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REVIEW

Microbial processes in the Athabasca Oil Sands and their potential applications in microbial enhanced oil recovery

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Abstract The Athabasca Oil Sands are located within the Western Canadian Sedimentary Basin, which covers over 140,200 km^2 of land in Alberta, Canada. The oil sands provide a unique environment for bacteria as a result of the stressors of low water availability and high hydrocarbon concentrations. Understanding the mechanisms bacteria use to tolerate these stresses may aid in our understanding of how hydrocarbon degradation has occurred over geological time, and how these processes and related tolerance mechanisms may be used in biotechnology applications such as microbial enhanced oil recovery (MEOR). The majority of research has focused on microbiology processes in oil reservoirs and oilfields; as such there is a paucity of information specific to oil sands. By studying microbial processes in oil sands there is the potential to use microbes in MEOR applications. This article reviews the microbiology of the Athabasca Oil Sands and the mechanisms bacteria use to tolerate low water and high hydrocarbon availability in oil reservoirs and oilfields, and potential applications in MEOR.

Keywords Bacteria · Bitumen · Biosurfactant · Degradation · Environmental · Methods · Oil sands · Water stress

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Introduction

Environmental challenge

Over the past 100 years, the Earth's global mean temperature has increased by about 0.6°C [121]. Current scientific consensus attributes the Earth's rising temperatures to an increased abundance of atmospheric greenhouse gases (GHGs) [47]. A point of concern and debate over the past 20 years has been the projected increase in emissions through rapid development of Canada's oil sands [30], which is also a contributor to GHG emissions in Canada [75].

The oil sands environment

The Athabasca Oil Sands in Alberta, Canada are comprised of a sand, clay, water, and bitumen mixture. Bitumen is a sticky, tar-like form of crude oil. It is viscous and does not flow until diluted or heated (Table 1). Mineral sand grains represent about 82% of the bulk composition of oil sands, with water being about 2% [110]. Quartz comprises 95% of the mineral grains, with the remaining being 2–3% feldspar grains, 2–3% mica flakes and clay [78]. The highest grade of oil sands has 18% (by weight) bitumen and 2% (by weight) water [78]. Rich oil sands have greater than 10% (by weight) bitumen, while moderate oil sands have 6–10% (by weight), and lean oil sands have less than 6% (by weight). The depth of the oil sands vary from 0 to about 500 m [137].

Water present in the Athabasca bitumen contains little to no dissolved oxygen [5] because it has no contact with the atmosphere or surface water. Expectedly, bacteria indigenous to oil sands and reservoirs are anaerobic [5]. Water occupies 10% of the pore space between mineral grains,

Table 1 Composition of the Athabasca oil sands

Constituent	Value	Reference
Bitumen		
Asphaltenes	15.59 wt%	[133]
Maltene	84.42 wt%	
Saturated aromatics	67.97 wt%	
Resin	16.44 wt%	
Elements		
C	82.84 wt%	
H	10.40 wt%	
S	4.78 wt%	
N	1.63 wt%	
Ni	68.50 ppm	
V	174 ppm	
Fe	16.80 ppm	
Water	2 wt%	[110]
Temperature	10°C	[137]
Density	8–12° (API)[a]	
Viscosity	2×10^6 cP	
pH	9	[91]
Porosity	25–35%	[78]
Grain size	62.5–250 μm	
Deposit depth	0–500 m (27 m avg.)	

[a] American Petroleum Institute gravity (a measure of how heavy or light a petroleum liquid is compared to water)

which results in a water-stressed environment for native microorganisms [110]. Microorganisms live at the oil–water interface on mineral surfaces [59]. Microbial adhesion to the oil–water interface is an important parameter in biodegradation of hydrocarbons to enhance uptake and metabolism of compounds with low aqueous solubility [1]. Bitumen provides carbon and energy for microbial growth and increases microbial adherence. The hydrophobic surface of the bitumen substrate appears to present no obstacle to bacterial adhesion. Bacteria use a glycocalyx for adhesion to the hydrophobic subsurface of bitumen; and bacteria within a few micrometers of the bitumen surface may also adhere via hydrophobic interactions [129].

The bulk oil sands material is an emulsion of quartz sand grains, hydrocarbons, and water. However, the location of the water on a grain-scale level has been the subject of discussion. The primary viewpoint is that the Athabasca Oil Sands are water wet, meaning water is present in a thin layer surrounding the sand grains [20, 23, 78, 110]. Conversely, the Athabasca Oil Sands have been postulated to be oil wet, meaning oil surrounds the sand grains and water exists as an emulsion within bitumen [136]. However, the ability to use the hot water extraction process provides evidence for the Athabasca Oil Sands being water wet, as the hot water extraction process will not work on oil-wet sands [78]. The water film surrounding the grains facilitates the separation of oil from the sand using a hot water extraction process. Since the oil is not in direct contact with the sand grains it is easier to liberate the oil. During the hot water extraction process the mined oil sands are agitated with water causing a separation of oil from the sand and water. The stability of the water film around sand grains has been postulated to be stabilized by pH neutral or alkaline conditions [23]. Interestingly, a sample from the Athabasca Oil Sands had a pH of 9 [91].

The origin of oil sands

Oil sands are created through the microbial biodegradation of light oils over millions of years, resulting in a decline of oil quality through increasing viscosity, sulphur, resin, asphaltenes, and metal content [137]. The pathways of biodegradation have only recently become understood, as scientists are able to isolate and identify microbial communities and their biodegradation metabolites. As biodegradation occurs the removal of linear *n*-alkanes occurs at a greater rate than pristane or phytane removal [8, 41]. The number of aromatic rings increases resistance to biodegradation [41]. The isolation of anaerobic hydrocarbon-degrading bacteria in the 1990s started to shift the scientific consensus away from aerobic pathways to anaerobic, which was further validated when metabolites characteristic of anaerobic hydrocarbon degradation were detected in oil, core, and drill cutting samples from biodegraded reservoirs [5].

Hydrocarbon degradation does not occur uniformly within oil deposits. A number of biodegraded reservoirs have a gradient whereby the least biodegraded oil is at the top of the oil column and the oil–water transition zone [40, 44, 45, 62]. Electron donors come from the oil column, while nutrients and electron acceptors are provided from the surrounding water underneath the lowest part of the column [40]. The breadth of the water and the availability of nutrients impact the rates of biodegradation observed in a reservoir [3, 45, 61, 62].

The level of degradation observed is impacted by the historical temperature range. When deposits are exposed to temperatures above 80°C, a process called paleo-pasturization occurs, resulting in the inactivation or death of the hydrocarbon-degrading bacteria [3]. This process explains why some oil deposits with ideal degradation conditions are minimally or not degraded at all [125].

Hydrocarbon degradation is anaerobic near surface sediments and has been linked to nitrate reduction, iron reduction, sulphate reduction, and methanogenesis [124]. As a result of the detection of similar metabolites in low-oxygen petroleum reservoirs it has been postulated that some or all of these processes could occur under anaerobic conditions [41]. Nitrate has been ruled out as a primary

oxidant in hydrocarbon degradation because it is uncommonly detected in petroleum reservoirs, and is likely consumed in near-surface sediments before reaching oil deposits [41]. Oxidized iron is commonly found in oil deposits, but like nitrate it has no major importance in the degradation of hydrocarbons, as it is likely oxidized during diagenesis [95]. Sulphate reduction and methanogenesis are the primary processes responsible for hydrocarbon oxidation.

This review will provide a microbiology perspective of the Athabasca Oil Sands, with an emphasis on microbial processes, and methods used to detect microbial species. Biosurfactant production by microbes under low water availability and the potential of biosurfactants in microbial enhanced oil recovery (MEOR) will also be examined.

Microbial communities in the Athabasca Oil Sands

The Athabasca Oil Sands contain a variety of microorganisms including methanogens, sulphate-reducing bacteria (SRB), fermentative microorganisms, acetogens, nitrate-reducing bacteria (NRB), and iron-reducing bacteria (Table 2). Microbial metabolism of organic compounds within the oil sands results in the production of methane (CH_4), hydrogen sulphide (H_2S), and carbon dioxide (CO_2). Understanding key microbial processes in oil sands such as methanogenesis and sulphate reduction is useful for oil sand tailing pond management strategies. Identification of community composition can allow for prediction and manipulation of metabolic interactions to inhibit or enhance process outcomes. Several factors influence species dominance within oil sand tailings including carbon source, temperature, redox potential, availability of terminal electron acceptors, depletion of trace nutrients, and accumulation of toxic compounds (e.g. naphthenic acids) [83].

Methanogens and SRB bacteria compete for electron donors [126] allowing methane production in marine sediments to occur only after sulphate has been depleted from pore water [72]. This is shown in the following reactions [97]. Sulphate reduction

$$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O \; \Delta G^\circ = -152\,\text{kJ}$$

Methanogenesis

$$4H_2 + HCO_2^- + H^+ \rightarrow CH_4 + 3H_2O \; \Delta G^\circ = -135\,\text{kJ}$$

Sulphate reduction

$$CH_3COO^- + SO_4^{2-} \rightarrow HS^- + 2HCO_3^- \Delta G^\circ = -47\,\text{kJ}$$

Methanogenesis

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \Delta G^\circ = -31\,\text{kJ}$$

Utilization of H_2 or acetate by SRB generates more energy than methanogens using these same compounds. Accordingly, SRB obtain more energy per unit of substrate than methanogens, and in environments abundant in sulphate, they outcompete methanogens for available substrates [32].

Methanogens

Methanogenic bacteria and archaea are strict anaerobes with various metabolic pathways, utilizing H_2, CO_2, acetate, methylamines, and dimethylsulphides in the oil sands to produce methane [134]. Methane accounts for 60–80% of gas flux across the surface of the Athabasca Oil Sands [43]. Enumeration of methanogens has found 10^4–10^5 most probable number (MPN)/ml within the fine tailings of oil sand waste settling basins [83]. Samples obtained from the Mildred Lake Settling Basin (MLSB) contained NRB and SRB, while methanogens were below detection limits ($<10^1$ MPN/ml) [33, 107]. Enumeration of samples from the MLSB in 1996 showed the presence of methanogens [106], and 3 years later 40–60% of the 12-km^2 MLSB water surface area was considered an active bubbling zone, with bubbles of gas observed. The estimated methane flux emitted from the MLSB is 43 million l/day [43].

Samples from two mature fine tailing deposits in the Athabasca Oil Sands (MLSB and West In-Pit) were analysed using clone libraries of amplified 16S rRNA, and were shown to contain prokaryotes responsible for methane production [83]. The closest matching archaeal sequences were methanogens, and 87% of clones were associated with *Methanosaeta* spp. Bacteria clone sequences were diverse, with about 55% related to *Proteobacteria*, including nitrate-, iron-, and sulphate-reducing bacteria (*Thauera*, *Rhodoferax*, and *Desulfatibacillum*). The effects of methanogens in oil sands extend beyond their biodegradation and methane production ability. Methanogens can slow sedimentation and densification in tailing ponds, hindering water recycling and increasing the amount of fresh water required for bitumen extraction [17, 31]. Through degradation of residual oil, methanogens can re-pressurize a petroleum reservoir allowing for greater recovery of residual oil [28]. Gypsum ($CaSO_4{\cdot}2H_2O$) is sometimes added to mature fine tailing ponds, where cation exchange between Ca^{2+} and Na^+ in clays increases slurry viscosity allowing for better water recycling [21]. In addition, sulphate from the gypsum can stimulate SRB by providing a sulphate substrate and therefore inhibit methanogenesis [32].

Sulphate-reducing bacteria

SRB were the first microorganisms recovered from oilfields [14]. These strict anaerobes utilize H_2, simple organic acids, or alcohols as electron donors for sulphate reduction.

Table 2 Species and functional groups found in Athabasca oil sands

Species/functional group	Sample taken from	References
Pseudomonas aeruginosa ATS-14	Soil from the Athabasca Oil Sands	[64]
Mycobacterium rhodochrous 7E1C	Bituminous hydrocarbons collected from the river sediment interface from the Athabasca and Steepbank rivers in the Athabasca Oil Sands Region	[129]
Pseudomonas spp. SB1SP, A4B, and A4F		
Rhodotorula spp. A4D2		
Mycobacterium spp. A55 and MK15A		
Nocardia spp. A4C		
Xanthomas spp. A4E1		
Gram-negative rod A41		
Methanogens	Mature fine tailings waste from the Mildred Lake Settling Basin, the Base Mine Lake and Demonstration Pond, the Athabasca Oil Sands	[43]
Sulphate-reducing bacteria		
Thiosulphate-reducing bacteria		
Arthrobacter oxydans	The Athabasca Oil Sands	[52]
Arthrobacter spp. CF46 (ASP243243)		
Denitirifying bacteria	Mature fine tailings from Mildred Lake Settling Basin in the Athabasca Oil Sands	[32]
Iron(III)-reducing bacteria		
Sulphate-reducing bacteria		
Methanogens		
Methanomethylovorans hollandica	Oil sands tailings and tailings sediments from the Athabasca Oil Sands	[17]
Archaeal *Methanosaeta* spp.	Mature fine tailings from Mildred Lake Settling Basin and West In-Pit in the Athabasca Oil Sands	[83]
Archaeal *Methanomethylovorans* spp.		
Archaeal *Methanocalculus* spp.		
Acidovorax spp.		
Thiobacillus spp.		
Thauera spp.		
Thiobacillus denitrificans		
Rhodoferax ferrireducens		
Desulfobacterium spp.	Oil sands tailings (tailing pond six) in the Athabasca Oil Sands	[93]
Desulfocapsa spp.		
Desulfurivibrio spp.		
Desulfuromonas spp.		
Pelotomaculum spp.		
Smithella spp.		
Syntrophus spp.		

SRB were isolated from MLSB in the order of 10^3–10^4 MPN/ml [83], which is comparable to a 1985 study with sample detections of 10^4 MPN/ml SRB [33]. Although addition of sulphate to mature fine tailing ponds inhibits methanogenesis [97], it is not feasible in the MLSB because of insufficient mixing of sulphate and the large volume of the tailing ponds [43]. Oil sand tailings are left over from the extraction process and are stored in large ponds. Over time materials settle and separate producing a middle layer, the mature fine tailings, which is comprised of 70% water and 30% fine silt.

Samples from Canadian oilfields were probed for SRB by reverse sample genome probing of the microbial community. In reverse sample genome probing, labelled environmental DNA is hybridized to genomes of target microorganisms. This technique allows the total DNA from a community to be quantitatively analysed in one step. Of 34 microorganisms detected, 10 were unique to the fresh water, 18 were unique to the saline water, and 6 microorganisms were cultured from both oilfield environments [119]. Aside from their preventative role in inhibiting methanogenesis, SRB have many detrimental effects on oil sand tailing ponds, such as accelerated corrosion, and undesirable production of H_2S. Additionally, thermophilic SRB are responsible for reservoir souring, which results from the production of H_2S, and occurs during water

flooding in secondary oil recovery [68]. SRB reduce sulphate in the injection water to sulphide, while oxidizing degradable organic electron donors present. Since large volumes of water (10,000 m^3/day) are injected, substantial sulphides are produced [46]. Hydrogen sulphide is a toxic oil-souring gas, and its production causes contamination of natural oil, corrosion, and reservoir plugging due to precipitation of metals [79].

Addition of nitrate is an approach used to control the accumulation of sulphides. Nitrate injection changes the microbial community enabling nitrate-reducing sulphide-oxidizing (NRSOB) bacteria and heterotrophic NRB (HNRB) to outcomplete SRB [26], because nitrate reduction to nitrogen or ammonia provides more free energy than sulphate reduction. HNRB compete with SRB for degradable organic electrons and nitrate production has also been shown to inhibit SRB metabolism [46, 118]. Nitrate-mediated sulphide control is most effective when sulphide oxidation by NRSOB is complemented by processes that inhibit sulphidogenesis [48].

Microbial detection in oil sites

The study of the function and dynamics of environmental oil site ecosystems requires the characterization of microbial communities. The study of microbial populations in these environments allows for the evaluation of long-term effects of petroleum pollution, development of waste remediation approaches, tracking the enrichment of microorganisms during remediation, control of deleterious microbial activities during petroleum production, and measurement of microbial interactions as affected by extraction processes [115]. Culture-dependent and -independent approaches are used to characterize microbial communities.

Culture-dependent approaches

Culture-dependent approaches have been used to study environmental microorganisms. Isolation can be on the basis of morphological, metabolic, or physiological characteristics and common techniques include growth on selective medium, MPN approaches, and the Biolog system. Plate counts and MPN are useful for enumerating specific culturable strains, while Biolog plates can distinguish changes in the metabolic activity profile of a microbial community [36].

Growth media commonly used for isolation of environmental microorganisms (including those in oil reservoirs) often contain more carbon and nitrogen than the environment from which the samples were taken [134]. To evaluate the microbial diversity in water samples from the North Sea Ekofisk oilfield, media were enriched with metabolites corresponding to metabolic requirements, allowing for bacterial fermenters, nitrogen reducers, acetogens, methanogens, and sulphate reducers to be isolated from samples [51]. In a growth medium with a sulphide electron donor and a nitrate as an electron acceptor, a *Campylobacter* sp. was shown to be present in different Western Canadian oilfields. This sulphide oxidizer may play an important role in the oilfield sulphur cycle by reoxidizing the sulphides formed by microbial reduction of sulphate or sulphur, and contributing to reduced oil souring [118]. To analyse the microbial community in the Pelican Lake Oil Field (Western Canadian Sedimentary Basin), a culturing approach included media amended with various carbon compounds to cultivate aerobic and anaerobic bacteria [35].

Culture methods for enumeration based on the MPN technique have been used in Alberta's oil sands [43, 83, 128]. The oil industry typically uses the MPN method to enumerate bacteria in oil reservoirs despite the time and labour required [112]. The MPN method has been used to enumerate methanogens and SRB in fine tailing ponds [43]. In a study of several cultivation-dependent and cultivation-independent methods to assess potential microbial activities in produced waters from Alberta oilfields, MPN analysis detected NRSOB and HNRB at numbers too low to be detected by fluorescent in situ hybridization (FISH) or denaturing gradient gel electrophoresis (DGGE) [60].

The Biolog system generates a fingerprint of the overall metabolic capabilities of the culturable microbial community by testing different molecules as sole carbon sources [39]. The Biolog system has been used to compare microbial community structures in wetlands of the Athabasca Oil Sands [39]. It has been estimated that only 0.1–5% of microorganisms can be cultured from environmental samples or detected using culture-based methods. Some microorganisms are considered non-culturable and a representative community profile is not attainable [51, 65]. Culturing also does not capture changes in the microbial community that may occur. Despite microorganisms being present in oil sands and other oil reservoirs for millions of years, our understanding of phylogenetic diversity, metabolic activity, ecological roles, and community dynamics in these environments is limited [134].

Culture-independent techniques

Molecular techniques have been used to assess the microbial diversity of environmental communities (Table 3). Only a fraction of microorganisms can be cultured in the laboratory and molecular techniques have allowed for the discovery of new phylogenetic groups of microorganisms [71].

Table 3 Molecular methods for detection of bacteria and archaea in environmental oil samples

Sample type	Detection method	Extraction method	Isolated microorganisms	References
Oilfields, India	qPCR assay DGGE	Extraction from isolated pure cultures	Sulphate-reducing bacteria	[4]
Oilfields, India	PCR amplification of 16S rRNA	Extraction from isolated pure cultures	Sulphate-reducing, thiosulphate-reducing bacteria	[4]
North Sea oilfield	PCR amplification of 16S rRNA	Custom extraction from environmental samples	Thermophilic and fermentative bacteria, thermophilic methanogenic archaea	[24]
Wetlands of Athabasca Oil Sands, Alberta, Canada	FAME DGGE	Custom extraction from environmental samples	NE	[39]
Coleville oilfield, Saskatchewan, Canada.	Dot blot cross hydridization PCR amplification of 16S rRNA	Extraction from isolated pure cultures	Sulphate-reducing and nitrate-reducing bacteria	[46]
Oil reservoir water, Teikoku Oil, Niigata, Japan	PCR amplification of 16S rRNA	Extraction from isolated pure cultures	Psychrotrophic bacteria	[52]
Ekofisk oil reservoir, Norwegian sector of the North Sea	PCR amplification of 16S rRNA DGGE	Custom extraction from environmental samples	Thermophilic bacteria	[51]
Oilfields, Alberta, Canada,	PCR DGGE FISH	Commercial extraction kit	Nitrate-reducing, sulphate-reducing bacteria	[60]
Long-term water-flooded petroleum reservoir, Huabei Oilfield, China	PCR amplification of 16S rRNA	Custom extraction from environmental samples	Thermophilic bacteria	[65]
Oil-containing thermal spring in Uzon volcano caldera, Kamchatka Peninsula	Parallel pyrosequencing of 16S rRNA gene fragments	NE	Thermophilic bacteria	[71]
Oil sands tailings, Alberta, Canada	PCR amplification of 16S rRNA	Commercial extraction kit	Methanogenic bacteria	[83]
Production water Schrader Bluff petroleum field, Alaska	PCR amplification of 16S rRNA Fosmid clone libraries	Custom extraction from environmental samples	NE	[84]
Athabasca Oil Sands tailing pond, Alberta, Canada	Pyrosequencing of 16S rDNA	Commercial extraction kit and skim milk powder	Syntrophs, sulphate- and sulphur-reducing bacteria, and methanogens	[93]
Berkel oilfield, Netherlands	PCR amplification of 16S rRNA DGGE	Commercial extraction kit	NE	[114]
Oilfield, Alberta, Canada	Reverse sample genome probing Southern blotting	Extraction from isolated pure cultures	20 different sulphate-reducing bacteria	[119]
Oilfield, Alberta, Canada	PCR amplification of 16S rRNA	Extraction from isolated pure cultures	Sulphate-reducing, fermentative, and sulphide-reducing bacteria	[118]
Production waters of oil wells, Japan	PCR amplification of 16S rRNA	Method for preparing DNA from crude oils using isooctane	Thermophilic, mesophilic bacteria	[131]

qPCR quantitative polymerase chain reaction, *DGGE* denaturing gradient gel electrophoresis, *FAME* phospholipid fatty acid analysis, *FISH* fluorescent in situ hybridization, *NE* not evaluated in study

Reverse sample genome probing was used for the detection and quantification of sulphate-reducing microorganisms in oil reservoir samples [119, 120]. 16S rRNA gene-based surveys provide an overall view of the composition of communities in a specific ecosystem, regardless of the metabolic abilities of the community members [134]. Studies of production water from low temperature oil reservoirs in Western Canada have featured 16S rRNA and culture-based enrichment techniques. In one study, 36 16S rRNA gene clones were analysed and groups of sulphate-

reducing, sulphide-reducing, and fermentative bacteria were identified [118]. Bacterial and archaeal phylotypes found in a production water sample from the mesothermic Schrader Bluff petroleum field in Alaska were found by two independent molecular methods. PCR and fosmid clone libraries of the same source DNA allowed for a more thorough account of the microbial diversity within the population [84]. Dot blot hydridization with functional gene probes and 16S rRNA gene sequence analysis have been applied to identify fermentative, acetogenic, anaerobic, sulphate oxidizers, and SRB populations (members of *Desulfovibrionaceae* and *Desulfobacteriaceae*) inhabiting a low temperature, water-flooded well in Western Canada [118, 119].

Molecular methods require efficient and high purity DNA extraction. Numerous extraction procedures have been developed to isolate bacteria from environmental oil samples. A method of extracting DNA from crude oil using high concentrations of 2,2,4-trimethylpentane (isooctane) as a DNA precipitator was developed [132] and used with PCR of 16S rRNA to identify indigenous bacterial and archaeal microorganisms in oil deposits and wellheads of Japanese oil wells [131]. Oil sand tailing samples are rich in clay, and strongly absorb DNA making extraction difficult. An improved extraction method using skim milk was developed for soils strongly absorbing DNA [109]. The skim milk competes with DNA in binding to clay, thereby aiding in the precipitation of DNA from clays and increasing extraction efficiency [109].

DGGE can be used to compare microbial diversity in oil-impacted sites [51], whereas phospholipid fatty acid analyses are useful as a measure of the general metabolic potential of the communities [39]. DGGE allows for the rapid comparison and phylogenetic analysis of microbial communities, and is now a common technique to study the ecology and dynamics of bacterial populations in environmental samples [122].

Molecular techniques are essential for the study of microbial population dynamics in oil reservoirs. Certain populations of microorganisms can impact the quality of petroleum reservoirs, but cannot be identified through traditional culturing techniques. A limitation of 16S rRNA gene sequences is that they do not give any indication of the metabolic properties of the species, which is the direction of future research [114]. Metagenomics can be applied to establish genetic profiles of native communities and to examine their potential applications in MEOR.

Tolerance to hydrocarbon exposure

Exposure to high concentrations of hydrocarbons causes stress on bacterial species, which is dealt with by changes in the lipid bilayer of the cytoplasmic membrane and related mechanisms. The microbial cytoplasmic membrane functions as a selective barrier for the uptake of substrates and excretion of products, and modifications or damage can impair the survival of cells. Hydrophobic compounds in the environment interact with microorganisms at the cytoplasmic membrane and tend to reside in the acyl chains of phospholipids in the hydrophobic area between the membrane monolayers. The penetration of hydrocarbons into cellular membranes is a function of the compound's octanol–water partition coefficient [115]. Compounds most stressful for microbial cells have $\log P_{\text{octanol–water}}$ values of 1–5 [42]. Hydrophobic compounds partitioning deep into interior membranes typically have a $\log P_{\text{octanol–water}}$ coefficient less than 2 [74].

Petroleum hydrocarbons alter the microbial cytoplasmic membrane by influencing membrane fluidity and protein composition [42, 105]. Hydrocarbons interact with cells in a non-specific manner but toxicity arises from lipid–lipid and lipid–protein interactions [117]. Some dissolved hydrocarbon molecules that come into contact with the cytoplasmic membrane passively enter the hydrophilic phase of the bilayer following the solubility-diffusion mechanism while others flow through transient channels [115]. In the transient-channel mechanism, fluctuations in the bilayer lead to the formation of a transient channel followed by solute diffusion through this water channel. In the solubility-diffusion mechanism, the solute enters and leaves the bilayer through short-lived cavities in the headgroup regions of the bilayer [37, 82]. The changes to cell membranes differ greatly and depend on the type of hydrocarbon substance interacting with the membrane.

The presence of polar and/or hydrophobic protein domains within a lipid bilayer assist in the structural stability of the membrane [74]. Cells that are highly tolerant to hydrophobic compounds have been shown to use active solvent efflux pumps to remove these stressful compounds from cells [57, 92]. Cytoplasmic membrane alterations and adaptations may be important for tolerance to hydrophobic substances, particularly changes in fatty acid composition, phospholipid headgroups, and protein content [42]. One of the key processes in the adaptation of some *Pseudomonas* strains to organic solvents is the isomerization of *cis*- into *trans*-unsaturated fatty acids. This decreases membrane fluidity by increasing membrane ordering [42, 49]. These alterations serve to produce a physical barrier to the intercalation of hydrocarbons into membranes, thus offsetting the passive influx of hydrocarbons into the cell [74]. Extreme environments are stressful for microorganisms and how they adapt and survive in oil sands usually depends on their capability to grow and divide under low-water conditions.

Tolerance to low water availability

Numerous species of bacteria have been shown to survive prolonged periods of desiccation, usually by slowing metabolism or through survival mechanisms such as inhibiting DNA replication, damaged DNA exportation, multiple genome copies, and efficient DNA repair processes [15, 73, 85]. However, since bacterial species found in oil sands encounter ongoing water stress they may use additional mechanisms to cope with prolonged low water availability. The continual water stress inherent in oil sands poses an interesting problem, considering the view that water is a necessity for all life, and proposed as necessary for the beginning of life [86]. How do bacteria survive in oil sands that harbour only 2% water content by weight?

Because of the low water availability in oil sands, past studies have sought to identify the location of bacteria within the oil sand matrix. It has been observed that bacteria tend to congregate within the oil–water interface, but the exact mechanisms of their survival in this extreme low-water environment have not been conclusively identified [7]. The Athabasca Oil Sands are unique from other oil deposits as they are water wet, the bulk of which consists of bitumen, sand, and water, which together form an emulsion [67].

An emulsion is created by two immiscible liquids, such as water and oil, and an emulsifier [50]. In nature, colloidal solids such as clay particles may act as emulsifiers by lowering interfacial tension and facilitating the dispersion of one liquid in a second liquid [50]. A good emulsifier provides repulsive forces between dispersed droplets of one liquid in the other, thereby preventing coalescence and phase separation of the two liquids [29, 50]. Emulsions create an increase in the surface area of the oil–water interface, and just as fine solid particles such as clay and sand can act as stabilizing agents within the emulsification, so can hydrophobic bacteria [29, 50]. Both solid colloids and biotic colloids (bacteria) attach strongly to the oil–water interface through hydrophobic interactions, which may give rise to adsorbed layers, resulting in increased emulsion stability [29, 50].

Bacteria tolerant to low-water stress have been isolated from oil sands and other extreme environments such as the Sonoran Desert, the Sahara Desert, and Antarctica [16, 90]. Proposed mechanisms of low-water tolerance have included energetic adjustments, namely the reduction of metabolic activity [7], efficient DNA repair mechanisms [15], adjustments of cell walls or unique extracellular structures [101], changes in cell surface hydrophobicity [34], and the biosynthesis of osmolytes [7] and extracellular biosurfactants that regulate hydraulic potential gradients [94].

Owing to their biosurfactant production, some species have been utilized for various industrial, cosmetic, and food processes, and there is increased emphasis on their use for MEOR projects [103]. Regardless of the many potential applications of biosurfactants within biotechnology and industry, an interesting biological aspect of microbial biosurfactants is how they benefit bacteria, particularly bacteria under low-water stress.

Researchers have studied the diverse array of biosurfactants produced microbially, including low molecular mass glycolipids and high-molecular mass bioemulsifiers [9, 103]. Biosurfactants act extracellularly, and are amphiphathic in nature, thereby stabilizing oil–water emulsions [9]. The stabilization of oil–water emulsions through biosurfactant production results in reduced surface tension and an increased surface area of water available for microorganisms [103]. Additionally, as many biosurfactants are stable at high salt concentrations, high temperatures, and within a wide pH range, it appears their production—as found in oil sands—could be considered a mechanism used to deal with low-water stress and high hydrocarbon exposure [101, 102].

Microbial production of exopolysaccharides creates a protective matrix around soil bacteria, which can hold several times its weight in water, thereby acting as a buffer against low soil water and increasing the diffusional ability of nutrients to bacteria in times of severe water stress [94]. Researchers have observed that the low-water-tolerant species *Pseudomonas aeruginosa* could survive on a 99% triglyceride, 1% water emulsion as a result of lipase production, from which they implied that the species might have a uniquely stable membrane or specific extracellular structures which may also contribute to the species' unique low-water tolerance [101, 102]. The ability of *Brevibacterium* to grow in oil may be influenced by a high lipid content of the cellular envelope. For example, after 6 days of growth lipids comprised 32% of the dry weight [100].

An extracellular polyanionic heteropolysaccharide bioemulsifier, emulsan from *Acinetobacter venetianus*, which was shown to have hydrocarbon substrate specificity and amphiphathic properties, has also been studied in *A. radioresistens* [9]. Emulsan aids in the formation and stabilization of oil–water emulsions, which may spatially increase bioavailability of water and nutrients to microorganisms [9].

Rhodococcus opacus strain PD630 belonging to the *Actinomycetes*, known for their presence in arid environments, has also been studied in response to water stress. *R. opacus* strain PD630 had a wide range of responses at different water levels, including decreased metabolic activity, synthesis and intracellular accumulation of compatible solutes, and the production of CO_2 in the absence of an added carbon source [7]. This indicated that the species was able to transform hydrocarbons into extracellular storage lipids. Upon further analysis, *R. opacus* PD630 regulated its cellular lipid content under low-water stress, and a matrix of extracellular polymeric substances was

observed through scanning electron microscopy, surrounding colony surfaces after induced water stress [7].

Previous studies have also shown extracellular polymer (or biosurfactant) production in other bacterial species, including *Pseudomonas aeruginosa* strains, known for their production of rhamnolipids [27]. The first stage of biosurfactant production in *P. aeruginosa* UG2 and PG201 was correlated with an increase in cell surface hydrophobicity, which may facilitate cell adhesion in the oil–water interface and therefore substrate access [27]. Additionally, a recent study examining three strains of *P. aeruginosa* found that increases in cell surface hydrophobicity occurred initially when grown on diesel fuel, glucose, and dodecane and hecadecane mixtures, with a decline in cell surface hydrophobicity after the initial increase [34]. These increases were substrate-dependent, and the presence of rhamnolipids was a key factor in the ability of *P. aeruginosa* to degrade larger amounts of different hydrocarbons [34].

Bacterial species inhabiting the Athabasca Oil Sands have evolved to grow and divide under extremes, including continual low-water stress. One mechanism to combat this stress that seems to have been adopted by some species is the production of extracellular surfactants.

Overview of enhanced oil recovery from conventional oil reservoirs

Enhanced oil recovery (EOR) is used to extract residual oil from wells left unrecovered from primary and secondary extraction methods. For a better understanding of the challenges faced during EOR a brief explanation of the first two stages of conventional oil recovery methods is necessary. Primary extraction methods involve drilling an oil well into an oil reservoir. Initially natural pressures drive the oil up to the surface; after the pressure dissipates, pumps are used. Secondary extraction focuses on improving the flow of oil to the wellhead by injecting water (waterflooding) throughout the reservoir. Once the ratio of water to oil pumped out of the well becomes too great, it is too costly to separate the water from the oil and secondary extraction is discontinued. However, more than two-thirds of the oil in the reservoir is left unrecovered after primary and secondary extraction [19, 54]. Residual oil is difficult to recover because it is often located in areas inaccessible to fluids used for flooding, or the oil is adhered to sand or carbonate particles in the reservoir [99]. High oil viscosity can also impede recovery.

Conventional EOR methods (also known as tertiary extraction) make use of chemicals (solvents, polymers, surfactants), injected gases (CO_2, N_2, flue gas), and thermal methods (steam flood, hot water, combustion) to extract remaining oil from stagnant reservoirs [99]. Similarly, MEOR utilizes solvents, gases, organic acids, polymers, biofilms, and biosurfactants produced by microbes to aid in the extraction of unrecovered oil [54–56, 63, 134] (Table 4).

Both conventional and MEOR methods focus on improving the mobility of oil through decreasing oil viscosity, dissolution of carbonates in the reservoir, physically displacing oil, and plugging of highly permeable areas in the reservoir to increase the sweep efficiency of waterflooding. MEOR is more economical [77] and environmentally considerate [63] compared to conventional EOR. Bacteria used for MEOR are inexpensive and are easy to maintain, whereas the solvents, polymers, and surfactants derived from petroleum sources are dependent on the increasing cost of crude oil [134]. Solvents produced by bacteria do not rely on the cost of crude oil, and represent a cost-effective alternative. Additionally, using solvents, acids, and biosurfactants produced by bacteria instead of petrochemicals or thermal recovery methods reduces the total input of energy used for extraction. Furthermore, bacterial activity in the oil well should become more effective as the microbes multiply and grow, whereas the effects of chemical additives will diminish over time [63]. MEOR is environmentally friendly as microbial products are biodegradable and have low toxicity.

MEOR and oil sands

Oil sand extraction methods decrease the viscosity of the bitumen through steam, chemical solvents, or hot air injection. There have been few studies on using MEOR for oil extraction from oil sands. However, MEOR still has potential for use in oil recovery from oil sands, especially processes focusing on decreasing the viscosity of oil and reducing the interfacial tension between oil and water interfaces. Of particular interest are MEOR strategies involving biosurfactants. Biosurfactant-producing bacteria are likely candidates for in situ MEOR from oil sands since they are equipped to cope with the low water availability and high hydrocarbon concentrations, such as those found in oil sands. To our knowledge little if any research on using biosurfactant-producing microbes in oil sands has been done yet. However, this does not invalidate the potential application of biosurfactants, or of MEOR in general to oil sands, as increasing concern over the amount of GHG emissions from conventional methods of oil sand recovery necessitates exploration of more environmentally friendly recovery methods.

Biosurfactants in MEOR

Biosurfactants have an important role in MEOR and have been reviewed [54, 63, 99]. Biosurfactants can be used in

Table 4 Biotechnological application of microbes in MEOR

Biosurfactants produced by microbes		
Applications: lower interfacial tension between oil and water, reduce oil viscosity		
Microbe	Biosurfactants produced	References
Acinetobacter sp.	Emulsan	[9, 22]
Bacillus sp.	Lichenysin	[66, 76]
	Surfactin	[38, 123]
	Lipopolysaccharide	[58]
Pantoea sp.	Glycolipid	[116]
Pseudomonas sp.	Rhamnolipid	[80, 87, 127]
	Glycolipid	[18]
Rhodococcus sp.	Trehalose lipids	[12, 111]
Biopolymer and biofilm produced by microbes		
Applications: selective plugging of oil-depleted zones, increases sweep efficiency of water flooding		
Microbe	Biopolymer or biofilm produced	References
Xanthomonous sp.	Xanthan gum	[53]
Alcaligeness sp.	Curdlan	[10]
Cytophaga, Arcobacter, and *Rhizobium* sp.	Biofilm formation	[96]
Acids produced by microbes		
Applications: carbonate dissolution in rocks by acids enhances oil migration by increasing porosity and permeability		
Microbe	Acids	References
Clostridium sp.	Acetate and butyrate	[55, 56, 134]
Bacillus sp.	Acetate, formate, lactate	[134]
Solvent produced by microbes		
Application: dissolution of rocks releases oil from porous matrix, also lowers oil viscosity		
Microbe	Solvents	References
Clostridium sp.	Acetone, butanol, propan-2-diol	[99]
Zymomonas sp.	Acetone, butanol, propan-2-diol	[99]
Klebsiella sp.	Acetone, butanol, propan-2-diol	[99]
Gases produced by microbes		
Application: increased pressure of oil, swelling of oil, and reduced viscosity of oil		
Microbe	Gases	References
Clostridium sp.	CO_2 and H_2	[134]
Bacillus sp.	CO_2	[6]

MEOR for conventional oil reservoirs in three different ways. They can be produced *ex situ* in fermentors and then injected into the well [98]. Biosurfactants can also be produced in situ by exogenous microorganisms injected into the well [135]. Also, the production of biosurfactants by indigenous biosurfactant-producing bacteria can be stimulated by injection of nutrients into the well [13, 113, 123, 130] (Table 5).

Concluding comments

The Athabasca Oil Sands are a product of millions of years of biodegradation processes which continue today. Microbial communities, including sulphate-reducing bacteria, fermenters, methanogens, acetogens, nitrate-reducing bacteria, and iron-reducing bacteria, interact in the oil sands environment and metabolize crude oil compounds. The mechanisms bacteria use to tolerate the low-water and hydrocarbon stresses inherent in the oil sands matrix vary between species and functional groups. Some of these mechanisms, such as the production of biosurfactants, influence the oil sands environment and have applications in MEOR. Bacterial communities can displace oil from mineral surfaces, and reduce oil viscosity through the production of biosurfactants, solvents, gases, and acids. Some research has been completed regarding bacterial species specific to the Athabasca Oil Sands, and the potential use of microbial biosurfactants in MEOR applications. As molecular methods progress within the fields of transcriptomics, proteomics, genomics, and metabolomics, these advances could be applied to the study of indigenous

Table 5 Laboratory-scale results of residual oil recovery from sand-packed columns using biosurfactants

Microorganisms	Biosurfactants	% Residual oil recovered	References
Acinetobacter calcoaceticus	Unidentified	36.4	[104]
Arthrobacter protophormiae	Unidentified	90	[88]
Bacillus subtilis DM03 and DM04	Lipopeptide	56–60	[25]
Bacillus sp. AB-2	Unidentified	90–100	[11]
B. subtilis MTCC1427	Lipopeptide	34–39	[70]
B. subtilis MTCC 2423	Surfactin	62	[69]
Bacillus licheniformis K125	Lipopetide	43	[108]
B. subtilis PT2	Unidentified	61	[87]
Engineered *P. aeruginosa* PEER02	Rhamnolipid	42	[123]
P. aeruginosa SP4	Unidentified	57	[87]
P. aeruginosa	Glycolipid	50–60	[18]
Pseudomonas strain	Glycolipid and phospholipids	52	[81]
Rhodococcus	Glycolipid	86	[2]
Serratia marcescens	Unidentified	82	[89]

Adapted from Youssef et al. [134] and Sen [99]

bacterial populations within oil sands to investigate their potential in new biotechnological applications.

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