

## MINIREVIEW

# The microbiology of oil sands tailings: past, present, future

Julia M. Foght<sup>1,\*</sup>, Lisa M. Gieg<sup>2</sup> and Tariq Siddique<sup>3</sup>

<sup>1</sup>Department of Biological Sciences, University of Alberta, Edmonton, AB, Canada T6G 2E9, <sup>2</sup>Department of Biological Sciences, University of Calgary, Calgary, AB, Canada T2N 1N4 and <sup>3</sup>Department of Renewable Resources, University of Alberta, Edmonton, AB, Canada T6G 2G7

\*Corresponding author: Julia Foght, CW405 Biological Sciences Bldg, University of Alberta, Edmonton, AB, Canada T6G 2E9. Tel: +001-780-492-3308; E-mail: [julia.foght@ualberta.ca](mailto:julia.foght@ualberta.ca)

**One sentence summary:** Oil sands tailings harbor diverse microbes that generate greenhouse gases, biodegrade hydrocarbons, transform minerals, consolidate fine tailings in ponds and impact tailings management in wet and dry reclamation scenarios.

Editor: Marcus Horn

## ABSTRACT

Surface mining of enormous oil sands deposits in northeastern Alberta, Canada since 1967 has contributed greatly to Canada's economy but has also received negative international attention due largely to environmental concerns and challenges. Not only have microbes profoundly affected the composition and behavior of this petroleum resource over geological time, they currently influence the management of semi-solid tailings in oil sands tailings ponds (OSTPs) and tailings reclamation. Historically, microbial impacts on OSTPs were generally discounted, but next-generation sequencing and biogeochemical studies have revealed unexpectedly diverse indigenous communities and expanded our fundamental understanding of anaerobic microbial functions. OSTPs that experienced different processing and management histories have developed distinct microbial communities that influence the behavior and reclamation of the tailings stored therein. In particular, the interactions of *Deltaproteobacteria* and *Firmicutes* with methanogenic archaea impact greenhouse gas emissions, sulfur cycling, pore water toxicity, sediment biogeochemistry and densification, water usage and the trajectory of long-term mine waste reclamation. This review summarizes historical data; synthesizes current understanding of microbial diversity and activities *in situ* and *in vitro*; predicts microbial effects on tailings remediation and reclamation; and highlights knowledge gaps for future research.

**Keywords:** bitumen; anaerobic hydrocarbon biodegradation; methanogenesis; sulfidogenesis; fluid fine tailings; tar sands

## INTRODUCTION TO OIL SANDS

Western Canadian oil sands (historically called 'tar sands') in the Athabasca region of northeastern Alberta (Supplementary Fig. S1) represent one of the world's largest proven petroleum reserves in the form of bitumen, a heavily biodegraded crude oil (Larter and Head 2014) that is too viscous to flow to a conventional oil wellbore. Bitumen extraction, upgrading and export represent a large component of Canada's economy and domestic energy security. Environmental consequences of this

endeavor have drawn public scrutiny (Gosselin *et al.* 2010; Jordaan 2012; Hodson 2013) but also provide unique scientific opportunities to examine the role of microbes in this massive engineered environment. Extraction of bitumen from surface-mined oil sands ores presents diverse environmental issues including disturbed land areas and fragmented habitat for wildlife; enormous tailings impoundment volumes and surface areas requiring reclamation; river water usage (Jordaan 2012); processed water toxicity (e.g. Clemente and Fedorak 2005; Brown and Ulrich 2015) that precludes release of water to the environment;

emission of biogenic greenhouse gases (Siddique et al. 2008; Burkus, Wheler and Pletcher 2014) and other emissions such as mercury and volatile hydrocarbons (Simpson et al. 2010; Kirk et al. 2014; Small et al. 2015); and potential groundwater contamination beneath tailings ponds (Holden, Donahue and Ulrich 2011). Legacy issues affecting reclamation efforts include H<sub>2</sub>S production from constructed wetlands containing sulfate-rich tailings (Reid and Warren 2016), acid mine drainage from surface deposition of tailings (Kuznetsov et al. 2015; Dean, Xiao and Roberts 2016; Kuznetsova et al. 2016) and acceptance of end pit lakes (EPLs; see Box 1) as remedial options (Charette et al. 2012). Microbes affect all of these aspects, yet their importance in tailings ponds was unappreciated until recently. Ironically, over geological time microbes were responsible for the very nature of the hydrocarbon reserves in the oil sands region (Hein et al. 2013) by depleting labile components of conventional crude oil and leaving high molecular mass bitumen that resists further biodegradation.

#### Box 1. Physical structure abbreviations

EPL: end pit lake; a tailings wet reclamation scenario currently being field-tested  
 MLSB: Mildred Lake Settling Basin; the largest, one of the oldest, and the most-studied OSTP  
 OSTP: oil sands tailings pond; man-made impoundment containing solid and fluid by-products of oil sands surface mining and bitumen extraction  
 OSPW: oil sands process-affected water, including the aqueous surface layer of OSTP

Currently microbes manifest as both beneficial and deleterious agents in the management and reclamation of oil sands mining wastes. To understand their roles in various aspects of oil sands tailings ponds (OSTPs) and reclamation practices, it is necessary to understand the processes that generate oil sands mining wastes and influence tailings management strategies. In addition to the brief overview below and schematic diagrams in Fig. 1, additional details and citations are available in Supplementary data. The reader is directed to peer-reviewed documents hosted by the on-line Oil Sands Research Information Network (OSRIN; [www.osrin.ualberta.ca/](http://www.osrin.ualberta.ca/)), especially the technology overview by BGC Engineering (2010) and glossary of terms (OSRIN 2014). The electronic resource Oil Sands Magazine (<http://www.oilsandsmagazine.com/>) also provides schematic summaries and lay explanations for complex processes and technologies. It is also important to note that the ~20 major extant OSTPs differ in their histories, contents and management practices; no single review can succinctly capture this complexity, and many ponds have not been characterized microbiologically. Therefore this review focuses on several major ponds operated by Suncor Energy Inc., Syncrude Canada Ltd, Shell Albian Sands and Canadian Natural Resources Ltd, which are, in order, the longest-established surface mining operators. Among these, two OSTP archetypes are considered, both of which comprise mineral and aqueous phases: ponds that contain water-saturated semi-solid 'fluid fine tailings' (FFT; see Box 2) and 'mature fine tailings' (MFT) (COSIA 2012); and those that have received 'engineered' tailings generated by adding chemicals to produce low-water, high-sulfur, high-solids wastes (consolidated tailings; CT; Matthews et al. 2002). Thus, the review addresses the major types of tailings and bitumen recovery processes for

which microbiological data are available. Two general reclamation strategies (wet versus dry landscaping) are discussed from the perspective of microbial impacts. Tailings engineered by physical methods (centrifugation, filtration, freeze-drying, etc.) are not considered here.

#### Box 2. Tailings abbreviations

CT: consolidated tailings; tailings that have been chemically engineered to recover interstitial pore water and decrease solids volume by adding gypsum (CaSO<sub>4</sub>•2H<sub>2</sub>O) and tailings sand  
 FFT: fluid fine tailings, having low solids content (e.g. ≤8 wt%)  
 MFT: mature fine tailings, having solids content ≥30 wt%  
 TT: thickened tailings; tailings that have been mixed with organic polymer to flocculate the solids and recover water

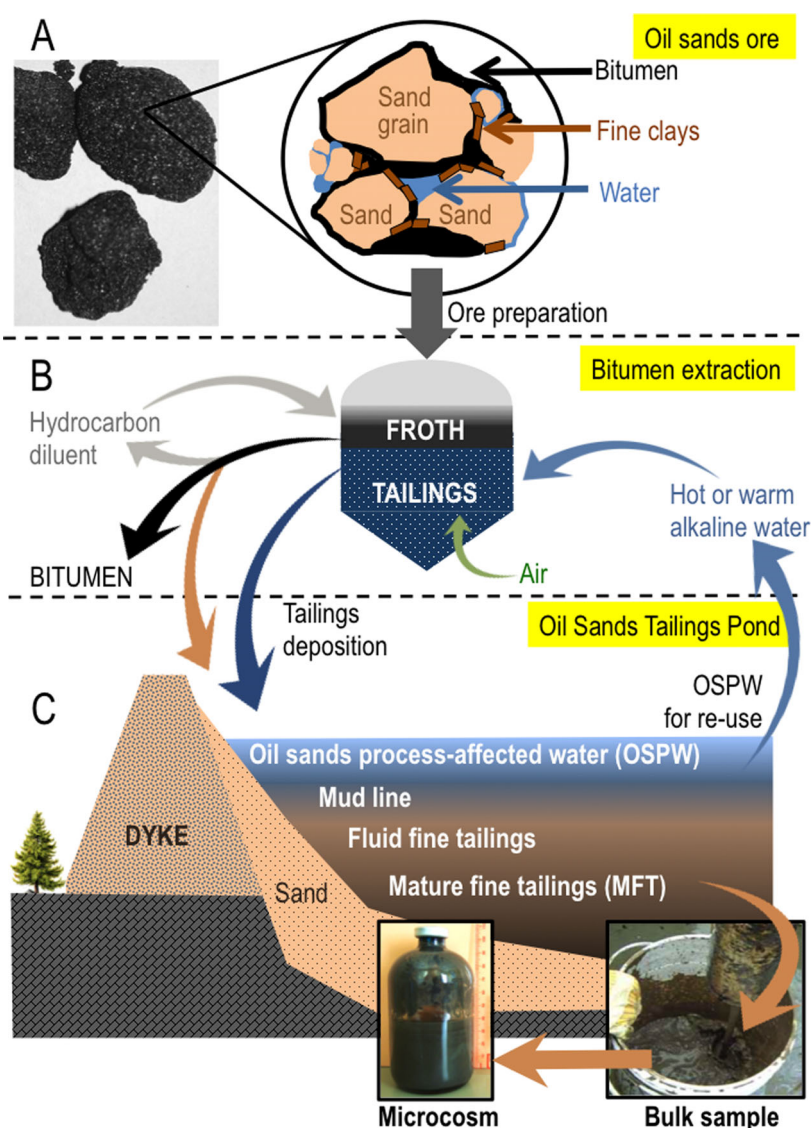
#### Oil sands ores, bitumen extraction and generation of oil sands tailings

The Athabasca oil sands region includes both shallow deposits (≤50–75 m below the surface; mbs) that are suitable for surface mining and deeper deposits that require unconventional methods such as steam injection to recover the bitumen; the latter do not generate fluid mine tailings and are not discussed in this review. The scale of operations is enormous and may represent the largest mining operation in the world: the total land area affected by active mining and tailings waste containment was estimated to be 895 km<sup>2</sup> in 2013 (Burkus, Wheler and Pletcher 2014), the volume of impounded mine tailings is currently estimated at ~1 billion m<sup>3</sup>, and new and future mining operations are in development.

Oil sands ores comprise sand grains that may be surrounded by microscopic films of formation water (Hall, Collins and Melrose 1983; Czarniecki et al. 2005; Doan et al. 2012) and complex organic material (Sparks et al. 2003). Bitumen occupies the void spaces with embedded fine silt and clay minerals ('fines', comprising primarily kaolinite, illite and mixed smectite clays, and iron-containing minerals; Gosselin et al. 2010) (Fig. 1A; see Box 3). Bitumen-rich ores co-occur with thin, discontinuous beds of clays so that oil sands ores average ~10 wt% bitumen, ~5% water and ~85% quartz sand, silt and clays (Chalaturnyk, Scott and Özüm 2002). To recover bitumen, ores are crushed and mixed with hot (historically 70–80°C but now ~50°C) or

#### Box 3. Other definitions

Biodensification: accelerated consolidation of the solid phase of MFT with attendant de-watering, assisted by biogeochemical reactions  
 Consolidation: reduction in the volume of tailings achieved through de-watering and flocculation of fines  
 De-watering: separation of interstitial water from the solids (mineral phase) in FFT and MFT by gravitational (self-weighted) settling or by physico-chemical engineering  
 Fines: an industry-defined term typically referring to mineral particles of ≤44 μm diameter  
 Mud line: a transition zone of sharply increasing specific density between low-solids and higher solids tailings  
 NAs: naphthenic acids; a family of organic chemicals, some of which are toxic solutes in OSPW



**Figure 1.** Highly simplified schematic of oil sands ore extraction and tailings pond structure. (A) Photograph of oil sands ore aggregates and (inset) enlarged representation of ore components. (B) generalized bitumen extraction process using aeration and froth flotation. Water re-use and diluent recovery are shown and two major tailings streams (sand and FFT) are indicated. (C) OSTP cross-section and photographs of MFT being collected in 20-L pail and after distribution to 75-mL anaerobic laboratory microcosm (insets). For additional details on processes and tailings streams, see Masliyah et al. (2004) and Gosselin et al. (2010).

warm (35–40°C) water at pH ~8.5. However, extraction processes and conditions are continually optimized by each operator according to the ore properties and bitumen processing infrastructure (BGC Engineering 2010) (Fig. 1B).

The ore–water slurry is agitated with aeration to produce a bitumen-rich froth that is recovered at the surface. A light hydrocarbon diluent is typically added during froth treatment to reduce water-in-bitumen emulsification, decrease bitumen viscosity and density, and enhance oil recovery from the sand and fine particles. Most operators use naphtha, a petroleum distillate comprising aliphatic hydrocarbons of ~C<sub>5</sub>–C<sub>16</sub> and monoaromatics (BTEX: benzene, toluene, ethylbenzene and xylene isomers) (Burkus, Wheler and Pletcher 2014) as the diluent. Others use a light paraffinic diluent comprising mainly C<sub>5</sub>–C<sub>6</sub> alkanes (Supplementary Table S1). Various chemicals that disperse or flocculate the fines may be used at different stages by some operators to alter the behavior of the slurry (BGC Engineering 2010) (Table 1).

Most but not all of the diluent is recovered from the froth treatment tailings (which are enriched in clays and diluent hydrocarbons but have little sand) before they are deposited, along with other tailings streams (Small et al. 2015), either at the pond surface or underwater in enormous mined-out pits to create OSTPs (Fig. 1C). The sand settles rapidly after deposition, typically forming surface or subsurface beaches of ‘coarse tailings’ at the pond margins, and can be reused for various purposes on-site. In contrast, after deposition the remainder of the fresh FFT (comprising ~8 wt% fines, ~5% unextracted bitumen, <1% unrecovered diluent and ~85% process water) form a colloidal suspension. Initially, de-watering (gravity-driven separation of interstitial water and clays that enhances consolidation of mineral particles; see Box 3) is relatively efficient, with FFT reaching ~15 wt% solids within a few weeks and ~20% solids after 3–9 months. Thereafter pore water expression is slower and another 5–10 years of settling by gravity is required to achieve ≥30 wt% solids, after which the tailings are called MFT.

**Table 1.** Pertinent characteristics of OSTP discussed in this review. Additional details can be found in Small et al. (2015) and Burkus, Wheler and Pletcher (2014).

Operator	OSTP name	Age (years)	Diluent <sup>a</sup>	Process details
Suncor	Pond 1A	~50 <sup>b</sup>	Light naphtha	Hot water ( $\geq 50^{\circ}\text{C}$ ), NaOH
	Pond 5 and Pond 6	~10	Light naphtha	Hot water ( $\geq 50^{\circ}\text{C}$ ) NaOH, gypsum, sand (CT process) <sup>c</sup>
Syn crude	Mildred Lake Settling Basin (MLSB)	~40	Heavy naphtha	Hot water ( $\geq 50^{\circ}\text{C}$ ), NaOH
	West In-Pit (now Base Mine Lake)	~20	Heavy naphtha	No additional chemicals <sup>d</sup>
	Aurora Settling Basin (Aurora North)	~15	None <sup>e</sup>	NaOH, warm water (35–40°C)
Albian Sands	Muskeg River Mine	~15	Paraffinic	Trisodium citrate, polymer, warm water (40–50°C)
CNRL	Horizon	~8	Medium naphtha	NaOH, hot water ( $\geq 50^{\circ}\text{C}$ ), CO <sub>2</sub> injection

<sup>a</sup>See Supplementary Table S1 for composition of diluents.

<sup>b</sup>No longer active; reclaimed after ~40 years of operation.

<sup>c</sup>CT, consolidated tailings (see Box 2).

<sup>d</sup>From 1995 to until December 2012 tailings were transferred from MLSB into West In-Pit without any additional chemical additives; no transfers have occurred since then, as West In-Pit tailings pond has become an experimental EPL called Base Mine Lake.

<sup>e</sup>Although naphtha is used in bitumen extraction, the naphtha-impacted froth treatment tailings from Aurora are transported to and deposited in MLSB.

Unassisted consolidation and de-watering sufficient to achieve a ‘trafficable’ material with substantial shear strength has been projected to take >125 years (Eckert et al. 1996). Throughout this settling period oil sands process-affected water (OSPW) is expressed from pore spaces and accumulates at the pond surface (Fig. 1C) to be reclaimed for re-use in ore processing. These individual OSTP compartments are described briefly below.

### Oil sands process-affected water

Water for processing the ores is drawn from the Athabasca River and augmented with alkaline, slightly brackish OSPW recovered from OSTPs. Fresh water use is minimized (e.g.  $\leq 15\%$  of demand) and use of recycled OSPW is maximized to reduce the net volume of tailings generated and to conserve natural surface water supplies.

The OSPW layer at the pond surface may vary from 2 to 10 m deep depending on the rate of removal for re-use. Hydrocarbon sheens and suspended or floating bitumen globules are common. The uppermost layer of water may be oxidic due to wind and wave action, but the bulk water rapidly becomes anoxic with depth (Ramos-Padrón et al. 2011; Saidi-Mehrabadi et al. 2013; Stasik et al. 2014; see Supplementary data for more detail). At least two mechanisms may explain the sharp oxycline in the OSPW layer: aerobic and/or facultative microbial metabolism *in situ*; and chemical oxygen demand due to oxidation of microbially produced sulfides at depth after sulfate (gypsum) addition to fluid tailings, discussed below.

### Tailings: FFT, MFT and CT

Below the OSPW layer is a diffuse ‘mud line’ (Fig. 1C), an interface of variable depth and thickness between the water layer and solids-enriched FFT layer that has a rapidly increasing density gradient (pycnocline) from <0.1 wt% to >10% solids content. Below this zone the FFT gradually increase in solids content to become MFT, and the total depth below the mud line may be  $\geq 40$  m, depending on the operator’s practices, depth of the basin and age of the pond. Natural consolidation and de-watering lead to chemical, physical and microbial stratification of MFT (Guo 2009). Depth correlates roughly to age (time since deposition) and the strata reflect temporal variations in operational processes and ore quality as well as spatial changes in deposition sites (e.g. from moving barges). All these factors contribute to significant heterogeneity in mature ponds.

Besides increasing solids content, other physical and chemical properties also change with depth. For example, temperature typically increases with depth because fresh tailings when deposited are still warm (historically up to 60°C; MacKinnon 1989) and also possibly from retained heat of microbial metabolism in this dense, insulating material. Penner and Foght (2010) reported temperatures of 12°C at 6 mbs to 22°C at 30 mbs in Syn crude’s Mildred Lake Settling Basin (MLSB), and Ramos-Padrón et al. (2011) documented a temperature change from 7°C at the surface to 19°C at 18.3 mbs in Suncor Pond 6. Chemical gradients may also exist, with diluent hydrocarbons and soluble electron acceptors such as sulfate typically decreasing with depth (age) compared with surface layers where fresh input replenishes these components (Guo 2009; Penner and Foght 2010; Stasik et al. 2014; Supplementary Fig. S2). Sulfate and iron concentration patterns may be complex because of historical changes to process practices (such as CT implementation) and also because of microbial activity. Thermal and chemical gradients may be disrupted in ponds receiving tailings that have been physically manipulated, e.g. by transfer of sediments from one basin to another.

Some operators such as Suncor mix gypsum (CaSO<sub>4</sub>·2H<sub>2</sub>O) with fine tailings plus coarse sand to form CT. This treatment rapidly expresses process water and increases solids content to 70–80 wt% within 1 year (Mikula et al. 1996). The smaller volume of CT pore water then has greater concentrations of Ca<sup>2+</sup>, sulfate (Fedorak et al. 2002) and naphthenic acids (NAs; see Box 3) than MFT pore water, affecting microbial activity regardless of whether the CT is subsequently stored in tailings ponds or subjected to wet landscape reclamation.

Notably, each OSTP is unique in its history, bitumen extraction conditions, ore and tailings geochemistry, physical characteristics and management practices, all of which impart selective conditions for the development of unique microbiota in each pond.

## MICROBIAL COMMUNITIES IN OSTPs

### Probable sources of OSTP microbiota

All OSTPs sampled to date harbor complex microbial communities, yet the process of pond ‘inoculation’ has not been rigorously examined. Simplistically, the major sources of viable microbes are inferred to be indigenous microbiota in the ores, process water and fresh river water that survive the various bitumen

extraction processes, plus minor contributions from airborne microbial propagules.

#### Subsurface inocula

Subsurface microbial communities indigenous to the ores may be the major initial inoculum source for new OSTPs. These microbes may represent descendants of the original microbiota that colonized kerogen-rich sediments during burial and have persisted at the oil–water transition zone. Incursion of rainwater, snow melt and glacial melt waters over geological time may also have contributed viable aerobic and anaerobic taxa to shallow deposits (Larter and Head 2014). Indeed, metagenomic analysis of deep oil sands cores from the Athabasca River region (those >75 mbs, where bitumen is too deep for surface mining) revealed interbedded networks of aerobic, facultative and anaerobic taxa including hydrocarbon-degrading bacteria and methanogens (An et al. 2013a). Similarly, the metagenomes of bitumen outcrops exposed in river valleys in the Athabasca region comprise hundreds of fungal and bacterial taxa including putative aerobic hydrocarbon-degraders, anaerobes including methanogens (Wong et al. 2015), and methanotrophs (Saidi-Mehrabadi et al. 2013). The bitumen and/or associated water films may be minor contributors of hydrocarbon-tolerant microbes analogous to those detected in microscopic water droplets embedded in heavy oil (Meckenstock et al. 2014; Supplementary information).

As well as pore water in the ores, bitumen reservoirs often have associated groundwater and/or aquifer fluids that may be fresh or brackish due to the ancient marine sediments in the formation. Hubert et al. (2012) reported the dominance of *Epsilonproteobacteria* and lesser proportions of *Methanomicrobiales* in water samples from a basal aquifer underlying an oil sands reservoir, although this observation has not been confirmed at other mine sites.

#### Surface inocula

Fresh water from the Athabasca River and re-used OSPW are plausible surface sources of microbes in OSTPs. The Athabasca River and its tributaries have long been known to be influenced by exposed bitumen deposits as they pass through and erode the oil sands region, especially near shallow bitumen deposits conducive to surface mining; bitumen was used by indigenous peoples and hydrocarbon sheens on the rivers were noted by early explorers. Over thousands of years, this natural contamination seems to have enriched the river water and sediments with microbial communities capable of adhering to bitumen and degrading hydrocarbons (Wyndham and Costerton 1981a,b). More recently, next-generation sequencing methods have revealed that communities in river sediments geographically close to oil sands mining sites are more similar to OSTP communities than are sediments more distant from the mines (Yergeau et al. 2012), suggesting cross-inoculation.

The harsh processing conditions of froth treatment (40–60°C, pH 8.5, natural and synthetic surfactants, high aeration, and exposure to low molecular mass hydrocarbons) put negative selective pressure on microbes indigenous to ores or process water. To our knowledge, no peer-reviewed publications have described the microbiota of fresh froth treatment tailings immediately post-extraction. However, internal reports to operators (Foght and Siddique 2014; Foght, Li and Semple 2014) have documented the presence of viable cells in froth treatment tailings. Although the magnitude and composition of the microbial community in ore–water slurries prior to froth treatment is unknown, it is clear that some strict anaerobes can survive the process. Most proba-

ble number (MPN) analysis of samples from two operators that use naphtha diluent revealed viable anaerobes in froth treatment tailings, with general fermenters at  $10^5$ – $10^7$  cells/ml fresh tailings, nitrate reducers at  $10^4$ /ml, sulfate reducers at  $\sim 10^2$ /ml and methanogens at  $\sim 10^1$ /ml. Aerobic hydrocarbon-degrading microbes were present at concentrations below the MPN detection limit ( $<10^0$ /ml), yet sufficient *Gammaproteobacteria* (including *Pseudomonadaceae*) and *Betaproteobacteria* (including *Comamonadaceae*), among other taxa, survived to achieve hydrocarbon degradation in aerobic primary cultures established from froth treatment tailings (Foght, Li and Semple 2014). Therefore, recycling of OSPW may concentrate and enrich adapted microbiota through repeated selection during bitumen extraction followed by incubation in the ponds. Furthermore, the lower water temperatures currently used for bitumen extraction may permit survival of more (or different) microbes than previous hotter conditions (although Wong et al. (2015) detected thermophiles in bitumen outcrops that were capable of aerobic hydrocarbon metabolism at 55°C in the laboratory).

Thus, multiple potential sources of microbial inocula exist, including deposition of air-borne propagules, but most such external sources likely become insignificant after an indigenous OSTP microbiota becomes established. That is, re-used OSPW and fresh tailings likely have greater roles as sources of labile carbon than as sources of inoculum in mature ponds.

#### In situ microbial abundance, diversity and community structure in OSTPs

General observations about the microbiota of OSPW and semi-solid tailings are presented below, followed by brief descriptions of communities in individual ponds, organized by operator. As is evident from the literature cited, the majority of publications on oil sands microbiota describe Syncrude mining operations and particularly MLSB, the largest and one of the oldest OSTPs.

#### OSPW microbiota

Few reports have enumerated or examined the diversity of OSPW microbiota compared with MFT, possibly because OSPW is only a small volumetric proportion of the ponds and/or because the OSPW layer is constantly being recycled and experiences seasonal changes. In contrast, MFT is retained, often undisturbed for decades, essentially in a ‘closed’ and diffusion-limited environment that enables long-term studies. OSPW recycling concentrates toxic ions and organic solutes (e.g. NAs) from the ores (Allen 2008). Fugitive diluent, H<sub>2</sub>S and methane (CH<sub>4</sub>) arising from beneath the mud line may provide additional oxygen demand or toxic pressures on indigenous microbes. Unsurprisingly, low estimates of strict anaerobes (methanogens and sulfate reducers) have been reported in OSPW using MPN methods (Supplementary Table S2), but facultative anaerobes such as heterotrophic nitrate reducers are abundant in Syncrude OSPW (e.g.  $10^5$ /ml; Sobolewski 1995, 1997, 1999b). Stasik et al. (2014) also detected aerobic thiosulfate-oxidizing cells ( $10^4$ – $10^8$ /ml) in OSPW from Syncrude ponds, with lower abundance of sulfate reducers ( $10^4$ – $10^6$ /ml) and iron reducers ( $10^1$ – $10^2$ /ml).

Ramos-Padrón et al. (2011) used pyrotag sequencing of 16S rRNA genes to assess the prokaryotic community composition as a function of tailings depth in Suncor Pond 6, including a surface water sample. As expected, they found that the surface water harbored entirely different taxa (*Methyloversatilis*, *Azospirillum* and *Gemmata*) from samples analyzed from deeper strata where strictly anaerobic taxa were identified (see below).

Subsequently, An et al. (2013a) used pyrotag sequencing to analyze 15 OSPW samples from three ponds, collected between 2009 and 2011. The conglomerate OSPW communities clustered separately from MFT from the same ponds and from deep oil sands ores collected nearby, and were dominated by *Rhodocyclales* (*Thauera*), *Burkholderiales* (*Acidovorax*, *Hydrogenophaga*, *Alcaligenaceae*) and *Flavobacteriales* (*Flavobacterium*), each of which includes facultative species that might be suited to the sharp oxycline of the OSPW layer. Sulfur oxidizers such as *Chromatiales* (*Thiocapsa*) and *Desulfuromonadales* (*Geobacter*) were also prevalent. As expected, both methanogens and sulfate reducers were confirmed to be minor members of OSPW communities (An et al. 2013a) whereas in-depth analysis of pyrotag data, microarrays and a metagenome revealed the presence of aerobic methanotrophic bacteria (predominantly *Methylocaldum*) and characteristic particulate monooxygenase (*pmo*) genes (Saidi-Mehrabadi et al. 2013), the significance of which is discussed below.

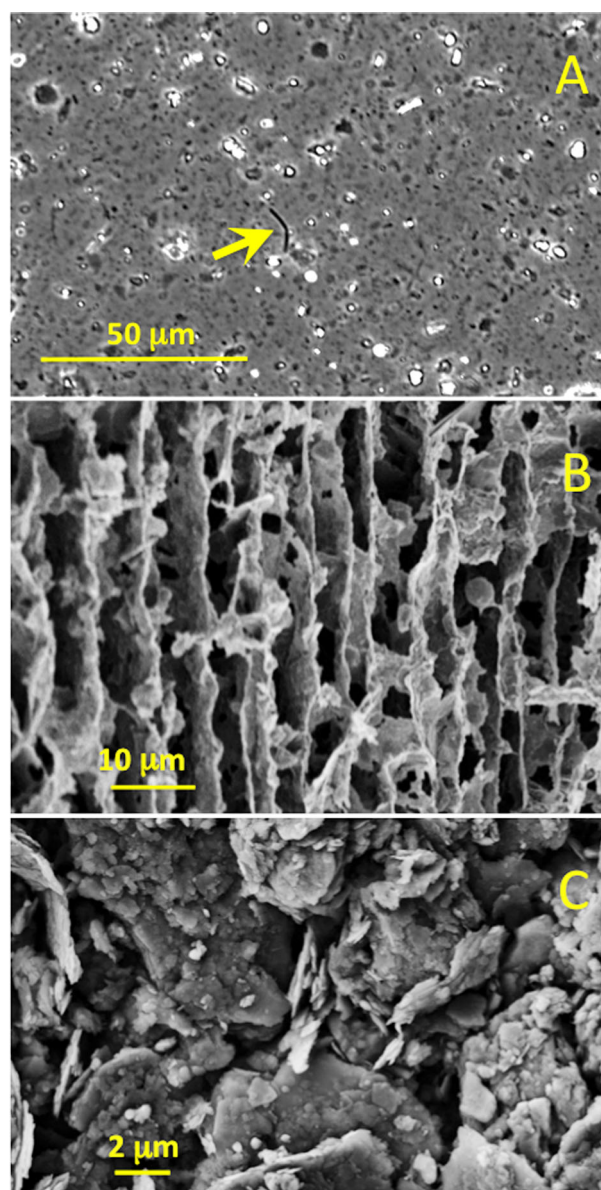
#### FFT and MFT microbiota in different operators' tailings ponds

Obtaining OSTP samples is seldom easy and their manipulation in the laboratory can be problematic (Supplementary data). Methods developed to overcome these problems have been compiled by Foght, Siddique and Gieg (2017). A particularly intractable problem is the heterogeneity of the ponds, since the subsurface distribution of tailings and their processing history is not precisely known, leading to sample variability with depth and lateral position and complicating inter-sample comparisons.

MPN estimates of the abundance of methanogens and sulfate reducers in MFT are summarized in Supplementary Table S2. Co-occurrence of these two metabolic types in all MFT samples tested suggests that they do not compete for substrates *in situ*, but rather may act synergistically in anaerobic hydrocarbon biodegradation (Supplementary Fig. S3), as discussed below. Some studies have also enumerated heterotrophic nitrate reducers (Sobolewski 1999b; Fedorak et al. 2002; Foght and Siddique 2014), sulfide-oxidizing nitrate reducers (Foght and Siddique 2014; Foght, Li and Semple 2014), iron reducers (Sobolewski 1997, 1999b; Fedorak et al. 2002; Stasik et al. 2014) and thiosulfate oxidizers (Stasik et al. 2014). In several of the studies the range of values given represents abundance along depth (age) profiles from newly deposited FFT to decades-old MFT (e.g. Penner and Foght 2010), highlighting long-term persistence of viable cells in the nearly closed environment of deep tailings.

Despite the relatively high abundance of cells in MFT, repeated attempts to use scanning electron microscopy (SEM) to directly observe arrangements of indigenous microbial communities in uncultivated MFT have been unsuccessful (Fig. 2B and C), underscoring the large surface area provided by the clays and the presumably patchy distribution of cells within the mineral matrix. Cells are also difficult to distinguish from clay minerals using phase contrast microscopy (Fig. 2A) because they have similar dimensions (with the exception of filamentous methanogens) and the mineral particles far outnumber cells. However, using SEM, Bordenave et al. (2010) observed microbial cells adhering to MFT that had been inoculated with pure cultures, amended with a carbon source and electron acceptor, and incubated under methanogenic, sulfate- or nitrate-reducing conditions. This illustrates the potential for microbial colonization of the fines surface.

Three of Syncrude Canada's tailings ponds are discussed here: MLSB, West In-Pit (now Base Mine Lake) and Aurora. MLSB was established in 1978 and for the first 15 years of its operation was not observed to produce CH<sub>4</sub> (Foght et al. 1985). Gas bubbles



**Figure 2.** Micrographs of three MFT samples (with scale bars). (A) Phase contrast light micrograph of MFT diluted in buffer. Presumptive methanogenic cells are indicated by arrow; other particles include fine mineral grains and (presumably) microbial cells. (B) Cryo-scanning electron micrograph of fractured methanogenic MFT exhibiting 'deck of cards' mineral alignment. Thin light grey lines are clay platelets viewed edge-on; darker grey voids between platelets are interstitial spaces occupied by pore water prior to cryo-ablation. Spherical structure at centre right may be a microbial cell. (C) Focused-ion beam scanning electron micrograph of younger MFT from a different operator, showing random 'house of cards' orientation of minerals. (A, C) courtesy of G. Southam, University of Queensland; (B) courtesy of C. Li, University of Alberta.

were first noted in the early 1990s at the surface of the south end of the pond where naphtha-enriched froth treatment tailings were being deposited (Holowenko, MacKinnon and Fedorak 2000; Guo 2009). The zone of active ebullition expanded slowly northward until, by 1999, 40–60% of its ~12 km<sup>2</sup> surface was releasing CH<sub>4</sub> and co-transporting CO<sub>2</sub> and volatile diluent hydrocarbons to the surface (Simpson et al. 2010). Considerable volumes of CH<sub>4</sub> also remained trapped within the MFT (Holowenko, MacKinnon and Fedorak 2000; Guo 2009), representing potential

future greenhouse gas emissions. The onset of CH<sub>4</sub> production coincided with increasing abundance of methanogens at depths from 5 to 25 mbs by 1996 (Supplementary Table S2). West In-Pit, which received methanogenic MFT transferred by pipeline from MLSB between 1995 and 2012, began bubbling within 1–2 years of initiating deposition. Aurora receives fresh tailings that have not been exposed to naphtha and it has not become methanogenic in ~15 years of operation.

Penner and Foght (2010) surveyed MFT depth profile samples (6–30 mbs) collected in 2004 from below the mud line near the centres of MLSB and West In-Pit. Because of the narrow suite of labile carbon substrates in the ponds (i.e. diluent hydrocarbons), it was expected that microbial diversity would be low. However, this report, the first to apply sequencing of 16S rRNA gene clone libraries to MFT, revealed surprising bacterial diversity, with 212 of 301 (~70%) operational taxonomic units (OTUs) being represented by single clones and many OTUs having no closely related cultivated affiliates (Penner and Foght 2010). Of the OTUs that were sequenced and presumptively identified, *Proteobacteria* dominated, including *Thauera*, *Rhodospirillum rubrum* and *Desulfatibacillum*. In contrast, the archaeal sequences primarily were affiliated with a small suite of cultivated methanogens, predominantly *Methanoseta* spp. (Penner and Foght 2010).

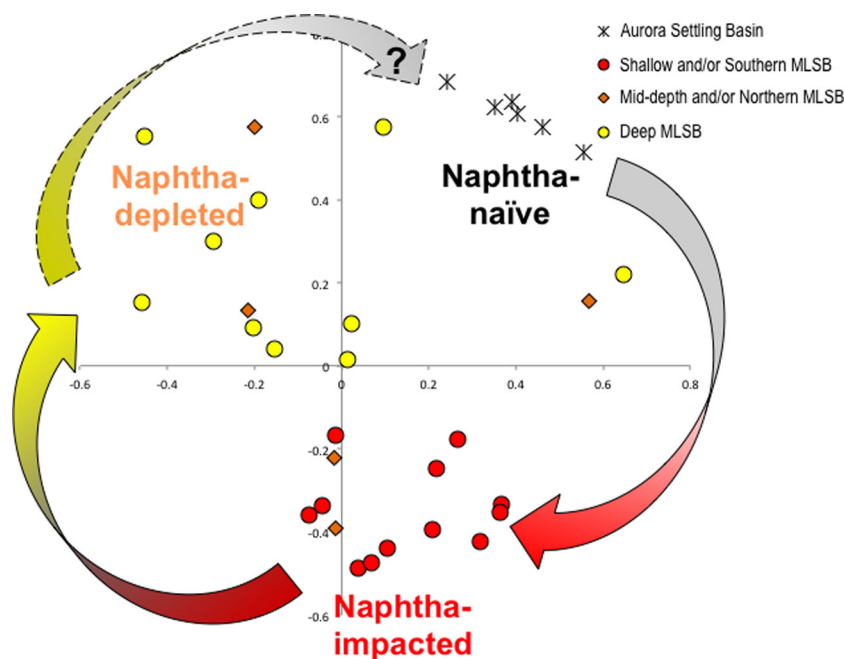
Subsequent pyrotag sequencing of 16S rRNA genes in 11 samples from MLSB at depths of 1–36 mbs expanded the detected prokaryotic diversity in uncultivated MFT. An estimated total of 2400 OTUs (at ≥95% identity) were detected, of which 36% were bacterial and 64% archaeal, representing 410 taxa that were mostly strict anaerobes (An et al. 2013a). The predominant bacteria in MFT were *Proteobacteria* including *Burkholderiales* and *Hydrogenophilales*, while *Methanomicrobiales* and *Methanosarcinales* dominated the archaea. Metagenomic analysis also provided information on the relative abundance of archaeal and bacterial 16S rRNA genes with increasing depth: bacterial OTUs increased with depth from 24% of total prokaryotic pyrotag sequences at 1.5 mbs to 91% at 31.5 mbs. Conversely, archaeal OTUs represented ~80% of pyrotag sequences in shallow (≤10 mbs) MFT samples having a broader range of residual diluent hydrocarbons and exhibiting active methanogenesis, but decreased to <10% in deep samples (>25 mbs) where labile hydrocarbons were depleted (Supplementary Fig. S2). The bacterial community structure also changed with depth: diversity decreased (fewer 'rare' bacterial OTUs in deeper samples) while alpha-, beta- and gammaproteobacterial 16S rRNA gene abundance increased at the expense of *Firmicutes* and *deltaproteobacterial* OTUs (Supplementary Fig. S2). Complementing these observations, Stasik et al. (2014) used terminal restriction fragment length polymorphism and denaturing gradient gel electrophoresis to demonstrate that the prokaryotic community structure in West In-Pit changed at the mud line: proportions of archaeal sequences decreased with depth whereas bacterial sequences increased. Chi Fru et al. (2013) also documented bacterial and archaeal community succession patterns in bioreactors containing MFT taken at 40 mbs from West In-Pit. This deep (old) MFT sample originally was dominated by bacterial OTUs but after manipulation (physical mixing, distribution into mesocosms and laboratory incubation), the onset of sulfidogenesis was accompanied by a shift to dominance by archaeal OTUs. This supports the hypothesis of 'dormant' MFT communities being bacteria-dominated whereas 'active' MFT is archaea-dominated.

Aguilar et al. (2016) were the first to report the presence of eukaryotic microbes in MLSB and West In-Pit ponds by reanalyzing metagenomic data (An et al. 2013a) in addition to generating and sequencing three 18S rRNA gene clone libraries from

total DNA extracts. They detected sequences from seven major eukaryotic groups within the metagenomic dataset, with 169 eukaryotic OTUs representing 11 higher orders from the amplicon libraries, of which 10 taxa were dominant (>90% of library). Sequences affiliated with phototrophic and heterotrophic eukarya were detected in both OSPW and MFT samples, including *Fungi*, *Metazoa*, *Chlorophyta*, *Alveolates*, *Stramenopiles* and *Excavata*. *Amoebozoa* and *Rhizaria* were detected only in the MFT sample. This first observation of (presumably extant) eukarya in OSTPs further expands our understanding of unanticipated microbial diversity even in 'closed system' anoxic tailings, as well as affording a glimpse of potential microbial community activity (e.g. grazing and photosynthesis) during future reclamation efforts.

Syncrude's Aurora Settling Basin, established in 2000, differs from MLSB and West In-Pit in that the tailings deposited therein have been subjected to warm water extraction but not froth flotation treatment (Burkus, Wheler and Pletcher 2014). That is, unextracted bitumen is a component of the tailings, but naphtha diluent is absent. Virtually no CH<sub>4</sub> emissions were measured in 2011–2012 (Burkus 2014), further differentiating Aurora from MLSB and West In-Pit, and supporting the premise that the major source of biogenic CH<sub>4</sub> in OSTPs is diluent hydrocarbons rather than bitumen or endogenous NAs. A pyrotag survey of the Aurora pond then should reflect a 'baseline' condition for indigenous microbes originating from process water and ores without the additional negative and positive selection pressures of exposure to naphtha hydrocarbons. Pyrotag analysis of an Aurora depth profile (10–35 mbs) revealed that methanogenic sequences were rare. Instead, *Beta-* and *Gammaproteobacteria* were dominant and generally increased with depth, whereas *Flavobacteriaceae* decreased with depth and *Chloroflexi* (*Anaerolineaceae*) were relatively constant (Supplementary Table S3). Notably, *Peptococcaceae* sequences that have been associated with methanogenic hydrocarbon degradation in MFT enrichment cultures (Tan 2014; Abu Laban et al. 2015a; Tan, Semple and Foght 2015b) were sparse. The Aurora depth profile differs from methanogenic pond profiles (e.g. Supplementary Fig. S2) and is consistent with the hypothesis that 'microbially inactive' MFT has low proportions of archaeal OTUs and a bacterial community predominated by *Beta-* and *Gammaproteobacteria*.

Combining pyrotag sequences from Aurora with those of depth profiles from a transect of MLSB (increasing in distance from the fresh froth treatment tailings outflow at the south end of the pond) provided a dataset for non-metric multidimensional scaling (NMDS) analysis (Fig. 3). The Aurora samples are considered 'naphtha-naïve', whereas shallow MLSB samples, particularly at the south end of the pond, have recently been exposed to selection by the extraction process and fresh naphtha diluent. Deeper MLSB samples were historically exposed to diluent but the hydrocarbon profiles have been selectively depleted due to biodegradation, with only the most recalcitrant components remaining, such as multiply substituted cyclo-alkanes and iso-alkanes (Supplementary Fig. S2). Samples near the north end, >3 km distal to the tailings deposition point, have been exposed to lower concentrations of naphtha that may already have been selectively altered due to biodegradation. NMDS analysis suggests that successive community adaptation to naphtha exposure, biodegradation of its labile components and accumulation of metabolic products occur in response to OSTP history. However, further examination of this possibility is needed to construct a sound hypothesis for pond microbiota maturation and progression through to reclamation sites.



**Figure 3.** Non-metric multidimensional scaling analysis of 16S rRNA gene pyrotag sequences detected in depth profile samples collected from a single site in Aurora Settling Basin and from three transect stations in MLSB at increasing distance from the southern tailings deposition site. Each symbol represents the prokaryotic community in a single sample. Solid arrows indicate hypothetical progression of community structure in response to exposure to fresh diluent in shallow samples and at southern site > northern site, and to depletion of specific naphtha components in deeper samples (Supplementary Fig. S2). Dashed arrow with question mark indicates the unknown potential for the MLSB communities to return to the inferred 'baseline' structure in Aurora samples that have not been exposed to naphtha.

The Suncor tailings ponds (e.g. Ponds 1, 5 and 6) have not been studied as extensively as Syncrude ponds but some details of microbial composition have been documented. Pond 1, recently reclaimed as a dry landscape (Wapisiw Lookout), was Suncor's oldest tailings pond and actively received extraction wastes from 1967 to 1997. Early reports documented microbial activity in this pond, including the presence of methanogens (MacKinnon and Sethi 1993; Sobolewski 1997, 1999c). MPN enumeration of MFT samples collected from this pond in the late 1990s from a depth of 3.5 mbs revealed the presence of nitrate reducers ( $10^9$  cells/g dry weight tailings), iron reducers ( $10^3$ /g), sulfate reducers ( $10^5$ – $10^9$ /g) and methanogens ( $10^2$ – $10^3$ /g) (Supplementary Table S2). While conducting mesocosm experiments with these samples, Fedorak et al. (2003) demonstrated that the methanogenic community was active, but further microbial research was not conducted on this pond prior to its closure and reclamation.

Tailings in Suncor Ponds 5 and 6 historically have been treated using CT technology and therefore differ chemically, physically and microbiologically from Syncrude MFT. MPN enumeration of anaerobes in samples of fresh CT revealed the presence of denitrifiers, iron reducers, sulfate reducers and methanogens that survived the CT process and pipeline transport at 55°C (Supplementary Table S2; Fedorak et al. 2002). Thus, microbes in fine tailings provided a diverse microbial community to CT that potentially could function under different redox conditions depending upon the availability of electron donor and different electron acceptors (Fedorak et al. 2002). Indeed, pyrotag surveys of these two ponds in 2009 and 2010, just before and after decommissioning, revealed the co-dominance of sequences affiliated with methanogens (*Methanosaeta*), sulfate reducers (e.g. deltaproteobacterial *Desulfocapsa*) and syntrophs (firmicute *Pelotomaculum* and deltaproteobacterial *Smithella* and *Syntrophus*). Both methanogenesis and sulfidogenesis have been

documented in these Suncor ponds (Ramos-Padrón et al. 2011; Ramos-Padrón 2013).

The tailings in Shell Albian Sands' Muskeg River Mine tailings pond differ from those of Syncrude and Suncor in several significant ways (Table 1): (i) the extraction water temperature is warm rather than hot; (ii) a low-molecular mass ( $C_5$ – $C_6$ ) paraffinic diluent is used rather than naphtha (Supplementary Table S1); (iii) the paraffinic diluent precipitates a large proportion of the asphaltenes from the bitumen, removing much of this material from the fluid tailings waste stream prior to deposition in the pond; (iv) trisodium citrate rather than NaOH has been used during extraction as a clay dispersant during extraction; and (v) tailings are thickened by addition of polyacrylamide before deposition onto surface 'beaches'. This pond began producing  $CH_4$  within a few years of establishment (versus the 10- to 15-year lag periods of Syncrude and Suncor ponds), likely because the easily fermentable citrate amendment enriched different fermentative bacteria compared with the naphtha-enriched methanogenic and sulfidogenic ponds. Measurements made in 2011–2012 indicated that  $CH_4$  emissions were substantially less than those of Syncrude or Suncor (Supplementary Table S4).

Li (2010) surveyed microbial abundance in Shell Albian Sands samples collected in 2007 and 2008 below the mud line at six different sites. MPN estimates of methanogens, sulfate reducers and fermenters were all in the range of  $10^5$ – $10^8$ /mL tailings (Supplementary Table S2), indicating a well-established anaerobic contingent. Clone libraries of 16S rRNA genes showed dominance by *Betaproteobacteria*, particularly *Albidiferax* (formerly *Rhodiferax*) and *Acidovorax* spp. The majority of archaeal sequences were affiliated with uncultivated methanogens, unlike Syncrude tailings. Subsequently, pyrotag sequencing of 16S rRNA genes revealed additional diversity, with *Betaproteobacteria* dominating the bacterial sequences but differing from those in Syncrude ponds: the Al-



bian Sands MFT harbored an increased abundance of fermenters within the *Chloroflexi* (*Anaerolineales*) and *Actinobacteria* (*Coriobacteriales*) and was dominated by bacteria (~75% of sequences) rather than archaea (Siddique et al. 2015; Collins, Foght and Siddique 2016; Mohamad Shahimin, Foght and Siddique 2016). The substantial differences between Shell Albian Sands and Syncrude tailings communities correlate with hydrocarbon substrate preferences and diluent composition, discussed below.

The Canadian Natural Resources Ltd Horizon Mine tailings pond, established in 2008, had produced very little CH<sub>4</sub> by 2012 (Burkus 2014) and is likely still developing its unique microbiota. This operation uses naphtha diluent like Syncrude and Suncor, but is unique in injecting CO<sub>2</sub> into fresh fluid tailings prior to deposition, to assist with FFT consolidation *in situ*. MPN analysis of tailings collected in 2013 estimated the abundance of methanogens, sulfate reducers and fermenters at 10<sup>3</sup>–10<sup>5</sup>/mL (Supplementary Table S2). 16S rRNA gene pyrotag analysis showed dominance of archaea (~80% of total reads) of limited diversity, with *Methanomicrobiales* (*Methanoregula* spp.) and *Methanosarcinales* (*Methanosaeta* spp.) accounting for >95% of the archaeal sequences. Among the bacteria, strict fermenters, particularly *Anaerolineaceae* and *Coriobacteriaceae*, were prevalent and a large proportion (~30–55%) of ‘rare’ bacterial OTU sequences (present at <2–5% abundance) were detected (Mohamad Shahimin, Foght and Siddique 2016; Mohamad Shahimin and Siddique 2017a,b). It would be valuable to follow development of the Horizon pond community structure with time and deduce the effect (if any) of CO<sub>2</sub> injection on diversity, taxonomy and activities of the microbiota.

It is clear that most OSTP studies to date have examined only individual ‘compartments’ or stages of the oil sands tailings enterprise, whereas an overarching survey might reveal a more integrated picture of microbial community patterns. Noah et al. (2014) attempted to survey the prokaryotic communities in the production chain from oil sands ores to MFT to engineered MFT to tailings undergoing dry reclamation. Unfortunately, the samples examined were collected from two different operators, one using naphtha and CO<sub>2</sub> injection and the other using aliphatic diluent plus sodium citrate in processing, so the sampling chain was not congruent. The analyses included oil sand samples from an open mine, but used only aerobic plate counts followed by 16S rRNA gene sequencing of isolated colonies to determine community diversity and structure, so the community profile is extremely biased and limited as it omits the anaerobes that predominate in OSTP. Direct microscopic counts were also performed, but on different sub-samples that had been stored differently, and so are not comparable with the plate count data. Thus, an overarching study is still needed to obtain a ‘big picture’ of oil sands microbiology. The genomic approaches described below have begun to address this knowledge gap.

### Metagenomics approaches to OSTP biodiversity

The Hydrocarbon Metagenomics Project (<http://www.hydrocarbonmetagenomics.com/>) provided resources for much of the metagenomic information gathered to date on OSTPs and has left a legacy of publicly available OSTP microbiome and enrichment culture sequence information with associated metadata. In addition to identifying dominant microbial community members and their physiologies in individual tailings ponds, comparative genomics approaches have been used to compare taxa and/or examine key functions across many hydrocarbon resource environments including

OSTPs managed by different operators. An et al. (2013a) used co-occurrence analysis to group prokaryotic taxa into two networks: Network A contained strict anaerobes, while Network B contained strict aerobes and facultative anaerobes. Aside from the surface water samples discussed above (dominated by aerobes and facultative anaerobes), microbiomes of all other OSTP samples were strongly associated with Network A, corresponding to the anoxic conditions in MFT and CT. Further comparison of the metagenomes of the pond samples (MLSB and Suncor Ponds 5 and 6) with other hydrocarbon-impacted environments (such as oil sands cores and coal beds) showed greater abundance in OSTPs of functional genes associated with anoxic processes including genes for anaerobic hydrocarbon degradation, syntrophy and methanogenesis (Supplementary Fig. S3). These findings were largely consistent with a subsequent, more in-depth analysis of functional genes in a Suncor Pond 6 metagenome (An et al. 2013b). Interestingly though, the tailings pond metagenomes also showed the presence of genes for aerobic aromatic hydrocarbon biodegradation and aerobic CH<sub>4</sub> oxidation, suggesting the potential for aerobic metabolism in the deep anoxic communities as well (An et al. 2013a), possibly harbored by facultative anaerobes such as *Pseudomonas* spp.

Using pyrotag sequences obtained from 95 tailings samples retrieved from six different OSTPs managed by Syncrude, Suncor and Shell, Wilson et al. (2016) identified the core microbiomes in each pond and across all the tailings ponds examined. Individually (as described above), all six ponds harbored diverse anaerobic microbial communities capable of various functions including element cycling (S and Fe), gas production (CH<sub>4</sub>, CO<sub>2</sub>, H<sub>2</sub>S) and hydrocarbon biodegradation. Such functional similarities may reflect close geographical locations, origins (oil sands formations) and processing (mechanical surface mining and hot alkaline extraction). However, the relative abundance of specific taxa can be quite dissimilar due to different pond management practices including processing chemistry and temperature, age of tailings after deposition, available electron donors (labile versus recalcitrant carbon sources), available electron acceptors (e.g. gypsum treatment) and water recycling regimes that influence microbial enrichment (Table 1). These factors presumably place unique selective pressures on the extant microbial communities. Wilson et al. (2016) found that individual OSTP core microbiomes comprised only 4–10 OTUs but that these core OTUs combined represented 39–54% of each pond’s total sequence reads. Each pond harbored distinct dominant taxa in their core biome and NMDS analysis showed that, in general, the pond from which samples were collected was the main determinant of the core biome. This finding supported an earlier report that also showed that Suncor and Syncrude tailings were distinct (based on one sample from each pond; Yergeau et al. 2012). Such differences in OSTP taxa indicate that future management and reclamation efforts based on microbial technologies should be pond-specific. The core microbiome across all OSTPs examined (wherein sequences from all six ponds were collectively analyzed) consisted of only 2–5 OTUs that included bacterial members of the *Comamonadaceae*, *Hydrogenophilaceae*, *Anaerolineaceae*, and the archaea *Methanosaeta* and *Methanoregula* (Wilson et al. 2016). These few taxa presumably have overarching roles in the various anaerobic processes occurring in the ponds, in comparison with the pond-specific taxa that are suited to individual tailings environments. However, another interpretation is that there is considerable functional redundancy in OSTP microbiota, with several taxa capable of filling the same ecological niches; chance may drive the selection and enrichment of

analogous yet taxonomically distinct isofunctional communities in OSTPs.

In addition to metagenomic analyses of uncultivated tailings, the metagenomes of various laboratory cultures enriched from MFT have been analyzed (e.g. Tan et al. 2013; Abu Laban et al. 2015a; Abu Laban, Dao and Foght 2015b; Tan, Semple and Foght 2015b; Collins, Foght and Siddique 2016; Mohammad Shahimin, Foght and Siddique 2016). A comparative study of two of these methanogenic enrichments (NAPDC, enriched with naphtha diluent, and SCADC, enriched with a C<sub>5</sub>–C<sub>6</sub> alkane mixture) plus a toluene enrichment not derived from OSTPs revealed that genes associated with syntrophy, anaerobic hydrocarbon biodegradation and methanogenesis were enriched compared with metagenomes from non-hydrocarbon-exposed environmental samples (Tan et al. 2015a). Further, the NAPDC and SCADC cultures that were enriched by growth on a broader range of hydrocarbons (as opposed to the culture enriched on pure toluene) showed a greater diversity of anaerobic hydrocarbon-degrading genes such as *assA* and *bssA* (for alkane and aromatic compound activation, respectively) (Tan, Semple and Foght 2015b). By binning sequences from such metagenomes and using single-cell sorting of the cultures followed by whole genome amplification, several draft genomes have been assembled from tailings enrichment cultures to further enhance our understanding of the potential activities in OSTPs (Saidi-Mehrabad et al. 2013; Tan and Foght 2014). For example, such enrichment studies have revealed that key anaerobic hydrocarbon-degrading taxa in MFT enrichments include members of the *Clostridia* (*Peptococcaceae*, *Desulfosporosinus*) and *Deltaproteobacteria* (*Desulfobulbaceae*, *Smithella*) (Tan et al. 2014a,b, 2015b; Abu Laban et al. 2015c,d).

## MICROBIAL ACTIVITIES OBSERVED IN SITU AND INFERRED FROM LABORATORY EXPERIMENTS

Field observations and analyses of OSTP samples and laboratory experiments, particularly in the past 10 years, have confirmed both beneficial and deleterious microbial activities *in situ* including (i) aerobic and anaerobic biodegradation of diluent hydrocarbons, some bitumen components, NAs, some process additives and other endogenous substrates; (ii) generation of metabolites and end products such as CH<sub>4</sub>, CO<sub>2</sub>, sulfides and partially oxidized hydrocarbons; (iii) reduction of CO<sub>2</sub>, sulfate and N<sub>2</sub>; (iv) biogeochemical reactions including sulfur (S) cycling, iron mineral transformations and acid rock drainage; and (v) physical changes to tailings including consolidation of fine minerals and creation of gas voids at depth in OSTPs.

It is likely that the majority of the microbial activity in most ponds is supported by metabolism of fugitive diluent in fresh tailings. Although the proportion of unrecovered diluent is small, the enormous volumes of fresh tailings deposited in OSTPs represent a large mass of potentially labile carbon for adapted microbiota. Burkus (2014) summarized company-reported total losses of diluent at ~723 000 tonnes over the 5-year period 2008–2012 (Supplementary Table S4). This large mass is exceeded by the mass of unextracted bitumen that enters the ponds with tailings (~2 million tonnes per year in Syncrude's MLSB alone; Burkus, Wheler and Pletcher 2014). However, bitumen is naturally depleted in labile substrates such as low molecular mass aliphatics and aromatics and, conversely, enriched in recalcitrant alkyl-polycyclic aromatic hydrocarbons, high molecular mass resins, asphaltenes and NAs (Strausz et al.

2010, 2011; Yang et al. 2011). Therefore, bitumen likely does not significantly support aerobic or anaerobic microbial growth (Holowenko, MacKinnon and Fedorak 2000), supported by the observed lack of CH<sub>4</sub> production from the Aurora Settling Basin, described above.

## Aerobic hydrocarbon biodegradation

Aerobic degradation of diluent hydrocarbons by OSPW microbes likely occurs in the uppermost OSPW layer where wind and wave action aerate the water during the summer. During winter months when ice covers much of the ponds, aerobic biodegradation would be slower. *In situ* aerobic biodegradation theoretically would alleviate some of the acute toxicity of OSPW by consuming mono- and polycyclic aromatic hydrocarbons and would reduce volatile organic carbon emissions from the ponds (Simpson et al. 2010; Small et al. 2015). Evidence supporting this potential activity was provided by An et al. (2013a) who detected genes encoding putative mono- and dioxygenases and O<sub>2</sub>-dependent ring cleavage enzymes in the metagenome of OSTP surface waters. As such, preliminary studies have shown that microbial communities within OSPW have the potential to aerobically biodegrade hydrocarbons such as benzene and naphthalene (Rochman 2016).

Saidi-Mehrabad et al. (2013) measured oxidation of the gaseous hydrocarbon CH<sub>4</sub> to CO<sub>2</sub> by methanotrophs in OSPW, which would mitigate some of the ponds' greenhouse gas emissions (discussed below). Rates of CH<sub>4</sub> oxidation determined in the laboratory were within the range measured in natural lakes and oceans. Further, the onset of CH<sub>4</sub> oxidation in the laboratory occurred without a lag, suggesting that this process was already established *in situ*. Extrapolation from conservative oxidation rates and pond surface areas indicated that ~17% of biogenic CH<sub>4</sub> at the pond surface may be microbially oxidized in the water layer, although CH<sub>4</sub> bubbles that are rapidly released may not be available to the methanotrophs and their activity may deplete dissolved oxygen, reducing potential methanotrophy in OSPW layers. Thus, further study of aerobic degradation of gaseous, dissolved and suspended hydrocarbons in OSPW is needed.

## Methanogenic hydrocarbon biodegradation

Anaerobic metabolism dominates OSTPs because of the overwhelming proportion of tailings versus OSPW. Sobolewski (1999b) measured redox potentials below –200 mV in numerous MFT samples from Syncrude ponds. Both methanogenic and sulfate-reducing activity have been observed, often concurrently, *in situ* and in laboratory microcosms. Methanogenesis appears to predominate in Syncrude, Albian Sands and CNRL ponds whereas sulfate reduction may dominate in engineered (gypsum-treated) tailings such as Suncor Ponds 5 and 6 and Syncrude's CT fen reclamation site (discussed below). Although facultative nitrate reducers are abundant and widely distributed throughout tailings depth profiles (Sobolewski 1999b; Fedorak et al. 2002), it seems unlikely that nitrate reduction is significant *in situ* because nitrate and nitrite concentrations typically are low (Fedorak et al. 2002; Penner and Foght 2010; Stasik and Wendt-Potthoff 2014).

Stasik, Wick and Wendt-Potthoff (2015) reported that BTEX concentrations (from fugitive naphtha) decreased with depth in West In-Pit, suggesting their biodegradation *in situ*; MLSB depth profiles similarly showed progressive, selective depletion of naphtha components with depth (Supplementary Fig. S2). The

pattern of loss, with simpler compounds disappearing first and more complex, presumably more recalcitrant, compounds remaining, is a presumptive biodegradation signature. Consistent with this observation, Penner and Foght (2010) reported that microcosms containing shallow (young) MFT samples (6 mbs) from MLSB produced more CH<sub>4</sub> than deeper samples (30 mbs), presumably because the endogenous naphtha profile was more complete in the younger tailings.

Laboratory incubations of MFT have repeatedly confirmed the potential for methanogenic degradation of naphtha and paraffinic diluents (Mohamad Shahimin and Siddique 2017a,b) and their individual components including short-chain n-alkanes (Siddique, Fedorak and Foght 2006; Tan et al. 2013; Tan, Semple and Foght 2015b; Mohamad Shahimin, Foght and Siddique 2016), iso- and cyclo-alkanes (Abu Laban et al. 2015a; Siddique et al. 2015; Tan, Semple and Foght 2015b) and BTEX (Siddique et al. 2007). Diauxic patterns of CH<sub>4</sub> production in Syncrude MFT from different diluent components have been observed in MFT enrichment cultures, with toluene being more labile than alkanes or other BTEX components (Siddique et al. 2007) and degradation of iso- or cyclo-alkanes being delayed until the preferred n-alkane substrates were depleted (Abu Laban, Dao and Foght 2015b; Tan, Semple and Foght 2015b). Only certain isomers were eventually biodegraded (2-methylpentane, 3-methylhexane, 4-methylheptane, methylcyclopentane) whereas other highly substituted molecules either were co-metabolized (2-methylhexane and 2-methylheptane) or were resistant (3-ethylhexane, 3-methylpentane) to biodegradation during several years of incubation in the laboratory (Abu Laban, Dao and Foght 2015b; Tan, Semple and Foght 2015b). These studies revealed the presumptive ability of previously undescribed fermentative bacteria, especially novel genera of uncultivated clostridial *Peptococcaceae*, to degrade iso- and cyclo-alkanes under methanogenic conditions using enzymes previously attributed to n-alkane degradation (Abu Laban et al. 2015a; Tan, Semple and Foght 2015b). This observation has expanded the general understanding of methanogenic hydrocarbon degradation by demonstrating expression of genes homologous to canonical alkylsuccinyl synthase (*ass*) genes and production of signature co-metabolites that were previously elusive under methanogenic conditions. However, the existence of additional alternative hydrocarbon metabolism pathways has not been ruled out.

Mohamad Shahimin and Siddique (2017a,b) recently investigated the biodegradation of whole naphtha and paraffinic diluents by Albian Sands and CNRL MFT. They reported the biodegradation of additional iso-alkanes in naphtha including 2-methyloctane, 3-methyloctane, 4-methyloctane and 2-methylnonane and implicated *Peptococcaceae*, *Anaerolineaceae* and *Syntrophaceae* in the biodegradation of diluents. A pattern of biodegradation was also observed wherein labile diluent hydrocarbons were sequentially biodegraded (n-alkanes > iso-alkanes > cyclo-alkanes) depending on the composition of diluents. Interestingly, while studying the biodegradation of a mixture of pure n-alkanes, MFT from Syncrude and CNRL showed a preference for n-alkane chain length C<sub>10</sub> > C<sub>8</sub> > C<sub>7</sub> > C<sub>6</sub> whereas Albian Sands MFT showed preference for short-chain alkanes C<sub>5</sub>, C<sub>6</sub> > C<sub>8</sub>, C<sub>10</sub> (Siddique, Fedorak and Foght 2006; Mohamad Shahimin, Foght and Siddique 2016). These preferences correspond to the predominant alkanes in the cognate naphtha and paraffinic diluents, respectively (Supplementary Table S1), implying selective adaptation of the microbiota to available labile hydrocarbon sources. This inference is supported by our recent study on paraffinic diluent biodegradation by Albian Sands and

CNRL MFT where the Albian MFT showed a shorter lag phase (~400 days) while biodegrading paraffinic diluent as a cognate diluent compared with CNRL MFT (~800 days) that had been previously exposed to naphtha diluent (Mohamad Shahimin and Siddique 2017b). However, the apparent requirement for adaptation can lead to extremely long adaptation periods in some cases: Siddique et al. (2015) noted lag times of 2–5 years for onset of methanogenesis in primary cultures of Albian Sands MFT collected early in the pond's history and incubated with n- or iso-alkane components of its paraffinic diluent. This extreme adaptation period may be due to the presence of citrate in fresh Albian Sands tailings (i.e. diauxic metabolism, with citrate being the preferred substrate for fermentation) and/or enrichment of a methanogenic community having a paucity of key bacterial syntrophs able to initiate anaerobic hydrocarbon degradation. In contrast, the MLSB microbiota long established on naphtha exhibited comparatively shorter lag times (5–9 months) for methanogenesis onset in primary cultures incubated with n- or iso-alkane components of paraffinic diluent (Siddique et al. 2015). Interestingly a much shorter lag phase (~80 days) was observed prior to biodegradation of n-alkanes when the same Albian Sands MFT was used in a subsequent study after 4 years of storage at 4°C (Mohamad Shahimin, Foght and Siddique 2016), implying microbial acclimation even at low temperature.

Suncor's Pond 1 was established in 1967 and was methanogenic by 1982 (Fedorak et al. 2002) and Syncrude's MLSB pond, established in 1978, was methanogenic by the mid-1990s (Holowenko, MacKinnon and Fedorak 2000). Holowenko, MacKinnon and Fedorak (2000) proposed that these ~15-year lags included the time required for sulfate depletion in pore water to reduce the competitive advantage of sulfate reducers over methanogens, followed by several years during which a methanogenic microbial community evolved. Certainly, methanogens initially were present in low numbers in both ponds (Supplementary Table S2) but increased thereafter. The paramount factor delaying the onset of methanogenic hydrocarbon degradation was likely the development of competent communities of syntrophs and methanogens (Supplementary Fig. S3), as CH<sub>4</sub> bubbles first appeared at the south end of MLSB near the fresh tailings outflow, subsequently progressing northward. The observation that volatile hydrocarbons inhibit methanogenic crude oil degradation (Sherry et al. 2014) also may help explain in part the length of the adaptation period. Another factor influencing the onset and extent of CH<sub>4</sub> production may be the proportion of diluent 'available' to microbes *in situ*. Availability appears to be influenced by the amount of residual bitumen present, since up to 30% of fugitive naphtha partitions into bitumen (Burkus, Wheler and Pletcher 2014) where its biological availability will be further limited by diffusion through the viscous material. Counteracting this scenario, however, are early observations by A. Sobolewski (pers. comm.) of micrometer- and sub-micrometer-sized oil droplets entrained in MFT, which would provide a high surface area for microbial access to any labile HCs in bitumen and for diffusion of such substrates to the bitumen–water interface.

In addition to diluent, minor components of bitumen (<2 wt%, representing acyclic and cyclic ≤C<sub>19</sub> and ≤C<sub>18</sub> hydrocarbons, respectively; Burkus, Wheler and Pletcher 2014) may serve as microbial substrates. Indeed, Siddique et al. (2011) observed methanogenic degradation of C<sub>14</sub>–C<sub>18</sub> n-alkanes added to MLSB tailings, although *in situ* these endogenous substrates may be less accessible to microbes relying on substrate diffusion through bitumen globules to the oil–water interface. Less is known about the ability of MFT microbiota to anaerobically

degrade polycyclic aromatic hydrocarbons and heterocycles in bitumen, although such activity has been demonstrated indirectly in heavy oil (Meckenstock et al. 2014). Folwell et al. (2016) recently reported slow anaerobic degradation of 2-methylnaphthalene (but not pyrene) by OSPW microbes and concluded that high molecular mass polycyclic aromatic hydrocarbons were likely to persist in OSPW under anaerobic conditions.

Efficient degradation of C-rich, N-deficient substrates such as hydrocarbons requires an external nitrogen source. In some ponds the OSPW irregularly receives small amounts of ammonium from processing inputs but, as noted above, MFT pore water generally has very low concentrations of ammonium, nitrate and nitrite. The resins fraction of bitumen and the organic matter associated with clays (Sparks et al. 2003) may contain organic N, but only as a component of complex, recalcitrant molecules. Thus, the ponds may be considered deficient in available nitrogen for microbial metabolism. Collins, Foght and Siddique (2016) recently used  $^{15}\text{N}_2$  uptake into biomass to demonstrate the potential for Albian Sands MFT microbes to fix gaseous nitrogen with citrate as the labile carbon source. Furthermore, pyrotag sequencing of N-deficient cultures under an anaerobic  $\text{N}_2$  headspace showed the presence of several  $\text{N}_2$ -fixing archaeal and bacterial genera (Collins, Foght and Siddique 2016). Haveroen, MacKinnon and Fedorak (2005) demonstrated that addition of polyacrylamide (as a tailings thickener) to MLSB tailings enhanced  $\text{CH}_4$  production, but only if added at a high concentration and after passage of cultures three times. The likely mechanism was deamination of the polymer as a nitrogen source rather than depolymerization to monomers as a carbon source, although the latter could not be measured due to analytical difficulties (Haveroen, MacKinnon and Fedorak 2005). Collins, Foght and Siddique (2016) found that the addition of polyacrylamide at process-relevant concentrations to primary Albian Sands MFT cultures did not enhance methanogenesis. Whether paucity of available nitrogen does limit hydrocarbon degradation in MFT *in situ* is an important question when considering long-term activity in the ponds and sustainability of reclamation efforts, and deserves further attention.

Contrasting with the potentially beneficial impacts of methanogenic hydrocarbon biodegradation in the ponds (e.g. mitigating acute diluent toxicity and accelerating pore water recovery from MFT, described below), there are several possible deleterious effects:

- (i) Incomplete oxidation and/or co-metabolism of hydrocarbons may generate water-soluble (and therefore mobile) NAs or other compounds that may be more toxic and/or recalcitrant than the parent hydrocarbons (Quagraine, Headley and Peterson 2005a). This has long-term implications for ecosystem health during tailings reclamation.
- (ii) Both complete and incomplete hydrocarbon biodegradation can produce biogenic  $\text{CH}_4$ , a potent greenhouse gas, plus  $\text{CO}_2$ . Siddique et al. (2008) used first approximations from laboratory cultures to estimate the theoretical yield of  $\text{CH}_4$  produced per unit of naphtha by Syncrude MFT microbes *in situ*. They estimated that 1 tonne of naphtha would yield  $280 \text{ m}^3$  of  $\text{CH}_4$ , although this figure was based only on biodegradation of labile BTEX components and *n*-alkanes, and did not consider contributions from iso- or cyclo-alkanes or recalcitrant BTEX components, which together represent a large proportion of naphtha (Abu Laban et al. 2015a). Siddique et al. (2008) also used a kinetics approach to predict  $\text{CH}_4$  emissions based on the residual

naphtha present in Syncrude OSTPs.  $\text{CO}_2$  emissions were not estimated in this model, but  $\text{CH}_4$  estimates were surprisingly similar to field estimates of  $\text{CH}_4$  flux from MLSB. Burkus (2014) corrected some assumptions made by Siddique et al. (2008) and, furthermore, expanded calculation of potential biogenic greenhouse gas emissions from different ponds (Supplementary Table S5). Not only are these gas volumes important for calculating the environmental footprint of active ponds, but also for predicting legacy emissions due to residual biodegradation in wet and dry landscaping scenarios, discussed below. A revised model accounting for methanogenic biodegradation of recalcitrant hydrocarbons is in preparation (J. Kong et al., pers. comm.).

- (iii) Ebullition of greenhouse gases from MFT may transport volatile organic compounds such as residual diluent hydrocarbons and biogenic  $\text{H}_2\text{S}$  (discussed below) from the mud line to the atmosphere and thus may contribute to atmospheric hydrocarbon emissions from the Athabasca oil sands region (Simpson et al. 2010; Small et al. 2015). Nix and Martin (1992) reported that early Suncor OSTPs were 'intermittently purged by gases from the underlying anaerobic sediments' and Guo (2009) photographed large areas of MLSB covered with gas bubbles.
- (iv) Ebullition also facilitates expression of pore water from MFT (Siddique et al. 2014a), thus transporting water-soluble metabolites, NAs and ions from sediment pore water to the OSPW layer where they may contribute to toxicity and biological and chemical oxygen demand, and may inhibit intrinsic methanotrophy.
- (v) Gases that are unable to escape from the dense sediments create voids in the MFT, ranging from millimeter-sized in shallow sediments to centimeter-sized voids at depth (Guo 2009), thus increasing the total volume of stored tailings; Holowenko, MacKinnon and Fedorak (2000) estimated that in the late 1990s the trapped gases in MLSB represented 2–5% of the already enormous pond volume.

Thus, methanogenesis *in situ* affects numerous chemical and physical characteristics of OSTPs and, by extension, reclamation outcomes.

### Sulfate reduction and S cycling

Chemical engineering of tailings to form CT not only decreases the volume of pore water retained in the tailings, but the added gypsum also provides substantial sulfate (e.g. 2–3 mM) as an electron acceptor for conversion to sulfide by extant sulfate reducers (Supplementary Table S1). Carbon sources driving microbial sulfate reduction in OSTPs include low molecular mass fatty acids (Stasik and Wendt-Potthoff 2016) and, potentially, labile hydrocarbons (Abu Laban, Dao and Foght 2015b; Tan, Semple and Foght 2015b). Because  $\text{H}_2\text{S}$  is a toxic, explosive and corrosive gas, concerns about its emission from tailings prompted investigation of this process, and the S cycle in general, in different ponds. In an examination of sulfate reduction processes in Suncor Pond 6, Ramos-Padrón et al. (2011) measured sulfate reduction rates from surface waters down to 18 mbs, and found that this process was occurring deeper within the anoxic zones of the pond but not at the surface (where no sulfide was detected). At discreet depths where sulfate reduction rates were highest, sulfide concentrations and relative abundances of sulfate reducers and sulfur reducers (deduced by pyrotag sequencing) were also highest. Sulfate concentrations were

greatest in the surface water (~6 mM), potentially generated through a combination of abiotic and biotic conversions of sulfide produced from deeper pond layers (Ramos-Padrón et al. 2011). Chen et al. (2013) used West-In Pit MFT-containing microcosms equipped with O<sub>2</sub>/sulfide sensors to show that sulfide fluxes mainly correlated with biological activity, with the generated sulfide precipitating as FeS, whereas O<sub>2</sub> diffusion was affected by both biological and abiotic processes across the water-sediment interface, further suggesting that both biotic and abiotic processes might diminish H<sub>2</sub>S emissions from OSTPs. Stasik et al. (2014) also determined sulfate reduction rates in West In-Pit MFT samples as a function of depth, similarly noting a lack of sulfate reduction in the surface oxic zone (where no sulfide was detected) and that rates were greatest approximately 1–3 m below the mud line in the anoxic zone. In addition, these authors showed that other S cycling processes were occurring in this anoxic zone, including microbiological sulfide and thio-sulfate oxidation processes that presumably aided in consuming any SRB-produced sulfide. Stasik et al. (2014) further demonstrated that microbial Fe(III) reduction producing Fe(II) was a significant process, and that sulfides (primarily existing as HS<sup>-</sup> in the slightly alkaline [pH~7.5] pond water) were precipitating as FeS. Thus, research to date has demonstrated that S cycling processes are ongoing in active OSTPs, and that abiotic or microbiological reactions such as sulfide/thiosulfate oxidation and Fe(III) reduction help to prevent any sulfide produced by sulfate reduction being released as harmful H<sub>2</sub>S.

Sulfate reduction processes within OSTPs may also play a positive role in reducing greenhouse gas (specifically CH<sub>4</sub>) emissions. Salloum, Dudas and Fedorak (2002) showed that sulfate addition to MFT inhibited methanogenesis, a finding that was confirmed by Ramos-Padrón et al. (2011) who observed that the presence of 2 mM sulfate (the average sulfate concentration in CT ponds) inhibited CH<sub>4</sub> production by approximately 50%. Based on a mean measured sulfate reduction rate of 10 mmol/m<sup>3</sup>/day in Suncor's Pond 6, this could theoretically reduce CH<sub>4</sub> emissions on the order of 2 million L/day for a 10<sup>7</sup> m<sup>3</sup>-sized tailings pond (Bordenave et al. 2009). Indeed, Suncor CT ponds historically have produced less CH<sub>4</sub> than Syncrude ponds but with a greater CO<sub>2</sub>:CH<sub>4</sub> ratio (Burkus, Wheler and Pletcher 2014; Supplementary Table S5). Recently, Stasik and Wendt-Potthoff (2016) predicted the prevention of >5 million liters of CH<sub>4</sub> emissions per day from Syncrude ponds, based on sulfate-reducing activities measured *in situ*.

All of these studies have demonstrated the complexity of the S biogeochemical cycles occurring in oxic surface waters and deeper anoxic zones that can affect H<sub>2</sub>S (and potentially CH<sub>4</sub>) emissions from OSTPs. The availability of electron acceptors in OSTPs can profoundly affect related processes, such as whether hydrocarbon biodegradation occurs under sulfate-reducing or methanogenic conditions (and therefore potentially the range of substrates consumed), and can influence microbial community composition (Stasik, Wick and Wendt-Potthoff 2015; Abu Laban, Dao and Foght 2015b; Tan, Semple and Foght 2015b). Understanding S cycling and biogeochemical cycling in general also has great importance for tailings reclamation scenarios. Additional studies examining the environmental consequences of CT storage (Warren et al. 2016) and reclamation in wetlands deposits (Reid and Warren 2016) are described below.

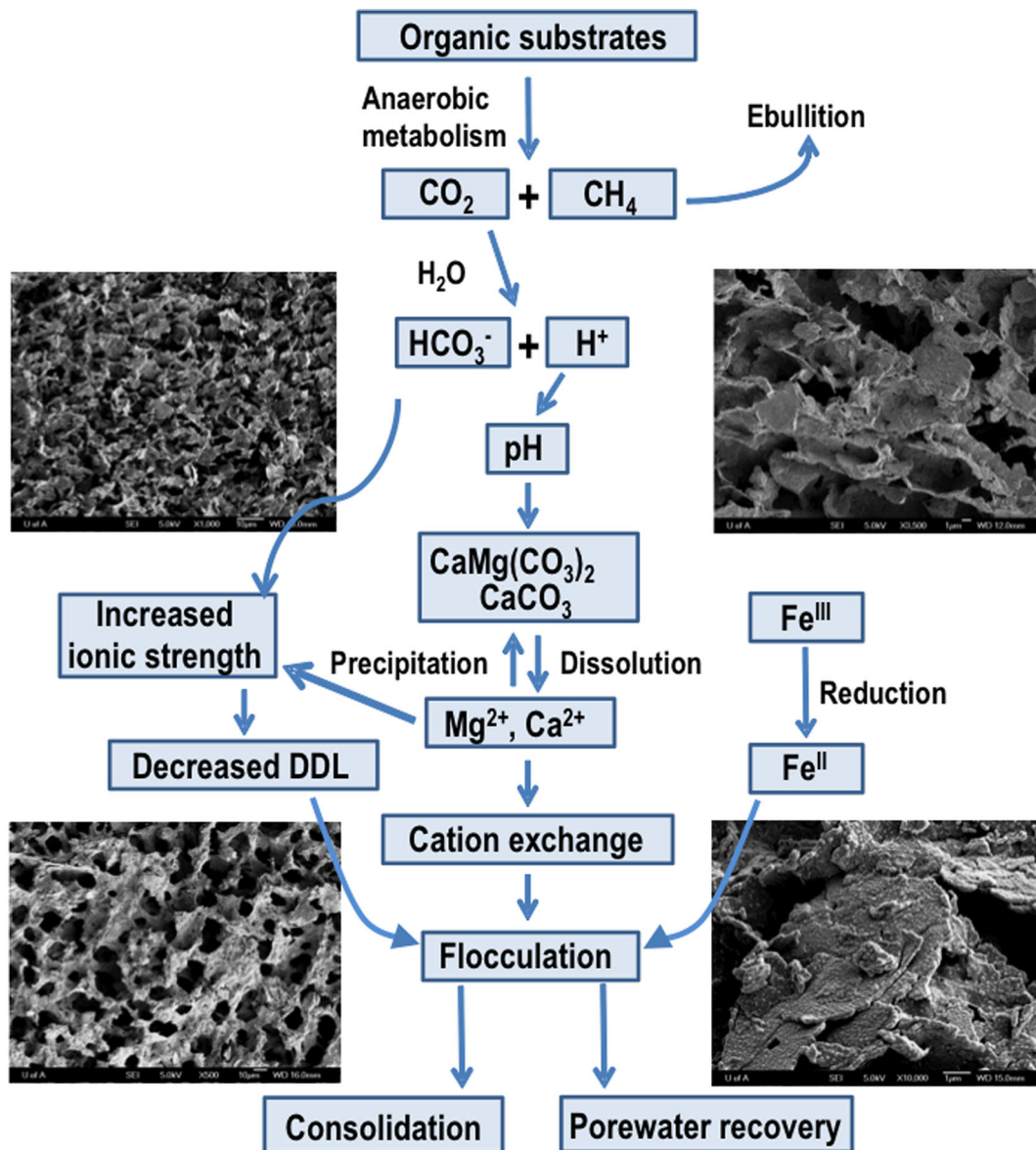
### Biodegradation of other endogenous substrates

Endogenous potential carbon sources in OSTPs other than hydrocarbons include humic acids and complex organics thought

to be intimately associated with ore minerals (Sparks et al. 2003), organics from river water and NAs. Process additives such as citrate, glycols, de-icing agents, demulsifiers and polymers likely contribute negligible amounts of fermentable carbon in proportion to other organics *in situ* (Burkus et al. 2014). Of these, NAs have been the most extensively examined as carbon source alternatives to diluent hydrocarbons. NAs are natural components of bitumen and it has been hypothesized that their presence is due to the partial oxidation of hydrocarbons over geological time. Partitioning of NAs from bitumen into tailings pore waters can account for their high concentrations in OSPW but it also has been speculated that additional NAs can arise *de novo* from incomplete metabolism of substrates in OSPW or MFT porewater (Quagraine, Headley and Peterson 2005a). Although NAs have been classically defined as cyclic alkanes containing a single carboxylic acid group (conforming to the canonical formula C<sub>n</sub>H<sub>2n+z</sub>O<sub>2</sub>; Clemente and Fedorak 2005), advanced analytical methods have revealed that these compounds can be highly complex and may contain N, S, and/or multiple O atoms (e.g. Grewer et al. 2010; Rowland et al. 2011a,b; Bowman et al. 2014; West et al. 2014; Wilde et al. 2015). Given their amphiphilic structures, NAs act as natural surfactants during the caustic hot water treatment process, enter tailings ponds in the processed water fraction, and may be concentrated by OSPW re-use. Thus, NA composition in OSPW can vary spatially and temporally in tailings ponds (Frank et al. 2016). NAs are compounds of environmental concern because they are toxic to many higher organisms and are commonly cited as being the most toxic component of OSTPs. Thus, there is great interest in their remediation by engineered or natural processes (reviewed by Clemente and Fedorak 2005; and Whitby 2010). Many studies have now shown that microorganisms derived from tailings have the ability to aerobically biodegrade NAs, especially monocyclic NAs (e.g. Whitby 2010). However, increasing complex molecular structure (e.g. branching and/or number of rings) greatly decreases NA biodegradability (Han et al. 2008; Smith et al. 2008). In contrast to aerobic studies, only a few studies have demonstrated biodegradation of single-ringed, classical NAs under anaerobic conditions (Gunawan, Nemati and Dalai 2014; Clothier and Gieg 2016). Even though NA degradation occurred under diverse anaerobic conditions (Clothier and Gieg 2016), the most extensive NA biodegradation occurred under nitrate-reducing conditions (Gunawan, Nemati and Dalai 2014; Clothier and Gieg 2016) that are likely irrelevant to OSTPs, as little nitrate is present.

### Microbially mediated consolidation of fine tailings (biodegradation)

Consolidation of fresh FFT and particularly of semi-solid MFT is an enormous technical challenge for the oil sands mining industry. A rapid and effective process would consolidate the solid fraction of fluid tailings for subsequent reclamation and recover more OSPW quickly for re-use in the bitumen extraction process. Fines suspended in tailings as colloidal gels hinder consolidation, and separation of fines and water by gravity is a slow process. Eckert et al. (1996) conducted laboratory and field experiments to evaluate the settling velocity of fine tailings and numerically modelled tailings consolidation. They calculated that 125–150 years would be required to consolidate tailings to ≥60 wt% final solids content in the absence of flocculant additions or other aides. This prediction was supported by observation of three 10-m tall (~6500 L) columns filled in 1982 with MLSB tailings collected prior to onset of



**Figure 4.** Proposed model explaining chemical and microbiological mechanisms affecting MFT consolidation and de-watering ('biodesiccation'). Ebullition of biogenic gases may create physical channels for pore water expression, in addition to larger pores created by biogeochemical transformation of minerals. Processes were inferred from observations and measurements of Syncrude MFT incubated in 50-L mesocolumns with dilute complex organic substrate amendment versus parallel unamended control. DDL, diffuse double layer. Scale bars on scanning electron micrographs on left are 10  $\mu\text{m}$ , on right are 1  $\mu\text{m}$ . See main text for general description and Siddique et al. (2014a,b) for details.

methanogenesis *in situ*; minimal consolidation by gravity was observed during >25 years of measurements in the laboratory (Jeeravipoolvarn, Scott and Chalaturnyk 2009). However, in the early 1990s field observations in MLSB revealed accelerated consolidation of tailings in the southern zone of the pond that had begun a period of intense methanogenic microbial activity and gas bubbling (Holowenko, MacKinnon and Fedorak 2000; Guo 2009). Rather than ebullition disturbing tailings consolidation (the expected effect), MFT de-watering and consolidation rates were accelerated in MFT experiencing biogenic gas generation in the laboratory (Fedorak et al. 2003; Guo 2009). After this unanticipated link was established, research focused on exploring mechanism(s) of 'biodesiccation' (MFT consolidation and de-watering aided by anaerobic microbial activity) so that technolo-

gies might be developed to help manage the large volumes of tailings.

A detailed biogeochemical model describing possible mechanisms of MFT biodesiccation was developed by Siddique et al. (2014a,b; Fig. 4) using Syncrude tailings in 50-L columns that were either unamended or amended with low concentrations of a complex carbon source to accelerate indigenous microbial activity. Biogenic endproducts ( $\text{CH}_4$ ,  $\text{CO}_2$  and organic acids) changed the chemistry of both porewater and solid (fine mineral) phases in several interrelated ways. Dissolution of  $\text{CO}_2$  lowered porewater pH, dissolved carbonate minerals in MFT, and increased calcium ( $\text{Ca}^{2+}$ ), magnesium ( $\text{Mg}^{2+}$ ) and bicarbonate ( $\text{HCO}_3^-$ ) ions in the porewater. Greater concentrations of soluble ions increased the ionic strength of porewater and de-

creased the thickness of the diffuse double layer of clay particles, causing rearrangement of clays from 'house of cards' to 'deck of cards' structures (Fig. 2) leading to accelerated densification of tailings (Siddique et al. 2014a). Arkell et al. (2015) reported similar observations in small-scale (2-L) column experiments and further revealed increased concentrations of exchangeable divalent cations, particularly  $Mg^{2+}$ , on clay exchange surfaces that decreased the thickness of the diffuse double layer. According to the model, indigenous tailings microbes concurrently influenced solid phase chemistry via biogeochemical processes to promote biodensification (Siddique et al. 2014a,b). MFT bacteria (predominantly *Clostridiales*, *Synergistaceae* and *Desulfobulbaceae*) and archaea (predominantly *Methanolinea*, *Methanoregula* and *Methanoseta*) transformed crystalline Fe(III) minerals (ferrihydrite and goethite) to amorphous Fe(II) minerals (amorphous iron sulfide, vivianite and possibly green rust) during metabolism. These minerals entrapped and masked electronegative clay surfaces and thereby increased their flocculation. Both porewater and solid phase biogeochemical changes aided aggregation of clays and formation of networks of pores (visualized by cryo-scanning electron microscopy; Fig. 4) that accelerated de-watering and consolidation of MFT. This model has been validated in part by subsequent field observations (Dompierre et al. 2016), discussed below.

Additional physical mechanisms may contribute to consolidation. Voordouw (2012) described interactions of oil sands tailings particles with polymers in TT and suggested that ebullition of biogenic gases creates channels in semi-solid MFT that allow upward passage of pressurized porewater. Brown et al. (2013) endorsed these findings and added that densification of tailings could also occur under nitrate-reducing and sulfate-reducing conditions, and that both microbial biomass and gas de-watering channels might be responsible for tailings densification. These proposed mechanical processes are consistent with the biogeochemical schema proposed by Siddique et al. (2014a,b) (Fig. 4).

A phenomenon related to biodensification may also be pertinent to clarification of the water layer in ponds by increasing the settling of fine particles through the water column (sedimentation). Bordenave et al. (2010) inoculated autoclaved Albian Sands tailings with pure cultures, amended them with lactate or acetate and incubated under nitrate-reducing, sulfate-reducing or methanogenic conditions. They observed increased sedimentation of fines under nitrate-reducing and methanogenic conditions but not under sulfate-reducing conditions, and attributed particle aggregation to microbial biofilms on the fine minerals, promoted by extracellular biogenic polymers. This observation may be important to wet landscape reclamation scenarios that rely on water clarity to establish functioning ecosystems, such as end pit lakes (described below).

Other microbial MFT consolidation processes have been proposed but their feasibility has yet to be validated. One such process is microbially induced calcite precipitation, which requires excess  $Ca^{2+}$  and addition of urea to promote calcite precipitates on the surface of microbial cells: these conditions should lead to cohesive bonding or cementation of fines within aggregates (Liang et al. 2015). This approach needs careful assessment to determine efficacy in tailings where naturally high concentrations of  $HCO_3^-$  already occur in porewater and might not require the addition of urea. As well, addition of sufficient  $Ca^{2+}$  via the proposed process could precipitate calcite in  $HCO_3^-$ -rich porewater even without microbial activity.

## LONG-TERM IMPLICATIONS FOR TAILINGS RECLAMATION

Because oil sands mining companies operate under a policy of zero effluent discharge to the environment, slow MFT consolidation rates and concomitant slow recovery of OSPW have contributed to an enormous tailings inventory, currently  $\sim 1$  billion  $m^3$  of stored tailings (Alberta Government 2016) with a surface area of  $\sim 220$   $km^2$  (Burkus, Wheler and Pletcher 2014). This environmental footprint has prompted regulations requiring operators to explore both 'wet' and 'dry' landscaping scenarios. Proposals include placing semi-solid tailings in a basin and capping with fresh water to create a viable 'end pit lake' (EPL) ecosystem or wetlands, or placing de-watered tailings in exhausted mine pits and covering them with sand, soil and vegetation to regenerate the boreal forest, respectively. For dry landscaping the sediments must be 'trafficable' (i.e. support the weight of vehicles) and therefore require chemical and/or physical manipulation of MFT to remove excess water and increase solids content, whereas MFT intended for wet reclamation (freshwater ecosystems) does not require additional de-watering. Both scenarios require the presence of suitable microbial communities to succeed.

### Wet reclamation scenarios

#### CT reclamation under wetlands

Wetlands, specifically bogs and fens, cover  $\sim 43\%$  of predisturbance boreal forest landscape in the Athabasca oil sands region (BGC Engineering 2010). Construction of wetlands provides a viable option for reclaiming CT and OSPW and for remediating toxic constituents, particularly organics (hydrocarbons and NAs). As described above, there can be concerns about  $H_2S$  emissions because CT contains hydrocarbons that can serve as electron donors for sulfate reduction (e.g. Abu Laban, Dao and Foght 2015b; Tan, Semple and Foght 2015b). Therefore, it is important to evaluate environmental consequences of CT deposition in wetlands particularly with respect to S cycling and greenhouse gas emissions, both being microbially-driven processes. Recently, Warren et al. (2016) performed a detailed microbial and mineralogical study of Syncrude's East In-Pit where CT has been deposited since 2000. By analyzing pyrosequence data they detected 131 bacterial OTUs comprising 19 phyla, including some common legacy phyla from FFT (notably *Gamma*- and *Betaproteobacteria*, *Firmicutes*, *Actinobacteria*, and *Chloroflexi*) and some OTUs distinct from OSTPs. Diversity and bacterial abundance were relatively low in the CT deposit. Although only 2% of the OTUs were affiliated with cultivated sulfate- or S-reducing bacteria, over 90% of the 131 OTUs identified were presumptively capable of S metabolism. This implies highly S-active communities in CT particularly at depths (22–24 mbs) where greater bacterial diversity correlated with highest porewater concentrations of total hydrogen sulfide (Warren et al. 2016).

The presence of diverse S-metabolizing taxa may therefore impact CT reclamation efforts. Reclamation of Syncrude CT beneath a pilot wetland was assessed regarding aqueous distribution of S compounds (i.e. S cycling) across different zones of the deposit (Reid and Warren 2016). The 17-ha instrumented research area within a 54-ha watershed consisted of a surface wetland (constructed with salvaged live peat material), a 10-m thick intermediary structural layer of coarse tailings sand equipped with underground de-watering drains to prevent upward migration of porewater into the freshwater wetland, and an underlying  $\sim 40$ -m thick CT zone. Although aqueous  $H_2S$  was

detected in porewater from the reducing zones of the wetland-sand interface, even greater concentrations of aqueous and gaseous  $H_2S$  were measured in the sand cap, a mixing zone where downward-migrating labile organic carbon from the wetland fueled sulfate reduction (Reid and Warren 2016). These findings warrant further examination of microbial dynamics, biogeochemical S transformations and hydrological transport in the wetlands system to ensure long-term sustainability of this wet reclamation landscape.

#### Reclamation of MFT and other tailings by-products in EPLs

EPLs represent a long-held wet reclamation concept for dealing with huge volumes of oil sands tailings by permanently retaining MFT and small amounts of other oil sands by-products (e.g. tailings sand, CT, TT, petroleum coke) below a water layer (surface water and/or OSPW) in exhausted mine pits (Nix and Martin 1992; Charette et al. 2012). The overarching objective is to store tailings (and possibly OSPW) until they are naturally attenuated and become a sustainable aquatic ecosystem with acceptable water quality, and capable of supporting economical, ecological and societal uses (Charette et al. 2012). It is expected that EPLs will be a common feature of the Athabasca oil sands region, with more than 30 such lakes currently in the planning phase.

Early bench- and small-scale field demonstrations simulating EPLs, coupled with toxicological monitoring of the overlying water layer, revealed progressive detoxification of water with time. However, the rate of detoxification was slowed by the presence of underlying MFT, suggesting that diffusion of toxicants from MFT to the water layer was faster than toxicant removal in these small-scale trials (Nix and Martin 1992). In 2013 the first full-scale ( $\sim 8 \text{ km}^2$  surface area) EPL demonstration trial was initiated at Syncrude's Base Mine Lake. This EPL (maximum depth 48 mbs) was called West In-Pit for the previous 18 years during which it received  $\sim 186$  million  $\text{m}^3$  of MFT transported by pipeline from MLSB, plus some coarse tailings sand and minor amounts of petroleum coke. The MFT was covered with 52 million  $\text{m}^3$  of water (average depth 6.5 m), mainly comprising OSPW from Syncrude's extraction process (Dompierre et al. 2016).

Initial geochemical characterization of Base Mine Lake revealed that porewater pH decreased with depth and concentrations of sulfate decreased across the water-MFT interface (Dompierre et al. 2016). Furthermore, methanogenesis has promoted dissolution of carbonate minerals, ion exchange reactions and precipitation of secondary minerals such as iron sulfide. These biogeochemical changes enhanced de-watering and densification of MFT in Base Mine Lake (Dompierre et al. 2016), endorsing the consolidation pathways proposed by Siddique et al. 2014a,b; Fig. 4). To determine mass transport of solutes from the MFT layer across the mud line to the water cap, Dompierre and Barbour (2016) used water temperature profiles and stable isotope signatures to reveal that the dominant mass transport mechanism was advection at a rate similar to the MFT de-watering rate. These observations suggest that solutes and contaminants could be transported from underlying MFT by the expressed porewater to the overlying cap water. This phenomenon would explain previous observations of toxicity persistence in small-scale laboratory and field trials, and *in situ* might retard the beneficial reclamation effects expected of the EPL scenario. To verify this possibility at fine-scale resolution, a comprehensive laboratory mesocolumn study is in progress to complement field observations of Base Mine Lake and to determine how organic and inorganic contaminants are mobilized or immobilized in MFT during methanogenesis. Observations include measuring microbial metabolism rates, characterizing the host phases in

MFT, determining how solutes are transported, and how such transport would affect the quality of overlying cap water (A. Ulrich et al. unpublished). Data from *in situ* monitoring of Base Mine Lake and from laboratory studies should provide geotechnical data and biogeochemical rates to improve existing EPL performance models (Vandenberg, MacKenzie and Buchak 2012) that currently lack sufficient information about microbial activities *in situ*. The duration of greenhouse gas emissions, particularly  $CH_4$ , is important to EPL success especially after the recent discovery that iso- and cyclo-alkanes can be metabolized under methanogenic conditions by MFT microbes long after depletion of labile *n*-alkanes (Siddique et al. 2015; Mohamad Shahimin and Siddique 2017a,b). The possibility of mitigating  $CH_4$  emissions via syntrophic anaerobic  $CH_4$  oxidation (Timmers et al. 2015) has been considered but 16S rRNA gene sequences closely related to archetypal anaerobic  $CH_4$ -oxidizing archaea (ANME clades) were not detected in any OSTPs surveyed (An et al. 2013a), despite the simultaneous presence of  $CH_4$ , sulfate, methanogenic archaea and sulfate-reducing bacteria involved in archetypal anaerobic methane oxidation (Timmers et al. 2015). This suggests that biogenic  $CH_4$  produced in EPLs might persist *in situ* until released by ebullition. However, very recently a relative of '*Candidatus Methanoperedens nitroreducens*' within the order *Methanosarcinales* was shown in a laboratory bioreactor to oxidize  $CH_4$  anaerobically by directly reducing Fe(III) or Mn(IV) without a bacterial partner (Ettwig et al. 2016). Given the significant proportions of *Methanosarcinales* OTUs that have been detected in MFT (although not the new candidate genus) and the presence of iron minerals in tailings, the possibility of microbial  $CH_4$  oxidation *in situ* by this mechanism warrants further investigation in future studies.

#### Dry reclamation scenarios

##### Evaporative drying of TT and the potential for microbially mediated acid rock drainage

TT are spread in multiple thin layers on gently sloping sand beaches constructed to undergo evaporative drying by drainage and natural evaporation so as to generate a high-solids, trafficable material for subsequent reclamation. The drainage runoff is collected for proper management (<http://www.suncor.com/>; BGC Engineering 2010). Variations of this evaporative drying process (called TRO™ by Suncor and 'atmospheric fines drying' or 'thin-lift drying' by other operators) can be accomplished over weeks versus years without thickeners, allowing dry reclamation of the tailings solids to begin sooner. However, this process exposes the tailings to atmospheric oxygen ( $O_2$ ) and natural precipitation (rainwater and snow) that may expedite microbially mediated acid rock drainage unless the deposits are constantly covered by new layers of wet tailings. Some oil sands tailings streams such as 'froth treatment tailings' are enriched in significant proportions of sulfide minerals like pyrite (Kaminsky et al. 2009) that are particularly prone to the generation of acid rock drainage during atmospheric drying. Kuznetsov et al. (2015) exposed two different froth treatment TT samples to controlled irrigation and airflow in the laboratory, simulating exposure to environmental conditions. One TT sample experienced acid rock drainage within 50 days, producing a leachate of  $pH \leq 2$ , and the other TT sample started generating acidic leachate by 250 days, corresponding to the initial concentrations of pyritic sulfide in the two samples. 16S rRNA gene pyrosequencing analysis revealed a rapid shift in microbial community composition when conditions became strongly acidic ( $pH \sim 2$ ), favoring the enrichment of indigenous S- and/or Fe-oxidizing bacteria (*Acidithiobacillus* and



*Sulfobacillus*) and strongly implicating them in development of acid rock drainage conditions.

Besides acidification of runoff water, a major consequence of evaporative drying and drainage of TT may be increased toxic metals in the leachate. Kuznetsova et al. (2016) analyzed the acidic leachates described above and found that soluble metal concentrations reached 10 000 ppb for Ni, 5000 ppb for Co, 3000 ppb for As, 2000 ppb for V and 1000 ppb for Cr. Therefore, using TT containing high-sulfide minerals directly in dry reclamation scenarios or for tailings volume reduction may unfavorably impact the environment and warrants scrutiny of appropriate strategies to manage pyrite-enriched oil sands tailings streams.

#### Tailings in uplands reclamation and revegetation

Only two reports are available wherein oil sands by-products were tested as substrates for plant growth and included examination of the roles of associated microorganisms. From a soil reclamation point of view, a reclaimed medium should support a healthy plant community and rhizosphere microbiota that could evolve toward an ecosystem comparable to that of neighboring natural areas. CT, coarse tailings sands (post-bitumen extraction) and mine overburden materials are low in nutrients for plant and microbial growth. In addition, CT can have high salt, high pH and phytotoxic hydrocarbons. Bois et al. (2005) studied the mycorrhizal inoculum potential of CT, tailings sand and overburden for revegetation with jack pine, hybrid poplar and red clover, and found that CT and tailings sand were devoid of active ectomycorrhizal propagules. On hostile substrates such as saline alkaline CT, controlled inoculation of seedlings in the nursery with selected strains of mycorrhizal fungi could compensate for the low natural inoculum potential and improve survival and growth of tree seedlings after out-planting (Bois et al. 2005). Bissonnette et al. (2014) tested the performance of alders (*Alnus* spp.) in association with their symbiont *Frankia* sp. against the stress of tailings sands. When symbioses occurred, the alders thrived and produced a large amount of biomass that was highly correlated to the total nodule biomass. The pre-inoculation of alder seedlings prior to their out-planting on tailings sands reclamation sites could significantly accelerate their growth and development (Bissonnette et al. 2014). However, much more research is needed to assess the roles that microorganisms play during such tailings reclamation strategies.

## KNOWLEDGE GAPS AND FUTURE OUTLOOK FOR MICROBIAL PROCESSES

### OSTP management and reclamation scenario knowledge gaps

Although considerable progress has been made in characterizing the presence and effects of microbes in oil sands development, several areas in particular need further study. The potential for *in situ* anaerobic biodegradation of organics in OSTPs, including NAs and complex petroleum components (e.g. polycyclic aromatic hydrocarbons and resins) has not yet been well defined. In fact, even the extent of aerobic biodegradation of simple diluent hydrocarbons *in situ* is currently unknown. Migration of porewater from OSTPs or EPLs into underlying formations and buried or surface river channels is known to occur (Abolfazl-zadehdoshanbehbazari et al. 2013), but the role of indigenous microbes in natural attenuation of contaminant migration has been inadequately studied. To achieve these necessary insights, process-wide studies combining laboratory and field measurements (from ores to tailings streams to ponds to reclamation

sites and receptor environments) are needed to document the presence and activities of microbes in oil sands extraction, atmospheric drying and remediation operations.

Industry holds great expectations for EPLs to be a viable wet reclamation scenario, but field demonstration of efficacy has only recently begun at a single site (Base Mine Lake) (Dompierre and Barbour 2016; Dompierre et al. 2016). It is likely that, similar to the uniqueness of OSTPs, each EPL will exhibit individual characteristics that are strongly influenced by indigenous microbiota. Therefore, considerable effort is needed to help predict (and possibly enhance or mitigate) microbial effects in EPLs, including (i) rate and duration of legacy biogenic greenhouse gas (CH<sub>4</sub> and CO<sub>2</sub>) production from residual recalcitrant organics in tailings; (ii) potential for co-transport of solute and liquid contaminants from EPL sediments to overlying cap water via ebullition of these gases; (iii) mobilization of metals and other organic constituents of concern from sediments into pore water; and (iv) generation of new NA or other toxic by-products from incomplete metabolism of organics.

In both wet and dry landscape reclamation scenarios, long term monitoring of subsurface microbial activities should be undertaken. For example, in the closed Suncor Pond 5, S cycling should be tracked to ensure that H<sub>2</sub>S production does not reach the levels observed in the fen landscape scenario (Reid and Warren 2016) and CT storage site (Warren et al. 2016). In dry landscape scenarios that include re-vegetation, the presence and effects of beneficial and deleterious rhizosphere microbes (e.g. Bois et al. 2005) have only begun to be examined and need more consideration for ecosystem sustainability.

### Potential biotechnology approaches

The unexpectedly high biodiversity and metabolic adaptability of microbes in oil sands ores and tailings provides biotechnological opportunities to utilize indigenous activities, stimulate or augment desirable outcomes, and prevent or ameliorate microbially influenced problems *in situ*. Potential approaches include:

- (i) Aerobic pre-treatment of froth treatment tailings to pre-emptively biodegrade diluent hydrocarbons prior to their deposition in OSTPs (Foght, Li and Semple 2014). In theory, this would decrease the toxicity of OSPW directly by removing low molecular mass hydrocarbons and indirectly by preventing production of NAs from incomplete metabolism of those hydrocarbons *in situ*. Secondly, it would decrease subsequent CH<sub>4</sub> emissions by decreasing labile carbon sources entering the tailings ponds and, later, reclamation sites. An added environmental benefit could be reduction of volatile C<sub>2</sub>–C<sub>10</sub> organic carbon emissions from the oil sands region (Simpson et al. 2010).
- (ii) *In situ* aerobic biological treatment of toxic organics such as NAs already present in OSPW, with or without chemical pretreatment. For example, one hybrid engineered—microbial intervention is partial oxidation of NAs using ozonation followed by biodegradation by OSPW microbes (e.g. Scott et al. 2008; Choi and Liu 2014a; Mahdavi et al. 2015; reviewed by Quagraine, Peterson and Headley 2005b; Quinlan and Tam 2015). This approach has shown promise for remediating more complex NA structures and thus reducing toxicity (e.g. Brown and Ulrich 2015). Other potential treatment methods that have shown promise include the use of biofilm-based bioreactors (wherein biofilms are enriched from OSPW: McKenzie et al. 2014; Demeter et al. 2015; Islam et al. 2015), and algal-based biotreatment of OSPW that

requires the addition of phosphate (Quesnel et al. 2015). A corollary is biosorption of metals from OSPW by algal cultures (Mahdavi, Liu and Ulrich 2013).

- (iii) Microbial sequestration and/or oxidation of fugitive gases such as aerobic CH<sub>4</sub> oxidation by OSPW methanotrophs (Saidi-Mehrabad et al. 2013) or vapors such as volatile organic carbon from diluent components in ponds or dry landscape reclamation sites using, for example, soil bed biofilters.
- (iv) Accelerated sedimentation of suspended particles in OSPW (Bordenave et al. 2010) and biodensification of MFT (Siddique et al. 2014a,b; Brigmon et al. 2016) by stimulating anaerobic metabolism.

Notably, none of these potential approaches has yet been examined at pilot scale or *in situ* in the oil sands, even though some may have been proven in other applications.

Some biotechnology processes at the conceptual stage include (i) improving bitumen recovery from oil sands ores by pre-treatment using 'microbial enhanced oil recovery' (MEOR) methods (Ding et al. 2014a,b; Langley 2015) and/or the use of biosurfactants produced by oil sands microbes to enhance bitumen recovery from oil sands ores or tailings (Huang et al. 2017); (ii) using encapsulated microbes to bioremediate OSPW (Pramanik 2016); and (iii) generating electricity from tailings by constructing microbial fuel cells (Jiang, Ulrich and Liu 2013; Choi and Liu 2014b) that utilize endogenous carbon sources in OSTPs.

Potential applications of oil sands microbiota to non-oil sands processes have also been proposed including (i) using synthetic biology-based DNA biosensors derived in part from OSTP metagenomes and fosmid libraries to identify unique enzymes and genes for nutraceutical and medicinal applications (e.g. MetaMixis Inc.); (ii) applying fundamental understanding of anaerobic microbial processes to other environments, such as contaminated harbor sediments, hydraulic fracturing ('fracking') operations and microbially enhanced coal bed CH<sub>4</sub> generation; and (iii) applying information about uncultivated microbes (e.g. syntrophs) to their isolation and characterization for greater understanding of environmental and industrial anaerobic consortia.

## CONCLUSIONS

Despite the technical difficulties of accessing and manipulating oil sands tailings, considerable progress has been made in the past 10 years towards understanding the magnitude and significance of microbes in OSTPs, which historically were considered barren. The unexpectedly rich taxonomic and metabolic diversity in oil sands ores, process streams and tailings has provided fundamental knowledge applicable to other environments, particularly regarding anaerobic hydrocarbon biodegradation, and possible biotechnological applications. Importantly, progress in this area has also made industry and regulators aware of potential beneficial and deleterious consequences of microbial activities in the enormous oil sands operations.

## SUPPLEMENTARY DATA

Supplementary data are available at [FEMSEC](http://FEMSEC) online.

## ACKNOWLEDGEMENTS

The authors thank the following oil sands operators for providing samples, expertise and funding for the authors' research: Syncrude Canada Ltd, Suncor Energy, Shell Albian Sands, Cana-

dian Natural Resources Ltd and Total E&P Canada. We also acknowledge Genome Canada, the Alberta Water Research Institute, Alberta Innovates Energy and Environment Solutions, the Helmholtz-Alberta Initiative, NSERC, the Institute for Oil Sands Innovation and COSIA for research funding, and the Oil Sands Technology Research Facility for access to research infrastructure.

We thank our many collaborators and co-authors, including Phil Fedorak, Gerrit Voordouw, Ania Ulrich, Camilla Nesbø and Peter Dunfield; industry advisors, particularly Mike MacKinnon and Tara Penner (Syncrude); André Sobolewski (Microbial Technologies) for providing insight and information from early studies; and Zvonko Burkus (Alberta Environment and Sustainable Resource Development) for valuable discussions. Finally, we gratefully acknowledge the contributions of Kathy Semple, Carmen Li and the invaluable laboratory members too numerous to list who have been essential to the authors' oil sands research.

**Conflict of interest.** None declared.

## REFERENCES

- Abolfazlzadehdoshanbehbazari M, Birks SJ, Moncur MC et al. Fate and transport of oil sand process-affected water into the underlying clay till: A field study. *J Contam Hydrol* 2013;**151**:83–92.
- Abu Laban N, Dao A, Foght J. DNA stable-isotope probing of oil sands tailings pond enrichment cultures reveals different key players for toluene degradation under methanogenic and sulfidogenic conditions. *FEMS Microbiol Ecol* 2015b;**91**:fiv039
- Abu Laban N, Dao A, Semple K et al. Biodegradation of C<sub>7</sub> and C<sub>8</sub> iso-alkanes under methanogenic conditions. *Environ Microbiol* 2015a;**17**:4898–915.
- Abu Laban N, Tan BF, Dao A et al. Draft genome sequence of uncultivated toluene-degrading *Desulfobulbaceae* bacterium Tol-SR, obtained by stable isotope probing using [<sup>13</sup>C<sub>6</sub>] toluene. *Genome Announc* 2015c;**3**:e01423–14.
- Abu Laban N, Tan BF, Dao A et al. Draft genome sequence of uncultivated *Desulfosporosinus* sp. strain Tol-M, obtained by stable isotope probing using [<sup>13</sup>C<sub>6</sub>] toluene. *Genome Announc* 2015d;**3**:e01422–14.
- Aguilar M, Richardson E, Tan B et al. Next-generation sequencing assessment of eukaryotic diversity in oil sands tailings ponds sediments and surface water. *J Eukaryotic Microbiol* 2016;**263**:732–43.
- Alberta Government. Oil sands information portal, 2016 <http://osip.alberta.ca/map/> (21 January 2017, date last accessed).
- Allen EW. Process water treatment in Canada's oil sands industry: I. Target pollutants and treatment objectives. *J Environ Eng Sci* 2008;**7**:123–38.
- An D, Brown D, Chatterjee I et al. Microbial community and potential functional gene diversity involved in anaerobic hydrocarbon degradation and methanogenesis in an oil sands tailings pond. *Genome* 2013b;**56**:612–8.
- An D, Caffrey SM, Soh J et al. Metagenomics of hydrocarbon resource environments indicates aerobic taxa and genes to be unexpectedly common. *Environ Sci Technol* 2013a;**47**:10708–17.
- Arkell N, Kuznetsov P, Kuznetsova A et al. Microbial metabolism alters pore water chemistry and increases consolidation of oil sands tailings. *J Environ Qual* 2015;**44**:145–53.

- BGC Engineering. Oil sands tailings technology review. OS-RIN Report No. TR-1. Edmonton, AB, Canada: Oil Sands Research and Information Network, University of Alberta, School of Energy and the Environment, 2010. <https://era.library.ualberta.ca/files/j098zc647#.WIOsCLYrLm0> (21 January 2017, date last accessed).
- Bissonnette C, Fahlman B, Peru KM et al. Symbiosis with *Frankia* sp. benefits the establishment of *Alnus viridis* ssp. *crispa* and *Alnus incana* ssp. *rugosa* in tailings sand from the Canadian oil sands industry. *Ecol Eng* 2014;**68**: 167–75.
- Bois G, Piché Y, Fung MYP et al. Mycorrhizal inoculum potentials of pure reclamation materials and revegetated tailing sands from the Canadian oil sand industry. *Mycorrhiza* 2005;**15**:149–58.
- Bordenave S, Kostenko V, Dutkoski M et al. Relation between the activity of anaerobic microbial populations in oil sands tailings ponds and the sedimentation of tailings. *Chemosphere* 2010;**81**:663–8.
- Bordenave S, Ramos E, Lin S et al. Use of calcium sulfate to accelerate densification while reducing greenhouse gas emissions from oil sands tailings ponds. *Proceedings of the Canadian International Petroleum Conference*, Calgary: Petroleum Society of Canada. 2009, PETSOC-2009-081, doi: 10.2118/2009-081.
- Bowman DT, Slater GF, Warren LA et al. Identification of individual thiophene-, indane-, tetralin-, cyclohexane-, and adamantane-type carboxylic acids in composite tailings pore water from Alberta oil sands. *Rapid Commun Mass Spectrom* 2014;**28**:2075–83.
- Brigmon RL, Berry CJ, Wade A et al. Bioprocessing-based approach for bitumen/water/fines separation and hydrocarbon recovery from oil sands tailings. *Soil Sed Contam* 2016;**25**: 241–55.
- Brown D, Ramos-Padrón E, Gieg L et al. Effect of calcium ions and anaerobic microbial activity on sedimentation of oil sands tailings. *Int Biodeterior Biodegrad* 2013;**81**:9–16.
- Brown LD, Ulrich AC. Oil sands naphthenic acids: A review of properties, measurement, and treatment. *Chemosphere* 2015;**127**:276–90.
- Burkus Z. GHG emissions from oil sands tailings ponds: Overview and modeling based on fermentable substrates. Part II: Modeling of GHG emissions from tailings ponds based on fermentable substrates. Edmonton: Alberta Environment and Sustainable Resource Development, 2014. <https://era.library.ualberta.ca/files/5x21tg04h#.WPELKBLyvm0> (21 January 2017, date last accessed).
- Burkus Z, Wheler J, Pletcher S. GHG emissions from oil sands tailings ponds: Overview and modeling based on fermentable substrates. Part I: Review of the tailings pond facts and practices. Edmonton: Alberta Environment and Sustainable Resource Development, 2014. <https://era.library.ualberta.ca/files/5x21tg04h#.WPELKBLyvm0> (21 January 2017, date last accessed).
- Chalaturnyk RJ, Scott JD, Özüm B. Management of oil sands tailings. *Petrol Sci Technol* 2002;**20**:1025–46.
- Charette T, Castendyk D, Hrynshyn J et al. End Pit Lakes Guidance Document, 2012. Cumulative Environmental Management Association Fort McMurray, Alberta, Canada 2010. <http://library.cemaonline.ca/ckan/dataset/2010-0016/resource/1632ce6e-d1a0-441a-a026-8a839f1d64bc> (21 January 2017, date last accessed).
- Chen M, Walshe G, Chi Fru E et al. Microcosm assessment of the biogeological development of sulfur and oxygen in oil sands fluid fine tailings. *Appl Geochem* 2013;**37**: 1–11.
- Chi Fru E, Chen M, Walshe G et al. Bioreactor studies predict whole microbial population dynamics in oil sands tailings ponds. *Appl Microbiol Biotechnol* 2013;**97**: 3215–24.
- Choi J, Liu Y. Biodegradation of oil sands process affected water in sequencing batch reactors and microbial community analysis by high-throughput pyrosequencing. *Int Biodeterior Biodegrad* 2014a;**92**:79–85.
- Choi J, Liu Y. Power generation and oil sands process-affected water treatment in microbial fuel cells. *Bioresour Technol* 2014b;**169**:581–7.
- Clemente J, Fedorak P. A review of the occurrence, analyses, toxicity, and biodegradation of naphthenic acids. *Chemosphere* 2005;**60**:585–600.
- Clothier LN, Gieg LM. Anaerobic biodegradation of surrogate naphthenic acids. *Water Res* 2016;**90**:156–66.
- Collins VCE, Foght JM, Siddique T. Co-occurrence of methanogenesis and N<sub>2</sub> fixation in oil sands tailings. *Sci Total Environ* 2016;**565**:306–12.
- COSIA. Technical guide for fluid fine tailings management, 2012. [http://www.cosia.ca/uploads/documents/id7/TechGuideFluidTailingsMgmt\\_Aug2012.pdf](http://www.cosia.ca/uploads/documents/id7/TechGuideFluidTailingsMgmt_Aug2012.pdf) (21 January 2017, date last accessed).
- Czarnecki J, Radoev B, Schramm LL et al. On the nature of Athabasca oil sands. *Adv Colloid Interface Sci* 2005;**114-115**: 53–60.
- Dean C, Xiao Y, Roberts DJ. Enriching acid rock drainage related microbial communities from surface-deposited oil sands tailings. *Can J Microbiol* 2016;**62**:870–9.
- Demeter MA, Lemire JA, Yue G et al. Culturing oil sands microbes as mixed species communities enhances ex situ model naphthenic acid degradation. *Front Microbiol* 2015;**6**:936.
- Ding M-S, Jia W-H, Lv Z-F et al. Improving bitumen recovery from poor processing oil sands using microbial pretreatment. *Energy Fuels* 2014b;**28**:7712–20.
- Ding M, Zhang Y, Liu J et al. Application of microbial enhanced oil recovery technology in water-based bitumen extraction from weathered oil sands. *AIChE J* 2014a;**60**:2985–93.
- Doan DH, Delage P, Nauroy JF et al. Microstructural characterization of a Canadian oil sand. *Can Geotech J* 2012;**49**:1212–20.
- Dompierre KA, Barbour SL. Characterization of physical mass transport through oil sands fluid fine tailings in an end pit lake: A multi-tracer study. *J Contam Hydrol* 2016;**189**:12–26.
- Dompierre KA, Lindsay MJB, Cruz-Hernández P et al. Initial geochemical characteristics of fluid fine tailings in an oil sands end pit lake. *Sci Total Environ* 2016;**556**:196–206.
- Eckert WF, Masliyah JH, Gray MR et al. Prediction of sedimentation and consolidation of fine tails. *AIChE J* 1996;**42**:960–72.
- Ettwig KF, Zhu B, Speth D et al. Archaea catalyze iron-dependent anaerobic oxidation of methane. *Proc Nat Acad Sci U S A* 2016;**113**:12792–6.
- Fedorak PM, Coy DL, Salloum MJ et al. Methanogenic potential of tailings samples from oil sands extraction plants. *Can J Microbiol* 2002;**48**:21–33.
- Fedorak PM, Coy DL, Dudas MJ et al. Microbially-mediated fugitive gas production from oil sands tailings and increased tailings densification rates. *J Environ Eng Sci* 2003;**2**:199–211.
- Foght JM, Fedorak PM, Westlake DWS et al. Microbial content and metabolic activities in the Syncrude tailings pond. AOSTRA J Res Edmonton: Syncrude Canada Ltd. 1985;**1**:139–46.
- Foght JM, Li C, Semple K. Microbial pre-treatment of Plant 6 froth treatment tailings before deposition. Report to Syncrude, December. Clagary: Publisher is Canadian Natural Resources Ltd. 2014.

- Foght JM, Siddique T. Microbial analysis of CNRL tailings. Report to Canadian Natural Resources Ltd, August 2014.
- Foght JM, Siddique T, Gieg LM. Protocols for handling, storing and cultivating oil sands tailings ponds materials for microbial and molecular biological study. In: McGenity TJ, Timmis KN, Nogales B (eds). *Hydrocarbon and Lipid Microbiology Protocols*. Berlin: Springer, 2017, 175–197, DOI: 10.1007/8623\_2014\_26.
- Folwell BD, McGenity TJ, Price A et al. Exploring the capacity for anaerobic biodegradation of polycyclic aromatic hydrocarbons and naphthenic acids by microbes from oil-sands-process-affected waters. *Int Biodeterior Biodegrad* 2016;**108**:214–21.
- Frank RA, Milestone CB, Rowland SJ et al. Assessing spatial and temporal variability of acid-extractable organics in oil sands process-affected waters. *Chemosphere* 2016;**160**:303–13.
- Gosselin P, Hruddy SE, Naeth MA et al. Environmental and health impacts of Canada's oil sands industry. **Expert Panel Report**. Ottawa: Royal Society of Canada, 2010.
- Grewer DM, Young RF, Whittall RM et al. Naphthenic acids and other acid-extractables in water samples from Alberta: What is being measured? *Sci Total Environ* 2010;**408**:5997–6010.
- Gunawan Y, Nemati M, Dalai A. Biodegradation of a surrogate naphthenic acid under denitrifying conditions. *Water Res* 2014;**51**:11–24.
- Guo C. Rapid Densification of the oil sands mature fine tailings (MFT) by microbial activity. PhD Thesis, University of Alberta, Edmonton, AB, Canada, 2009.
- Hall AC, Collins SH, Melrose JC. Stability of aqueous wetting films in Athabasca tar sands. *Soc Petrol Eng J* 1983;**23**:249–58.
- Han X, Scott AC, Fedorak PM et al. Influence of molecular structure on the biodegradability of naphthenic acids. *Environ Sci Technol* 2008;**42**:1290–5.
- Haveroen ME, MacKinnon MD, Fedorak PM. Polyacrylamide added as a nitrogen source stimulates methanogenesis in consortia from various wastewaters. *Water Res* 2005;**39**:3333–41.
- Hein FJ, Leckie D, Larter S et al. Heavy oil and bitumen petroleum systems in Alberta and beyond: The future is nonconventional and the future is now. In: Hein FJ, Leckie D, Larter S, Suter JR (eds). *Heavy Oil and Oil-sand Petroleum Systems in Alberta and Beyond*. AAPG Studies in Geology 64. Tulsa, OK: American Association of Petroleum Geologists, 2013, 1–21.
- Hodson PV. History of environmental contamination by oil sands extraction. *Proc Natl Acad Sci U S A* 2013;**110**:1569–70.
- Holden AA, Donahue RB, Ulrich AC. Geochemical interactions between process-affected water from oil sands tailings ponds and North Alberta surficial sediments. *J Contam Hydrol* 2011;**119**:55–68.
- Holowenko FM, MacKinnon MD, Fedorak PM. Methanogens and sulfate-reducing bacteria in oil sands fine tailings waste. *Can J Microbiol* 2000;**46**:927–37.
- Huang G, Yu H, Li G et al. Development of an innovative bioremediation technology for oil-sands tailings waste treatment. Report 002-00017-UOR to Petroleum Technology Research Centre, 2017. <http://steps.ptrc.ca/concrete/files/4014/0554/8430/UOR-002-00017.Final.Report.pdf> (21 January 2017, date last accessed).
- Hubert CRJ, Oldenburg TBP, Fustic M et al. Massive dominance of *Epsilonproteobacteria* in formation waters from a Canadian oil sands reservoir containing severely biodegraded oil. *Environ Microbiol* 2012;**14**:387–404.
- Islam MS, Zhang Y, McPhedran KN et al. Next-generation pyrosequencing analysis of microbial biofilm communities on granular activated carbon in treatment of oil sands process-affected water. *Appl Environ Microbiol* 2015;**81**:4037–48.
- Jeeravipoolvarn S, Scott JD, Chalaturmyk RJ. 10 m standpipe tests on oil sands tailings: long-term experimental results and prediction. *Can Geotech J* 2009;**46**:875–88.
- Jiang Y, Ulrich AC, Liu Y. Coupling bioelectricity generation and oil sands tailings treatment using microbial fuel cells. *Biore-sour Technol* 2013;**139**:349–54.
- Jordaan SM. Land and water impacts of oil sands production in Alberta. *Environ Sci Technol* 2012;**46**:3611–7.
- Kaminsky HAW, Etsell TH, Ivey DG et al. Distribution of clay minerals in the process streams produced by the extraction of bitumen from Athabasca oil sands. *Can J Chem Eng* 2009;**87**:85–93.
- Kirk JL, Muir DCG, Gleason A et al. Atmospheric deposition of mercury and methylmercury to landscapes and waterbodies of the Athabasca oil sands region. *Environ Sci Technol* 2014;**48**:7374–83.
- Kuznetsov P, Kuznetsova A, Foght JM et al. Oil sands thickened froth treatment tailings exhibit acid rock drainage potential during evaporative drying. *Sci Total Environ* 2015;**505**:1–10.
- Kuznetsova A, Kuznetsov P, Foght JM et al. Trace metal mobilization from oil sands froth treatment thickened tailings exhibiting acid rock drainage. *Sci Total Environ* 2016;**571**:699–710.
- Langley M. Method and apparatus for use of microbes and other gas stimulants in radial enhancement drilling fluid. US Patent 9,109,400, 2015.
- Larter SR, Head IM. Oil sands and heavy oil: Origin and exploitation. *Elements* 2014;**10**:277–83.
- Li C. Methanogenesis in oil sands tailings: An analysis of the microbial community involved and its effects on tailings densification. MSc Thesis, University of Alberta, Edmonton, AB, Canada, 2010.
- Liang J, Guo Z, Deng L et al. Mature fine tailings consolidation through microbial induced calcium carbonate precipitation. *Can J Civ Eng* 2015;**42**:975–8.
- McKenzie N, Yue S, Liu X et al. Biodegradation of naphthenic acids in oils sands process waters in an immobilized soil/sediment bioreactor. *Chemosphere* 2014;**109**:164–72.
- MacKinnon MD. Development of the tailings pond at Syncrude's oil sands plant: 1978-1987. *AOSTRA J Res* 1989;**5**:109–33.
- MacKinnon M, Sethi A. A comparison of the physical and chemical properties of the tailings ponds at the Syncrude and Sunco oil sands plants. In: Liu JK (ed). *Proceedings of Fine Tails Symposium: Oil Sands—Our Petroleum Future*, Paper F2. Ottawa: Environment Canada, 1993.
- Mahdavi H, Liu Y, Ulrich AC. Partitioning and bioaccumulation of metals from oil sands process affected water in indigenous *Parachlorella kessleri*. *Chemosphere* 2013;**90**:1893–9.
- Mahdavi H, Prasad V, Liu Y et al. In situ biodegradation of naphthenic acids in oil sands tailings pond water using indigenous algae-bacteria consortium. *Bioresour Technol* 2015;**187**:97–105.
- Masliyah J, Zhou Z, Xu Z et al. Understanding water-based bitumen extraction from Athabasca oil sands. *Can J Chem Eng* 2004;**82**:628–54.
- Matthews JG, Shaw WH, MacKinnon MD et al. Development of composite tailings technology at Syncrude. *Int J Surf Mining Reclam Environ* 2002;**16**:24–39.
- Meckenstock RU, Von Netzer F, Stumpp C et al. Water droplets in oil are microhabitats for microbial life. *Science* 2014;**345**:673–6.

- Mikula RJ, Kasperski KL, Burns RD et al. Nature and fate of oil sands tailings. In Schramm LL (ed). *Suspensions: Fundamentals and Applications in the Petroleum Industry*. Washington, DC: American Chemical Society, 1996, 677–723.
- Mohamad Shahimin MF, Foght JM, Siddique T. Preferential methanogenic biodegradation of short-chain *n*-alkanes by microbial communities from two different oil sands tailings ponds. *Sci Total Environ* 2016;**553**:250–7.
- Mohamad Shahimin MF, Siddique T. Sequential biodegradation of complex naphtha hydrocarbons under methanogenic conditions in two different oil sands tailings. *Environ Pollut* 2017a;**221**:398–406.
- Mohamad Shahimin MF, Siddique T. Methanogenic biodegradation of paraffinic solvent hydrocarbons in two different oil sands tailings. *Sci Total Environ* 2017b;**583**:115–122.
- Nix PG, Martin RW. Detoxification and reclamation of Suncor's oil sand tailings ponds. *Environ Toxicol Water Qual* 1992;**7**: 171–88.
- Noah M, Lappé M, Schneider B et al. Tracing biogeochemical and microbial variability over a complete oil sand mining and reclamation process. *Sci Total Environ* 2014;**499**:297–310.
- OSRIN. Glossary of terms and acronyms used in oil sands mining, processing and environmental management—December 2014 Update. OSRIN Report SR-1, 2014. <http://hdl.handle.net/10402/era.17544> (21, January 2017, date last accessed).
- Penner TJ, Foght JM. Mature fine tailings from oil sands processing harbour diverse methanogenic communities. *Can J Microbiol* 2010;**56**:459–70.
- Pramanik S. Review of biological processes in oil sands: a feasible solution for tailings water treatment. *Environ Rev* 2016;**24**:274–84.
- Quagraine EK, Headley JV, Peterson HG. Is biodegradation of bitumen a source of recalcitrant naphthenic acid mixtures in oil sands tailings pond waters? *J Environ Sci Health Pt A* 2005a;**40**:671–84.
- Quagraine EK, Peterson HG, Headley JV. In situ bioremediation of naphthenic acids contaminated tailing pond waters in the Athabasca oil sands region—Demonstrated field studies and plausible options: A review. *J Environ Sci Health Part A Toxic Hazard Subst Environ Eng* 2005b;**40**:685–722.
- Quesnel DM, Oldenburg TBP, Larter SR et al. Biostimulation of oil sands process-affected water with phosphate yields removal of sulfur-containing organics and detoxification. *Environ Sci Technol* 2015;**49**:13012–20.
- Quinlan PJ, Tam KC. Water treatment technologies for the remediation of naphthenic acids in oil sands process-affected water. *Chem Eng J* 2015;**279**:696–714.
- Ramos-Padrón E. Physiology and molecular characterization of microbial communities in oil sands tailings ponds. PhD Thesis, University of Calgary, Calgary, AB, Canada, 2013.
- Ramos-Padrón E, Bordenave S, Lin S et al. Carbon and sulfur cycling by microbial communities in a gypsum-treated oil sands tailings pond. *Environ Sci Technol* 2011;**45**:439–46.
- Reid ML, Warren LA. S reactivity of an oil sands composite tailings deposit undergoing reclamation wetland construction. *J Environ Manage* 2016;**166**:321–9.
- Rochman FF. Aerobic hydrocarbon-degrading microbial communities in oilsands tailings ponds. PhD Thesis, University of Calgary, Calgary, AB, Canada, 2016.
- Rowland SJ, Scarlett AG, Jones D et al. Diamonds in the rough: Identification of individual naphthenic acids in oil sands process water. *Environ Sci Technol* 2011b;**45**:3154–9.
- Rowland SJ, West CE, Scarlett AG et al. Identification of individual acids in a commercial sample of naphthenic acids from petroleum by two-dimensional comprehensive gas chromatography/mass spectrometry. *Rapid Comm Mass Spectrom* 2011a;**25**:1741–51.
- Saidi-Mehrabad A, He Z, Tamas I et al. Methanotrophic bacteria in oilsands tailings ponds of northern Alberta. *ISME J* 2013;**7**:908–21.
- Salloum MJ, Dudas MJ, Fedorak PM. Microbial reduction of amended sulfate in anaerobic mature fine tailings from oil sand. *Waste Manage Res* 2002;**20**:162–71.
- Scott AC, Zubot W, MacKinnon MD et al. Ozonation of oil sands process water removes naphthenic acids and toxicity. *Chemosphere* 2008;**71**:156–60.
- Sherry A, Grant RJ, Aitken CM et al. Volatile hydrocarbons inhibit methanogenic crude oil degradation. *Front Microbiol* 2014;**5**:131.
- Siddique T, Fedorak PM, Foght JM. Biodegradation of short-chain *n*-alkanes in oil sands tailings under methanogenic conditions. *Environ Sci Technol* 2006;**40**:5459–64.
- Siddique T, Fedorak PM, MacKinnon M et al. Metabolism of BTEX and naphtha compounds to methane in oil sands tailings. *Environ Sci Technol* 2007;**41**:2350–6.
- Siddique T, Gupta R, Fedorak PM et al. A first approximation kinetic model to predict methane generation from an oil sands tailings settling basin. *Chemosphere* 2008;**72**: 1573–80.
- Siddique T, Penner T, Semple K et al. Anaerobic biodegradation of longer-chain *n*-alkanes coupled to methane production in oil sands tailings. *Environ Sci Technol* 2011;**45**:5892–9.
- Siddique T, Kuznetsov P, Kuznetsova A et al. Microbially-accelerated consolidation of oil sands tailings. Pathway I: Changes in porewater chemistry. *Front Microbiol* 2014a;**5**:106.
- Siddique T, Kuznetsov P, Kuznetsova A et al. Microbially-accelerated consolidation of oil sands tailings. Pathway II: Solid phase biogeochemistry. *Front Microbiol* 2014b;**5**:107.
- Siddique T, Shahimin MF, Zamir S et al. Long-term incubation reveals methanogenic biodegradation of C<sub>5</sub> and C<sub>6</sub> iso-alkanes in oil sands tailings. *Environ Sci Technol* 2015;**49**:14732–9.
- Simpson IJ, Blake NJ, Barletta B et al. Characterization of trace gases measured over Alberta oil sands mining operations: 76 speciated C<sub>2</sub>-C<sub>10</sub> volatile organic compounds (VOCs), CO<sub>2</sub>, CH<sub>4</sub>, CO, NO, NO<sub>2</sub>, NO<sub>y</sub>, O<sub>3</sub> and SO<sub>2</sub>. *Atmos Chem Phys* 2010;**10**:11931–54.
- Small CC, Cho S, Hashisho Z et al. Emissions from oil sands tailings ponds: Review of tailings pond parameters and emission estimates. *J Pet Sci Eng* 2015;**127**:490–501.
- Smith BE, Lewis CA, Belt ST et al. Effects of alkyl chain branching on the biotransformation of naphthenic acids. *Environ Sci Technol* 2008;**42**:9323–8.
- Sobolewski A. Interactions between nitrogen-cycling bacteria and toxicants during detoxification of tailings water at the Syncrude and Suncor leases, September. Edmonton: Consultant's report to Syncrude. 1995.
- Sobolewski A. Anaerobic microbial populations at the Syncrude lease. Consultant's memo to Syncrude, February 1997.
- Sobolewski A. Evolution of microbial populations in process-affected aquatic ecosystems, June. Edmonton: Consultant's report to Syncrude. 1999a.
- Sobolewski A. Survey of anaerobic bacteria in tailings samples at the Syncrude lease, June. Edmonton: Consultant's report to Syncrude. 1999b.
- Sobolewski A. Hydrogen sulphide production in gypsum-amended MFT and NST, June. Edmonton: Consultant's report to Syncrude. 1999c:38 pp.

- Sparks BD, Kotlyar LS, O'Carroll JB et al. Athabasca oil sands: Effect of organic coated solids on bitumen recovery and quality. *J Petrol Sci Eng* 2003;**39**:417–30.
- Stasik S, Wendt-Potthoff K. Vertical gradients in carbon flow and methane production in a sulfate-rich oil sands tailings pond. *Water Res* 2016;**106**:223–31.
- Stasik S, Wendt-Potthoff K. Interaction of microbial sulphate reduction and methanogenesis in oil sands tailings ponds. *Chemosphere* 2014;**103**:59–66.
- Stasik S, Loick N, Knöller K et al. Understanding biogeochemical gradients of sulfur, iron and carbon in an oil sands tailings pond. *Chem Geol* 2014;**382**:44–53.
- Stasik S, Wick LY, Wendt-Potthoff K. Anaerobic BTEX degradation in oil sands tailings ponds: Impact of labile organic carbon and sulfate-reducing bacteria. *Chemosphere* 2015;**138**:133–9.
- Strausz OP, Lown EM, Morales-Izquierdo A et al. Chemical composition of Athabasca bitumen: The distillable aromatic fraction. *Energy Fuels* 2011;**25**:4552–79.
- Strausz OP, Morales-Izquierdo A, Kazmi N et al. Chemical composition of Athabasca bitumen: The saturate fraction. *Energy Fuels* 2010;**24**:5053–72.
- Tan BF, Charchuk R, Li C et al. Draft genome sequence of uncultivated Firmicutes (*Peptococcaceae* SCADC) single cells sorted from methanogenic alkane-degrading cultures. *Genome Announce* 2014a;**2**:e00909–14.
- Tan BF, de Araujo e Silva, R, Rozyki T et al. Draft genome sequences of three *Smithella* spp. obtained from a methanogenic alkane-degrading culture and oil field produced water. *Genome Announce* 2014b;**2**:e01085–14.
- Tan B, Dong X, Sensen CW et al. Metagenomic analysis of an anaerobic alkane-degrading microbial culture: Potential hydrocarbon-activating pathways and inferred roles of community members. *Genome* 2013;**56**:599–611.
- Tan BF, Foght J. Draft genome sequences of *Campylobacteriales* (Epsilonproteobacteria) obtained from methanogenic oil sands tailings pond metagenomes. *Genome Announce* 2014;**2**:e01034–14.
- Tan B, Fowler SJ, Laban NA et al. Comparative analysis of metagenomes from three methanogenic hydrocarbon-degrading enrichment cultures with 41 environmental samples. *ISME J* 2015a;**9**:2028–45.
- Tan BF, Semple K, Foght J. Anaerobic alkane biodegradation by cultures enriched from oil sands tailings ponds involves multiple species capable of fumarate addition. *FEMS Microbiol Ecol* 2015b;**91**:fv042.
- Timmers PHA, Widjaja-Greefkes HCA, Ramiro-Garcia J et al. Growth and activity of ANME clades with different sulfate and sulfide concentrations in the presence of methane. *Front Microbiol* 2015;**6**:988
- Vandenberg J, MacKenzie I, Buchak E. CEMA Oil Sands Pit Lake Model. 09-1336-1008, 2012. <http://library.cemaonline.ca/ckan/dataset/67a9f78b-eb47-4053-a7f5-4aeffee25d19/resource/5d22e341-aa10-49e8-a889-43bad0e97fa3/download/20090043cemaoilssandspitlakemodel.pdf> (21 January 2017, date last accessed).
- Voordouw G. Interaction of oil sands tailings particles with polymers and microbial cells: First steps toward reclamation to soil. *Biopolymers* 2012;**99**:257–62.
- Warren LA, Kendra KE, Brady AL et al. Sulfur biogeochemistry of an oil sands composite tailings deposit. *Front Microbiol* 2016;**6**:1533.
- West CE, Scarlett AG, Tonkin A et al. Diaromatic sulphur-containing 'naphthenic' acids in process waters. *Water Res* 2014;**51**:206–15.
- Whitby C. Microbial naphthenic acid degradation. *Adv Appl Microbiol* 2010;**70**:93–125.
- Wilde JM, West CE, Scarlett AG et al. Structural identification of petroleum acids by conversion to hydrocarbons and multi-dimensional gas chromatography-mass spectrometry. *Anal Chem* 2015;**87**:8457–65.
- Wilson SL, Li C, Ramos-Padrón E et al. Oil sands tailings ponds harbour a small core prokaryotic microbiome and diverse accessory communities. *J Biotechnol* 2016;**235**:187–96
- Wong M-L, An D, Caffrey SM et al. Roles of thermophiles and fungi in bitumen degradation in mostly cold oil sands outcrops. *Appl Environ Microbiol* 2015;**81**:6825–38.
- Wyndham RC, Costerton JW. Heterotrophic potentials and hydrocarbon biodegradation potentials of sediment organisms within the Athabasca oil sands deposit. *Appl Environ Microbiol* 1981a;**41**:783–90.
- Wyndham RC, Costerton JW. In vitro microbial degradation of bituminous hydrocarbons and in situ colonization of bitumen surfaces within the Athabasca oil sands deposit. *Appl Environ Microbiol* 1981b;**41**:791–800.
- Yang C, Wang Z, Yang Z et al. Chemical fingerprints of Alberta oil sands and related petroleum products. *Environ Forensics* 2011;**12**:173–88.
- Yergeau E, Lawrence JR, Sanschagrin S et al. Next-generation sequencing of microbial communities in the Athabasca river and its tributaries in relation to oil sands mining activities. *Appl Environ Microbiol* 2012;**78**:7626–37.

Reproduced with permission of copyright owner. Further reproduction prohibited without permission.