Presented at the
First International Congress on
Industrial and Harbour Scientific Research
Sept-Îles, Quebec, Canada
May 29, 2019

Bioremediation of oil spills in harbours and on shorelines: a promising cost effective way to minimize environmental damage

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Abstract

Hydrocarbons that are released into the environment either naturally or in accidental spills are degraded ultimately into non-toxic chemicals by biological processes. These processes occur even in the Arctic. Enhancing this natural bioremediation as a first-response tool has been used to clean up spilled oil for several decades now throughout the world. The various approaches for using bioremediation, their mechanisms, and rates of action will be discussed using examples from actual oil spills. Recent advances in this field and the pros and cons of bioremediation as a response tool for oil spills, including the use of enzymatic bioremediation, will be reviewed.

1 Background

1.1 Why Bioremediation as an approach

There are naturally occurring bacteria throughout the marine (and on-shore) environment that consume hydrocarbons as their preferred food. Such bacteria are also found throughout the marine waters of the Arctic and in cold regions of the world. There are limitations to the rate at which such bacteria break down hydrocarbons into carbon dioxide and water and there are a number of technologies designed to enhance the effectiveness of such oil spill remediation approaches. Enzymatic bioremediation (OSEI, 2016a), nutrient addition, and the actual addition of appropriate oil-consuming species of bacteria to the oil spill are three such technologies. Some of these technologies are approved for use in many jurisdictions throughout the world, e.g. the enzymatic bioremediant OSEII. Canada has particular expertise with such approaches in the Arctic and an active research community studying the technology (UToday, 2017; National Research Council Canada, 2016a). Figure 1 shows work being done in the High Arctic on Ellesmere Island which is making use of bioremediation to remove hydrocarbon contamination from soils.

Figure 1: Bioremediation Cleanup of Petroleum-Contaminated Soils on the Coast of Ellesmere Island in the Canadian Arctic, Project Director Dr. Charles Greer (National Research Council Canada, 2016a and b)

1.2 Objective of the paper

In the oil spill response field, especially in the Arctic or in cold regions where ice will be present, the challenge in a broad sense has been to balance public expectation and industry/government preparedness against the realities of the situation in the field. Of course every effort should be made to improve safety when handling hydrocarbon fuels to lower the risks of accidental spills, especially in the Arctic. In this paper, we suggest how bioremediation can be an attractive approach to the complex engineering, scientific and socio/economic issues of balancing the many factors that would constitute the optimum oil-spill response in the Arctic and cold regions. For background regarding oil-spill response in the Arctic and in cold regions, a number of references are included, such as Adams and Ives (2012); several Arctic Council reports (2015a and b, 2016a, b, and c), and the JIP reports (2017a, b, and c); Churchill Marine Observatory (2016); C-Core (2016); Environment and Climate Change Canada (2016); International Maritime Organization (2017a and b); International Tanker Owners Pollution Federation Limited (2017a and b); National Energy Board (2011); and World Wildlife Fund (2017a, b, and c).

The differences in use between chemical dispersants and bioremediation for response to oil spills will be discussed, since this is currently an active topic in the oil-response community.

Physically removing the oil from the environment as quickly as possible is the optimum solution, but this is often not practical or even possible due to weather conditions or the presence of ice and the lack of readily available labour or equipment resources. Oil rapidly spreads over water and unless it is contained in some manner, collecting it with skimmers within a short time becomes impractical. Thus the use of bioremediants that can ultimately remove the oil from the environment can be considered an option even if other methods are used, since no method removes all the oil released, and many collect only a small percentage. For example, it is estimated that less than 10% of the oil in the Gulf of Mexico spill was recovered despite massive efforts and the location of this accident in the heart of the American oil industry.

1.3 Monitoring the fate of spilled oil

In oil spills in Arctic and cold regions, when sea ice or fresh-water ice is present (unless the ice is shore-fast), movement of the ice creates a situation where ice-flows can move the oil long distances. So, although the oil may be localized by entrapment in the ice, it may move far from the site of the original spill location. It is therefore imperative to provide methods to mark and follow the oil until action can be taken to deal with the spill. In addition, oil undergoes physical degradation processes when exposed to the environment as the lighter fractions evaporate, soluble fractions enter the water, and chemical processes occur as the oil is exposed to sunlight and air during the spring melt. Such changes in the properties of the oil must be monitored to ensure that the appropriate technologies are applied to remove or treat it and to best remediate its impact on the environment.

If a spill occurs in winter under ice, the spring melt causes movement of the oil into the brine channels and ultimately to the surface of the ice where it forms ponds. The long periods of sunlight in the Arctic summer rapidly warm the dark oil, leading to the loss of lighter fractions. The spring sun melts the snow cover and the light penetrates the ice pack providing energy for the phytoplankton. Thus, this period is the one that is most critical for the biological systems associated with the sea ice or fresh-water ice when the interfaces of ice and water are extremely active, with microorganisms attracting all levels of the food chain, including sea mammals and birds (see Figures 2 and 3)

Figure 2: Spring appearance of sea ice during oil-spill tests at Balaena Bay near Cape Parry where 50 Tonnes (400 Barrels) of Norman Wells and Prudhoe Bay crude were heated and pumped under the ice in the winter of 1974 (Beaufort Sea Project, 2010) (Photo credit W.A. Adams).

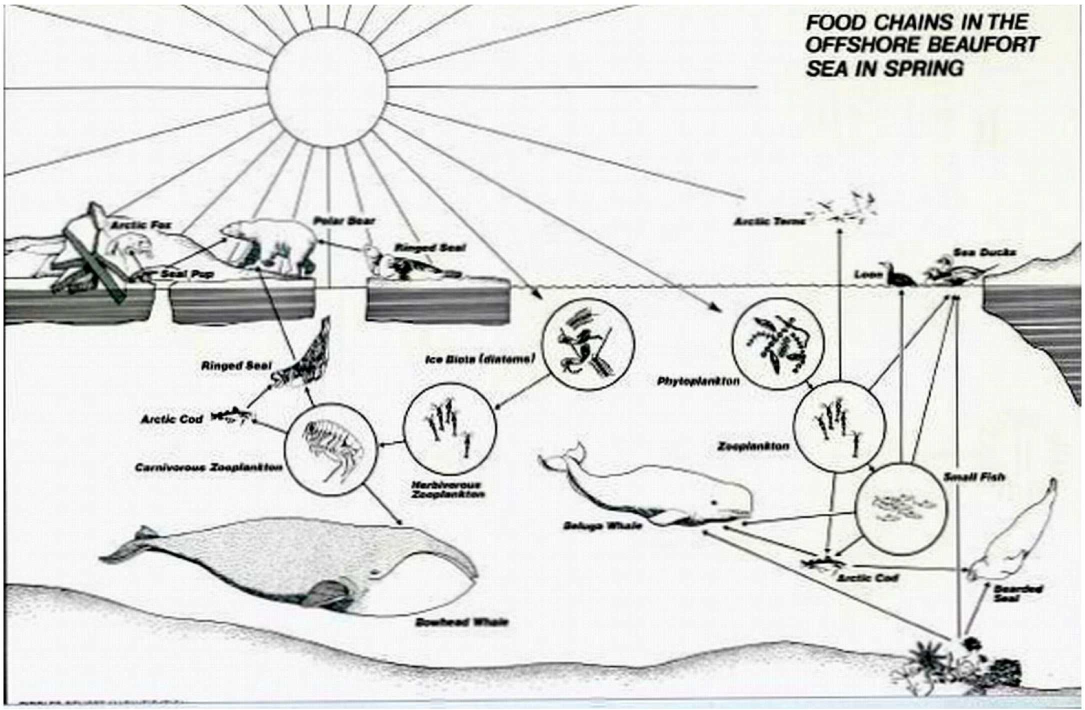

Figure 3: Food Chains in the Arctic Ocean (Beaufort Sea Project, 2010) and marine estuaries such as the Gulf of St. Lawrence.

Although some work has been done in this area, it is still a largely unexplored field where research is required to help understand what methods would be most appropriate to provide oil spill response over the longer term as oil appears from the ice throughout the seasons.

2 Review of bioremediation

2.1 Lawrence Anthony Earth Organization (LAEO) Review (2016)

This review, a comprehensive study of the current status of bioremediation, looked at papers published from 1991 to 2016. Even if both physical removal as well as dispersants are used to remove oil from the surface, the final stage of removing hydrocarbons from the environment is carried out by hydrocarbon-consuming bacteria, which respond to spills in large numbers and break down hydrocarbons into more bioavailable compounds. The processes used by these organisms to remove oil can be facilitated by bioremediation agents, which are better suited to promoting these ecological processes, and less toxic, than synthetic dispersants, and are naturally biodegradable. Topics covered in the review are efficiency, toxicity, and rate of action. The full review paper is provided as a separate file.

2.2 Bioremediation agents

The following is taken from the US EPA NRT Fact Sheet (2013) *Bioremediation in Oil Spill Response*, which is an information update on the use of bioremediation.

"Many compounds in crude oil are environmentally benign, but significant fractions are toxigenic or mutagenic. The latter are the ones we are most interested in removing or destroying in an oil spill. Bioremediation is a technology that offers great promise in converting the toxigenic compounds to nontoxic products without further disruption to the local environment. The primary reason for cleaning up oil spills is to reduce or eliminate the toxic and/or harmful components, thus enabling the survival of flora and fauna, including single-cell organisms, in each niche of the food chain. Although chemical dispersants commonly used today eliminate the visual and other damaging aspects of the spill on the surface, the spill's toxicity problem remains in the environment and at times, is worsened by the adding of chemicals contained in dispersants. The goal of the bioremediation process is to convert toxic compounds in oil/hydrocarbon-based material to nontoxic chemicals such as CO_2 and water, hereby permanently removing the hazardous components of oil/hydrocarbons from the environment and returning the affected spill area to pre-spill conditions."

The four main types of bioremediation are shown below along with their modes of action. Bioremediation is defined to include (based on LAEO Review, 2016):

(1) Addition of nutrients or other growth limiting substances to enhance the activities of indigenous pollutant-degrading microorganisms. In this approach, the population of biodegrading organisms is enhanced until the added chemicals are depleted or do not remain in the area of the spilled oil and then the oil degradation processes slow down as the nutrients are no longer available to the organisms.

(2) Enzymatic agents Oil degraders are present throughout marine environments: deep sea, open water, shoreline, etc. (Gao et al., 2015; Ali et al., 2016; Alonso-Gutiérrez et al., 2009. Addition of enzymatic agents is effective, since extracellular enzyme activity is a key step in degradation and utilization of organic polymers, and only compounds with molecular mass lower than 600 daltons can pass through cell pores. Hydrolytic enzymes disrupt major chemical bonds in the toxic molecules, thus reducing their toxicity (Chandrakant and Rao, 2011). Bioremediation in Arctic waters is attainable and follows the same mode of action, albeit at a lower rate, than in temperate waters (Garrett et al., 2003). Bioremediation strategies that can sustain high levels of bacterial diversity rather than selecting specific taxa may significantly increase the efficiency of hydrocarbon degradation in contaminated marine sediments (Dell'Anno et al., 2012).

(3) Bio-surfactant agents – Despite the extensive research on dispersants, there is very little on the use of bio-surfactants as bio-dispersants despite their potential benefits, particularly for enhancing oil biodegradation and solubilization (Mulligan 2005). The synthesis and application of microbial bio-surfactants are of practical significance for bioremediation of a range of petroleum pollutants. Bio-surfactants can be considered a key component in the cleanup strategy for petroleum hydrocarbon remediation due to

their biodegradability and low toxicity (Matvyeyeva et al., 2014). Bio-surfactants can make hydrocarbon complexes more mobile, and therefore of potential use in recovery, pumping and bioremediation of crude oil contaminants (Bordoloi and Konwar, 2009). Bio-surfactants were less toxic than the synthetic surfactants to some invertebrate species tested (Silva et al., 2014; Souza et al., 2004).

(4) Addition of naturally occurring non-indigenous microorganisms − This approach is not recommended and is prohibited in many jurisdictions where the addition of non-indigenous organisms into the environment is thought to be too risky.

Experimental evidence for the effectiveness of enzymatic bioremediants (type 2 above) has been presented in detail (see Clean Gulf*,* 2016). The composition of the particular enzymatic bioremediation agent reported on in this work, OSEII, consists of bio-surfactants, enzymes, and nutrients. Toxicology testing indicates that this agent is non-toxic to humans as well as to fish, invertebrates and other marine organisms such as plankton and bacteria. It acts rapidly to biodegrade hydrocarbons such as crude oil into smaller components and finally into CO_2 and water within 4 to 6 weeks depending on temperature and oxygen availability. It acts by stimulating the growth of naturally occurring oil-consuming bacteria in the waters being treated. Figure 4 shows test results from the above report for a variety of contaminated waters and soils that indicate how efficiently enzymatic bioremediation treatment can reduce the contamination.

The costs in US $s of conventional oil spill response vs. enzymatic bioremediation can be estimated based on the BP *Deepwater Horizon* spill in the Gulf of Mexico (Clean Gulf*,* 2016).

Estimated cost of treatment – using dispersants as was the response used in the Gulf spill

- Estimated spillage – 200M gallons (760M litres)
- Total estimated cost of remediation – US$42B
- Estimated cost of cleanup = $210/gal oil ($55/litre oil)

Cost of alternate approach – enzymatic bioremediation

- Total estimated cost of remediation – $800M
- Estimated cost of cleanup = $4/gal oil ($1/litre oil)

Resulting savings – 98.1%

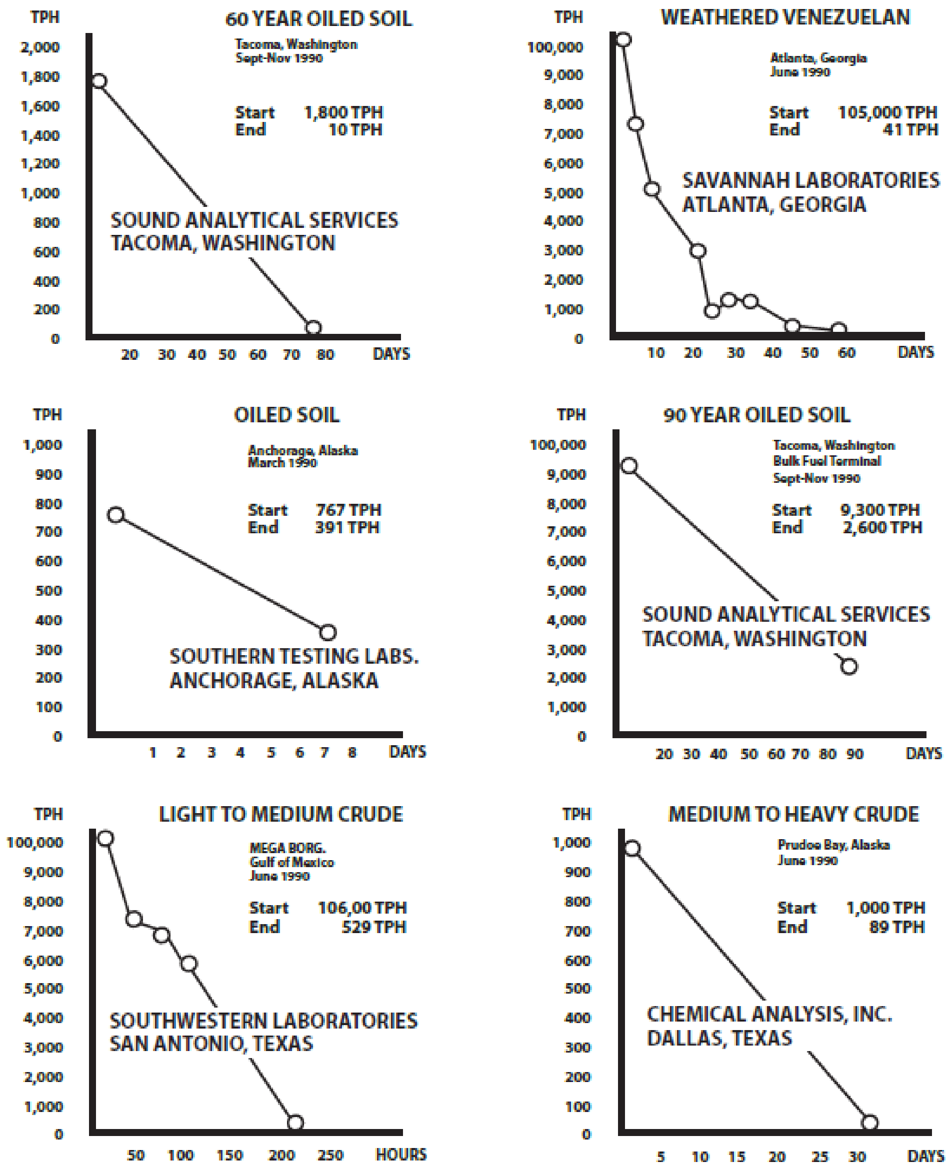

Figure 4: Results of Biodegradation of Total Petroleum Hydrocarbon (TPH) Tests Using Enzymatic (OSEII) Treatment of Crude Oil Performed by Independent Labs (Clean Gulf, 2016).

2.3 Canada's position on bioremediation and dispersants

In 2015, the Canadian Federal Government provided notification that there would be a 30-day public comment period on the proposed regulations related to the listing of spill-treating agents (STAs) under the Canada Oil and Gas Operations Act. Comments were sought on the proposed listing of two STAs:

Corexit® EC9500A and Corexit® EC9580A

A total of 3,553 submissions were received, including one from RESTCo (see

RESTCo letter, 2016). Despite the large response, which was mainly negative, based on the evidence available in the literature analysing environmental and health impacts where these STAs had been used in the treatment of past oil spills, the decision was made to list them for use in Canadian waters. They are now being stockpiled in such regions as Nova Scotia and Newfoundland for use in the event of a marine oil spill or blowout. The experience in large-scale use of these dispersants has led to considerable scientific work that provides ample evidence that there is not a net environmental benefit to their employment in the event of an oil spill. This is amply demonstrated by the references included in the RESTCo letter of 2016. The Canadian Government's response to the public submissions is provided in the document published to indicate that the above two dispersants were listed as available STAs for use in Canadian waters (see Regulations Establishing a List of Spill-treating Agents, 2016).

As a contrast to the use of chemical dispersants, the use of enzymes instead of synthetic chemicals or microbes presents the following advantages (Alcalde et al., 2006):

1. the biotransformation does not generate toxic side products as is often the case with chemical and some microbiological processes
2. the enzymes are digested, in situ, by the indigenous microorganisms after the treatment
3. the requirement to enhance bio-availability by the introduction of organic co-solvents or surfactants is much more feasible from an enzymatic point of view than using whole cells
4. the potential to produce enzymes at a higher scale, with enhanced stability and/or activity and at a lower cost, by using recombinant-DNA technology.

Despite the advantages of bioremediation, especially with enzymatic bioremediant-type STAs such as OSEII that have been in use throughout the world since the 1990s, this class of STA was not considered by the Canadian authorities in their work leading to the listing of the two chemical dispersants in 2016. However the Canadian published regulations of 2016 do indicate that other STAs will be considered for listing in future. It is to be hoped that the testing being done in Canadian Federal Laboratories will lead to the listing of additional STAs in the near future, including the enzymatic STA type such as the product OSEII.

3 Oil spill remediation technologies

3.1 Oil spill response technologies in general use, e.g. cold-water dispersants, herders, and in situ burning

The use of chemical dispersants is widely accepted in temperate regions of the world where the objective is to disperse the oil in a manner that allegedly permits it to be more readily biodegraded. Dispersed oil is also thought by some to be less damaging to marine mammals and birds and less likely to contaminate shorelines. However, there are also results showing that dispersed oil is more damaging to the environment and to workers exposed during cleanup operations. Research is on-going on this topic and there are active studies to determine the ultimate environmental benefit of using chemical dispersants, especially in cold water or in the presence of ice (Arctic Oil Spill Response Technology Joint Industry Programme (JIP), 2017a). Several jurisdictions, including Canada, have begun to plan for the use of dispersants and are stockpiling them in areas such as Halifax for possible use in oil spills during off-shore drilling programs.

Figure 5: Dispersant Trials in Ice-Covered Waters (SINTEF Photo from ITOPF, 2017b).

Rather than disperse the oil, herders, a category of dispersants/surfactants, are designed to thicken the oil on the water surface. The herder chemicals rapidly spread across a water surface to create a surfactant monolayer that reduces the water surface tension. When the surfactants reach the boundary of an oil slick, they cause the oil to contract to a new, thicker equilibrium state. The increased slick thickness produced by herders can improve ignition and allow oil to be burned without the need for containment booms or accelerants. Knowledge of the environmental impacts of the use of chemical or biological herders is also needed to assign the optimum role for their use in Arctic oil spill response. (Arctic Oil Spill Response Technology Joint Industry Programme (JIP), 2017b)

3.2 An example of the use of booms to contain an oil spill in ice-covered water

Figures 6 and 7 show booms being used to contain the spilled oil from a ship in a Norwegian oil spill. Physical skimmers can then be used to collect the oil. This process is labour-intensive and the percentage of oil collected is low, as can be seen from oil escaping over the booms in Figures 8 and 9 (see Centre of Documentation, 2015). Booms are only effective in relatively calm water.

Figure 6: Godafoss Oil Spill (Norway, 2011) with a Double Boom Deployed in Ice-covered Waters.

Figure 7: Workers Deploying a Boom during the Godafoss Oil Spill.

Figure 8: Double Boom Used During the Godafoss Oil-Spill Cleanup, 2011.

Figure 9: Booms in Ice During the Godafoss Oil Spill,2011.

3.3 Examples of oil-spill response using enzymatic bioremediation

Numerous countries (over 21), including the USA, have approved enzymatic bioremediation for use in their jurisdictions (see http://www.osei.us/published). Examples of oil spill response approvals for enzymatic remediation treatment in several countries follow.

3.3.1 USA

OSEII was listed as a spill-treating agent in the USA in 2009. Test results and recommended methods for its use are included in the material provided in OSE USA Listing (2009). The US Department of Defense and the US Coast Guard have successfully used OSEII for oil-spill cleanup on land and water.

3.3.2 UK

OSEII is listed as an approved oil spill treatment product in the UK on their most recent list, dated January 2019, by the Marine Management Organisation.

3.3.3 Saudi Arabia

Research was conducted by King Fahd University of Petroleum and Minerals, Centre for Environment and Water (2014), which included a review of published research with regard to the health and safety and the efficacy of OSEII for oil-spill remediation. The conclusions from the report are quoted directly below:

> Based on our evaluation, the OSE II product can be considered as an innovative addition for the biological treatment of spilled oil. The product is an economical solution to an oil spill of different origin with low operational cost and high treatment efficiencies. It is very effective for a wide range of oil spill remediation. This product can be used locally for the treatment of spilled oil in environments including river water, seawater and contaminated soil.

Testing of the enzymatic bioremediant OSEII on small, 1-litre test spills of Arabian crude oil in Saudi Arabia on both the water and the sandy shoreline indicated an immediate effect (see OSEI Saudi Arabian tests, 2016).

3.3.4 Australia

The Australian Maritime Safety Authority added OSEII to their register of oil spill control agents in 2013.

4 Approach to oil-spill response with an enzymatic bioremediation agent

4.1 Pre-planning

The speed of response is a critical element in the success in limiting the impact of an oil spill on the environment and keeping the overall cost of the spill under control. Therefore the preparation and pre-approval of response plans and coordination of the various elements of the response are key factors. In Arctic regions, their remoteness requires that equipment be pre-positioned close to the location of potential spills. In addition, due to the lack of readily available personnel to work on spill response, the training of local community personnel and the regular exercise of spill-response techniques will be an option for a fast initial response to an oil-spill emergency. This requirement has been recognized in Canada, where the Canadian Coast Guard has positioned oil spill response equipment in several coastal communities throughout the Canadian Arctic. However it has been found that much of this equipment is not in a fit state for rapid deployment in the case of an oil spill, e.g. the containers were locked up and the key(s) were not readily available in the hamlets. The role of local communities and aboriginal organizations is discussed in several Arctic oil spill response reports of the World Wildlife Fund (WWF) (WWF, 2017a, b, and c). Figure 16 from WWF(2017c) is a good example of a proposed community-based oil spill response plan. It is important that local harbour authorities (usually municipal) also prepare oil spill response plans, including the pre-positioning of equipment and supplies, and most importantly hold regular response exercises with the local emergency response personnel as well as with volunteers who will likely be called to assist in an emergency. Such activities should be coordinated with Territorial, Provincial and Federal levels of government who will be involved in response activities with regard to funding and provision of supplies and advice, depending on the size and seriousness of the spill accident.

4.2 Method of application

Bioremediation is a well-known method of treating contaminated soils or groundwater (for example, see EPA (2004) for information on the application of bioremediation on salt marshes). The following section provides examples of methods of applying bioremediation to open water or to the shoreline.

Instructions for the use of OSEII for oil-spill remediation are available (see OSEII procedures for cleanup, 2002). The basic procedure is to use the local water (to ensure that it includes local oil-consuming bacteria), then to mix in the OSEII in a ratio of 1 part OSEII with 50 parts water and apply it based on a one to one ratio of this mixture with the volume of oil to be treated. Small spills can be treated using a hand sprayer. For larger spills, a helicopter or barge equipped with spray booms, eductor system or hand sprayer is used to spray the mixed OSEII onto the perimeter of the oil spill, working toward the centre. Next, OSEII is sprayed over the entire surface of the spill. If the oil spill is very heavy (more than two or three inches deep), OSEII may have to be reapplied to achieve the one (1) part mixed OSEII to one (1) part heavy-end hydrocarbon. Figure 10 shows OSEII being used to clean up a crude oil spill by spraying the spill from a truck-based tank system.

Cold water reduces the rate at which OSE-II enhances biodegradation of crude oil. However, biodegradation will continue to 28°F in salt water and 32.5°F in fresh water. This STA can be used on contaminated beaches, on open water, both fresh and marine, and on intertidal zones.

Figure 10: Application of OSEII to a Texaco crude oil spill (see OSE oil spill cleanup, undated).

4.3 Monitoring

The following procedure is recommended for OSEII response monitoring when treating oil spilled on land (see OSEII procedures for cleanup, 2002).

A. Determine a grid formation for the spill area.

B. Take a 50-gram extraction from each grid. Mix in a plastic bag and shake to form a composite; then perform an EPA 8030 or 8100 TPH test to determine the initial TPH – and note.

C. Apply product.

D. On days 7, 15, and 30, and every 15 days thereafter until an acceptable TPH level is obtained, then take a 50-gram extraction from each treated grid. Mix in a plastic bag to form a composite and perform an EPA 8030 or 8100 TPH test to determine the extent of bioremediation. Testing should cease once the acceptable level of TPH reduction is obtained.

The following procedure is recommended for OSEII response monitoring when treating oil spilled on water (adapted from OSEII procedures for cleanup, 2002).

A. Items needed:

1. An extraction device that will hold 100 ml (3 ounces) of liquid and can be pushed 6 inches (60 cm) below the water surface.

2. 20 brown 100-ml bottles with Teflon sealed caps.

3. Ice chest and ice to transport samples to the laboratory.

B. Pre-OSEII Application Procedures:

1. Keep a daily log of observations.

2. Choose 3 areas of the spill, forming a triangle, and extract 3 samples, one from each area.

3. Extract the 3 samples with the extraction device, pushing the collection vessel just under the surface.

4. Place each extraction in a brown jar and seal with Teflon cap.

5. Mark jars (Initial Untreated Samples).

6. Place samples in the ice chest.

C. Control:

Perform the same steps as above, except pull one sample proximal to the spill but from an area not contaminated, affected, or impacted in any way by the spill. This is to determine the background level or pre-spill conditions. Note the time and date of extraction.

D. 10 minutes after applying OSEII, perform the next extractions:

1. If possible, using the same triangle of extraction points, push the extraction device approximately 2 to 3 inches below the surface and pull the extraction.

2. Decant the extracted sample into a brown jar and mark the initial sample 3 minutes after applying OSEII; note the time and date of extraction.

3. Place the brown jar with samples in the ice chest and transport to the laboratory.

E. Sampling Times

1. Using procedures as in D above, extract samples on days 7, 15, 30 and every 15 days thereafter until the acceptable level of cleanup is accomplished. Obviously, testing should cease once the acceptable levels are met.

2. In most cases, within 30 days the acceptable levels will have been achieved.

F. Laboratory Tests

1. If the spill is light-end hydrocarbons, then either EPA method 8015 or 8030 should be performed.

2. If the spill is heavy-end hydrocarbons, then either EPA method 8030 or 8100 should be utilized.

5 Summary

The viability of bioremediation for the treatment of oil spills has been reviewed and a particular product used to illustrate that this approach is not only viable but highly effective and less costly than conventional methods. Relevant literature references are provided to enable the topic to be studied in greater depth and with a broader look at the issues especially related to low temperatures and the presence of ice in Arctic and cold regions. Although the oil industry is researching oil-spill response in the Arctic, little attention has been given to the potential of bioremediation. Clearly there are opportunities to explore the use of bioremediation more broadly, beyond how it is currently employed.

6 Acknowledgements

The author would like to thank his RESTCo colleagues Darryl McMahon, Christopher Ives and Peter Russell for suggestions with respect to the manuscript. I greatly appreciated the editorial assistance of Rosemary Russell.

I would like to thank Diane Wagenbrenner from the Lawrence Anthony Earth Organization (LAEO) and Paul Sammarco from the Louisiana Universities Marine Consortium (LUMCON) for their continuous efforts to improve oil-spill response, and especially to introduce bioremediation agents into active use.

7 References

Adams, W.A. and C. Ives. Operating in the Arctic Ocean: Environmental Realities and Technical Challenges, in Proceedings of the Northern Lights Conference, 2012,
Session III – Arctic Ocean Technology: Utilizing R&D to Overcome Resource Development Challenges, 2012, and also reported to the UK Environmental Audit Committee of Parliament – Protecting the Arctic, written evidence submitted by the Remote Energy Security Collaborative (RESTCo) in 2012, see
https://www.publications.parliament.uk/pa/cm201213/cmselect/cmenvaud/171/171vw02.htm

Alcalde, M., M. Ferrer, F.J. Plou and A. Ballesteros **(2006).** Environmental biocatalysis: from remediation with enzymes to novel green processes, *Trends in Biotechnology*, 24(6): 281–287.

Ali, N., N. Dashti, S. Salamah, N. Sorkhoh, H. Al-Awadhi and S. Radwan **(2016).** Dynamics of Bacterial Populations During Bench-Scale Bioremediation of Oily Seawater and Desert Soil Bioaugmented with Coastal Microbial Mats. *Microbial Biotechnology,* 9: 157−171.

Alonso-Gutierrez, J., A. Figueras, J. Albaiges, N. Jimenez, M. Vinas, A.M. Solanas and B. Novoa. **(2009).** Bacterial Communities from Shoreline Environments (Costa da Morte, Northwestern Spain) Affected by the Prestige Oil Spill. *Applied and Environmental Microbiology,* June 2009: 3407–3418.

Arctic Council. General background and history, see http://www.arctic-council.org/index.php/en/about-us/arctic-council, 2016a.

Arctic Council. Description of working groups, see http://www.arctic-council.org/index.php/en/about-us/working-groups, 2015a.

Arctic Council. Emergency Prevention, Preparedness and Response (EPPR) Working Group, see http://www.arctic-council.org/index.php/en/about-us/working-groups/eppr, 2016b.

Arctic Council. Protection of the Arctic Marine Environment (PAME) Working Group, see http://www.arctic-council.org/index.php/en/about-us/working-groups/pame, 2015b.

Arctic Council. Open Access Repository, for information about the Standards report expected to be presented in spring 2017 in Fairbanks see https://oaarchive.arctic-council.org/bitstream/handle/11374/1823/EDOCS-3847-v1A-ACSAOUS203_Portland_2016_InfoDoc1-1_AMAROK-Mini-report.PDF, 2016c.

Arctic Oil Spill Response Technology Joint Industry Programme (JIP). See http://www.arcticresponsetechnology.org/about-the-jip, 2017a.

Arctic Oil Spill Response Technology Joint Industry Programme (JIP). Field Research using Herders to Advance In Situ Burning, http://www.arcticresponsetechnology.org/research-projects/field-research-using-chemical-herders-to-advance-in-situ-burning/at-a-glance, 2017b.

Arctic Oil Spill Response Technology Joint Industry Programme (JIP). Dispersant Testing Under Realistic Conditions, http://www.arcticresponsetechnology.org/research-projects/dispersant-testing-under-realistic-conditions/at-a-glance, 2017c.

Beaufort Sea Project. Summary Report reprints on RESTCo website, see http://www.restco.ca/BSP_Reprints.shtml, 2010.

Bordoloi, N.K. and B.K. Konwar **(2009).** Bacterial biosurfactant in enhancing solubility and metabolism of petroleum hydrocarbons. *Journal of Hazardous Materials*, 170: 495–505.

Chandrakant S.K. and S.S. Rao **(2011).** Role of Microbial Enzymes in the Bioremediation of Pollutants: A Review. *Enzyme Research*, 2011.

Churchill Marine Observatory. Oil spill research program plans, see http://umanitoba.ca/ceos/research/CMO.html, 2016.

C-Core. Building Arctic Capacity for Arctic Oil Spill Monitoring and Response, https://www.c-core.ca/ArcticOilSpill, 2016.

Centre of Documentation, Research and Experimentation on Accidental Water Pollution, "Godafoss", http://wwz.cedre.fr/en/Our-resources/Spills/Spills/Godafoss, 2015.

Dell'Anno, A., F. Beolchini, L. Rocchetti, G.M. Luna and R. Danavaro **(2012).** High Bacterial Biodiversity Increases Degradation Performance of Hydrocarbons During Bioremediation of Contaminated Harbor Marine Sediments. *Environmental Pollution,* 167: 85–92.

Clean Gulf. (2016) Enzymatic Bioremediation of Petroleum Hydrocarbons by Paul Sammarco, Elizabeth Montgomery, William A. Adams, and Diane Wagenbrenner (2016). Available at http://www.osei.us/wp-content/uploads/Enzymatic-Bioremediation-Efficacy-and-Toxicology-Analysis-Dr.-Paul-Sammarco.pdf.

Environment and Climate Change Canada. Agreement on Cooperation on Marine Oil Pollution Preparedness and Response in the Arctic, **2016,** https://www.ec.gc.ca/international/default.asp?lang=En&n=7772EA0E-1.

EPA/600/R-04/074, Guidelines for the Bioremediation of Oil-contaminated Salt Marshes, Xueqing Zhu, Albert D. Venosa, Makram T. Suidan, and Kenneth Lee (2004).

Gao, X., W. Gao, Z. Cui, B. Han, P. Yang, C. Sun and L. Zheng **(2015).** Biodiversity and degradation potential of oil-degrading bacteria isolated from deep-sea sediments of South Mid-Atlantic Ridge. *Marine Pollution Bulletin,* 97: 373–380.

Garrett, R. M., S.J. Rothenburger, and R.C. Prince **(2003).** Biodegradation of Fuel Oil under Laboratory and Arctic Marine Conditions. *Spill Science & Technology Bulletin,* 8: 297–302.

International Maritime Organization (IMO), Polar Code text as adopted, http://www.imo.org/en/MediaCentre/HotTopics/polar/Documents/POLAR%20CODE%20TEXT%20AS%20ADOPTED.pdf , 2017a.

International Maritime Organization (IMO), Shipping in Polar Waters, http://www.imo.org/en/mediacentre/hottopics/polar/pages/default.aspx , 2017b.

International Tanker Owners Pollution Federation Limited (ITOPF), Technical Library on Ship Source Pollution, http://www.itopf.com/knowledge-resources/documents-guides/technical-information-papers/, 2017a.

International Tanker Owners Pollution Federation Limited (ITOPF). At Sea Response to Arctic Oil Spills, http://www.itopf.com/knowledge-resources/documents-guides/arctic-cold-climates/at-sea-response-to-arctic-oil-spills/, 2017b.

King Fahd University of Petroleum and Minerals, Centre for Environment and Water. A report on the evaluation of oil spill eater II (OSE-II), 2014. http://www.osei.us/wp-content/uploads/Saudia-Arabia-King-Fadh-University-peer-review-of-OSE-II-large-report-2014-Report-on-the-Evaluation-of-Oil.pdf.

Lawrence Anthony Earth Organization (LAEO). Oil Spill Treatment: Enzymatic Agents, Bio-surfactants, and Toxicity Elimination in Hydrocarbon Clean-up in Aquatic Environments - A Literature Overview, Paul W. Sammarco, E.A. Montgomery, and William (Bill) Adams (2016).

Matvyeyeva, O.L., O.A. Vasylchenko and O.R. Aliieva **(2014).** Microbial Biosurfactants: Role in Oil Products Biodegradation. *International Journal of Environmental Bioremediation & Biodegradation*, 2(2): 69–74.

Mulligan, C. (**2005).** Environmental applications for biosurfactants. *Environmental Pollution*, 133: 183–198.

National Energy Board. Review of Offshore Drilling in the Arctic, December 2011, see https://www.neb-one.gc.ca/nrth/rctcffshrdrllngrvw/2011fnlrprt/2011fnlrprt-eng.pdf.

National Research Council Canada. Using Bacteria to Clean Up Canada's Waterways, http://www.nrc-cnrc.gc.ca/eng/achievements/highlights/2016/bioremediation.html, 2016a.

National Research Council Canada. Cleaning Up the Arctic, http://www.nrc-cnrc.gc.ca/eng/achievements/highlights/2009/arctic_cleaning.html, 2016b.

OSE oil spill cleanup. http://www.osei.us/photoalbums/crude-oil-spill-cleanup (undated)
OSE USA Listing (2009). http://www.osei.us/ncp-listing-information-and-disclosures

OSEI OSEI Library of Technical Documents, http://osei.us/technical-library-documents, 2016a.

OSEI. List of countries where enzymatic bioremediant OSEII is listed for use as an oil spill treatment agent, http://www.osei.us/published, 2016b.

OSEII. Procedures for cleanup (2002). http://www.osei.us/tech-library-pdfs/2011/22-OSEI%20Manual_CleanupProcedures(CompleteSet).pdf

OSEI. Saudi Arabian tests https://www.youtube.com/watch?v=_u9alKeWwvI&feature=youtu.be, 2016.

Regulations Establishing a List of Spill-treating Agents (Canada Oil and Gas Operations Act, http://www.gazette.gc.ca/rp-pr/p2/2016/2016-06-15/html/sor-dors108-eng.html**,** June 15, 2016.

RESTCo. Letter to Dr. Carl Brown, Manager, Emergencies Science and Technology Section, Environment and Climate Change Canada, from Darryl McMahon, RESTCo, "*Request to Delay Approval of Corexit Products as Spill Treating Agents Pending Further Review*" (2016). https://www.restco.ca/RESTCo%20to%20Carl%20Brown%20re%20Corexit%20Approval.pdf

Silva, F.S., D.G. Almeida, R.D. Rufino, J.M. Luna, V.A. Santos and L.A. Sarubbo **(2014).** Applications of Biosurfactants in the Petroleum Industry and the Remediation of Oil Spills. *International Journal of Molecular Science*, 15: 12523–12542.

Souza, E., T.C. Vessoni-Penna, R.P. de Souza Oliveira **(2004).** Biosurfactant-enhanced hydrocarbon bioremediation: an overview. *International Biodeterioration & Biodegradation,* 89: 88–94.

Technology guide: Bioremediation, Version 0.1: August 2018, https://www.crccare.com/files/dmfile/CTechguide_Bioremediation_Rev0.pdf.

US EPA. NRT Fact Sheet – *Bioremediation in Oil Spill Response*, see https://www.epa.gov/sites/production/files/2013-07/documents/nrt_fact_sheet_bioremediation_in_oil_spill_response.pdf, 2013.

UToday. U of Calgary and U of Manitoba partner to understand how marine microbes help clean up Arctic oil spills, https://www.ucalgary.ca/utoday/issue/2016-12-08/ucalgary-and-umanitoba-partner-understand-how-marine-microbes-help-clean-arctic-oil, 2017.

World Wildlife Fund (WWF). Ship-based Oil Spill Response Capacity in Nunavut and the Beaufort Sea, Ship-Based Oil Spill Response Capacity in the Beaufort Sea, http://awsassets.wwf.ca/downloads/170405___oilspillresponsecapacitybeaufort_web_1.pdf?_ga=1.136400688.1829272820.1475691822, March, 2017a.

World Wildlife Fund. Ship-based Oil Spill Response Capacity in Nunavut and the Beaufort Sea, Background Information for Community Oil Spill Response Planning in Pond Inlet, Resolute, Grise Fiord, and Arctic Bay, http://awsassets.wwf.ca/downloads/170405___oilspillresponsecapacitynunavut_web.pdf?_ga=1.60976172.1829272820.1475691822, March, 2017b.

World Wildlife Fund. Ship-based Oil Spill Response Capacity in Nunavut and the Beaufort Sea: Framework for the Development of Nunavut Community Oil Spill Response Plans, http://awsassets.wwf.ca/downloads/170405___oilspillresponseframeworknunavut_web.pdf?_ga=1.136400688.1829272820.1475691822, March 2017c.