MICROBIOLOGICAL AND GEOTECHNICAL CHARACTERIZATION OF OIL SANDS TAILINGS IN RELATION TO ACID ROCK DRAINAGE

by

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Abstract

Traditional disposal of oil sands fine tailings has been a challenge to the industry, and developing new disposal technologies, such as surface deposition, poses alternative challenges. Unsaturated surface deposits of paraffinic froth treatment (TSRU) tailings have the potential to generate acid rock drainage (ARD). To develop environmentally-sound prevention measures to mitigate this risk, such as phage therapy, knowledge of the microbial and geotechnical characteristics of TSRU tailings is essential. To investigate the native microbial population potentially related to ARD generation in surface deposits of these tailings, enrichment cultures were used to isolate sulfur-oxidizing microbes at three stages of ARD development: pH 7, 4.5 and 2.5. Microbial growth studies were performed to establish the conditions for phage growth. The geotechnical properties and unsaturated behaviors were established of four variations of the tailings: untreated, polymer-amended, and two sand-mixed tailings. The findings indicate that TSRU tailings host native microbial communities potentially capable of playing a role in ARD development, and that the unsaturated tailings behavior would likely expedite this process upon surface deposition and subsequent desaturation. These results highlight the need for the development of ARD prevention technologies in the event of surface deposition of TSRU tailings. This research adds to the limited knowledge of microbial communities in oil sands tailings, and to the geotechnical performance of TSRU tailings.
Preface

When I joined this research team, Dr. Roberts and Dr. Siddiqua had initiated the direction for this research. I helped progress the direction of the research during the experimental phase.

A version of chapter 2 will be submitted to a journal for publication. Dean, C., Xiao, Y., Roberts, D.J. Enriching acid rock drainage related microbial communities from surface-deposited oil sands tailings. Roberts, D.J., and Xiao, Y. contributed to experimental design and manuscript editing. Xiao, Y. also performed the reactor set-up and some of the microbial growth studies. I performed various aspects of the lab work, including sampling, bacterial isolation, DNA extraction, growth studies, as well as helping supervise undergraduate students also performing some of these duties. I compiled and analyzed all of the data presented, and wrote the manuscript.

A portion of chapter 3 was published. Dean, C., Siddiqua, S., Roberts, D., Siemens, G. Characterization of oil sands tailings containing a significant hydrocarbon content and a synthetic polymer. In the 67th Canadian Geotechnical Conference Proceedings. Siddiqua, S., Roberts, D., and Siemens, G., contributed to the analysis of the results and editing the manuscript. I performed all of the laboratory experiments, analyzed the data and wrote the manuscript.

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manuscript. I performed all of the laboratory experiments, analyzed the data and wrote the manuscript.
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List of Symbols

H’: Shannon-Weaver diversity index

J’: Evenness

C_u: Coefficient of uniformity

C_c: Coefficient of curvature

Ck: cracking

d_{10}: Effective grain size (μm)

d_{50}: Mean grain size (μm)

G_s: Specific gravity

ρ_s: Density of solids (g/cm^3)

θ: Volumetric water content

Δ: Change

ε_o: Initial void ratio

ε_n: Void ratio for a given period

w: Gravimetric water content
$S$: Degree of saturation

$\psi$: Suction (kPa)

$M_d$: Dry mass (g)

$V_{Tn}$: Total volume for a given period (cm$^3$)

$e(w)$: Void ratio based on gravimetric water content

$a_{sh}$: Minimum void ratio

$b_{sh}$: Slope of the line of tangency

$c_{sh}$: Shape of the shrinkage curve

$w_s$: Saturated water content

$a_{fb}$: Fitting parameter related to the air entry value

$j_{fb}$: Fitting parameter related to the air entry value

$n_{fb}$: Fitting parameter related to the steepest part of the curve

$k_{fb}$: Fitting parameter related to the steepest part of the curve

$m_{fb}$: Fitting parameter related to the shape of the curve

$l_{fb}$: Fitting parameter related to the shape of the curve
List of Abbreviations

16SrRNA: 16S ribosomal ribonucleic acid

ABI: Applied Biosystems

AER: Alberta Energy Regulator

AEV: Air Entry Value

AFD: Atmospheric Fines Drying

AM: Acidocella Medium

ARD: Acid Rock Drainage

ASTM: American Society for Testing and Materials

ATCC: American Type Culture Collection

BLAST: Basic Local Alignment Search Tool

bSOM: Basal Sulfur-oxidizing Medium

bSOM-PA: Basal Sulfur Oxidizing Medium with Pure Agar

bSOM-PC: Basal Sulfur Oxidizing Medium with Plate Count Agar

C1D: Cell 1 Downstream
C1U: Cell 1 Upstream

C5D: Cell 5 Downstream

C5U: Cell 5 Upstream

CFU: Colony Forming Units

COSIA: Canada’s Oil Sands Innovation Alliance

CST: Consolidated Tailings

CT: Composite Tailings

D-074: Directive 074

DNA: Deoxyribonucleic acid

DO: Dissolved Oxygen

DSMZ: Deutsche Sammlung von Mikroorganismen und Zellkulturen (German Collection of Microorganisms and Cell Cultures (GmbH))

EC: Electrical Conductivity

ERCB: Energy Resource Conservation Board

FDS: Total Fixed Solids

FFT: Fluid Fine Tailings
FTT: Froth Treatment Tailings

IO: Iron-oxidizing Medium

IOM: Iron-oxidizing Microbe

LL: Liquid Limit

MFT: Mature Fine Tailings

MPN: Most Probable Number

NP100/0: No Polymer 100% tailings/0% sand

NST: Non-segregating Tailings

OD520: Optical Density at 520 nm

OD_{600}: Optical Density at 600 nm

P100/0: Polymer 100% tailings/0% sand

P50/50: Polymer 50% tailings/50% sand

P30/70: Polymer 30% tailings/70% sand

PA: Pure Agar

PCA: Plate Count Agar
PCR: Polymerase Chain Reaction

PL: Plastic Limit

PSD: Particle Size Distribution

RDP: Ribosomal Database Project

RNA: Ribonucleic acid

RO: Reverse Osmosis

SEM: Scanning Electron Microscope

SFR: Sands to Fines Ratio

SL: Shrinkage Limit

SOM: Sulfur-oxidizing Microbe

SWCC: Soil-water characteristic curve

TDS: Total Dissolved Solids

TM: *Thiobacilli* Medium

TSRU: Tailings Solvent Recovery Unit

TT: Thickened Tailings
USCS: Unified Soil Classification System

VDS: Volatile Dissolved Solids

WT: Whole Tailings
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Dedication

To my family; past, present and future.
Chapter 1: Introduction

The environmental impact of tailings disposal into ponds from mining operations in the Athabasca oil sands has become a critical issue for the operating companies due to the vast area, toxicity and reclamation issues. Therefore, significant research is being conducted to develop new technologies for tailings disposal, including ground surface deposition where unsaturated conditions could develop. This poses a potential for the generation of acid rock drainage (ARD), where sulfide mineral surfaces are oxidized producing sulfuric acid. ARD is a long term, worldwide problem affecting areas with exposed sulfide mineral surfaces. Although there are some prevention and treatment methods for ARD, all of them have drawbacks and are not sustainable for operations at the scale of the oil sands. Therefore, novel methods are needed to control ARD in this environment.

1.1 Oil Sands Tailings

The Athabasca oil sands in northern Alberta, also known as the Fort McMurray formation, underlay an area of 140, 200 km$^2$ with reserves estimated to contain 170 billion barrels of bitumen (www.oilsands.alberta.ca/about.html). In 2014 the oil sands were producing an average of 1.9 million barrels of bitumen per day (www.oilsands.alberta.ca/about.html), generating 262,000 m$^3$ of tailings per day (http://www.energy.gov.ab.ca). The bitumen is primarily extracted using the Clark hot water extraction process, where the crushed ore is mixed with hot water (Xu et al., 2013a). This process produces extraction tailings which are separated into two streams based on particle sizes; the coarse sand tailings (CST), or whole tailings (WT), which
are beached and the fluid fine tailings (FFT) which are stored in tailings ponds (Xu et al., 2013a). A froth layer containing approximately 60% bitumen, 30% water and 10% mineral solids is also produced during primary extraction, which is further treated to purify the bitumen content (Sobkowicz, 2012). Froth treatment involves the addition of a solvent, conventionally naptha and more recently a paraffinic solvent (pentane or pentane-hexane), which separates the bitumen from impurities in the froth, such as clay particles, water and unwanted hydrocarbons like asphaltenes (i.e. the froth treatment tailings (FTT), a form of fluid fine tailings) (Sobkowicz, 2012). The added solvent decreases the density of the bitumen, allowing for the separation of water and mineral solids by gravity or mechanical separation (Sobkowicz, 2012). The FTT are processed in a tailings solvent recovery unit (TSRU) in order to recover solvent from the tailings, producing tailings containing residual solvent, fine particles and residual hydrocarbons. Conventional froth treatment results in a more viscous bitumen product compared to paraffinic froth treatment because the latter treatment removes a higher amount of asphaltenes from the bitumen (Shelfantook, 2004), which are retained in the tailings. Therefore, paraffinic froth treatment tailings are referred to as TSRU tailings (Xu et al., 2013a). Because paraffinic froth treatment is a relatively new technology employed by one operator in the oil sands, there is little published information regarding this material.

Traditionally FFT (including TSRU tailings) are deposited in tailings ponds, which currently occupy an area of 170 km², where they can form mature fine tailings (MFT) (Siddique et al., 2011a). Mature fine tailings result when the settled fines in a tailings pond reach 30% solids content, which takes approximately 2 years after deposition. These tailings retain water giving
them a gel-like consistency and are relatively unstable (Chalaturnyk et al., 2002), making reclamation of tailings ponds a significant challenge for the oil sands industry. Due to the nature and vast amount of tailings produced in the oil sands, the Alberta Energy Regulator (AER; formerly the Energy Conservation Resources Board, ERCB) established tailings management requirements in 2009 known as Directive 074 (D-074) (ERCB, 2009). D-074 aims to reduce the production of FFT and create trafficable deposits with undrained shear strength requirements of 5 kPa after one year of deposition and a minimum of 10 kPa after 5 years of inactive deposition (ERCB, 2009). These strengths would be achieved by self-weight consolidation, drying, drainage and/or capping (ERCB, 2009). Various technologies have been or are being developed to meet these requirements including tailings manipulation and alternative disposal methods.

Manipulating the properties of tailings has focused on the fluid fine tailings fraction, producing composite/consolidated tailings (CT) which is a mix of CST with MFT, thickened tailings (TT) which is FFT mixed with a flocculent, and non-segregating tailings (NST) which is a mix of CST and TT (Shell Canada Energy, 2011). The goal of these technologies is to produce a tailings feed that has a higher solids content and does not segregate upon deposition. The CT technology has limitations based on the need for sand, which is used for dyke construction, and also exhibits segregation upon certain disposal methods (Sobkowicz, 2012). Thickened tailings are produced by mixing tailings with a flocculent in a hydrocyclone, allowing for the separation of the thickened tailings as the underflow and water as the overflow. Tailings thickeners have been used at two of Shell’s mines in the oil sands (Muskeg River and Jackpine), which has allowed for bench and pilot scale studies for the TT deposition, however non-segregating deposition has
not been achieved with this method at the commercial scale (Sobkowicz, 2012). These technologies are used in combination with new disposal technologies to meet the requirements of D-074. The depositional technologies include in-line thickening with thin lift dewatering or accelerated dewatering, centrifuging MFT and atmospheric fines drying (AFD) (Sobkowicz, 2012). The first two involve the addition of an anionic polyacrylamide polymer to MFT during pipe transport and then deposition as a thin lift (10 - 30 cm) or in shallow (< 5 m) or deep (20 - 30 m) pits. The thin lifts are allowed to settle, drain and then are reworked to increase the solids content by atmospheric drying before removal to a surface storage area (Sobkowicz, 2012). The pit deposition allows the tailings to expel water during settling which can then be manually decanted, allowing cracks to develop and drying to accelerate (Sobkowicz, 2012). Centrifugation of MFT allows for a high solids content cake to form, which can then be disposed of at a designated facility (Sobkowicz, 2012). Atmospheric fines drying involves mixing MFT with a flocculent and then spreading the mixture in thin layers on the ground. The water is expelled from the MFT-polymer mix and drains off the surface. Subsequent MFT-polymer layers are added once the original layer has completely dried (Shell Canada Energy, 2011).

In 2011, a pilot scale study at the Muskeg River Mine considered the application of AFD with thickened TSRU tailings (Masala et al., 2012). The TSRU tailings were hydraulically deposited into dug out pits, 2 - 3 m wide and 7.5 m long with side slopes of 2:1. They considered the segregation, freeze/thaw settlement, strength and hydraulic conductivity of untreated TSRU tailings, in-line flocculated TSRU tailings and TSRU tailings thickened with a synthetic polymer. The results showed that the untreated and in-line flocculated TSRU tailings segregated,
with the larger particles settling out faster and closer to the pipe (upstream), and a slurried fines pool at the downstream end, creating spatially heterogeneous deposition. The thickened TSRU tailings showed no spatial segregation and dewatered faster than the other tailings. These results indicated the possibility of alternative disposal options for TSRU tailings.

1.1.1 Tailings Composition

The oil sands consist of bitumen (0 – 19 wt %), water (3 – 6 wt %) and mineral solids (84 – 86 wt %), predominantly quartz sand, silts and clays (Chalaturnyk et al., 2002). The clay components consist of kaolinite (40 – 70 wt %), illite (28 – 45 wt %) and montmorillonite (1 – 15 wt %) (Chalaturnyk et al., 2002). Non-clay minerals are also present in the oil sands, with heavy minerals concentrated in the froth during bitumen extraction (Kotlyar et al., 1999; Omotoso and Mikula, 2004). Two of these heavy minerals containing titanium and zirconium are recovered from these tailings as an economically viable resource (Liu et al., 2006). Other minerals include muscovite, anatase, brooklite, ilmenite, schorl, amphibole, pyroxene, tourmaline, garnet, staurolite, kyanite, biotite, carbonates and pyrite (Liu et al., 2006; Kaminsky et al., 2008). Pyrite, or iron sulfide (FeS₂), is less valuable economically but is of environmental concern due to potential acid-generating reactions. Although pyrite is present in the coarse (45 – 106 μm) fraction (Kaminsky et al., 2008), the majority is associated with the fine (<45 μm) fraction, constituting 4-5% of the FTT (Omotoso and Mikula, 2004; Liu et al., 2006). Pyrite has been shown to be associated with quartz, making it more difficult to remove from the tailings (Liu et al., 2006; Kaminsky et al., 2008). The pyrite associated with the quartz was reported to be present in small particle sizes, only visible with a scanning electron microscope (Liu et al., 2006).
suggesting that there is a relatively large surface area present in the FTT. TSRU tailings have been found to contain varying amounts of pyrite (6.0 – 13.4 %) along with siderite, kaolinite, quartz and muscovite (Kuznetsov et al., 2015).

1.2 Acid Rock Drainage

Acid rock drainage arises from the oxidation of sulfide minerals releasing acid, heat and toxic metals into the surrounding environment (Baker and Banfield, 2003). Pyrite is the most commonly studied sulfide mineral, and is present in many ARD sites as well as the TSRU tailings from the Athabasca oil sands; therefore there is potential for ARD generation in surface-deposited unsaturated oil sands tailings. A study by Kuznetsov et al., (2015) showed that these TSRU tailings did in fact generate ARD, reducing the leachate pH from 5 to 2 within 75 days for polymer-treated TSRU tailings and 250-300 days for untreated TSRU tailings, results which were consistent with their acid-base accounting predictions for these tailings. The chemical equations for pyrite oxidation include (Johnson and Hallberg, 2003):

\[
\text{FeS}_2(s) + \text{H}_2\text{O} + 3.5\text{O}_2 \rightarrow 2\text{H}^+ + 2\text{SO}_4^{2-} + \text{Fe}^{2+} \quad [1.1]
\]

\[
\text{FeS}_2(s) + 6 \text{Fe}^{3+} + 3\text{H}_2\text{O} \rightarrow 7\text{Fe}^{2+} + 4\text{S}_2\text{O}_3^{2-} + 6\text{H}^+ \quad [1.2]
\]

\[
4 \text{Fe}^{2+} + \text{O}_2 + 4 \text{H}^+ \rightarrow 4 \text{Fe}^{3+} + 2\text{H}_2\text{O} \quad [1.3]
\]

\[
\text{S}_2\text{O}_3^{2-} + 2\text{O}_2 + \text{H}_2\text{O} \rightarrow 2\text{H}^+ + 2\text{SO}_4^{2-} \quad [1.4]
\]

\[
\text{Fe}^{3+} + 3\text{H}_2\text{O} \rightarrow \text{Fe(OH)}_3 + 3\text{H}^+ \quad [1.5]
\]

These reactions can occur both chemically and through microbial metabolic reactions. Chemically, these reactions are typically slow and self-limiting due to the spontaneous oxidation of ferrous (Fe\(^{2+}\)) to ferric (Fe\(^{3+}\)) iron and subsequent precipitation of iron (III) hydroxide.
(Fe(OH)$_3$) at neutral pH (Ehrlich and Newmann, 2009a). The precipitate forms a white crust on the surface of the pyrite, creating a barrier to oxygen, ceasing any subsequent oxidation of the mineral surface. However, when microbially-mediated, the rate of equation 1.3 can increase up to $10^6$ times (Singer and Stumm, 1970), accelerating the production of sulfuric acid, decreasing the pH to acidic conditions which solubilizes Fe$^{2+}$ and Fe$^{3+}$ ions (Ehrlich and Newmann, 2009a). The solubility of Fe$^{3+}$ also tends to increase with increasing ionic strength, acidity and organic matter, and decreasing temperature (Liu and Millero, 1999); potential conditions of surface-deposited oil sands tailings. Because Fe$^{3+}$ is a stronger oxidant of pyrite compared to oxygen, the oxidation of Fe$^{2+}$ is often the rate-limiting step for acid production (Singer and Stumm, 1970).

1.2.1 Microorganisms and ARD Development

Pyrite oxidation is mediated by iron- and sulfur-oxidizing microbes (IOM/SOM) and occurs either directly or indirectly (Ehrlich and Newmann, 2009b). Direct oxidation occurs because IOM/SOM contain an exopolymer that allows them to attach to and oxidize sulfide minerals (Ehrlich and Newmann, 2009b). Indirect bacterial-mediation occurs in equation 1.3, where IOM oxidize Fe$^{2+}$ to Fe$^{3+}$, which then chemically oxidizes sulfide minerals. However, several other biologically driven processes occur in ARD environments including iron, sulfur and organic carbon oxidation, iron and sulfur reduction, carbon and nitrogen fixation and exopolymeric slime production (Baker and Banfield, 2003). These processes are carried out by both heterotrophic and autotrophic organisms, resulting in a symbiosis between the two groups, where autotrophs produce organic compounds for heterotrophic survival, and heterotrophs remove the organic compounds which are toxic to autotrophs in high concentrations (Baker and Banfield, 2003).
This results in a diverse collection of organisms that inhabit ARD environments from all domains (Eukarya, Bacteria and Archea). Although responsible for some of these processes, the Eukaryotes, including yeast, protists and fungi, and Archaea, are not within the scope of this study. The bacterial groups that have been found in ARD environments include the Proteobacteria, Nitrospira, Firmicutes, Actinobacteria and Acidobacteria (Baker and Banfield, 2003).

Acid rock drainage develops through a microbial community succession of neutrophilic to acidophilic organisms (Harrison, 1978). Acid production can originate from several sources, including autotrophic and heterotrophic neutrophilic SOM, the heterotrophic hydrolysis of organic sulfate esters and filamentous neutrophilic IOM (Harrison, 1978). The rate of acid production can be influenced by the pH of the environment, where Fe$^{2+}$ is oxidized to Fe$^{3+}$ biotically and abiotically above pH 4, and only biotically below pH 4 (Johnson and Hallberg, 2005). The microbial succession is driven by the environmental pH, where organisms can co-exist in small overlapping ranges. Once the neutrophiles have started to decrease the pH enough, the environmental conditions become tolerable for more acidophilic organisms. At this point, the acidophiles can decrease the pH further, creating an inhospitable environment for the acid-intolerant neutrophiles, and reducing the overall microbial diversity of the ARD sites, which is generally reported to be low (Baker and Banfield, 2003).

To study ARD development in oil sands tailings, the contributing microbial populations and their ecologies need to be understood. Because the rate-limiting step is the production of Fe$^{3+}$, it seems logical to focus on iron-oxidizing microbes. This is problematic due to the lack of
knowledge and limited ability to culture neutrophilic IOM (Emerson and Moyer, 1997) and because of the spontaneity of Fe$^{2+}$ to oxidize to Fe$^{3+}$ at neutral pH (Ehrlich and Newmann, 2009a). Due to this, and the nature of the environment of oil sands tailings, a better approach may be to study the native neutrophilic organisms which have the ability to reduce the pH, allowing for the transition to an ARD-generating microbial community.

The knowledge of the microbes which naturally inhabit oil sands tailings is fairly limited to studies on napthenic acid and hydrocarbon degraders (Herman et al., 1994; Siddique et al., 2006), and methanogens (Holowenko et al., 2000; Penner and Foght, 2010; Siddique et al., 2011a). Furthermore, the majority of the studies are based on tailings origins where ARD is unlikely to occur; namely MFT or tailings pond water. Neither of these environments can substitute for unsaturated tailings deposits due to the differences in phase (liquid vs solid for tailings pond water) and oxygen availability (MFT constitute an anaerobic environment) (Salloum et al., 2002).

Because surface-deposited oil sands tailings is a relatively new technology, little is known about the microbial communities that inhabit them. A study by Golby et al. (2012) considered the microbial communities of a biofilm grown from the sludge of an oil sands tailings pond. Due to the decreasing oxygen availability with depth of a biofilm, this study was able to characterize the aerobic, microaerophilic and anaerobic microbial communities. The principal group found in the biofilms were within the *Proteobacteria* (65-73%), dominated by the *Betaproteobacteria*, and followed by the *Gamma-, Alpha- and Delta-proteobacteria*. There were 16 genera identified in the aerobic biofilm, which showed a relatively even population distribution and good diversity.
Among the organisms found was one alkali sulfur-oxidizing organism, as well as Pseudomonas, of which some mixotrophic species can oxidize sulfur (Ehrlich and Newmann, 2009b). The presence of acid-producing neutrophiles suggests that given the correct environmental conditions (i.e. unsaturated tailings), ARD generation could occur.

Another approach is to consider the microbial communities that typically inhabit ARD sites. The difficulty is that most studies focus on sites that already have established ARD. This is not representative of the surface-deposited oil sands tailings because they have not developed ARD, having a pH near neutral to slightly basic (Penner and Foght, 2010; Moore et al., 2013). Few studies have considered the microbial communities at neutral pH in relation to ARD. Harrison (1978) studied coal and overburden from the Richmond Mine and found that heterotrophs initially dominated between pH 5-7 until a decrease in pH to 2.6 where IOM dominated. The maximum growth of SOM, identified to be from the *Thiobacillus* and *Halothiobacillus* genera, occurred between pH 5.2 - 6.5. This supports the suggestion by Baker and Banfield (2003) that acid production is likely controlled by elemental sulfur oxidation due to the complete loss of protons during this process. Therefore, SOM are of key interest in the study of neutrophiles that contribute to ARD generation.

1.2.2 Prevention and Treatment of ARD

Microbially-mediated ARD generation has been estimated to account for 75% of ARD production (Edwards et al., 1998; Baker and Banfield, 2003), making ARD difficult to treat and/or prevent. The most commonly used methods to treat or prevent ARD, respectively, are neutralization with a neutralizing reagent or sealing sulfide-rich deposits with water. The
complexities of bacterial ecosystems add a level of difficulty when trying to target these communities in treatment or prevention options. Chemical treatments used for targeting the bacterial communities often result in more widespread damage, as it is difficult to target specific bacteria with chemicals.

Prevention measures for ARD include the exclusion of oxygen from the system by underwater deposition, sealing with water and using clay covers for surface deposits (Johnson and Hallberg, 2005). The application of these types of prevention is limited by the inability to control environmental factors such as re-suspension by turbulence for underwater storage and cracking of the clay covers (Johnson and Hallberg, 2005). Studies using biocides to prevent ARD showed that this method did not significantly reduce the sulfate production and was potentially only viable as a short-term option (Johnson and Hallberg, 2005; Sand et al., 2007). In theory, biological control of ARD is possible using biological methods to reduce Fe$^{3+}$ and SO$_4^{2-}$, as well as generating alkalinity with processes such as denitrification, methanogenesis or manganese reduction (Johnson and Hallberg, 2005). However, these processes are dependent on multiple factors, such as anaerobic conditions which are not suitable for many environments, including surface deposited oil sands tailings. This highlights the need for novel methods to control ARD.

1.3 Applications of Phage Therapy

A currently proposed method to control ARD development in unsaturated oil sands tailings surface deposits by biological means is to use bacteriophages (phages; viruses that infect bacteria) to control the neutrophilic acid-producing organisms native to the tailings. Phages, non-living segments of genetic material protected by a protein coat, evolve with their hosts making
them highly host-specific. This application is termed phage therapy, meaning the use of phages to control microbial populations at an industrial level. The first application of phage therapy was in the medical industry, where phages were used to control bacterial infections before antibiotics were discovered (Summers, 2012). However with the discovery of antibiotics, the use of phage therapy for medicinal practices in North America disappeared (Summers, 2012). More recently, phages have been applied in agriculture (Wall et al., 2010; Frampton et al., 2012). The use of phage therapy to control microbial populations in oil sands tailings offers benefits to existing ARD control measures, including the potential for prevention of ARD and limited downstream effects due to the highly specific nature of phages to their hosts.

1.4 Motivations and Objectives

Although many treatment options are available for ARD, no one is universal and their implementation is site specific, making ARD a continuing worldwide challenge. Based on the knowledge of the oil sands tailing composition and depositional strategies, it is possible that ARD could develop in unsaturated deposits over time. This realization has prompted an initiative to develop sustainable and environmentally sound novel methods for the prevention and/or treatment of ARD at the scale of the oil sands.

One of the proposed methods is the use of naturally-occurring viruses to control the native microbial populations involved in ARD generation in the oil sands tailings. This involves the characterization of the native microbial community of the oil sands tailings and the isolation of viruses that would infect the microbes known in the generation of ARD. Furthermore, knowledge of the geotechnical properties of the tailings is necessary for the experimental
analysis and modelling of the viruses’ transport in the tailings. All of these aspects are necessary
to perform pilot studies and determine mode of application.

This overall project is a collaborative effort among various researchers at multiple institutions
and integrates various disciplines. The specific goals of this thesis were to:

1) characterize the geotechnical properties and unsaturated behavior of TSRU oil sands
tailings;
2) isolate and characterize the native microbial community of the TSRU tailings which
would likely play a role in the development of ARD.

The hypotheses associated with these goals were:

1) the geotechnical properties and unsaturated behavior of the TSRU tailings will vary
   with polymer and sand content and will be different than what is known for other
types of oil sands tailings;
2) neutrophilic SOM are native to the TSRU tailings and are culturable and able to
   produce acid.
Chapter 2: Microbiology of TSRU Tailings

2.1 Background

During the development of ARD, the environmental pH shifts from circumneutral (7 - 8.5) to acidic (2-3) over time, influencing a succession in the microbial community from neutrophiles to acidophiles (Harrison, 1978). The majority of the microbial-ARD studies focus on acidophiles from well-established ARD sites (Fortin et al., 1996; Edwards et al., 1999, 2000b; Baker and Banfield, 2003) while few consider the potential microbial contribution to ARD at circumneutral pH (Harrison, 1978). Therefore, assessment of ARD potential in fresh minerals is typically chemical, including trace element content, acid-base accounting, mineralogy, reaction rates and drainage chemistry (www.empr.gov.bc.ca), and often do not incorporate the possible microbial contribution.

Microbes typically associated with ARD generation are capable of autotrophic or heterotrophic iron or sulfur oxidation (or both) (iron-oxidizing microbe/sulfur-oxidizing microbe; IOM/SOM) (Baker and Banfield, 2003). Iron oxidizing microbes and SOM typically dominate at acidic pHs, along with other acidophilic microbes (Baker and Banfield, 2003). There are few IOM that exist at neutral pH due to the lack of ferrous iron which is spontaneously oxidized at neutral pH; therefore these organisms are typically found at oxic-anoxic interfaces (Emerson and Moyer, 1997; Johnson and Hallberg, 2003). The indirect role of IOM in iron oxidation is well established, where they oxidize Fe^{2+} to Fe^{3+} which subsequently oxidizes sulfide minerals more strongly than oxygen (Singer and Stumm, 1970). The direct role of IOM is less clear, but is
thought to involve the action of exopolymers rich in Fe\(^{3+}\) which can directly oxidize the surface of pyrite (Schippers et al., 1996). Research regarding the role of microbe attachment to mineral surfaces has shown that although planktonic and attached organisms solubilize similar amounts of Fe\(^{2+}\), the minerals with attached microbes are pitted, increasing the available surface area for oxidation by both IOM and Fe\(^{3+}\) (Edwards et al., 1998).

Although most SOM cannot oxidize iron, and therefore cannot directly increase the rate of pyrite oxidation (Edwards et al., 2000a), they do produce acid when they oxidize sulfur in turn decreasing the pH to create a more suitable environment for IOM. Because more SOM are found at circumneutral pH compared to IOM, they may play a critical role in the early formation of ARD. Sulfur-oxidizing microbes are also thought to be important in increasing the exposed surface area of the pyrite and producing acid by the oxidation of elemental sulfur compounds that cover the pyrite surface at lower pH, which has been proposed as the rate limiting step in acid generation at low pH (Dopson and Lindström, 1999; Edwards et al., 2000a; b; McGuire et al., 2001). Further, they are thought to supply organic substrate for heterotrophic IOM based on studies of pure and mixed cultures of IOM and SOM where the rate of pyrite dissolution was consistently higher in mixed cultures, suggesting a sort of facultative symbiotic relationship between the two groups for ARD generation (Clark and Norris, 1996; Bacelar-Nicolau and Johnson, 1999; Dopson and Lindström, 1999).

Studies on the microbial communities native to oil sands have focused largely on the tailings ponds water and sediments and the organisms involved in hydrocarbon degradation, sulfate reduction and methane production (Siddique et al., 2006; Penner and Foght, 2010; Kendra, 2013;
Siddique et al., 2011b; Golby et al., 2012; Siddique et al., 2012, 2014). These studies have shown that these communities are diverse (Penner and Foght, 2010), and can include substantial aerobic communities in environments previously thought to be anaerobic (i.e. tailings pond sediments, oil sands deposits) (An et al., 2013). Because surface deposition of oil sands tailings is relatively new, little is known about the microbial communities that inhabit the aerobic zone where ARD would occur.

Given the scale of the surface mining in the oil sands, the development of ARD would have catastrophic impacts on the surrounding environment. Therefore, ARD prevention measures for this environment are necessary yet undeveloped. This study is part of a research initiative for the application of phage therapy as an ARD prevention measure for surface-deposited oil sands tailings. The development of phage therapy requires culturable target bacteria; in this case native microbes of tailings that play a role in ARD generation. Because of the lack of knowledge regarding these microbial communities, this study aimed to provide an initial characterization of them at various stages of ARD development (i.e. neutral to acidic pH conditions). The results highlight the microbial potential for ARD generation in these tailings and the need for development of prevention technologies.
2.2 Methods

2.2.1 Tailings Samples

The oil sands tailings used in this study originated from a tailings solvent recovery unit (TSRU) which produces fluid fine tailings containing a high asphaltene (hydrocarbon) content (Xu et al., 2013a). The environment where the tailings were deposited had average summer (Jun-Sept) and winter (Nov-Feb) temperatures of 14°C and -14°C, respectively (www.agriculture.alberta.ca). Initially the tailings were at circumneutral pH and were slightly saline (0.5%) (Kuznetsov et al., 2015).

Four tailings samples were collected from two surface deposits (Cell 1 upstream (C1U) and downstream (C1D) and Cell 5 upstream (C5U) and downstream (C5D)) from a pilot study at the Muskeg River Mine in June 2012. Details of the pilot study are reported by Masala et al. (Masala et al., 2012). The samples were collected and shipped in 20 L buckets and kept at 4°C prior to any analyses. The C5 samples had an anionic polyacrylamide flocculent (polymer) added for thickening (Masala et al., 2012; Xu et al., 2013a).

2.2.2 Reactors

To investigate the ARD-related SOM communities native to the tailings, enrichment methods were used to obtain three cultures (pHs: 2.5, 4.5 and 7). The three 3L batch reactors (Bioflow 1500, New Brunswick Scientific, Enfield, CT, USA) were used to control the environmental conditions of each reactor, including dissolved oxygen (DO), temperature, agitation and pH. The DO was set to 100% saturation with minor fluctuations between 20 – 70 %, the agitation was set
between 100 – 200 rpm and the temperature was held constant at 20°C. The pH was the only differing condition among the reactors and was controlled by the automatic addition of 5N NaOH. The start-up time was considered the time it took for the pH of each reactor to stabilize at the desired pH, while the operation time was the entire period of operation of each reactor (Table 2.1).

Table 2.1. Conditions of each reactor.

<table>
<thead>
<tr>
<th>Reactor Name</th>
<th>Initial pH</th>
<th>Set-point pH</th>
<th>Start-up time (days)</th>
<th>Operation time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARD2.5</td>
<td>4.46</td>
<td>2.5</td>
<td>63</td>
<td>344</td>
</tr>
<tr>
<td>ARD4.5</td>
<td>5.21</td>
<td>4.5</td>
<td>14</td>
<td>346</td>
</tr>
<tr>
<td>ARD7</td>
<td>5.78</td>
<td>7</td>
<td>7</td>
<td>352</td>
</tr>
</tbody>
</table>

ARD7 was first inoculated with 4.2 g of C1D tailings and 2 L of basal sulfur-oxidizing microbial (bSOM) medium (without the Na$_2$S$_2$O$_3$•5H$_2$O; recipe: Table 2.2; modified from (Nica et al., 2000)). After one day, 5 g of Na$_2$SO$_3$•5H$_2$O was added to it to stimulate SOM growth. After 5 days, 20 g of C5U were added to it as both inoculum and a source of reduced sulfur (pyrite). The other two reactors were each inoculated with 60 mL of 13 day-old enrichment culture from the ARD7 reactor, as well as 25 g of C5U tailings and 2 L of bSOM medium.

During the start-up period of the ARD7 reactor, the SOM presence was determined by measuring the sulfate and thiosulfate ion concentrations, and the SOM concentrations were estimated using the most probable number (MPN) technique. The sulfate and thiosulfate concentrations were monitored using a Dionex (Sunnyvale, CA, USA) ICS-2100 ion chromatograph equipped with an AS10H column. This was to ensure SOM were growing and producing SO$_4^{2-}$ while reducing the concentration of S$_2$O$_3^{2-}$. For SOM concentrations, five
replicates of 1 mL samples from ARD7 were diluted into 9 mL autoclaved bSOM medium in a 10-fold series of $10^{-2}$ to $10^{-7}$ and incubated in a shaker at 30°C and 110 rpm for 13 days. The optical densities at 600 nm ($\text{OD}_{600}$) were measured on days 6, 8 and 13, and the pH was measured on day 13 to determine positive or negative growth (Roberts et al., 2002). The MPN was determined using the standard methods table (Greenberg et al., 1992).

Over the course of the operation times, the reactors were given 5 g of Na$_2$S$_2$O$_3$•5H$_2$O weekly or biweekly. The salinities of the three reactors were approximated by the total dissolved solids (TDS). Although the TDS often represents an overestimate of the salinity, it is commonly used for a salinity index. A single sample from each reactor was centrifuged and the supernatant was vacuum filtered through a glass microfiber filter into a test tube. A known volume of the filtrate was pipetted onto a pre-weighed aluminum weigh boat and heated to 98°C for 24 hours. The dried samples were weighed and then heated to 550°C for 1 hour and subsequently weighed. These masses were used to determine the fixed and volatile dissolved solids (FDS and VDS, respectively).

2.2.3 Bacterial Isolation

Four types of growth media were used to isolate bacteria from the reactors. Two types used the bSOM medium as the base, with the addition of plate count agar (bSOM-PC) to isolate heterotrophs and pure agar (bSOM-PA) to isolate autotrophs. The third medium was a *Thiobacilli* medium (TM) that was modified from the ATCC recipe for medium 290 S6. The fourth was a medium used to target iron-oxidizers (IO), modified from a *Leptospirillum ferrooxidans* medium (Atlas, 1997). The recipes for each medium are in Table 2.2.
Table 2.2. Recipes for the microbial media used for isolation and growth experiments.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Mass Concentration (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>bSOM</td>
</tr>
<tr>
<td>Na$_2$S$_2$O$_3$•5H$_2$O</td>
<td>5</td>
</tr>
<tr>
<td>FeSO$_4$•7H$_2$O</td>
<td>----</td>
</tr>
<tr>
<td>NH$_4$Cl</td>
<td>0.1</td>
</tr>
<tr>
<td>CaCl$_2$•2H$_2$O</td>
<td>0.1</td>
</tr>
<tr>
<td>MgCl$_2$•6H$_2$O</td>
<td>0.14</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>1.5</td>
</tr>
<tr>
<td>(NH$_4$)$_2$SO$_4$</td>
<td>----</td>
</tr>
<tr>
<td>FeCl$_3$•6H$_2$O</td>
<td>----</td>
</tr>
<tr>
<td>MnCl$_2$•4H$_2$O</td>
<td>----</td>
</tr>
<tr>
<td>Na$_2$HPO$_4$•7H$_2$O</td>
<td>----</td>
</tr>
</tbody>
</table>

Solid Media

<table>
<thead>
<tr>
<th>Plates</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure agar (PA)</td>
<td>10</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Plate count agar (PCA)</td>
<td>15</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

During the operation times of the reactors, 3 attempts were made to isolate bacterial cultures: after 6 days, 110 - 139 days and at the end of operation (344 – 352 days). These three isolations are referred to as isolation 1, 2 and 3, respectively. No autotrophic medium was used in isolation 1, therefore only bSOM-PC was used. For isolation 2, bSOM-PC and bSOM-PA were used for bacterial isolations and in isolation 3, bSOM-PC, bSOM-PA, TM and IO plates were used. The media used in isolations 1 and 2 had a pH of 6-6.5 but for isolation 3 was adjusted to 4.5 by the addition of either 1N sulfuric acid (H$_2$SO$_4$) or 1N sodium hydroxide (NaOH).

The bacterial culture isolation methods for each isolation period differed slightly. For isolation 1, 9 mL of bSOM liquid medium was inoculated with 1 mL of reactor sample, and then serially diluted from $10^{-1}$ – $10^{-6}$, each of which was spread onto bSOM-PC plates. The same method was used for isolation 2, but the dilutions were spread onto bSOM-PC and bSOM-PA plates. For
isolation 3, 9 mL of phosphate buffer solution was inoculated with 1 mL of reactor sample, and then serially diluted from $10^{-2} – 10^{-6}$, each of which was spread onto bSOM-PA, bSOM-PC, TM and IO plates. The plates were incubated at room temperature (22°C) and plates with colony forming unit (CFU) ranges of 30-300 were enumerated within 6-8 days of being spread. For isolation 3, colony morphologies, including colour, clarity, texture, form, elevation and margin were recorded.

For all isolations, individual colonies were purified based on differences in morphologies by two consecutive streak plates. These isolates were then grown in their respective liquid media; positive growth was determined by optical density at 520 nm (OD$_{520}$) using a Spectronic 20D+ spectrophotometer (Thermo Electron Corp.). The pH was monitored during this growth with either an Orion 9156BNWP pH probe with an Orion 5 Star multi-meter (Thermo Scientific) or pH paper. Organisms that were capable of decreasing the pH (producing acid) relative to the blank media were selected as isolates of interest for further growth studies and sequencing.

2.2.4 Growth Experiments

For all liquid media growth experiments, positive growth was determined by the OD$_{520}$ and/or a decrease in pH, measured as described above. All test tubes contained 9 mL of media that were inoculated with 1 mL of culture and incubated in a shaker (110 rpm) at room temperature (~22°C).

Growth experiments using different sulfur sources in the bSOM medium were performed to determine the best sulfur substrate for the heterotrophs from isolation 1 and the autotrophs from
isolation 2. The four sulfur sources tested were: thiosulfate (Na$_2$S$_2$O$_3$, 5 g/L), tetrahionate (K$_2$S$_4$O$_6$, 3 g/L), iron sulfide (FeS, 5 g/L) and elemental sulfur (S$^\circ$, 5 g/L). To sterilize the elemental sulfur separately from the liquid media, it was placed in a loosely capped glass bottle with 2 drops of dH$_2$O, which was placed in hot reverse osmosis (RO) water (92-92°C) for 3 hours for 3 consecutive days. The sulfur-source growth experiments were performed in liquid media only and the pH and OD$_{520}$ were measured every 2-3 days for 20 days.

Growth curves for the autotrophic isolates from isolation 2 were determined in both bSOM and TM liquid media. Growth curves for the heterotrophic isolates from isolation 2 were determined using the bSOM liquid medium and only pH was measured once per week for 3 weeks. Autotrophic growth on solid phase was determined by spreading 100 μL of pure culture onto bSOM-PA and TM plates.

Growth curves for one of the isolates related to Acidocella sp. were determined using bSOM and Acidocella liquid medium (AM). The AM medium followed DSMZ medium 269 (www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium269.pdf) which contained 2.0 g/L (NH$_4$)$_2$SO$_4$, 0.1 g/L KCl, 0.5 g/L K$_2$HPO$_4$, 0.5 g/L MgSO$_4$x7H$_2$O, 0.3 g/L yeast extract, 1.0 g/L D-glucose. The yeast and glucose were autoclaved separately as 3% and 10% solutions, respectively. After sterilization, the three solutions were mixed together and the pH was adjusted to 4 with 1N H$_2$SO$_4$. The tubes were incubated at room temperature in a shaker (110 rpm) and the OD$_{520}$ and pH were monitored for 9 days.
2.2.5 DNA Extraction and 16S rRNA Gene Sequencing

The 16SrRNA gene sequences of organisms from isolation 1 were sequenced at the University of Alberta. For isolations 2 and 3, the DNA was extracted from fresh cultures of each of the isolates with the MO Bio Power Soil™ DNA extraction kit (Carlsbad, CA). Using the polymerase chain reaction (PCR), the DNA was targeted and amplified with the universal 16S rDNA primers 341F: 5’-CCTACGGGAGGCAGCAG-3’ and 907R: 5’-CCGTCAATTCMTTTGAGTTT-3’, along with AmpliTaq Gold® 360 Master Mix (Invitrogen). The target sequence was amplified under the following conditions: 94°C for 3 minutes, followed by 28 cycles of: 94°C for 30 seconds, 58°C for 45 seconds, 72°C for 45 seconds. The final elongation step was at 72°C for 10 minutes, after which the PCR products were sized by gel electrophoresis prior to sequencing.

The DNA was sequenced by the Fragment Analysis and DNA Sequencing Services Lab (Kelowna, BC, CA). Sequencing was performed using the ABI 3130xl Genetic Analyzer with the ABI BigDye v3.1 Terminator chemistry (Applied Biosystems, California, USA). Forward and reverse sequences were analyzed using the Sequencher software (Gene Codes Corp. MI, USA) and combined to obtain a 450 – 650 bp sequence for each isolate.

The gene sequences were compared with known 16S rRNA gene sequences using the Basic Local Alignment Search Tool for nucleotides (BLASTn) and the Ribosomal Database Project (RDP) to determine the closest relative identities. Percent identity >95% was accepted as the same genus and >98% as the same species. If multiple species of the same genus had >98% similarity, the isolate was placed within the genus only. Isolates with <95% similarity to any organism were labelled as unknown, and placed in the lowest taxonomic category possible at a
95% confidence threshold. All gene sequences were aligned to each other using BLAST Align to determine if any of the isolates were the same organism.

The genera richness, evenness and diversity using the Shannon-Weaver index (H’) were evaluated for each reactor. Because the H’ is not linear, the true diversity was evaluated by using the exponential function of the H’ to provide comparable results between the reactors (Chao and Jost, 2012). The true diversity, also known as the number of effective species, is the number of equally abundant species required to produce a given H’ (Chao and Jost, 2012).
2.3 Results

2.3.1 Enrichment Cultures

Figure 2.1 shows the pH and base addition during the start-up period for the ARD7 reactor. Five days after the initial inoculation with C1-U, the pH of ARD7 slowly increased to about 7.5. At this point, C5-U tailings were added, which was followed by a decrease in pH. On day 7, enough acid was produced in the reactor to trigger the base pump in order to maintain the pH at 7. Base addition stopped at day 15 suggesting that acid production had also stopped.

![Figure 2.1](image.png)

Figure 2.1. pH (solid line) and cumulative NaOH volume (dotted line) added during the ARD7 reactor start-up period. The filled circles indicate the addition of inoculum, the filled triangles
indicate the addition of sodium thiosulfate and the numbered circles indicate the samples that were removed for other studies.

Three samples were taken from ARD7 at the times indicated in Figure 2.1 to determine the sulfate and thiosulfate concentrations during the initial start-up process. The thiosulfate concentration gradually decreased to about 5 mg/L on day 15, while the sulfate concentration increased from about 3400 to 4800 mg/L (Figure 2.2). In order to confirm the sulfur in the oil sands tailings was oxidized by the SOM in the reactor, the sulfur mass balance between sample 2 and sample 3 was calculated and the presence of SOM was quantified. During this time frame, 460 mg/L SO$_4^{2-}$ was produced in the reactor. However, if the thiosulfate utilized by SOM was all converted to sulfate, the generated SO$_4^{2-}$ would be 291 mg/L. The SOM densities were quantified using the MPN method resulting in SOM densities of 5 x 10$^5$ MPN/100 mL with confidence limits of 2 x 10$^5$ - 1.5 x 10$^6$ MPN/100 mL (equivalent to 9 x 10$^8$ MPN/kg dry tailings with confidence limits of 3.6 x 10$^8$ – 2.7 x 10$^9$ MPN/kg dry tailings).
Figure 2.2. Sulfate and thiosulfate anions concentrations in reactor ARD7 during the start-up period.

Figure 2.3 shows the pH change and the cumulative addition of base solution in reactors ARD4.5 (A) and ARD2.5 (B). The initial pH of the two reactors was around 5. It was expected that with more sulfate generated, the pH of the two reactors would eventually reach the set point. ARD4.5 reached the target pH of 4.5 on day 10 after the first spike feed with sodium thiosulfate. The pH in the ARD2.5 reactor was unstable for the 63 day start-up period (Figure 2.3B). Attempting to bring the pH down in ARD2.5, inoculum from low pH (<2) batch cultures enriched from the same oil sands tailings were added to the reactor on day 32 and 41. However, these additions led to an increase in pH. Thus, NaOH had not been added to ARD2.5 until after the 63 day start-up.
Figure 2.3. (A) pH (solid line) and the cumulative volume of NaOH (dotted line) added in reactors ARD4.5 (A) and ARD2.5 (B). The filled circles indicate the addition of inoculum and the filled triangles indicate the addition of sodium thiosulfate.
Figure 2.3 also shows that after each spike of thiosulfate, the pH of the two reactors increased and the NaOH addition ceased. This is because in the first step of thiosulfate oxidation by SOM hydroxyl ions are produced (Equation 2.1):

$$2\text{Na}_2\text{S}_2\text{O}_3 + \text{H}_2\text{O} + \frac{1}{2}\text{O}_2 \rightarrow \text{Na}_2\text{S}_4\text{O}_6 + 2\text{OH}^-$$  \hspace{1cm} [2.1]

The tetrathionate formed is unstable and will transform to penta- and trithionate, which will eventually be oxidized to sulfate, generating acid as seen by the resumed NaOH addition once the pH stabilized. The overall reaction is shown in Equation 2.2 (Starkey, 1935):

$$\text{Na}_2\text{S}_2\text{O}_3 + \text{H}_2\text{O} + 2\text{O}_2 \rightarrow \text{Na}_2\text{SO}_4 + \text{H}_2\text{SO}_4$$  \hspace{1cm} [2.2]

The salinities of the reactors, approximated by the TDS, along with the FDS and VDS are presented in Table 2.3. When compared to seawater with an average salinity of 35 g/L (Boerlage, 2012) the salinities in the reactors were high, representing a moderately saline environment (Ventosa and Nieto, 1995). For all measures, ARD7 was lower than ARD4.5 and ARD2.5, which were similar.

Table 2.3. Dissolved solids present in each reactor at the end of operation.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>TDS (g/L)</th>
<th>FDS (g/L)</th>
<th>VDS (g/L)</th>
<th>Salinity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARD2.5</td>
<td>104</td>
<td>97</td>
<td>7</td>
<td>~10</td>
</tr>
<tr>
<td>ARD4.5</td>
<td>110</td>
<td>100</td>
<td>10</td>
<td>~10</td>
</tr>
<tr>
<td>ARD7</td>
<td>82</td>
<td>81.5</td>
<td>0.5</td>
<td>8.2</td>
</tr>
</tbody>
</table>
2.3.2 Isolation, Identification and Growth of Native Microbes

During 344, 346 and 352 days of enrichment for pH 2.5, 4.5 and 7 respectively, 3 attempts to isolate microorganisms were made using serial dilutions spread on four media types. The 16S rRNA genes from these isolates were sequenced for identification of the most closely related organisms (Table 2.4).

2.3.2.1 Isolations 1 and 2

Organisms from isolation 1 were only isolated from ARD 7, six days after its start-up, and were most closely related to the genera *Burkholderia sp.* and *Pseudomonas sp.*. After 110 – 139 days of operation (isolation 2), only organisms related to *Halothiobacillus neapolitanus* were isolated from ARD7; they were also isolated from ARD4.5 along with relatives of *Pseudomonas sp.* There were no organisms isolated from ARD2.5 during isolation 2.

Growth experiments with different sources of sulfur were used to determine which sulfur source allowed for the most acid production from the isolates of interest. Isolates related to *Pseudomonas sp.* from isolation 1, and isolates related to *H. neapolitanus* from isolation 2 were tested. The relatives of *Pseudomonas sp.* from isolation 1 were only able to decrease the pH using tetrathionate, while the pH using the other sulfur sources increased with time (Figure 2.4). However, organisms related to *Pseudomonas sp.* from isolation 2 were capable of acid production using thiosulfate (Figure 2.5). The other sulfur sources were not tested with these isolates.
Table 2.4. Identity of the partial 16S rRNA gene sequences from the isolates.

<table>
<thead>
<tr>
<th>Reactor pH</th>
<th>Isolation</th>
<th>Isolate name</th>
<th>16S rRNA gene length (bp)†</th>
<th>Most closely related organism</th>
<th>Strain</th>
<th>GenBank accession no.</th>
<th>% Gene homology</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>1</td>
<td>2A</td>
<td>-</td>
<td>Burkholderia sp.</td>
<td>AY367010</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>2B</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>99</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>6</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>99</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>Pseudomonas sp.</td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>7B</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>7B</td>
<td>-</td>
<td>Pseudomonas sp.</td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>8</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>4.5</td>
<td>2</td>
<td>7B</td>
<td>579</td>
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<td></td>
<td></td>
<td>99</td>
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<tr>
<td>4.5</td>
<td>2</td>
<td>7D</td>
<td>586</td>
<td></td>
<td></td>
<td></td>
<td>99</td>
</tr>
<tr>
<td>4.5</td>
<td>2</td>
<td>2E</td>
<td>573</td>
<td></td>
<td></td>
<td></td>
<td>99</td>
</tr>
<tr>
<td>4.5</td>
<td>2</td>
<td>4PA4S2</td>
<td>572</td>
<td></td>
<td></td>
<td></td>
<td>99</td>
</tr>
<tr>
<td>4.5</td>
<td>2</td>
<td>7PA1</td>
<td>575</td>
<td></td>
<td></td>
<td></td>
<td>99</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>7PA1</td>
<td>574</td>
<td></td>
<td></td>
<td></td>
<td>99</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>7PA1S1</td>
<td>574</td>
<td></td>
<td></td>
<td></td>
<td>99</td>
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<tr>
<td>7</td>
<td>2</td>
<td>7PA1a</td>
<td>580</td>
<td></td>
<td></td>
<td></td>
<td>99</td>
</tr>
<tr>
<td>4.5</td>
<td>3</td>
<td>7PA2</td>
<td>581</td>
<td>H. neapolitanus</td>
<td></td>
<td></td>
<td>99</td>
</tr>
<tr>
<td>4.5</td>
<td>3</td>
<td>PA4.1</td>
<td>556</td>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>4.5</td>
<td>3</td>
<td>TM4.1</td>
<td>541</td>
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<td></td>
<td>100</td>
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<tr>
<td>7</td>
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<td>PA7.1</td>
<td>555</td>
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<td>100</td>
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<td>7</td>
<td>3</td>
<td>TM7.1</td>
<td>531</td>
<td></td>
<td></td>
<td></td>
<td>99</td>
</tr>
<tr>
<td>2.5</td>
<td>3</td>
<td>PCA2.1</td>
<td>526</td>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>4.5</td>
<td>3</td>
<td>PCA4.1</td>
<td>483</td>
<td></td>
<td></td>
<td></td>
<td>99</td>
</tr>
<tr>
<td>4.5</td>
<td>3</td>
<td>PCA4.2</td>
<td>556</td>
<td>Achromobacter sp.</td>
<td></td>
<td></td>
<td>99</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>PCA7.1</td>
<td>520</td>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>PA7.2</td>
<td>468</td>
<td></td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>TM7.2</td>
<td>554</td>
<td></td>
<td></td>
<td></td>
<td>95</td>
</tr>
<tr>
<td>4.5</td>
<td>3</td>
<td>TM4.2</td>
<td>515</td>
<td>Acidocella sp.</td>
<td>NR.114266.1</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>3</td>
<td>PCA4.4</td>
<td>555</td>
<td>Curtobacterium sp.</td>
<td>NR.025461.1</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>2</td>
<td>4PA1</td>
<td>562</td>
<td>Unknown</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>2.5</td>
<td>3</td>
<td>PA2.1</td>
<td>558</td>
<td>Unknown 1*</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>2.5</td>
<td>3</td>
<td>TM2.1</td>
<td>563</td>
<td>Unknown 2*</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>4.5</td>
<td>3</td>
<td>PCA4.3</td>
<td>565</td>
<td>Unknown 3**</td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>FE7.1</td>
<td>615</td>
<td>Unknown 4*</td>
<td></td>
<td></td>
<td>-</td>
</tr>
</tbody>
</table>

†DNA sequenced at the University of Alberta; 16S rRNA gene lengths not provided.

*Placed in the Chitinophagaceae family at a 95% confidence threshold using RDP.

**Placed in the Alcaligenaceae family at a 95% confidence threshold using RDP.
Figure 2.4. Growth of isolates related to *Pseudomonas sp.* from isolation 1 with different sulfur sources.

Figure 2.5. Growth of isolates related to *Pseudomonas sp.* from isolation 2 in bSOM liquid medium.
For isolation 2, all of the isolates related to *H. neapolitanus* were able to decrease the pH using thiosulfate (bSOM medium) as an energy source in the sulfur source growth experiments (Figure 2.6A). Two of the isolates (7PA1a, 7PA2) were able to decrease the pH in the tetrathionate medium, while only one isolate (7PA2) was able to decrease the pH in the FeS medium. All of the isolates were able to decrease the pH in the S\(^0\) medium, however after 5 days, only one isolate (7PA2) was able to maintain the low pH in this medium (Figure 2.6 B, C, D).

![Figure 2.6](image)

Figure 2.6. Growth of isolates related to *H. neapolitanus* from isolation 2 with different sulfur sources. (A) bSOM. (B) Tetrathionate. (C) FeS. (D) Elemental Sulfur.
Growth curves for the isolates related to *H. neapolitanus* from isolation 2 in bSOM and TM are presented in Figure 2.7. These autotrophs were able to grow and decrease the pH in both media types. Although the overall absorbance reading was higher for TM, the growth was more consistent in bSOM. The log phase growth for most isolates in bSOM and TM was estimated to occur at 4 days. For solid substrate growth, the plate counts show that isolates related to *H. neapolitanus* grew better on bSOM medium compared to TM (Figure 2.8).

Figure 2.7. Growth of isolates related to *H. neapolitanus* from isolation 2 in bSOM (A) and TM (B) medium. Final pH between 2-3 (bSOM) and 3-3.5 (TM).
2.3.2.2 Isolation 3

At the end of the reactor operations, organisms isolated were most closely related to *H. neapolitanus*, *Achromobacter sp.*, and one unknown (unknown 4) from ARD7; *H. neapolitanus*, *Achromobacter sp.*, *Acidocella sp.*, *Curtobacterium sp.* and one unknown (unknown 3) from ARD4.5, and *Achromobacter sp.* and two unknowns (unknowns 1 and 2) from ARD2.5. The four isolates labelled as unknown were not closely related to any organisms at the genus level in the reference database (Table 2.4). These unknowns were placed at the family taxonomic level into *Chitinophagaceae* (unknowns 1, 2 and 4; 90–91% similarity between the three sequences).
and Alcaligenaceae (unknown 3). A total of 11 colony morphologies were described for the three reactors (Table 2.5). The majority of the isolates were Gram negative rods with the exception of a few Gram positive isolates. The isolates were grouped according to similar colony morphologies; two for ARD2.5, six for ARD4.5 and four for ARD7.

The three reactors yielded different microbial community structures (Figure 2.9A). Two isolates related to Achromobacter sp. and one related to H. neapolitanus were identified in ARD7. Unknown 4 was also isolated from ARD7, however numbers were not tabulated due to the fuzzy texture and lawn margin resulting in indistinguishable colonies. The relative of Achromobacter sp. which dominated over the other was isolated on both heterotrophic (PCA7.1) and autotrophic (PA7.2) media showing different colony morphologies on each media type. However the 16S rRNA gene sequences were 100% identical, therefore the colony morphologies were likely influenced by media type. The Achromobacter sp. related isolate with low plate count numbers (TM7.2) (Figure 2.9B) was isolated only on an autotrophic medium (TM) and had the same colony morphology as the other relative of Achromobacter sp. isolated on autotrophic medium (PA7.2). The 16S rRNA gene sequences, however, were <96% similar, so they were considered different species of this genus in this study. An isolate related to H. neapolitanus represented almost half of the community composition of the ARD7 reactor.
Table 2.5. Phenotypic characteristics of the organisms from isolation 3.

<table>
<thead>
<tr>
<th>Reactor pH</th>
<th>Isolate name*</th>
<th>Colony morphology</th>
<th>Isolation media metabolism</th>
<th>Closest genetic relatives</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>PA2.1</td>
<td>cream opaque shiny punctiform convex entire</td>
<td>Autotroph</td>
<td>Unknown 1</td>
</tr>
<tr>
<td>2.5</td>
<td>TM2.1</td>
<td>pink opaque smooth circular convex entire</td>
<td>Heterotroph</td>
<td>Achromobacter sp.</td>
</tr>
<tr>
<td>2.5</td>
<td>PCA2.1</td>
<td>cream translucent smooth irregular flat entire</td>
<td>Heterotroph</td>
<td>Achromobacter sp.</td>
</tr>
<tr>
<td>4.5</td>
<td>PCA3.0</td>
<td>cream translucent smooth punctiform umbonate entire</td>
<td>Heterotroph</td>
<td>Unknown 3</td>
</tr>
<tr>
<td>4.5</td>
<td>PCA3.1</td>
<td>cream translucent smooth punctiform umbonate undulate</td>
<td>Heterotroph</td>
<td>Halothiobacillus neapolitanus</td>
</tr>
<tr>
<td>4.5</td>
<td>PCA3.2</td>
<td>clear transparent smooth punctiform convex entire</td>
<td>Heterotroph</td>
<td>Acidocella sp.</td>
</tr>
<tr>
<td>4.5</td>
<td>PCA3.3</td>
<td>cream translucent smooth punctiform convex entire</td>
<td>Heterotroph</td>
<td>Achromobacter sp.</td>
</tr>
<tr>
<td>7</td>
<td>PA7.1</td>
<td>cream opaque rough punctiform umbonate entire</td>
<td>Autotroph</td>
<td>Halothiobacillus neapolitanus</td>
</tr>
<tr>
<td>7</td>
<td>TM7.1</td>
<td>cream opaque rough punctiform umbonate entire</td>
<td>Autotroph</td>
<td>Achromobacter sp.</td>
</tr>
<tr>
<td>7</td>
<td>PA7.2</td>
<td>cream opaque rough punctiform umbonate entire</td>
<td>Autotroph</td>
<td>Achromobacter sp.</td>
</tr>
<tr>
<td>7</td>
<td>TM7.2</td>
<td>cream opaque rough punctiform umbonate entire</td>
<td>Autotroph</td>
<td>Achromobacter sp.</td>
</tr>
<tr>
<td>7</td>
<td>IO7.1</td>
<td>white/grey fuzzy - - - lawn</td>
<td>Autotroph</td>
<td>Unknown 4</td>
</tr>
</tbody>
</table>

*Isolate name includes the isolation media (PA: bSOM-PA; PCA: bSOM-PCA; TM, IO).
ARD4.5 showed the highest number of isolates of all the reactors, the community of which was dominated by relatives of *H. neapolitanus*, followed by relatives of *Acidocella sp.*, an unknown isolate (placed in the *Alcaligenaceae* family), and similar densities of *Achromobacter sp.* and *Curtobacterium sp.* The growth of the organism related to *Acidocella sp.* is presented in Figure 2.10. Based on absorbance, this isolate had better growth in AM compared to bSOM, where the log growth was established at 2 days and 3 days, respectively, and the overall absorbance was higher in the AM. The pH for both media decreased from 4 to 2, with an initial decrease occurring in the AM after 2 days, and in the bSOM medium after 4 days.

The plate counts of organisms found in ARD2.5 were 2 orders of magnitude lower than the other two reactors so are shown in Figure 2.9B because they were not visible in Figure 2.9A. Three organisms, two unknown and one related to *Achromobacter sp.*, all with similar densities were isolated from ARD2.5. The two unknown organisms were isolated on different autotrophic media, had the same colony morphologies and similar densities, however their 16S rRNA sequences were only 91% similar. Therefore, they were considered to be isolates from two genera.
Figure 2.9. (A) Plate counts of organisms isolated in each reactor. (B) Plate counts of the lower abundance organisms in ARD2.5 and ARD7. Error bars represent +/- one standard deviation.
Using the plate counts from isolation 3, the overall genera richness and diversity were determined to be low in all three reactors (Table 2.6). ARD4.5 hosted the highest number of culturable organisms (highest richness) followed by ARD2.5 and ARD7 which had the same genera richness. The Shannon-Weaver diversity index and the true diversity decreased with increasing pH; however the difference in magnitude of the true diversity between the three reactors was low. The culturable microbial community was even in the ARD2.5 reactor and was not even in the other two reactors.
Table 2.6. Genera richness and diversity in the three reactors.

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Richness</th>
<th>Shannon Index (H’)</th>
<th>Evenness (J’)</th>
<th>True Diversity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARD2.5</td>
<td>3</td>
<td>1.10</td>
<td>1.00</td>
<td>3.00</td>
</tr>
<tr>
<td>ARD4.5</td>
<td>5</td>
<td>1.04</td>
<td>0.58</td>
<td>2.83</td>
</tr>
<tr>
<td>ARD7</td>
<td>3</td>
<td>0.71</td>
<td>0.65</td>
<td>2.03</td>
</tr>
</tbody>
</table>
2.4 Discussion

Microbially-generated ARD poses a high risk in surface deposits of oil sands tailings highlighting the need to characterize the native microbial communities and their possible role in ARD generation. Enrichment cultures were used in this study to provide an initial characterization of SOM communities. The start-up periods for the enrichment cultures tended to increase with decreasing pH. This may be due to the initial pH of oil sands tailings being circumneutral; therefore the microbial community in ARD7 did not need a long period of time to equilibrate to the change in environmental conditions. The microbial community in the ARD4.5 reactor took only 3 days longer than the ARD7 reactor to establish the desired pH conditions, indicating that some of the organisms comprising the native microbial community of the tailings are capable of growing and producing acid in the pH range of 4.5 – 7. The SOM densities measured during the start-up of ARD7 were higher than the neutrophilic SOM densities but lower than the acidophilic SOM densities reported by Kuznetsov et al., (2015) for these tailings, and were sufficient to produce enough acid to require NaOH addition to maintain the reactor at a neutral pH. This highlights the native microbial community’s ability to produce acid at neutral pH in the tailings, suggesting that they may be a part of the microbial succession in the process of ARD generation.

Excess sulfate ions produced in the ARD7 reactor compared to the thiosulfate addition during the start-up period suggests that the oxidation of other reduced sulfur compounds, such as pyrite, was taking place. The source of this oxidation was not established in this study (abiotic vs. biotic), however the presence of SOM and the production of acid during this time period
suggests there was some biotic sulfur oxidation occurring. These results support the theory for
the potential of ARD generation in unsaturated deposits of these tailings.

The microbial diversities of culturable organisms in the three reactors were low and similar to
each other, decreasing by only one effective species from pH 2.5 to pH 7. Interestingly, even
though the microbial abundances were lower in ARD2.5 by two orders of magnitude, this reactor
had the highest diversity, due to the high genera evenness. Recently, a whole-genome approach
via pyrosequencing was performed on similar tailings, revealing a generally low diversity, which
decreased at a pH < 2 (Kuznetsov et al., 2015). This supports the current findings, as it is
expected that enrichment and culture-based approaches would inherently decrease the diversity.

The low diversity could also be representative of the diversity in ARD environments, which is
generally low compared to other natural systems (Bond et al., 2000). A study on the diversity of
primary producers as a function of pH after exposure to ARD showed that the H’ decreased with
decreasing pH (Niyogi et al., 2002) which contradicts the trend presented in the current study.
However, the H’ values were similar between the two studies, and Niyogi et al. (2002) showed a
high variation in the H’ values at circumneutral pH, encompassing values as low as reported here
for the corresponding pH. This suggests that the diversity of culturable organisms found in these
enrichment cultures, although low, falls within the range reported for other ARD environments.

Oil sands tailings have been shown to host a diverse bacterial community (Penner and Foght,
2010; Kendra, 2013; Golby et al., 2012), and a less diverse archaeal community (Siddique et al.,
2012; An et al., 2013; Penner and Foght, 2010). Golby et al. (Golby et al., 2012) reported H’
values ranging from 4.1 – 4.9, equivalent to 60 – 134 effective species, which is 20 – 45 times
higher than the present study. This difference is partly due to the inclusion of molecular-based approaches in this study to evaluate microbial diversity, as culture-based methods generally underestimate microbial diversity. Furthermore, the enrichment techniques used in the present study selected for SOM and IOM, decreasing the native microbial diversity.

The method of enrichment did not include replacement of the microbial media in the reactors, resulting in the salinity of the reactors increasing to ~ 8 - 10% over time as the sodium and sulfate ion concentrations increased from the addition of sodium thiosulfate and its subsequent oxidation by SOM. Because of this, these reactor systems may have also selected for halophiles and/or halotolerant organisms. However, Golby et al. (2012) also performed culture-based studies, revealing only 4 - 5 colony morphologies and abundances between 10^5 to 10^6 CFU from a tailings pond sludge sample. These culture-based results are similar to the results of the present study, suggesting that the increased salinity may not have significantly affected the microbial abundances.

Although the tailings have a deposition salinity lower than seawater (~0.5%; seawater average 3.5%) (Boerlage, 2012), studies on oil sands tailings have shown an increased ionic strength near the surface of a deposit, partly due to microbial activity, which is where ARD would occur (Kendra, 2013; Siddique et al., 2014). Furthermore, the salinity of the tailings was shown to increase by an order of magnitude after the onset of ARD in the tailings during a bench-scale experiment, which resulted in final salinities of approximately 1.5% (Kuznetsov et al., 2015). These observations indicate that the salinity would likely increase in surface deposits of these tailings and the results suggest that microbial communities capable of acid production would
Although the organisms identified are native to these tailings, the overall community compositions and diversities are likely underestimates of the *in situ* communities of tailings surface deposits due to the low representation of culture-based methods and the potential selection for halotolerant organisms. Regardless, the results provide insight into the microbial community composition as a function of pH at moderately saline conditions. This is important because salinity has been shown to be a key determinant in the microbial community structure in oil sands composite tailings (CT) deposits and natural soils (Lozupone and Knight, 2007; Kendra, 2013).

The three phyla identified in the tailings, the *Actinobacteria*, the *Bacteroidetes* and the *Proteobacteria*, have all previously been found in oil sands tailings (CT and mature fine tailings (MFT)) and tailings pond water, with the *Proteobacteria* being the dominant group (Penner and Foght, 2010; Golby et al., 2012; Kendra, 2013). These three phyla have also been found in ARD environments (Baker and Banfield, 2003; Johnson and Hallberg, 2003).

ARD7 hosted members of the genera *Pseudomonas* and *Burkholderia* in isolation 1 and *H. neapolitanus* in isolation 2. ARD4.5 also hosted members of *Pseudomonas* and *H. neapolitanus* in isolation 2. Some members of *Pseudomonas* and *Burkholderia* are known to degrade hydrocarbons (Ghevariya et al., 2011) and some are halotolerant (Ventosa and Nieto, 1995; Wani et al., 2007; De la Rosa-García et al., 2007). However, relatives of these two genera were not isolated at the end of reactor operation in any of the reactors, suggesting that the organisms isolated in the first isolation were not halotolerant or they were outcompeted by other organisms. *Pseudomonas spp.* have been found in ARD environments (Hallberg et al., 2006) and oil sands
environments, including similar tailings (An et al., 2013; Golby et al., 2012; Kuznetsov et al., 2015). Some species belonging to the *Pseudomonas* genus are capable of growth by sulfur oxidation and subsequent acid production (Schook and Berk, 1978; Palleroni, 2005). When grown on reduced sulfur compounds, the relatives of *Pseudomonas spp.* from isolation 1 were only able to produce acid using tetrathionate. The isolates related to *Pseudomonas spp.* from isolation 2 were able to produce acid using thiosulfate, suggesting that over time the composition of the enrichment cultures changed, selecting for organisms capable of thiosulfate oxidation. Members of the *Burkholderiales* order have been found in similar tailings and in many other hydrocarbon resource environments, however none of the isolates were identified to the *Burkholderia* genus (An et al., 2013; Kuznetsov et al., 2015). *Burkholderia spp.* were found in an ARD environment where they were thought to play a syntrophic role in arsenite oxidation (Duquesne et al., 2008).

Isolations 2 and 3 resulted in multiple isolations of the well-known halotolerant SOM *H. neapolitanus*, suggesting a shift in the reactor environment to moderately saline. *H. neapolitanus* is a neutrophile that can grow between pH 4.5 – 8.5 (Kelly and Wood, 2000), supported by its isolation from ARD7 and ARD4.5 and not from ARD2.5. However, in isolation 3 it was found to exist in higher densities at a pH of 4.5 compared to 7, suggesting that its optimum pH for growth in these tailings may be either lower than what was previously known for this organism (6.5-8) (Kelly and Wood, 2000) or that the optimal growth pH is a function of the media or environment hosting the organism. Although members of the *Halothiobacillus* genus are known to grow on most reduced sulfur compounds, except thiocyanate (Kelly and Wood, 2000), the growth studies
of the relatives of *H. neapolitanus* showed variable results, with the most consistent growth on thiosulfate as the sulfur source. The measurement of growth in the TM liquid medium may have been affected by the insoluble FeCl$_3$ content which increased the variability of the absorbance readings due to the effect of mixing. The bSOM medium had lower concentrations of all of the chemical constituents (thiosulfate, phosphate, ammonia, magnesium) than the *H. neapolitanus* medium from DSMZ (medium 68), and lacked trace elements, indicating that the relatives of *H. neapolitanus* were able to grow on minimal medium. The variable growth of these isolates suggests that they may be different strains, or perhaps the partial sequences were not different enough to tell these organisms apart.

*H. neapolitanus* has not previously been reported as an isolate in oil sands tailings (Penner and Foght, 2010; Golby et al., 2012; An et al., 2013), although members of the same order have (An et al., 2013). *H. neapolitanus* has been found to inhabit ARD environments (Harrison, 1978; Johnson et al., 2002; Hallberg and Johnson, 2003). It is known to contribute to the production of ARD when grown on reduced sulfur compounds, where the pH decreased from circumneutral to 2.5-3 (Kelly and Wood, 2000). Further, an isolate capable of iron oxidation with >99% 16S rRNA identity to *H. neapolitanus* was isolated from a moderate ARD mine environment (pH 3.4), suggesting that the metabolic function of this organism is more complex than previously thought, or that there are closely related species or subspecies with variable metabolic pathways (Hallberg and Johnson, 2003). The apparent metabolic variation of *H. neapolitanus* and its ability to survive at a range of acidic to neutral pH make *H. neapolitanus* a candidate transition organism for ARD generation in the tailings.
Organisms closely related to *Achromobacter sp.* were found in all three reactors, suggesting this genus hosts species that inhabit a wide range of pHs from neutral to acidic. The highest densities were obtained from ARD7, which may have been from the lack of competition (only one other isolate from this reactor) or that while they are capable at growing at a wide range of pHs, they thrive at circumneutral pH. *Achromobacter spp.* are mostly chemoorganotrophs, with some members that are facultative lithoautotrophic hydrogen oxidizers (Busse and Auling, 2005), and some members capable of sulfur oxidation (Vitolins and Swaby, 1969; Graff and Stubner, 2003). This suggests that the organisms related to *Achromobacter spp.* isolated from ARD7 may have been capable of either of the latter metabolisms since they were isolated on both autotrophic and heterotrophic media. The heterotrophic isolates related to *Achromobacter spp.* could have contributed to acid production since CO$_2$ is a bi-product of heterotrophic metabolism. Some species of *Achromobacter* are known to use biphenyls (aromatic hydrocarbons) as their sole carbon and energy source (Ahmed and Focht, 1973; Furukawa and Matsumura, 1976), making oil sands tailings a suitable environment for their growth. Although they are known to be non-halophilic (Busse and Auling, 2005), at least one species is halotolerant and degrades hydrocarbons (Ghevariya et al., 2011). They are also known to be metal-resistant (Schmidt and Schlegel, 1994) making them suitable microbes to inhabit ARD sites, although they are not typically reported in these environments.

There seemed to be no difference in organisms isolated between the two autotrophic media types (bSOM-PA and TM), with the exception of the isolate closely related to *Acidocella sp.* which was isolated only on TM. This was interesting because *Acidocella spp.* are known
chemoorganotrophs, and none of the known species can independently grow chemolithotrophically on sulfur energy sources (Hiraishi, 2005). *Acidocella* spp. are able to reduce Fe$^{3+}$ producing acid, explaining the decrease of pH in the AM during the growth study. However, Fe$^{3+}$ and organics were not present in the bSOM medium but the relative of *Acidocella* sp. was still able to grow and produce acid in this medium. This suggests that this isolate was capable of mixotrophic growth and potentially sulfur oxidation.

*Acidocella* spp. have been shown to be capable of enhancing sulfate reduction syntrophically in co-culture with a sulfate-reducing bacteria (SRB) by converting acetic acid to carbon dioxide and hydrogen (Kimura et al., 2006). This produced acid (CO$_2$) and the hydrogen were used as an electron donor for the SRB which was used for further sulfate reduction (Kimura et al., 2006). Although no SRB were isolated from the enrichment cultures, it is likely that some aero-tolerant SRB existed due to the abundance of sulfate. This example of mixotrophic growth, and the fact that *Acidocella* spp. are capable of iron reduction (Coupland and Johnson, 2008), suggest that they likely played a key role in the iron-sulfur cycling in the enrichment cultures, although the exact role is undetermined.

*Acidocella* spp. are not known inhabitants of the oil sands tailings, but some of the species are known to degrade hydrocarbons including naphtha and toluene, common to oil sands tailings (Stapleton et al., 1998; Dore et al., 2003). Further, *Acidocella* spp. have commonly been isolated from ARD environments (Johnson et al., 2001, 2002; Hallberg et al., 2006), and growth studies during the current study showed that the isolate related to *Acidocella* sp. was capable of reducing
the pH from 4 to 2. This suggests that \textit{Acidocella spp.} could be key organisms in the development of ARD in oil sands tailings.

One organism closely related to the \textit{Curtobacterium} genus was isolated from the ARD4.5 reactor. These chemoorganotrophs are known to weakly produce acid and are capable of reductive dissolution of ferric hydroxide in anaerobic conditions (Saddler and Messenberg-Guimarães, 2009; Ňancucheo and Johnson, 2011). Although the organism related to \textit{Curtobacterium sp.} was isolated on a heterotrophic medium in the present study, it may have been capable of sulfur oxidation, as seen by a \textit{Curtobacterium sp.} isolate from a biofilter that was able to oxidize thiosulfate (Bessa et al., 2011). Members of this genus have also been isolated from acidic (pH 4) sulfate- and metal-rich mineral environments (Saddler and Messenberg-Guimarães, 2009; Ňancucheo and Johnson, 2011). Members belonging to the same order, \textit{Actinomycetales}, have been identified in similar tailings (Kuznetsov et al., 2015).

Three of the unknown isolates (unknowns 1, 2 and 4) were identified with a 95% threshold using RDP to be related to members of the \textit{Chitinophagaceae} family, in the \textit{Bacteroidetes} phylum. New species are being added to this family somewhat frequently (Kämpfer, 2010), indicating that little is known about these organisms and their diversity. Recent studies have shown that members of the \textit{Chitinophagaceae} may be linked to hydrocarbon degradation (Zhang et al., 2011; Lladó et al., 2012) and the genus which most closely matched the current isolates (\textit{Sediminibacterium}; RDP 50% confidence threshold) of this family also represents one of the 22 most prevalent genera found in the oil sands (An et al., 2013). \textit{Sediminibacterium spp.} are IOM involved in cast iron corrosion (Wang et al., 2012) and members of this family have also been
found in high abundance in microbially-induced concrete corrosion but little is known about their role (Cayford et al., 2012). Another genus, *Chitinophaga*, have been linked to dimethyl sulfide degradation by way of carbon cycling, and not direct growth on dimethyl sulfide (Hayes et al., 2010). Members of this family have also been found in alkaline, high-salinity environments (Schmalenberger et al., 2013) but have not been identified in ARD environments. The organisms isolated in the present study grew only on autotrophic media and were isolated at pH 2.5 on bSOM-PA and TM and at pH 7 on IO, suggesting that they may have been capable of iron oxidation at these two pHs. The presence of IOM at circumneutral pH could increase the ARD potential in surface deposits of oil sands tailings.

The factors of interest for ARD generation of the final microbial communities are summarized in Table 2.7. Most of the organisms isolated from the tailings were closely related to groups involved in hydrocarbon degradation and acid production by sulfur oxidation or other mechanisms. Both the heterotrophs and autotrophs native to the oil sands tailings were capable of acid production at the laboratory scale. Further, iron reduction and iron oxidation are known metabolic pathways for 4 of the close relatives of the isolates. Three of the related genera have previously been isolated from established ARD sites. All of the relative groups identified in this study have been found in the oil sands at the Order operational taxonomic unit (An et al., 2013). The presence of both IOM and SOM in the tailings suggests that surface deposits of these tailings host native microbial communities which may aid in the generation of ARD.

Even though culture-based methods tend to represent a small portion of the actual microbial community, these results show that the native microbial communities of the tailings are capable
of acid production at circumneutral pH, exhibit community succession with decreasing pH and host stable populations of hydrocarbon degraders in the acidic to neutral range. The results of this study are limited by the use of enrichment cultures and culture-based isolation methods, which select for certain microbial populations and isolates only capable of growing on the provided media, respectively. Despite these limitations, the results demonstrate that there exists a native microbial community in the tailings with acid generating potential. Further, these organisms were culturable at neutral and moderately acidic pHs, suggesting that microbial-mediation of ARD could exist in unsaturated deposits of oil sands tailings and that development of phage therapy for ARD prevention is theoretically possible. The concept to use phage therapy at an industrial scale has been proven for the mitigation of microbial pipeline corrosion in the petroleum industry (Summer, 2012). These findings provide a starting point for the development of phage therapy as an ARD prevention measure in unsaturated oil sands tailings. Further studies, including molecular techniques to evaluate a wider scope of the microbial ecology are underway. These results contribute to the knowledge of the types of culturable organisms that inhabit aerobic, moderately saline, hydrocarbon-rich environments, such as areas affected by oil brines, and may have implications for remediation.
Table 2.7. Known metabolic functions and habitats of interest of the relative groups of the tailings isolates after enrichment (R: reduction; O: oxidation).

<table>
<thead>
<tr>
<th>Metabolic function/habitat</th>
<th>Achromobacter sp.</th>
<th>Chitinophagaceae</th>
<th>Acidocella sp.</th>
<th>Curtobacterium sp.</th>
<th>H. neapolitanus</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>2.5, 4.5, 7</td>
<td>2.5, 7</td>
<td>4.5</td>
<td>4.5</td>
<td>4.5, 7</td>
</tr>
<tr>
<td>Hydrocarbon degradation</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Acid production</td>
<td>No</td>
<td>No</td>
<td>Yes*</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Iron Redox</td>
<td>No</td>
<td>O</td>
<td>R</td>
<td>R</td>
<td>O</td>
</tr>
<tr>
<td>Sulfur Redox</td>
<td>O</td>
<td>No</td>
<td>R**</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>ARD</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Oil sands</td>
<td>This study</td>
<td>(An et al., 2013)</td>
<td>This study</td>
<td>This study</td>
<td>This study</td>
</tr>
</tbody>
</table>

*Acid generation is dependent on the composition of the iron-containing substrate.

**Increases sulfur reduction syntrophically as described by (Kimura et al., 2006), but does not directly reduce sulfur.
Chapter 3: Geotechnical Analysis of TSRU Tailings

3.1 Background

Due to the challenges surrounding tailings ponds, surface deposition is being investigated as an alternative disposal method for slurried oil sands tailings. Much of the research around surface deposited tailings has involved flocculent addition promoting flocculation, thickening, and dewatering of the tailings (Masala et al., 2012; Bajwa and Simms, 2013; Beier et al., 2013; Dunmola et al., 2013; Farkish and Fall, 2013; Moore et al., 2013; Soleimani et al., 2013; Xu et al., 2013a). These studies tend to focus on the strength, consolidation and hydraulic conductivity of MFT (Bajwa and Simms, 2013; Dunmola et al., 2013; Farkish and Fall, 2013; Moore et al., 2013; Soleimani et al., 2013), with some research on whole tailings and tailings solvent recovery unit (TSRU) tailings (Masala et al., 2012; Beier et al., 2013; Xu et al., 2013a).

Relatively few studies have considered the properties and deposition of TSRU tailings, which are estimated to represent ~4% of the tailings that will be produced at the Muskeg River Mine between 2011 - 2059 (Shell Canada Energy, 2011; Masala et al., 2012; Xu et al., 2013a). TSRU tailings are the waste produce from the paraffinic froth treatment process, which results in higher pyrite and residual asphaltene content compared to the conventional naptha-treated FTT (Xu et al., 2013a; b). Therefore, TSRU tailings are likely to exhibit unique behaviour upon deposition, both underwater and surface. Current practices of disposal in tailings ponds results in TSRU tailings segregation with larger asphaltene particles settling with the coarse particles, and finer asphaltene particles remaining in suspension and creating a MFT layer (Xu et al., 2013a). A
recent study found that this segregation, which also occurred in surface deposited TSRU tailings, could be eliminated in surface deposits by the addition of a synthetic polymer for flocculation (Masala et al., 2012). These studies were the first to explore the alternative deposition options for TSRU tailings.

Meeting the strength requirement of a trafficable tailings surface deposit poses the potential for unsaturated conditions to develop during halted operations, or once a deposition site is at capacity. This could lead to the development of acid rock drainage (ARD), which occurs when sulfide minerals exposed to oxygen and water are oxidized, producing sulfuric acid and leaching toxic heavy metals. These toxins could potentially seep into the groundwater or nearby surface water systems, posing environmental and human health risks. This process is catalyzed and often controlled by sulfur- and iron-oxidizing microbes, both of which exist in the native microbial community of unsaturated TSRU tailings (Dean et al., submitted). Although ARD development is not normally considered a risk associated with conventional oil sands tailings, the paraffinic froth treatment process tends to concentrate sulfide minerals, namely pyrite, in the TSRU tailings. Therefore, in an effort to prevent the development of ARD, research into a novel technology is underway, where phages, viruses specific to these microbes, are applied to unsaturated TSRU tailings deposits to control their populations. This technology, called phage therapy, is already used in the food, agriculture and medical industries (Summers, 2012; Wall et al., 2010; Frampton et al., 2012), and is being explored for controlling environmental microbial populations involved in pipeline corrosion (Summer, 2012). The development of this technology for TSRU tailings requires the characterization of the geotechnical properties, soil-water
behaviour and consolidation behaviour. This paper explores these aspects of untreated, polymer-amended and sand-mixed TSRU tailings to provide baseline information of these tailings for further experimental design.
3.2 Methods

TSRU tailings samples were obtained from pilot-scale surface deposits from the External Tailings Facility at the Muskeg River Mine site in northern Alberta. The primary difference between TSRU tailings compared to other extraction tailings is the concentrated pyrite and high fraction of hydrocarbons in the solids (Xu et al., 2013a; Masala et al., 2012). These hydrocarbons are primarily made up of asphaltenes (>85 wt %) (Xu et al., 2013b) but will be referred to as hydrocarbons in this study. TSRU tailings also contain residual solvent (Masala et al., 2012) made up of pentane and/or hexane (Xu et al., 2013b).

Samples were collected from Cells 1 and 5, upstream and downstream (C1U, C1D, C5U, C5D) as outlined in (Masala et al., 2012). The C1U and C1D tailings were untreated (no polymer; NP) and displayed grain size separation upon deposition, resulting in larger grain sizes in the C1U tailings, and smaller grain sizes in the C1D tailings. The C5U and C5D tailings were treated with a synthetic polymer (P) for thickening, which resulted in uniform grain size deposition between C5U and C5D.

Four subsamples were prepared for the geotechnical analyses presented. The untreated (NP) sample consisted of a C1U:C1D mix (48:52, dry wt %), aiming for similar properties to the polymer-amended 100% tailings sample. The other three treated (P) samples consisted of C5U tailings:sand mixes with the following compositions (% w/w): 100/0, 50/50, 30/70 by dry mass. The four samples are named based on their compositions: NP100/0, P100/0, P50/50, P30/70.
The silica sand (0.420 – 0.850 mm) was obtained from Target Products Ltd. The sand had a specific gravity of 2.65, an effective grain size (d_{10}) of 0.45-0.55 mm and a uniformity coefficient (C_u) of <1.6 (www.targetproducts.com/UserContent/SpecSheets/fltwatw1AB.pdf).

Using de-aired RO water, the four samples were made into slurries from the initial gravimetric water contents of 53% and 55% for the NP100/0 and P100/0 samples, respectively. To ensure initial saturation, the samples were placed on a vibrating table for 2 minutes to remove any air trapped in the voids (Péron et al., 2006). Prior to the analyses, the samples were stored at 4°C to slow any chemical reactions that may have been taking place.

3.2.1 Geotechnical Index Properties

Average particle size distributions (PSD) for the four samples were obtained using the wet-sieve (2 mm – 0.08 mm mesh) and hydrometer analyses, performed in triplicate (ASTM, 2007). The water contents were determined in triplicate following standard methods (ASTM 2010a). The specific gravities (G_s) of the NP100/0 and P100/0 samples were determined following the ASTM D854-10 procedure (ASTM, 2010b). The experimental G_s for the P100/0 sample was used to calculate the specific gravities of the P50/50 and P30/70 samples based on the respective percentage of materials used. There was difficulty in determining the liquid and plastic limits of these materials due to their lack of cohesion, which was also previously identified (Masala et al., 2012). The three samples were classified using the Unified Soil Classification System (USCS) (ASTM, 2011a).
3.2.2 Tailings Pore Water Salinity

The salinities of the NP100/0 and P100/0 samples were approximated using total dissolved solids (TDS) and electrical conductivity (EC). The pore water from three ~ 50 g samples was extracted using centrifugation (3500 rpm for 15 minutes). The pore water was vacuum filtered through a glass microfiber filter to isolate the dissolved solids. The TDS was determined using triplicate samples, where possible, of 5 mL of pore water that was pipetted onto a pre-weighed aluminum dish and dried at 98°C for 24 hours and weighed again. The volatile portion (VDS) and fixed portion (FDS) of the TDS were determined by heating the TDS sample to 550°C for 1 hour and determining the change in mass.

The EC of the unused pore water was measured with an Orion 013605MD Conductivity probe and Orion 5 star bench top meter (Thermo Scientific, Waltham, MA). The EC values were converted to g/L for comparison with the TDS results. Two conversion were used: the standard conversion and the sulfate-dominant conversion (Chang et al., 1983). The standard conversion was based on the conversion of the NaCl standard used to calibrate the EC probe (Equation 3.1)

\[
TDS = \frac{7230 + EC}{12.9} \quad [3.1]
\]

The sulfate-dominant conversion was established for soils containing high concentrations of sulfate ions (Chang et al., 1983) (Equation 3.2).

\[
TDS = 765.1 * EC^{1.087} \quad [3.2]
\]
The pH was measured for the EC samples with an Orion 9156BNWP pH probe and Orion 5 star bench top meter (Thermo Scientific, Waltham, MA).

3.2.3 Shrinkage Curve

The shrinkage curves were developed following the laboratory techniques outlined by Fredlund et al. (2011) and Fredlund & Houston (2013). Briefly, the slurries were gently mixed by hand to homogenize the sample and then poured into stainless steel rings which were underlain by parafilm to prevent leakage from the bottom of the ring. The rings were 1.2 cm in height and 4.1 cm in diameter.

Once poured, each ring was weighed and the loss in height of the slurry sample was measured using a digital micrometer with a level attached to the top. Due to difficulties in obtaining accurate dimension measurements while the sample was relatively wet, correction factors were applied to the height loss measurements as follows: P100/0 and P50/50: -1mm; P30/70: -0.25mm. These correction factors were based on the differences in height and diameter measurements between the relatively wet sample and dry sample, when the stainless steel ring could be removed for more accurate measurements. Furthermore, diameter measurements were difficult to obtain until sufficient shrinkage had occurred that the stainless steel rings could be removed prior to measurement. Therefore, observations of diameter change were made and the corresponding data points were not used in the analysis. This only applied to the P100/0 and P50/50 samples in the water content ranges of 6-9% and 4-5%, respectively. The diameter of the P30/70 sample did not adequately shrink during the entire testing period for the stainless steel ring to be removed. These measurements gave the total volume of the sample.
At the end of the testing period, the samples were oven dried to obtain their final water contents and void ratios. The volume of solids was assumed to be constant throughout the test; therefore the change in mass was only attributed to the water content. The water contents for each time step were back calculated using the oven dry mass. The initial water contents were calculated assuming the samples were 100% saturated (i.e. \( e_0 = wG_s \)). The total volume in \( \text{cm}^3 \) (\( V_{Tn} \)) at each time step (n) combined with the oven dry mass in g (\( M_d \)) and solids density in g/cm\(^3\) (\( \rho_s \)) gave the void ratio of each sample for each time step (\( e_n \)) with the following equation:

\[
e_n = \frac{V_{Tn}\rho_s}{M_d} - 1
\]  

[3.3]

The room temperature and relative humidity were recorded during each measurement and were used to determine the final suction value based on the Kelvin equation. This suction value was associated with the final water content of each sample prior to oven-drying. The average temperatures and relative humidities were 21.8 ± 0.9°C and 38.1 ± 4.5% for NP100/0 and 22.4 ± 0.4°C and 45 ± 10% for P100/0, P50/50 and P30/70. The shrinkage limits were produced using SoilVision (2009), the software used to analyze the data, as well as calculated based on the method in ASTM D4943-08 (ASTM, 2008).

3.2.4 Soil-Water Characteristic Curve

The soil-water characteristic curve (SWCC) tests were performed using tempe cells equipped with 500 kPa ceramic plates at the base. The four slurry samples were gently stirred to ensure homogeneous mixtures and then poured into the tempe cells in an attempt to represent deposition in the field. The sides of the tempe cells were held down to prevent slurry leakage through the
bottom until the tops of the tempe cells were in place. Once loaded, the samples were allowed to equilibrate for 6-11 days before suction was applied. Suctions between 0-15 kPa were applied via hanging columns, while suctions applied between 15-500 kPa were applied through a pressurized air port on the top of the tempe cell applying the axis translation method. The suctions were increased once the mass was stable for 2 or more consecutive measurements over a period of 2-4 days. Water was collected in a small vial at the end of the hanging column, which was weighed each day. Suction levels were increased in small increments (2-12 kPa) below 200 kPa to minimize sample cracking.

The loaded tempe cells were weighed and the sample heights were recorded daily for a period of 5 months. Air bubbles were removed daily from the tempe cells by pushing de-aired RO water through the base with a syringe and needle. For the P30/70 sample, the air bubbles were difficult to remove from the tempe cell at pressures higher than 200 kPa, so this sample was only tested to 300 kPa. During the first 1-2 suction applications, a water layer persisted on top of the tailings layer. The height of the water layer was monitored each day until it was gone. Measurements from this time period only occurred for the P50/50 and P30/70 samples and were not included in the analysis due to the inability to accurately calculate the water content. Upon completion of the SWCCs, the samples were extracted from the tempe cells and oven dried to obtain the final water contents. To confirm the volume and mass measurement accuracies, segments from the shrinkage and SWCC tests were wax-coated to determine their bulk densities (ASTM, 2009).
3.2.5 Consolidation

Consolidation tests were performed following the ASTM D2435/D2435M-11 standard (ASTM, 2011b) in a fixed ring consolidometer (Hoskin Scientific, BC, CA). The samples were placed between two porous stones lined with filter papers. The NP100/0 and P100/0 samples were packed at their natural gravimetric water contents of 53% and 50% to densities of 1.30 g/cm$^3$ and 1.50 g/cm$^3$, respectively to emulate the field conditions reported in Masala et al. (2012). Incremental loads were applied in the range of 1 – 160 kPa to represent self-weight consolidation as described by Owolagba and Azam, (2014). The load increment was changed once the vertical deformation was within 0.01 mm over 24 hours. The tailings dewaterability or volume reduction was calculated as $\Delta e/(1+e_o)$.

3.2.6 Image Analysis

Image analysis to qualitatively compare the microstructure of the untreated and treated TSRU tailings was performed using scanning electron microscopy (SEM). Samples for SEM analysis included the fraction less than 80 μm from the particle size distribution for the NP100/0 and P100/0 samples. The fractions were oven dried at 98°C for 24 hours, and subsequently burned at 550°C for 1 hour in a muffle furnace to remove any organics. The samples were carbon-mounted on to aluminum studs and sputter-coated with 10 nm of a platinum-palladium alloy to increase the sample conductivity. A Tescan MIRA3 XMU SEM (beam intensity of 15 nm) was used to generate images from secondary electrons. Images were collected at 5000X magnification at a beam energy of 20.0 kV.
3.2.7 Data Analysis

Experimental results were analyzed using SoilVision software (Soil Vision Systems Ltd., 2009), including the index properties, PSDs, shrinkage curves, SWCCs and consolidation tests. The volume-mass characteristics of the initial slurries were calculated based on the water contents, specific gravities, and the assumption that they were at 100% saturation. The shrinkage curves were fitted with a hyperbolic function (Equation 3.4) which calculated the void ratio based on the water content \( e(w) \) by using fitting parameters (\( a_{sh} \): minimum void ratio, \( b_{sh} \): slope of the line of tangency, and \( c_{sh} \): shape of the shrinkage curve) (Fredlund et al., 2002).

\[
e(w) = a_{sh} \left[ \frac{w c_{sh}}{b_{sh} c_{sh} + 1} \right]^{1/c_{sh}}
\]  

[3.4]

The SWCC data were fit with the Fredlund bimodal equation (Equation 3.5) for the gravimetric \((w\)-based) and volumetric \((\theta\)-based) water contents and the degree of saturation \((S\)-based) representations. This fit is based on the saturated water content \( w_s \), the bimodal split \( s \), the suction \( \psi, \text{kPa} \) and various fitting parameters. The \( a_{fb} \) and \( j_{fb} \) parameters are related to the two AEVs associated with bimodal fits, the \( n_{fb} \) and \( k_{fb} \) parameters are related to the steepest part of the curve and the \( m_{fb} \) and \( l_{fb} \) are related to the shape of the curve (Fredlund, 1999). The air entry values were estimated based on the intersection of the tangent lines to the curves near the breaking point for each type of SWCC.
\[
w(\psi) = w_s \left\{ s \left[ \frac{1}{\ln\left(\exp(1) + \left(\frac{a_{fb}^m}{\psi}\right)^{m_{fb}}\right)} \right] + (1 - s) \left[ \frac{1}{\ln\left(\exp(1) + \left(\frac{j_{fb}^k}{\psi}\right)^{j_{fb}}\right)} \right] \left[ 1 - \left( \frac{\ln\left(1 + \frac{\psi}{3000}\right)}{\ln\left(1 + \frac{1000000}{3000}\right)} \right) \right] \right\}
\]
3.3 Results

3.3.1 Geotechnical Index Properties

The geotechnical properties of the samples are summarized in Table 3.1. The P100/0 and P50/50 samples meet the criteria for well graded sand based on the $C_u$ and coefficient of curvature ($C_c$) ($C_u \geq 6, 1 < C_c < 3$; Holtz & Kovacs, 1981) while the NP100/0 and P30/70 samples do not, thus are classified as poorly graded. Fines are typically defined as <44 μm in the oil sand industry (ERCB, 2009), so the values reported were interpreted from the PSDs (Figure 3.1). The NP100/0 and P100/0 samples both showed a higher fines content than the two samples mixed with sand.

Table 3.1. Index properties of the oil sands tailings slurries.

<table>
<thead>
<tr>
<th>Property</th>
<th>NP100/0</th>
<th>P100/0</th>
<th>P50/50</th>
<th>P30/70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water content (%)</td>
<td>104.67 ± 1.11</td>
<td>78.10 ± 2.08</td>
<td>48.04 ± 0.35</td>
<td>34.03 ± 0.80</td>
</tr>
<tr>
<td>Solids content (%)</td>
<td>48.86 ± 0.26</td>
<td>56.24 ± 0.65</td>
<td>68.36 ± 0.16</td>
<td>75.33 ± 0.45</td>
</tr>
<tr>
<td>Specific gravity ($G_s$)</td>
<td>2.06 ± 0.02</td>
<td>2.04 ± 0.03</td>
<td>2.35 ± 0.03</td>
<td>2.46 ± 0.03</td>
</tr>
<tr>
<td>USCS</td>
<td>Silt with sand (ML)</td>
<td>Sandy silt (ML)</td>
<td>Silty sand (SM)</td>
<td>Silty sand (SM)</td>
</tr>
<tr>
<td>Effective grain size ($d_{10}$) (μm)</td>
<td>13.6</td>
<td>5</td>
<td>9.5</td>
<td>12.8</td>
</tr>
<tr>
<td>Mean grain size ($d_{50}$) (μm)</td>
<td>38.9</td>
<td>26.6</td>
<td>271.1</td>
<td>537.3</td>
</tr>
<tr>
<td>Coefficient of uniformity ($C_u$)</td>
<td>3.67</td>
<td>6.47</td>
<td>39.09</td>
<td>48.20</td>
</tr>
<tr>
<td>Coefficient of curvature ($C_c$)</td>
<td>0.89</td>
<td>1.30</td>
<td>1.41</td>
<td>11.64</td>
</tr>
<tr>
<td>% fines (&lt;44 μm)</td>
<td>55%</td>
<td>58%</td>
<td>31%</td>
<td>20%</td>
</tr>
<tr>
<td>Sands:fines ratio (SFR)</td>
<td>0.82</td>
<td>0.72</td>
<td>2.2</td>
<td>4</td>
</tr>
</tbody>
</table>
The sands:fines ratios (SFRs) for each sample were determined from the PSDs, and are plotted on a ternary tailings classification diagram (Figure 3.2; adapted from Azam & Scott 2005; COSIA 2014). The labelled zonings represent saturated conditions, where the arrows represent the direction of dewatering and end-product targets (COSIA, 2014). All of the tailings samples fall within the non-segregating and fines matrix categories, with all but the P30/70 being pumpable. The 100% tailings samples fall within the zoning known for slightly desaturated thickened tailings and the P30/70 samples falls within the unsaturated CT/NST zoning. The P50/50 sample does not fall within any of the zones for any tailings type outlined by COSIA (2014).
Figure 3.2. Ternary diagram for tailings classification showing the distribution of the samples used in relation to other tailings and tailings properties. MFT: mature fine tailings; WT: whole tailings; CT: composite tailings; NST: non-segregating tailings; BT: beached tailings. Arrows represent the desaturation direction for end product targets. Adapted from Azam and Scott (2005); COSIA (2014).

3.3.2 Slurry Water Salinity

The salinity of the slurry water for the NP100/0 and P100/0 samples was approximated by both TDS and EC. The VDS, FDS and pH were also determined. Table 3.2 shows that the directly
measured TDS was considerably higher than both the TDS converted from the EC using the NaCl standard and the sulfate-dominant conversion. For all measures, the NP100/0 had higher values compared to the P100/0 sample.

Table 3.2. Slurry water characteristics for untreated and treated TSRU tailings (error is one standard deviation).

<table>
<thead>
<tr>
<th>Measure</th>
<th>NP100/0</th>
<th>P100/0</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDS (g/L)</td>
<td>5.41</td>
<td>4.59 ± 0.21</td>
</tr>
<tr>
<td>EC (mS/cm)</td>
<td>4.94</td>
<td>3.88</td>
</tr>
<tr>
<td>EC (g/L) standard</td>
<td>2.77</td>
<td>2.18</td>
</tr>
<tr>
<td>EC (g/L) sulfate</td>
<td>4.34</td>
<td>3.34</td>
</tr>
<tr>
<td>VDS (g/L)</td>
<td>1.19</td>
<td>0.97 ± 0.02</td>
</tr>
<tr>
<td>FDS (g/L)</td>
<td>4.22</td>
<td>3.62 ± 0.21</td>
</tr>
<tr>
<td>pH</td>
<td>6.9</td>
<td>6.14</td>
</tr>
</tbody>
</table>

*Only the polymer-amended sample had enough extractable liquid portion to perform replicate samples.

3.3.3 Shrinkage Curves

The shrinkage tests took 4 months to complete, with the majority of the desaturation occurring within the first 100 hours. As expected due to larger particle and pore sizes, the polymer-amended sand-mixed samples showed the fastest desaturation (~50 hours) during the shrinkage tests, compared to the water contents of the 100% samples, which stabilized around 80 hours. The untreated sample showed the most complete desaturation, while the P100/0 sample had the least desaturation (Figure 3.3). The untreated sample also had a slightly higher rate of desaturation, while the polymer-amended samples exhibited similar rates compared to each other. Quadruplicate samples of the untreated tailings showed little variation in the rate of
desaturation, therefore single samples were tested for the polymer-amended tailings. None of the samples exhibited cracking during desaturation.

Figure 3.3. Desaturation of TSRU tailings with time during the shrinkage test. The markers for the NP100/0 represent one standard deviation of the mean gravimetric water content.

The shrinkage curves for all samples followed the pattern of drying from a slurry, described by Fredlund and Houston (2013) as a decrease in void ratio along the line of saturation where the mass of water loss is equal to the volume of water lost (Figure 3.4). Air enters the sample at the point where the curve deviates from the line of saturation, and the volume change becomes negligible with respect to the mass of water lost. The shrinkage limit (SL) is the water content at the intersection between the line of saturation and a horizontal line from the minimum void ratio.
Two values were obtained for each sample; one produced from SoilVision and one calculated based on the ASTM method (ASTM, 2008) (Table 3.3). The two estimates produced similar results, and since the SoilVision estimate was used in the production of the SWCCs, it was also the value used for further comparisons. The shrinkage limits were similar for the NP100/0 and P100/0 tailings samples and decreased in the P50/50 and P30/70 samples. All samples were fitted reasonably well with a hyperbolic equation (Equation 3.4).

Figure 3.4. Shrinkage curves for the NP100/0 (R²: 0.9886), P100/0 (R²: 0.9689), P50/50 (R²: 0.9641), P30/70 (R²: 0.9931) samples.
Table 3.3. Shrinkage limits for the TSRU tailings samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Shrinkage Limit (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SoilVision</td>
<td>ASTM D4943-08 (2008)</td>
</tr>
<tr>
<td>NP100/0</td>
<td>54</td>
<td>50</td>
</tr>
<tr>
<td>P100/0</td>
<td>50</td>
<td>52</td>
</tr>
<tr>
<td>P50/50</td>
<td>33</td>
<td>35</td>
</tr>
<tr>
<td>P30/70</td>
<td>25</td>
<td>27</td>
</tr>
</tbody>
</table>

The polymer-amended tailings exhibited significantly less shrinkage compared to the NP100/0 sample. Among the polymer-amended samples, the P100/0 sample showed the highest change in void ratio followed by the P50/50 and then the P30/70 sample, indicating that the presence of sand reduced the shrinkage in these tailings. The NP100/0 and P100/0 showed early deviation from the line of saturation which was not observed in the sand-mix samples.

3.3.4  Soil-Water Characteristic Curves

The SWCC testing was completed over 5 months, and all of the samples exhibited cracking during desaturation. Upon deconstruction, a vertical moisture gradient was apparent in the polymer-amended samples, with the intensity decreasing with increasing sand content. This moisture gradient was not present in the untreated sample. All of the samples had an orange coloured ring approximately 1-2 mm deep which developed at about 1 kPa; it was termed the line of oxidation.

The soil-water behaviour for all samples was best described with the Fredlund bimodal fit (Equation 3.5) for the three SWCC representations (w-based, θ-based and S-based) (Figure 3.5, 3.6 and 3.7). The fitting parameters for the w-based fits are presented in Table 3.4. For the w-based and θ–based SWCC, the polymer amended samples all exhibited similar shapes with
different initial water contents, tending to have a lower water content at a given suction when compared to the untreated tailings. The S-based SWCC showed that the sand-mixed samples behaved similarly, while the NP100/0 sample had a higher degree of saturation at a given suction. The P100/0 sample behaved similarly to the NP100/0 sample at suctions below the AEV, after which the P100/0 sample behaved comparable to the sand-mixed samples.

Figure 3.5. Gravimetric water content SWCCs of the polymer tailings represented with the Fredlund bimodal fit (NP100/0 $R^2$: 0.9984; P100/0 $R^2$: 0.9977; P50/50 $R^2$: 0.9980; P30/70 $R^2$: 0.9981).
Figure 3.6. Volumetric water content SWCCs represented with the Fredlund bimodal fit.
Figure 3.7. Degree of saturation SWCC for all samples each represented with the Fredlund bimodal fit.

The $\theta$-based SWCCs were developed using the $w$-based SWCCs and the shrinkage as a function of volume change of the whole sample. Fredlund et al., (2011) and Fredlund and Houston (2013) argue that integrating the $w$-based SWCC with the shrinkage as a function of volume change of the void space, giving the $S$-based SWCC, is necessary to estimate the true AEV for materials that shrink upon desaturation. The estimated AEVs for each sample from the three SWCC representations and the shrinkage tests, along with the suctions at cracking conditions (Ck) are presented in Table 3.5. Of the four samples, only the NP100/0 sample showed a slight and
consistent increase in AEV, from 40 kPa to 48 kPa to 54 kPa for the \( w \)-based, \( \theta \)-based and \( S \)-based SWCCs, respectively. The polymer-amended samples had relatively stable AEVs among the three SWCCs; the P100/0 and P50/50 samples had the same AEVs for all SWCCs while the P30/70 sample had lower AEVs. The corresponding \( w \) and \( \theta \) water contents were highest in the NP100/0 sample and decreased with sand content in the polymer-amended samples. The corresponding degrees of saturation were highest in the P100/0 sample, followed by NP100/0, P30/70 and P50/50. The Ck, SWCC, and SL suctions were similar for the P100/0 sample, while they decreased from Ck to SWCC to SL for the sand mixes, and showed the opposite trend for the untreated tailings. The corresponding water contents were similar for all samples.

The estimated AEVs for the sand-mixed samples corresponded well with the fitting parameter \( j_{fb} \) from the Fredlund bimodal fit, which is related to the second air entry value. The estimated AEV for the P100/0 sample is 5 kPa lower than the \( j_{fb} \) fitting parameter, while the estimated AEV for the NP100/0 sample is 25 kPa lower than the fitting parameter. The AEVs corresponding to the shrinkage limits were determined using the \( w \)-based SWCC (Table 3.5). Only the values for the P100/0 sample were the same as the SWCC test, while the SL-AEV was higher in the NP100/0 and was lower in the sand mixes.
Table 3.4. Bimodal fitting parameters for the w-based SWCCs for each sample.

<table>
<thead>
<tr>
<th>Fitting Parameter</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NP100/0</td>
</tr>
<tr>
<td>(a_{fb}) (kPa)</td>
<td>2.8019</td>
</tr>
<tr>
<td>(n_{fb})</td>
<td>0.9320</td>
</tr>
<tr>
<td>(m_{fb})</td>
<td>6.4323</td>
</tr>
<tr>
<td>(j_{fb}) (kPa)</td>
<td>65.1995</td>
</tr>
<tr>
<td>(k_{fb})</td>
<td>1.3126</td>
</tr>
<tr>
<td>(l_{fb})</td>
<td>0.8359</td>
</tr>
</tbody>
</table>

Table 3.5. Comparison of the air entry values from the shrinkage test (SL), the three different SWCCs and the cracking conditions (Ck) for each sample (values rounded to nearest whole number).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Air Entry Values (kPa)</th>
<th>Water Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SL* w (\theta) S Ck</td>
<td>SL(_w) w (\theta) S Ck (\text{Ck}_w)</td>
</tr>
<tr>
<td>NP100/0</td>
<td>64 40 48 54 35</td>
<td>54 58 52 91 56</td>
</tr>
<tr>
<td>P100/0</td>
<td>22 22 22 25 23</td>
<td>50 52 50 95 49</td>
</tr>
<tr>
<td>P50/50</td>
<td>1.4 22 22 25 31</td>
<td>33 32 39 86 28</td>
</tr>
<tr>
<td>P30/70</td>
<td>0.1 16 15 13 20</td>
<td>27 22 33 88 22</td>
</tr>
</tbody>
</table>

*Values obtained from the w-based SWCC.

3.3.5 Consolidation Test

One-dimensional consolidation tests were performed to assess the behaviour of the TSRU tailings to loading and unloading conditions. The consolidation tests revealed no obvious preconsolidation behaviour in either of the 100% samples studied which is in agreement with Masala et al (2012) for remoulded TSRU tailings (Figure 3.8). The consolidation behaviour in this range of effective stresses was linear for the untreated tailings, and non-linear for the polymer-amended tailings. The \(C_c\) and \(C_r\) values were determined to be 0.3346 and 0.055 for
NP100/0 and 0.2812 and 0.042 for P100/0. The swelling indices were 0 for both samples. The tailings dewatering with respect to volume change was calculated to be 19% and 23% for NP100/0 and P100/0, respectively. The change in gravimetric water content during the consolidation test was negligible for the NP100/0 sample, but decreased by 13% in the P100/0 sample.

Figure 3.8. Consolidation behaviour of the untreated (A) and treated (B) TSRU tailings.

Comparing the overall void ratio change normalized to the sample volume from each test shows that the shrinkage test consistently had the highest change in void ratio (Table 3.6). This also shows that the sand-mixed samples had the least amount of volume change, and that the untreated tailings had more volume change compared to the P100/0 sample upon desaturation for the shrinkage and SWCC tests. However, for the consolidation test, the P100/0 sample had slightly higher volume change compared to the NP100/0 sample.
Table 3.6. Overall void ratio change for each sample during the shrinkage, SWCC and consolidation tests (not including rebound). Values are normalized to the dry volume (cm$^3$) from each test.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total $\Delta e$ (x$10^{-2}$ cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shrinkage</td>
</tr>
<tr>
<td>NP100/0</td>
<td>8.61</td>
</tr>
<tr>
<td>P100/0</td>
<td>5.45</td>
</tr>
<tr>
<td>P50/50</td>
<td>3.09</td>
</tr>
<tr>
<td>P30/70</td>
<td>2.17</td>
</tr>
</tbody>
</table>

3.3.6 Image Analysis

Figure 3.9 shows the SEM images of the untreated and treated 100% TSRU tailings samples, of grain sizes less than 80 $\mu$m. These images qualitatively show the floc structure retained in the fine portion of the polymer-amended tailings.
Figure 3.9. SEM images of TSRU tailings < 80 um at 5000X magnification. A. NP100/0. B. P100/0.
3.4 Discussion

The unsaturated geotechnical properties of untreated and polymer-amended TSRU tailings and tailings-sand mixes were characterized through index properties, shrinkage, soil-water and consolidation behaviour. The initial samples for these tests were slurries, with the exception of the consolidation tests, which were performed at the natural water content.

Overall the NP100/0 and P100/0 samples had similar index properties, allowing for comparison between the two samples. The $G_s$ values for the NP100/0 and P100/0 tailings were lower than previously reported values for oil sands tailings ($G_s$: 2.1 – 2.5, (Suthaker and Scott, 1996); $G_s$: 2.60, (Qiu and Sego, 2001); $G_s$: 2.34 and 2.39, (Owolagba and Azam, 2014)). Based on the similar $G_s$ values for the samples with and without polymer, the overall low $G_s$ of these tailings is likely due to the significant asphaltene content (Xu et al., 2013a) and residual solvent (Masala et al., 2012), which may decrease the settling capacity of these materials in tailings ponds producing more FFT.

The SFR of the NP100/0 and P100/0 samples were similar to each other and to polymer-amended MFT but higher than that for untreated MFT which is typically 0.1 (Jeeravipoolvarn et al., 2009; Bajwa and Simms, 2013). Polymer treatment has been shown to significantly increase SFRs in MFT (Bajwa and Simms, 2013), however this was not observed in the TSRU tailings, where the NP100/0 sample had a slightly higher SFR compared to the P100/0 sample. Although the NP100/0 sample was prepared to achieve similar properties to the P100/0 sample, this difference in the SFRs is due to the slightly lower fines content in the NP100/0 sample. The SFR
of the P50/50 sample is not similar to known values for oil sands tailings while the P30/70 sample approached the typical values for CT (Beier et al., 2013; Jeeravipoolvarn, 2010).

The PSDs for the untreated and treated 100% TSRU tailings samples show a similar trend at particle sizes >45 μm, and differed at particle sizes smaller than this, with the polymer-amended sample containing higher fines. This is unexpected as the function of the polymer is to agglomerate fine particles into flocs, creating larger particle sizes, resulting in a lower fines content. This effect has been shown for polymer-amended MFT (Bajwa and Simms, 2013) and may be a function of shearing (Dunmola et al., 2013). The higher amount of fine particles in the polymer-amended TSRU tailings (< 45 μm), and in the sand-mixed samples (< 10 μm), is likely due to the use of a dispersing agent during the hydrometer testing, which may cause floc dissociation, releasing fines into the solution. Dispersing agents have been shown to both increase (Jeeravipoolvarn, 2010) and decrease (Bajwa and Simms, 2013) the fines content of cyclone overflow and MFT, respectively, from hydrometer testing. Therefore, the effect of dispersing agents on PSDs of oil sands tailings is unclear, and may be a function of material type.

Overall, the salinity estimates and pH were higher for the untreated tailings; the salinities for both materials were within the range known for oil sands (An et al., 2013), while the pHs were 1-2 units lower than typical oil sands tailings values (Fedorak et al., 2003). The directly measured TDS were higher than the EC-converted TDS, suggesting that the mass of hydrocarbons may have increased the direct measure, but may not have influenced the EC measurements since their electrical conductivities are 6 – 7 orders of magnitude smaller than the
measured values (Zeng et al., 2009). These results suggest that the polymer may have decreased the ionic and organic fractions of the pore water, possibly by absorption and trapping during flocculation and/or agglomeration. Therefore, the EC-converted TDS may offer a more accurate measure of salinity for these materials. Since the ion concentrations were not within the scope of this study and these methods are only approximations for estimating salinity, the two EC conversions were interpreted as the approximate salinity range for these materials.

Salinities of the same order of magnitude found for the TSRU tailings have been shown to increase cracking, water storage and the shrinkage behaviour of soils (Lima and Grismer, 1994). Salinity has also been shown to increase the hydraulic conductivity of bentonite-sand mixed materials (Siddiqua et al., 2011). Although these effects are dependent on material type and the effects of salinity on the geotechnical behaviour of the TSRU tailings were not investigated in this study, it is an important parameter to characterize as phage transport decreases with increasing salinity in porous media. Therefore this knowledge is critical for future experimental design for studying phage transport in these tailings as a means of ARD prevention.

The shrinkage limits for the untreated and treated 100% TSRU tailings were high compared to typical values for oil sands tailings (10 – 20 %) (Yao, 2012; Fredlund et al., 2011; Owolagba and Azam, 2014). The P30/70 sample was just above this range, and the P50/50 was similar to that of flocculated MFT (Yao, 2012). This suggests that the higher coarse particle content of the TSRU tailings will reduce the shrinkage, which may decrease the rate of dewatering at water contents below the shrinkage limit as shrinkage expels water from the pore spaces.
The shrinkage tests revealed that although the untreated and treated 100% TSRU tailings had similar final void ratios, the untreated tailings exhibited greater shrinkage, suggesting that the polymer reduced shrinkage upon desaturation. This is inconsistent with what has been shown for flocculated MFT, where the overall shrinkage between untreated and flocculated MFT was similar, but the flocculated MFT had a significantly higher final void ratio (Yao, 2012). The shrinkage was further reduced with increasing sand content, as is expected for materials with high SFRs. During the shrinkage tests, the untreated tailings showed a higher rate and more complete desaturation compared to the polymer amended samples. This suggests that the polymer may trap water in the tailings, which would increase the residual water content and decrease the residual suction.

The NP100/0 and P100/0 showed early deviation from the line of saturation which was not observed in the sand-mix samples. This was shown for flocculated MFT but not for untreated MFT (Yao, 2012), suggesting flocculent may produce this effect, but it has also been attributed to the residual hydrocarbon content of oil sands tailings (Owolagba and Azam, 2014). Because this behaviour was exhibited in both treated and untreated TSRU tailings, it is more likely that it’s a function of the hydrocarbon content, which would have been diluted in the sand-mixed samples, rather than a function of the polymer for these materials.

The w- and θ-based SWCCs showed that the polymer-amended tailings had similar shapes and tended to have a lower water content at a given suction compared to the untreated tailings. The S-based SWCC showed that the sand-mixed samples behaved similarly to each other, i.e. deviating from saturation upon the first applied suction. They also had a lower degree of
saturation at a given suction and a lower AEV compared to the 100% tailings samples, attributed to the sand content increasing the drainage capacity. The P100/0 sample behaved similarly to the NP100/0 sample at suctions below the AEV, after which the P100/0 sample behaved more similar to the sand-mixed samples. This suggests that the polymer may reduce the AEV of the TSRU tailings, possibly by promoting cracking due to the agglomeration of flocs in the microstructure during desaturation (Bajwa and Simms, 2013). The S-based SWCC also shows that after the AEV has been reached, polymer-amended tailings require less energy (lower suction) to desaturate compared to untreated tailings. This result is inconsistent with what has been shown for flocculated MFT, where at suctions below 200-400 kPa, flocculated MFT hold more water than untreated MFT (Dunmola et al., 2013; Yao, 2012).

The AEVs for the polymer-amended samples were similar to those found by Dunmola et al. (2013) for both flocculated and untreated MFT. Although higher and increasing with the w-based, θ-based and S-based SWCCs, the AEV for the untreated TSRU tailings was still 2 orders of magnitude lower than what has been shown when incorporating shrinkage to produce the S-based SWCC for untreated oil sands tailings (Fredlund et al., 2011; Fredlund and Houston, 2013). This may be the result of sample cracking allowing for preferential flow paths in the samples, producing lower AEVs. Péron et al. (2009) have shown that the SL, AEV and cracking onset were similar, as they were for the water content values of the TSRU tailings. However, the suction values for these three parameters were only similar for the P100/0 sample, while the sand-mixed samples showed descending suctions from Ck > AEV > SL, and the untreated tailings had the opposite trend. This implies that in the sand-mixed samples, the sample stopped
shrinking before the AEV, and was prone to cracking after the AEV, indicating that air entry may have promoted crack formation. The crack formation was also likely influenced by the moisture gradient present in these samples (Péron et al., 2009). In the untreated tailings, the sample cracked close to air entry and while the sample was still undergoing shrinkage, suggesting that shrinkage may have promoted cracking and air entry in this material. The effect of shrinkage on the soil-water behaviour of the untreated TSRU tailings was also demonstrated by the change in AEV with SWCC representation. This was not the case for the polymer-amended samples, indicating that the polymer may negate this effect. As this may also be the result of sample cracking, further studies, including shrinkage-SWCC coupled pressure plate extractor tests, should be performed to validate this result.

The Fredlund bimodal equation produced the best fit to represent the SWCCs for all of the TSRU tailings samples. The line of oxidation, which formed at low suctions (~1 kPa) suggests that air entered the surface of the sample early on and caused oxidation of the sulfide minerals. Since this occurred in all of the samples, it suggests that the surface of these tailings desaturate at low suctions and could lead to the development of ARD soon after surface deposition. This surface air entry may also have resulted in the low first air entry value (related to the fitting parameter $a_{fb}$) and subsequent bimodality observed for the soil-water behaviour of these tailings.

The fitting parameter related to the second air entry value was only close for the polymer-amended tailings and was considerably higher for the untreated tailings. This implies that interpretation of the AEVs for the polymer-amended TSRU tailings may be mathematically obtained with some accuracy but not for untreated TSRU tailings.
Although the SWCC data are limited to 500 kPa, the fitting curves, along with the shrinkage behaviour, allow for prediction of the residual conditions. The data suggest that although the polymer-amended tailings require less energy for desaturation, they will likely have an overall higher residual water content at a lower suction. This is potentially due to water being trapped in the floc structures, which would increase the overall water content of these tailings once residual conditions were achieved. Further studies that include a higher suction range are required to establish the residual conditions for unsaturated TSRU tailings.

The untreated tailings exhibited normally consolidated behaviour while the polymer-amended tailings showed non-linear behaviour. This non-linear behaviour has previously been shown for polymer-amended MFT (Farkish and Fall, 2013), however, linear behaviour has also been shown by others (Dunmola et al., 2013; Yao, 2012). This discrepancy may be influenced by flocculent type, concentration, and testing conditions. Polymer content has been shown to produce lower $C_c$ values (Farkish and Fall, 2013) as seen with the TSRU tailings. Both the untreated and treated TSRU tailings $C_c$ values were in agreement with previously reported values for oil sands tailings (Yao, 2012; Owolagba and Azam, 2014).

The overall volume change per unit volume during the shrinkage and SWCC tests was highest in the untreated tailings, and decreased with increasing sand content in the polymer-amended tailings. However, in the consolidation test, the P100/0 had a higher volume change compared to the NP100/0 sample. Further, the change in gravimetric water content during the consolidation test was negligible for the NP100/0 sample, but decreased by 13% in the P100/0 sample, which also had a dewaterability with respect to volume 4% higher than the untreated tailings. This
water loss resulted in the overall void ratio change per unit volume being slightly higher in the polymer-amended tailings. These results suggest that under self-weight consolidation in the field, the polymer could significantly enhance tailings dewatering with only a slight effect on the volume change associated with compression and loss of water.
Chapter 4: Conclusions

Disposal of oil sands tailings poses challenges to the industry due to the difficult nature of the materials, and the potential for hazardous environmental effects. This research is among the first to look at the microbial and geotechnical characteristics of TSRU tailings and the application of the results to the potential generation of ARD, meeting the goals of this thesis. The hypotheses associated with these goals were accepted for both the microbial and geotechnical aspects. The conclusions for this thesis are as follows:

1. TSRU tailings host a native microbial population that is capable of acid generation which could play a role in the development of ARD.

2. The results provide potential microbial targets for the development of phage therapy as a means of ARD prevention.

3. The results add to the scientific knowledge about the types of microbes native to oil sands tailings.

4. The specific gravity of TSRU tailings is lower than other oil sands tailings, indicating potentially more FFT production during deposition in tailings ponds. As Directive 074 requires the reduction in FFT, alternative disposal methods, such as surface deposition, should be investigated for these tailings.

5. All forms of studied TSRU tailings had high shrinkage limits, and the untreated tailings exhibited a higher degree of shrinkage compared to the polymer-amended samples. The SWCCs showed that at suctions greater than the AEVs, the polymer-amended samples desaturate with less energy than the untreated tailings.
6. All of the TSRU tailings had relatively low AEVs for high volume change materials. Compared to the untreated tailings, the AEVs of the polymer-amended samples were lower and were not affected by shrinkage. The $S$-based SWCC provided the best representation for assessing the soil-water behaviour of the TSRU tailings.

7. The consolidation behaviour of the polymer-amended TSRU tailings was non-linear whereas the untreated tailings had linear behaviour. The polymer-amended tailings exhibited a higher dewaterability during self-weight consolidation.

8. Sand addition reduced shrinkage of the polymer-amended TSRU tailings. Polymer-amended samples with 0% and 50% sand had the same AEV with the latter having slightly better drainage. The lowest AEV was achieved at 70% sand content, however this also produced a non-pumpable material, which is inadequate for hydraulic transport and disposal.

9. At low suctions, air enters the surface of the TSRU tailings and pyrite oxidation occurs.

This research was limited by the lack of publically available information on the methods currently used by the oil sands industry for geotechnical characterization, and general characteristics of TSRU tailings. Further, the samples were only taken from one area, and may not have been sufficiently representative of the TSRU tailings. Sample collection was performed by the oil operators with no detailed account of the collection procedures, limiting the ability to control for biases from sampling.

This research could possibly be applied to other environments affected by crude oil, such as areas affected by oil spills, as well as applications in ARD sites. Since ARD is a world-wide
problem, research into sustainable, environmentally-friendly prevention technologies will be applicable and benefit any formative technologies.

Possible directions for future research could include further geotechnical analyses to refine the methods most appropriate for these materials, performing a coupled soil-water-shrinkage characterization using the pressure-plate extractor method to validate these results, hydraulic conductivity testing, and performing strength testing on the TSRU tailings to inform on the likelihood of achieving the requirements for surface deposition in D-074. Hydraulic conductivity estimates were attempted from the consolidation data, however due to the little amount of time required to achieve full consolidation at each load, the curves could not produce adequate data. Further microbial analysis, including molecular-based approaches, would help identify the target microbial populations involved in ARD generation in these tailings. This would aid in the isolation of viruses for developing phage therapy as a means to control ARD-generating microbial populations.
References


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