

BIODEGRADATION POTENTIAL OF OIL IN ARCTIC FIRST-YEAR SEA ICE

WHITNEY H. BLANCHARD

BS, University of New Hampshire, 2006

PROJECT PAPER

Submitted to the University of New Hampshire

In Partial Fulfillment of

The Requirements for the Degree of

Master of Science

in

Civil Engineering

May, 2008

## ABSTRACT

### BIODEGRADATION POTENTIAL OF OIL IN ARCTIC SEA ICE

by

Whitney H. Blanchard

University of New Hampshire, May, 2008

Massive amounts of petroleum enter the sea worldwide as a result of human activity (i.e., from extraction (38,000 metric tonnes), transportation (150,000 metric tonnes) and consumption (480,000 metric tonnes); NRC, 2003). The releases can be in catastrophic spills such as the T/V *Exxon Valdez* in Prince William Sound, AK in 1989 or small chronic releases. As oil reserves around the world dwindle, there is increasing pressure to drill in politically and environmentally sensitive Arctic regions. The United States Geological Survey (USGS) has predicted approximately 25 % of the world's remaining petroleum resources are in the Arctic. As the Arctic warms twice as fast as the global average, the Arctic Climate Impact Assessment has predicted by 2050 only a small part of its ocean will have sea ice coverage. New shipping routes will be created in the Arctic with the potential to become the main summer passageways between North America, Asia and Europe.

Even if the best available technology is used to extract and transport oil in the Arctic, there will still be accidental releases to the marine environment including into sea ice infested waters. Microbes in the sea play a major role in the degradation of oil; these processes are well studied in warmer waters. Recently, there have been studies of oil

degrading bacterial communities in cold seawater and sea ice. It is important to compile the existing information on sea ice, sea ice ecology, and brine channels to better understand the fate and transport of oil if it becomes trapped in, on, or under ice. For my project paper, I reviewed the literature on sea ice ecology (i.e., sea ice formations and brine channels; microorganisms in ice, microhabitat dynamics between sea ice algae and bacterial production), and oil degrading bacterial communities (i.e., in cold seawater, brash ice, sea ice). Based on the synthesis of this literature, I constructed a conceptual model of the microbial communities in and on the surface of sea ice and the conditions where biodegradation of oil in ice could potentially occur.

Brine channels within sea ice provide a habitat where microorganisms thrive. Petroleum hydrocarbons from encapsulated oil may become bioavailable to bacteria. The exposure of oil would likely create a shift in the microorganisms present. Algae would die due to toxicity effects which would stop the production of oxygen. The bacterial community would shift where the majority of bacteria would be hydrocarbon degraders, possibly under anoxic conditions. Parameters that will affect bacterial biodegradation of the petroleum hydrocarbons are temperature, salinity, nutrient availability, and ice conditions.

## TABLE OF CONTENTS

	PAGE
ABSTRACT.....	i
ACKNOWLEDGEMENTS.....	iv
LIST OF TABLE.....	v
LIST OF FIGURES.....	v
A. INTRODUCTION.....	1
I. EXISTING ARCTIC CONDITIONS AND SEA ICE CHARACTERISTICS.....	2
II. SEA ICE ECOLOGY.....	11
III. PETROLEUM SOURCES AND SHIPPING ACTIVITY IN THE ARCTIC.....	16
IV. BEHAVIOR OF OIL IN THE MARINE AND FREEZING ENVIRONMENTS..	22
V. REQUIREMENTS FOR BIODEGRADATION.....	28
VI. BIODEGRADATION OF PETROLEUM HYDROCARBONS IN THE.....	31
MARINE ENVIRONMENT	
VII. BIODEGRADATION OF HYDROCARBONS IN COLD AND FREEZING.....	36
ENVIRONMENTS	
VIII. CONCEPTUAL MODEL: BIODEGRADATION POTENTIAL OF OIL IN.....	39
ICE	
B. CONCLUSIONS.....	44
C. LIST OF REFERENCES.....	46
APPENDIX A DEFINITIONS.....	52

## ACKNOWLEDGEMENTS

Funding for this research was provided by grant NA04NOS4190063 from the National Oceanic and Atmospheric Administration Office of Response and Restoration to the University of New Hampshire for the Coastal Response Research Center and from a 2007-2008 Fulbright fellowship grant from the United States - Norway Fulbright Foundation. I would like to thank SINTEF (Trondheim, Norway) for providing resources and facilities in order to complete this paper.

## LIST OF TABLE

	PAGE
1. O <sub>2</sub> , DOC, DON, and DIP of natural sea ice brines from the Weddell Sea .....	15

## LIST OF FIGURES

1. Structure of first-year sea ice.....	2
2. Temperature, brine salinity and brine density profile in sea ice.....	3
3. Schematic of brine expulsion with decreasing temperatures having brine..... ‘squeezed’ to underlying seawater	4
4. Schematic of gravity drainage in sea ice.....	5
5. Spacing of brine inclusions in ice.....	6
6. Arctic sea ice extent.....	16
7. Northwest Passage through the Canadian Arctic Archipelago and Northern..... Sea Route along the Russian coast	17
8. Annual and cumulative oil and gas production in Arctic areas, by country.....	19
9. Political map of the Arctic Region.....	21
10. Fate of spilled oil with weathering processes and their time window.....	22
11. Oil-ice interactions: oil droplets under the ice, new ice growth below the oil, oil..... appearing on the surface in the spring, wind herding of oil on melt pools, and the appearance of emulsified oil on top of the ice	23
12. Cross-section of oil-ice sandwich in laboratory experiment.....	24
13. Schematic of mass flux across static boundary layer for surface associated..... and free floating bacteria	29
14. Schematic of aromatic ring cleavage with molecular oxygen.....	33
15. Conceptual model of clean ice and biodegradation encapsulated oil in first-year..... sea ice	43

## A. INTRODUCTION

While there have been relatively few reported investigations of biodegradation of oil in sea ice, the topic has become of great interest with increased petroleum development and shipping activity in the Arctic. The purpose of this document is to serve as a comprehensive literature review and present a conceptual model on biodegradation of oil in Arctic sea ice. The literature review covers: Arctic conditions and sea ice characteristics (i.e., the environment in which biodegradation would occur should there be an oil release to the marine environment); the behavior of oil in freezing environments; and petroleum development and shipping activity in the Arctic. The literature review also covers biodegradation of oil, including aliphatic hydrocarbons and polycyclic aromatic hydrocarbons (PAHs), in cold and freezing marine environments. The information is synthesized into a conceptual model for the potential of biodegradation of oil in Arctic sea ice. [N.B., For the purpose of this paper, oil refers to crude oil.]

## CHAPTER I

### EXISTING ARCTIC CONDITIONS AND SEA ICE CHARACTERISTICS

The Arctic is commonly defined as the region around the Earth's North Pole and above the Arctic Circle (66° 33'N). The temperature of the Arctic Ocean is close to freezing year-round. Surface water temperatures in winter and summer in the shelf areas are below -1°C and 4-5 °C, respectively, with greater seasonal variability in the peripheral areas due to the influence of the Atlantic and Pacific Oceans (AMAP,

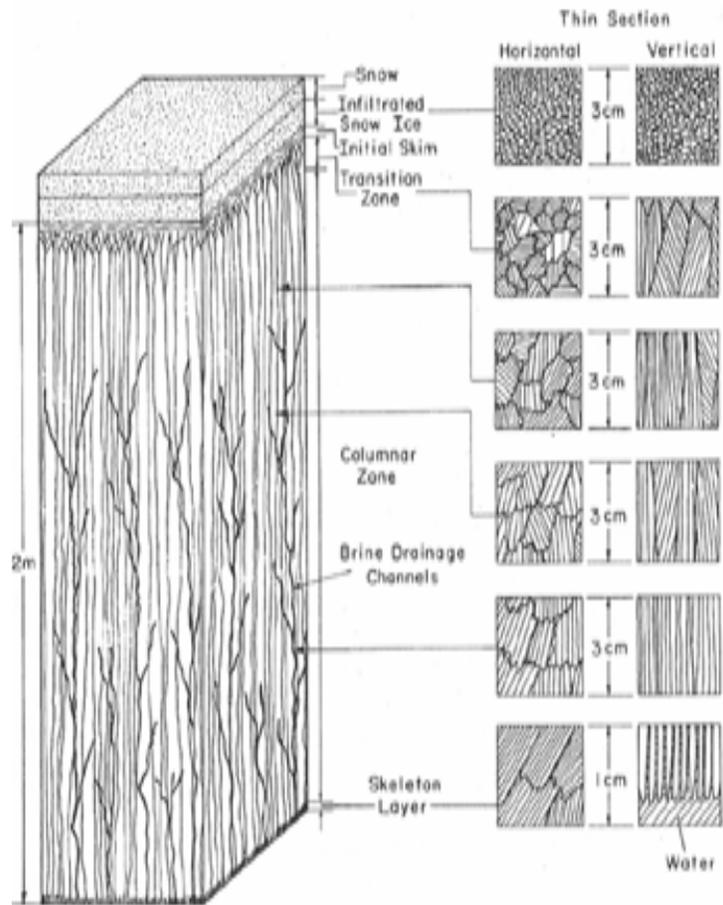


Figure 1. Structure of first-year sea ice (from Løset, 2007)

1998). [N.B., The salinity in seawater creates a freezing point depression.] The salinity of the Arctic Ocean ranges from 30-33 psu and is generally less saline in summer than winter due to the freshwater input from rivers and ice melt (AMAP 1998). [N.B., Salinity is reported in practical salinity units (psu) when possible, otherwise, salinity is reported in parts per thousand (ppt). Between 0 and 42 ppt, ppt

and psu are equivalent assuming standard seawater composition (Unesco, 1978).] Sea ice [N.B., The underlined terms in text are explained in Appendix A DEFINITIONS.] is formed as the seawater begins to freeze (e.g., -1.86 °C at 34 psu). The ice is a semisolid matrix permeated by a network of channels and pores filled with brine. Sea ice in the Arctic may last several years with the average thickness > 2 m (Haas, 2003). Between 60 % and 80 % of the ice cover in the Arctic Ocean is composed of congelation ice found in the columnar zone (Figure 1; Eicken, 2003). Major ions present in seawater ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{CO}_3^{2-}$ ) are not incorporated into the ice crystal lattice and are rejected by the ice-water interface during ice crystal growth (Eicken, 2003). The salts are trapped between the lamellae (i.e., skeletal layer (Figures 1 and 4))

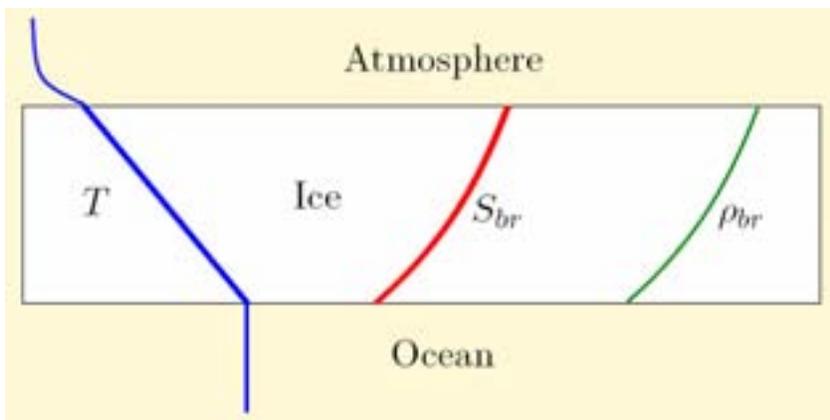


Figure 2. Temperature, brine salinity ( $S_{br}$ ) and brine density ( $\rho_{br}$ ) profile in sea ice (from Notz, 2007)

during ice growth and form brine that remains within the ice matrix, or are expelled into the underlying water (Eicken, 2003). Trapped brine in the sea ice matrix becomes more concentrated and the freezing point is

depressed. As temperature decreases, the ice grows downward. The major salts by percent in the brine are approximately:  $\text{Na}^+$  and  $\text{Cl}^-$  (85 %);  $\text{SO}_4^{2-}$  (8 %); and  $\text{Mg}^{+2}$ ,  $\text{Ca}^{+2}$ ,  $\text{K}^+$  (6 %; Eicken, 2003). Figure 2 is a qualitative density profile of temperature, salinity and density of brine in ice with an upper and lower boundary of the atmosphere and ocean, respectively. As the salts are rejected from the ice crystal lattice, the concentrations of salts increases while the freezing point of the brine decreases (i.e., the salts cause a freezing point depression effect in the brine). For

example, in a closed system where the mass fraction of components remains constant, seawater is cooled below the freezing point at  $-1.86^{\circ}\text{C}$  with a salinity of 34 ppt to  $-5^{\circ}\text{C}$  with brine salinity ( $S_{br}$ ) of 87 ppt and the mass of ice accounts for 65 % while the remaining 35 % is the liquid impurities. At  $-8.2^{\circ}\text{C}$ , the concentration of salts is at supersaturation, and the major constituent of seawater,  $\text{Na}_2\text{SO}_4$ , begins to precipitate in the form of mirabilite ( $\text{Na}_2\text{SO}_4 \times 10\text{H}_2\text{O}$ ; Eicken, 2003), followed by hydrohalite ( $\text{NaCl} \times 2\text{H}_2\text{O}$ ) at  $-22.9^{\circ}\text{C}$ . The total mass of hydrohalite precipitated from seawater is more than four times that of mirabilite (Light *et al.*, 2003). The size of hydrohalite crystals in liquid inclusions is 1 to 9  $\mu\text{m}$ . Mirabilite crystals have been observed in piles at the bottom of brine tubes, in clusters throughout the tubes, or at constrictions in the tubes. Observed crystal diameter is 0.015 to 0.14 mm and may be too small to resolve. Light (1995) infers an effective crystal size of 0.009 mm (as cited in Light *et al.*, 2003). Mirabilites appear to remain separate from ice, while the formation of hydrohalite crystals is observed to be closely associated with ice crystal formation (Light *et al.*, 2003). It is unclear whether hydrohalite crystals nucleate on the ice surface or on suspended particles or bubbles within the brine. When warmed, brine pockets enlarge and precipitated salts gradually redissolve in the water.

The volume of brine channels in ice is proportional to temperature and the brine concentration (Eicken *et al.*, 2000) until  $\sim -40^{\circ}\text{C}$  where a small liquid fraction remains (Eicken, 2003). At  $-6^{\circ}\text{C}$ ,  $-10^{\circ}\text{C}$ , and

$-21^{\circ}\text{C}$ , the brine salinities are 100, 145 and 216 ppt, respectively (Mock and Thomas, 2005). For  $T >$

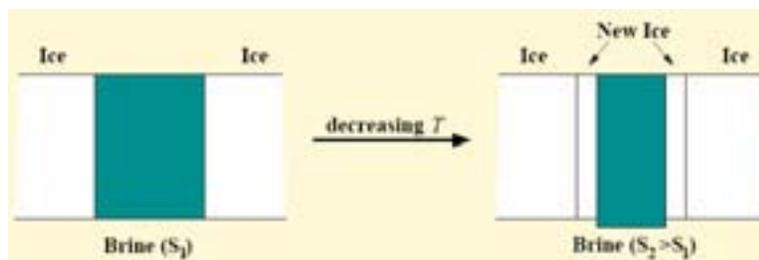


Figure 3. Schematic of brine expulsion with decreasing temperature as brine is ‘squeezed’ to underlying seawater (from Notz, 2007)

-23°C,  $S_{br}$  is approximated as  $S_{br} = \left(1 - \frac{54.11}{T}\right)^{-1} \times 1000$  ppt (Eicken, 2003) where T is temperature (°C).

The two processes by which sea ice desalinates to the underlying seawater are gravity brine expulsion and gravity drainage. Brine expulsion occurs when ice is formed with a volume greater than the original seawater and results in brine being 'squeezed' to the underlying seawater (as cited in Cox and Weeks (1988)). The volume of brine lost to brine expulsion is much less than in gravity

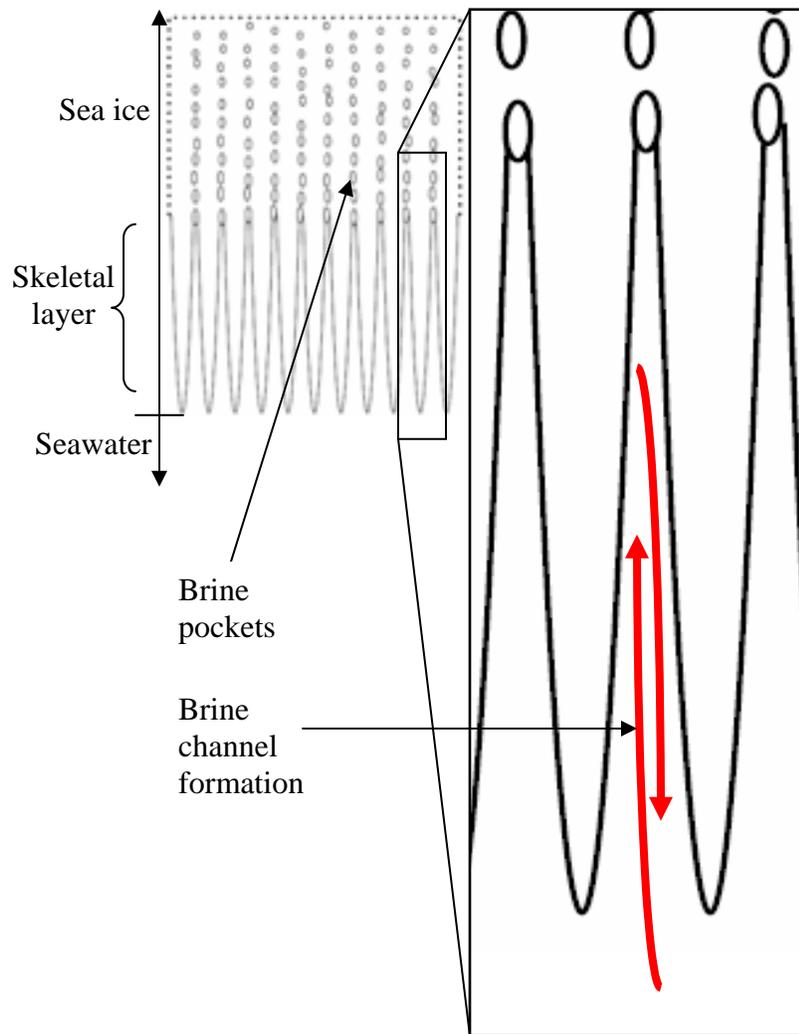


Figure 4. Schematic of gravity drainage (adapted from Petrich, 2004)

drainage (Cox and Weeks, 1988). Gravity drainage results from an unstable brine density profile where the denser saline brine in the ice is in contact with the less dense underlying seawater which results in a convective overturn where the brine is flushed out with seawater (Cox and Weeks, 1988). Cox and Weeks (1975) observed in laboratory experiments that gravity drainage

ceases when  $S_{br} > 50$  ppt. For  $S_{br} < 50$  ppt,  $\left(\frac{\Delta S_i}{\Delta t}\right) = 1.68 \times 10^{-5} \left(\frac{\Delta T}{\Delta z}\right) - 3.37 \times 10^{-7} V_b \left(\frac{\Delta T}{\Delta z}\right)$

where  $\frac{\Delta S_i}{\Delta t}$  is the rate of change in salinity due to gravity drainage ( $\text{mL}^{-1}\text{s}^{-1}$ );  $\frac{\Delta T}{\Delta z}$  is the temperature gradient ( $\text{K cm}^{-1}$ ); and,  $V_b$  is the brine volume ( $\text{mL}^{-1}$ ; as cited in Cox and Weeks, 1988).

Brine channels are vertically elongated channels ( $\leq 1$  m) with horizontal spacing between them in the ice matrix; channel diameters range from  $< 1$  mm to several centimeters. However, the permeability of fully consolidated, cold sea ice is too low for convection of brine through the entire volume (Eicken, 2003); columnar sea ice is impermeable to brine transport with porosity  $\sim < 5\%$  (Weeks and Ackley, 1986). There is a vertical temperature gradient throughout the ice matrix as the underside of the ice floe is in contact with the underlying seawater which is at or

near freezing, while the surface of the ice is at or near atmospheric temperature. This creates a gradient in brine salinity, and column distribution of brine channels and pores throughout the matrix (Mock and Thomas, 2005).

The gradient disintegrates as ice begins to warm and melt in spring and early summer (Eicken, 2003). Pores enlarge and join together with increasing temperature, which results in the

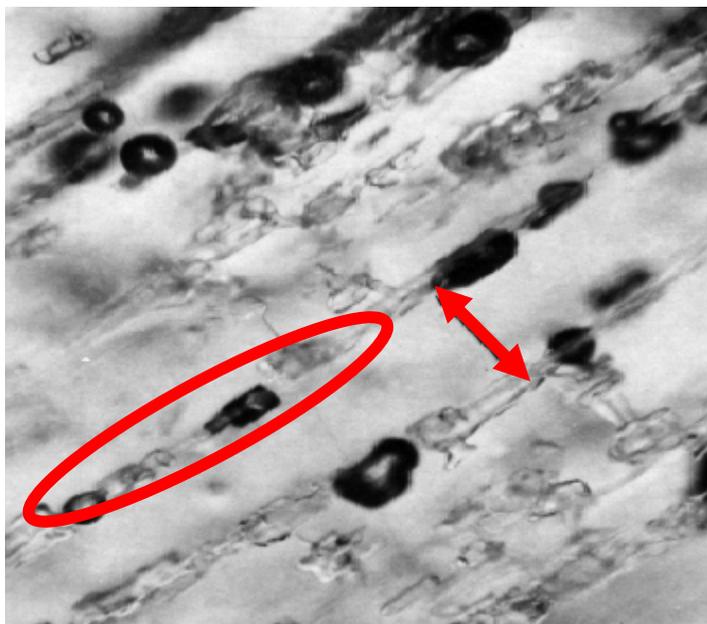


Figure 5. Spacing of brine inclusions in ice are indicated by the arrow and brine inclusions are circled (from Løset, 2007)

vertical elongation of pores into channels. Major brine channel growth occurs in the spring when

the air and sea ice approach the freezing point (Martin, 1979). [N.B., The actual freezing point is dependent on salinity and temperature.]

Light *et al.* (2003) classified inclusions into two categories: pockets and tubes (i.e., channels) whose length ( $l$ )  $< 0.50$  mm and  $l \geq 0.50$  mm, respectively. Pockets frequently appear in clusters or vertical strings and channels have a near-vertical orientation. Pockets with  $l < 0.03$  mm are generally assumed spherical. At  $-15$  °C, ~80 % of the brine inclusions are pockets, but the tubes present may contain  $>90$  % of the brine by volume. Although much smaller by volume, brine within pockets plays a role in light scattering (e.g., at  $-15$  °C, 10 % of the brine volume is within the pockets and accounts for 25 % of the light scattering; Light *et al.*, 2003). Brine channel distribution is typically one channel per cross-sectional area of  $10 \text{ cm}^2$  (Eicken, 2003; Figure 5). As temperature decreases, inclusions shrink while maintaining the same length to diameter ratio. Freezing equilibrium relationships predict at  $-20$  °C, the brine inclusion is 82 % of its volume at  $-15$  °C; and at  $-25$  °C the volume is reduced to 42 % of the original (Light *et al.*, 2003).

Bubbles can be entrained into sea ice at the growth interface during the freezing process when dissolved gas comes out of solution. Bubbles may form in brine inclusions as ice warms. They fill the void created as ice with a lower density melts into a higher density liquid. Air can be introduced in ice above the freeboard level due to meltwater drainage during the summer (Light *et al.*, 2003). This suggests there should be bubbles in the ice lattice as well as in the inclusions, however, bubbles have only been observed within inclusions. Gas bubble radius ( $r_{\text{gb}}$ ) ranges from 0.004 to 0.07 mm with an average of 1.3 bubbles  $\text{mm}^{-3}$  at  $-15$  °C, which is 5 % of the density of brine inclusions. Other observations indicate  $r_{\text{gb}}$  ranges from 0.1 to 2 mm with an

occurrence of 0.03 bubbles  $\text{mm}^{-3}$  (Light *et al.*, 2003). These observations are for bubbles entrained in rapidly growing, young (i.e., first-year) ice during initial formation.

Darcy's Law describes fluid migration through a porous medium:  $u = \frac{k}{\eta} \nabla p$ , with specific discharge;  $u$  ( $\text{ms}^{-1}$ ), pressure gradient,  $\nabla p$  ( $\text{Nm}^{-3}$ ); permeability,  $k$  ( $\text{m}^2$ ); and dynamic viscosity,  $\eta$  ( $\text{kg m}^{-1} \text{s}^{-1}$ ). The permeability ( $k$  is represented as  $\Pi$  in sea ice literature) of Arctic sea ice during the summers of 1995 and 1996 in the Siberian and central Arctic ranged from  $10^{-10}$  to  $10^{-8} \text{ m}^2$  (Freitag and Eicken, 2003). The permeability of sea ice affects transport of particulate and dissolved matter through the matrix influencing nutrient supply to microorganisms (Hudier and Ingram, 1994) and transport of contaminants (e.g., oil; Martin, 1979). The critical permeability ( $k_{crit}$ ) is the point that separates meltwater retention and pooling at the ice surface from complete downward draining of melt. For Darcian flow,  $k_{crit} = 1.5 \times 10^{-10} \text{ m}^2$ , suggesting that in mid- to late summer there is a downward draining of meltwater in sea ice cover (Freitag and Eicken, 2003). Meltwater migration through sea ice is dominated by individual large pore channels. Hence, potential freezing and thawing changes the pore size distribution in ice (Freitag and Eicken, 2003). Although there is interannual variability (i.e., the highest variability at the sea ice surface) the permeability of the middle and lower ice layers may vary by a factor of two (Freitag and Eicken, 2003). [N.B., Hydraulic conductivity, the capability of a porous medium to pass water, is a function of formation and fluid properties:  $K = \frac{k \times \rho \times g}{\mu}$ , with hydraulic conductivity,  $K$  [ $\text{m s}^{-1}$ ]; permeability,  $k$  [ $\text{m}^2$ ]; density of water,  $\rho$  [ $\text{kg m}^{-3}$ ]; the acceleration due to gravity,  $g$  [ $\text{m s}^{-2}$ ]; and, dynamic viscosity,  $\mu$  [ $\text{kg m}^{-1} \text{s}^{-1}$ ].  $K$  is a fluid characteristic not commonly found in sea ice literature, however, is often used with fate and transport of pollutants (e.g., oil spills).]

The brine volume fraction (e.g., the total porosity ( $f_t$ )) is often reported; however, the effective porosity ( $f_e$ ), which describes the fluid flow through the porous medium, may be of more interest. Petrich *et al.* (2006) developed a computational fluid dynamics (CFD) model of permeability-porosity appropriate for growing sea ice. They used a Monte Carlo percolation model to assess the relationship between  $f_t$  and  $f_e$ . The vertical permeability component ( $k_v$ ) is:  $k_v = 7 \times 10^{-10} \text{ m}^2 (f_t - 0.054)^{1.2}$ . Freitag (1999) gave the relationship (based on bailer measurements in the Arctic) as:  $k_v = 3 \times 10^{-8} \text{ m}^2 f_e^{3.9}$  for  $0.05 \leq f_e \leq 0.2$  for young first-year ice and  $k_v = 6 \times 10^{-10} \text{ m}^2 f_e^{1.6}$  for  $6 \times 10^{-4} \leq f_e \leq 0.6$  for older first-year, multi-year and ridged sea ice.

There is scatter among measurements as sea ice salinity and permeability may be dependent on growth conditions. The numerical relationship has been approximated by simple analytical methods and has only been compared to experimental data for calcite aggregates. Currently, there is insufficient data available on sea ice for comparison.

Temperature changes in ice can affect the size, number, distribution, and chemistry of the inclusions, and change the optical properties and radiative transfer in ice. Ice albedo feedback involves absorption, transmission, storage, and redistribution of solar heat and depends on the way short wave radiation is absorbed, transmitted, and backscattered by the ice cover. Optical properties of sea ice vary with ice type, temperature throughout annual cycles, and the number and distribution of inhomogeneities within the ice. Sea ice is composed of brine, gas, precipitated salts crystals, and impurities surrounded within a matrix of nearly pure ice (Light *et al.*, 2003). Changes in chemistry of sea ice are determined by bulk properties (e.g., temperature, salinity, density, brine composition, ice thickness, surface conditions). Inherent optical properties (IOPs) describe absorption and light scattering in the ice. The magnitude and

variation of IOPs are dependent on brine, gas, precipitated salt crystals and other impurities in the ice matrix.

With respect to biodegradation of oil in first-year sea ice, the key points from Chapter I include:

- The sea ice matrix consists of pure water molecules with vertically aligned concentrated impurities (brine) in the form of pockets or channels (formation of which is temperature dependent).
- Sea ice is permeable. There is downward drainage of meltwater via brine channels. The permeability of sea ice affects transport of particulates (e.g., oil droplets).
- Gravity drainage is the primary mechanism for brine transport when  $S_{br} > 50$  ppt; columnar sea ice is impermeable to brine transport with porosity  $\sim < 5\%$ .

## CHAPTER II

### SEA ICE ECOLOGY

The pore and channel spacing within the sea ice matrix forms a microhabitat in which there are temperature, salinity, and light gradients. In addition, there are large (Eicken *et al.*, 1992) and small scale variations in morphology (i.e., spatial heterogeneity) within a single ice floe (Thomas and Dieckmann, 2002). The brine channel walls offer a large surface area that may be colonized by bacteria and algae and used for protistan attachment and grazing (Krembs, 2000). The total surface area of internal brine channels ranges from 0.6 to 4 m<sup>2</sup>/kg of sea ice. At -2°C, it is estimated between 6 % to 41 % of the brine channel surface area may be covered by microorganisms (Krembs, 2000) which is higher than the total surface area of soil where < 1 % is covered by microorganisms (as cited in Krembs, 2000). Cooling sea ice from -2 to -6 °C increases the percent coverage of microorganisms due to a surface reduction in the brine channels and pores (Krembs, 2000). Brine filled pore space within the ice matrix can be isolated in brine pockets and connected with brine tubes at -20 °C and is habitable with densities of over 150 cells/mm<sup>3</sup> (Huston, 2003).

The ice-water interface is the most dynamic part of sea ice with respect to changing microstructure (Mock and Thomas, 2005). At the interface, there is the localized transport of heat and salt which influences sea ice porosity, and brine channel structure and distribution. There is also a diffusive boundary layer at the interface, where at -3°C, there is a dynamic flux of

dissolved oxygen from the ice into the brine (Mock *et al.*, 2003). Brine channels are often hyperoxic (Gleitz *et al.*, 2005) and dissolved organic matter (DOM) in oxic zones within sea ice may provide a favorable growth substrate for bacteria (Amon *et al.*, 2001). Oxygen is produced in brine channels by diatoms and is present in the form of gas bubbles. Over time, dissolved oxygen concentrations decrease and gaseous oxygen is released to the atmosphere and seawater (Mock *et al.*, 2002).

There is a ratio between primary production by sea ice algae and bacterial production: bacterial production ranges from ~ 10 % of ice algal production to circumstances where bacterial consumption of organic carbon exceeds primary production (Mock and Thomas, 2005). Most sea ice bacterial strains are cold-adapted, halotolerant with both free living and surface associated species (Brown and Bowman, 2001; Junge *et al.*, 2002), however, surface associated bacteria are more dominant with decreasing temperature. Over half of the cells found in sea ice cores are associated with particles or surfaces (i.e., sediment, detritus, ice crystal boundaries; Junge *et al.*, 2004). The percentage of active cells associated with particles or surfaces increases with decreasing temperature; nearly all active cells are particle associated at -20 °C. Free living bacteria have diffusive limitations on nutrient uptake, enzymatic reactions, and exchange of metabolites in highly viscous fluids (i.e., brine in sea ice during winter) due to limited mass transfer flux while surface associated bacteria have direct access to adsorbed organic substrates (Junge *et al.*, 2004). There is little known about how organic substrates are sorbed on brine channel walls versus in the liquid brine. [N.B., Mass transfer is discussed in Chapter V.]

Studies in the Antarctic have shown sea ice favors psychrophilic microorganisms (Jakosky *et al.*, 2003). There is a higher diversity of bacterial phylotypes in Arctic sea ice than in Antarctic ice due to ice formation and possibly to the land masses surrounding Arctic

(Brinkmeyer *et al.*, 2003). The similarity of phylotypes in ice at the poles implies that bacteria have the same selective mechanisms (Brinkmeyer *et al.*, 2003). Additionally, Junge *et al.* (2002) found a 100 % sequence similarity between an Arctic and Antarctic isolate suggesting bipolar distribution and Brinkmeyer *et al.* (2003) suggests mixing of bacterial populations globally.

Measurements of sea ice assemblages in Arctic sea ice can be more active than those in seawater (Junge *et al.*, 2002). These assemblages are confined to a small habitat and may be protected from predators that are larger than the size of the brine space (i.e., size exclusion of predators). The high concentration of DOM in Arctic sea ice also may contribute to the relatively high bacterial concentrations (Thomas *et al.*, 1995). The DOM has been largely uncharacterized and may be extracellular polymeric substances (EPS) produced by algae and/or bacteria (Krembs *et al.*, 2002). EPS is primarily used for cell attachment (i.e., biofilm; Sievers *et al.*, 2004) aiding survival in cold environments (Junge *et al.*, 2004) where less energy is expended swimming and seeking food. Particulate EPS is densely colonized by bacteria, serving as a carbon source when dissolved organic matter (DOM) is not otherwise available.

Additionally, it may serve as microhabitats for bacteria which increase sea ice diversity (Mock and Thomas, 2005) and may protect the microorganisms from sea ice crystal damage, pH and salinity changes, and offer protection from predators. EPS alters the morphology of brine pore spaces which changes the permeability of solutes (Krembs *et al.*, 2001). In dense accumulations, EPS may change the physicochemical environment (Thomas and Dieckmann, 2002). Biofilms are irreversibly associated with a surface which includes a matrix of bacterial cells, mineral crystals, particles and polysaccharide material associated with EPS (Donlan, 2002). Prior to growth of a biofilm, the surface is conditioned by polymers from the medium within minutes, modifying the surface and extent of attachment (Loeb and Neihof, 1975 as cited in Donlan,

2002). The walls of the brine channels and pockets are preconditioned with polymers from the brine or underlying seawater prior to cell attachment.

The bacterial community transitions with decreasing temperature from the diverse species inhabiting in seawater to psychrophilic species. Psychrophilic microorganisms feed on carbohydrates produced by death and lysis of sea ice organisms, and exuded algal and bacterial organic polymers (e.g., emulsan, EPS; Thomas *et al.*, 2001; Krembs *et al.*, 2001). The maintenance of functional lipid membranes is a critical metabolic requirement at low temperatures. Cell membrane fluidity is regulated by the fatty acid composition of the structural phospholipids. At low temperatures, the percent of unsaturated fatty acids in the membrane increases and the average chain length is decreased. Polyunsaturated fatty acids (PUFAs) also increase to retain membrane fluidity (Nichols *et al.*, 1992; Russel, 1997) and allow physical transport of compounds across the membrane. PUFAs support primary and secondary electron acceptors and the velocity of electron flow (i.e., electron transport). At low irradiances of light in sea ice, there is an increase in proportion of PUFAs (Thomas and Dieckmann, 2002).

Dehydration, caused by the increase in brine salinities, stresses sea ice microorganisms. When ice melts, the organisms are exposed to hyposaline conditions. Psychrophilic bacteria regulate their lipid packing and fatty acids and have salt-tolerant enzymes that function over a range of salinities (Nichols *et al.*, 2000). The concentrations of osmolytes are adjusted in order to survive osmotic stress, including inorganic ions and organic solutes, which accumulate in hypersaline conditions or degrade in hyposaline shock (Thomas and Dieckmann, 2002).

There may be an increased dependence on  $\text{NH}_4^+$  as an inorganic nitrogen source for algae and bacteria at low temperatures because with increasing pH there is increasing dissociation of  $\text{NH}_4^+$  to  $\text{NH}_{3(g)}$  which can diffuse into the cell (Reay *et al.*, 1999; Raven *et al.*, 1992). In sea ice,

levels of  $\text{NH}_4^+$  may be as high as 150  $\mu\text{M}$  (Thomas and Dieckmann, 2002). Papadimitriou *et al.* (2007) characterized the biogeochemical composition of natural sea ice brines from the Weddell Sea with temperatures ranging from  $-2.1$  to  $-3.4$   $^\circ\text{C}$  in early austral summer in first-year ice. Dissolved oxygen ( $\text{O}_2$ ), dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and dissolved inorganic phosphorus (DIP) are of particular interest for biodegradation [N.B.,  $\text{O}_2$ , C, N, P are further discussed in Chapter V]. The concentrations (M/V) are converted (Table 1)

assuming a brine density of  $\sim 1040$   $\text{kg/m}^3$ . The biogeochemical characteristic of the brine fits within the Redfield

Table 1.  $\text{O}_2$ , DOC, DON, DIP, and C:N:P ratio of natural sea ice brines from the Weddell Sea (adapted from Papadimitriou *et al.*, 2007)

$\text{O}_2$	319 to 602 $\mu\text{mol/kg}$	10.62 to 20.03 $\mu\text{g/mL}$
DOC	95 to 183	1.186 to 2.284
DON	5 to 14.8	0.073 to 0.215
DIP	0.36 to 1.31	0.012 to 0.042
C:N:P	139.7:1.1:1 to 263.9:13.9:1	

ratio (C:N:P = 106:16:1) which is an empirical ratio for plankton. However, there is not such a clear ratio relationship between other species (e.g., S, O; Papadimitriou *et al.*, 2007).

With respect to biodegradation of oil in first-year sea ice, the key points from Chapter II include:

- Brine volume in sea ice is a suitable habitat for microorganisms at low temperatures and high salinities.
- Bacteria use the oxygen produced from sea ice algae in the brine; brine channels are often hyperoxic because of photosynthesis.
- Surface associated bacteria are predominant in brine channels because of diffusive limitations. EPS enables cell attachment, buffers the surrounding environment and protects bacterial cells from predation.
- There is N and P available in the brine channel. The brine C:P:N ratios mirrors the Redfield ratio.

## CHAPTER III

### PETROLEUM SOURCES AND SHIPPING ACTIVITY IN THE ARCTIC

Petroleum hydrocarbons may be introduced into the Arctic by natural sources (e.g., oil seeps), blow outs and operational discharge from oil and gas production; and transportation (including the transport of petroleum products and release from ships; AMAP, 1998). The US Geological Survey (2000) has predicted ~ 25 % of the remaining petroleum reserves worldwide are located in Arctic regions. *Arctic Oil and Gas 2007* (AMAP, 2007) presents several findings with respect to the Arctic marine environment:

- Oil and gas activity (OGA) has occurred, with much produced and much more remaining;
- In the marine environment, an oil spill is the largest threat from OGA; and
- Responding to oil spills in remote, icy environments remains a challenge.

OGA in Arctic

regions is partly possible due

to the recent decline in summer sea ice extent (Figure 6). The *Arctic Climate Impact Assessment*

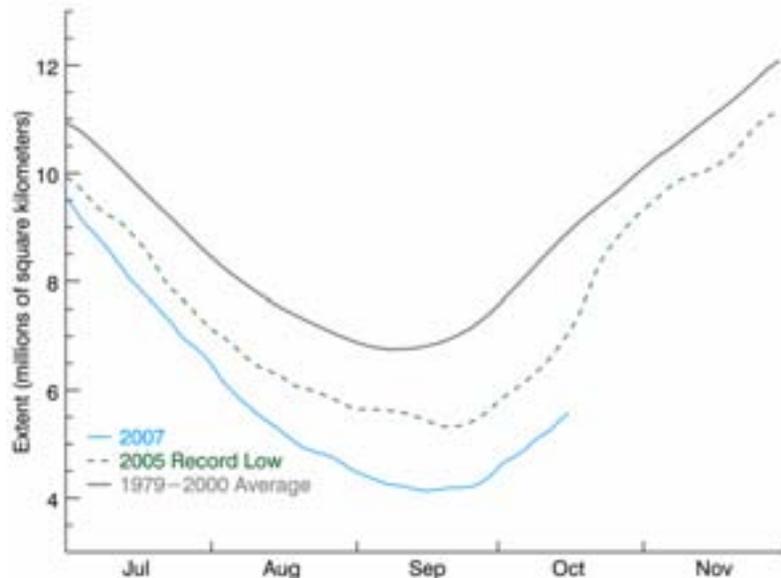


Figure 6: Arctic sea ice extent (area of ocean  $\geq 15\%$  sea ice; from National Snow and Ice Data Center, 2007)

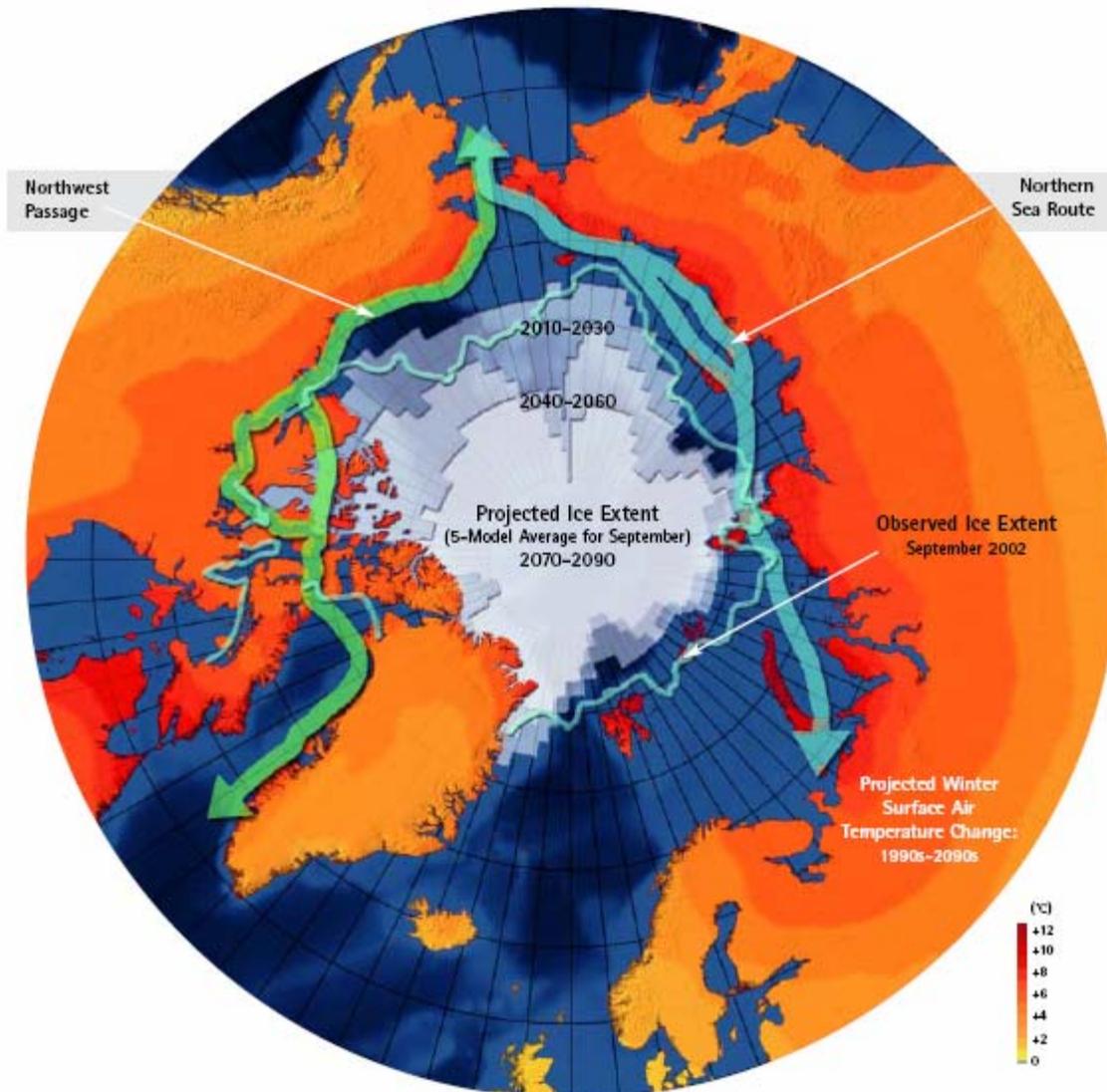


Figure 7: Northwest Passage ( left) through the Canadian Arctic Archipelago and Northern Sea Route (NSR) along the Russian coast (right) (from ACIA, 2004)

(ACIA; 2004) Key Finding #6 states that in response to a reduction in sea ice cover and thickness, there is high probability (> 90 %) that there will be an increase in maritime shipping activity and access in the Arctic because it connects the Atlantic and Pacific Oceans (Figure 7). This includes the Northern Sea Route (NSR) along the Russian coast of the Far East and Siberia and the previously impassable Northwest Passage through the Canadian Arctic Archipelago (Figure 7). In general, navigable conditions in the Arctic require < 50 % sea ice cover. The NSR navigation season is projected to increase from the current 20-30 days to 90-100 days per year by

2080 (ACIA, 2004). Ships with ice breaking capability have the capacity to pass through 75 % ice covered waters, extending the estimated NSR navigation season an additional 40-50 days. Increased navigation implies the opening of shipping routes and access to natural resources (ACIA, 2004).

In addition to decreased sea ice extent, there has also been less predictable year-to-year variability in sea ice (ACIA, 2004). Increased development and transportation along with increased variability in environmentally harsh conditions increase the risk for an oil spill in the Arctic. More open water may create larger waves and greater wave stress on facilities and vessels (AMAP, 2007). Icebergs maybe become more common with calving glaciers (e.g., in Greenland) affecting tanker traffic. A comprehensive Arctic Marine Shipping Assessment (AMSA) will be released in 2009 which will assess current and future (i.e., 2020, 2050) social, economic and environmental impacts of Arctic marine shipping.

The Arctic region currently produces ~ 10 % and ~ 25 % of the world's oil and gas, respectively. Approximately 80% of this oil and 99% of the gas come from the Russian Arctic (AMAP, 2007; Figure 8). Russian petroleum activity prior to 2002 had been insignificant; however, in that year alone, 4 million tonnes of oil were transported along the Russian and Norwegian coasts (Bambulyak and Frantzen, 2005) as Russia began to transport oil to Europe by tanker (AMAP, 2007). Shipping and transportation expanded from 8 million tons in 2004 to ~ 12 millions tons in 2005 in the form of oil and related products exported from the Barents Sea along the Norwegian coast. The predicted annual export of Russian oil along the Norwegian coast may reach 50-150 million tonnes in the next decade (Bambulyak and Frantzen, 2005). Norway's total recoverable potential includes sources from the North, Norwegian and Barents Seas. Norway is the second largest gas exporter to Europe after Russia. Norwegian and Russian

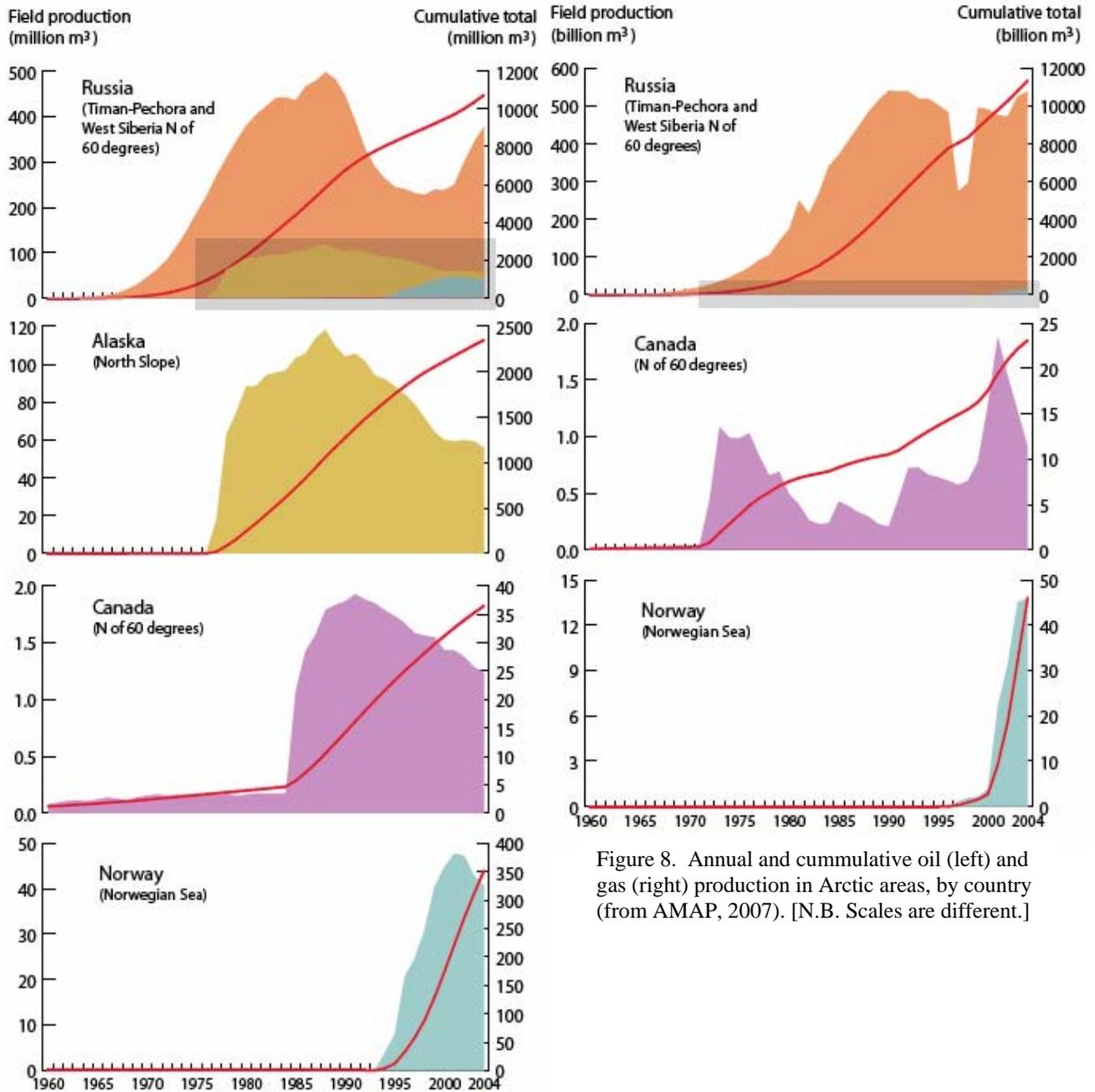


Figure 8. Annual and cumulative oil (left) and gas (right) production in Arctic areas, by country (from AMAP, 2007). [N.B. Scales are different.]

oil and gas production will be most active in Arctic regions including transportation in Arctic waters.

Canada's discovered recoverable resources in the Yukon, Northwest Territories and Nunavut, include 1,665 million barrels (MMbbl) of oil and 31,252 billion cubic feet (Bcf) natural gas (Drummond, 2006). Most oil and gas exploration in Canada is south of the Canadian North;

oil that is exported in Canada goes predominantly to the U.S. and therefore, does not require transportation in Arctic waters. Alaska's Arctic oil and gas development is on the North Slope. Development in the Alaskan National Wildlife Refuge may occur; but this is politically controversial. There is currently a production of 475,000 bbl/day in the Greater Prudhoe Bay area (BP, 2006). Most of Alaska's oil is transported to the ice free Port Valdez via the Trans Alaskan Pipe Line system and is therefore does not cause oil spills in the Arctic seas.

New areas for development include new locations in Greenland, Iceland, and the Faroe Islands (AMAP, 2007). OGA is projected to occur in the Arctic for decades to come (AMAP, 2007). As OGA and transportation the Arctic continues, oil spills in the marine environment and ice infested waters remain a risk.

With respect to biodegradation of oil in first-year sea ice, the key points from Chapter IV include:

- There has been, is, and will be OGA in the Arctic.
- A decrease in summer sea ice extent will result in an increase in marine transportation in the Arctic seas.
- Increased marine traffic, seasonal variability, wave stress, and frequency of icebergs increase the risk of an oil spill to the Arctic marine environment.

# ARCTIC REGION



Figure 8. Political map of Arctic Region  
(source: [http://www.lib.utexas.edu/maps/islands\\_oceans\\_poles/arctic\\_region\\_pol\\_2007.jpg](http://www.lib.utexas.edu/maps/islands_oceans_poles/arctic_region_pol_2007.jpg))

## CHAPTER IV

### BEHAVIOR OF OIL IN THE MARINE AND FREEZING ENVIRONMENTS

When oil is spilled into the marine environment, it spreads to form a slick microns thick (i.e., sheen; Berridge *et al.*, 1968, Floodgate, 1995). Both water-in-oil emulsification and oil-in-water dispersion are driven by wave and wind action. Water in oil emulsions form a mixture often referred to as a “mousse”, which becomes stable (Floodgate, 1995; Colwell and Walker, 1977) with increased volume and viscosity, and low surface-to-volume ratio preventing biodegradation (Davis and Gibbs, 1975). Depending on the temperature and composition of the oil mixture, evaporation may remove 30 % to 40 % of the oil within days (Floodgate, 1995). Immediate surface evaporation results in the volatilization of small C<sub>5</sub>-C<sub>15</sub> alkanes and

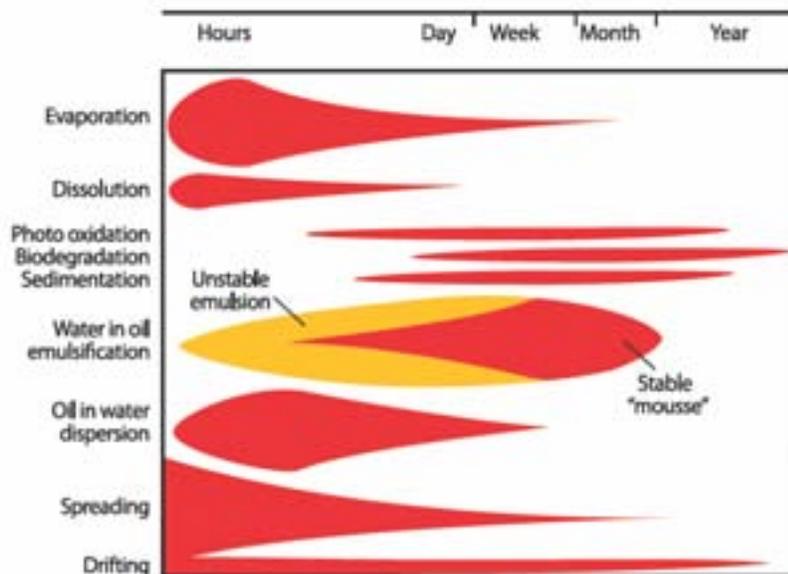


Figure 10. Fate of spilled oil resulting from weathering processes and their time window (from AMAP, 2007).

monoaromatics (e.g., benzene, toluene, ethylbenzene, xylenes).

The remaining compounds in the sheen may be biodegraded (Brakstad and Faksness, 2000).

These processes (i.e., emulsification, dispersion, evaporation, biodegradation)

contribute to the weathering of

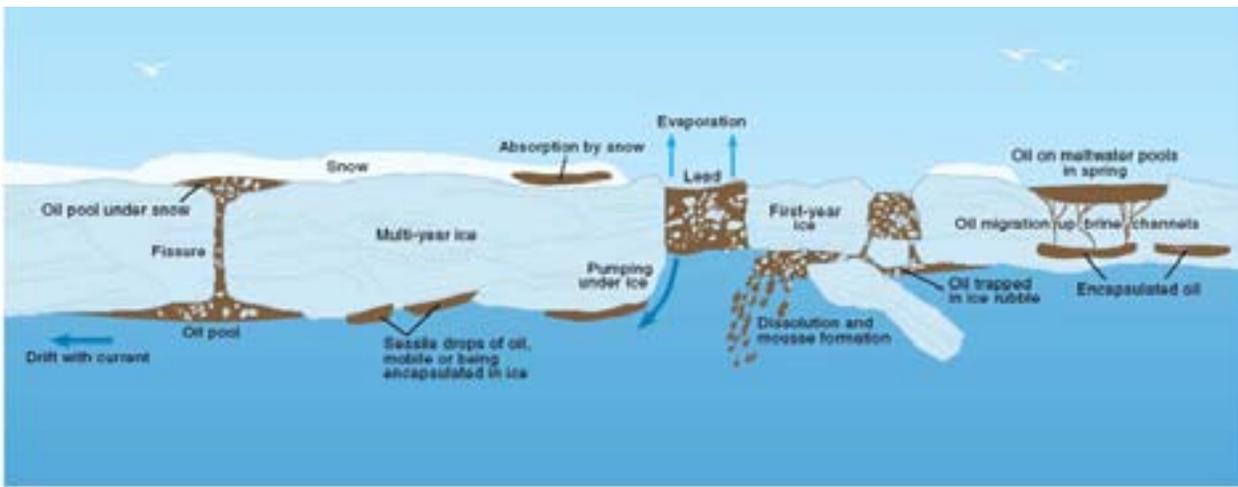


Figure 11. Oil-ice interactions: oil droplets under the ice, new ice growth below the oil, oil appearing on the surface in the spring, wind herding of oil on melt pools, and the appearance of emulsified oil on top of the ice (from AMAP, 1998).

oil and each process has a time window in which it occurs (Figure 10).

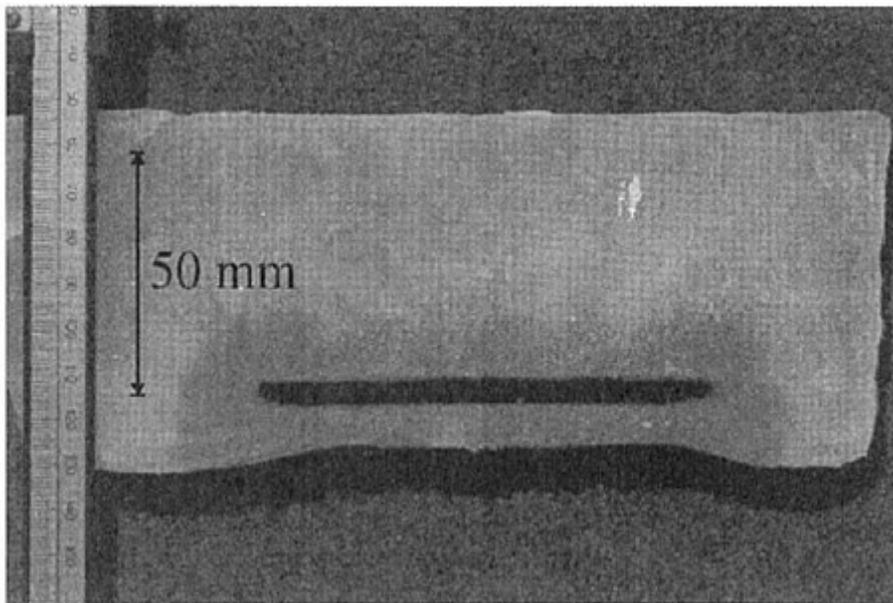
The oil's behavior in freezing environments differs from that in environments where there is seawater alone. Spreading on smooth ice is influenced by an oil's gravity and viscosity. Below  $-19\text{ }^{\circ}\text{C}$ , oil's viscosity is so high it does not spread (Chen, 1972). Oil may be distributed in several ways in ice (Figure 11), however, this paper focuses on encapsulated oil and its migration through brine channels in first-year ice.

Sea ice has under roughness ( $R_s$ ), so an oil's behavior under ice is dominated by under ice topography (Fingas and Hollebone, 2003). Oil under ice spreads systematically filling the nearest depression before moving onto the next one (Fingas and Hollebone, 2003). The ice conditions, including under ice configuration, play a more dominant role than the microscale oil spreading behavior (Fingas and Hollebone, 2003). The storage volume of oil under ice ranges from  $0.01$  to  $0.06\text{ m}^3$  of oil /  $\text{m}^2$  of ice surface (Goodman *et al.*, 1987). Its thickness is greater under ice than in an open water slick because of the forces of interfacial tension acting on the oil (oil-ice, oil-water and ice-water interface). The sum of these forces acts as a retarding force to the spreading oil under ice. The final thickness ( $h_T$ ) of the oil is reached when its buoyancy force equals the interfacial tension acting on it (Izumiyama *et al.*, 2004).  $h_T$  is dependent on the under ice roughness. With little roughness ( $R_s < h_T$ ), the impacted area has limited dependence on the

under ice topography; interfacial tension is the dominant mechanism to hold oil under the ice. In rough regions ( $R_s > 50h_T$ ), the oil impacted area is highly dependent on the ice roughness and pooling is the dominant mechanism holding the oil (the relative roughness height:  $R_s/h_T$ ; Izumiyama *et al.*, 2004). [N.B., Izumiyama *et al.* (2004) used freshwater in their studies.]

When oil is released under ice, it generally forms small droplets (< 1 cm dia) and spreads to a thickness of 0.8 to 20 cm. Over a period of hours, a lip forms around the oil and within days it is completely encapsulated in the matrix (NORCOR, 1975), as ice continues to accrete

Figure 12. Cross-section of oil-ice sandwich in laboratory experiment (from Izumiyama *et al.*, 2004)



downwards (i.e., an “oil ice sandwich”; Figure 12). Ice growth under the oil layer is smaller than elsewhere (Izumiyama *et al.*, 2004). The ice on the bottom and around the perimeter of the oil grows simultaneously

and the two join to encapsulate the oil. The time of entrapment does not depend on the size of the slick (Izumiyama *et al.*, 2004). The oil under ice does not appear to have a measurable effect on ice growth (NORCOR, 1975).

The encapsulated oil is exposed to the internal brine channel network. Brine channels with diameters ranging up to 10 mm are the most important ice features for oil entrainment and transport (Martin, 1979). The coefficient of permeability of oil is  $\sim 10^{-3}$  that of water (Otsuka *et*

*al.*, 2004). Trapped oil can permeate upwards in sea ice in melt season during spring and summer. The distribution of oil (by volume) is greatest at the injection point and decreases towards the surface of the ice (Otsuka *et al.*, 2004). In a study conducted in the Beaufort Sea, oil encapsulated over winter remained in first-year ice until February. By March, it moved vertically approximately 20 cm, and the rate increased to 150 cm per hour in April (NORCOR, 1975) [N.B., NORCOR (1975) reported oil migration by month/season]. Migration through the brine channels and ice ablation move oil to the surface (Dome Petroleum Ltd., 1981). During experimental spills under first-year ice, encapsulated oil was released to the surface the next melt season (Fingas and Hollebone, 2003). In addition to the migration of bulk oil, there is a migration of the water soluble compounds (WSCs) downward into the ice. The concentration of WSCs in ice was reduced from 30 ppb to 6 ppb of brine over 20 cm from the encapsulated oil from February to June in fjord ice in Svalbard, Norway (Faksness and Brandvik, 2005).

Oil and oil-in-water emulsions encapsulated under first-year ice in the Southern Beaufort Sea migrate to the surface, however, only the oil-in-water emulsion appears on the surface by ice ablation and not through brine channel migration (Buist *et al.*, 1983). With a combined oil and gas spill, gas will be released and reach the surface before the oil (Fingas and Hollebone, 2003) due to the density difference of gas, oil, and brine. The relative quantities of oil released from ice depend on the depth of the oil lens, the rate of brine channel opening, and the configuration of the oil in the ice (e.g., discrete droplets vs a pool; Fingas and Hollebone, 2003).

The transport of encapsulated oil in ice in the melt season may be similar to the vertical transport of oil in soil. The movement and distribution of oil in soil differs from the marine environment primarily by vertical movement instead of slick formation. Infiltration of oil through the soil prohibits evaporation of volatile hydrocarbons. However, particulate matter may

also play a role by adsorbing hydrocarbons, reducing toxicity. In addition, humic substances may absorb oil constituents and form persistence residues (Bossert and Bartha, 1984). [N.B., The concentration of humic substance in sea ice is unknown.] Bacteria are the key agents degrading oil in marine environments (Leahy and Colwell, 1990) yet biodegradation of oil in ice has not been reported.

The effect of temperature and salinity on polycyclic aromatic hydrocarbon (PAH) solubility has not been reported in the range of temperature and salinities in sea ice. Changes in salinity between 3 to 36 ppt do not have a significant impact on the solubility of PAHs (i.e., phenanthrene, anthracene, 2-methylantracene, 2-ethylanthracene, 1,2-benzanthracene, and benzo(a)pyrene) in seawater (Whitehouse, 1983) however, this may not be the case at salinities in brine ( $S_{br}$ )(e.g., at -6 °C, -10 °C, and -21°C,  $S_{br}$  is 100, 145 and 216 ppt, respectively (Mock and Thomas, 2005)). Solubility of PAHs decreases with decreasing temperature from 25.3 °C to 3.7 °C (Whitehouse, 1983). The time to establish equilibrium in the water accommodated fraction (WAF) preparation is greater at lower temperatures (2 °C to 13 °C; Faksness *et al.*, 2008). It is important to understand the dissolution of PAHs because they may not be bioavailable to microorganisms.

[N.B., Articles referenced in this chapter did not examine the same petroleum products, however, the oil behavior described below applies to most crude oils.] With respect to biodegradation of oil in first-year sea ice, the key points from Chapter IV include:

- Oil released under ice will spread filling depressions as it spreads.
- If oil is released under ice during freezing, ice will freeze around the oil forming an 'oil-ice sandwich'.
- The oil is preserved in the oil-ice sandwich and weathering processes are limited.

- Oil moves to the surface through brine channel migration and ice ablation during melt season.
- WSCs are transported downwards towards the underlying seawater through advection/dispersion.
- The solubility of PAHs in sea ice brine at cold temperatures and low salinities has not been reported.

## CHAPTER V

### REQUIREMENTS FOR BIODEGRADATION

Alexander (1973) described the conditions needed for biodegradation: (i) organisms must have access to the necessary enzymes; (ii) a sufficient mass of organisms that can degrade the compound must be present; (iii) the compound must be accessible to the organisms (i.e., bioavailable); (iv) if the initial enzyme is extracellular, the bonds acted upon by the enzyme must be exposed for it to interact with them or if intercellular degradation occurs, the compound must penetrate the cell's surface; and, (v) the environment must be conducive to allow maintenance and/or growth of the microorganisms.

Microorganisms need organic carbon (e.g., aromatic hydrocarbons) and a terminal electron acceptor (TEA) to produce energy (i.e., for cell maintenance and growth) and CO<sub>2</sub> as a byproduct: Organic C + TEA → CO<sub>2</sub> + Reduced Electron Acceptor + Energy. In aerobic conditions, O<sub>2</sub> is the TEA used by bacteria. In anaerobic conditions, other TEAs include, but are not limited to, Fe<sup>3+</sup>, NO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, and CO<sub>3</sub><sup>2-</sup>. The acclimation period, also known as the adaptation or lag period, is the length of time between the introduction of the chemical and evidence of detectable biodegradation (Alexander, 1999). Factors controlling the length of the acclimation period include: the structure of the molecule, temperature, pH, dissolved oxygen concentration, and concentration of nutrients (e.g., N, P).

The specific growth rate of a bacterium ( $\mu$ ) was mathematically described by Monod

(1949) as:  $\mu = \frac{\mu_{\max} S}{K_S + S}$  where  $\mu_{\max}$  is the maximum specific growth rate (M/M·T) and S is the

substrate present (M/V).  $K_S$  (M/V) is a constant that represents the concentration of the growth limited substrate where the  $\mu$  is half the maximum rate ( $\mu_{\max}$ ). The lower the value of  $K_S$ , the greater the bacterium's affinity for the contaminant (Alexander, 1999).

Free floating bacteria in the brine channel use organic C, a TEA, and nutrients available in the bulk liquid, whereas surface associated bacteria create a biofilm and adhere to the brine channel wall. The substrate (e.g., organic C, TEA, nutrients) will diffuse across a the static boundary layer (SBL; Figure 13) and reach free floating

bacteria and the biofilm of surface associated bacteria. The flux is the mass of S that moves through the SBL or biofilm per unit surface area per unit of biofilm per time. Based on Fick's Law, the mass transfer flux ( $N_S$ ) [M/L<sup>2</sup>T] is defined as:

$$N_S = K_L(S_B - S_S) \text{ and } K_L = \frac{D_{S,\text{liquid}}}{L} \text{ where } K_L \text{ is the mass}$$

transfer coefficient [L/T],  $D_{S,\text{liquid}}$  is the molecular diffusion coefficient of S [L<sup>2</sup>/T] in the liquid, L is the thickness of the static boundary layer (SBL),  $S_B$  is the concentration of S in the bulk liquid (not to be confused with brine salinity,  $S_{br}$ ) [M/L<sup>3</sup>], and  $S_S$  is the concentration of S at the biofilm surface [M/L<sup>3</sup>].

The SBL is the layer of the liquid where there is no advective

flow so the mass transfer is due solely to diffusion (as opposed to advection with flow). If there is turbulence in the bulk liquid, the SBL is small and vice versa. In sea ice, the biofilm, or bio

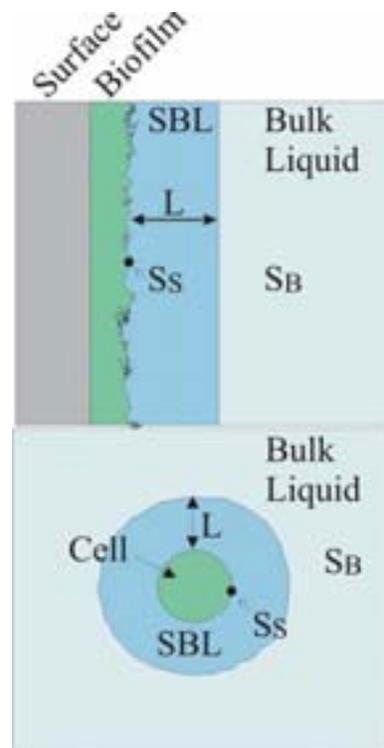


Figure 13. Schematic of mass flux across static boundary layer (SBL) for surface associated (top) and free floating bacteria

patches, may be a monolayer (i.e., pore diffusion and tortuosity does not apply) and there is no mass transfer. Free floating bacteria may have a larger SBL than surface associated bacteria in an environment with laminar flow. This leads to more diffusive limitations for free floating bacteria and a predominance in surface associated bacteria.

With respect to biodegradation of oil in first-year sea ice, the key points from Chapter V include:

- Organic C + TEA  $\rightarrow$  CO<sub>2</sub> + Reduced Electron Acceptor + Energy  
For hydrocarbon degrading bacteria, hydrocarbons are the organic C source. O<sub>2</sub> is the preferred TEA (i.e., the most energy generating), but biodegradation may still occur in anaerobic conditions.
- Factors that influence biodegradation are temperature, pH, and concentrations of nutrients (i.e., N, P)
- Both surface associated and free floating bacteria have an SBL where the limiting substrate (most often nutrients or the TEA) diffuses across the static layer to reach the biofilm or cell. The SBL for surface associated bacteria is smaller than for free floating (i.e., less time for substrate to reach cells).

## CHAPTER VI

### BIODEGRADATION OF PETROLEUM HYDROCARBONS IN THE MARINE ENVIRONMENT

Petroleum hydrocarbons are divided into four classes including saturates, aromatics, asphaltenes, and resins (Colwell and Walker, 1977). Most petroleum hydrocarbons are biodegradable; there are nearly 200 bacterial species known to degrade hydrocarbons (Prince, 1993 and 2005). In general, microbial communities are the least inhibited by cyclic alkanes and have increasing problems with low molecular weight aromatics, branched alkanes and n-alkanes, respectively (Perry, 1984). In general, saturates have the highest biodegradation rate followed by light aromatics, and high molecular weight aromatics, while polar compounds have low biodegradation rates (Fusey and Oudot, 1984; Jobson *et al.*, 1974; Walker *et al.*, 1976). The greater the complexity of the hydrocarbon, the slower the rate of biodegradation, and the greater the formation of intermediate metabolites (Altas and Cerniglia, 1995). Several polynuclear aromatics and high molecular weight aliphatic hydrocarbons are not bioavailable (i.e., not soluble or chemically bound in the context of biodegradable) to microorganisms and hence, biodegrade very slowly, if at all. Compounds can also be recalcitrant if the indigenous microorganisms lack adequate enzymes to degrade them (Altas and Cerniglia, 1995). Often, the presence of a hydrocarbon can induce production of the enzyme.

Hydrocarbon degrading microorganisms are ubiquitous in the marine environment (Altas and Cerniglia, 1995). These microbes biodegrade hydrocarbons (i.e., using them as

electron donors) producing energy for cell maintenance and growth (i.e., production of cell biomass), CO<sub>2</sub>, and many intermediate oxygenated metabolites. In decreasing order, the most common hydrocarbon degrading bacteria belong to the genera: *Pseudomonas*, *Achromobacter*, *Flavobacterium*, *Nocardia*, *Arthobacter* and other coryneforms, *Vibrio*, *Bacillus*, *Micrococcus* and *Acinetobacter* (Atlas, 1995).

Hydrocarbon bioavailability begins when oil disperses into the water column and forms oil-in-water emulsions. The release of biosurfactants by microorganisms is an important preparatory step in biodegradation (Singer and Finnerty, 1984). Biosurfactants are extracellular substances that have a hydrophilic and hydrophobic component which emulsify hydrocarbons (hydrophobic) and allow transport into the cell (hydrophilic) for biodegradation.

Temperature has a physical and chemical effect on oil, as well as changing the rate of microbial metabolism and the composition of the microbial community (Atlas, 1981). As oil viscosity increases, the volatilization of toxic short chain alkanes is reduced, increasing water solubility which prolongs the lag phase of microbial degradation (Atlas and Bartha, 1972). In general, rates of degradation decrease with decreasing temperature, in part because there is a decrease in enzyme activity (Atlas and Bartha, 1972; Gibbs *et al.*, 1975).

In order for bacteria to begin degradation of a hydrocarbon, the molecule must be transformed. For example, double bonds (i.e., in aromatics) must be cleaved into single bonds (i.e., alkanes). Under aerobic conditions for a benzene ring, O<sub>2</sub> is inserted to form functional groups on the ring and ultimately to form catechol. Bacteria convert catechols to aliphatics using aromatic ring cleavage dioxygenases (Atlas and Cerniglia, 1995, Figure 14). The catechol ring is cleaved eventually forming an aliphatic with a carboxyl group which is used by the cell in the

tricarboxylic acid cycle (TCA or Krebs Cycle) which is a series of reactions critical for cellular respiration (Figure 14).

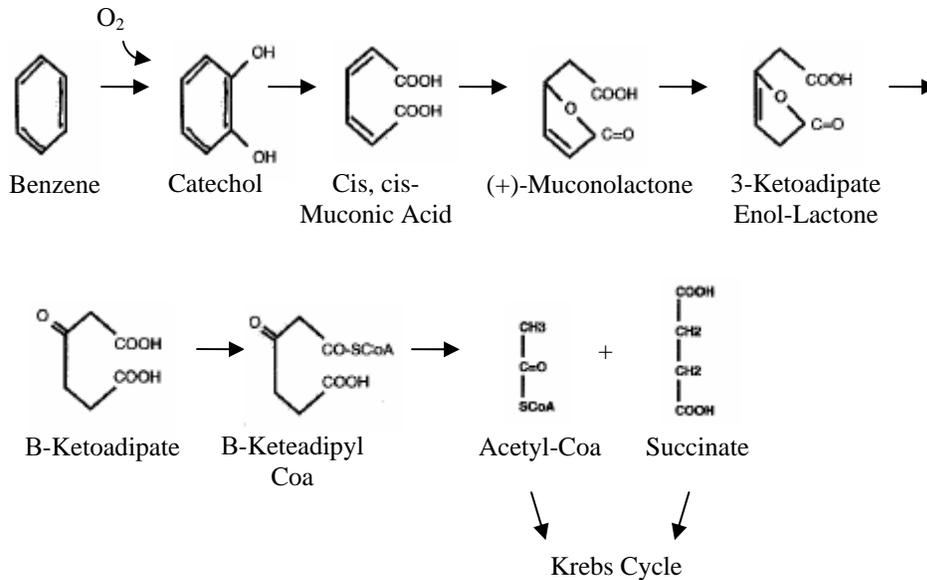


Figure 14. Schematic of aromatic ring cleavage with molecular oxygen (adapted from Baker and Herson, 1994).

Molecular oxygen is the preferred terminal electron acceptor (TEA) to oxidize oil compounds because it generates the most energy for a cell and it is present in sea ice brine. The theoretical oxygen demand is 3.5 mg  $O_2$ /mg hydrocarbon (Brakstad, 2008). Nitrate can act as an electron acceptor under microaerophilic denitrifying conditions (Mihelcic and Luthy, 1988; Zeyer *et al.*, 1986). Alkanes, monocyclic and polycyclic aromatic compounds can be degraded in anoxic conditions with  $NO_3^-$ ,  $Fe^{3+}$ , or  $SO_4^{2-}$  as an electron acceptor (Harayama *et al.*, 2004).

Nitrogen and phosphorus availability limit microbial degradation in the marine environment (Leahy and Colwell, 1990). There have been numerous studies of enhancement of carbon/nitrogen/phosphorus ratios (i.e., using oleophilic fertilizers) to stimulate the biodegradation of oil. For petroleum hydrocarbons, the ideal C:N:P ratio is 50:5:1 (Alexander, 1999). Although effective in closed systems, the addition of nutrients may be limited in the

environment because they dissolve in seawater and are rapidly diluted (Leahy and Colwell, 1990).

When oil is introduced into a previously pristine environment, there is an initial decrease in the total heterotrophic (i.e., organic C degrading) bacteria suggesting an inhibitory effect. There is a concomitant increase in the abundance of oil degraders, confirming that some members of the indigenous bacterial community have the capacity to mineralize oil compounds to CO<sub>2</sub> (Floodgate, 1995). Oil is comprised of many hydrocarbons and each compound is degraded separately. The adaptation of the bacterial community to favor hydrocarbon degraders determines how rapidly subsequent oil compounds will be mineralized (Leahy and Colwell, 1990). Three mechanisms by which adaptation occurs are: (i) induction and/or depression of specific enzymes, (ii) new metabolic capabilities resulting from genetic change, and (iii) selective enrichment of microorganisms able to transform the compound (Spain *et al.*, 1980; Spain and van Veld, 1983). In general, the abundance of hydrocarbon degrading microorganisms in the environment correlates to the degree of contamination present (e.g., abundance of hydrocarbon degraders increases after petroleum exposure; Leahy and Colwell, 1990). Adapted communities that have previously been exposed to hydrocarbons show evidence of higher biodegradation rates than microbial communities with no prior history (Leahy and Colwell, 1990).

While many of the compounds in crude oil are biodegradable depending on their size and complexity and the TEA used, some individual hydrocarbons or mixtures can be inhibitory. For example, shorter alkanes (C<sub>5</sub>-C<sub>10</sub>) inhibit many hydrocarbon degraders (Bartha, 1986). Juhasz *et al.* (1997) found that *Burkholderia cepacia* ceases to use pyrene as a carbon source after ~ 400 mg/L had been catabolized. They suggest this is due to the accumulation of toxic intermediates

which reach inhibitory concentrations in the medium. Degradation of pyrene, fluoranthrene and phenanthrene by *Rhodoccus sp.* is inhibited when phenanthrene is added to cultures containing pyrene or fluoranthrene (Bouchez *et al.*, 1995) possibly as a result of blocking the induction of PAH degrading enzymes (Juhasz *et al.*, 1997). Although there is an inhibition effect on individual strains, the community composition may include strains that are not inhibited by the PAHs and may still be biodegraded. Due to the complexity of the hydrocarbon mixtures and the presence of different bacterial species, little is known about the individual roles of bacteria (Boyd *et al.*, 2007).

With respect to biodegradation of oil in first-year sea ice, the key points from Chapter VI include:

- Most petroleum hydrocarbons are biodegradable.
- The more complex the hydrocarbon, the slower the rate of biodegradation. Some hydrocarbons inhibit bacterial degradation.
- Hydrocarbons must be bioavailable to the microbes in order for biodegradation to occur.
- Biosurfactants are extracellular substances which emulsify hydrocarbons making them bioavailable and hence enabling bacteria to degrade them.
- After hydrocarbon exposure, there is a shift in the bacterial community to hydrocarbon degrading species.
- O<sub>2</sub> is the preferred TEA for hydrocarbon biodegradation because it generates the most energy for the cell. Nutrients (i.e., N, P) are the growth limiting substrate (S) for bacteria.

## CHAPTER VII

### BIODEGRADATION OF HYDROCARBONS IN COLD AND FREEZING ENVIRONMENTS

The Arctic and Antarctic oceans and sea ice have an abundance of bacteria in the classes of  $\alpha$ -proteobacteria and  $\gamma$ -proteobacteria and the phylum of Cytophaga-Flavobacter-Bacteroides (CFB) (Bowman *et al.*, 1997; Brown and Bowman, 2001; Bano and Hollibaugh, 2002; Junge *et al.*, 2004; Brinkmeyer *et al.*, 2003; Gerdes *et al.*, 2005; Yakimov *et al.*, 2004).

Hydrocarbon volatilization is reduced in cold and freezing seawater, increasing the concentration of toxic hydrocarbons *in situ* (i.e., the more volatile fractions are more toxic). If oil reaches ice covered waters, the slick is thicker since the oil is partly restricted by ice cover. These scenarios account for the prolonged lag phase and delayed onset of biodegradation (Atlas and Bartha, 1972; Hokstad *et al.*, 1999). Waxy oils with high pour points may reduce dilution and dispersion in cold seawater since precipitated wax may build a matrix, limit mixing, and act as a diffusion barrier (Brakstad, 2008). Microbes in cold seawater degrade soluble oil compounds at the oil water interface of the small droplets (Brakstad and Bonnaunet, 2006).

Members of the several bacterial genera able to degrade petroleum hydrocarbons are found in seawater and marine ice in the polar regions. Studies of psychrophilic and psychrotolerant hydrocarbon degrading marine bacteria have shown a high degree of phylogenetic diversity: *Sphingomonas*, *Marinobacter*, *Marinomonas*, *Halomonas*, *Psychrobacter*, *Psychromonas*, *Colwellia*, *Oleispria*, *Acinetobacter*, *Shewanella*, *Pseudoalteromonas*, *Pseudomonas*, *Cytophaga*, *Agreia*, *Arthrobacter*, and *Rhodococcus*

(Michaud *et al.*, 2004; Van Hamme *et al.*, 2003; Deppe *et al.*, 2005; Gerdes *et al.*, 2005; Powell, 2005; Brakstad and Bonaunet, 2006). Hydrocarbonoclastic marine bacteria are present in low numbers in pristine seawater, but when oil is released can account for  $\leq 90$  % of the microbial community (Deppe *et al.*, 2005). The lag phase depends on several factors (e.g., hydrocarbon type, pristine vs. polluted environment, temperature). In a laboratory experiment, there was a 2-4 day lag phase at 0 °C for winter seawater collected at the sea surface (<10 °C) and from 90 m depth (6-8 °C) with 1 % crude oil supplied with inorganic nutrients (Brakstad, personal communication).

Water soluble hydrocarbons can dissolve from a pool of oil-in-ice into brine channels. The channels normally contain high concentrations of bacteria. The oil transports through the ice in the spring as melting occurs; oil may vertically rise through the brine channels or the ice surface may ablate down to the oil (Fingas and Hollebone, 2003).

A consortium of psychrotolerant Arctic bacteria are capable of degrading crude oil at -4 °C using the hydrocarbons as electron donors except for o-xylene and the methylnaphthalenes (Deppe *et al.*, 2005). The bacterial strains are closely related to the genera: *Pseudoalteromonas*, *Pseudomonas*, *Shewanella*, *Marinobacter*, *Psychrobacter*, and *Agreia* (Deppe *et al.*, 2005).

Oil droplets are usually covered by a biofilm of bacteria before effective biodegradation occurs. Short chain alkanes (C<sub>8</sub>-C<sub>15</sub>) are the first metabolized, followed by longer n-alkanes (C<sub>15</sub>-C<sub>34</sub>) in parallel with isoprenoids, pristane and phytane (Deppe *et al.*, 2005). *Pseudoalteromonas atlantica* has the ability to switch the production of EPS on and off and may play a role with biofilm formation prior to biodegradation (Deppe *et al.*, 2005).

Hydrocarbon degrading species often produce extracellular emulsifying agents, usually consisting of high molecular weight polysaccharides associated with proteins (Bach *et al.*, 2003).

Polysaccharides generate extracellular emulsan proteins which emulsify hydrophobic hydrocarbons (Zuckerberg *et al.*, 1979). Emulsan contains 10-20 % protein and is primarily active with a mixture of aliphatic and aromatic hydrocarbons (Bach *et al.*, 2003). It is released from the bacterial cell surface by esterase, a key component in the active emulsan protein complex (Shabtai and Gutnick, 1985).

Some PAHs (e.g., phenanthrene, dibenzothiophene, acenaphthene) along with saturated cyclic compounds (i.e., hopanes, which are one of the most persistent components from oil spills in the environment) are not attacked by microbial communities (Deppe *et al.*, 2005). The more complex the PAH (e.g., polycyclic, double bonds) the more difficult it is for the microorganisms to emulsify the molecule and use the molecule as an electron donor. Complex components found in oil that are too complex will not be degraded by bacteria and may remain persistent in the environment.

With respect to biodegradation of oil in first-year sea ice, the key points from Chapter VII include:

- Several bacterial genera found in seawater or marine sea ice in the polar regions are able to degrade petroleum hydrocarbons.
- Oil droplets are covered by biofilms before biodegradation occurs.
- EPS may play a role with biofilm formation.
- Simpler hydrocarbons are metabolized first.

## CHAPTER VIII

### CONCEPTUAL MODEL: BIODEGRADATION POTENTIAL OF OIL IN ICE

Most investigations of hydrocarbon catabolism in cold environments have been performed in Arctic or Antarctic (frozen) soils (Brakstad, 2008). Sea ice may be a better environment for biodegradation than frozen soil because the brine volume enables microbial motility, respiration, and a matrix for transport of hydrocarbons and nutrients (Brakstad, 2008).

Perhaps a better analog for a sea ice biodegradation conceptual model is non-frozen soil because the sea ice matrix with vertical flow in the brine channels may be compared to a soil column in which there is vertical transport of pollutants. Both sea ice and soil favor surface associated microorganisms. In addition, oil introduced to soil and sea ice remains more toxic than in open water because: (i) low molecular weight compounds that can volatilize in open water are trapped in soil or sea ice; and (ii) sufficient mixing energy is not present, reducing dispersion of the petroleum hydrocarbons. This matrix concept may be applied to computer modeling of oil transport through ice.

Individual hydrocarbons and dissolved components (e.g., WSCs) are transported downwards advecting with the brine as it flows through the channels and may reach the ice-seawater interface. Oil components become bioavailable to other organisms (e.g., algae) at the ice ocean interface and in the underlying seawater additional biodegradation may occur. The bulk oil migrates upwards due to the density difference of oil and brine leaving residual oil

constituents and small oil droplets behind. Bulk oil is toxic to many microorganisms, however, the dissolved components and small droplets are probably bioavailable to hydrocarbon degrading bacteria. Small oil droplets may be encapsulated under the bulk oil during ice accretion due to entrainment from turbulence (i.e., currents) in the water column. If the buoyancy of the small oil droplets is less than the advection in the brine flow then the droplets will migrate downwards with other individual hydrocarbons and dissolved components.

In order for biodegradation to occur, there must be hydrocarbon bioavailability and a TEA, most preferably,  $O_2$ . Brine channels have been characterized as hyperoxic from photosynthesis. Therefore,  $O_2$  would not be, at least initially, the limiting factor for biodegradation. The bacteria and algae are often associated with the surfaces of the brine channels. As  $O_2$  is produced by algae, the bacteria can immediately use it as a TEA for hydrocarbon biodegradation. In this case, there is not a mass transfer flux across a SBL as the concentration of  $O_2$  at the cell surface would be equal to (if not greater than) the concentration in the bulk liquid. There would be a mass transfer flux of nutrients (i.e., N, P) from the brine and they may thus be the limiting substrate.

There are common similarities between bacteria found in brine channels in sea ice and hydrocarbon degrading bacteria. Surface associated microorganisms dominate in sea ice with decreasing temperatures and hydrocarbon degrading bacteria adhere to the surface of oil droplets in order to access the electron donor. EPS in sea ice produced by bacteria may serve as a biofilm matrix and may be used for cell attachment by Arctic bacteria. It is also found over the surface of oil droplets after it is synthesized by hydrocarbon degraders before they begin effective biodegradation.

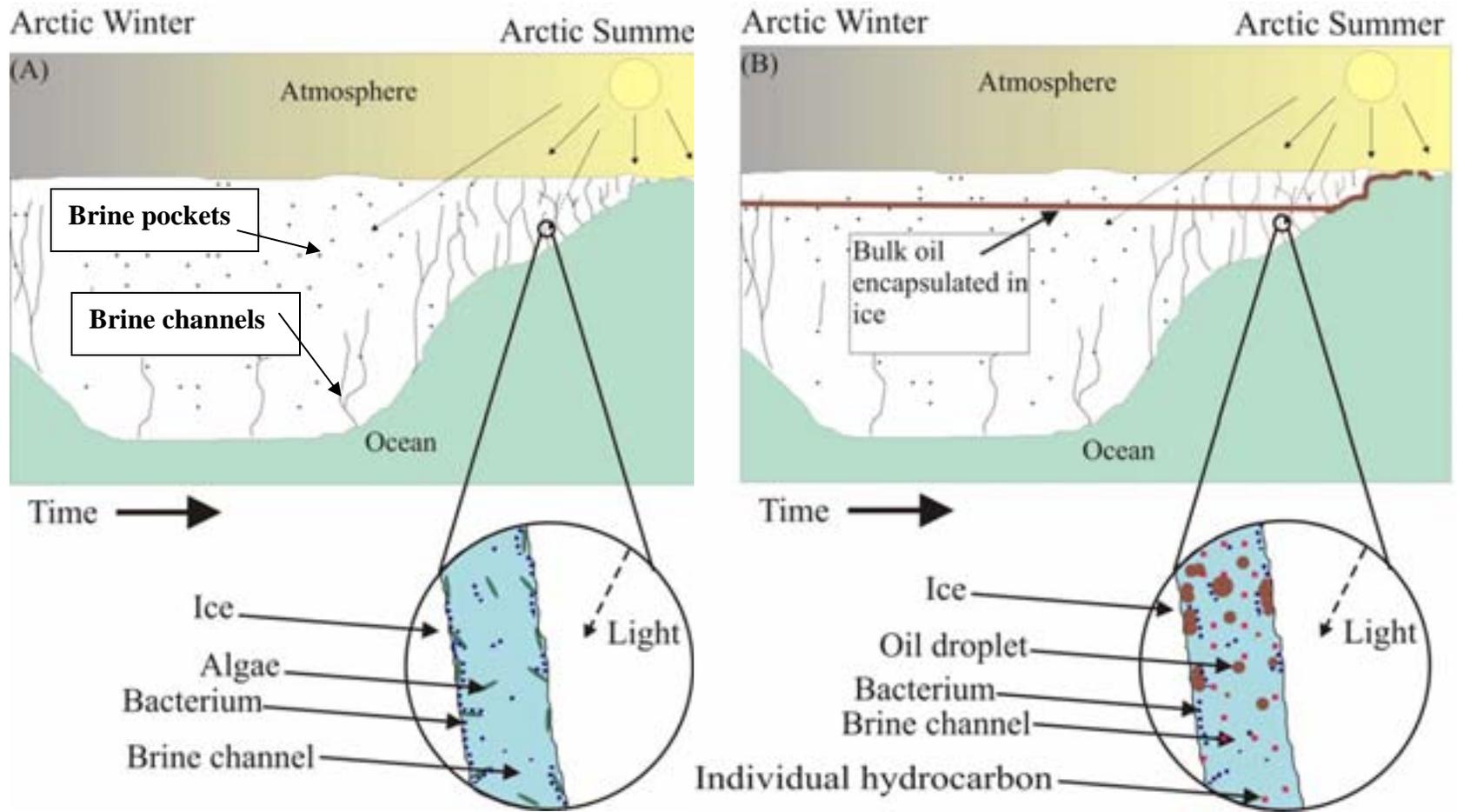
The solubility of petroleum hydrocarbons in the highly saline brine at low temperatures is unknown. However, the transport of dissolved oil or droplets through the brine channel may be modeled as a plug flow reactor with the brine channels serving as a continuous flow system. This environmental engineering reactor theory-based approach has not been applied to brine channel biodegradation. It is unclear if there is a bacterial preference for oil compounds adhering to the brine channel walls or ice crystals, or dissolved in the brine. For surface associated bacteria, the hydrocarbons are transported downwards from the encapsulated oil in the brine and would need to diffuse across the SBL to the bacteria.

The toxicological effect of crude oil or PAHs on sea ice diatoms are unknown, however, the growth of *Scenedesmus subspicatus*, a freshwater green algae test organism for ecotoxicological bioassays, was inhibited by PAHs (i.e., benzo(a)pyrene, pyrene, anthracene, phenanthrene, naphthalene yielded EC<sub>50</sub> values of 1.48, 18.72, 1.04, 50.24, 68.21 µg/L, respectively for a period of seven days)(Djomo *et al.*, 2004). If oil is encapsulated in ice and small oil droplets or dissolved oil constituents come in contact with the brine channels, algae will likely die or be inhibited by the toxic compounds in the oil and there will no longer be a production of O<sub>2</sub>. The system will remain aerobic until the bacteria use all the O<sub>2</sub> as a TEA at which point the brine channels will become microaerophilic and then anoxic. If there is no O<sub>2</sub> present, the nitrate and sulfate reducing bacteria may use NO<sub>3</sub><sup>-</sup> or SO<sub>4</sub><sup>2-</sup> as a TEA. The NO<sub>3</sub><sup>-</sup> in brine from sea ice in the Weddell Sea is up to 1.5 µmol/kg (Papadimitriou *et al.*, 2007); and as discussed in Chapter 1, SO<sub>4</sub><sup>2-</sup> accounts for ~ 8 % of the dissolved salts in brine. There will be a lag phase while the bacterial community shifts from bacteria that use organic carbon produced by the algae and/or EPS as the electron donor to those that use hydrocarbons as the electron donor.

A conceptual model of oil-in-ice is shown in Figure 15. There are free floating and surface associated bacteria in the brine channel. Surface associated bacteria typically dominate in the system, however, there will no longer be algae producing O<sub>2</sub> or an organic carbon source. With the absence of algae and O<sub>2</sub>, surface associated bacteria will rely on the mass transfer flux of the TEA and nutrients across the SBL in order to degrade the hydrocarbons. Hydrocarbon degrading bacteria will degrade individual hydrocarbons that have dissolved into the brine and to small oil droplets.

Biodegradation of oil in first-year ice is the focus of this conceptual model because it is the ice most likely to come into contact with and encapsulate oil spilled due to transportation in the NSR and other routes in the Arctic. [N.B., First-year ice melts before the second winter; otherwise the ice is classified as multi-year ice.] Should there be a spill encapsulating oil in ice (e.g., in autumn), then the potential for biodegradation of the oil in the ice would occur in winter and spring, before the break up of the ice the following summer, when the bulk oil and oil components would be released into the seawater. Where brine channels are formed, the brine flow is due to the unstable density difference driven by the atmospheric temperature. Dissolution and transport of hydrocarbons into the brine from the encapsulated oil would most likely be significant in spring and summer when the ice warms.

Figure 15. Conceptual model of (A) pristine sea ice and (B) biodegradation encapsulated oil in first-year sea ice. Note lack of algae in (B).



## B. CONCLUSION

If there is encapsulated oil in sea ice, biodegradation of hydrocarbons should occur. Abundances of bacteria in the brine channel are greater than in non-frozen soils and seawater. However, the amount of biodegradation that occurs will be a function of the product of cell abundance times the biodegradation rate (activity per cell). There is evidence that hydrocarbon degrading bacterial communities are capable of degrading oil at low temperature (0 to 5 °C) in the marine environment. Within the brine channels, there are O<sub>2</sub> and nutrients which are available to the microorganisms. The microbes that survive the pristine, hyperoxic, high saline, and cold environment may be well adapted to use oil as a carbon source and continue to survive in harsh conditions.

The length of the lag phase and the biodegradation rate of oil in ice remain unknown. Relative to the cycle of first-year ice, oil that is not biodegraded would be exposed to seawater when the ice melts. Encapsulated bulk oil will resurface, however, oil constituents in brine would most likely be transported to the underlying seawater and be further transported and biodegraded in the water column. If there was oil encapsulated in ice that did not melt, the ice would become multi-year ice and biodegradation could continue [N.B., Multi-year ice characteristics are not discussed in this paper and are different than those of first-year ice.].

The biodegradation potential of oil in sea ice should be further investigated with laboratory and/or field experiments. The physical/chemical characteristics of oil interactions in brine (i.e., low temperatures, high salinities) are poorly understood and without this information the bioavailability of oil to the microbes is unknown. In addition, understanding the fate (i.e., in

addition to biodegradation) and transport of oil encapsulated in ice allows a better prediction of the fate of oil released into the seawater column and the sea surface after the melting cycle of first-year ice. The kinetics and hydraulic transport would aid the development of a biodegradation performance model that could predict the removal of oil over time and space.

## C. LIST OF REFERENCES

- Alexander, M. *Biodegradation and Bioremediation*. 2 ed. Academic Press: San Diego. 1999.
- Alexander, M. Nonbiodegradable and other recalcitrant molecules. *Biotechnology and Bioengineering*. 1973. 15:611-647
- Amon, R.M.W. *et al.* Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. *Limnology and Oceanography*. 2001. 46:287-297.
- Arctic Climate Impact Assessment (ACIA). *Impacts of Warming Arctic*. Cambridge University Press: Cambridge. 2004.
- Arctic Oil and Gas 2007. Arctic Monitoring and Assessment Programme (AMAP). Oslo. 2007
- Arctic Pollution Issues. Arctic Monitoring and Assessment Programme (AMAP). Oslo. 1998.
- Atlas, R.M., 1995. Petroleum Biodegradation and Oil Spill Bioremediation. *Marine Pollution Bulletin*. 1995. 31:178-182.
- Atlas, R.M. Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Critical Review Microbiology*. 1981. 5:371-386
- Atlas, R.M. and Bartha, R. Biodegradation of petroleum in seawater: limitation by nitrogen and phosphorus. *Biotechnology Bioengineering*. 1972 14:309-317
- Atlas, R.M. and Cerniglia, C.E. Bioremediation of petroleum pollutants. *Bioscience*. 1995. 5:332-336.
- Bach, H., Y. Berdichevsky, and D. Gutnick. An exocellular protein from the oil-degrading microbe *Acinetobacter venetianus* RAG-1 enhances the emulsifying activity of the polymeric bioemulsifier emulsan. *Applied Environmental Microbiology*. 2003. 69:2608–2615.
- Baker, K.H. and D.S. Herson. *Bioremediation*. McGraw-Hill. New York. 1994
- Bambulyak, A. and Frantzen, B. Oil transport from the Russian part of the Barents Region: status per January 2005. Svanhovd Environmental Centre. 2005.
- Bano, N. and Hollibaugh, J.T. Phylogenetic composition of bacterioplankton assemblages from the Arctic Ocean. *Applied Environmental Microbiology*. 2002. 68:505-518.
- Bartha, R. *Biotechnology of petroleum pollutant biodegradation*. *Microbial Ecology*. 1986. 12: 155-172.
- Berridge, S.A. *et al.* The properties of persistent oils at sea. In *Scientific aspects of pollution of the sea by oil*. Hepple (eds). London, UK: Institute of Petroleum. 1968. 35-59.
- Brakstad, O.G. Natural and stimulated biodegradation of petroleum in marine environments. In *Psychrophiles: from Biodiversity to Biotechnology*. Springer Berlin Heidelberg. 2008. 23.

- Brakstad, O.G. and Bonaunet, K. Biodegradation of petroleum hydrocarbons in seawater at low temperatures (0-5 °C) and bacterial communities associated with degradation. *Biodegradation*. 2006. 17:71-82
- Brakstad, O.G. and Faksness, L-G. Biodegradation of water-accomodated fractions and dispersed oil in the seawater column. In Proceedings to the SPE Conference on Health, Safety and Environment in Oil and Gas Exploration and Production. Society of Petroleum Engineers. Stravanger, Norway. 26-28 Jun 2000. Paper 61466.
- Brakstad, personal communication.
- Brown, M.V. and Bowman, J.P. A molecular phylogenetic survey of sea-ice microbial communities (SIMCO). *FEMS Microbial Ecology*. 2001. 35: 267-275.
- Brinkmeyer R *et al.* Diversity and structure of bacterial communities in Arctic versus Antartctic pack ice. *Applied Environmental Microbiology*. 2003 69:6610-6619.
- Bowman, J.P. *et al.* Diversity and association of psychrophilic bacteria in Antarctic sea ice. *Applied Environmental Microbiology*. 1997. 63: 2448-2462.
- Bossert, I and Bartha, R. The fate of petroleum in soil ecosystems. In *Petroleum microbiology*. Atlas (eds). New York, New York: Macmillan Publishing Co. 1984. 434-476.
- Bouchez, M *et al.* Degradation of polycyclic aromatic hydrocarbons by pure strains and by defined strain association: inhibition phenomena and cometabolism. *Applied Microbiology and Biotechnology*. 1995. 43:156-164. *as cited in Juhasz, A.L., et al. 1997.*
- Boyd, A. *et al.* Determining the identity and roles of oil-metabolizing marine bacteria from the Thames estuary, UK. *Environmental Microbiology*. 2007. 9(1): 165-176.
- British Petroleum (BP). Fact Sheet: Prudhoe Bay. 2006.
- Buist, I. *et al.* Oil spills in leads: tank tests and modeling. Manuscript Report EE-95. Environmental Canada, Ottawa, ON. 1983. 126.
- Chen, E.C. Arctic winter oil spill test. Technical Bulletin No. 68, Environmental Canada. 1972. 20.
- Colwell, R.R. and Walker, J.D. Ecological aspects of microbial degradation of petroleum in the marine environment. *Critical Review of Microbiology*. 1977. 5:423-445.
- Cox, G.F.N. and W.F. Weeks. Numerical simulations of the profile properties of undeformed first-year sea ice during growth season. 1988. 93: 12449-12460.
- Davis, S.J. and Gibbs, C.F. The effect of weathering on crude oil residue exposed at sea. *Water Research*. 1975. 9:275-285.
- Deppe, U. *et al.* Degradation of crude oil by an arctic microbial consortium. *Extremophiles*. 2005. 9: 461-470.
- Djomo, J.E. *et al.* Toxic effects of some major polyaromatic hydrocarbons found in crude oil and aquatic sediments on *Scenedesmus subspicatus*. *Water Research*. 2004. 38:1817-1821.
- Donlan, R.M. Biofilms: microbial life on surfaces. *Emerg Infect Dis* [serial online]. 2002 [2008]. <<http://www.cdc.gov/ncidod/EID/vol8no9/02-0063.htm>>.
- Dome Petroleum Ltd. Oil and gas under sea ice. Report CVI, Canadian Offshore Oil Spill Research Association. Calgary, AB I and II. 1981. 286.

- Drummond, K.J. Canada's Discovered Oil and Gas Resources North of 60. Search and Discovery Article #10102 2006. 7p.
- Eicken, H. *et al.* The role of sea ice in structuring antarctic ecosystems. *Polar Biology*. 1992 12: 3-13.
- Eicken, H. *et al.* Magnetic resonance imaging of sea-ice pore fluids: methods and thermal evolution of pore microstructure. *Cold Regions Science Technology*. 2000. 31: 207-225.
- Eicken, H. From the microscopic, to the macroscopic, to the regional scale: growth, microstructure, and properties of sea ice. In *Sea Ice – an Introduction to its Physics, Chemistry, Biology and Geology*. Thomas and Dieckmann (eds). Oxford, UK: Blackwell Science. 2003. 22-81.
- Faksness, L-G and Brandvik, P.J. Dissolution of water soluble components from oil spills encapsulated in ice. In Proceedings to the 2005 Arctic and Marine Oil Spill Program Technical Seminar. Calgary. 2005. 59-73.
- Faksness, L-G, P.J. Brandvik, and L.K. Sydes. Chemical composition of water accommodated fractions as a function of mixing times and temperatures. University of Bergen. Thesis. 2008. 63-84.
- Fingas, M.F. and Hollebone, B.P. Review of behaviour of oil in freezing environmental. *Marine Pollution Bulletin*. 2003. 47: 33-340.
- Floodgate, G.D. Some environmental aspects of marine hydrocarbon bacteriology. *Aquatic Microbial Ecology*. 1995. 9:3-11.
- Freitag, J. and Eicken, H. Meltwater circulation and permeability of Arctic summer sea ice derived from hydrological field experiments. *Journal of Glaciology*. 2003. 49:349-358.
- Fusey, P and Oudot, J. Relative influence of physical removal and biodegradation in the depuration of petroleum contaminated seashore sediments. *Marine Pollution Bulletin*. 1984. 15:136-141.
- Gerdes, B *et al.* 2005. Influence of crude oil on changes of bacterial communities in Arctic sea ice. *FEMS Microbial Ecology*. 53:129-139.
- Gibbs, C.F. *et al.* Quantitative studies on marine biodegradation of oil. II. Effect on Temperature. *Proceeding Royal Society London Series B*. 1975. 199:83-94
- Gleitz, M. *et al.* Comparison of summer and winter inorganic carbon, oxygen and nutrient concentrations in Antarctic sea ice brine. *Marine Chemistry*. 2005 51: 81-91.
- Glossary of Meteorology. American Meteorological Society. 2e. 2000.  
< <http://amsglossary.allenpress.com/glossary> >.
- Goodman, R.H. *et al.* A technique for the measurement of under-ice roughness to determine oil storage volumes. In *Proceeding of 1897 International Oil Spill Conference*. American Petroleum Institute. Washington, DC. 1987. 395-398.
- Haas, C. Dynamics versus thermodynamic. In *Sea Ice – an Introduction to its Physics, Chemistry, Biology and Geology*. Thomas and Dieckmann (eds). Oxford, UK: Blackwell Science. 2003. 82-111
- Harayama, S *et al.* Microbial communities in oil-contaminated seawater. 2004. 15: 205-214.
- Huston, A.L. Bacterial adaptation to the cold: in situ activities of extracellular enzymes in the North Water polynya and characterization of a cold-active aminopeptidase from *Colwellia psychrerythraea* strain 34H. Doctoral dissertation, School of Oceanography, University of Washington. 2003. 153p.

- Hudier, E. and Ingram, G. Small scale melt processes governing the flushing of nutrients from first-year sea ice, Hudson Bay, Canada. *Oceanologica acta*. 1994. 17: 397-403. *Cited from Freitag and Eicken 2003*.
- Hokstad *et al.* Chemical and ecotoxicological characterization of oil-water systems. *Spill Science Technology Bulletin*. 1999. 5:75-80
- Izumiyama, K. *et al.* Prediction of Oil-Ice Sandwich Formation. Proceedings of the 13th International Offshore and Polar Engineering Conference. 2004. 268-272.
- Jakosky, B.M. *et al.* Subfreezing activity of microorganisms and the potential habitability of Mars' polar regions. *Astrobiology*. 2003. 3:343-350.
- Jobson, A.M. *et al.* Effect of amendments on the microbial utilization of oil applied to soil. *Applied Microbiology*. 1974. 27:166-171.
- Juhasz, A.L., et al. Degradation of fluoranthene, pyrene, benz[a]anthracene and dibenz[a,h]anthracene by *Burkholderia cepacia*. *Journal of Applied Microbiology*. 1997. 83: 198-198. from Boyd, A. et al. 2007.
- Junge, K. *et al.* Phylogenetic diversity of numerically important Arctic sea-ice bacteria at subzero temperature. *Microbial Ecology*. 2002. 43:315-328.
- Junge, K. *et al.* Bacterial activity at -2°C to -20°C in Arctic wintertime sea ice. *Applied Environmental Microbiology*. 2004. 70:550-557.
- Krembs, C. *et al.* ASLO 2001 Aquatic Sciences Meeting, Albuquerque, NM. 12-16 Feb 2001. [www.also.org/albuquerque2001/cs25.html](http://www.also.org/albuquerque2001/cs25.html).
- Krembs, C. *et al.* Implications of brine channel geometry and surface area for the interaction of sympagic organisms in Arctic sea ice. *Journal of Experimental Marine Biology and Ecology*. 2000. 243: 55-80.
- Krembs, C. *et al.* High concentration of exopolymeric substances in Arctic winter sea ice: implication for the polar ocean carbon cycle and cryoprotection of diatoms. *Deep Sea Research, Part 1*. 2002. 49: 2163-2181.
- Leahy, J.G and Colwell, R.R. Microbial Degradation of Hydrocarbons in the Environment. *Microbiological Reviews*. 1990. 54:305-315.
- Light, B. G.A. Maykut and T.C. Grenfell. Effects of temperature on the microstructure of first-year Arctic sea ice. *Journal of Geophysical Research*. 2003. 108: 16p.
- Loeb G.I. and R.A. Neihof. Marine conditioning films. *Advances in Chemistry*. 1975;145:319-335 *as cited in Donlan, 2002*.
- Løset, S. TBA4265 Marine Physical Environment. Class notes. Autumn 2007.
- Martin, S. A field study of of brine drainage and oil entrainment in first-year sea ice. *Journal of Glaciology*. 1979. 22:473-504. *Cited from Freitag and Eicken 2003*.
- Michaud *et al.* The biodegradation efficiency on diesel oil by two psychrotrophic Antarctic marine bacteria during a two-month-long experiment. *Marine Pollution Bulletin*. 2004. 49: 405-409.
- Mihelcic, J.R. and Luthy , R.G. Microbial degradation of acenaphthlene and naphthalene under denitrification conditions in soil-water systems. *Applied Environmental Microbiology*. 1988
- Mock, N and Thomas, D.N. Minireview–Recent advances in sea-ice microbiology. *Environmental Microbiology*. 2005. 7:605-619.

- Mock *et al.* A new microcosm to investigate oxygen dynamics at the sea-ice water interface. *Aquatic Microbiology Ecology*. 2003. 29:197-205.
- Mock, T. *et al.* Micro-optodes in sea ice: a new approach to investigate oxygen dynamics during sea ice formation. *Aquatic microbial ecology*. 2002. 29: 297-306.
- National Research Council. *Oil and the Sea III: Inputs, Fates, and Effects*. National Academy Press, Washington D.C. 2003.
- Nichols, D.S., P.D. Nichols, and T.A. McMeekin. Anaerobic production of polyunsaturated fatty acids by *Shewanella putrefaciens* strain ACAM 342. *FEMS Microbiology Letters*. 1992. 98:117-122.
- Nichols, D.S. *et al.* Effect of Temperature and Salinity Stress on Growth and Lipid Composition of *Shewanella gelidimarina*. *Applied Environmental Microbiology*. 2000. 66:2422-2429.
- NORCOR Engineering and Research Ltd. The interaction of crude oil with arctic sea ice. Beaufort Sea Technical Report, No. 27. Beaufort Sea Project, Department of the Environment, Victoria, BC. 1975. 201.
- Otsuka, N. *et al.* Experimental Study on the Characteristics of Oil Ice Sandwich. *Oceans 2004. MTS/IEEE Techno-Ocean 2004*. 1470-1475.
- Papadimitriou, S. *et al.* Biogeochemical composition of natural sea ice brine from the Weddell Sea during early austral summer. *Limnology and Oceanography*. 2007. 52: 1809-1823.
- Petrich, C. Growth, Structure, and Desalination of Refreezing Cracks in Sea Ice. Doctoral thesis, University of Otago. 2004. 386p.
- Petrich, C. *et al.* Modelling the interrelationships between permeability, effective porosity and total porosity in sea ice. *Cold Regions Science and Technology*. 44:131-144. 2006.
- Prince, R.C. Petroleum spill bioremediation in marine environments. *Critical Review Microbiology*. 1993. 19:217-242.
- Prince, R.C. The microbiology of marine oil spill bioremediation. In *Petroleum microbiology*. Olliver and Magot (eds). Washington DC: ASM Press. 2005. 317-335.
- Powell, S.M. *et al.* A comparison of the short term effects of diesel fuel and lubricant oils on Antarctic benthic microbial communities. *Journal of Experimental Marine Biology and Ecology*. 2005. 322: 53-65.
- Raven, J.A. *et al.* A comparison of ammonium and nitrate as nitrogen-sources for photolithotrophs. *New Phytologist*. 1992. 121:19-32
- Reay, D.S. *et al.* Temperature Dependence of Inorganic Nitrogen Uptake: Reduced Affinity for Nitrate at Suboptimal Temperatures in Both Algae and Bacteria. *Applied. Environmental Microbiology*. 1999. 65:2577-2584.
- Russel, N.J. Psychrophilic bacteria—Molecular adaptations of membrane lipids. *Comparative biochemistry and physiology*. 1997. 118:489-493.
- Shabtai, Y. and Gutnick, D.L. Exocellular esterase and emulsan release from the cell surface of *Acinetobacter calcoaceticus*. *Journal of Bacteriology*. 1985. 161:1176–1181.
- Sievers, A. *et al.* The ribosome as an entropy trap. *Proceedings of the National Academy of Sciences USA*. 2004. 1001:7897-7901.
- Singer, M.E. and Finnerty, W.R. Microbial metabolism of straight-chain and branched alkanes. In *Petroleum microbiology*. Atlas (eds). New York, New York: Macmillan Publishing Co. 1984. 1-69

Spain, J.C. *et al.* Effects of adaption on biodegradation rates in sediment/water cores from estuarine and freshwater environments. *Applied Environmental Microbiology*. 1980. 40:726-734.

Spain, J.C. and van Veld, P.A. Adaptation of natural microbial communities to degradation of xenobiotic compounds: effects on concentration, exposure time, inoculum, and chemical structure. *Applied Environmental Microbiology*. 1983

Thomas, D.N. *et al.* Dissolved organic matter in Arctic multi-year ice sea ice during winter: major components and relationship to ice characteristics. *Polar Biology*.1995. 15:477-483.

Thomas, D.N. *et al.* Dissolved organic matter in Antarctic sea ice. *Annals of Glaciology*. 2001. 33:297-303.

Thomas, D.N. and Dieckmann, G.S. Antarctic Sea Ice – a Habitat for extremophiles. *Science*. 2002. 295:641-644.

Unesco. Eighth report of the joint panel on oceanographic tables and standards. Unesco technical papers in marine science 28, Unesco, Paris. 1978.

Van Hamme, J.D., A. Singh, and O.P. Ward. Recent advances in petroleum microbiology. *Microbiology and Molecular Biology Reviews*. 2003. 67: 503-549.

Walker, J.D. *et al.* Biodegradation rate of components of petroleum. *Canadian Journal Microbiology*. 1976. 22:1209-1213.

Weeks, W.F. and S.F. Ackley. The growth, structure, and properties of sea ice. In *The Geophysics of sea ice*. Ed. By N. Utersteiner. Plenum: New York. 1986. p 9-164.

Whitehouse, B.G. The effect of temperature and salinity on the aqueous solubility of polynuclear aromatic hydrocarbons. *Marine Chemistry*. 1983. 14: 319-332.

Yakimov, M.M *et al.* Crude oil induced structural shift of coastal bacterial communities of Rod Bay (Terra Nova Bay, Ross Sea, Antarctica) and characterization of cultured cold-adapted hydrocarbonoclastic bacteria. *FEMS Microbial Ecology*. 2004. 49:419-432.

Zeyer, J.E. *et al.* Rapid microbial mineralization of toluene and 1,3-dimethylbenzene in the absence of molecular oxygen. *Applied Environmental Microbiology*.1986. 52:944-947.

Zuckerberg, A.A. *et al.* Emulsifier of *Arthrobacter* RAG-1: chemical and physical properties. *Applied Environmental Microbiology*. 1979. 37:414–420.

## APPENDIX A DEFINITION OF TERMS

Ablation: is the sum of the processes that remove ice, snow, or water from a glacier, snowfield or ice floe (e.g., melting, evaporation; Glossary of Meteorology, 2000)

Anaerobic: is the biological process in the absence of O<sub>2</sub>.

Anoxic: is depleted of O<sub>2</sub>.

Biodegradation: is the biologically-catalyzed reduction in complexity of chemicals. In case of organic compounds, biodegradation frequently leads to the conversion of C, N, P, S and other elements in the original compound to inorganic products (Alexander, 1999).

Columnar zone: is the region of ice under its surface. In order for ice to accrete, heat is conducted through the ice layer to the atmosphere. This creates a more orderly process than the initial ice growth at the surface.

Congelation ice: is found in the columnar zone and is the area in which ice is the strongest. There is a strong tendency for the ice plates from the crystal formation to extend downward in a parallel manner (Stinger *et al.*, 1984).

Freeboard: is the height of the ice floe above the water surface.

Hydrocarbonoclastic: hydrocarbon degrading

Hyperoxic: is a high concentration of dissolved oxygen.

Hypersaline: is high salinity (i.e. <0.2 mol/L NaCl)

Ice cover: is the ratio of an area of ice of any concentration to the total area of sea surface (WMO, 1970). It is commonly expressed in tenths (i.e., 80 % ice cover is 8/10).

Osmolyte: is a solute that protects the cell from drying out in response to salinity change

Psychrophilic: cold-loving.

Psychrotolerant: cold-tolerant.

Sea ice: is found at sea and has originated from the freezing of seawater (WMO, 1970).

Water accommodated fraction (WAF): is the amount of oil dissolved in water. WAF is prepared in the laboratory by mixing oil in water and is mostly free of the droplets of bulk oil. WAF can

include some particulates whereas 'water soluble fraction' has no particulates (e.g., these are removed by filtration or centrifugation). The Chemical Response to Oil Spills: Ecological Effects Research Forum (CROSERF) group has standardized WAF laboratory preparations (Singer *et al.*, 2000).