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Critical Review

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Bioremediation of Unconventional Oil Contaminated Ecosystems under Natural and Assisted Conditions: A Review

Seyyed Mohammadreza Davoodi[1,2], Saba Miri[1,2], Mehrdad Taheran[1], Satinder Kaur Brar[1,2*], Rosa Galvez-Cloutier[3], Richard Martel[1]

[1]INRS-ETE, Université du Québec, 490, Rue de la Couronne, Québec, Canada G1K 9A9

[2] Department of Civil Engineering, Lassonde School of Engineering, York University, North York, Toronto, Ontario Canada M3J 1P3.

[3] Département de Genie Civil, Université Laval, Québec G1K 7P4, Canada

(*Phone: 1 418 654 3116; Fax: 1 418 654 2600; E-mail: satinder.brar@lassonde.yorku.ca; satinder.brar@ete.inrs.ca;)

Abstract:

It is a general understanding that *unconventional oil* is petroleum-extracted and processed into petroleum products using unconventional means. The recent growth in the United States (US) shale oil production and the lack of refinery in Canada built for heavy crude processes have resulted in a significant increase in U.S imports of unconventional oil since 2018. This has increased the risk of incidents and catastrophic emergencies during the transportation of unconventional oils using transmission pipelines and train rails. A great deal of effort has been made to address the remediation of contaminated soil/sediment following the traditional oil spills. However, spill response and clean-up techniques (e.g., oil recuperation, soil-sediment-water treatments) showed slow and inefficient performance when it came to unconventional oil, bringing larger associated environmental impacts in need of investigation. To the best of our knowledge, there is no coherent review available on the biodegradability of unconventional oil, including Dilbit and Bakken oil. Hence, in view of the insufficient information and contrasting results obtained on the remediation of petroleum, this review is an attempt to fill the gap by presenting the collective understanding and critical analysis of the literature on bioremediation of products from the oil sand and shale (e.g., Dilbit and Bakken oil). This can help evaluate the different aspects of hydrocarbon biodegradation and identify the knowledge gaps in the literature.

Keywords: Unconventional oil, Diluted Bitumen, Bakken Oil, Microbial Community Structure, Bioremediation

1 Introduction

Unconventional oils are generally defined as hydrocarbons obtained by unconventional means and they are classified into the following groups: heavy oil, extra-heavy oil, oil sand (bitumen) and oil shale (Kerogen).[1] In Canada, oil sands are found in the form of bitumen in three main geological zones, named Canada's oil sands region (COSR), including the Athabasca, Peace River and Cold Lake which make up the third-largest proven oil reserves in the world, after Venezuela and Saudi Arabia.[2-4]The technology of unconventional oil extraction from the rock, the costs of production and management of wastes and residues together with the oil transportation from sources are generally more complex and expensive than traditional petroleum e.g. North Africa and Persian Gulf. Despite these difficulties, the production of unconventional oil has increased with the rising price of crude oil after the economic recession in the US since the beginning of 2009. It is the case in the US with light oil that has largely come from tight resource formations in regions of the Bakken Permian Basin with a prevision of 2 M barrels per day (bbl/day) in 2025 or in Canada with bituminous sands (as Dilbit from Athabasca) with previsions exceeding the 3M bbl/day in 2024.[5, 6]

Multiple factors including aging infrastructure, ground failures, such as densifications, pipeline incidents and increased rail transport that use unsafe tanker cars have increased the risk of unconventional oil spill incidents during the transportation of these hydrocarbons. With this rapid development of new supply sources, the other environmental problems associated with unconventional oils that have raised concerns over oil spills include waste generation and leakage from the streamer, underground tanks, and abandoned bitumen refinery sites. The aftermath of recent high impact oil spill incidents (e.g., spill of Bakken oil in Lac Megantic and Dilbit from Alberta's oil in Kalamazoo) highlights the lack of preparedness of governments to deal with unconventional oil emergencies.[7] The inability to timely control the hazards; to constrain the spread of oil and to efficiently protect polluted zones has been attributed to the differences between unconventional oil and traditional petroleum characteristics and their behavior in the environment. In order to address these issues, novel Dilbit/Bakken spill response techniques classified as chemical and physical/chemical have been recently applied to decrease the remediation time by: (1) promoting biodegradation and limiting the movement of surface oil slicks using high-temperature oil booms,[8] (2) reducing the water/oil interfacial tension using dispersants,[9] (3) uptake of unconventional oil using superhydrophobic sorbents and magnetic particles applied in novel absorbent techniques,[8] and (4) separating oil and water *in-situ* without additional energy input using hydrophobic meshes.[10] Moreover, microbial metabolism of unconventional oil has also been considered as a cost-effective process in both microbially-enhanced recovery and upgrading of bitumen and bioremediation.[11] However, it was generally thought that hydrocarbon-degrading microorganisms isolated from hydrocarbon polluted sites can only grow on the lighter components of bitumen/Bakken, not on the recalcitrant asphaltene, resin fractions and high molecular weight (HMW) alkylated polynuclear aromatic hydrocarbon (PAH).[12] There has been a great need to gain in-depth knowledge on the following aspects: a) fate of dangerous compounds during environmental emergencies, b) innovative attenuation measures and the recuperation of oil, c) integrated methods for evaluating residual toxicity, d) methods for understanding biological degradation while treating soils, and e) the specific mechanism by which microorganisms degrade hydrocarbons, biodegradation patterns, chemistry of transformation products and their residual toxicity.

In this review, we present the current state of knowledge about the biodegradability of unconventional oil in aquatic and terrestrial environments. It is necessary to apply multidisciplinary strategic research to address technical and economic challenges regarding the biodegradability of Dilbit and Bakken petroleum. This review, thus, discusses the significance of microbes in unconventional oil biodegradation and risk-based assessment through responses of environment receptors (eco-toxicity and ecological impact) coupled with chemical analyses to study the bioremediation efficacy.

2 Problems of Unconventional Oil

2.1 The High Risk of Unconventional Oil spill Incidents

As mentioned previously, technological advancement in hydraulic fracturing and horizontal drilling caused a spike in unconventional oil well development in 2009 for oil-producing states of the US.[13] Subsequently, it raised concerns over oil spills at unconventional oil wells. For example, A. Patterson *et al.* analyzed databases of spills related to 31,481 unconventional oil wells located in the US and reported a spike in annual spill rates (14 and 16 % increase in spill rates in Pennsylvania and North Dakota). Given the present situation, the transport of these hydrocarbons will also increase, raising the risk levels of spills or releases of chemicals and wastes.[14, 15] Table S1 shows spills attributed to unconventional oils that occurred in very close proximity to streams.

2.2 Oil Spill Location

2.2.1 Soil

The condition of the spill location might adversely affect the bioremediation of unconventional oil spills. For example, the predominant soil types in COSR are chernozems and organic-rich lucidols. [6]For weathered or crude unconventional oil, the adsorption of higher molecular weight compounds to these organic fractions of soils increased retention of oil in soils. Even though the retained oils could be easily removed by reclamation measures or bioremediation, the microbial degradation of adsorbed compounds largely decreased. The region where the hydraulic conductivity is very high might also cause ground-water contamination even if the unconventional oil spill is low. Price *et al.* studied landscape restoration in dry Western Boreal Plains near Fort McMurray, Alberta. They reported the areas underlying a sloping layer of fine-grained materials with low hydraulic conductivity maintained a concentrated plume of crude unconventional oil in a sand layer with the conductivity of 10^{-5} m/s. This retained concentrated plume is very concerning because it can act as a long-term source of pollution.[16] [6, 17]

2.2.2 Ecosystem

Cleanup and recovery from an oil spill is difficult and depends on the ecosystem involved. For example, wetland areas that cover approximately 21 percent of Alberta are ecologically sensitive to the oil spill, owing to slow anaerobic biodegradation of substrates, such as PAHs and polar hydrocarbons.[6] Anaerobic environments limit the number of microbial species and slow down the natural attenuation by preventing oxygen from acting as the most favorable electron acceptor. Further, the presence of the water might decrease the permeability of the subsurface so that the retained oil can act as a long-term source of pollution in the subsurface. [16, 18]

2.2.3 Climate

Exploration and production facilities, as well as transportation activities including pipelines, are often located in cold regions where Dilbit spills from ruptured pipelines cause more serious environmentally damaging pollution problems. For many cold region sites, natural attenuation is probably not a satisfactory option in these circumstances and petroleum contaminants rapidly migrate off-site. On the other hand, the environmental consequences of bulk extraction and the cost of excavation varies with a wide range of factors and the removal of contaminated soil and media for off-site treatment as well as disposal might cause more damage to the fragile wetland than the oil itself. [19, 20]

2.2.4 Behavior of Unconventional oil in the Environment

The pattern of ecotoxicity and biodegradation might be different from traditional petroleum in aquatic and terrestrial environments due to the physicochemical differences between conventional and unconventional oils properties and characteristics as presented in Table S2.[21] With regard to Dilbit, the US NAS reported that in comparison to other transported crudes, the properties relevant to environmental impacts of the bitumen component such as exceptionally high density, viscosity, acidity and adhesion differ from traditional crudes when the oil is subjected to weathering.[22] Moreover, there is no good understanding of the fate of toxic and recalcitrant fractions in an environment following oil spills. Saint *et al.* demonstrated that the most toxic components in Bakken oil (e.g., the trace metals and alkylated PAHs) were very low in the river sediments due to the river currents that prevented the accumulation of contaminated sediments during spring floods.[7] But, Hossain *et.al* reported that gravel sediments with large pore spaces can trap oil for a longer period of time and are considered as a source of contamination.[23] Figure 1 shows the ultimate fate of the plumes for fresh (A) and weathered (B) diluted bitumen, influenced by the density of the fluid and the hydraulic conductivity of the subsurface, which might change the environmental engineering option for remediation of these contaminants. In the case of the weathered Dilbit spills on land regarded as dense nonaqueous phase liquids (DNAPL), the plume of oil will sink and when it interacts with the water table, less concentrated plumes move into the water table, but the main plume continues to sink into very deep subsurface. From an environmental health perspective this plume acts as a long-term source of pollution for the aquifer (Figure.1. B). When the plume reaches the bedrock surface, it might flow in a direction opposite to the flow of ground water. As a result, it can spread in unexpected directions from the leaking zone.[24]

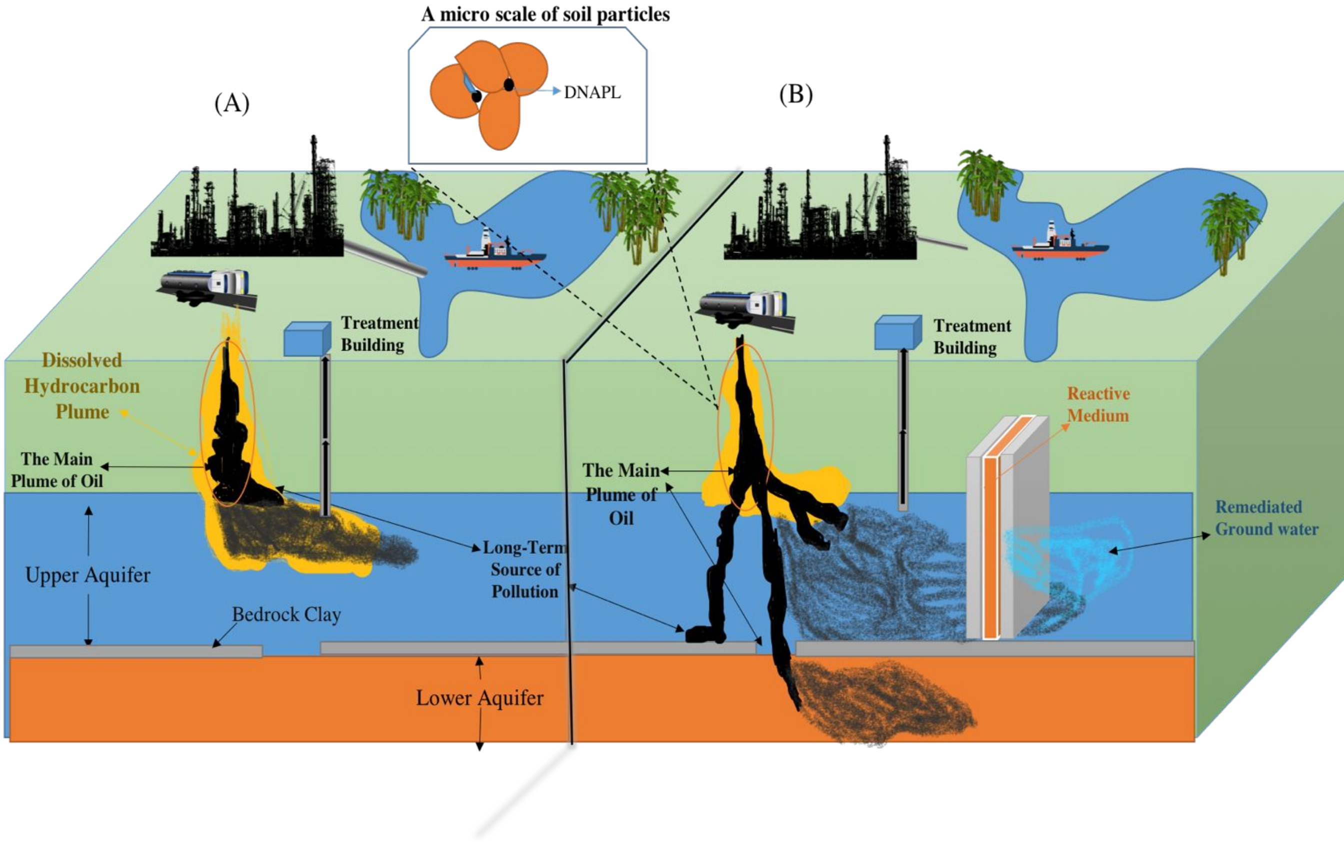


Figure 1. Subsurface contamination and transport of: (A) light non-aqueous phase liquid, such as traditional petroleum, gasoline and Dilbit; (B) dense non-aqueous phase liquid, such as weathered Dilbit,

3 Potential Solutions

The presence of complex mixtures of petroleum hydrocarbons, trace metals, volatile compounds have been reported in polluted sites after spills of unconventional oils, all of which presented high risks to the ecosystems and human health.[25] A great deal of effort has been made to investigate the feasibility of applying new technologies of tar sand recovery (mass transfer practices such as vapor extraction, solvent extraction) to the remediation of soils contaminated with bitumen and other heavy oils.[8] An environment agency survey conducted in 2009 indicated that about 90 % of the remediation techniques used on highly contaminated soils, particularly with heavy oils, were civil engineering methods and biological treatments were not considered as a treatment option in most cases.[26] Still no single remediation practice is considered the best option for removal of two main classes of the major constituents of unconventional oils including polar nonhydrocarbons (heavy non-volatile compounds) and PAH from the environment.[27, 28] Figure S1 shows a three-component research project that will be required to make a decision concerning the technologies to remediate the unconventional oil-contaminated site. In fact, a multiple lines of evidence approach is needed to study: a) advanced physical and chemical characterization of unconventional oil (i.e. Dilbit and Bakken oils)[7] ; b) development of innovative efficient oxidants and non-chemical oil adsorbents;[29] and finally, c) evaluation of *in-situ* toxicity (eco-toxicity bioassay); natural degradation and improvement of assisted oil-biodegradation. The assessment of the contamination characterization, ecotoxicity and the impact of unconventional oil on the indigenous microbial community is required to determine whether unconventional oil spill could be the worst-case scenario of all oil spills.[30] For example, following the Kalamazoo River incident, local officials did not discover that pipeline was carrying bitumen and not conventional oil. The submerged oil surprised them and the cost of the oil spill cleanup ($700 million) exceeded the company's $ 650 million insurance policy that it had for the pipeline in the event of a rupture.[28] Multidisciplinary research, thus, could deliver innovative assessment tools (genomics), eco-engineering sustainable cleaning processes and the ecological impact on the microbial community (analysis of 16s rRNA gene sequence or stable carbon isotope fractionation) as given in Figure S1. [31]

3.1 Physical and Chemical Treatment

To enhance the efficiency of remediation, a series of physicochemical techniques, such as chemical oxidation, extraction, washing, and microbial biosorption has been developed for soil and water remediation.[32, 33] As seen in Figure 2, some of the physical treatment methods designed to remove unconventional oils do not remediate or detoxify toxic components.

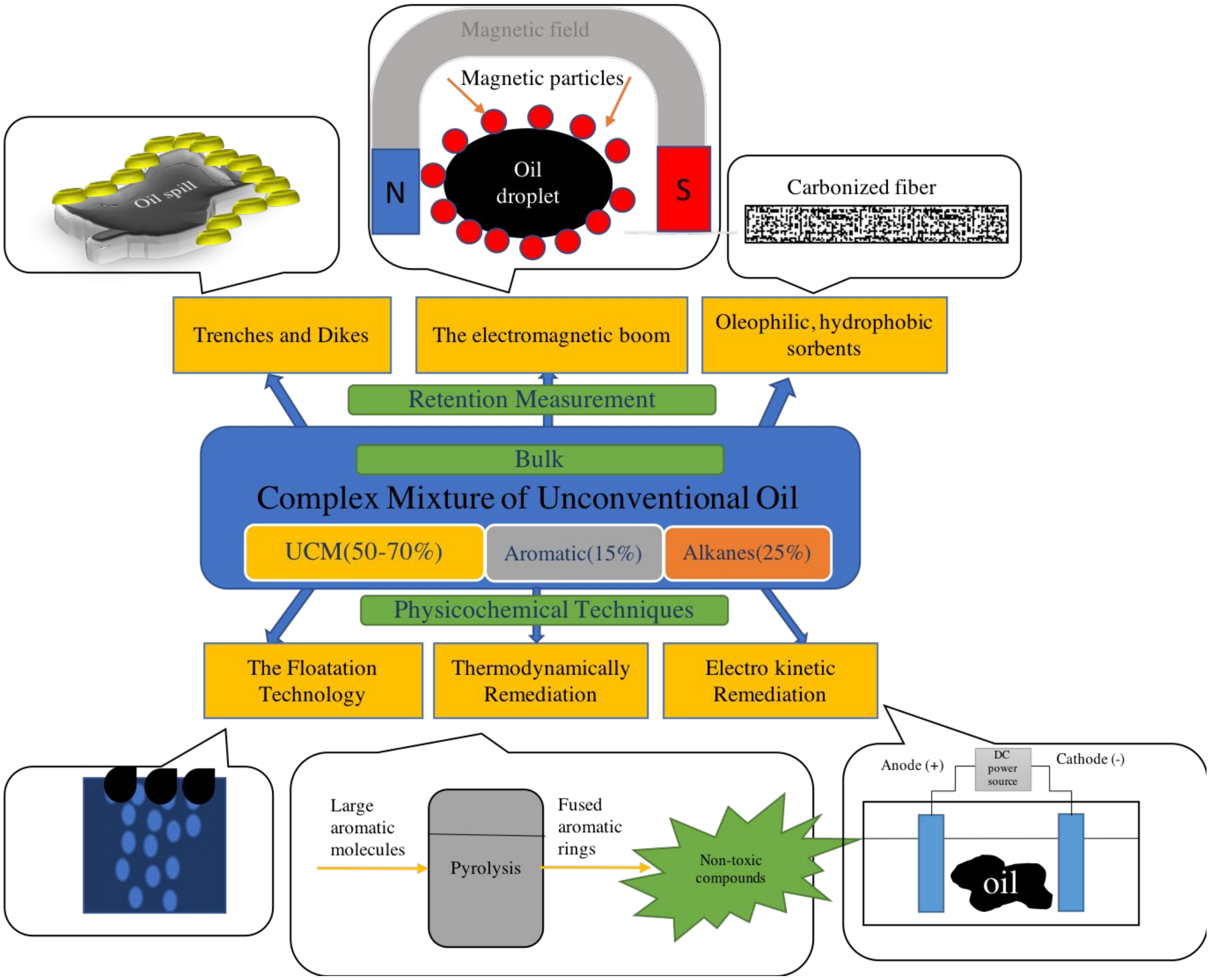
3.1.1 Aquatic Ecosystem

Recently, the application of functionalized meshes, membranes or granular adsorbents has been studied to improve existing oil/water separation systems for oil recovery following a marine oil spill.[34] The other treatment method to remediate an unconventional oil spill on the water is the use of a hybrid oil sorbent/boom. Warner *et al* recommended magnetizing oil spill which could then be magnetically manipulated and captured.[35] They implemented the simple principle, the addition of naturally-occurring magnetic minerals might form some sort of a bond with the oil, into an electromagnetic boom. This technique, unlike traditional boom and skimmers, target the unconventional oil below the water surface.[36] For weathered or crude oils that are unable to flow rapidly into a sorbent material, the available external surface area will determine the performance of adsorbents. Thus, loose strands of sorbent such as treated peat moss with a greater surface area than a boom might be expected to be more effective with these hydrocarbons. [37]

In order to remediate groundwater pollution, hydraulic control of unconventional oil movement and oil removal using discharge and recharge wells is the first option. The second option is to treat the pumped groundwater using *ex-situ* treatment, such as treatment columns. The pump-and-treat method was very common until 2000, but as the understanding of bioremediation increased, this method is less favored today. It might be difficult to pump out oil-contaminated water at a higher depth. Moreover, the entrapped oil between soil particles is never removed and this entrapped unconventional oil can disperse a low level of contamination for a long time (Figure 1). [38]

3.1.2 Soil System

Even though physicochemical methods (e.g. dispersants, in-situ burning, and mechanical recovery) are the fastest treatments, they have not been considered eco-friendly and sustainable approached compared to bioremediation of oil spills. Recently, a great deal of effort has been invested in making these methods more environmentally friendly by applying pyrolysis techniques. [39] A. Dominguez *et.al* studied the application of microwave irradiation method for drying, pyrolyzing and gasification of valuable sources (e.g. sewage sludge and used adsorbents that are abundant in the volatile matter) to produce useful products, such as gas, oil or char.[40, 41] Thus, the sorbed oil might be used and recover as a source of energy and input for the production of lightweight compounds.[41] Figure 3 shows oil adsorbent applications as an environmentally friendly technique and methods that allow the recovery of oil, sorbents, and energy. As can be seen in Figure 3, modification of surface properties is needed to enhance the sorption capacity of oil adsorbents so that it can be further channelized to a small-scale pyrolysis plant for making fuel. Sustainable, reusable and recyclable oil adsorbents are recommended for the removal of land-based and marine oil spills that might be applicable to the treatment of spilled unconventional oil.[42] To the best of our knowledge, the feasibility of applying oil adsorbents to remove unconventional oils from contaminated soils and using them as valuable sources to produce gas, oil or char is not yet explored.



UCM: Unresolved Complex Mixture;

Figure 2. Measurements to reduce remediation time following an unconventional oil spill

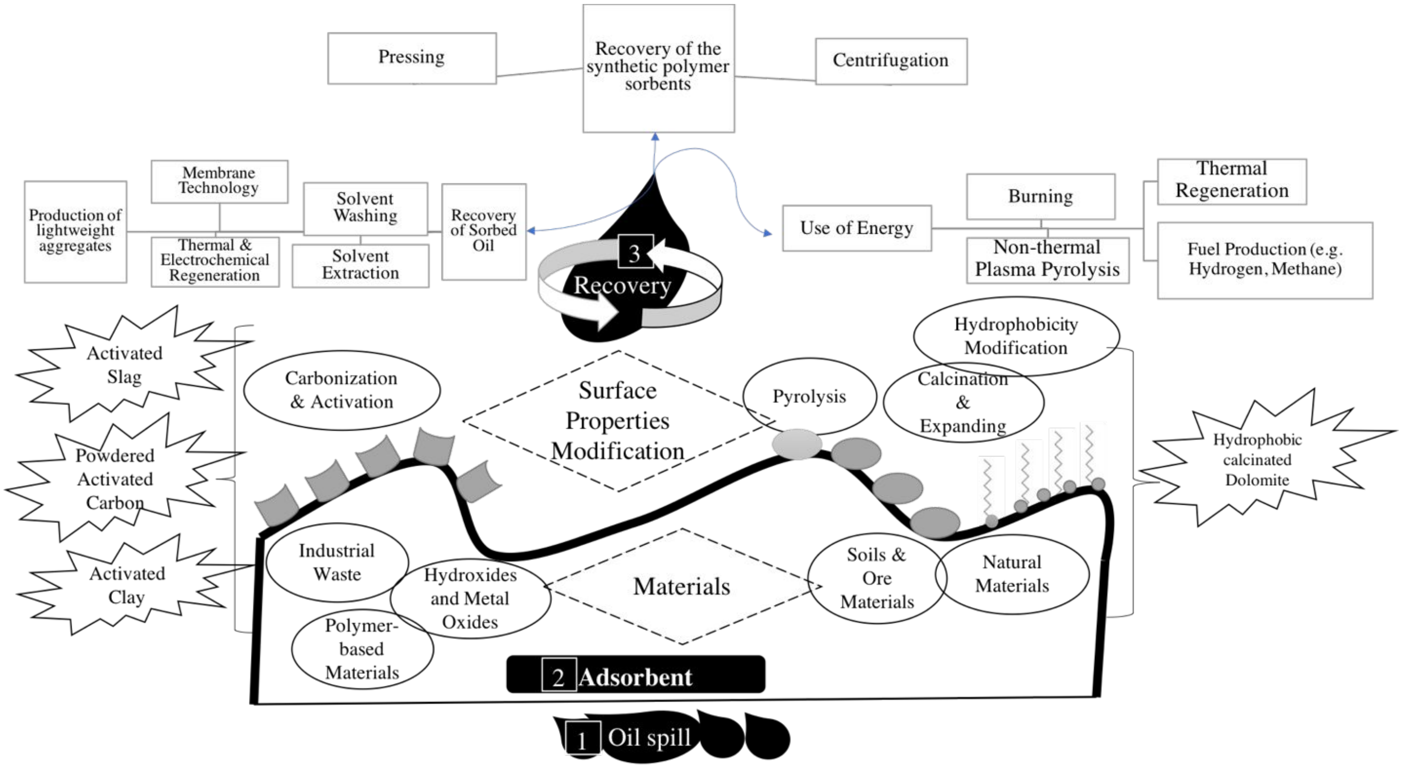


Figure 3. Schematic illustration of oil adsorbent applications as an environmentally friendly technique

3.2 Biological Remediation Approach

The organic nature of petroleum hydrocarbons including unconventional oil and traditional petroleum makes these contaminants suitable for biological degradation. One important requirement for the biological process is the presence of micro or macroorganisms with the appropriate metabolic capabilities for contaminant removal. Biodegradation of hydrocarbons can be performed by ensuring adequate concentrations of oxygen and nutrients and optimal pH. [43] In addition to indigenous microorganisms that might have the capability to degrade petroleum hydrocarbons, there are situations where the use of an exogenous microbial inoculum may enhance petroleum hydrocarbon biodegradation. This method can introduce a new degradation pathway for enhancing pollutant removal. However, using exogenous microorganisms can increase the competition between endogenous (native) and exogenous microbial populations. It can also result in the risk of introducing pathogenic microorganisms and the possibility of survival of exogenous microorganisms in the new environment; making it a very skeptical approach. [44, 45]

4 Biological Degradation of Unconventional oils

Unconventional oils bioremediation can be problematic due to the presence of recalcitrance compounds and their toxicity.

4.1 Unresolved Issues

To date, traditional soil remediation, such as organic amendments using activated sludge, dead plant biomass, and other lignocellulosic substrates have been applied to enhance the biodegradation of residual fraction of TPH and highly recalcitrant PAHs fractions. [6, 46, 47] However, these conventional means (e.g. bio-sludge) are not considered a viable stand-alone response option for the recovery of discharged oil and unconventional oil biodegradation. For example, with regard to spill of Dilbit into the Kalamazoo River near Marshall, the analytical methods described in the United States Environment Protection Agency (USEPA) report showed that even under optimum biodegradation conditions, approximately 25% of the viscous sample was degraded in the measured TPH concentration. [48]

Less disruptive strategies, such as natural attenuation and phytoremediation, that reduce the contaminants by naturally occurring processes, are crucial when managing unconventional oil-contaminated soils. However, region-specific research is required to determine the capacity of natural degradation to reach cleanup goals. These processes are considered as a management strategy for low-level soil contamination. [52, 53] The assessment of degradation at high concentrations failed to meet strict regulatory standards for reuse of legacy brownfield sites with residual heavy hydrocarbon contaminants. Schreiber *et al.* evaluated the natural attenuation potential of Dilbit by microbial communities from Douglas Channel waters to mitigate the impacts of a potential unconventional oil spill. [36] They reported that microbial communities were effective at removing alkanes, while no significant changes were observed for the overall concentration of aromatic fractions as biodegradation progressed.

4.1.1 Toxicity of Unconventional oils

The study of the actions undertaken after the Lac-Meganctic's Bakken oil spill revealed other issues regarding the unconventional oil degradation including some disadvantages and limitations on the effectiveness of unconventional oil bioremediation. The potential formation of intermediate compounds which are more toxic than the parent compounds, as well as the presence of other toxic contaminants, such as heavy metals are some examples of these limitations. [49] Moreover, bioremediation is not applicable in sites where a high concentration of inorganic salts, and organic compounds hinder microbial growth and Bakken oil spill. Thus, bioremediation should be applied with a thorough understanding of the metabolic, pathways and the microbial processes involved to prevent the production of more toxic substances.[7] Santiago *et al.* mentioned unresolved issues and requirements that may lead to higher initial costs for site characterization and feasibility evaluation for bioremediation. [25] They suggested that the chemical monitoring associated with chemical/physical remediation, as well as microbiological assays are required during the implementation of bioremediation. Saint *et. al* studied the behavior of toxic and recalcitrant fractions of Bakken oil to address the above-mentioned issues. They carried out a full characterization of riverbed sediments and river-bank soils in the Chaudière River to measure the concentration of petroleum hydrocarbons (C_{10}-C_{50}), PAHs, and trace metals (Cd, Cu, Ni, Pb, and Zn).[7] They mentioned new issues regarding the Bakken oil remediation monitoring including the threshold of certain pollutants (i.e. some derivatives of PAH). Even though they measured the concentration of around 60 PAH and alkylated PAH, yet some of these compounds did not have classification criteria. In other words, they were not considered in the criteria for the protection of the environment due to their lower water-solubility and a little was known about the bioaccumulation and the adverse effects of higher mass PAHs on marine living organisms (Table S3). [19, 55] Most of the previously mentioned literature was restricted to test the feasibility of attenuation and fertilization for biodegradation using chemical analysis such as gas chromatography, microbial respiration (physiological and biogeochemical approaches) and community analysis (e.g., genomics analysis). Thus, the fate and toxicity of both parent compounds and metabolites, in a mixture or alone has not been studied much.[7] Recent reports have considered ecotoxicological analyses and bioassay to study the effect of hydrocarbon transformation. For example, Logeshwaran *et.al* studied constructed microcosm and mesocosm to investigate the effect of treated sediments on biological receptors and provided more information on the pattern of toxicity during the biodegradation process.[31] The bioassays applied during the biodegradation have been reported to estimate the effect of mixed contaminants and intermediate metabolites during the clean-up of contaminated sites. Likewise, Yu *et.al* measured acute toxicity to study the effect of UltraZyme-amended treatments.[50] However, analytical technologies used in this study could not track the detailed changes in the composition of organic compounds in water residual bitumen and they were unable to explain the reduction of toxicity when DOC including naphthenic acid reduction did not occur .[51] The effectiveness of soil/sediment remediation can also be determined using toxicity evaluation (e.g., phytotoxicity) as well as other bioindicators such as microbial community composition.

4.1.2 Other Challenges- The Need for Multidisciplinary Strategic Research

A noteworthy fact about the unconventional oil studies is that all have reached different conclusions for the rate and level of Dilbit and Bakken degradation. For example, Cobanli *et al.* and Deshpande *et al.* suggested the biodegradability of Dilbit which is in contrast with the report of USEPA and other literature that reported limited degradation in the presence of sufficient nutrients.[52] Moreover, they reported high degradation rates measured for the freshwater communities (Degradation rate: 0.44-1.53 day^{-1}) due to their previous exposure to Dilbit and availability of inorganic nutrients (Table 1). However, the genome analysis of some marine hydrocarbonoclastic bacteria such as *A.borkumensis*, showed their capacity to degrade a large number of alkanes, branched aliphatic, as well as isoprenoid hydrocarbons,and alkyl cycloalkanes.[52-54] Inconsistent information and contrasting results regarding biodegradability of unconventional oil have been attributed to the lack of treatability results throughout all these studies.[7, 49] It might also be associated with the difference in the experimental setup, and varied nutrition concentrations which have been reported to be important to reach maximum biodegradation rates. Yu *et al.* reported that inconsistent information could be attributed to the different types of inoculum source, the composition of microbial enrichment and surfactants applied that could affect the enrichment of microbial communities. [51] As can be seen in Table 1, some literature has reported the biodegradation of bitumen and Bakken oil as not being consistent with others suggesting limited degradation

Table 1. Constant Rate Results of Bitumen Biodegradation

Oil	Consortium	Temperature (˚C)	Time	K (day^{-1})/ Removal%			Ref
				Alkanes	PAHs	Total	
Bitumen	NPK-amended soil (2.1 g)	25		-	-	0.039/53.2%	55
	Hydrogen peroxide amended Soil (0.5 g)	25		-	-	0.032/50.1%	
	Unamended soil (Natural attenuation)	25		-	-	0.014	
	NPK+ Hydrogen peroxide-amended soil (2.60 g)	25		-	-	0.042/55%	
Dilbit (WCS)	AF	5		0.44/98.1%	0.04/55.74%	-	12
		25		0.9/99.18%	0.18/60.88%	-	
	KR	5		0.48/99.92%	0.07/74/19%	-	
		25		1.14/99.95%	0.16/97.56%	-	
Dilbit (CLB)	AF	5		0.24/99.23%	0.03/79.18%	-	
		25		1.26/99.85%	0.31/84.46%	-	
	KR	5		0.56/99.64%	0.15/85.13%	-	
		25		1.53/99.89%	0.24/98.47%	-	
Dilbit (AWB)	Non-Amended seawater	22	13	0.0014	0.0011	ND	

Dilbit (CLB)	Non-Amended seawater	22	13	0.0011	0.0005	ND	52
Dilbit (AWB)	Seawater/ BH/Dispersant (AWB chemically dispersed)	22	42	0.14/99%	0.002/20%	ND	
	Seawater/BH (AWB naturally dispersed)	22	42	0.11/99%	0.0011/10%	ND	
Dilbit (CLB)	Seawater/ BH/Dispersant	22	42	0.099/ND	0.004/ND	ND	
	Seawater/BH (CLB naturally dispersed)	22	42	0.099/ND	0.005/ND	ND	
Dilbit (AWB)	Costal microbes/Ammonium/Phosphate	4	5	0.023	ND	ND	52
Dilbit (AWB)	Coastal microbes/Nirate/Phosphate	4	5	0.0235	ND	ND	

ND: Not Determined.
AF: Culture isolated from Ohio River;
KR: Culture isolated from Kalamazoo River
CLB: Cold Lake Blend
AWB: Access Western Blend

4.2 Bio-Stimulation

One of the bioremediation approaches to address the above-mentioned issues regarding the removal of two main classes of the major constituents of unconventional oils including polar hydrocarbons and PAH from the environment is bio-stimulation. The bio-stimulation is a method to adjust environmental conditions (optimization of C-N-P relationships) so that the transformation of contaminants can be increased by indigenous microbes.[56] Unlike the food industry where extensive sterilization is required to minimize the level of microbial contamination before inoculating them with appropriate bacteria, the bioremediation is usually carried out by applying physiologically adapted native microorganisms present in the affected areas to remove petroleum products by adding nutrients, dispersants, surfactants, and fertilizers.

4.2.1 Oxygen Accessibility and Nutrient Content

Bioventing is an example of *in-situ* biodegradation for bio-stimulation of indigenous aquifer microorganisms by drawing oxygen through the soil. This technology is primarily designed to treat soil contamination by non-halogenated volatile organic compounds, pesticides, and herbicides. However, following the Bakken oil spill in Lac-Megantic (Canada), bioventing along with, nutrient addition and inoculation with oil-degrading bacterium was used to clean up Bakken oil in unsaturated soil in several platforms due to low concentration of heavy metals, and inorganic salts in the sites.[25] This study also indicated that while physicochemical factors (e.g. metals, nutrition, and temperature) are important in the activity of microbial groups, the rate and extent of unconventional oil degradation strongly depends on previous exposure of the consortia to hydrocarbon contaminants. Agarry *et.al* studied the effect of inorganic NPK fertilizer and oxygen release compound (hydrogen peroxide) and their combination on the kinetics and the extent of bitumen biodegradation using autochthonous microorganisms in the soil resulting in 55 % TPH removal.[55] The introduction of pure oxygen to soil and injection of hydrogen peroxide has been suggested to address the issues regarding the inefficient air permeability of contaminated soil which provided sufficient oxygen for aerobic biodegradation. In this study, they assumed that the remediation of soil contaminated with bitumen can be expressed in terms of reduction in TPHs rather than risk reduction.[57] However, the ecotoxicological assessment revealed that a reduction in TPH load could not be linked to a reduction in residual toxicity. They also applied gravimetric analysis to estimate the extent of utilization of the TPH in the bitumen. Nevertheless, the gravimetric analysis cannot be accurate due to the fact that it does not indicate whether compounds of aromatic, asphaltene, alkanes have been degraded or not.

The effect of temperature on the efficiency of the bio-stimulation method for biodegradation of Bakken oil was studied on affected urban soils in Lac-Megantic. Diaz Sanz *et al.* reported that the bio-stimulation method enhanced the extent of Bakken oil degradation, especially for mesophilic microorganisms.[58] However, they did not compare the effectiveness of other commercial fertilizers and other common methods, such as bioventing. Recently, Cobanli *et al.* compared the biodegradation rate of Dilbit using microbes enriched from seawater and freshwater and studied the effect of inorganic nutrient concentrations on coastal microbial community response to Dilbit following an oil spill (Table 2).[59] They reported that the coastal microbial community enriched by marine ecosystems degraded Dilbit (20% PAH removal) less effectively than freshwater microbial communities which were exposed to Dilbit following a pipeline spill in 2010 (up to 98 % PAH removal) (Table 2). Yu *et al.* evaluated the impact of labile organic substrates that are considered to stimulate hydrocarbon degradation.[51] They studied the microbial activity and the change in dissolved inorganic carbon, as well as insoluble hydrocarbons to demonstrate the effect of the addition of acetate on the degradation of heavy and light compounds. [42] It had been reported that acetate accumulation and competition for electron acceptors resulted in a delay in biodegradation. However, Yu *et.al* did not observed that the inhibitory effect of acetate on recalcitrant degradation due to the difference in redox level or several metabolic pathways of various hydrocarbons in bitumen.[42, 60] Table 2 summarizes the results from various recent research studies that have investigated the effect of nutrient addition on the extent and rate of unconventional oil degradation.

Table 2. The efficiency of bio-stimulation treatment on bioremediation of unconventional petroleum

Nature of Pollutant	**Effect on biodegradation**	**Nutrients Addition**	**Removal Efficiency**	**Reference**
Extracted Bitumen	Removal of toxic hydrocarbons originated from bitumen	Nitrogen addition (ammonium nitrate)	53% toxicity	[61]
Residual Bitumen	Simulation of bitumen/hydrocarbon-degrader growth	Sodium acetate	34% heavy fraction $> C_{50}$	[62]
Nigerian bitumen	Significantly enhancement of kinetics and extent of bitumen degradation compared to being used singly	NPK fertilizer + Hydrogen Peroxide	61% TPH	[63]
Bakken petroleum shale oil	Fertilization could manage polluted urban soils when the temperature dropped below 10 ^{O}C due to a limitation of macronutrients N and P	$NH_4NO_3 + K_2HPO_4$	20 %TPH @ 10^{O}C 12%TPH @ 20 ^{O}C	[64]
Asphalt in bitumen mixture	The addition of glucose, malt extract, and polypeptide did not induce enzyme production significantly.	$Mn^{2+} + H_2O_2$	81% Asphalt in liquid medium 24% Asphalt in Soil	[65]
Dilbit	Freshly collected surface seawater used to set-up microcosm reduced any possible effects of changes in nutrients	Inorganic micronutrients in seawater	96% Alkane	[66]

4.2.2 Surfactants (Chemical and Biological) and Dispersant

The addition of dispersant might stimulate the growth of hydrocarbon degraders by increasing the bioavailability of hydrocarbon compounds. For example, Bookstaver *et.al* reported that non-ionic surfactants can enhance the growth of *A.borkumensis* on crude oil.[67] Moreover, the main ingredients of dispersants (e.g. glycols, dioctyl sulfosuccinate, and light petroleum distillates) might serve as a substrate for microbial growth. Interestingly, Schreiber *et al.* reported that the dispersant appeared to stimulate the enrichment of genera associated with Dilbit degradation. [36] As mentioned earlier, the biodegradation using microbial communities might not target toxicity that is due to methylated or more than three-ring PAHs fractions of oils. Thus, future studies are necessary to study the evolution of Bakken oil and Dilbit toxicity during the course of incubation in the presence of dispersants. Experiments were also recently carried out to characterize the optimal conditions for biosurfactant production by microorganisms growing on heteroatomic polyaromatic compounds to enhance aromatic component degradation. For example, Eric *et al.* reported favorable growth conditions for the production of surface-active compounds for a soil *Pseudomonas* strain requiring an elevated C-to-N ratio and limiting iron concentration.[68]

4.3 Bioaugmentation

This strategy may be used in different situations, for instance in areas requiring longer acclimation period e.g. cold regions [69] where the number of specific petroleum hydrocarbon degraders is low, and in the presence of recalcitrant compounds. In the case of unconventional oil, the commonly used methods for bioaugmentation are the addition of: (1) recalcitrant degrader strains; (2) consortium of different macro and micro-organisms; (3) genetically modified microorganism; and (4) emulsifier-producing strains. The effectiveness of this method is variable due to the fact that the survival ability of introduced microbial communities, enzyme activity and stability depend on environmental conditions [70].

4.3.1 Unconventional Oil-Degrading Microorganisms

Microorganisms (e.g., fungi, yeast and bacteria) may have different preferences during biodegradation. It was reported that the bacterial communities were responsible for the degradation of saturated and partially aromatic hydrocarbons [12, 71]. As for the fungal community, it was reported to transform HMW PAHs (six or more aromatic rings) as well as the asphaltenes [72].

4.3.1.1 Bacterial Strains

Most of the literature on Dilbit/Bakken oil degradability has mainly focused on the growth of hydrocarbon-degrading microbes in the lighter components of bitumen/Bakken oil (not on the recalcitrant asphaltene fraction resins).[36] Thus, the assumption is that the polar oil compounds were not biodegradable and recalcitrant fractions in bitumen did not support bacterial growth. For example, Wyndham demonstrated bacterial colonization of bitumen surfaces but microbial activity and degradation of bitumen have not been experimentally observed as reflected in a number of microorganisms.[73] A potential cost-effective solution that is greatly needed to increase the detoxification of unconventional oils might be to detect and isolate bacterial from oil reservoirs for degradation of fractions with structural complexity and high viscosity.[36, 37] Some of these bacteria, such as *A.borkumensis* SK2 might be enriched to Dilbit and Bakken oils under natural and assisted conditions to analyze the TPHs in the enrichment cultures. Most recently, Gao *et al.* and Zhou *et al.* demonstrated the ability of *P. aeruginosa* and thermophilic *Geobacullus Stearothermiphilus* strains to degrade HMW fractions including resins, asphaltenes and alkyl derivatives PAHs that are considered as subfraction of unconventional oils. These microorganisms can break the chemical bonds between monomers, such as naphthenic or aromatic rings in fused-ring compounds or by secreting enzymes producing surfactant and bio-emulsifier. Earlier literature has proven the biodegradability of asphaltenes and resin using bacterial strain (*Bacillus* sp*., Corynebacterium* sp*., Bacillus* sp*., Revibacillus* sp*., and Staphylococcus* sp.). However, the efficiency of asphaltenes biodegradation (e.g., the percent removal of asphaltene and biodegradation kinetics) was low compared with that of light fractions of the crude oil.[74, 75] *Gao* *et.al* reported that the dominant groups of bacteria with a significant application in microbially-enhanced oil recovery, that belong to the genera *Pseudomonas, Clostridium, Bacillus*, and *Acinetobacter,* might emulsify and degrade heavy oil fractions more efficiently (59-73% of crude oil asphaltenes). They reported that some of these bacteria could be cultured under aerobic conditions on individual growth substrates (i.e. BTEX and PAHs). [75, 76]

4.3.1.2 Fungal Community

The fungal community can remove recalcitrant polar hydrocarbons, such as asphaltene and resins by applying enzymatic and/or non-enzymatic reactions.[77] Hernandez-Lopez *et al* for the first time investigated the fungal transformation of asphaltene and various PAHs with six aromatic rings including benzo (g, h, i) perylene, indeno (1,2,3-cd) pyrene, and coronene by *Neosartorya fischeri*. They showed the role of cytochrome P450 system (CYP) as a monooxygenase in the oxidation of the recalcitrant compound.[78] According to the assessment of PAHs contamination in the Riverbanks of the Chaudière River three years after the Lac-Megantic Railway disaster, the concentration of these PAHs exceeded the acceptance criterion B level (not the criterion C limit which is considered a high level of contamination).[7, 78, 79] Thus, this fungus might be applied in biodegradation of heavy oil containing HMW PAHs. Hernandez *et al.* also reported the involvement of intracellular CYP enzymatic system in the transport mechanism of *Neosartorya fischeri*.[78] Most recently, it has been reported that newly formed potential microbes (i.e. white-rot ligninolytic fungi) might be more efficient than those applied conventionally for unconventional oil biodegradation due to their strong capabilities for the initial transformation of heavy PAHs and other complex structures, such as hetero-polyaromatic hydrocarbons and alkyl chains. For example, Li *et.al* reported 45% biodegradation of phenanthrene and 90% of benz[a] anthracene under *in vivo* conditions by white-rot fungi non-selective peroxide enzymes. This is probably due to its mechanism of lignin-degrading, via unique extracellular, and nonspecific oxidative lignin-modifying enzymes (i.e. lignin peroxidase, manganese peroxidase, versatile peroxidase, and laccase). [80]

It is reasonable to make the hypotheses that unconventional oils might select different microorganisms than conventional oil. Biodegradative gene analysis might help us to gain an overview of the restriction of oil-degrading bacteria in geographic distribution.

4.3.1.3 *Biodegradative Gene Analysis*

The recent improvement in the science of gene expression of microbes (i.e. metagenome, metatranscriptomics, microarray gene expression data, and real-time PCR) shows that metabolically versatile and pluripotent bacteria genera (e.g., *Alcanivorax*, *Rhodococcus, Pseudomonas, Corynebacterium,* and *Bacillus*) can be considered as unconventional oil-degrading microorganisms.[81] Most recent studies have applied metatranscriptomics to provide evidence suggesting the capacity of indigenous bacteria in the tailings to degrade bitumen aerobically. The gene sequence of hydrocarbon-degrading bacteria might yield unprecedented insights into the bacterial capacity for unconventional oil degradation. Susanne et.al showed that *A.borkumensis* SK2 has a streamlined genome with a plethora of genes accounting for its efficient oil-degradation capabilities.[82] Table S4. summarizes microorganisms and microbial genes involved in unconventional oil sub-fractions degradation

4.3.2 Microbial Consortium of Different Micro and Macro-Organisms

The literature presents strong evidence that the introduction of a large number of exogenous microorganisms into a bio-treatment system will promote the biodegradation of high molecular weight hydrocarbons [83-85]. Soil and water community analysis using shotgun metagenomic and metatranscriptomic data provides the required knowledge and pertinent information about the composition of microorganisms present in the environment. For example, Wong *et.al* studied microbial community compositions in outcrop bitumen-saturated sandstone from outcrop cliffs (i.e. an ecosystem that contains comparable highly diverse HMW hydrocarbons) by pyrosequencing of 16S/18S rRNA genes. The role of fungi to attack PAHs with soluble extracellular enzymes including lignin peroxidase, laccase, and manganese peroxidase together with cytochrome p450 mono-oxygenase was confirmed using metagenomic data.[86] They showed that fungal-bacterial cocultures can degrade HMW PAHs faster than pure cultures of *Stenotrophomonas, Burkholderia, Rhodococcus,* and *Sphingomonas*. Application of different bacterial consortia, fungi and plant roots for the biodegradation of high-molecular-weight PAHs and heavy polar hydrocarbons in liquid media and soil has been investigated by Boonchan, Tamas, Garcia-Sanchez.[84, 87, 88] They investigated the hypotheses that non-ligninolytic (i.e. *Penicillium janthinellumm* and *Cunninghamella elegans)* and ligninolytic fungi might promote the degradation of heavy PAHs by their non-selective and extracellular peroxide enzymes and plant root would stimulate new microbes that have the potential to mineralize the residual metabolic intermediates of the degradation. Table S5. summarizes the results from various recent research studies that have investigated profitable approaches for cleaning up unconventional oil-contaminated soils and liquid media using the synergistic effect of different macro- and microorganisms. As seen in Table S5, only few studies focused on mycoremediation, fungal contaminant removal, as a new branch of bioremediation that uses the similarity between recalcitrant aromatic hydrocarbons (asphaltenes, 4-,5-and 6-ring and their alkyl derivatives PAHs that represented more than 50% of Dilbit/Bakken petroleum) and the components of the lignin macromolecules to imply fungal mycelia and their enzymes in the presence of rhizosphere microbiome. Moreover, to the best of our knowledge, there is no sufficient literature available on bioremediation of unconventional oil-contaminated soil and liquid media using white-rot fungi and rhizosphere microbiome. [75]

Recently, literature utilized multiple remedial approaches including physical, such as excavation, and an array of biological processes associated with phytoremediation, such as nutrient exchange through root exudates to bio stimulate hydrocarbon-degrading microorganisms in the rhizosphere or stimulate the expression of oxygenase genes to degrade heavy hydrocarbons to less toxic compounds. This approach is especially suggested for bioremediation of soil co-contaminated with heavy metal and bitumen. To the best of our knowledge, the application of phyto-treatment to stimulate degradation of the contaminants and its transformation products present in Dilbit or Bakken oil-contaminated soils has not been investigated.

4.3.3 The Use of Genetically Engineered Microorganisms (GEMs)

The components of unconventional oil are structurally diverse causing each compound to be present at low concentration, which compromises biodegradation of high molecular weight components. Thus, the addition of external microbial consortia and nutrients may not necessarily increase the efficiency of pollutant removal due to the fact that specific enzymes (e.g. oxidative enzymes) synthesis is endergonic and requires energy.[36] This energy might be recouped only when a sufficient concentration of pollutant molecules is present in the complex mixture of oils. Shi *et.al* suggested the use of random oxidation such as chemical ozonation to generate less diverse substrates for subsequent biodegradation.[7, 65, 86] Recently, the application of the state-of-the-art tools to modify the expression of different sets of genes has been recommended as a promising method for increasing bioremediation yield and bringing together desirable biological oxidants (i.e. enzymes), biodegradation pathways and perform specific reactions. Genetic engineering also might enhance:(1) detoxification of toxic metals using construction of plasmids with combination of different metal resistant genes such as NiCoT , merA.[54] (2) expression of multiple genes such bacterial laccase (CotA), endoxylanase (Xyl) and pectate lyase (Pel) in a single host,[66] and (3) overexpression of hydrocarbon degrading enzymes such as laccase from *Thermus thermophilus* that might yield high laccase activity for oxidization of PAHs, asphaltene and resins.[89] There is not enough literature available on biodegradation of unconventional oil using this method that might be attributed to the fact that the ecological and environmental risks of genetically engineered microorganisms need to be considered to make this method successful. For instance, bioaugmentation with non-indigenous or GEM is banned in Iceland, Sweden, Antarctica and Norway. [90, 91]

4.3.4 Biosurfactant and Degradative Enzymes Mediated Unconventional Oil Degradation

The mass transfer problem of recalcitrant chemicals with large, tightly packed molecule structures, including resins and asphaltenes in the unconventional oil matrix as well as the bioavailability of soil-bound PAHs, can probably be overcome through surface-active compounds. Production of biosurfactants by bacteria, fungi, and yeasts is considered an important strategy that influences the bioavailability of hydrophobic chemicals, as well as releasing the trapped hydrocarbons from the porous medium in contaminated soils.[92, 93] Recently, the capability of different types of biosurfactants has been reported to enhance the metabolism of aged PAH, asphalt and heavy vacuum gas oil. *In-situ,* biosurfactant production through nutrient-only bio-stimulated treatment can serve as a biological tool to overcome problems in limited bioavailability of unconventional oil sub-functions, especially in soil. The bacteria isolated from bitumen contaminated soil contained efficient PAH and asphalt degrading, biosurfactant producing species like P.*aeruginosa*, *Ochrobac-trumps*, Bacilli–i.e., Bacil-*lusstratosphericus*, *Bacillus subtilis*, and *B. megateriumi* demonstrated good reduction in the surface tension (under 40 nM/m) and emulsification index values (55 %).[92, 94] The slow rate of bioremediation for unconventional crude oil might be addressed by using enzymes instead of the whole microorganism. Enzymatic technologies for remediation unconventional oils can be especially suitable for conditions where rapid bioremediation is needed to mitigate the adverse effects of indigenous microbes. A great deal of effort has been made to address issues regarding the application of enzymes for soil and water remediation.[95] However, the production of purified target enzymes is a costly process; thus, recombinant strains are usually constructed to overproduce the specific enzymes. The synergy between specific enzymes is more important than simply pursuing a higher enzyme activity due to the structural complexity and high viscosity of unconventional oil composition. Beilen *et.al* studied other practical issues and challenges in the application of enzyme remediation methods such as co-factor regeneration rates, oxygen mass transfer, overoxidation and substrate uptake. Moreover, Redox enzymes including oxygenases (e.g. monooxygenase and dioxygenase) require expensive co-factors, such as NAD(P)H and improved cofactor regeneration that increase the specific oxygenase activity of whole-cell oxygenase biocatalysts.[96, 97]

A combination of extracellular enzymes with enzymes that are more stable such as chloroperoxidase seems promising to result in faster degradation. Abolafia *et.al* determined an optimal mixture of enzymes that would reduce asphaltene aggregation in bitumen. [92, 98] The application of enzyme cocktail containing specific enzymes, such as laccases, lipase, alkane hydroxylase, monooxygenase, and esterase might be explored in the future.

4.3.5 Immobilization Technique

The immobilization method has been proposed to overcome challenges for enzyme-based processes applicable to the oil industry including the processing and upgrading of unconventional oil and heavy hydrocarbons.[99] Most recently, with the advent of nanotechnology, nanostructured materials (e.g. nanofibrous materials) with high porosity, well-designed selective wettability, and large specific surface area have been developed for highly efficient oil-water separation.[100] These findings inspired researchers to combine the electrospinning methods and enzyme immobilization. For example, fabrication of electrospun fibrous membranes might be applied for adsorption and degradation of remaining heterocyclic compounds in soils and groundwater after applying non-biological treatment methods. Dai *et.al* demonstrated that the synergistic effect of the membrane adsorption and laccase immobilization increased PAH degradation.[101] Further work is necessary in this field to investigate potential application of portable oil spill cleaning mop with enzyme-immobilized nanofibers that mimic the octopus' long sticky tentacles grab food particles to adsorb contaminant and remediate contaminated water.

In the case of immobilized microbial cells, permeable reactive bio-barriers (PRB) have been suggested to remove pollutants from groundwater in full-scale projects with promising results (Figure.1). A wide range of reactive medium such as activated carbon and several materials as support to biofilm formation can be used in PRB. [102] Cobas *et.al* studied the performance of a permeable reactive bio-barrier filled with immobilized fungus as a potential useful method to adsorb and degrade PAH.[102] However, they did not provide details about the mechanism that cells attached to the supports and did not suggest the approach to immobilize microorganism on hard surface instead of porous support.[102] Surface engineering of cell walls of hydrocarbon-degrading bacteria might be applied to magnetically modify biofilm formation of hydrocarbon-degrading microorganisms on hard surface such as stainless steel and concentrate the cells using magnetic field.[102, 103] Further work is necessary in this field to investigate the combination of electromagnetic-based approach used for oil spill remediation and magnet-responsive oil-degrading bacteria to gather contaminant and degraders in a common location. [104]

5 Future Research Needs

Bioremediation of unconventional oil-contaminated soil, sediment, seawater, and groundwater are confronted with special challenges that include:

- Biodegradation of unconventional oil petroleum by means of microorganisms in seawater with high salinity is slower compared to other locations due to the harsh environmental conditions. The use of a material carrier to immobilize hydrocarbon-degrading bacteria consortium culture to improve extra heavy petroleum and unconventional oil in seawater might be an effective approach. Nevertheless, case studies on immobilized-microbial cells and enzymes for cleaning up unconventional oil-contaminated sites are still limited.
- Limited bioavailability of heavy hydrocarbons inspired researchers to study the use of surfactants (chemical and biological) to improve the dispersion of asphaltene transport in a liquid medium and release the trapped hydrocarbons from the porous medium in contaminated soils. Thus, *in-situ* biosurfactant production through nutrient-only bio-stimulated treatment can serve as a biological tool to overcome issues regarding increased viscosity of unconventional oil sub-fractions.
- The presence of structurally diverse high molecular weight components at ultra-low concentrations that act as competitive inhibitors has been reported to avoid the action of specific enzymes, compromising unconventional oil biodegradation. The ultra-low concentration of very toxic compounds, for example, NAs in a bitumen mixture might act as competitive inhibitors and adversely affect the activity of specific enzymes. Therefore, the co-remediation of unconventional oil sub-fraction using enzymes, such as laccase and

locally isolated potential microbes, such as white-rot fungi might result in a higher rate of degradation due to their synergistic effect on laccase activity.

6 Conclusion

Due to the increased use of unconventional oil, its transportation by pipelines and subsequently the potential for leaks and spills is growing. This review has highlighted the fact that non-biotechnological (e.g. pump and treat method) might remove contamination from subsurface or surface of ecosystems, but biotechnological tools should be applied to remediate and detoxify unconventional oil (e.g. Dilbit and Bakken oil) under moderate temperature conditions and even cold climate sites. Even though biodegradation of these hydrocarbons has been extensively improved using bioaugmentation and bio-stimulation (such as organic/active sludge amendments), integrated methods and mechanisms including bioaugmentation, bio-stimulation and phytoremediation are necessary to be applied to improve the performance of bioremediation of high concentrations of weathered hydrocarbons and bitumen. Other biotechnological approaches (such as genetically engineering bacteria, immobilization method, and enzyme remediation technology) and newly found potential microbes might also promote the degradation of recalcitrant components. To date, only a few scattered studies of these mentioned approaches have been carried out to effectively clean-up these hydrocarbons. Moreover, there are only a few studies on the hypothesis that unconventional oils would select different microorganisms than traditional petroleum due to the presence of metal, oxidizing compounds, asphaltene, resin fractions and high molecular weight alkylated PAH. Thus, further research is required to demonstrate the applicability of the above-mentioned methods in the mixture of unconventional oil. Nevertheless, it becomes all the more important to mention that unconventional oils remediation entails hybrid versus single methods.

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8 Supporting Information

Table: Table S1, the location and causes of the unconventional oil incidents S2; Table S2, Comparison of selected properties for traditional petroleum, Dilbit and Bakken crudes S3; Table S3, Hydrocarbons and metal element concentrations in contaminated environments S4; Table S4, Degradative gene(s), enzymes and detective methods involved in the degradation of unconventional oil sub-fractions S6, Table S5, Synergistic contribution of different organisms in the degradation of unconventional oil-contaminated ecosystems S8; Figures: Figure S1, Illustration of a multidisciplinary strategic and structuring research approach to address unconventional oil contaminants S9.

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