-pheanthrene, D\textsubscript{10}-pyrene, and 5\textbeta-cholestane) was added to evaluate extraction recovery (acceptable range of 100 ± 30%). Sixty mililiters of DCM was used to rinse the flask and then transferred to the separatory funnel. Each funnel was sealed and shaken vigorously for 1 min with periodic venting to release excess pressure, then kept stationary for 10 min to allow the organic layer to separate from the GP2. The solvent extract was collected in an Erlenmeyer flask. Subsequently, the extraction was repeated two more times with 50 mL of DCM. After the liquid-liquid extraction, the oil extracts were filtered by passing through a funnel containing a glass wool plug and anhydrous sodium sulfate (10 g) to remove the remaining water and then concentrated to 1-2 mL final volume in a TuoboVap II workstation. Finally, a solvent-exchanged into hexane was performed with a final volume of 10 mL. For analysis, 1 mL of
the oil extract was dispensed into auto-sampler vials to which 10 µL of the internal standard solution (including D22 n-dencane, D34 n-Hexadecane, D42 n-Eicosane, D62 n-Triacontane, D8-Naphthalene, D10-Anthracene, D12-Chrysene and 5α-Cholestane) was added.

Analysis of oil components was performed on an Agilent (Palo Alto, CA) 7890A GC coupled with an Agilent 7000 mass selective detector triple quadrupole and an Agilent 7693 series auto sampler. It was equipped with a DB-5 capillary column by J&W Scientific (30 m × 0.25 mm, and 0.25 µm film thickness) to achieved chromatographic separation of the alkanes and aromatics. A modified method based on EPA Method 8270D (2007) was followed. Table 2.2 showed the instrumental conditions for GC-MS/MS.

An initial calibration was running by using six working standards with all the target compounds at the following concentrations: 0.05 mg/L, 0.1 mg/L, 0.2 mg/L, 0.5 mg/L, 1 mg/L, 2.5 mg/L and 5 mg/L. Once the calibration curve had been validated, samples were analyzed. A check standard was run once in every 10 samples to ensure QA consistency.

Table 2.2 Instrumental Conditions for Crude Oil Analysis

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Agilent 7890A Gas Chromatograph - 7000 MS triple quadrupole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>J&amp;W Scientific DB5-MS column (30 m × 0.25 mm, 0.25 µm)</td>
</tr>
<tr>
<td>Carrier Gas</td>
<td>Helium, ultra-high purity grade (99.999%)</td>
</tr>
<tr>
<td>Inlet Temperature</td>
<td>300°C</td>
</tr>
<tr>
<td>Transfer Line (detector) Temperature</td>
<td>300°C</td>
</tr>
<tr>
<td>Oven Temperature Program</td>
<td>35°C for 2 minutes to 310°C at 10°C/minute for 5 min</td>
</tr>
<tr>
<td>Flow Rate</td>
<td>Constant flow at 1mL/min.</td>
</tr>
<tr>
<td></td>
<td>Linear velocity: 36.2 cm/sec.</td>
</tr>
<tr>
<td>Injection Volume</td>
<td>1 µL</td>
</tr>
<tr>
<td>Split/splitless Mode</td>
<td>Splitless</td>
</tr>
<tr>
<td>Total Run Time</td>
<td>34.5 minutes</td>
</tr>
</tbody>
</table>
2.4.3 DOSS analysis

According to the ASTM D7730 Standard Method (ASTM D7730, 2011), DOSS was measured by using a 1200 series liquid chromatograph coupled with a 6410 tandem mass spectrometer (LC-MS/MS) from Agilent (Palo Alto, CA). To prepare the DOSS sample, 20 mL subsamples were collected from the flasks for dispersant alone or dispersed oil treatment. The surrogate, D$_{17}$-DOSS, and NH$_4$CO$_2$H were added to the sample. Subsequently, the samples were diluted to 40 mL final volume with ACN and mixed thoroughly for 1 min. Finally, about 1 mL portions were filtered through 1 mL syringe fitted with PTFE membrane syringe tip filter (0.2 μm pore size) into 2 mL auto sampler vials.

A 2.1 × 50 mm Zorbax Eclipse XBD C18 analytical column was used for the chromatographic separation of the analytes. The pump flow rate and the gradient conditions for the liquid chromatograph are listed in Table 2.3. The mobile phase composition was (A) 99% water +1% ACN with 0.1% formic acid and (B) 99% ACN + 1% water with 0.1% formic acid. The Mass Spectrometer parameters are presented in Table 2.4. For the purpose of quantification, the detection of DOSS and D$_{17}$-DOSS were based on the reactions $m/z$ 421 $\rightarrow$ $m/z$ 81 and $m/z$ 445.4 $\rightarrow$ $m/z$ 81, respectively. Quantification of DOSS and its deuterated surrogate were conducted at the beginning of each sequence, via a seven-point (3, 10, 20, 60, 100, 150 and 200 μg/L) calibration curve. A calibration standard check, which required a percentage deviation less than 35% from the nominal concentration, was analyzed every 10 samples.
Table 2.3 Gradient Conditions for DOSS Liquid Chromatography

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow (mL/min)</th>
<th>%A (Water with 0.1% Formic)</th>
<th>%B (ACN with 0.1% Formic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>0.4</td>
<td>98</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>0.4</td>
<td>60</td>
<td>38</td>
</tr>
<tr>
<td>7</td>
<td>0.4</td>
<td>20</td>
<td>80</td>
</tr>
<tr>
<td>12</td>
<td>0.4</td>
<td>2</td>
<td>98</td>
</tr>
<tr>
<td>12.1</td>
<td>0.4</td>
<td>98</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 2.4 Mass Spectrometer Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Settings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scan Mode</td>
<td>Negative</td>
</tr>
<tr>
<td>Capillary Voltage</td>
<td>4.0 kV</td>
</tr>
<tr>
<td>To Waste</td>
<td>Until 2.6 min</td>
</tr>
<tr>
<td>Segments</td>
<td>3</td>
</tr>
<tr>
<td>Gas Temperature</td>
<td>350 °C</td>
</tr>
<tr>
<td>Gas Flow</td>
<td>6 L/min</td>
</tr>
<tr>
<td>Nebulizer</td>
<td>15 psi</td>
</tr>
</tbody>
</table>

2.5 PCR Analysis

On each sampling day, 2-mL of the collected sample was centrifuged at 10,000 rpm for 10 min, and the biosolids stored at -80 °C for PCR analysis. PCR assays amplified the DNA and generated the sequencing libraries after the total DNA isolation from original collections. PCR assays were conducted in 25 μL volumes, containing Ex Taq Polymerase kit (Takara), 200 nM each of the forward and reverse primer and 2 μL of template. The cycling reaction consisted of the following three steps: a 5 min denaturation at 95 °C to open double strand melts, a 35 cycles annealing (45 s at 95°C, 60 s at 50 °C, 90 s at 72 °C) for the primers to bind the single-stranded DNA template, and a 10 min extension step at 72 °C to synthesize the new DNA by adding dNTPs (Takara Mirus Bio, Madison, WI).
Chapter 3 Biodegradability of Alaska North Slope Crude Oil Dispersed with Corexit 9500 and Finasol OSR 52

3.1 Introduction

First Generation chemical dispersants (industrial detergents) were primarily used in marine oil spills after the first major tanker catastrophe, the Torrey Canyon oil spill, in which large amount of alkylphenol surfactants were delivered to the contaminated area (Clayton, 1993; Committee on Effectiveness of Oil Spill Dispersants, 1989). The addition of dispersant lowered the interfacial tension between spilled oil and water, accelerate the breakdown of oil slicks, and prevent the resurfacing of small droplets (European Maritime Safety Agency, 2010). Nevertheless, it has been claimed that dispersed oil could be more toxic than crude oil itself because of the increased PAH concentration in the water column (Gardiner et al., 2013; Gulec et al., 1997; Goodbody et al., 2013). Moreover, discussions regarding dispersant utilization in deep-sea condition has come to the forefront after the GOM oil spill in 2010.

As one of the most common dispersants, C9500 has been widely studied for decades. Finasol OSR 52, another product that is widely stockpiled in Europe and European Free Trade Association countries, has not been completely evaluated for its toxicity and biodegradability. According to a test by Steffek (2015), the effectiveness of Finasol OSR 52 is similar to that of C9500 when comparing the volume of oil dispersed into the water column and particle size distribution of oil droplets. Aurand et al. (2010) reported the effectiveness of nine different dispersants (Corexit EC9500A, Corexit EC9527A, Finasol OSR 52, JD 2000, Dispersit SPC 1000, Nokomis 3AA, Nokomis 3F4, SAF-RON Gold, and Sea Brat #4) on fresh Mississippi Canyon 252 crude oil. It was found that the Corexit 9500, Corexit 9527, and Finasol OSR 52 outperformed the rest products at all conditions.
In the aquatic environment, biodegradation is one of the predominant oil removal mechanisms in which bacteria utilize the spilled oil as a carbon source and thus degrade the oil components. While chemical dispersants increase the bioavailability of the oil, the degradation of the dispersants themselves is a challenge for the microbial community. As aforementioned, DOSS persisted for months at the GOM subsurface after the injection at the BP oil spill (Kukawinski et al., 2011). Several studies assessed the biodegradation of C9500, while only Bergueiro-Lopez et al. (1997) examined the metabolism of Finasol OSR 52 by employing a mixture of bacteria called BIOLEN IG 30. In order to characterize the degradation rate of DOSS and hydrocarbons, an experiment was set up to study the degradation of Finasol alone, ANS alone, and ANS dispersed with Finasol under two temperature conditions which represented the surface (25 °C) and deepwater (5 °C) environments, respectively. Moreover, these results will be compared with Phase I experiments conducted with ANS and C9500 (Zhuang, 2016).

3.2 Results and Discussion

3.2.1 DOSS degradation

Panels A and B in Fig. 3.1 present the concentration of DOSS, the active anionic surfactant in Finasol, versus sample time with live cultures. At 25 °C, biodegradation was fast in the replicates with or without ANS. The removal extents exceeded 95% after 24 and 48 days, respectively (open symbols). The biodegradation rate coefficients were calculated by fitting the DOSS degradation data to the first-order decay model:

\[ Y = (Y_0 - \text{Plateau}) \times \exp(-k \times X) - \text{Plateau} \]

where \( Y \) is the real-time concentration of target compound, \( \mu g/L \); \( X \) is the time, day; \( Y_0 \) is the \( Y \) value (\( \mu g/L \)) when \( X \) (time) is zero; \( \text{Plateau} \) is the \( Y \) value at infinite times, \( \mu g/L \); \( k \) is first-
order rate coefficient, expressed in reciprocal of the time units, 1/d. As the final concentration of DOSS at 25 °C approached zero, Plateau were constrained to zero.

The first-order rate coefficients were - 0.068 ± 0.006 /d (Finasol + ANS) and - 0.060 ± 0.008 /d (Finasol alone). The addition of ANS did not significantly affect the degradation of DOSS based on the removal rates and extents ($p = 0.2318$). For the KCs of both treatments (open symbol in panels C and D of Fig. 3.1), significant abiotic loss of DOSS was observed. Less than 10% of the initial DOSS concentration remained as the result of hydrolysis (Campo, 2013) by the end of the experiment. The reduction of DOSS followed zero-order kinetics:

$$Y = k_z \times X + Y_0$$  \hspace{1cm} (3-2)
where $k_z$ is the zero-order rate coefficient, $\mu g/L/d$.

The rate constants were $-5.9 \pm 0.4 \mu g/L/d$ and $-5.4 \pm 0.4 \mu g/L/d$ for the KCs of Finasol with ANS and Finasol alone treatments, respectively. In order to identify the microbial uptake from the hydrolysis loss, DOSS concentration from each sampling event were compared with the values of corresponding KCs treatments by a $t$-test ($\alpha = 0.05$). Significant differences were measured after day 12 ($p = 0.029$) and 16 ($p = 0.025$) in the treatment with and without ANS, respectively. Therefore, the biological reaction prevailed over the hydrolysis in the overall degradation process.

Conversely, DOSS remained in both live and KCs treatments at 5 °C (close symbols in Fig. 3.1). These factors together suggest that bioremediation in subsea or cold surface waters is not as effective as in warm surface waters. As Campo et al. (2013), hydrolysis resulted in the abiotic loss at the surface temperature. Hence, the breakdown of DOSS were inhibited in the low temperature.

3.2.2 Hydrocarbon Degradation

As showed in Fig. 3.2, hopane concentration was unchanged through the whole experiments in all the treatments. Hence, all the targets compounds were normalized by hopane concentration in the following discussion. Hopane concentration in ANS alone (Fig. 3.2, circle symbols) was higher than ANS dispersed with Finasol (Fig. 3.2, triangle symbols) due to the preparation method at corresponding temperatures. Samples at 25 °C contained more hopane than those at 5 °C due to the residual oil content in the cultures (Table 3.1).
The contribution of hopane and PAHs by the meso culture is considerably larger than that by cryo culture. Table 3.1 summarized the concentration of hydrocarbons groups in two cultures. The ratios of initial oil concentration and dispersed oil were also calculated to evaluate the effects on each treatment. For the total alkanes, the ratios were 5 (M1), 12 (M2), 6 (C1) and 13% (C2). However, the initial PAHs concentration in the treatments, especially at 25 °C, were considerably affected by the residual oil from the cultures in term of the ratios: 59 (M1), 111 (M2), 5 (C1) and 9% (C2). Hopane from meso were 88% (M1) to ANS alone and 174% (M2) to dispersed ANS, whereas cryo contained 7 (C1) and 16% (C2), respectively.
Table 3.1 Oil Residual in Cultures (phase II)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Meso Cultures (25 °C), µg/L</th>
<th>Cryo Cultures (5 °C), µg/L</th>
<th>ANS without Culture, µg/L</th>
<th>ANS+Finasol without Culture, µg/L</th>
<th>M1</th>
<th>M2</th>
<th>C1</th>
<th>C2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hopane</td>
<td>6.83</td>
<td>0.61</td>
<td>8.87</td>
<td>3.92</td>
<td>77%</td>
<td>174%</td>
<td>7%</td>
<td>16%</td>
</tr>
<tr>
<td>nC10-16</td>
<td>24.47</td>
<td>25.37</td>
<td>684.40</td>
<td>247.52</td>
<td>4%</td>
<td>10%</td>
<td>4%</td>
<td>10%</td>
</tr>
<tr>
<td>nC 17-21</td>
<td>28.27</td>
<td>33.57</td>
<td>430.28</td>
<td>200.97</td>
<td>7%</td>
<td>14%</td>
<td>8%</td>
<td>17%</td>
</tr>
<tr>
<td>nC 22-29</td>
<td>27.66</td>
<td>20.14</td>
<td>393.73</td>
<td>201.22</td>
<td>7%</td>
<td>14%</td>
<td>5%</td>
<td>10%</td>
</tr>
<tr>
<td>nC 30-35</td>
<td>0.00</td>
<td>0.00</td>
<td>89.84</td>
<td>52.50</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>PH+PR</td>
<td>7.56</td>
<td>15.39</td>
<td>111.52</td>
<td>50.58</td>
<td>7%</td>
<td>15%</td>
<td>14%</td>
<td>30%</td>
</tr>
<tr>
<td>Total Alkanes</td>
<td>87.95</td>
<td>94.47</td>
<td>1709.78</td>
<td>752.79</td>
<td>5%</td>
<td>12%</td>
<td>6%</td>
<td>13%</td>
</tr>
<tr>
<td>NAP Group</td>
<td>164.91</td>
<td>4.76</td>
<td>255.31</td>
<td>108.91</td>
<td>65%</td>
<td>151%</td>
<td>2%</td>
<td>4%</td>
</tr>
<tr>
<td>PHE Group</td>
<td>54.45</td>
<td>0.67</td>
<td>96.70</td>
<td>55.83</td>
<td>56%</td>
<td>98%</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>FLN Group</td>
<td>27.75</td>
<td>0.42</td>
<td>34.99</td>
<td>17.90</td>
<td>79%</td>
<td>155%</td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td>DBT Group</td>
<td>36.84</td>
<td>14.87</td>
<td>69.45</td>
<td>47.08</td>
<td>53%</td>
<td>78%</td>
<td>21%</td>
<td>32%</td>
</tr>
<tr>
<td>BNT Group</td>
<td>1.18</td>
<td>0.00</td>
<td>16.30</td>
<td>12.97</td>
<td>7%</td>
<td>9%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>PYR Group</td>
<td>3.16</td>
<td>0.00</td>
<td>9.60</td>
<td>7.86</td>
<td>33%</td>
<td>40%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>CHY Group</td>
<td>2.82</td>
<td>1.89</td>
<td>15.32</td>
<td>10.70</td>
<td>18%</td>
<td>26%</td>
<td>12%</td>
<td>18%</td>
</tr>
<tr>
<td>Total PAHs</td>
<td>293.61</td>
<td>22.91</td>
<td>499.07</td>
<td>263.81</td>
<td>59%</td>
<td>111%</td>
<td>5%</td>
<td>9%</td>
</tr>
</tbody>
</table>

\[
M1 = \frac{\text{Hydrocarbon compound concentration in Meso}}{\text{Hydrocarbon compound concentration in ANS without cultures}}
\]

\[
M2 = \frac{\text{Hydrocarbon compound concentration in Meso}}{\text{Hydrocarbon compound concentration in ANS with Finasol without cultures}}
\]

\[
C1 = \frac{\text{Hydrocarbon compound concentration in Cryo}}{\text{Hydrocarbon compound concentration in ANS without cultures}}
\]

\[
C2 = \frac{\text{Hydrocarbon compound concentration in Cryo}}{\text{Hydrocarbon compound concentration in ANS with Finasol without cultures}}
\]
Alkanes

In Fig. 3.3, panels A and B present the hopane-normalized concentration of total alkanes through the whole experiment. The initial hopane-normalized concentration for 5 °C was almost twice as that of 25 °C because of the lower background hopane concentration from cryo. At 25 °C, alkanes were degraded faster than at 5 °C, regardless of the presence or absence of Finasol. The biodegradation occurred without any lag phase. By day 4, the removal extent was achieved 79% in the dispersed oil treatment, while 41% of alkanes were metabolized by the meso culture in the oil alone samples. In both cases, over 90% of the total alkanes were removed by the end of experiment (day 48). Thus, it is plausible to infer that the addition of Finaosl favored the alkanes’ uptake at the preliminary stage.

As presented in Fig. 3.4, only $nC_{10}$ was completely biodegraded in both treatment at two temperatures. The first-order coefficients for this compound were $-0.43 \pm 0.05 /d$ for ANS alone and $-0.46 \pm 0.04 /d$ for dispersed oil treatment at 25 °C. The rates were similar both in the presence or absence of dispersant. Due to the persistence till the completion of the experiment and extremely rapid depletion after acclimation, a first-order model could not be fitted as previously done for high oil and dispersant concentration studies (Campo et al., 2013; Abulikemu, 2015). Thus, removal extent of individual aliphatic was presented to compare the degradation effectiveness (Fig. 3.4). As for oil alone treatment (part A of Fig. 3.4), the degradation rate declined with the increasing of carbon numbers. However, the uptake of long chain alkanes was enhanced in the presence of Finasol at 25 °C. As Table 3.2 displayed, the initial concentration of heavy alkanes are lower than those of light alkanes in dispersed oil treatment at 25 °C. Thus, the removal extents of the long chain alkanes, especially $nC_{28-35}$, reached 100% since these compounds were easier to approach the undetectable concentration.
Figure 3.3 Biodegradation of hopane-normalized total alkanes (A, B) and PAHs (C, D) at 5 and 25 °C without (A,C) and with (B, D) Finasol dispersant.

As seen in Fig. 3.3, at 5 °C, the biodegradation occurred followed a 4-day acclimation period for ANS + Finasol treatment, whereas oil metabolized without a significant lag for the oil alone samples. As for the individual alkane, only \( nC_{10-11} \) dropped markedly by day 2. A 4-day lag phase was observed for other alkanes except \( nC_{34-35} \) which took longer. By the last sampling event (day 56), 85% and 92% of the total alkane fraction were metabolized for the ANS with and without dispersant treatments (close symbols in panels A and B of Fig. 2). As displayed in Fig. 3.4 (black bars in panels A and B), similar degradation trends were observed in both treatments which reveals that the effect of dispersant is insignificant at 5 °C. The above finding also contrasts with those of Abulikemu (2015), who conducted experiments with ANS crude oil at a higher concentration (~ 0.7 g/L) and C9500 (DOR 1:25) and reported statistically significant enhancement of alkane degradation based on the first
order rate coefficients. In addition, the uptake extent for $nC_{30-35}$ was favored in dispersed oil in our case. However, higher residuals of alkanes was present in all the treatments in this study when comparing with the experiment using higher oil concentration (Abulikemu, 2015).

* $nC_{30-35}$ were completely removed in dispersed oil at 25 °C because those alkanes approached undetectable concentration of GC-MS/MS (0.05 mg/L).

**Figure 3.4** Removal extent of individual alkane by the end of experiment in the absence (A) and presence (B) of Finasol at two temperatures.

Panels A and B of Figure 3.5 show total alkane concentration in parallel KCs treatments at two temperatures for the oil alone and dispersed oil treatments. For the oil alone treatment, KCs were set up only on the final sampling day. A slight loss of $nC_{10-13}$ took place after 8 days at 25 °C, whereas the all the alkanes persisted at 5 °C. The possible reason of this biotic loss was the volatilization of light molecular weight paraffins at high cultivation temperature.
Table 3.2 Initial Concentration of individual alkanes in dispersed oil at 25 °C

<table>
<thead>
<tr>
<th>Alkane Compounds</th>
<th>Initial Concentration (mg/L)</th>
<th>Alkane Compounds</th>
<th>Initial Concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>nC10</td>
<td>0.216 ± 0.014</td>
<td>nC22</td>
<td>0.376 ± 0.009</td>
</tr>
<tr>
<td>nC11</td>
<td>0.312 ± 0.018</td>
<td>nC23</td>
<td>0.356 ± 0.008</td>
</tr>
<tr>
<td>nC12</td>
<td>0.335 ± 0.025</td>
<td>nC24</td>
<td>0.358 ± 0.012</td>
</tr>
<tr>
<td>nC13</td>
<td>0.438 ± 0.023</td>
<td>nC25</td>
<td>0.318 ± 0.010</td>
</tr>
<tr>
<td>nC14</td>
<td>0.443 ± 0.061</td>
<td>nC26</td>
<td>0.288 ± 0.011</td>
</tr>
<tr>
<td>nC15</td>
<td>0.460 ± 0.059</td>
<td>nC27</td>
<td>0.223 ± 0.008</td>
</tr>
<tr>
<td>nC16</td>
<td>0.494 ± 0.010</td>
<td>nC28</td>
<td>0.194 ± 0.005</td>
</tr>
<tr>
<td>nC17</td>
<td>0.504 ± 0.046</td>
<td>nC29</td>
<td>0.166 ± 0.006</td>
</tr>
<tr>
<td>pristane</td>
<td>0.343 ± 0.018</td>
<td>nC30</td>
<td>0.135 ± 0.004</td>
</tr>
<tr>
<td>nC18</td>
<td>0.528 ± 0.025</td>
<td>nC31</td>
<td>0.109 ± 0.006</td>
</tr>
<tr>
<td>phytane</td>
<td>0.241 ± 0.006</td>
<td>nC32</td>
<td>0.084 ± 0.006</td>
</tr>
<tr>
<td>nC19</td>
<td>0.437 ± 0.008</td>
<td>nC33</td>
<td>0.090 ± 0.003</td>
</tr>
<tr>
<td>nC20</td>
<td>0.424 ± 0.009</td>
<td>nC34</td>
<td>0.062 ± 0.003</td>
</tr>
<tr>
<td>nC21</td>
<td>0.400 ± 0.006</td>
<td>nC35</td>
<td>0.061 ± 0.004</td>
</tr>
</tbody>
</table>

Figure 3.5 Biodegradation of hopane-normalized total alkanes (A, B) and PAHs (C, D) at 5 and 25 °C without (A,C) and with (B, D) Finasol dispersant in KCs samples.
Aromatics (PAHs)

Panels C and D of Figure 3.3 present total PAH degradation data for all the treatments at two temperatures. Similar to alkanes, the hopane-normalized PAH concentration at 5 °C was higher than at 25 °C because of the higher hopane residual from the meso cultures. At 25 °C, PAHs persisted in both oil alone and dispersed oil treatment, except for naphthalene and C₁₀-naphthalene. Similar results were obtained after performing the whole experiment at 25 °C again to eliminate any operational error. Such findings are contrary to the previous studies conducted by Zhuang with the same oil and cultures (Zhuang, 2016). To address this problem, the microbial community structure was analyzed by PCR analysis. (See Section 3.3.2)

![Figure 3.6](image)

**Figure 3.6** Loss rates of individual PAHs by the end of experiment in the absence (A) and presence (B) of Finasol at two temperatures.

At 5 °C, a 12-day lag phase occurred before observable degradation ensued in both dispersed and non-dispersed oil treatments. The trends of PAH degradation were similar for ANS alone and ANS with Finasol (p = 0.525), which indicated that the effect of dispersant
on total PAHs microbial uptake was negligible. At the last sampling event, 25% and 28% of the initial loaded concentration persisted for the ANS and ANS + Finasol treatment, respectively.

For the individual aromatic compounds, Fig. 3.6 depicts the removal extent after 56 days. Most of the 2-ring PAHs disappeared in both treatments except the C3-nap in non-dispersed oil. Among the 3-ring aromatics, C0-2-phe, C0-2-fln and C1-dbt were completely degraded, while negligible reduction of 4-ring compounds occurred. However, a first-order decay model could not be fitted due to the long lag phase period (16 days) and rapid decrease. In the case of C3-4-phe and C2-3-dbt, the addition of the dispersant accelerated the biodegradation.

Similar to the alkanes, total PAHs data for corresponding KCs were displayed in Fig. 3.5, which reveals that non-biotic loss occurred through the experiment period. Hence, microbial uptake of cryo completely dominated the PAHs degradation at low temperature.

3.2.3 Bacterial Community Effect (PCR analysis)

As mentioned previously, meso cultures showed significant differences in PAH uptake between Phase I (ANS + C9500) and II (ANS + Finasol) experiments which were performed under same experiments condition, but varying only the dispersant product. PCR analysis of these bacterial consortia provided a clearer understanding of the microbial makeup and an in-depth explanation for the inconsistent result. DNA and RNA compositions of 25 °C ANS alone treatment were investigated to clarify the community structures of the microorganism and microbial taxa actively participating in degradation.
Figure 3.7 Changes in microbial community structures of meso cultures on DNA genus level in ANS alone treatment of Phase I (A) and Phase II (B) experiments.

Figure 3.8 Changes in microbial community structures of meso cultures on RNA genus level in ANS alone treatment of Phase I (A) and Phase II (B) experiments.
DNA and RNA distributions of Phase I (part A) and II (part B) were depicted in Fig 3.7 and 3.8, respectively. According to the DNA community, meso from Phase I and II were dominated by the genera of *vibrio*, *pseudoidiomarina*, *marinobacter*, *alcanivorax*, and *thallassospira*. However, *thallassospira* was remarkably lower in Phase II. Furthermore, several species in the active community including *alcanivorax*, *pseudoidiomarina* and *thallassospira* were abundant in Phase I but not in Phase II.

In the active community, *alcanivorax*, one of the predominant hydrocarbon-degrading bacteria in the contaminated seawater, (Cappello et al, 2007; Harayama et al, 2004; Wang et al, 2014) increased from 0.74% to a maximum of 69.3% of total consortium on day 40 in phase I (red symbols in part A of Fig. 3.9); *alcanivorax* is a ubiquitous bacteria that was found in the GOM during the DWH oil spill (Kostka et al., 2016). However, same species in Phase II presented a constant pattern lower than 20% (part B, Fig. 3.9). Besides, microbes classified as *pseudoidiomarina* comprised of 22 and 32% of reads in DNA and RNA of Phase I samples (part C, Fig. 3.9). On the contrary, *pseudoidiomarina* showed lower abundance between 2-8% (DNA) and 8-14% (RNA) of recovered reads in Phase II (part D, Fig. 3.9). To our knowledge, *pseudoidiomarina* has been isolated and identified as one of the PAH-degrading bacteria from enrichment consortium by using flu and phe as carbon and energy source (Moghadam et al., 2014). Additionally, *thallassospira*, which were absent in both total and active community of Phase II (part B of Fig. 3.7 and 3.8), represented a much higher proportion (~26%) of the active community in Phase I (part F, Fig. 3.9).
Figure 3.9 DNA and RNA Changes in individual species on genus level in ANS alone treatment of Phase I (A, C, E) and Phase II (B, D, F) experiments.
The increase in *pseudidiomarina* and *thallassospira* occurred between day 12 to 24, which corresponded with the PAHs degradation at 25°C in Phase I. This observation also support the role of these species in PAH metabolism among meso cultures. Hence, it is reasonable to infer this loss of active community resulted in the unusual persistence of aromatics at 25 °C.

### 3.3 Effect of dispersant comparison

Effect of temperatures on biodegradability of Finasol, ANS dispersed with Finasol has been discussed in this chapter. The results are also compared with previous study for another dispersant (C9500) with same active surfactant dispersing ANS to further study the effectiveness of Finasol.

#### 3.3.1 Biodegradability of Dispersants

Table 3.3 summarizes the initial concentration, biodegradation rates and removal extents of DOSS at 25 °C. Although the initial concentration of primary surfactant (DOSS) in Finasol was around 20% higher than in C9500, over 95% of the anionic surfactant fraction was metabolized for both types of dispersant products by the end of the experiment at 25 °C while it persisted at 5 °C. The presence of ANS remarkably favored the biodegradation first order rate of C9500 by approximately 2.4-fold, but no such effect was observed for the Phase II experiment with Finasol. In terms of the abiotic loss of DOSS, both rates and extents of two dispersants were slight higher in the presence of ANS at 25 °C. But the removal extents for Finasol were 17 and 12% higher than the corresponding dispersant alone and dispersant oil samples.
Table 3.3 Summary of Biodegradability of two Dispersants at 25 °C

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Initial DOSS Concentration (µg/L)</th>
<th>Degradation rate /d</th>
<th>Removal extent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C9500 alone</td>
<td>203.97±6.98</td>
<td>-0.07 ± 0.009</td>
<td>96</td>
</tr>
<tr>
<td>C9500 + ANS</td>
<td>228.16±11.94</td>
<td>-0.16 ± 0.015</td>
<td>98</td>
</tr>
<tr>
<td>C9500 Alone KCs</td>
<td>203.97±6.98</td>
<td>-4.1 ± 0.3</td>
<td>74</td>
</tr>
<tr>
<td>C9500 + ANS KCs</td>
<td>228.16±11.94</td>
<td>-5.1 ± 0.4</td>
<td>81</td>
</tr>
<tr>
<td>Finasol Alone</td>
<td>280.87±21.19</td>
<td>-0.060 ± 0.008</td>
<td>96</td>
</tr>
<tr>
<td>Finasol + ANS</td>
<td>234.11±5.38</td>
<td>-0.068 ± 0.006</td>
<td>98</td>
</tr>
<tr>
<td>Finasol Alone KCs</td>
<td>280.87±21.19</td>
<td>-5.4 ± 0.4</td>
<td>91</td>
</tr>
<tr>
<td>Finasol + ANS KCs</td>
<td>234.11±5.38</td>
<td>-5.9 ± 0.4</td>
<td>93</td>
</tr>
</tbody>
</table>

3.3.2 Dispersant effect on hydrocarbon degradation

Similar to DOSS degradation, the impact of two dispersants on petroleum hydrocarbons biodegradation were also compared. As for the alkane depletion, higher temperature considerably improved the biodegradation extent of from 85 (5 °C) to 94% (25 °C) for ANS + Finasol samples, while around 85% of aliphatic fraction were removed from ANS dispersed with C9500 at both temperatures. Left panels of Fig. 3.10 show that the degradation trends of all the alkane groups were similar at 5 °C, except for a slightly higher initial loaded concentrations of oil dispersed with C9500. However, at 25 °C, less alkanes remained with the addition of Finasol than those in the ANS dispersed with C9500 treatment, especially nC_{17-29} and iso-alkanes.

Since meso cultures failed to degrade the PAHs in the Finasol experiment, the difference between the two dispersants was only analyzed for the 5 °C condition. As presented in Fig. 3.11, several low molecular weight aromatics (2- and 3-ring), namely, C_{0-4}-nap, C_{0-2}-phe, C_{0-2}-fln and C_{1}-dbt, were completely degraded in both dispersed oil. Other aromatic analytes, including C_{3-4}-phe, C_{3}-fln, C_{0}-dbt and C_{2-3}-chy, reached higher loss extents in the presence of Finasol.
Figure 3.10 Biodegradation of $n$-alkanes (A-H) and branched alkanes (I, J) with two dispersants at 5 °C (left panels) and 25 °C (right panels).
The effectiveness of Finasol and C9500 on biodegradability of DOSS and hydrocarbons are different, although their major active anionic surfactant is the same. These differences could be caused by the following factors. Firstly, the initial DOSS concentration is higher in Finasol based on our results, which facilitated the decreasing of interfacial tension. Additionally, it is possible that the biodegradability varied due to the differences in composition. Riehm and McCormick (2014) studied the role of anionic and nonionic surfactants on oil-water interfacial tension which explained the effect of DOSS on interfacial tension, oil droplet-size distribution and hence microbial degradation potential. Hence, tiny droplet-size, large surface area and low interfacial tension provided a conducive circumstance for adherence of bacteria to oil droplets and enhanced alkane degradation (Venosa and Holder, 2007; Bruheim et al., 1997). Moreover, the rapid loss of DOSS to the aqueous phase, due to hydrolysis and biodegradation, also governed the hydrocarbons uptake. In our case, the first order decay coefficient is much higher for C9500. Finally, the low activity of several aromatic-degrading species in meso cultures most likely reduced the nutrients competition and favored the cultivation condition for alkane-degraders.
Chapter 4 Biodegradability of Alaska North Slope and Endicott Crude Oil
Dispensed with Corexit 9500

4.1 Introduction

Corexit 9500, a well-known formula capable of dispersing heavy and weathered oils, is listed on the U.S. EPA National Contingency Plan Product Schedule and stockpiled around the world (Lessard and Demarco, 2010). Large quantities of C9500 and C9527 were used during the GOM Deep Water Horizon spill, making it the highest dispersant application in U.S. history. In certain countries such as United Kingdom, C9500 was banned as one of the countermeasures for oil spill incidents because of concerns about possible threats to marine life and human health (D’Andrea M.A. and Reddy G.K., 2013). However, the pressing demand for deep-sea drilling requires continuing assessment of biodegradability of dispersants and dispersed oil (Zhuang et al. 2016).

To date, the biodegradability of various crude oils dispersed with C9500 have been assessed in laboratory experiments, such as Prude Bay crude oil (Venosa and Holder, 2007), Macondo crude oil (Wang et al. 2016), and South Louisiana crude oil (Campo et al. 2013). Results varied due to the differences in oil properties and microbial community structure. Nonetheless, only a limited number of studies have been conducted where the biodegradability of dispersed Endicott crude oil was evaluated, and with only one publication evaluated the biodegradation by monitoring total petroleum hydrocarbon concentration under anaerobic conditions (Personna et al., 2014).

In this study, meso and cryo cultures, isolated from surface and deep-sea waters of GOM, were added to Endicott dispersed with C9500, Endicott alone, and C9500 alone at 5 and 25 °C, following the procedure described in Chapter 2. The degradation rates and extents
of DOSS and hydrocarbons were evaluated and compared with a previous experiment conducted with ANS dispersed with C9500 under same experimental conditions.

4.2 Results and Discussion

4.2.1 DOSS degradation

DOSS was rapidly degraded in live dispersed oil samples after a 2-day lag phase at 25 °C (Open symbols, panel B, Fig. 4.1). The concentration decreased following the first-order decay model, with a constant rate of - 0.077 ± 0.007 /d. In the absence of Endicott (Open symbols, panel A, Fig. 4.1), DOSS uptake occurred after an acclimation period of 4 days, with a rate coefficient of - 0.069 ± 0.005 /d, which was not statistically different from dispersed oil treatment ($p = 0.1825$). Removal extents surpassed 90% by day 24 and 40 in the presence and absence of oil, respectively. Panels C and D of Fig. 4.1 present the corresponding KCs for C9500 alone and Endicott + C9500 treatments at 5 and 25 °C. Only 10% of the initial concentration of DOSS was remaining in the two sets after 45 days at 25 °C. The zero order coefficient rates of the abiotic loss were - 4.9 ± 0.2 µg/L/d (Endicott + C9500) and - 4.7 ± 0.2 µg/L/d (C9500 alone). According to the Student’s $t$-test, significant differences between live and killed samples were observed after 8 days for with ($p = 0.024$) and without ($p = 0.004$) oil treatments, which implied the role of microbial uptake in DOSS degradation.

Similar to the Finasol experiment, the effect of temperature is evident as DOSS persisted until the end of experiment (56 days) regardless of the treatment at 5 °C. Thus, the low temperature condition greatly inhibited both the microbial uptake and abiotic loss of DOSS.
Figure 4.1 Disappearance of DOSS in the absence (A,C) and presence (B, D) of Endicott at 5 and 25 °C. Live treatments are in panels A and B, whereas KCs are in panels C and D.

4.2.2. Hydrocarbon degradation

Fig. 4.2 displays the hopane concentration of all the live samples, which revealed that the hopane persisted throughout the run regardless of the temperature and treatment. Similar results were obtained in the phase I and Phase II experiments as well. For both dispersed and undispersed oil samples, the amount of hopane was higher at 25 °C than at 5 °C, since meso cultures showed higher background concentration of hopane (Table 4.1). As in the previous experiment, concentrations of all the hydrocarbon analytes were normalized to hopane. As listed in Table 4.1, the concentration of PAHs contributed by the meso culture was 30% (Endicott) and 65% (Endicott + C9500) when compared with the initial oil concentration. Conversely, the cryo culture contributed much less alkanes (< 10%) and PAHs (< 1%), but added 20 and 47% hopane in the oil alone and dispersed oil treatments, respectively. Hence,
at time zero, the total alkane and PAH concentration normalized to hopane varied between the various treatments.

**Figure 4.2** Time series concentration of hopane

**Table 4.1** Oil Residual in Cultures (phase III)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Meso Cultures (25 °C), µg/L</th>
<th>Cryo Cultures (5 °C), µg/L</th>
<th>Endicott without Culture, µg/L</th>
<th>Endicott + C9500 without Culture, µg/L</th>
<th>M1</th>
<th>M2</th>
<th>C1</th>
<th>C2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hopane</td>
<td>0.03</td>
<td>0.01</td>
<td>0.07</td>
<td>0.03</td>
<td>51%</td>
<td>121%</td>
<td>20%</td>
<td>47%</td>
</tr>
<tr>
<td>nC10-16</td>
<td>15.19</td>
<td>26.31</td>
<td>432.10</td>
<td>190.44</td>
<td>4%</td>
<td>8%</td>
<td>6%</td>
<td>14%</td>
</tr>
<tr>
<td>nC17-21</td>
<td>9.52</td>
<td>7.55</td>
<td>304.16</td>
<td>195.14</td>
<td>3%</td>
<td>5%</td>
<td>2%</td>
<td>4%</td>
</tr>
<tr>
<td>nC22-29</td>
<td>19.27</td>
<td>15.23</td>
<td>408.95</td>
<td>220.79</td>
<td>5%</td>
<td>9%</td>
<td>4%</td>
<td>7%</td>
</tr>
<tr>
<td>nC30-35</td>
<td>0.00</td>
<td>3.03</td>
<td>86.80</td>
<td>59.63</td>
<td>0%</td>
<td>0%</td>
<td>3%</td>
<td>5%</td>
</tr>
<tr>
<td>PH+PR</td>
<td>4.70</td>
<td>11.25</td>
<td>143.42</td>
<td>45.50</td>
<td>3%</td>
<td>10%</td>
<td>8%</td>
<td>25%</td>
</tr>
<tr>
<td>Total Alkanes</td>
<td><strong>48.68</strong></td>
<td><strong>63.38</strong></td>
<td><strong>1375.42</strong></td>
<td><strong>711.50</strong></td>
<td>4%</td>
<td>7%</td>
<td>5%</td>
<td>9%</td>
</tr>
<tr>
<td>NAPs</td>
<td>70.86</td>
<td>0.04</td>
<td>279.33</td>
<td>122.49</td>
<td>25%</td>
<td>58%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>PHEs</td>
<td>32.60</td>
<td>0.12</td>
<td>70.65</td>
<td>36.31</td>
<td>46%</td>
<td>90%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>FLNs</td>
<td>13.06</td>
<td>0.01</td>
<td>33.03</td>
<td>14.57</td>
<td>40%</td>
<td>90%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>DBTs</td>
<td>9.35</td>
<td>0.04</td>
<td>45.19</td>
<td>20.97</td>
<td>21%</td>
<td>45%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>BNTs</td>
<td>3.07</td>
<td>0.03</td>
<td>13.93</td>
<td>9.41</td>
<td>22%</td>
<td>33%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>PYRs</td>
<td>3.65</td>
<td>0.02</td>
<td>4.44</td>
<td>1.73</td>
<td>82%</td>
<td>211%</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>CHYs</td>
<td>4.74</td>
<td>0.04</td>
<td>12.50</td>
<td>6.24</td>
<td>38%</td>
<td>76%</td>
<td>0%</td>
<td>1%</td>
</tr>
<tr>
<td>Total PAHs</td>
<td><strong>137.34</strong></td>
<td><strong>0.30</strong></td>
<td><strong>459.07</strong></td>
<td><strong>211.72</strong></td>
<td>30%</td>
<td><strong>65%</strong></td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>
Alkanes

At 25 °C, alkanes from the meso cultures were 4 and 7% for the oil alone and dispersed oil samples, respectively. The temperature affected the biodegradation significantly, as there was shorter lag phase at 25 °C. In the dispersed oil treatment (open symbols, panel B, Fig. 4.3), biodegradation ensued without any lag phase with removal efficiency exceeding 76% by day 4, after which there was no further degradation. In the absence of C9500, a notable decline in total alkanes was observed after an acclimation period of 2 days (open symbols, panel A, Fig. 4.3), and, subsequently degraded up to 89% by day 8.

At lower temperature, degradation occurred between days 8 and 12 in oil alone treatment, after an initial lag for 8 days (- 2.05 ± 0.13 /d). In the presence of dispersant, the lag phase lasted only 4 days, after which degradation occurred (- 3.88 ± 0.71 /d). Hence, the addition of dispersant considerably shortened the length of the acclimation period. Moreover, the extent of biodegradation for total alkanes were 89 (undispersed oil) and 91% (dispersed oil) at day 56.

Fig. 4.4 shows the removal extents for individual alkanes at the end of experiment, which demonstrates that the effect of C9500 on the removal rates was negligible except for \( nC_{31-33} \) at 25 °C. As the concentration of most aliphatics approached a nonzero asymptote, a first-order decay model for each alkane was unable to be fitted. However, for the light molecular weight compounds, \( nC_{10-16} \), the first-order rate coefficients were - 0.20 ± 0.03 /d for Endicott alone treatment and - 0.33 ± 0.04 /d for Endicott + C9500 treatment. These values suggested that the addition of dispersant enhanced \( nC_{10-16} \) degradation by a factor of 1.65 at the higher temperature.
Figure 4.3 Biodegradation of hopane-normalized total alkanes (A, B) and PAHs (C, D) at 5 and 25 °C without (A, C) and with (B, D) C9500 dispersant.

Figure 4.4 Removal extent of individual alkanes by the end of experiment in the absence (A) and presence (B) of C9500 at two temperatures.
The addition of dispersant stimulated the biodegradation rates for \( n \)-alkanes with over 27 carbons at 5 °C. Since over 95% of \( nC_{10-16} \) disappeared in both treatments, the time series concentration was fitted to the first order decay model; the rate coefficients were \(-0.1 \pm 0.02 \) /d (without dispersant) and \(-0.13 \pm 0.01 \) /d (with dispersants) which exhibited that the degradation of light molecular weight \( n \)-alkanes was not significantly altered in the presence of C9500 (\( p = 0.0808 \)).

**Figure 4.5** Biodegradation of hopane-normalized total alkanes (A, B) and PAHs (C, D) at 5 and 25 °C without (A,C) and with (B, D) C9500 dispersant in KCs samples.

The time series concentration of total alkanes in the KCs were also measured for the abiotic loss (Fig. 4.5). Distinct reduction of \( nC_{10-12} \) were detected in the high temperature treatment, while no decrease was observed at 5 °C. Hence, microbial degradation played an important role in alkane removal.
Aromatics

As shown in panels C and D of Fig. 4.3, more than 80% of initial PAH concentration remained until the end of the 25 °C experiment (45 days) and none of the PAHs degraded (expect C_{0.2}-nap) in both the treatments. Such results are similar with those observed in phase II experiments. Samples were collected for PCR analysis to investigate this issue.

At 5 °C, the cryo culture began degrading PAHs after 32 days for both dispersed and undispersed oil, after which the biodegradation efficiencies promptly exceeded approximately 80% for the two treatments. The 2-ring compounds (C_{0.4}-nap) were completely removed regardless of the presence or absence of dispersant, as well as several 3-ring compounds (C_{0.2}-phe, C_{0.1}-fln, and C_{1}-dbt) as shown in Fig. 4.6. Aromatic compounds, including C_{3.4}-phe, C_{2.3}-dbt, C_{0}-nbt, and C_{1}-pyr, showed significant increase in biodegradation rates in the presence of C9500, while the other of 4-ring PAHs were removed to a much lower extent (~20%).

Similar to alkanes, the corresponding KCs (panels C and D, Fig. 4.5) presented no decrease in total PAHs for either treatment or temperature which eliminated the effect of abiotic loss.

![Figure 4.6](image-url)  
**Figure 4.6** Removal extent of individual PAHs by the end of experiment in the absence (A) and presence (B) of C9500 at two temperatures.
4.3 Effect of oil properties comparison

In this study, the biodegradability of Endicott dispersed by C9500 was evaluated. Furthermore, these results were compared with phase I experiment which was conducted to study the effect of C9500 on ANS crude oil (Zhuang, 2016). To my knowledge, ANS is a mixture of crude oils from several oil fields in the Alaska North Slope region, and Endicott is also one of the major Alaskan crude oils. Both of these two crude oil are medium oil (Table 4.2). The density of two oils measured in this study are 0.8733 g/L (ANS) and 0.8838 g/L (Endicott). Moreover, Fig. 4.7 and 4.8 present every alkane and aromatic analyte in the two crude oils. The proportion of alkane decrease as carbon number increases for the both oils. ANS contained more short paraffins (nC10-20) and branched alkanes, whereas n-alkanes over 20 carbons showed higher concentration in Endicott. As for PAHs composition, most of the target compounds are higher in Endicott except for C4-nap and C1-chy.

<table>
<thead>
<tr>
<th>Property</th>
<th>ANS</th>
<th>Endicott</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density, ρ (g/mL)</td>
<td>0.8733</td>
<td>0.8838</td>
</tr>
<tr>
<td>Dynamic Viscosity, μ (cP, at 15 °C)</td>
<td>35</td>
<td>120</td>
</tr>
<tr>
<td>Kinematic Viscosity, ν (cSt, at 15 °C)</td>
<td>40</td>
<td>134</td>
</tr>
<tr>
<td>API gravity</td>
<td>28.24</td>
<td>23.00</td>
</tr>
</tbody>
</table>

* General crude oil categories: Heavy (API < 22.3°), Medium (22.3° ≤ API < 31.1°), and Light (API ≥ 31.1°).
Figure 4.7 Alkanes distribution for ANS and Endicott Crude oils

Figure 4.8 PAHs distribution for ANS and Endicott Crude oils
Fig. 4.9 shows that the degradation trends of the aliphatic fraction ($nC_{10-29}$ and iso-alkanes) for the two crude oils are similar. However, the removal extents of Endicott chemically dispersed with C9500 were slightly higher than those of ANS with C9500. At 5 °C, the long chain alkanes ($nC_{30-35}$) were degraded faster in dispersed Endicott than dispersed ANS. At time zero, the hopane-normalized values of this group were higher in ANS + C9500 because of the contribution from the cultures. The removal extents of some 2- and 3-ring aromatics (Fig. 4.10), including C$_0$-nap, C$_0$-2-phe, C$_0$-2-fln and C$_1$-dbt, were complete (100%) in both the experiments. As for the 4-ring aromatics, dispersed Endicott showed a higher degradation rate, which possibly resulted from the lower initial concentration of 4-ring compounds in Endicott.
Figure 4.9 Biodegradation of n-alkanes (A-H) and branched alkanes (I, J) with two oils dispersed by C9500 at 5 °C (left panels) and 25 °C (right panels).
Figure 4.10 Loss rates of individual PAHs for two dispersed oils after 56 days at 5 °C.
Chapter 5 Summary and Future Research

The goal of this research was to evaluate the effect of temperature on the biodegradability of two oil dispersants (Finasol OSR 52 and C9500), two oils (ANS crude oil and Endicott) and two dispersed oils (ANS dispersed with Finasol and Endicott dispersed with C9500). Additionally, we also compared these results with a previous study that was conducted with ANS and C9500 (Zhuang, 2016). This research not only evaluated the effect of dispersant type (Finasol and C9500) on ANS biodegradation, but also analyzed the biodegradation potential of two types of crude oil (ANS and Endicott) when dispersed with C9500.

In the phase II experiment with ANS and Finasol, DOSS disappeared promptly at 25 °C, while it persisted throughout the experiments at 5 °C. Additionally, the biodegradation of DOSS was not statistically significantly different in the presence of ANS. However, the length of the acclimation period and removal extent were highly impacted by the addition of the dispersant. The long chain aliphatic degradation was favored when ANS was chemically dispersed with Finasol. Conversely, C9500 did not have a significant impact on alkane reduction in dispersed Endicott at 25 °C. The 2- and 3-ring aromatics degraded at 5 °C whereas most PAHs persisted at 25 °C, which possibly resulted from the loss of several oil degrading microbial species such as *alcanivorax*, *pseudoidiomarina* and *thallassospira*. The difference in hydrocarbon degradation between phase I and II experiments is possibly due to the higher initial concentration of DOSS in Finasol, differences in dispersant composition and variations in the activity of several aromatic-degrading species.

For phase III experiment with Endicott and C9500, DOSS degradation results are similar to that of phase II. Higher DOSS uptake was observed at 25 °C and the presence of
Endicott did not affect the degradation rates. Although the composition of Endicott considerably differs from ANS, especially the short chain paraffin and 2-ring PAHs, the degradation trends were similar to ANS, which indicated that the effect of C9500 on the two oils were similar.

Future work includes analysis of PCR samples from phase III experiment to evaluate the effect of Endicott on microbial community. Since the PAHs persisted at 25 °C, and low activity of critical degrading species in meso cultures was observed, it is recommended that different cultures should be evaluated under the same test conditions. Since the primary active surfactant for Finasol and C9500 are the same, the effectiveness might be similar. Hence, another dispersant with a different anionic surfactant, such as JD-2000, can be studied since JD-2000 displayed a significant advantage over C9500 (Zhuang et al., 2016).
References


Committee on Effectiveness of Oil Spill Dispersants, 1989. Using Oil Spill Dispersants on the Sea. 6-7.


Deepwater Horizon Incident Joint Information Center, 2010. Coast Guard and EPA Approve Use of Dispersant Subsea in Further Effort to Prevent Oil from Reaching U.S. Shoreline / Agencies Reserve Authority to Stop the Use of the Dispersant At Any Time. [https://yosemite.epa.gov/opa/admpress.nsf](https://yosemite.epa.gov/opa/admpress.nsf)


Gutierrez T., Singleton D.R., Berry D., Yang T., Aitken M.D., Teske A., 2013. Hydrocarbon-degrading bacteria enriched by the Deepwater Horizon oil spill identified by cultivation and DNA-SIP. *2013 International Society for Microbial Ecology* 7, 2091-

Hemmer M.J., Barron M.G. and Greene R.M., 2011. Comparative toxicity of eight oil dispersants, Louisiana sweet crude oil (LSC), and chemically dispersed LSC to two aquatic test species. Environmental Toxicology and Chemistry 30(10), 2244-2252.


Lee K., Nedwed,T., Prince, R.C., Palandro, D., 2013. Lab tests on the biodegradation of
chemically dispersed oil should consider the rapid dilution that occurs at sea. *Marine Pollution Bulletin* 73, 314-318.


National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2011. Washington, DC.


U.S. Environmental Protection Agency, 2010. Comparative Toxicity of Eight Oil Dispersant Products on Two Gulf of Mexico Aquatic Test Species.


