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Review of Biological Processes in Oil Sands – A Feasible Solution for Tailings Water Treatment

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Abstract

Bitumen extraction process from Athabasca oil sands ore produces large quantities of toxic processed water as tailings. The oil industry has reduced the demand of fresh water in the extraction process by recycling the tailings water. Continual recycling increases the toxicity of tailings water many folds and poses a serious threat to surface and groundwater quality. For a sustainable expansion of Canada's oil sands industry, it is essential to develop a technically practicable and economically feasible tailings water treatment technology. A review was carried out to describe the integral role of biological processes in oil sands history for identifying a successor tailings water treatment technology. This study proposes the application of entrapped cells system as a feasible solution for tailings water treatment. Bio-augmentation followed by entrapment of microbial community indigenous to tailings ponds can be a promising tailings water treatment technology.

Keywords: Oil sands tailings water treatment, Naphthenic acids, Biological process, Cell entrapment, Microbial community

1. Introduction

The Athabasca oil sands, located in the northeastern Alberta, Canada are one of the large petroleum reserves in the world. The oil sands have very important contribution to the Canadian economy and labour forces. Oil sands have been recognized by an ongoing controversy because of environmental and health issues despite the economic benefits. Bitumen extraction process from the oil sands ores requires huge amounts of water and generates substantial quantity of tailings water; highly toxic in nature. Government of Alberta has established a zero effluent discharge policy for the oil sands industries by the Alberta Environmental Protection and Enhancement Act 1993 (Giesy *et al.* 2010). This requirement has resulted in several hundred million cubic meters of tailings water stored in man-made lakes called tailings ponds. More than 80% of the tailings water recycles back in the extraction process to reduce the fresh

water intake. The toxic contaminants in the tailings water intensify over time due to repeated recycling and the tailings water become unsuitable for reclamation. Management of oil sands tailings water has raised issues of adverse environmental impacts including depletion of fresh water, greenhouse gas and chemical emissions, toxicity of tailings water on environment and human health. Recent studies found that the toxic compounds of tailings ponds water also migrate in the groundwater of the region by infiltration through the underlying clay (Holden *et al.* 2011; Hodson P.V. 2013; Frank *et al.* 2014). The infiltration of tailings water reveals that the storage of tailings water in onsite ponds seems fundamentally a non-sustainable process and requires attention. Implementation of a sustainable tailings water treatment technology is very much necessary for the future development of the oil sands industries. This study presents a brief overview of the oil sands and environmental impacts of oil sands development. Next, it outlines the previous research works on tailings water treatment and explores the biological processes involved at different stages of the oil sands resources. Finally, a feasible solution for tailings water treatment has been proposed which may overcome the limitations of the existing treatment technologies in a more sustainable way.

2. Overview of Oil Sands

The Athabasca oil sands cover approximately 140,000 km² area of northeastern Alberta, Canada. The oil sands contain approximately 170 billion barrels of recoverable bitumen (Allen 2008 I; Holden *et al.* 2011; Frank *et al.* 2014). The oil sands ores consist of bitumen (~12 wt%), sand, silts, clays (mineral content ~85 wt%) and water (3-6 wt%). Typical bitumen recovery ranges from 88 to 95% depending on the ore grade and origin (Beier and Sego 2008; Beier *et al.* 2013). It has been estimated that the oil sands crude oil production will increase from 1.5 million barrels per day in 2010 to 3.5 million barrels per day in between 2020 and 2025, approximately (Kelly *et al.* 2010; Kurek *et al.* 2013). The bitumen extraction process of

Athabasca oil sands is mainly based on the hot water extraction method invented by Dr. Karl Clark at the Alberta Research Council (Clark and Pasternack, 1932). The water from the bitumen extraction process stores in the tailings ponds to meet the zero effluent discharge policy (Quagraine *et al.* 2005; BGC Engineering Inc. 2010). Extents of these tailings ponds cover an area of more than 170 km² and are easily visible on recent satellite photographs (Sobkowicz 2013). Currently, the tailings ponds approximately contain about 720 million m³ of process water which is expected to increase to more than 1.1 billion m³ by 2020 (Parajulee and Wania 2014; Schindler 2014). The tailings water undergoes consolidation in the pond over long period. Consolidation releases pore water from tailings which recycles back to the bitumen extraction process. Finally reduction and reclamation of the consolidated tailings are required.

3. Tailings Water

Oil sands tailings water approximately contains 70 to 80 wt% water, 20 to 30 wt% solids (sand, silt, and clays) and 1–3 wt% bitumen. In tailings pond, the coarse sand particles settle rapidly at the bottom and finer solids (clay and silts) remain in a floating layer called fluid fine tailings. The fine tailings settle to 20 wt% solids within a few weeks but require several years to reach 30–35 wt% solids. At this point, the consolidation process of fine tailings reduces considerably and the sludge is designated as mature fine tailings (MFTs) (Allen 2008 I; Beier *et al.* 2013). The bulk properties of MFTs mostly depend on a small fraction of nano-sized clay particles (20 to 300 nm). The consolidation of MFTs depends on the concentration of nanoparticles and electrolytes in the tailings water (Kotylar *et al.* 1996). MFTs take several years for complete settlement; starting from few decades to 150 years (Eckert *et al.* 1996). Currently, oil industry uses the consolidated tailings (CT) process which separates water from MFTs in a relatively short time (Renault *et al.* 1998; Jeeravipoolvarn 2005). Oil sands tailings water is highly alkaline in nature with elevated concentrations of salts, dissolved solids,

suspended solids and toxic compounds. The toxic compounds include heavy metals, naphthenic acids, organic compounds and residual bitumen (Renault *et al.* 1998; Allen 2008 I; Liang *et al.* 2011).

The major toxicity contributor in tailings water is naphthenic acids (NAs). The background concentration of NAs in surface water is approximately less than 1.0 mg/L. NAs are a complex mixture of alkyl-substituted acyclic and cycloaliphatic carboxylic acids and are negatively charged at neutral and alkaline pH values (Yen *et al.* 2004; Scott *et al.* 2005; Headley and Peru 2007; Merlin *et al.* 2007; Scott *et al.* 2008, Giesy *et al.* 2010; Sun *et al.* 2014). The degradation half-lives of NAs are approximately more than 13 years (Drzewicz *et al.* 2010). The low molecular mass NAs ($C \leq 17$) are more easily biodegradable than the high molecular mass ($C \geq 18$). Commercial naphthenic acids do not accurately reflect the potential biodegradability of NAs present in the tailings water. The structure of NAs in tailings water consists of branched alkyl chains, which are less available for microbial degradation (Scott *et al.* 2005; Johnson *et al.* 2011).

4. Environmental Impacts of Oil Sands

The oil sands industry is responsible for the largest concentration of mining activity in the world. Developments of the oil sands industry contribute significant impacts on the environment. The major adverse impacts are habitat destruction by surface mining and tailings disposal, depletion of fresh water resources, greenhouse gas and chemical emissions, and effects of toxic tailings water on environment and human health (Hodson 2013; Wislesky *et al.* 2013).

Management of the large quantities of tailings water is one of the major challenges of oil sands industry. After CT process, approximately 80% of the tailings water recycles back in the extraction process. Although CT process reduces the requirement of fresh water, it releases water with elevated concentrations of dissolved ions and toxic compounds (Allen 2008 I; BGC

100 [Engineering 2010](#)). The concentrations of dissolved ions and toxic compounds gradually
101 increase due to continual recycling of tailings water. High concentrations of dissolved ions and
102 toxic compounds may lead to poor extraction recovery of bitumen ([Allen 2008 I](#)). Eventually
103 the intake of fresh water increases in order to reduce the dissolved ions concentrations to levels
104 suitable for extraction process ([Allen 2008 I](#); [Beier and Sego 2008](#)). In addition, continual
105 recycling turns the tailings water highly saline and acutely toxic; unsuitable for releases to the
106 surface water ([Kotylar et al. 1996](#); [Drzewicz et al. 2010](#)).

107 Recent studies have monitored the presence of several priority pollutants of the oil sands
108 industry in the Athabasca River and its watershed ([Kelly et al. 2010](#); [Hodson 2013](#)). Pollutants
109 present in the tailings water are infiltrated into groundwater through the underlying clay and
110 eventually reached in the river system. The estimated concentrations of those pollutants are up
111 to the toxic limit ([Frank et al. 2014](#)). It has been evaluated that approximately 0.09 to 5.6
112 million liters of seepage water from tailings ponds travels to the Athabasca River daily; in spite
113 the presence of the seepage-capture facilities at the sites ([Parajulee and Wania 2014](#)).

114 Tailings ponds currently release approximately 40 million liters of methane gas per day,
115 although methane producing bacteria were not detected in the microbiological assessment of
116 the tailings water in 1978 ([Holowenko et al. 2000](#); [Penner and foght 2010](#)). Tailings water has
117 started to produce the methane gas nearly after 15 years of storage in the ponds when it turned
118 into anaerobic in nature. With time tailings water harbored active and diverse microbial
119 communities responsible for methane production. The representative bacterial community is a
120 large metabolically diverse group of Betaproteobacteria which includes chemoheterotrophs,
121 photolithotrophs, methylotrophs, and chemolithotrophs ([Salloum et al. 2002](#); [Penner and foght](#)
122 [2010](#)). In tailings ponds, anaerobic biodegradation of low molecular weight (C₆–C₁₀) aliphatic
123 and aromatic hydrocarbons produces the methane gas. These hydrocarbons present in

unrecovered naphtha/residual bitumen which deposits in the ponds with fresh tailings (Siddique *et al.* 2006; Siddique *et al.* 2007; Penner and foght 2010). According to a recent study, methane also produces from the biodegradation of higher molecular mass n-alkanes (C₁₄, C₁₆, and C₁₈) under methanogenic conditions after a lag phase of 180 days (Siddique *et al.* 2011). Continual recycles of tailings water accumulate large amounts of hydrocarbons and reduced chemicals like sulfides which build extremely reduced conditions favorable for methanogenesis (Quagraine *et al.*, 2005; Mehrabad *et al.* 2013).

The oil sands industry currently accounts for approximately 0.1% of global emissions which is 5% of Canada's total green house gas emissions (Government of Alberta 2009). Greenhouse gas emissions from oil sands have increased by 121%, between 1990 and 2008. Future development plan of oil sands industry indicates that the greenhouse gas emissions will continue to rise nearly three times from 2008 to 2020 (Droitsch *et al.* 2010). In addition, approximately 663 km² of land areas have been disturbed due to oil sands mine operations as of year 2009, which are about the size of the City of Edmonton (Government of Alberta 2009). Decontamination of tailings water, reduction of biogenic greenhouse gas emissions, and rapid consolidation of fine tailings are identified as the most important aspects of consideration for the sustainable development of the oil sands industry (Siddique *et al.* 2011). Implementation of an appropriate water treatment process for the management of tailings water may overcome the challenges of the oil sands industry.

5. Overview of Tailings Water Treatment Processes

There are no water quality guidelines for the oil sands industry since at present no tailings water releases to the surface water according to zero effluent discharge policy. High concentrations of salts and naphthenic acids are the target contaminants of tailings water. A water treatment process is always necessary to improve the quality of tailings water. Tailings

water can be restored to a quality acceptable for reuse within the bitumen extraction plant or for future discharge to the surface water (Sobkowicz 2012).

The only commercially operating consolidated tailing (CT) process reduces the fresh water demand in bitumen extraction process by increasing the amount of recycle water. In this process, coarse sands and high doses of gypsum (CaSO_4) (800 – 1200 g/m³ of extraction tailings) mix with fluid fine tailings to form a non-segregating mixture which undergoes self-weight consolidation and releases pore water (Renault *et al.* 1998). CT process itself contributes significant amount of calcium and sulfate ions in the recycle water which detrimentally affect the bitumen extraction efficiency and lead to a secondary increase of fresh water demand. Moreover, CT process potentially causes H₂S emissions as a result of anaerobic reduction of sulfate ions, in the presence of residual bitumen. Emissions of H₂S cause bulking of CT materials with poor consolidation characteristics (Allen 2008 I; BGC Engineering 2010; Scott and Ozum 2010). Consolidated tailing is a relatively low cost treatment process with fast consolidation rate and large scale operational capacity. The process is still under review despite over 10 years of commercial use (BGC Engineering Inc. 2010; Devenny 2010).

In recent years, issues related to oil sands tailings management acquire international attention. Major changes to current tailings management techniques need to be developed and implemented with the development of oil sands industry in order to prevent an environmental mishap. The impacts of tailings water on surface and groundwater quality have been described as one of the major upcoming issues of oil sands by the Royal Society of Canada in 2010 (Royal Society of Canada Expert Panel 2010; Sobkowicz 2012). One of the major challenges that the industry and academic communities will face soon is to find out a way of removing contaminants and reducing toxicity from the tailings water to release it safely into the environment (Giesy *et al.* 2010). Attempts have been made to reduce the toxicity of tailings

water by various wastewater treatment technologies. However, none of them seemed a technically practicable and economically feasible treatment process for the large quantities of oil sands tailings water.

Investigated treatment processes mainly include adsorption (Mohamed *et al.* 2008; Small *et al.* 2012; Zubot *et al.* 2012), membrane processes (Kim *et al.* 2012; Alpatova *et al.* 2014; Dong *et al.* 2014; Moustafa *et al.* 2014), conventional/advanced oxidation processes (Scott *et al.* 2008; Martin *et al.* 2010; Mishra *et al.* 2010; Liang *et al.* 2011; Wang *et al.* 2013; Shu *et al.* 2014; Afzal *et al.* 2015; Chen *et al.* 2015; Boudens *et al.* 2016), freeze separation (Gao *et al.* 2003; Gao *et al.* 2004), reverse-osmosis (Loganathan *et al.* 2015), biological processes (Rio *et al.* 2006; Martin *et al.* 2010; Choi *et al.* 2014; Demeter *et al.* 2014; Islam *et al.* 2014; Islam *et al.* 2015) and treatment wetlands (Werker *et al.* 2002; Allen 2008II). Some of the treatment processes such as, membrane and reverse-osmosis have been studied in pilot-scale whereas most of the other processes were investigated at the laboratory/bench-scale level for tailings water treatment (Sobkowicz 2012).

Significant fouling problems have been observed in adsorption and membrane processes because of the presence of high concentrations of dissolved solids, suspended solids and inorganic constituents in the tailings water. Fouling increased the reactivation costs of adsorbents or membranes and eventually shortened their lifetime. Pretreatment of water by coagulation-flocculation-sedimentation process generally used to overcome the fouling problems. However, pilot-scale works indicated that the coagulation-flocculation pretreatment of high volume of tailings water is difficult to control because of very high chemicals demands (Allen 2008II; Sobkowicz 2012; Kim *et al.* 2012; Alpatova *et al.* 2014; Dong *et al.* 2014).

Conventional/advanced oxidation processes did not appear to be an economically and technically viable treatment process for tailings water because of incomplete oxidation and

very high energy consumption. The oxidation processes for tailings water treatment were interrupted by the presence of high concentration of radical scavengers (chloride and bicarbonate ions) and UV attenuator (suspended particles) in tailings water. These were the primary limiting factors for the oxidation processes for tailings water treatment (Allen 2008 II; Scott *et al.* 2008; Liang *et al.* 2011). In a very recent study, gamma irradiation (GI) was used for the first time to reclaim the oil sands tailings water. In this process, GI produces very reactive species ($\text{OH}\cdot$, $\text{H}\cdot$), which have the energy to oxidize the organic compounds present in tailings water. Gamma irradiation process reduced the concentration of NAs in oil sands tailings water and fluid fine tailings by 97% and 85%, respectively. Post treatment like inoculation of native microbial communities back into the tailings water was required since gamma irradiation eliminates all biological species from water during reclamation (Boudens *et al.* 2016). The spray freezing/freezing separation process was also found to be less effective at temperatures less than -24°C although the treatment process can be used only in the cold regions. Efficiency of the spray freezing treatment process was optimum only within a very narrow temperature ranges (Gao *et al.* 2003, Gao *et al.* 2004, Allen 2008 II).

In reverse-osmosis process, different types of fouling problems such as, colloidal/particulate fouling, organic fouling, inorganic fouling and bio-fouling were observed as major limitations. Bacterial adhesion and growth were the most serious forms of membrane fouling in this process. Reverse-osmosis process can be used only after extensive pre-treatment of tailings water. Detoxification of tailings water is also required along with the reverse osmosis process, to ensure the complete removal of soluble toxic organic compounds from tailings water. The reverse-osmosis process appeared as an expensive treatment method for the large quantity of tailings water because of high treatment costs like, cost of chemicals, resin regeneration, cleanings, energy, labors, maintenance, and capital-related items (Allen 2008 II; Sobkowicz

220 [2012; Loganathan *et al.* 2015](#)). Biological processes of tailings water treatment will be
221 discussed in section 6.5.

222 Among the various treatment options for tailings water, treatment wetlands or end pit lake
223 approaches are the cheapest reclamation choice for the oil sands industry but incomplete
224 detoxification and high hydraulic retention time were reported as major limitations of
225 natural/constructed wetlands. Moreover, treatment wetlands require larger area compared to
226 other treatment options and do not function effectively in cold climates ([Werker *et al.* 2002](#);
227 [Allen 2008II](#)). Recent studies also showed that the seepage of toxic tailings water from the end
228 pit lakes or tailings ponds can cause groundwater contamination in the long run ([Frank *et al.*](#)
229 [2014; Parajulee and Wania 2014](#)).

230 **6. Biological Processes Involved in Oil Sands Resources**

231 Biological process is an integral part of oil sands starting from the ore formation and up to the
232 consolidation and reclamation of oil sands tailings. These are either naturally occurring or
233 developed biological processes. This study reviews the various biological processes involved
234 at different stages of the oil sands resources.

235 **6.1. Oil sands formation**

236 Biological process can be considered as a crucial process in oil sands history where bitumen
237 itself is the result of prolonged biological activities. The bitumen composition in the Athabasca
238 oil sands deposit is heterogeneous in nature. The formation theory of the oil sands is
239 controversial, although it has been agreed that oil sands bitumens are the natural end products
240 of the microbial degradation of conventional oil over extremely long periods of time in a
241 geological environment. Biomarker analyses of oil sands samples showed a moderate to severe
242 biodegradation level according to the Peters and Moldowan scales. The heterogeneity of
243 bitumen may have resulted from the complex and diverse biodegradation of conventional oil
244 which possibly followed different altered degradation pathways ([Fustic *et al.* 2006](#)).

6.2. Bitumen extraction

Clark hot water bitumen extraction process is a complex and capital intensive process which consumes huge amount of water. In this process, two to four barrels of water are required to extract one barrel of bitumen and four cubic meters of tailings water are produced from the extraction process of one cubic meter of oil sands ore (Holowenko *et al.* 2002; Giesy *et al.* 2010). The bitumen extraction process is a key to current oil sands economy. Bitumen recovery from poor weathered ores is very low by the Clark hot water bitumen extraction process. The poor weathered ores are abundant in oil sands deposits. Biological processes may help to overcome the limitation of hot water bitumen extraction process. According to recent studies, microbial pretreatment of poor weathered ores can improve the recovery of bitumen. Microbial pretreatment of poor weathered ores with *Bacillus subtilis* has improved the bitumen recovery by 90-94% whereas no bitumen was recovered from the untreated ores. In this process, bacterial metabolic products or biosurfactants might altered the surface wettability of the oil sands solid particles from hydrophobic to hydrophilic state which decreased the adhesion between bitumen and solids (Ding *et al.* 2014 a).

In another study, bitumen recovery from the poor weathered ores was improved by 98% after the pretreatment with *Pseudomonas aeruginosa* for 20 days. Microbial activities such as, production of biosurfactants, degradation of the asphaltene component of the bitumen and reduction of the bitumen viscosity due to gas production might be solely or collectively responsible for the enhanced bitumen recovery from the surface of sand grains. Choice of microorganisms and application of the pretreatment process at field scale level are the issues of concern for large scale industrial application (Ding *et al.* 2014 b). Biological processes can be used to develop more versatile and effective bitumen extraction process, especially for the poor weathered ores of oil sands deposits.

6.3. Tailings pond *in-situ* processes

Microorganisms are naturally active throughout the entire process of oil production beginning from the formation of oil sands deposits and up to the tailings ponds. Tailings ponds contain a wide variety of active microbial communities both aerobic and anaerobic in nature (Golby *et al.* 2012; An *et al.* 2013 a; An *et al.* 2013 b; Fru *et al.* 2013; Mehrabad *et al.* 2013). *Pseudomonas*, *Thauera*, *Hydrogenophaga*, *Rhodoferrax*, and *Acidovorax* were found as the dominant species in the mixed biofilm communities developed from the direct culturing of microbes from a tailings pond sample (Golby *et al.* 2012).

The microbial activities in an active tailings pond were investigated in previous studies. An active tailings pond routinely receives fresh tailings water after CT treatment. The microbial communities were distributed as a function of depth in an active tailings pond. The activities of aerobic bacteria were limited only at the surface waters of the pond. Naphthenic acid degradation, methane oxidation and sulfide oxidation were recognized as three major microbial processes at the surface waters of the pond (Padron E.R. 2013). Microbial gene sequences indicated that the aerobic surface layer of the pond water is habitat of an unusual mixture of obligate aerobes, obligate anaerobes and facultative anaerobes which are very much different from the bacterial community found in the rest of the pond (Padron *et al.* 2011; Mehrabad *et al.* 2013). The predominant operational taxonomic unit identified in the surface water of the pond was belonged to Betaproteobacteria, particularly in Comamonadaceae and Rhodocyclaceae families, which are mostly facultative anaerobes (Mehrabad *et al.* 2013). These metabolically diverse species are known for their ability of phototrophy, lithotrophy, organotrophy, aerobic respiration, fermentation, iron reduction and denitrification. Identified species were hydrocarbon degraders such as *Acinetobacter*, *Geobacter*, *Rhodoferrax*, *Methylibium*, *Hydrogenophaga*, *Pseudomonas*, *Acidovorax*; methanotrophs like *Methylocaldum*, *Methylomonas*; methylotrophs like *Methyloversatilis*, *Methylophilaceae*, *Xanthobacter*; N₂-

fixing *Azospirillum*; Planctomycetes like *Gemmata*; and several other species such as *Pedomicrobium*, *Alcaligenes*, *Porphyrobacter* etc (Padron *et al.* 2011; Mehrabad *et al.* 2013; Padron E.R. 2013).

An active tailings pond is mostly anaerobic in nature except the limited aerobic surface layer. Very high chemical and biological oxygen demands of the pond water due to continuous addition of the hydrocarbons and gypsum limited the activity of aerobic bacteria only at the surface waters of the pond. A wide variety of anaerobic microbial species were identified in the Pyrosequencing survey of microbial genes collected from different depths of a tailings pond. Identified species were belonged to Proteobacteria, Euryarchaeota, Firmicutes, Actinobacteria, Chloroflexi, and Bacteroidetes phyla (An *et al.* 2013 a). Methanogenesis and sulfate reduction were identified as the major anaerobic microbial processes in an active pond (Padron E.R. 2013). An active tailings pond often receives sulfate with fresh tailings from the gypsum addition in CT process. In active tailings pond, methanogenesis process were inhibited due to the addition of sulfate in the tailings water (Padron *et al.* 2011). *Desulfocapsa* and *Desulfurivibrio* species were the most prevalent sulfate reducing bacteria (SRB) in the pond. An active tailings pond gradually turned into methanogenic after the depletion of sulfate and methanogenesis dominated the anaerobic zone of the pond (Padron *et al.* 2011; Padron E.R. 2013; Liu *et al.* 2015).

The acetoclastic pathway was found as the predominant methanogenic pathway in the tailings pond. However, it was also suggested that both hydrogenotrophic and acetoclastic pathways occur simultaneously in different areas or micro-sites of the tailings ponds (Penner and foght 2010). Several methanogenic species were active as a function of depth in the anaerobic zone of the pond. Methane producing achaea like *Methanosaeta* and *Methanolinea* were predominant at the upper portion of the anaerobic zone, *Methanospirillum* and *Methanosarcina*

were active in the middle and *Methanomethylovorans* and *Methanobacterium* were observed mainly in the lower or deeper zones of the pond (Padron *et al.* 2011). In the deeper anaerobic zones of the pond, the methanogens were depended on the syntrophs for their survival. *Pelotomaculum*, *Syntrophus*, and *Smithella* species were found as syntrophs; among them *Pelotomaculum* was the most abundant genus (Padron *et al.* 2011). SRB, syntrophs, and methanogens were active throughout the anaerobic zones of the tailings pond with a complex interplay among them. In addition, other varieties of anaerobes such as, hydrocarbon degrader *Pseudomonas*, *Acinetobacter*, *Acidovorax* and iron and sulfur cycling species *Thiobacillus*, *Acidovorax*, *Rhodoferrax* were also present in the anaerobic zones of the pond. Abundant presence of *Brachymonas*, *Thiobacillus*, and *Cellulomonas* were identified only in the deepest zones of the pond which apparently assisted in the consolidation of the fine tailings (Padron *et al.* 2011; An *et al.* 2013 b; Padron E.R. 2013).

During long term study of an active tailings pond, same microbial species were observed at every sampling time although their relative abundance was changed with time depending on the availability of electron acceptors and fresh tailings input (Padron E.R. 2013). The microbial activity in an active tailings pond decreased with the reduction of fresh tailings input. An active tailings pond turned into inactive state after stopped receiving the fresh tailings input. A shift in the major microbial community structure and functions was observed in inactive tailings pond from its active state. Methanogens no longer dominated the inactive pond and a few putative hydrocarbon degraders became prevalent. *Pseudomonas* and *Acidovorax* species were mainly identified in the inactive tailings pond. Unlike the active pond, microbial activity was concentrated only near the surface water of the inactive pond (Padron *et al.* 2011; Padron E.R. 2013).

6.4 Consolidation of fine tailings

The hot water bitumen extraction process has a great impact on the tailings properties. Caustic

soda used in this process promotes the flotation of fine particles in the tailings (Jeeravipoolvarn S. 2005). Consolidation of oil sands tailings considerably reduces after reaching a solid content value of about 30-35% (Jeeravipoolvarn S. 2005). CT process is used by the oil industries to accelerate the settling of tailings. The commercial CT process is based on self-weight consolidation theory and does not consider the contribution of biodensification in the consolidation of fine tailings (Siddique *et al.* 2014 I). However, studies revealed that the methanogenesis process in the tailings pond enhanced the densification of fine tailings by changing its pore water chemistry. Consolidation of fine tailings due to microbial activity or biodensification was reported along with the self-weight consolidation of tailings. An increase in the consolidation of fine tailings was observed with the increase of methanogenic activity of microorganisms. Production of bacterial extracellular polymeric substances was observed in methanogenic condition which might help to aggregate the fine tailings. It is not fully known which factors of the methanogenesis process are responsible for the biodensification of tailings (Fedorak *et al.* 2003; Bordenave *et al.* 2010; Padron *et al.* 2011).

Recent studies proposed the fundamentals of biodensification and two biodensification pathways which might increase the consolidation of MFTs. Pathway I is an indirect process in which microbes alter the pore water chemistry (pH, ionic strength) which accelerates the consolidation of tailings (Siddique *et al.* 2014 I). Pathway II is more important and direct biogeochemical pathway in which a group of anaerobic bacteria (predominantly *Clostridiales*, *Synergistaceae*, and *Desulfobulbaceae*) and Archaea (*Methanolinea/Methanoregula* and *Methanosaeta*) transform the Fe (III) minerals into amorphous Fe (II) minerals during methanogenic metabolism of an external labile organic carbon source in the MFTs. The amorphous Fe (II) minerals entrap the electronegative clay particles. As a result, surface charge potential (repulsive forces) of the clay particles reduces and thereby helps in aggregation of

clay in MFTs (Siddique *et al.* 2014 II). Several other microbes responsible for the biodensification of fine tailings were also found in the tailings pond. These various microbially-mediated consolidation processes occurred simultaneously in MFTs and enhanced the consolidation (Bordenave *et al.* 2010; Padron *et al.* 2011; Siddique *et al.* 2014 II).

6.5 Treatment of tailings water

The major challenge of oil sands industry is to reduce the NAs toxicity in tailings water. In previous studies, attempts have been made to develop biological processes for tailings water treatment. It was observed that some fractions of NAs present in tailings water were recalcitrant toward biodegradation because of their complex cyclical molecular structures (Quagraine *et al.* 2005; Martin *et al.* 2010; Loganathan *et al.* 2015). Naphthenic acids are the major toxic substances of tailings water and NAs present in the tailings water are very much different from the commercially available NAs. Microorganisms indigenous to tailings ponds were able to degrade only 25% of tailings water NAs under aerobic condition while fully degraded the low molecular mass commercial NAs (Scott *et al.* 2005). In a study, researchers isolated two co-occurring bacterial species, *Pseudomonas putida* and *Pseudomonas fluorescens* from tailings pond sediments by using commercial NAs as the sole carbon source. The isolated co-cultures were able to remove more than 95% of the commercial NAs whereas only a small portion of tailings water NAs was removed. Moreover, none of the species was able to degrade commercial NAs (only 15%) individually. The synergistic effect of these species might be the result of a co-metabolism process (Rio *et al.* 2006). In anaerobic zones of the tailings pond, methanogens only degraded the simple NAs and the carboxylated side chains of NAs into methane and inhibited by the presence of high molecular mass NAs (Holowenko *et al.* 2001).

Various biological processes of tailings water treatment have been developed and studied to increase the NAs degradation efficiency of bacterial community indigenous to tailings ponds

and are still ongoing. The performances of biofilm or biofilm reactor in degradation of NAs were investigated; no such difference in NAs degradation efficiency was observed between biofilm and suspended cultures of indigenous bacteria (Demeter *et al.* 2014). Ozonation of the tailings water prior to biodegradation was carried out in several studies to increase the degradation rate of NAs (Martin *et al.* 2010; Wang *et al.* 2013; Choi *et al.* 2014; Islam *et al.* 2014; Islam *et al.* 2015). Ozonation oxidized the biopersistent NAs fraction in tailings water and thereby accelerated the biodegradation of NAs. However, a residual NAs fraction was detected in the tailings water even after the extensive treatment (Martin *et al.* 2010). The effects of different ozone pre-treatment doses on the performance of endogenous microbial populations for the treatment of oil sands tailings water were evaluated. It was observed that at optimal ozone pre-treatment dose of 50 mg/L; maximum organic compounds were removed through biodegradation (Dong *et al.* 2015). In toxicity test, ozonation followed by 28-day biodegradation reduced or completely removed the toxicity of tailings water toward mice and *Vibrio fischeri*, respectively (Wang *et al.* 2013). Various types of biofilm reactors were studied to remove toxicity from oil sands tailings water. The performances of batch and continuous biofilm reactors to remove NAs toxicity from ozone-treated oil sands tailings water were evaluated. Continuous flow biofilm reactors performed better compared to the batch flow biofilm reactors. In both the reactors, low molecular mass NAs (carbon number less than 16) were preferentially degraded compared to the high molecular mass NAs. The biodegradation processes in the biofilm reactors were not able to remove NAs toxicity completely; but transformed the NAs into simpler forms. Ozonation killed certain microbial strains in the tailings water and facilitated the growth of remaining viable strains in the biofilm reactors by providing less complex NAs after oxidization (Choi *et al.* 2014). Sequencing batch reactors (SBR) with two different inoculums

of activated sludge (AS) and mature fine tailings (MFT) were used to remove the acid-extractable organics (AEO) from tailings water. The study demonstrated that the maximum removal of acid-extractable organics (AEO) was 8.7% and 16.6 % in the AS-SBR and the MFT-SBR, respectively with a hydraulic retention time of one day. Beta- and gamma-Proteobacteria were found as the predominant microorganisms in both the reactors (Choi and Liu 2014). Pre-treatment of oil sands tailings water with 30 mg/L ozone dose increased the NAs removal rate of moving bed biofilm reactor (MBBR) from 34% up to 79% after a treatment period of 210 days. Ozonation also increased the removal rate of acid-extractable organics (AEO) from 18% up to 41%. In ozonation combined MBBR process, 68% of NAs were removed by ozonation and only 11% of NAs were removed by the following MBBR (Shi *et al.* 2015). The integrated fixed-film activated sludge (IFAS) reactor consisting of both suspended growth and attached biofilm system was also used for the treatment of tailings water. The IFAS reactor inoculated with aerobic municipal wastewater activated sludge removed 43% of NAs from tailings water after 11 months of operation while ozone pre-treatment enhanced the NAs removal efficiency of IFAS reactor up to 80%. It was observed that the biodegradation of NAs decreased significantly as the cyclization number of NAs increased (Huang *et al.* 2013; Huang *et al.* 2015). In another study, immobilized soil/sediment bioreactors (ISBRs) containing biofilm established from oil sands sediment microorganisms, achieved a steady removal rate of NAs (2.32 mg/L/day) at a residence time of 160 hours (McKenzie *et al.* 2014). In recent study, a modified Ludzacke Ettinger membrane bioreactor (MLE-MBR) with a submerged ceramic membrane was inoculated with mixture of wastewater anoxic sludge and mature fine tailings sediments and used for the treatment of tailings water. The reactor achieved the NAs removal of 24.7% after 361 days of operation (Xue *et al.* 2016). Adsorption of NAs by granular activated carbon (GAC) and biodegradation by endogenous

bacteria were studied in fluidized bed biofilm reactors for the treatment of raw and ozone-treated oil sands tailings water. Fluidized bed biofilm reactors improved the removals of classical and oxidized NAs (96.2% and 77.1%) from ozone-treated tailings water compared to raw tailings water (93.3% and 73.7%). In granular activated carbon (GAC) biofilm reactor, a combination of adsorption and biodegradation process was involved in the treatment of tailings water. In GAC biofilm reactor, biodegradation and adsorption process removed 14% and 63% of NAs from raw tailings water, respectively. Pre-ozone treatment of tailings water improved the NAs removal efficiency of biodegradation and adsorption process of the reactor to 18% and 73%, respectively. Ozone dose (30 mg/L) combined with GAC biofilm treatment significantly increased NAs removal rates. Ozone pre-treatment decreased the microbial diversity in raw tailings water while increased the microbial diversity in the GAC biofilm. The 16S rRNA gene pyrosequencing technique showed the predominance of Alpha- and Gammaproteobacteria in the biofilm. Diverse microbial communities of *Burkholderia multivorans*, *Polaromonas jejuensis* and *Roseomonas* sp were found in the biofilm colonized on the surface of GAC. An addition of exogenous NA-degrading microorganisms was recommended for the further enhancement of the treatment process (Islam *et al.* 2014; Islam *et al.* 2014a; Islam *et al.* 2015; Islam *et al.* 2015a; Islam *et al.* 2016). Ozone oxidation prior to biodegradation converted some of the NAs into less toxic form but could not remove all the recalcitrant NAs from the tailings water. Ozonation is also not an economical process for the treatment of wastewater containing high amounts of suspended solids, biochemical oxygen demand, chemical oxygen demand, or total organic carbon (USEPA 1999; Multani and Shah 2014). Ozonation cost of huge tailings water, formation of ozonation byproducts from metals and inorganic substances present in tailings water and their toxicity are the main disadvantages (Martin *et al.* 2010).

Apart from different biofilm reactors, microbial fuel cells were used for the treatment of tailings water with simultaneous generation of electricity from the mixture of mature fine tailings and oil sands process-affected water. The microbial fuel cells treatment removed 27.8% of the total COD, 81.8% of the soluble COD and 32.9% of the total acid extractable organics from tailings water along with the continuous generation of electricity (Jiang *et al.* 2013).

Although algal communities have always been associated with the tailings ponds, the NAs degradation abilities of these algae were rarely studied and very less is known compared to the indigenous bacteria. Marine alga *Dunaliella tertiolecta* was used for NAs degradation study. The alga was found to be capable of degrading model NAs followed by the β -oxidation pathway; however could not degrade the recalcitrant fractions of tailings water NAs like other biodegradation studies (Quesnel *et al.* 2011). Another study exhibited the potential of indigenous algae in bioremediation of toxic acid-extractable organics (AEOs) present in oil sands tailings water. Phosphate biostimulation process was used to promote the growth of algae indigenous to oil sands tailings water which significantly reduced the toxicity of AEOs in 12 weeks (Quesnel *et al.* 2015).

7. Proposed Feasible Solution for Tailings Water Treatment

Several wastewater treatment processes have been studied since 1980 for the detoxification of the oil sands tailings water (Allen 2008 II); however none of those processes has turned out to be a technically practicable and economically feasible option. Cost of treatment per unit volume of tailings water is one of the most important factors of consideration because of the substantial volumes of tailings water. Ozonation is not economically feasible for treatment of oil sands tailings water due to a heavy demand of energy and chemicals, especially when considering the huge amount of tailing water stored in Athabasca region (976 million m³).

Moreover, by-products of ozonation are still being evaluated for their associated toxicity issues. Increase of toxicity was also observed during ozonation of some types of organic compounds (Xue *et al.* 2016).

Biological process is known as a simple, environment-friendly, energy efficient and most economical process of wastewater treatment. Review of biological processes involved at different stages of oil sands revealed that the process is an integral and natural part of the oil sands history. Consequently, biological processes may have potential to remove the toxicity of tailings water successfully.

Dealing with the recalcitrant fraction of NAs and its toxic effects on microorganisms are the major limitations of any traditional biological processes. Review of recent developments on oil sands tailings water treatment have anticipated the use of advance emerging water treatment technologies for successful tailings water treatment. Advances in biological treatment may lead to new technologies for tailings water treatment (Allen 2008 II, Scott *et al.* 2008). Thus, selection of microorganisms likely capable of degrading NAs and adequate protection to those microorganisms against tailings water toxicity may successfully deal with the limitations of the conventional biological processes.

Biofilm-based bioprocesses have been mostly used for the treatment of oil sands tailings water until now. Biofilm-based bioprocess is difficult to control because of the intrinsic diversity and dynamic properties of microbial biofilm. Entrapment of cells in polymeric material has proposed as a better alternative of biofilm-based bioprocess to achieve long-term robust performance in engineering applications (Zhang *et al.* 2014). It was also observed that the entrapment of cells in polymeric matrices showed significantly higher metabolic activity in comparison with immobilization of cells as biofilm on a support material (Mathew *et al.* 2013). It is now known that the extracellular DNA (eDNA), produced by active secretion or

513 controlled cell lysis is a major structural component of many different microbial biofilms. The
514 self-organization of cells in an actively expanding biofilm takes place through the formation of
515 a complex network of interconnected trails which is facilitated by eDNA (Gloag *et al.* 2013;
516 Kahrstrom C.T. 2013). Studies revealed the various important role of eDNA in biofilm such as,
517 disseminating genes amongst different micro-organisms, storing nutrients and strengthening of
518 biofilm matrix by adhering cells to abiotic surfaces through acid–base interactions. Anionic
519 eDNA also chelates cations and restricts the diffusion of cationic antimicrobials. Moreover,
520 chelation of eDNA with magnesium ion (Mg^{2+}) aggravates the genetic response of cells to
521 resist against adverse conditions (Jakubovics *et al.* 2013; Okshevsky and Meyer 2015). It was
522 found that the production of extracellular DNA (eDNA) in entrapped cells was substantially
523 higher than that in biofilms (Zhang *et al.* 2014) which might provide more robustness to
524 entrapped cells compared to biofilms. This study proposes the use of entrapped cells system as
525 a successor feasible technology for the treatment of oil sands tailings water.

526 Entrapment of microbial cells in polymer matrices is one of the most popular approaches of
527 cell immobilization (Scott 1987). Cell entrapment is considered as one of the emerging
528 technologies in wastewater treatment (Siripattanakul and Khan 2009). The use of entrapped
529 cells in wastewater treatment is drawing attention as it offers several advantages over the
530 suspended cells. One of the frequently reported advantages is the enhanced production
531 efficiency or enhanced metabolic activity (Junter *et al.* 2002). Entrapment of cells within
532 polymer matrix increases the biological strength by improving the tolerance or protection of
533 cells from physicochemical challenges, such as temperature, pH, toxic substrates or end-
534 product inhibition. High biomass concentration, easy separation of cells from the treatment
535 system, and prevention of cells washout are the added advantages of entrapped cells in
536 wastewater treatment applications (Pramanik and Khan 2008). Entrapped cells system may be

an efficient and cost effective process for oil sands tailings water treatment.

Variations in the temperature of tailings water due to the hot water bitumen extraction process can decrease the efficiency of the suspended microbial cells. In entrapped condition, polymer matrices protect the microbial cells from the direct exposure of such changes. The choices of entrapment media and cell-to-media ratio (cell loading) are the crucial parameters for the performance of an entrapped cells-based wastewater treatment process. An appropriate entrapment media can provide necessary protection to the microbial cells against tailings water toxicity while facilitates the adequate supply of substrates for the growth of the cells and the rejection of metabolites from cells (Pramanik and Khan 2008; Pramanik and Khan 2009). It creates a protective micro-environment condition for the microbial cells which promotes physiological changes of the cells. Altered physiology of entrapped cells might be responsible for its different behavior than free cells. The nature of physiological changes of entrapped cells also depends on the characteristics of the polymeric matrices used for entrapment (Pramanik *et al.* 2011a). Physiological changes associated with certain entrapment media are strategies for the cells to survive or grow well under particular conditions that are related to key operational and economical factors in wastewater treatment (Nedovic and Willaert 2004; Pramanik *et al.* 2011a; Pramanik *et al.* 2011b). Typical representative image of entrapped cell beads, SEM (scanning electron microscope) images of beads cross-sections and the development of the microbial population inside the beads are presented in *Figure 1*. Judicious selection of entrapment media and cell loading can be able to increase the efficiency of entrapped cells for tailings water treatment, significantly.

Analysis of denaturing gradient gel electrophoresis (DGGE) data indicated that the content of naphthenic acids greatly influenced the structure of the indigenous bacterial community in the tailings pond (Hadwin *et al.* 2006). Exposure to slightly changed NAs content than usual

caused changes to the bacterial community structure in the tailings pond ([Hadwin *et al.* 2006](#); [Padron E.R. 2013](#)). This can lead fluctuations in the efficiency of the tailings water treatment process by indigenous bacterial community. Entrapment provides stability to the bacterial community structure against such changes and thereby maintains steady treatment efficiency. It was found that the microbial communities indigenous to tailings water were not much efficient to remove its toxicity. In bioremediation, sometimes indigenous microorganisms do not have the ability or take unreasonably long time to degrade contaminants. Bioaugmentation is very much useful in these situations to enhance the treatability of contaminants. Treatment efficiency of entrapped cells system can further increase by the bioaugmentation of microbial community indigenous to tailings ponds ([Quagraine *et al.* 2005](#)).

Studies were carried out to get an overview about the changes in microbial community structure of oil sand ores in response to the bitumen production processing. Prominent changes in the aerobic cultivable communities were observed between the processed and the original oil sands samples; although all the analyzed samples were originated from the same geological setting. The original aerobic microbial community of oil sands ores was almost completely replaced by new community which established during the bitumen production processing ([Noah *et al.* 2014](#)). High abundance of Actinobacteria and the low abundance of Betaproteobacteria were found in the original oil sands ores whereas high abundance of Betaproteobacteria and particularly Comamonadaceae were found in the tailings ponds. Bitumen extraction process and continual recycle of tailings water are responsible for the changes in aerobic microbial community structures ([An *et al.* 2013 b](#); [Noah *et al.* 2014](#)). The predominant anaerobic microbial communities of tailings ponds could not successfully degrade the recalcitrant fractions of tailings water NAs in previous studies. NAs are natural component of petroleum reserves and are thought to have originated from unprecedented degree of aerobic

microbial degradation of petroleum hydrocarbons (Scott *et al.* 2005; Rowland *et al.* 2011). Past experimental evidences indicate that NAs degradation is predominantly an aerobic process and NAs degradation rate decreases with the depletion of dissolved oxygen in the tailings water (Lai *et al.* 1996; Rio *et al.* 2006). Thus, the original aerobic microbial community of oil sands ores may have the potential to further degrade the recalcitrant fractions of NAs in tailings water. Bioaugmentation with the original aerobic microbial community of oil sands ores may enhance the NAs degradation efficiency of the entrapped bacterial community indigenous to tailings ponds or entrapped indigenous cells system.

Increase of alkyl side chain branches in the structure of tailings water NAs is responsible for its recalcitrant nature against biodegradation (Whitby 2010; Johnson *et al.* 2011). Biodegradation study of aromatic alkanolic acids with branched alkyl side chains was conducted to identify the predominant microbial communities capable to degrade the recalcitrant fractions of NAs. Identified key microorganisms which might be involved in the aromatic alkanolic acid transformations were *Burkholderia*, *Pseudomonas* and *Sphingomonas* species (Johnson *et al.* 2011). Addition of appropriate recalcitrant naphthenic acids degrading species along with the original aerobic microbial community of oil sands ores may further strengthen the bioaugmentation process. Bioaugmentation of tailings water indigenous species with the chosen microorganisms followed by entrapment may provide an efficient and cost effective oil sands tailings water treatment method. *Table 1* summarizes the application potential of the reviewed and proposed tailings water treatment technologies.

8. Conclusion

The oil industry is a major driver of Canada's economy and accounts for about 5% of Canada's gross domestic product (GDP); although a continuing controversy is always there because of environmental and health issues associated with it. The Royal Society of Canada identified the

609 impacts of tailings water on surface and groundwater quality as a major upcoming issue of the
610 oil sands. It is necessary to come up with a water treatment technology which is technically
611 and economically applicable to manage the large volume of tailings water for sustainable
612 development of the oil industry. Emerging water treatment technologies like entrapped cells
613 systems have been used to overcome limitations of the conventional treatment processes.
614 However, there is no study on the application of entrapped cells system in tailings water
615 treatment. This study introduces the application of entrapped cells system for tailings water
616 treatment after identifying the integral part of biological processes in oil sands history and
617 limitations of the existing tailings water treatment technologies. Entrapped cells system may
618 use as a technically and economically approachable process of tailings water treatment. Bio-
619 augmentation of microbial community indigenous to tailings water with carefully selected
620 microorganisms followed by an appropriate entrapment procedure can be a promising
621 technology for biodegradation of the recalcitrant NAs in the tailings water. Investigations on
622 optimization and successful implementation of the proposed entrapped cells system for tailings
623 water treatment is recommended for future work.

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Figure 1: (a) Optical image of **alginate-chitosan** beads ([Westman *et al.* 2012](#)). SEM images of the cross-section of (b) alginate, and (c) polyvinyl alcohol (PVA) bead. SEM images of the development of microbial population inside (d) alginate ([Pramanik *et al.* 2011a](#)), and (e) PVA bead.

Treatment Technology	Disadvantages	Benefits
Consolidated tailing (CT) process	Contribute dissolved ions and toxicity, H ₂ S emissions	Low cost, large scale operational capacity
Adsorption	Fouling problems, high operational cost, reactivation cost of adsorbent, requirement of pre-treatment	-
Membrane processes	Fouling problems, high operational cost, reactivation cost of membrane, requirement of pre-treatment, energy intensive	-
Conventional/advanced oxidation processes	Incomplete oxidation, very high energy consumption, high operational cost	-
Freeze separation	Effective only within a very narrow temperature range	-
Reverse osmosis	Several types of fouling problems, requirement of extensive pre-treatments, high treatment cost	-
Biological processes	Incomplete toxicity removal, substrate (tailings water) toxicity, requirement of pre-treatment like ozonation, additional treatment cost of ozone oxidation, formation of toxic ozonation by-products	Large scale operational capacity
Entrapped bio-augmented cells system	No background information, preliminary studies are needed for optimization and implementation of the technology	Enhanced metabolic activity, protection of cells against tailings toxicity, cost effective, large scale operational capacity

Table 1: Application potential of the tailings water treatment technologies

Figure 1: (a) Optical image of **alginate-chitosan** beads ([Westman *et al.* 2012](#)). SEM images of the cross-section of (b) alginate, and (c) polyvinyl alcohol (PVA) bead. SEM images of the development of microbial population inside (d) alginate ([Pramanik *et al.* 2011a](#)), and (e) PVA bead.