Application of Rhamnolipid and Microbial Activities for Improving the Sedimentation of Oil Sand Tailings

Soroor Javan Roshtkhari

A Thesis

In the Department of

Building, Civil and Environmental Engineering

Presented in Partial Fulfillment of the Requirements

For the Degree of

Doctor of Philosophy (Building, Civil and Environmental Engineering) at

Concordia University

Montreal, Quebec, Canada

April 2016

CONCORDIA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

This is to certify that the thesis prepared

By: Soroor Javan Roshtkhari

Entitled: Application of Rhamnolipid and Microbial Activities for Improving the Sedimentation of Oil Sand Tailings

and submitted in partial fulfillment of the requirements for the degree of

PhD (Civil Engineering)

complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the final examining committee:

Dr. Robin A.L. Drew Chair

Dr. Satinder Kaur Brar External Examiner

Dr. J. Patterson External to Program

Dr. S. Li, Examiner

Dr. F. Haghighat Examiner

Dr. C. Mulligan Thesis Supervisor

Approved by

Chair of Department or Graduate Program Director

Dean of Faculty

Abstract

Application of Rhamnolipid and Microbial Activities for Improving the Sedimentation of Oil Sand Tailings

Soroor Javan Roshtkhari, PhD.

Concordia University, 2016

Densification of oil sand tailings deposited in the tailing ponds and recovering water from them are two major challenges issues in the oil sands surface mining industry. A small increase in the tailing settlement rate (which normally is very slow) can improve the densification of tailings and significantly, reduce water consumption and the volume of tailing ponds. Currently most of the industrial methods for consolidation of oil sand tailings are based on clay particle flocculation methods which use different kinds of agents such as calcium sulfate (gypsum), and polymeric flocculants. In this work, rhamnolipid (JBR 425) was investigated as a flocculating agent and microbial activities by performing the sedimentation experiments to increase the sedimentation of fine tailing particles. It has been found that rhamnolipid increased sedimentation by improving the hydrophobic interaction between the particles. The feasibility of in situ biosurfactant production by indigenous microorganisms, *Bacillus subtilis* strain and two microbial strains isolated from weathered oil was investigated and it was found that all strains could produce very low amounts of biosurfactant. A mixed culture of two microbial strains isolated from weathered oil increased the sedimentation. Different concentrations of rhamnolipid together with these two microbial strains at 23 ºC ± 2 ºC could lead to significant increases in sedimentation (by a maximum factor of 3.04), increases in the concentration of larger particles (by a maximum factor of 1.9), particle mean diameter (by a maximum factor of 2.11) and flocculation in the tailings samples amended with them compared to the control. Rhamnolipid (0.5%) together with these two microbial strains at 15 ºC ± 2 ºC showed significant increases in sedimentation (by a factor of 5.1), the concentration of larger particles (by a factor of 2.63), particle mean diameter (by a factor of 2.70) and flocculation in the tailings samples compared to the control. while the zeta potential is still negative. According to the pH measurements (during the 50 days) increase in the ionic strength (I) of the pore water and reduction in the thickness of the DDL of clay particles is not responsible for increasing the sedimentation as dissolution of MFT carbonate minerals and releasing divalent cations could not occur at pH higher than 7.5. However there might be a small amount of CH_4 production at 15 ºC ± 2 ºC in the deeper layer of mud. This means that the mechanism of flocculation in these cases could be probably due to increasing the hydrophobicity of the particles, due to the interaction of biosurfactant and high molecular weight microbial organic compounds through a bridging mechanism with clay particles, and to due to forming transient canals from small amounts of CH_4 production. Using rhamnolipid as a flocculating agent could bring the remaining oil and also a small amount of insoluble heavy metals from the tailing sediment into the water. Dissolved heavy metal ions and rhamnolipid in water could be removed through micellar- enhanced ultrafiltration (MEUF) process (between 30% for Cd and 100% for V, and 97.5% for rhamnolipid). This work shows the potential of using rhamnolipid and microbial culture in order to increase the oil sand sedimentation through flocculation and microbial activity in a more environmental friendly and densification process.

Acknowledgements

I would like to say thanks to my supervisor Professor Catherine Mulligan for her help, her support and her understanding during this work. Also thanks to the faculty and staff in the Building Civil and Environmental Engineering department specially Ms. Hong Guan. Thanks to my friend Nayereh Saborimanesh who helped and supported me as a friend during this work.

Contents

List of Figures

List of Tables

List of Abbreviation

CHWE	Clark Hot Water Extraction
CMC	Critical Micelle Concentration
COSIA	Canada’s Oil Sands Innovation Alliance
Cp	Concentration in the permeate
Cs	Concentration in the supernatant (feed)
CSH	Cell Surface Hydrophobicity
CSS	Cyclic Steam Stimulation
CT	Consolidated Tailings
CTAB	Cetyl trimethyl ammonium bromide
Da	Daltons
DDL	Diffuse Double Layer
EPAs	Environmental Priority Areas
EPS	Exopolysaccharide or Extracellular Polymeric Substances
ERCB	Energy Resources Conservation Board
ES-SAGD	Expanding Solvents Steam-Assisted Gravity-Drainage
FID	Flame Ionization Detector
GC	Gas Chromatography
GCOS	Great Canadian Oil Sands
HAA	Hydroxyalkanoyloxy Alkanoic Acid
I	Ionic strength
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
LDHs	Layered Double Hydroxides
LOI	Loss on Ignition
LPS	Lipopolysaccharide
MEUF	Micellar Enhanced Ultrafiltration

MFT	Mature Fine Tailings
MWCO	Molecular Weight Cut-Off
NRB	Nitrate-Reducing Bacteria
PAHs	Polycyclic Aromatic Hydrocarbons
PVA	Poly- Vinyl Alcohols
R	Rejection percent
RLs	Rhamnolipids
S	Sedimentation
SAGD	Steam-Assisted Gravity-Drainage
SDS	Sodium Dodecyl Sulphate
SRB	Sulfate-Reducing Bacteria
SRD	Sustainable Resource Development
TFT	Thin Fine Tailings
TRO	Tailings Reduction Operations
TT	Thickened Tailings
VOCs	Volatile organic compounds
ζ	Zeta potential

1. INTRODUCTION

Global demand for unconventional energy sources such as coal bed methane, heavy oil, and bitumen has grown in recent years. Bitumen is extracted from oil sands exist naturally as a mixture of bitumen, sand, clay and water. Bitumen can be upgraded to crude oil which is the main natural resource for energy. Canadian oil sands are mainly located in the Athabasca region in Alberta, with the rest in Peace River and Cold Lake (Government of Alberta 2008). There are two methods for bitumen production from oil sands; surface mining (truck and shovel open pit mining) and in situ separation of the bitumen (Patterson 2012). Two to four barrels of fresh water are required per barrel of oil produced from the surface mining method (Alberta Energy 2010).

"Tails" or "tailings" are the by-products from the extraction of bitumen from the sand by surface mining method which are pumped into tailings ponds for storage (Fig 1-1). This tailing suspension is a mixture of process affected water, sand, clays, salts, metals, residual bitumen and hydrocarbon diluents. It has been reported that between 1992 and 2008, the extent of tailings ponds grew by 422% while the extent of mine pits, facilities, and infrastructure grew by 383% (Timoney and Lee 2009). The government regulated the tailing ponds (Bordenave et al. 2010; Government of Alberta 2010; WWF 2010). In February 2009, the Alberta Energy Resources Conservation Board issued Directive 074 with aggressive criteria for managing tailings (Government of Alberta 2013). According to it, companies are required to reduce tailings and provide target dates for closure and reclamation of ponds. The Directive also lays out timelines for operators to process fluid tailings at the same rate they produce them (which will eliminate growth in fluid tailings). According to the Alberta government's Tailings Management Framework, industry must have effective bird deterrence systems (are designed to prevent birds from landing on the ponds) in place under the government's approval requirements for tailings ponds. All tailings ponds are constructed with groundwater monitoring and seepage capture facilities. Where seepage is detected, government requires a recapture system to return the process-affected water to the pond (Government of Alberta 2013).

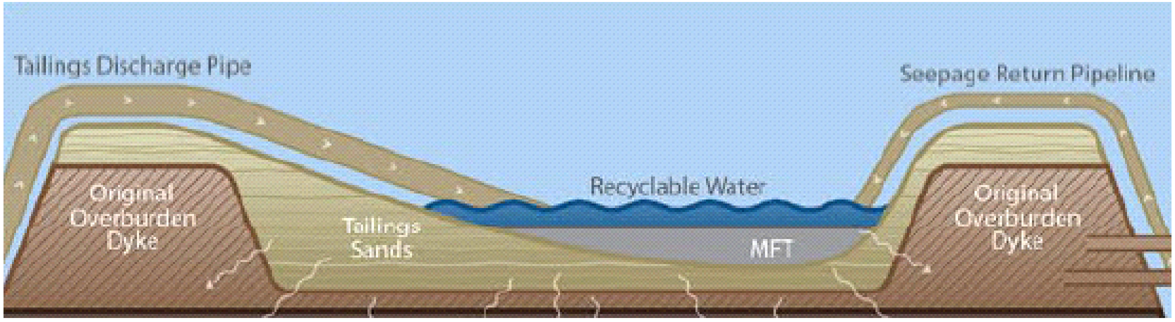


Figure 1-1 Schematic Diagram of a Tailings Pond (Government of Alberta 2013)

In the pond there are different layers according to the weight of the material for settling to the bottom of the pond. The toxic water forms the top layer which can be recycled into the extraction process (Mamer 2007; Masliyah 2007).

In oil sands there are specific definitions of “fines” and “sand” (BGC Engineering Inc 2010; ERCB 2009; OSRIN 2013; Sobkowicz 2012). Fines are defined as minerals which include silt (2µm-0.05 mm), and clay (<2 µm) smaller than about 44 µm (BGC Engineering Inc 2010; ERCB 2009; OSRIN 2013; Sobkowicz 2012). Coarse sand is defined as mineral solids with particle sizes greater than 44 µm (BGC Engineering Inc 2010; ERCB 2009) or a soil particle between 0.05 mm and 2.0 mm in diameter (OSRIN 2013).

Coarse sand grains (larger than 44 microns) settle out quickly. Thin fine tailings (TFT) which are a combination of fines and water with less than 30% solids will start immediately. TFT will settle and within two or three years a layer of mature fine tailings (MFT) develops which is a mixture of fine clay particles (under 44 microns in size) and water, with approximately 30%-60% solids and has a consistency similar to yogurt. Complete settling of MFT is very slow (Mamer 2007; Masliyah 2007; WWF 2010) (almost a century). Figure 1-2 shows the segregation of tailings within a tailings pond.

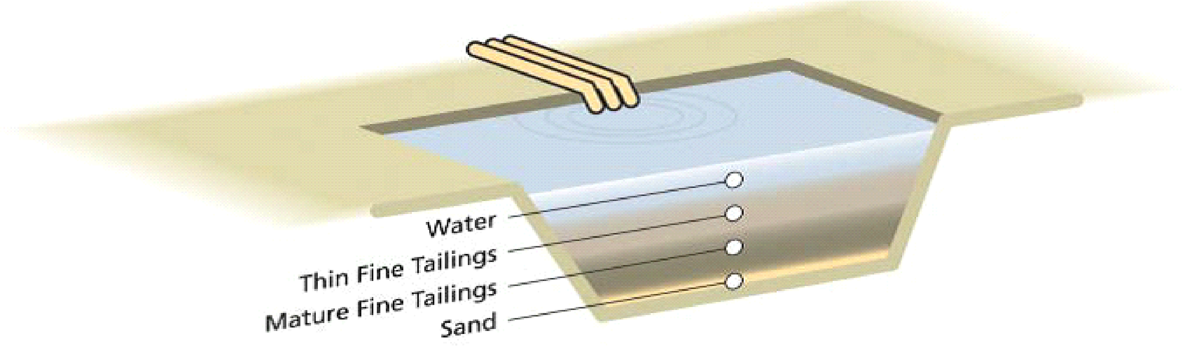


Figure 1-2. Segregation of tailings within a tailings pond (Mamer 2007)

The more active clays, perhaps somewhat degraded by weathering or the action of caustic soda, and coated with bituminous residues appear to be the main cause of the gel-like structure formation in the tailings and for the ion exchange mechanism in the tailings ponds (Chalaturnyk et al. 2002). The fundamentals of the formation of low density fine tailings deposits are still poorly understood, despite enormous research efforts (BGC Engineering Inc 2010).

It is known that clay minerals, in the presence of caustic soda, possess an enhanced negative surface charge which promotes dispersion of the particles, inhibiting their sedimentation and consolidation (Mikula et al. 2009). Dispersion of the clays, which is necessary for efficient bitumen extraction by flotation, prevents rapid dewatering (sedimentation and consolidation) of the tailings clays. Adding sodium ions (as caustic soda) to the oil sands extraction process exacerbates this undesirable condition as far as tailings disposal is concerned. The dispersant effect of these monovalent sodium ions can be counteracted and controlled to some extent by the addition of divalent calcium ions. This cation exchange process and the affinity of calcium ions for the clay surface play an important role in many tailings treatment strategies (Mikula et al. 2009).

In a study which investigates the methanogens and sulfate-reducing bacteria in oil sands fine tailings waste, Holowenko et al. (2000) stated that generally the temperature of the fine tailings (found at depths greater than 5m) does not fluctuate significantly, averaging between 11°C and 15°C year round (Holowenko et al. 2000). Their samples were collected from the large tailings pond (Mildred Lake Settling Basin) on the Syncrude Canada Ltd. lease near Fort McMurray, Alberta. Temperature also increases with depth due to a lack of surface cooling and retention of heat at 30-60˚C by deposited tailings (about 22˚C at depth 20 m and 16.9˚C at depth 10 m) (Penner and Foght 2010). Siddique at al. (2014) assumed 20 °C, for the tailings pond in situ temperature. (Foght and Dunfield (2013) report that the in situ temperature of MFT was ~12–20°C all year round at a depth of between10 m-25 m (Foght and Dunfield 2013). According to a study by Wells (2011), which investigated the long term in-situ behaviour of oil sands fine tailings in the Suncor Pond 1A, the in situ temperature of MFT was between 13.1°C-17°C at a depth of between 10.8-37.8 m year round. Temperature (thermodynamic effects) is one of several factors which may influence the species dominant in the MFT. For example, acetogens often compete better with hydrogenotrophic methanogens for limiting H_2 at low temperatures (<15°C), whereas at moderate temperatures (>20°C) hydrogenotrophic methanogens partner with syntrophic bacteria to oxidize fatty acids (Kotsyurbenko et al. 2004).

Suncor currently has eight oil sands tailings ponds covering a total area of 2,689 hectares. Most are close to 46 metres in depth (Suncor Energy 2011). Figure 1-3 shows the typical depth for each layer in the tailing pond.

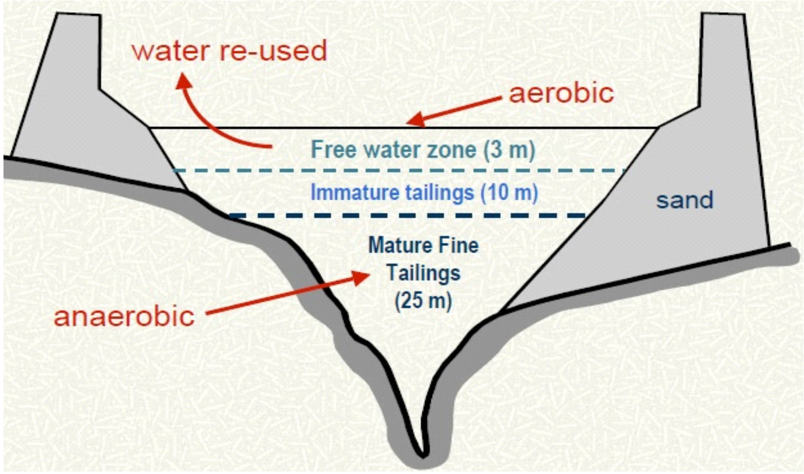


Figure 1-3. Different depths of tailing layers within a tailings pond (adapted from (MacKinnon 1989)

As of 2008, about 750 million cubic meters of MFT exist within the tailings ponds. If there is no change in tailings management, the inventory of fluid tailings is forecast to reach one billion cubic meters in 2014 and two billion in 2034 (Houlihan and Haneef 2008). Now more than 170 km^2 of tailings ponds exist in Alberta and the government is trying to significantly reduce these tailings ponds by strict legislation for limiting fresh water intake and increasing the recycling of water from tailings (Government of Alberta 2010; Masliyah 2007; WWF 2010). Toxic impacts of tailing ponds can affect ecosystems and human health. It means that there is an urgent need for the attention of the world's scientific community and systematic study (Timoney and Lee 2009).

An increase in the tailings settlement rate can increase the efficiency of water recycling and reduce the volume of tailings ponds. The large volume of MFT requiring safe containment and the vigilant management of capping waters represents a significant management challenge and liability for the industry (BGC Engineering Inc 2010). It means that management and increase in the sedimentation of oil sand tailings is an important environmental and economical issue.

There are many methods in order to treat tailings ponds and increase sedimentation. These methods can be natural, physical or chemical/biological. Natural treatments include freeze-thaw technology and plant dewatering (the planting of grasses, shrubs or trees). Plants may assist in dewatering (plant transpiration) and consolidation by the fibrous roots that withdraw water for growth (BGC Engineering Inc 2010). However the high salinity of tailings can inhibit establishment and healthy growth. Physical methods include centrifugation, filtration, electrophoresis and electro-coagulation (BGC Engineering Inc 2010). In chemical/biological methods, densification can be achieved by addition of agents such as calcium sulfate (gypsum), sodium silicate, organic flocculants, inorganic coagulants, oxidizing and reducing agents and most recently carbon dioxide and some microbial activity such as methanogenesis (Bordenave et al. 2010; Masliyah 2007). These methods will be discussed in the next chapter.

Currently most of the industrial methods for oil sand tailings densification are based on clay particle flocculation (BGC Engineering Inc 2010). The flocculation process refers to the macroscopic aggregation of suspended particles into loosely packed flocs by addition of polymeric flocculants (Crittenden 2005; Hunter 2001b). Microbial densification is a low cost method for increasing sedimentation (Quagraine et al. 2005; Siddique et al. 2007). Methane formation (methanogenesis) by anaerobic microbes can improve tailings densification.

1.2 Statement of the Problem

The polymeric flocculation methods which are now the most effective method for oil sand tailings sedimentation and the microbial densification methods have their own limitations which will be discussed in the next chapter. The performance of the flocculants is an important issue in flocculation based methods. However the recycle water quality, startup and operational costs, experienced operators and careful operational control, in some cases should be considered (BGC Engineering Inc 2010). In the microbial activity and methanogenesis method, methane and carbon dioxide emissions (up to 104 m^3 day^{-1} for methane) from tailing ponds should be controlled as they are greenhouse gases (Fedorak et al. 2003; Voordouw 2013).

It has been reported that surface active agents (surfactants) can be applied for increasing sedimentation and dewatering. Surfactants can reduce surface and interface tension by forming molecular film at the interface of air and water or two liquid phases (i.e. oil/water). There are some surfactants which are produced by living natural sources (*i.e.*, from a plant, animal or microorganism) and known as biosurfactants (Chhatre et al. 1996; Mulligan 2005).

In most of the work which has been done on increasing sedimentation and dewatering, the effect of synthetic surfactants on flocculation and dewatering of different clay particles was investigated. They showed great ability for dewatering slurries when they are combined with polymers as flocculants. They can change surface wetting characteristics of particles and lead to an increase in flocculation and dewatering.

Biosurfactants have more advantages over synthetic surfactants such as low or non-toxicity and biodegradability. They are also more economic than the other surface active agents in some cases due to high efficiency. In addition to these advantages which make them attractive for many environmental applications and protection, they have potential to decrease the environmental impacts of oil sands (Banat et al. 2000; Mulligan 2005; Rahman et al. 2002a; Rodrigues et al. 2006; Urum and Pekdemir 2004; Xu et al. 2011). Rhamnolipids (RLs) are the most intensively studied biosurfactants. Many studies show their potential for remediation of oil contaminated soil and water (Mulligan 2014).

1.3 Objectives

In this work the main objective is to evaluate the use of biosurfactants (rhamnolipid) as flocculating agents and microorganisms (by inoculation or naturally present) together with rhamnolipid biosurfactant to enhance the sedimentation in tailing ponds, which could reduce the need for fresh water and tailings ponds volume, and understanding the mechanism of sedimentation within this approach. The sub objectives of this work are:

-To determine the effect of various parameters on sedimentation of amended samples and the kinetic rate of sedimentation with rhamnolipid and microbial cultures.

-To determine the relation between biosurfactant/microbial flocculation and the clay portion.

-To determine the effect of the treatment of the remaining sediment and recovered water and to evaluate Micellar Enhanced Ultrafiltration as a treatment of the water for potential recycling.

- To evaluate the potential for in situ biosurfactant production.

2. LITERATURE REVIEW

2.1. Alberta's Oil Sand

Alberta’s oil sands deposits have 171.3 billion barrels of remaining oil reserves (Government of Alberta 2010). From these reserves 34 billion barrels are recoverable through mining (ERCB 2010). Oil sands production is expected to increase from 2.3 million barrels per day in 2014 to 4 million barrels per day in 2024(Alberta Energy 2015).

Commercial extraction of oil sands began in 1967 by Great Canadian Oil Sands (GCOS) on the banks of the Athabasca River north of Fort McMurray. Today four operators (Suncor, Syncrude, Shell and Canadian Natural Resources Ltd.) extract and process bitumen from oil sands in a region in Northern Alberta. In addition to the existing government policies, the Government of Alberta also released a 20-year strategic plan for the oil sands industry in February 2009 (Government of Alberta 2010). Furthermore, a number of organizations (including the Alberta Environment, the Alberta Sustainable Resource Development (SRD), the Energy Resources Conservation Board (ERCB), Canada’s Oil Sands Innovation Alliance (COSIA) and others) have been formed to monitor the environmental impacts and to ensure an efficient development of oil sands resources. For example COSIA is an alliance of oil sands producers focused on accelerating the pace of improvement in environmental performance in Canada's oil sands. Through COSIA, participating companies capture, develop and share the most innovative approaches and best thinking to improve environmental performance in the oil sands, focusing on four Environmental Priority Areas (EPAs) (tailings, water, land and greenhouse gases) (Alberta Energy 2010; Government of Alberta 2006; https://www.cosia.ca).

2.1.1. Oil production methods

In both surface mining and in situ separation of the bitumen methods, produced bitumen needs to be upgraded to the lighter hydrocarbon fluid before going to refining units (CAPP 2009). In the upgrading units, the viscosity and the sulfur, nitrogen, and metal contents will decrease (Humphries 2008; Hyndman and Luhning 1991; Isaacs 2007).

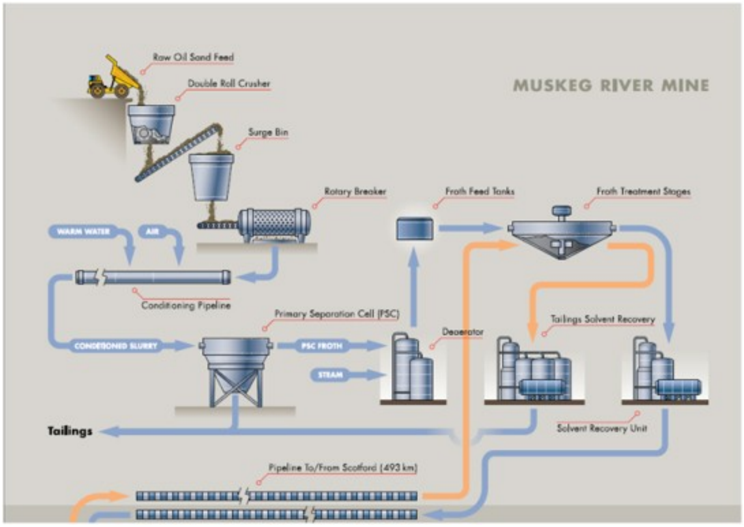


Figure 2-1. Schematic flow chart of bitumen production (Shell Canada 2016)

2.1.2. Separation processes

There are two main industrial processes for bitumen extraction: the Clark Hot Water Extraction (CHWE) and the Steam-Assisted Gravity-Drainage (SAGD). CHWE is the original extraction process and is used by the majority of the oil sands industry as the standard extraction process from surface mining (Hyndman and Luhning 1991). SAGD is the most commonly used in-situ operations (Chow et al. 2008). Cyclic Steam Stimulation (CSS) is another in-situ extraction process used in the industry in Cold Lake, Alberta areas (Chow et al. 2008).

2.1.2.1 Clark Hot Water Extraction (CHWE) process

In open-pit mined oil sands sites after mining, crushing and slurring, the oil sands slurry is delivered through hydro transport pipelines with warm recycled process water and small amounts of air (Isaacs, 2007). The tar-like bitumen is extracted from the ore with hot water and chemicals such as NaOH and steam (Holowenko et al., 2000). The Clark Hot Water Extraction (CHWE) process for bitumen extraction was patented in 1929 by Dr. Karl Clark and has been in commercial use since 1967 (Chow et al. 2008). Mixing heated water (heated to between 35 and 80 degrees Celsius) with crushed ore and NaOH led to reduction of the viscosity of the bitumen and the mechanical shear helps to separate the bitumen from the sandy slurry (Chow et al. 2008; Isaacs 2007). This extraction is an iterative process and separated bitumen floats to the top of the mixture and is skimmed off and the remaining sand slurry is sent to the tailing ponds (Alberta Chamber of Resources 2004; Chow et al. 2008; Patterson 2012). The CHWE process works based on the fact that the coarse Athabasca sands are hydrophilic (grains of sand in the ore attract a thin film of water) while the bitumen in the ore is hydrophobic and repels wetted surfaces.

2.1.2.2 Steam-Assisted Gravity-Drainage (SAGD)

About 80% of the oil sands resource is deposited underground at a depth that is not feasible for open pit mining and needs to be recovered by in situ extraction. Roger Butler and his colleagues at Imperial Oil developed the Steam-Assisted Gravity-Drainage (SAGD) for bitumen recovery from oil sands in the late 1970s (Chow et al. 2008). This technology was first employed in Cold Lake (Masliyah 2007).

In this process a pair of horizontal wells is drilled, with a separation of 5 m to each other, one above the other (Masliyah 2007). The upper well injects steam, and the loosened bitumen is collected in the lower production well (Isaacs 2007). Expanding solvents also can combine with the steam in the injection process. This new technology is called Expanding Solvents Steam-Assisted Gravity-Drainage (ES-SAGD) (Chow et al. 2008; Patterson 2012).

2.2. Oil Sands Tailings Properties

2.2.1 Oil sands tailing clays and their properties

Bitumen remaining in the tailings stream can cause major issues with respect to settlement of solids (BGC Engineering Inc 2010; Irwin Wislesky et al. 2013). It is believed that illite and mixed layers of clays are largely responsible for the processing and compaction problems in oil sands extraction and fine tailings disposal (BGC Engineering Inc 2010). Existence of these particles makes the tailing pond a colloidal system and strongly inhibits the settling of particles. Oil sands tailings contain a significant amount of clay particles (Masliyah 2007).

Clay particles are electrically charged and the process of cation exchange is an important phenomenon of clays. There are different types of interactions between particles in a colloidal system. These interactions are closely related to the surface properties of particles. Interaction of charged species in an electrolyte solution is usually explained by the formation of an electrical double layer. The electrical double layer is formed near the charged surface which attracts oppositely charged ions in the solution. Forming this layer makes the whole system electrically neutral (Fuerstenau 1982).

The net interaction between the particles resulting from the attraction and repulsion between the particles is responsible for the stability of the system (Masliyah and Bhattacharjee 2005). The repulsive net interaction makes the system stable and particles stay as dispersions, while the attractive net interaction makes the system unstable and the particles will coagulate. The repulsion between particles comes from the presence of an electrical double layer while the attraction is due to van der Waals forces (Masliyah and Bhattacharjee 2005). Compressing the electrical double layer, which favors sedimentation of oil sands tailings, might be considered for increasing the overall attraction between clay particles. Zeta potential (ζ) is used for estimating the degree of electrical double layer charge (Figure 2-2).

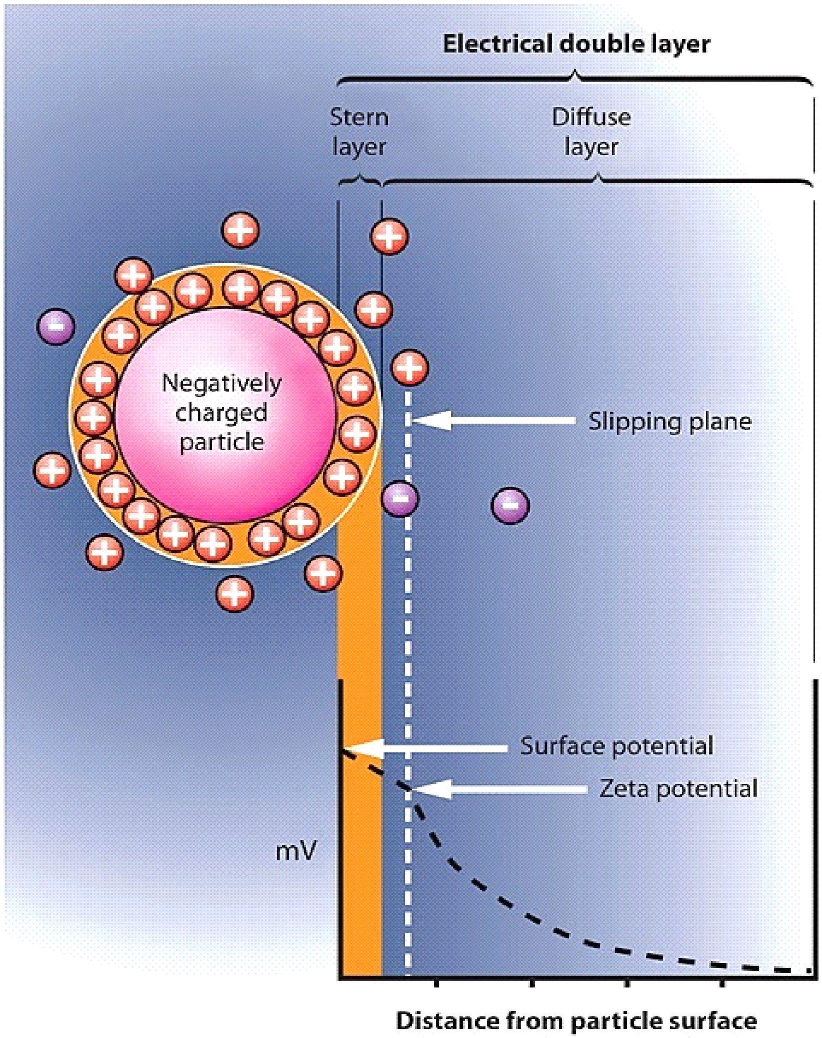


Figure 2-2. Schematic representation of the electrical double layer and its corresponding electrical potential profile (http://www.highbeam.com/doc/1G1-223907507.html)

In the Stern layer, the ions are believed to be bound to the particle surfaces (Masliyah and Bhattacharjee 2005). The potential in the Stern layer decreases linearly from the particle surface due to the presence of counter ions (Masliyah and Bhattacharjee 2005). The potential decreases exponentially away from the slipping plane (Masliyah and Bhattacharjee 2005). The slipping plane refers to the plane that the ions and the ion bearing solution move around the particles (Masliyah and Bhattacharjee 2005). The slipping plane is known as the zeta potential (ζ) (Masliyah and Bhattacharjee 2005). The potential within the double layer region, including the zeta potential, depends on the type of electrolytes and their concentrations (Masliyah and Bhattacharjee 2005).

2.2.2 Toxic impacts of oil sands tailing ponds

Pollution from the oil sand industries can affect the ecosystem and human health (Timoney and Lee 2009). However its extent is a subject of growing international concern. The contamination levels into the natural and industrial sources still cannot be quantitatively determined accurately (Timoney and Lee 2009).

The most abundant polycyclic aromatic hydrocarbons (PAHs) in the Syncrude Aurora North tailings pond correspond closely with the Muskeg River PAHs whose concentration increased the most downstream of the pond(Timoney and Lee 2009). C2 phenanthrene/anthracene were the most abundant PAHs in the Aurora North tailings pond, C3 dibenzothiophene was the second, C2 dibenzothiophene was the fourth, and C2 fluorene was the sixth most abundant PAH in the tailings pond. Sediments from the lower Athabasca River and its delta have been found to be toxic to several species of invertebrates (RAMP 2001) and contain high levels of PAHs and metals (RAMP 2006).

Tailings pond seepage can introduce toxins into the ecosystem. The Suncor's Pond 1 (also known as Tar Island Pond One) seepage increases the concentration of C2 naphthalene, barium, beryllium, boron, strontium, thallium, titanium, and uranium in the sediment pore water of the Athabasca River by a factor of 2-4 fold which exceeds maximum ambient concentrations. The concentration of beryllium, chromium, manganese, strontium, vanadium and naphthenic acids at an Athabasca River surface water site were found to exceed either water quality guidelines or maximum ambient (rivers, lakes or groundwater) concentrations (Komex 2005; Timoney and Lee 2009). It has been quantified that seepage into ground water hydraulically connected to the Athabasca River is about 5.5-5.7 million L/day (Barker et al. 2007; Hunter 2001a).

As part of an assessment of the ecological risk posed by Tar Island Pond One, (Komex 2005) chemicals of potential ecological concern were identified. These were arsenic, ammonia, barium, chromium, bismuth, iron, lithium, manganese, naphthenic acids, selenium, strontium, tin, vanadium, zinc, methylnaphthalene and C2 naphthalene (Komex 2005).

Birds

Large numbers of birds die each year due to exposure to tailings ponds (Timoney and Lee 2009) (43 species and 51 taxa of birds have died so far). Development of the Athabasca tar sands can change migration patterns and have a serious threat to migratory birds and to the Peace-Athabasca Delta (Schick and Ambrock 1974). During the spring migration, waterfowl and shorebirds were attracted to the warm effluent in tailings ponds while natural water bodies are still frozen (Timoney and Lee 2009). A variety of deterrents have been used to discourage water birds from landing in tailings ponds such as floating and beach effigies, propane scare cannons, and sound-producing systems (Boag and Lewin 1980; Golder-Associates-Ltd 2000; Ronconi and Cassady St. Clair 2006; Timoney and Ronconi 2010).

Some birds that land in the tailings ponds become oiled and a proportion of the oiled birds later die (Timoney and Ronconi 2010). It was observed that at least 16,000 birds were flying over one tailings pond during this time (Ronconi and Cassady St. Clair 2006) and at (natural) McClelland and Kearl Lakes, 1,154 and 2,700 ducks by single-day counts (Ronconi 2006). Tailing ponds require effective systems to deter birds (Ronconi and Cassady St. Clair 2006).

Waterfowl and shorebirds are the most affected by the tailing ponds. However there are birds of prey, gulls, passerines, and other groups that die due to tailing ponds impacts (Dyke et al. 1976; Gulley 1980; Ronconi 2006; Sharp et al. 1975). The annual bird mortality due to tailings pond exposure is not known with certainty, but it has been estimated to range from 458 to 5,037 birds (Timoney and Ronconi 2010).

Air

The main source of releases of volatile organic compounds (VOCs), such as benzene, xylene, ammonia, and formaldehyde and hydrogen sulphide is the evaporation from tailings ponds. Methane production due to the bacterial activities in tailings ponds also can lead to an increase in greenhouse gas levels. This may impact tailings reclamation options (Holowenko et al. 2000). It has been reported that the pond at the Mildred Lake Settling Basin (MLSB), produces a large amount of methane; as 60-80% of the gas flux across the pond’s surface, is due to methane (Holowenko et al. 2000).

Fish and aquatic life

Leakage of toxins from tailings ponds may be a concern for decades if not for centuries. Four metals commonly exceed fish protection threshold effects levels in the Athabasca River for walleye and lake white fish which are aluminum, selenium, silver, and vanadium (RAMP 2006). Of these metals, selenium may present the largest risk to fish health. Selenium can contribute to reproductive failure, deformities, and death among aquatic organisms and water birds, and can adversely affect people (CRBSCF 1999). Athabasca River natural bitumen and oil-refining wastewater pond sediments caused significant risk for fish (Colavecchia et al. 2004). Increases in mortality, hatching alterations malformations, and reduced size were observed in fish which were exposed to Athabasca River PAHs (Colavecchia et al. 2006; Colavecchia et al. 2007; Tetreault et al. 2003).

Humans

Increased levels of PAHs in sediment, of arsenic in water and sediment, and of criteria air contaminants such as VOCs, and sulphur dioxide are concerns. Arsenic is a known carcinogen linked with human bile duct, liver, urinary tract, and skin cancers, vascular diseases, and Type II diabetes (Guo 2003). Aquatic biota and the people who depend upon aquatic life for food are exposed to both arsenic and PAHs. Co-exposure to arsenic and the PAH benzo(a)pyrene can increase rates of genotoxicity 8-18 times above rates observed after exposure to either carcinogen in isolation (Fischer et al. 2005; Maier et al. 2002). Exposure to environmental contaminants such as arsenic and PAHs, particularly in "country foods", is a plausible factor for the apparent elevated rates of human cancers and other diseases in Fort Chipewyan (Chen 2009; Timoney and Lee 2009).

2.3 Technologies for Treatment of Oil Sands Tailings

2.3.1 Chemical/biological treatment

These methods are mainly based on clay particle coagulation and flocculation.

2.3.1.1 Tailings coagulation

Coagulation is the process in which destabilization is achieved by the addition of a coagulant. Coagulants which are added into the tailings pipeline cause particle destabilization by reducing particle charges and/or compressing electrical double layers, forming doublets, triplets, etc., upon collision driven by Brownian motion (Hunter 2001b), allowing the particles to come closer together and form large clumps (BGC Engineering Inc 2010). There are different chemicals, both organic and inorganic which can be used for coagulation of the clays. Depending on the each coagulant aid, various changes in pH, salinity, cation and anion levels, buffering capacity and toxicity have been observed. Each coagulant aids also can impact water quality. The most common coagulants are mineral salts including aluminum sulfate, ferric chloride, lime, calcium chloride, and magnesium chloride.

Whole tailings coagulation technology is not very robust for operating oil sands plants because of the low initial solids content in the tailing stream which leads to segregation of coarse particles (>44 µm) from the whole tailings and fines particles flowing into a pond. However coagulation can be developed to CT technology using cycloned sand which increases the solid content and reduces segregation (BGC Engineering Inc 2010). In this technology recycled water quality is also an important concern as coagulant and chemical reagents can induce impacts (BGC Engineering Inc 2010).

2.3.1.2 Tailings flocculation

The most efficient way to remove the solids in the oil sands tailing is by flocculation. Using flocculants can treat MFT without adding divalent ions (BGC Engineering Inc 2010). Flocculants cause chemical bonding of colloids and form flocs. In the flocculation technique polymeric materials are used as flocculants. They form bridges between individual particles and (bridging) help particles aggregate(Suncor Energy 2011). Flocculants carry active groups with a charge which will counterbalance the charge of the particles (charge neutralization).

Most bridging flocculants carry either a positive (cationic) or a negative (anionic) charge. These charges serve two purposes: (a) They provide a means of adsorption onto the particle surface by electrostatic attraction and (b) They cause the polymer molecule to extend and uncoil due to charge repulsion along the length of the polymer chain, so that the molecule is more nearly linear and can therefore accommodate more particles (Moss and Dymond 1978).

A polymer is considered nonionic if less than 1% of the monomer units are charged. In aqueous systems, polymers function as flocculants primarily by the bridging mechanism. Hence, they must be of high or very high molecular weight for practical applications (Tripathy and Ranjan De 2006). Polyacrylamide is the most important nonionic polymer (Tripathy and Ranjan De

2006). In general, the higher the molecular weight, the better the flocculation and the faster the sedimentation rate (Moss and Dymond 1978).

It is essential to monitor the particle size distribution before and after the flocculation. A high concentration of nano to sub micron particles before the flocculation process and a much lower concentration of large flocculates after the process occurs (http://www.anms.ca/products/tailing-ponds-of-oil-sands). Generally, flocs produced by polymers are much stronger than those formed by coagulation; the particles are held together with elastic bonds, not merely by weak Van der Waals forces (BGC Engineering Inc 2010). Three groups of flocculants are currently available: mineral (silica, bentonite, alum, ferric hydroxide), natural (starch derivatives) and synthetic (polyacrylamides) (BGC Engineering Inc 2010; Tripathy and Ranjan De 2006).

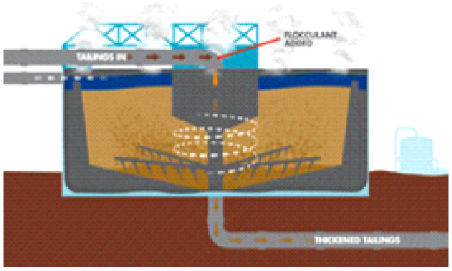
Ongoing research is focusing on various kinds of polymeric flocculants. Polymeric materials used as flocculant aids are mostly water-soluble linear polymers of very high molecular weight. Polymeric flocculants are widely used in water treatment (BGC Engineering Inc 2010; Crittenden 2005; Tripathy and Ranjan De 2006). A cationic hybrid polymer was found to be more effective than the corresponding organic flocculants (Yang et al. 2004). Use of anionic polyacrylamide in flocculation of lime treated oil sands tailings has been reported by (Hamza et al. 1996).

The performance of flocculants has a significant effect on this technology. Wang et al. (2010) compared two polymer aids for settling and filtration of oil sands tailings: commercial Magnafloc 1011 (Percol 727) polymer (a high molecular weight, anionic co-polymer of acrylamide and acrylates (Sworska et al. 2000a; Sworska et al. 2000b) and an in-house synthesized Al-PAM polymer. Both polymers increase tailings sedimentation, but Al-PAM was very effective as a filtration aid which showed its potential as an oil sands tailings disposal approach that can eliminate tailings ponds (Alamgir et al. 2012; Wang et al. 2010). In the flocculation method for tailing densification, recycle water quality also is a concern (BGC Engineering Inc 2010). This technology also has high operational costs (BGC Engineering Inc 2010).

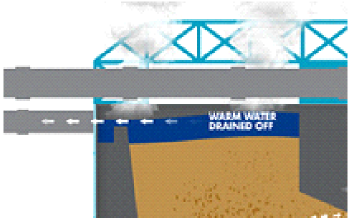
2.3.1.3 Thickening process

Thickened Tailings (TT) technology, which is currently used exclusively by Shell and Syncrude is also known as paste technology (BGC Engineering Inc 2010; Fair 2008). After extraction, instead of the tailings going straight to the tailings area, they go to a thickening plant. In this process, at the thickening plant, a flocculent or thickener is added to bind the particles together which leads to rapid settling and sedimentation of suspended fines before they reach the tailings pond (BGC Engineering Inc 2010; Shell 2012).

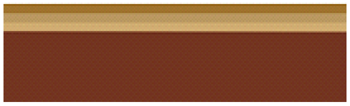
A large amount of producing water which is suitable for recycle to the extraction process is drained off at the plant. The drain off water is still warm so it does not need to be re-heated to the same degree as normal tailings water. In this way energy can be saved, resulting in reduction of imported energy costs and greenhouse gas emissions (BGC Engineering Inc 2010; Shell 2012), The sediment which remains without water is called thickened tailings (BGC Engineering Inc 2010; Masliyah 2007; Shell 2012). This process yields a density of about 30% solids content and the typical residence time in a thickener is half an hour (Devenny 2009), while it takes a few years to achieve 30% solid content through gravity settlement of TFT in a tailings pond. Thickened tailings can be disposed of in a disposal area, where they are again spread in layers, and allowed to consolidate with less land disturbance and high potential for land reclamation (BGC Engineering Inc 2010; Shell 2012).



a



b



c

Figure 2-3 Thickened Tailings (TT) technology consists of (a) adding a flocculent at the thickening plant which leads to rapid sedimentation and (b) draining off the producing warm water at the plant while (c) remaining thickened tailings can be disposed of in a disposal area (Shell 2012).

Higher densities can be achieved by applying super flocculating agents. However the effect of the super floc which remains in the recycled water and consequently in the extraction process should be considered (Devenny 2010). However this technology has low imported energy costs but still needs high startup and operational costs, and experienced operators and careful operational control. Bitumen accumulation in the thickener feed well can impair flocculation efficiency.

There are some challenges in the TT technology. It generally includes the thickener feed preparation process, thickener type selection and thickening process, flocculant selection and flocculation process, understanding the mechanism of dewatering in order to increase its rate, thickened tailings transport (conveyor and positive displacement pump), deposition and consolidation and the strategy for reuse of thickener overflow water. Recycled water quality and impacts on the environment and plant operation need more studies. Cost also should be competitive relative to other tailings management technologies (BGC Engineering Inc 2010; Yuan and Lahaie 2013).

2.3.1.4 Composite/Consolidated tailings (CT)

Consolidated Tailings (CT) technology is used by Syncrude, Suncor and CNRL (Mamer 2007; Syncrude) for treatment of MFT. It was developed at the University of Alberta (Caughill et al. et al. 1993). In this process gypsum (containing calcium ions) is added to the mixture of MFT and coarse sand tailing sediments to produce a mixture with a sedimentation rate which is much quicker (BGC Engineering Inc 2010; Caughill et al. 1993). After adding gypsum the mixture is pumped to a pond, where it densifies over time and becomes solid and releases clear water which can be recycled (BGC Engineering Inc 2010).

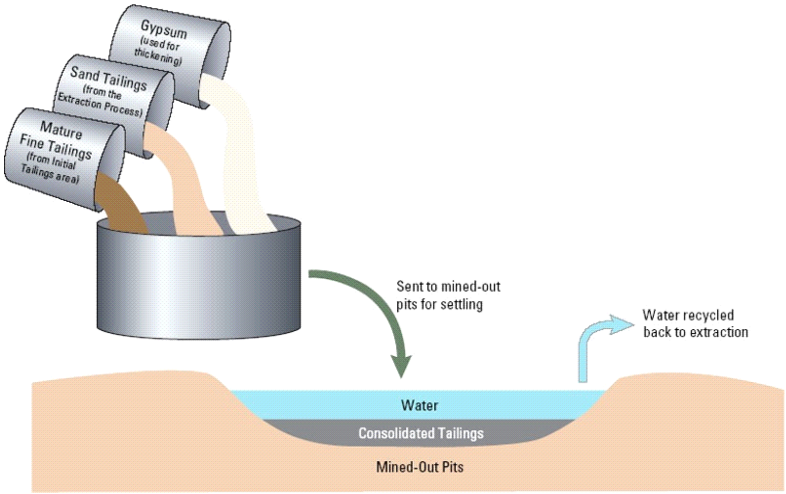


Figure 2-4. Consolidated Tailings (CT) technology (BGC Engineering Inc 2010)

This mature technology is relatively low cost and flexible for tailings management (BGC Engineering Inc 2010). There are some challenges in CT technology. It requires a large containment area until it solidifies (containment area is expensive) and also a large amount of sand while the energy efficiency is low.

The calcium ion concentration in the recycle water is high and can be harmful to the extraction process (BGC Engineering Inc 2010). There are H_2S emissions by anaerobic reduction of SO_4^{-2} with the residual bitumen in the tailings (BGC Engineering Inc 2010).

2.3.1.5 Drying tailings reduction operations (TRO)

There is also a possibility to use MFT drying as a tailings technology. MFT drying is known as Tailings Reduction Operations (TRO) and is used by Suncor (Mamer 2007). In TRO, a polymer flocculent is added to the MFT and the process is similar to CT. The MFT/flocculent mixture is then deposited in thin layers (10-15 cm thick). Under self-weight consolidation (aided by a high proportion of sand grains), particle-free water is released from the deposit leaving the fines solids and some water trapped within the voids of the coarse solids matrix. After about a month, the sediment is like “solid earth” and can be reclaimed in place or moved for final reclamation (MacKinnon 1989) and dry landscape. TRO will reduce the need to build more tailings ponds, and also it is quicker in reclamation than CT (a few weeks are required compared to around 30 years) (Mamer 2007).

2.3.1.6 In-situ biological treatment

This technology is based on inoculation or enhancement of microbial metabolism in the tailing ponds to increase MFT or fine tailing densification and sedimentation. It is clear that microbial action and metabolism of resident hydrocarbons can catalyze hydrocarbon biodegradation (Quagraine et al. 2005; Siddique et al. 2007) in the tailing ponds. However it can produce a large amount of methane (up to 104 m^3 day^{-1}) and carbon dioxide emissions which are not desirable, but biological methane formation (methanogenesis) by anaerobic microbes can improve tailings densification and increase its rate (Fedorak et al. 2003; Voordouw 2013).

The rising of gas bubbles in the tailings can produce channels which make it easy for water to drain due to excess pore pressures within the tailings (Guo 2009). However this is a low cost technology, but the generation of methane from oil sands tailings ponds which is a greenhouse gas should be controlled.

It has been reported that microbial cells and exopolysaccharides or extracellular polymeric substances (EPS) can improve the clay aggregation and flocculation (Li et al. 2007). EPS are compounds secreted by microorganisms into their environment. These compounds are important in biofilm formation and cell attachment to surfaces. There are also interactions between soil bacteria and clay particles which can form clay aggregates with the appearance of 'hutches' housing the bacteria (Lünsdorf et al. 2000).

There are different classes of microorganisms growing in the tailings ponds that contribute to increased tailings aggregation and sedimentation. In addition to methanogen bacteria, sulfate-reducing bacteria (SRB) and nitrate-reducing bacteria (NRB) have also been found in tailing ponds environment (Holowenko et al. 2000; Salloum et al. 2002). The tailings pond microbial communities are dynamic and change rapidly when the input of fresh tailings and/or gypsum is stopped (Golby et al. 2012).

Bordenave et al. (2010) observed that microbial cells can absorb the clay particles on their surfaces and within the EPS, causing the aggregation of fine particles. In this way the tailings sedimentation under gravity will increase (Bordenave et al. 2010). According to their report, the active cultures of *M. barkeri* (methanogen) or *Thauera sp*. strain N2 (NRB) show strong bonds to clay particles and an increase in sedimentation. Nitrate addition also can reduce methane production by methanogenic bacteria. These observations show the potential for increasing tailing sedimentation by using microbial biomass to aggregate and reduce methane production by in situ addition of nitrate (Bordenave et al. 2010).

Addition of calcium ions in the form of $Ca(NO_3)_2$ and lactate to tailing pond samples can increase the densification rate by 15% (v/v). Lactate significantly boosted microbial activity with increased methanogenesis, sulfate reduction or nitrate reduction (Brown et al. 2013).

In one study, the adhesion to a sandy soil and a clay loam soil of a series of *Lactobacillus* strains with various cell surface characteristics were investigated (Huysman and Verstraete 1993). Bacterial cell surface hydrophobicity, as determined by the bacterial adherence to octane and polystyrene, was the major parameter influencing the adhesion to the sandy soil. The cell surface charge of the bacteria was of minor importance in the adhesion to the sandy soil (Huysman and Verstraete 1993). It has been reported that the attachment to the benthos is facilitated by the common action of both coflocculation and hydrophobic interactions. EPS also can help the bacteria to adhere to the surface and can serve as flocculants to bind small clay particles (Rehm 2009).

Ramos-Padron et al. (2010) found that methanogenesis was inhibited in laboratory incubations by nearly 50% when sulfate was added at pond-level concentrations. It suggests that in situ sulfate reduction can significantly reduce methane emissions. The sulfide emissions by SRB activity in the gypsum treated pond are also limited as they are highly soluble and will oxidize in surface waters which suggests that the production of hydrogen sulfide might be a self-limiting process, which will begin to decrease after a period of time (Chen et al. 2013; Ramos-Padrón et al. 2010; Voordouw 2013).

2.4. Biosurfactants

Surface active agents (surfactants) are amphiphilic chemical compounds. They have a hydrophobic chain or tail and a hydrophilic head group. The hydrophobic tail is the hydrocarbon (linear or branched) portion and the hydrophilic part is polar or ionic portion of a surfactant. The hydrophobic portion may concentrate at the air water interface or into the oil phase, while the hydrophilic portion orients towards the bulk water. They can produce molecular films at the interface of air and water or two liquid phase with different polarity and hydrogen bonds (i.e. oil/water) and reduce the surface and interface energy (surface and interface tension) (Mulligan 2005).

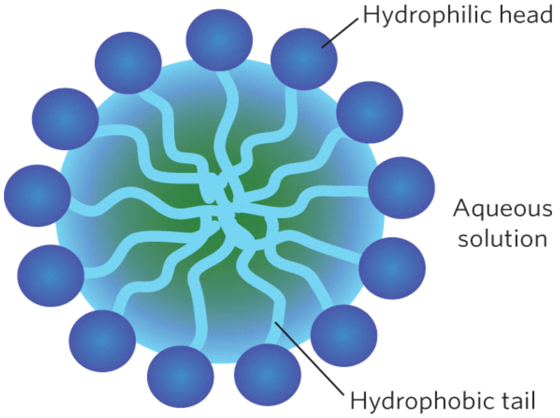


Figure 2-5. Surfactant monomers form a spherical micelle in aqueous solution (Pasquali 2010)

Surface tension is dependent on the surfactant concentration as long as the surfactant concentration is below the Critical Micelle Concentration (CMC) (Figure 2-6). In other words, the CMC is the minimum concentration necessary to initiate micelle formation. Efficient surfactants have low CMC. It means that small amounts of surfactant can reduce surface tension. The CMC is dependent on pH, temperature and ionic strength.

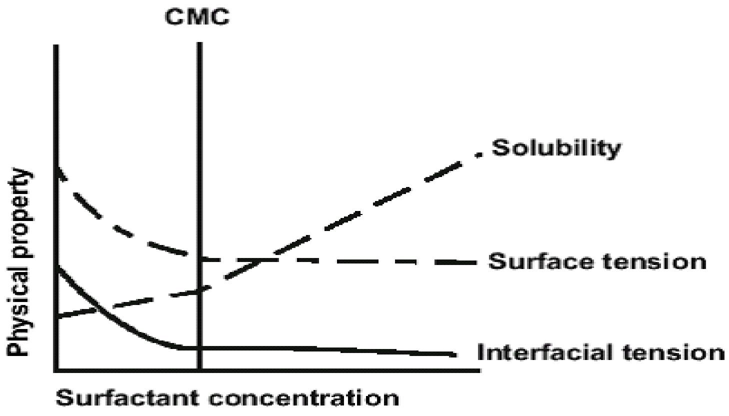


Figure 2-6. Surface tension, interfacial tension and solubilization as a function of surfactant concentration (CMC represents critical micelle concentration)(Mulligan 2005)(p.184).

There are different types of surfactants. Generally they can be classified based on their head group charge into four classes: Cationic surfactants, anionic surfactants, nonionic surfactants and amphoteric and zwitter-ionic surfactants (which have both cationic and anionic charges in the same molecule) (Mulligan 2014). Surfactants which are produced from living natural sources (*i.e.*, from a plant, animal or microorganism) are known as biosurfactants (Chhatre et al. 1996; Mulligan 2005).

Microbial biosurfactants are produced either on the surfaces of microbial cells or excreted extracellularly (Mulligan 2005). Some biological substances which have high emulsifying activity but cannot reduce surface tension are also categorized with biosurfactants. Their classification is based mainly on the categorization of their microbial origin and their chemical composition. In this way they can be classified as glycolipids, fatty acids, phospholipids, surface active antibiotics, polymeric microbial surfactants (biosurfactants which are a combination of many chemical types), and particulate surfactants (Chhatre et al. 1996).

Most biosurfactants are anionic or neutral; only a few of them which contain amine groups are in cationic form (Mulligan et al. 2001). The hydrophobic part of biosurfactants is composed of a long chain of fatty acids, hydroxy fatty acids or α-alkyl-β-hydroxy fatty acids. The hydrophilic part can be a carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid or alcohol. As mentioned in Table 2-1, there are a wide range of microorganisms that can produce these compounds (Mulligan 2005).

Table 2-1. Type and microbial origin of biosurfactants (Mulligan 2005)(p. 185)

Type of biosurfactant	Microorganism
Trehalose lipids	*Arthrobacter paraffineus*
	Corynebacterium sp.
	Mycobacterium sp.
	Rhodococcus erythropolis, *Norcardia* sp.
Rhamnolipids	*Pseudomonas aeruginosa*
	Pseudomonas sp., *Serratia rubidea*
Sophorolipids	*Candida apicola*, *Candida bombicola*
	Candida lipolytica
	Candida bogoriensis
Glycolipids	*Alcanivorax borkumensis*
	Arthrobacter sp., *Corynebacterium* sp.
	R. erythropolis, *Serratia marcescens*
	Tsukamurella sp.
Cellobiose lipids	*Ustilago maydis*
Polyol lipids	*Rhodotorula glutinus*
	Rhodotorula graminus
Diglycosyl diglycerides	*Lactobacillus fermentii*
Lipopolysaccharides	*Acinetochacter calcoaceticus* (RAG1)
	Pseudomonas sp., *Candida lipolytica*
Arthrofactin	*Arthrobacter* sp.,
Lichenysin A, Lichenysin B	*Bacillus licheniformis*
Surfactin	*Bacillus subtilis*, *Bacillus pumilus*
Viscosin	*Pseudomonas fluorescens*
Ornithine, lysine peptides	*Thiobacillus thiooxidans*
	Streptomyces sioyaensis
	Gluconobacter cerinus
Phospholipids	*Acinetochacter* sp.
Sulfonylipids	*T. thiooxidans*
	Corynebacterium alkanolyticum
Fatty acids	*Capnocytophaga* sp.
(Corynomycolic acids,	*Penicillium spiculisporum*
spiculisporic acids, etc.)	*Corynebacterium lepus*
	Arthrobacter paraffineus
	Talaramyces trachyspermus
	Norcadia erythropolis
Alasan	*Acinetobacter radioresistens*
Streptofactin	*Streptomyces tendae*
Particulate surfactant (PM)	*Pseudomonas marginalis*
Biosur PM	*Pseudomonas maltophila*

The CMC of biosurfactants varies from 1 mg/l to 20 mg/l depending on solution ionic strength and their molecular weights are in the range of 500 -1500 Daltons (Da) (Mulligan 2005).

The increasing demand for surfactants is mainly supplied by chemical surfactants. Many chemical surfactants have high toxicity, are not degradable and accumulate in the environment. Their production and their by-products also have significant impacts on environment. Considering these problems, biosurfactants offer advantages over synthetic surfactants. Interest in biosurfactants has been steadily increasing in recent years due to their derivation from renewable resources, diversity, environmentally friendly nature, possibility of large-scale production, low or non-toxicity, biodegradability, excellent surface activity, possible reuse through regeneration, selectivity, performance under extreme temperature and pH conditions, and potential applications in environmental protection (Banat et al. 2000; Mulligan 2005; Rahman et al. 2002a; Rodrigues et al. 2006; Urum and Pekdemir 2004; Xu et al. 2011).

Most microbial-derived biosurfactants are produced from hydrocarbon substrates (Syldatk and Wagner 1987). They can also be produced from carbohydrates which are very soluble. Biosurfactants are very effective for in situ reduction of interfacial surface tension of oil and water, the oil viscosity, water removal from the oil, and release of bitumen from oil sands (Mulligan 2014). For this purpose, emulsan which is a high molecular weight biosurfactant has been commercialized (Mulligan 2014).

Enhancing solubilization and biodegradation, soil treatment (in situ and ex situ), and water and waste treatment are environmental applications of biosurfactants. Among different types of biosurfactants, rhamnolipids, sophorolipids and surfactin which have low molecular weights are well studied (Mulligan 2014). Their research trends and applications are reviewed in detail by Mulligan (2014).

2.4.1 Rhamnolipid biosurfactant

Rhamnolipids (RLs), the glycolipid biosurfactants produced mainly by *Pseudomonas aeruginosa*, are the most intensively studied biosurfactants (Lang and Wullbrandt 1999; Soberón-Chávez et al. 2005). They are the only biosurfactants thus far that have been approved by US Environmental Protection Agency for use in food products, cosmetics and pharmaceuticals (Nitschke and Costa 2007).

Pseudomonas aeruginosa species has the ability to produce four different rhamnolipid analogs (R1-R4) (Mulligan 2005; Syldatk et al. 1985). They have a glycosyl head group, in this case a rhamnose moiety, and a 3(hydroxyalkanoyloxy)alkanoic acid (HAA) fatty acid tail (Cabrera-Valladares et al. 2006; Ochsner et al. 1994). Specifically there are two main classes of rhamnolipids, mono-rhamnolipids and di-rhamnolipids; consisting of one or two rhamnose groups respectively (Figure 2-7) (Rahim et al. 2001).

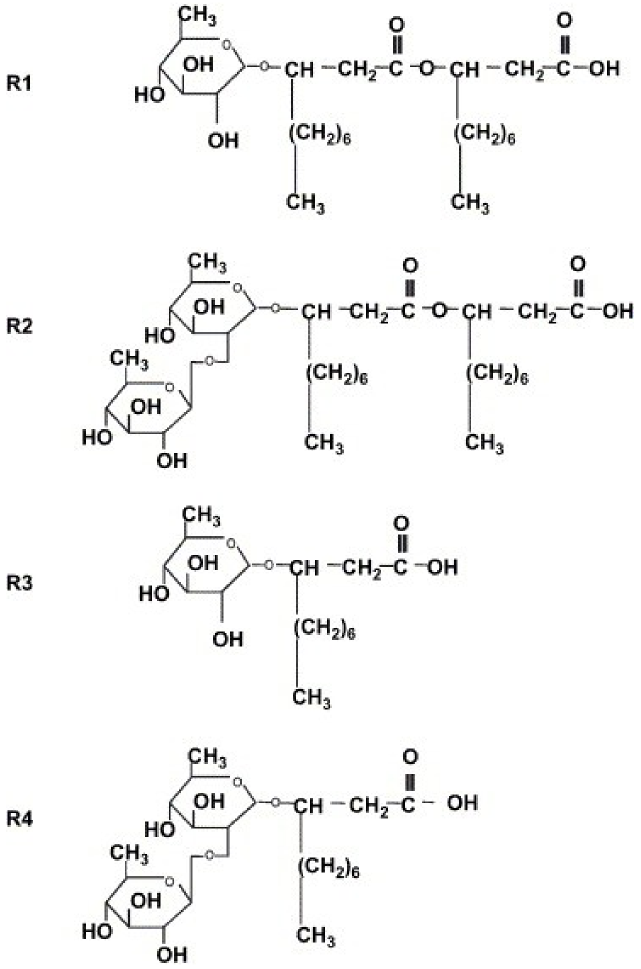


Figure 2-7. Rhamnolipids type I or mono-rhamnolipids contain one rhamnose group while rhamnolipids type II or di-rhamnolipids; contain two rhamnose groups (Mulligan 2005)(p.185)

Rhamnolipids are also heterogeneous in the length and degree of branching of the HAA moiety (Desai and Banat 1997) which varies with the growth media used and the environmental conditions (Matsufuji et al. 1997; Mulligan 2005). They are anionic and are capable of reducing water surface tension from 72 mN/m to 29 mN/m (Mulligan 2005).

2.4.2 Rhamnolipid important environmental applications

Rhamnolipids have the potential to decrease the environmental impacts of oil sands as many studies have shown their potential for remediation of oil contaminated soil and water.

2.4.2.1 Enhancing solubilization and biodegradation of petroleum hydrocarbon and PAH

Rhamnolipid can enhance diesel-contaminated soil remediation by enhancing biomass production and diesel biodegradation (Whang et al. 2008). Vasefy and Mulligan (2008) used rhamnolipid with two commercial biological products which contain bacteria consortia and nutrients (which can enhance biodegradation rate) to investigate the effectiveness of rhamnolipid on the biodegradation of weathered light crude oil, heavy oil, heavy crude oil and diesel fuel which spilled on saline water. Rhamnolipid solutions showed 65% removal of diesel fuel, 70% of light crude oil, and 59% of heavy crude oil (Vasefy and Mulligan 2008). Rhamnolipid can enhance bioremediation of refinery oil sludge (Ju et al. 2011). It has been reported that rhamnolipid can stimulate hydrocarbon biodegradation when *P. aeruginosa* and a rhodococcal strain were inoculated into a soil contaminated with an oily sludge (Cameotra and Singh 2008). *Pseudomonas* strain isolated from the marine environment could reduce the crude oil amount by 83% (Mehdi and Giti 2008). Washing of oil-based drill cuttings (petroleum exploration and production wastes) by a rhamnolipid before biodegradation by a mixed culture can result in a significant decrease of organic content (however the rhamnolipid concentration should be at a certain level) (Yan et al. 2011).

Rhamnolipid addition can enhance ex situ bioremediation of gasoline-contaminated soil (Rahman et al. 2002b). Rhamnolipid addition can enhance degradation of phenanthrene by *P. aeruginosa* as it solubilized the phenanthrene and increased its bioavailability (García-Junco et al. 2001). A significant increase in the solubility of phenanthrene under a range of pH and salinities by adding rhamnolipid also has been reported (Yin et al. 2009). So it has potential for bioremediation of crude oil. Straube et al. (2003) showed that the biosurfactant produced by *P. aeruginosa* strain 64 in the soil enabled PAH biodegradation (Straube et al. 2003).

2.4.2.2 Soil treatment (in situ and ex situ) and removal of heavy metals and organics

Rhamnolipids can enhance the release of low solubility compounds from soil and other solids. They have the ability to enhance washing of a crude oil contaminated soil (Urum et al. 2003). They increase oil release from the beaches in Alaska after the Exxon Valdez tanker spill (three times as much as water do alone) (Harvey et al. 1990). However the removal efficiency varied according to contact time and biosurfactant concentration (Mulligan 2014).

Hydrocarbon removal from sandy loam soil has been reported. Hydrocarbon type and rhamnolipid concentration can change removal degree (from 23% to 59%) (Scheibenbogen et al.

1994). 5 g/L of rhamnolipids can remove 10% more hydrocarbons from a sandy loam soil than a slit loam soil. Rhamnolipid is more effective than SDS (Dyke et al. 1993). They could remove oil from contaminated sandy soil while the composition of the aromatic and paraffinic oil did not change and could be recycled (Santa Anna et al. 2007).

Rhamnolipids are more effective than synthetic surfactants for desorption of phenanthrene from marine sediment (Zhu et al. 2011) and washing of low level and high TPH-contaminated soil (Lai et al. 2009). Styrene also could be removed from contaminated soil by rhamnolipid (Guo and Mulligan 2006). A mixture of rhamnolipid and synthetic surfactants and biosurfactants show the ability to be used for environmental remediation applications or enhanced oil recovery (Nguyen et al. 2008). The anionic nature and complexation ability of rhamnolipids enable them to remove metals from soil and ions such as cadmium, copper, lanthanum, lead, and zinc (Herman et al. 1995; Mulligan 2014; Ochoa Loza 1998; Tan et al. 1994).

2.4.2.3 Dispersing oil in oil contaminated water and water treatment

Rhamnolipid can act as biodispersant for oil biodegradation and solubilization (Mulligan 2014). It has been reported that chemical and biosurfactants were also intercalated with layered double hydroxides (LDHs) to remove organic pollutants from water (Chuang et al. 2010). Rhamnolipids can be useful for oil spills since they could be less toxic and degrade more than synthetic surfactants (Lang and Wagner 1987). They facilitate emulsification of oil and then oil can be then remove by demulsification or remediate by biodegradation (Nakata and Ishigami 1999). Three different studies showed that emulsifier produced by *P. aeruginosa* SB30, rhamnolipids produced by isolated bacteria and rhamnolipids together with ethanol and octanol could effectively disperse oil into fine droplets and in this way increase biodegradation (Chhatre et al. 1996; Holakoo and Mulligan 2002).

Rhamnolipid also can significantly increase the low molecular weight heavy metal removal from water when combined with ultrafiltration (El Zeftawy and Mulligan 2011). Micellar-enhanced ultrafiltration (MEUF) which use the micellar properties of surfactant solutions to effectively remove low molecular weight dissolved ions (i.e. copper) and/or organics (i.e. benzene molecules) has shown the potential for wastewater treatment (Ridha and Mulligan 2011).

2.4.3 Surfactants and flocculation

Adsorption of surfactants on the particle surface can change surface wetting characterization of particles which leads to an increase in flocculation and dewatering of clay slurries. In a study performed by Besra et al. (2003) the influence of surfactants on settling rate, flocculation and dewatering of clay particles was investigated. They applied surfactants (anionic sodium dodecyl sulphate (SDS), cationic cetyl trimethyl ammonium bromide (CTAB) and non-ionic Triton X 100) on kaolin suspensions and cationic polyacrylamide (PAM-C) flocculant. The results show that pre-treating the kaolin with surfactants can further increase or decrease the settling rate depending on the type of surfactant used. However, the flocs which formed in the presence of surfactants show better filtration and dewatering behavior and low cake moisture in comparison with PAM-C alone. It has been argued that simultaneous addition with surfactants might reduce the entrapment of excess water in the smaller flocs and increase the hydrophobicity due to adsorption of surfactants (Besra et al. 2003).

Hydrophobic agglomeration is originated from the hydrophobic attraction between particles, which is essentially different from electrolyte coagulation and polymer flocculation. It is applied to mineral processing in floc-flotation process to improve the recovery of mineral fines (Yang and Song 2014). Linag et al., (2016) show that Polyethylene oxide (PEO) is an efficient flocculant for the settling of various minerals including coal, quartz, and phyllosilicate through hydrophobic flocculation (Liang et al. 2016).

Enhancement in filtration dewatering due to surfactants has been also reported by Singh et al. (1998). They investigated vacuum filtration of clean coal slurries and flocculated slurries with cationic, anionic and nonionic surfactants. All surfactants significantly reduced the filter cake moisture. They find that surface tension by itself does not give much indication of the effectiveness of surfactants as dewatering agents. It is believed that the wetting characteristics of the coal surface were changed due to the surfactant adsorption. These changes enhance the filtration dewatering characteristics (Singh et al. 1998).

Flocculation and sedimentation of aqueous kaolin suspension was investigated by another study performed by Nasim and Bandyopadhyay (2012). They used different molecular weight poly-vinyl alcohols (PVA) as new eco-friendly flocculants and performed sedimentation experiments at different pH and presence of different surfactants in the suspension media. Results show that sedimentation is strongly dependent on pH, molecular weights of PVA and the type of surfactants. However in their cases, the presence of surfactants delayed sedimentation but it can lead to less residual turbidity. The best performance was achieved by using low molecular weight PVA at pH 4 in the presence of a cationic surfactant (Nasim and Bandyopadhyay 2012). The effect of sodium oleate and sodium dodecyl sulfate (SDS) as anionic surfactants at different suspension pH, surfactant concentrations, stirring speeds, and flocculation times on shear flocculation of colemanite mineral has been investigated by Ucbeyiay et al. (2011). It was observed that sodium oleate has shown more effective flocculation than SDS and the colemanite particles could be flocculated by oleate in a broad pH range. They also report that the flocculation degree will increase in the presence of some inorganic salts (cations). Flocculation behavior of colemanite mineral was determined to be dependent on the pH, cation concentration, type of surfactant and inorganic salt (Ucbeyiay Sahinkaya and Ozkan 2011).

According to the literature using biosurfactnat for enhancing the sedimentation of oil sand tailings has not been done before. Considering the significant potential of biosurfactant for environmental application this would lead to developing a more environmentally friendly densification method for oil sands tailings without having the limitations of other methods, without producing large amounts of CH_4 and taking advantage of the biosurfactants for remaining water and sediment bioremediation. The remaining sediment will have a lower concentration of heavy metals and hydrocarbons as it extracts the remaining oil from the sediment into the water. Oil in the recycled water at the top of sediment can be dispersed in to fine drops and be ready for microorganisms for biodegradation.

2.4.4 Micellar enhanced ultrafiltration (MEUF)

Ultrafiltration has been used to purify and concentrate both surfactin and rhamnolipids from culture supernatant fluids by Mulligan and Gibbs (1990) using commercially available membranes in a one-step method (Mulligan and Gibbs 1990). It suggests that purification could be achieved for large volumes directly from culture medium (Baker and Chen 2010). At concentration above the critical micelle concentration (CMC) surfactant molecules tend to form micelles which can be isolated from the bulk solution by high molecular weight cut off membranes due to their larger size (Baker and Chen 2010; Mulligan and Gibbs 1990; Witek-Krowiak et al. 2011). In this way the concentrate contained biosurfactant micelles and proteins while the permeate contained the lower molecular weight impurities such as mineral salts, free amino acids, peptides and small proteins (Baker and Chen 2010; Mulligan and Gibbs 1990; Witek-Krowiak et al. 2011).

Micellar enhanced ultrafiltration (MEUF) (a surfactant-based version of ultrafiltration membrane (Ghosh and Bhattacharya 2006)) has been used for the removal of copper, chromate, zinc, nickel, cadmium, selenium and arsenate from aqueous phase (Bade and Lee 2011; Ghosh and Bhattacharya 2006; Gzara and Dhahbi 2001; Samper et al. 2009). This process works based on the increasing the size of pollutant molecules by forming a complex with anionic or cationic surfactant micelles at the critical micelle concentration (CMC). Anionic or cationic pollutants (such as heavy metal ions) can be bonded to the surface of opposite charged micelles due to the electrostatic forces (Rahmanian et al. 2010). The ultrafiltration membrane could isolate micelles and bound pollutants from the solution while the unbound ions and surfactant monomers pass through the ultrafiltration membrane to the permeate side (Abbasi-Garravand and Mulligan 2014; Bade and Lee 2011; Chaudhari and Marathe 2010; Landaburu-Aguirre et al. 2010). It has been reported that rhamnolipid as the biosurfactant could remove more than 99% of heavy metals such as zinc, nickel, and cadmium (El Zeftawy and Mulligan 2011), 100% of copper (Ridha 2010) and 96% of chromium from water in the MEUF system (Abbasi-Garravand and Mulligan 2014). There are high concentrations of biosurfactant in the retentate. Separation of surfactant from the bulk solution is an important concern for reuse purposes and economizing the MEUF process (Bade and Lee 2011). Characteristics and concentration ratio of surfactant and metals, pH, flow rate, and membrane pore size would affect the efficiency of heavy metals removal by MEUF (Abbasi-Garravand 2012).

In an oil sand tailing sedimentation approach, water quality is a very important concern. MEUF process can be applied for separation of biosurfactant and heavy metals from recycled water after oil sand tailing sedimentation as there are high level of heavy metals in recycled water. Using rhamnolipid for increasing the sedimentation will also bring more heavy metals from remaining sediment into the recycled water. For this propose the change of heavy metal concentrations and biosurfactant concentration in the recycled water needs to be investigated.

3. MATERIALS AND METHODS

3.1 Origin of the Oil Sand Tailings

The tailings pond sample was provided by Maria Demeter (Lab Manager / Environmental Engineering Technologist), Civil & Environmental Engineering Department, University of Alberta. It is a by product of the extraction of bitumen from oil sand which was prepared by Industrial Hygiene. Tailings samples were provided in 20 L plastic pail and stored at room temperature. It is comprised of bitumen (1-2 wt%), naphtha (<0.1 wt%), clay (30-60 wt%), and water with the pH in the range of 7.3-7.8. The clay content of 30-60 wt% shows that the samples are taken from mature fine tailing layer from the depth below 10 m of the tailing pond (Foght and Dunfield 2013).

3.2 Rhamnolipid

Rhamnolipid biosurfactant (JBR 425 from Jeneil Biosurfactant Co., USA) was used to investigate its effect on oil sands tailings. It is a mixture of two forms of rhamnolipid, at 25 wt% in water, with the CMC value of 30 mg/l at the lowest surface tension of 28 mN/m (Abbasi-Garravand 2012; Clifford et al. 2007; Wang and Mulligan 2009).

3.3 Microbial Cultures

A Bacillus subtilis strain and cultures of two microbial strains isolated (by growing on R2A nutrient agar medium (Sigma-Aldrich, for microbiology) and Bushnell Hass medium by one of my colleagues in the lab for her own research) from weathered oil (including light crude oil, diesel and biodiesel/B 100) were used for this study (Saborimanesh and Mulligan 2015). Characterization of the natural microbial communities was conducted by pyrosequencing of 16S rRNA. Characterization of bacteria isolated from the BD, D and L oil by 16S rRNA pyrosequencing showed that the *Firmicutes* was the dominant phylum in biodiesel (100%) and diesel (53%). The *Actinobacteria* was dominant in the diesel (47%) and the *Proteobacteria* (97%) and *Actinobacteria* (3%) were the two dominant phyla in the light crude oil (Saborimanesh and Mulligan 2015). The strains used in this study belongs to *Firmicutes* and *Proteobacteriaphyla* and were identified as orders of *Bacillales* and *Sphingomonadales* (Saborimanesh and Mulligan 2015). The microbial cultures were grown aerobically in 25 ml of medium containing mineral salts of nitrogen (sodium nitrate) and phosphorus (monobasic and dibasic potassium phosphates) at C:N:P ratio of 100:10:1 (Cookson 1995), at 37ºC for 24 hours without shaking (Youssef 2006).

3.4 Experimental Approach

The effect of rhamnolipids and microbial cultures on sedimentation was evaluated through sedimentation experiments. The feasibility of biosurfactant production was evaluated through batch experiments. The sedimentation experiments were performed in cylinder glass tubes however the real tailing ponds could have different shape which could induce the effect in sedimentation.

3.4.1 Sedimentation experiments

In a suspension of solid particles smaller particles have slower sedimentation rate. In addition to particle size, density and concentration, and fluid viscosity, other less obvious factors affect the sedimentation rate. When the effects of mutual interference are negligible, free settling conditions are said to prevail. Density also has a direct relationship with sedimentation rate as dense particles settled more rapid than less dense particles (Troy 2005).

The tailings densification process includes consolidation and sedimentation processes near the bottom and top of a tailings column, respectively (Eckert et al. 1996). The sedimentation process can be easily monitored as the downward movement of the boundary between clear liquid and suspended tailings. Its rate of movement, the ‘‘hindered settling velocity” (Eckert et al. 1996), is orders of magnitude smaller than the Stokes’ single particle settling velocity (35 cm/day for a 2 micron diameter particle with a density of 2.65 g/cm^3 (Bordenave et al. 2010); tailings sedimentation rates in the experiments (about 0.1 cm/day) were much smaller than this and the mean diameter of particles in this work even after adding the rhamnolipid and microbial culture is not greater than 2.32 micron). In this way, the sedimentation (S) was determined according to the following equation (Bordenave et al. 2010):

$S(\%)=1-h/H$

where h is the position of the boundary and H is the total height of the liquid column

(Bordenave et al. 2010).

Each test was repeated three times (triplicate) and the average data are reported.

3.4.1.1 Sedimentation Experiments at 23 ºC ± 2 ºC

All the sedimentation experiments were performed with 13-15 g of tailings diluted in 5 mL of deionized water in 20 mL glass tubes (15 cm) closed with a compressed layer of paper towel in order to prevent liquid evaporation. However there are still small amounts of liquid lost due to evaporation but it resembles the aerobic conditions at the surface of actual tailing ponds Sedimentation experiments were performed at room temperature (23 ºC ± 2 ºC).

3.4.1.1.1 Sedimentation experiments at different rhamnolipid concentrations

Five ml of rhamnolipid at different concentrations (0.5%, 1%, 2%) were added into the glass tubes containing a diluted tailing sample. Five ml of deionized water were added to a 20 mL glass diluted tailing sample tube as control. Sedimentation tubes were homogenized by 10 repeated inversions and were left without agitation at room temperature. Sedimentation (S) was measured every 5 days in unshaken tubes over a period of 25 days. All experimental pH values were adjusted by NaOH (0.1N) to 8.

3.4.1.1. 2 Sedimention experiments by microbial cultures

Sedimentation experiments were also performed with diluted tailing pond samples inoculated with cultures of the biosurfactant producer *Bacillus subtilis* strain, and a mixed culture of two microbial strains isolated from weathered oil. Five mL of microbial culture were added to the diluted tailing pond samples. Sedimentation tubes with diluted tailings samples and 5 mL of deionized water served as the control. pH was adjusted to 8 by adding 0.1N NaOH. Six homogenized sedimentation tubes were incubated at room temperature (23 ºC ± 2 ºC) and the measurement of the sedimentation in unshaken tubes was performed every 10 days for a period of 50 days.

3.4.1.1.3 Sedimentation experiments by rhamnolipid and microbial cultures

The sedimentation of tailings pond samples was evaluated in the presence of microbial cultures and rhamnolipid. One mL of mixed culture of two microbial strains isolated from weathered oil and 5 mL rhamnolipid biosurfactant at different concentrations (0.5%, 1%) were added to the diluted tailing pond samples. Sedimentation tubes with diluted tailings samples and 6 mL of deionized water served as the control. pH of the samples were adjusted 8 by adding NaOH (0.1N). Homogenized sedimentation tubes were incubated at room temperature (23 ºC ± 2 ºC) and measurement of the sedimentation in unshaken tubes was performed every 10 days over a period of 50 days.

3.4.1.2 Sedimentation Experiments at 15 ºC ± 2 ºC

3.4.1.2.1 Sedimentation experiments by rhamnolipid and microbial cultures

These experiments were performed in four 500 mL glass columns. 100 ml rhamnolipid at 0.5% concentrations, 100 ml of deionized water and 10 ml of mixed culture of two microbial strains isolated from weathered oil (grown previously aerobically in 25 ml of NB medium) were added to 200 g of oil sand tailing pond samples (three columns). A sedimentation column with 200 g tailings samples and 210 mL of deionized water served as the control. The columns were covered with a paper towel in order to prevent liquid evaporation. pH of the samples were measured at the starting time. Homogenized sedimentation columns were incubated at 15ºC ± 2 ºC and measurement of the sedimentation in unshaken columns was performed every 10 days over a period of 50 days.

3.4.2 Feasibility of in situ biosurfactant production

The feasibility of biosurfactant production by the indigenous microorganisms of oil sand tailings pond, the biosurfactant producer *Bacillus subtilis* strain, and two microbial strains isolated from weathered oil was evaluated through batch experiments.

3.4.2.1 Indigenous microorganisms of oil sand tailings pond

In this set of experiments there are two batches: control batches and nutrient amended batches. The control batches are tailings and deionized water only, and were performed to evaluate the natural ability of tailings to produce biosurfactants.

The nutrient amended batches were performed to improve the natural biosurfactant production in tailings. In this approach mineral salts of nitrogen (sodium nitrate) and phosphorus (monobasic and dibasic potassium phosphates) were added to the tailings to attain a C:N:P ratio of 100:10:1 (Cookson 1995; Jalali 2007). A buffer solution was added to maintain a constant pH in accordance with that of the control batches. The concentration of added phosphorus was doubled to account for its precipitation (Jalali 2007; Walworth and Reynolds 1995). For each batch, approximately 20 g of tailings and 100 mL of deionized water were placed in a 250 mL Erlenmeyer flask. Foam stoppers were used to fit the flasks top in order to prevent the entry of microorganisms and dust into the samples while allowing aeration. Flasks and media were sterilized by autoclaving at 121 ºC for 20 min before each experiment run. Aeration was achieved by rotating the flasks at 200 rpm on an orbital shaker (Thermolyne AROS 160) for 50 days. Samples covering days 0, 5, 15, 30, 45 and 50 were taken to measure the surface tension. All tests were performed in triplicate.

3.4.2.2 Bacillus subtilis strain and two microbial strains isolated from weathered oil

In this set of experiments there were two batches: tailings and deionized water batches inoculated with 5 mL of microbial culture of biosurfactant producer *Bacillus subtilis* strain and tailings and deionized water batches inoculated with 5 mL of two microbial strains isolated from weathered oil. For each batch, approximately 20 g of tailings and 100 mL of deionized water were placed in a 250 mL Erlenmeyer flask. Foam stoppers were used to fit the flasks top in order to prevent the entry of microorganisms and dust into the samples while allowing aeration. Flasks and media were sterilized by autoclaving at 121 ºC for 20 min before each experiment run. Aeration was achieved by rotating the flasks at 200 rpm on an orbital shaker (Thermolyne AROS 160) for 50 days. Samples covering days 0, 5, 15, 30, 45 and 50 were taken to measure the surface tension. All tests were performed in triplicate.

3.5 Analysis

Settled tailings and tailing process water in each set of experiments were separated using a pipette in order to perform analysis on them. The analytical procedures are described in the following subsections.

3.5.1 Settled tailings

3.5.1.1 Particle size distribution

Fines are defined as mineral particles smaller than 44 µm. The fines to solids ratio was the weight percent of fines in the whole solid mass. The size distribution of tailings was measured using a Horiba model LA-950V2 laser scattering particle size analyzer which uses Mie Scattering (laser diffraction) to measure particle size in the span of 0.01-3000 µm (http://www.horiba.com).

Light scattered from the particles is dependent on their size. It has been shown that bigger particles scatter incident light at lower angles when compared with scattered light from finer particles. So by carefully calculating the intensity of the scattered light, the percentage of different particles size particles and their respective quantities can be estimated (http://www.horiba.com).

This equipment has a standard centrifugal pump to suspend and circulate the particles in the cell. Both the circulation and the agitation speeds were adjusted to 5. The in-built 130 watt ultrasonic probe delivers highest dispersing capability during the measurements. Both the ultrasonic power and the ultrasonic times were adjusted to 5. Dried tailings samples from all parts of the sediment column (Rock crystal with refractive index (R): 1.540) were dispersed in deionized water (Water with Refractive Index (R): 1.333) before introducing them into the particle size analyzer.

3.5.1.2 Zeta potential measurement

The zeta potential (or electrophoresis mobility) of the oil sands tailings particles in a diluted suspension (after using rhamnolipid and/or microbial cultures, and before it) was measured by Zeta-Meter System 3.0+ (USA). It works based on energizing the electrodes and watching and tracking one of the colloid particles (which are placed in a viewing chamber called an electrophoresis cell) as it moves across a grid in the microscope.

The cell was filled with the sample (about 20 ml). Electrodes were inserted and were connected to the Zeta-Meter 3.0+ unit. The specific conductance of the sample was determined and according to it the appropriate voltage would be selected. The electrodes were energized and moving colloids across a grid were watched in the microscope. One moving colloid was tracked by simply pressing a button and holding it down while the colloid moves across the grid. When the button was released, the colloid's zeta potential (or electrophoretic mobility) was instantly displayed (http://www.somatco.com/ZM3-U-G_D390-2.pdf). Fifteen measurements were done for each sample and the average value is presented.

3.5.1.3 Organic matter content

The organic matter content of the settling tailings was determined by the weight loss on ignition method (Rowell 1994), as well as the digestion by hydrogen peroxide (H_2O_2) method (American Public Health Association (APHA) 1998).

Loss on ignition (LOI) method

Loss on ignition (LOI) is a simple method to determine total organic content of a given soil, presented in percent. Loss on ignition provides an estimation of organic content and is most useful for determining the organic content of sandy soils (Brickner 2013). Soils containing high percentages of clay or silt particles may fracture at high temperatures, resulting in the loss of some structural water (Brickner 2013; Rowell 1994).

The loss on ignition was determined using a standard method described in D.E. Rowell's Soil Science: Methods and Applications (1994). Tailings samples were oven-dried overnight in an oven set at 105ºC. Then the oven-dried tailings were ground with a mortar and pestle to ensure an even consistency. A small ceramic crucible was weighed and 5 g of oven dried tailings were placed in it. The weight of oven-dried sample together with small ceramic crucible was recorded as initial "oven-dried" mass of the sample and the sample was then placed in a furnace at 550ºC for a minimum of 4 hours. Sample was cooled in a desiccator and then weighed to determine the amount of mass lost in the furnace (record the change in mass (mass LOI)). The loss on ignition was calculated with the following relationship (Brickner 2013):

LOI (%) = (Mass LOI / Initial "oven-dried" mass) x 100%

Hydrogen peroxide (H_2O_2) digestion method

A clean and dry beaker was weighed. Five g of dry settling tailings were added to the beaker. Under the fume hood, 10 mL aliquots of a 30% hydrogen peroxide (H_2O_2) solution were added to the beaker. It started to bubble. Adding of H_2O_2 was continued until the bubbling stopped. The beaker was left under the fume hood over night and was weighed again. The difference between the initial weight and final weight is the weight of organic matter.

The difference in weight was divided on the initial weight of the soil and multiplied by 100 to give the percentage of organic matter. It can be represented with the following relationship:

Organic Matter (%) = [(initial sample weight - final sample weight)/initial sample weight] x 100%

3.5.1.4 Heavy metal contents and ICP-MS analysis

To determine the heavy metal content, the settled tailings were prepared by digestion using an EPA method 3050b (1996). 1-2 g sample (wet weight) or 1 g sample (dry weight) of settled tailing sample was transferred to a 250 ml beaker (digestion vessel). Ten mL of 1:1 HNO_3 were added and mixed with the sample. The beaker was covered with an elevated watch glass to prevent sample contamination from the fume hood environment and was placed on the hot plate for solution evaporation. The hot plate was located in a fume hood and was previously adjusted to provide evaporation at a temperature of approximately 95°C ± 5°C and refluxed for 10 to 15 minutes without boiling (EPA 1996).

The sample was allowed to cool and 5 mL of concentrated HNO_3 were added. The cover was replaced, and refluxed for 30 minutes. If brown fumes are generated, this step was repeated (addition of 5 mL of concentrated HNO_3) over and over until no brown fumes are given off by the sample (indicating the complete reaction with HNO_3). Using a watch glass, the solution was heated at 95°C ± 5°C without boiling for two hours.

Then the sample was cooled, and 2 mL of water and 3 mL of 30% H_2O_2 were added (for samples which contain rhamnolipid and microbial culture 10 mL of H_2O_2 were added to make sure that all organic matter is removed). The vessel was covered with a watch glass and was returned to the heat source for warming and to start the peroxide reaction (care must be taken to ensure that losses do not occur due to excessively vigorous effervescence). It was heated until effervescence subsides and then was cooled. Addition of 30% H_2O_2 in 1 mL aliquots with warming was continued until the effervescence is minimal or until the general sample appearance was unchanged.

The sample was covered with a watch glass and heating the acid-peroxide digestate was continued at 95°C ± 5°C without boiling for two hours. After cooling, the sample was diluted to 100 mL with deionized water. Particulates in the digestate then were removed by filtration through Whatman No. 41 filter paper (EPA 1996). The prepared sample solution was analyzed by Inductively Coupled Plasma- Mass Spectrometry (ICP-MS) (Agilent 7500ce).

3.5.2 Tailings process water

3.5.2.1 Surface tension measurement

The surface tension of the filtered supernatant was measured using a Fisher Scientific Tensiometer. This device works based on the ASTM method D1331-89 (ASTM 2006) which employs the du Noüy ring method for direct results with no calculations (McInerney et al. 1990). The method involves slowly lifting a ring, often made of platinum-iridium, from the surface of a liquid. The force required to separate the ring from the liquid's surface is measured and related to the liquid's surface tension. The accuracy of this method is ±0.25 mN/m. The device was calibrated as instructed by the manufacturer and its accuracy checked by measuring the surface tension of deionized water at room temperature and comparing it to the 72 mN/m which is reported in the literature (Mulligan 2005). The reduction in surface tension is related to the biosurfactant concentration below the defined CMC. The CMC can be used as an indicator for biosurfactant production levels. Above the CMC the surface tension does not change with rhamnolipid concentration. The concentration in this range was determined by serial dilution which brings the concentration below the CMC (Figure 3-1). This figure shows the surface tension versus rhamnolipid concentration and its CMC (Abbasi-Garravand and Mulligan 2014).

Below the CMC the concentration was determined according to the Figure 3-1 which was obtained by (Abbasi-Garravand 2012).

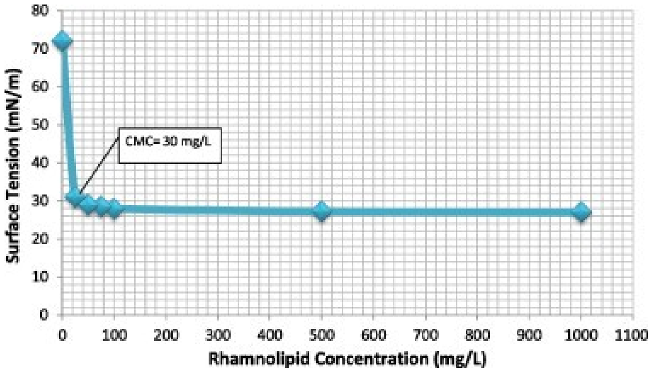


Figure 3-1. Surface tension versus rhamnolipid concentration and its CMC (Abbasi-Garravand and Mulligan 2014)

3.5.2.2 Total petroleum hydrocarbon and gas chromatography analysis

Total petroleum hydrocarbon in the tailing process water was determined using the EPA standard method 1664 (n-hexane extractable material) (EPA 2010). This method uses n-hexane and a separatory funnel to extract relatively non-volatile hydrocarbons from water, waste water, and aqueous wastes.

For extraction, 4 mL of the filtered process water and 2 mL of n-hexane (Sigma-Aldrich, 95%) were transferred to a 60 mL separatory funnel. The mixture was vigorously shaken for two minutes and then allowed to settle for 10 minutes until the two phases were completely separated. The water was then eluted through the stopcock into a 100 mL Erlenmeyer flask and the process repeated three times. The collected hexane was dried over 2 g of sodium sulfate (granular anhydrous ACS grade) to remove residual water, and filtered through a Whatman No.40 filter. The extracts were then transferred to a 20 mL amber vial and stored at 4°C until the time of analysis by gas chromatography (GC). Liquid/liquid extraction blanks (only deionized water with hexane in a separatory funnel) were prepared using the same procedure.

Gas chromatography analysis

Extracted samples were analyzed by a CP-3800 VARIAN gas chromatograph equipped with a Flame Ionization Detector (GC-FID) with auto sampler. Chromatographic separations were conducted using a DB-5 fused silica column with 30 m long, an inner diameter of 0.25 mm, 0.25 µm film thicknesses and the temperature range of 60°C to 325°C. Helium was used as the carrier gas at a constant flow rate of 2 mL/min and makeup gas flow rate of 30 mL/min. Hydrogen gas and air flow rates were 30 mL/min and 300 mL/min, respectively in the FID detector. Injector and detector temperatures were kept constant at 250°C. One µL of the sample was injected to the column in the split/splitless mode. The oven temperature program was set at 50°C for 2 minutes, increased to 250°C at a rate of 8°C/min, and held at 250°C for 6 minutes (total run time of 33.25 minutes). The total petroleum hydrocarbon was determined from the area under the GC-FID chromatogram.

3.5.2.3 Heavy metal contents

The heavy metal content of the process water was determined using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent 7500ce). For the determination of dissolved analytes in supernatant waters, approximately 1 ml was taken from filtered supernatant using a pipette. This sample was diluted 100 times to bring the concentration of metals into the measuring range of the machine. An appropriate volume of 1:1 HNO_3:H_2O was added to adjust the acid concentration of the aliquot to approximate a 1% (v/v) nitric acid solution (e.g., add 0.4 mL 1:1 HNO_3 to a 20 mL aliquot of sample) (EPA 2007).

For samples which contain rhamnolipid, acid digestion was done in order to make sure that rhamnolipids were removed from the samples. For the acid digestion according to the EPA method 200.8 (2007), a 100 mL (±1 mL) aliquot from a well mixed, acid preserved sample was transferred to a 250 mL beaker. 2 mL 1:1 HNO_3 and 2.0 mL of 1:1 hydrochloric acid (HCl:H_2O) was added to the beaker containing the measured volume of sample (the concentration of hydrochloric acid was doubled due to rhamnolipid presence in the solution). The beaker was covered with an elevated watch glass and placed on the hot plate for solution evaporation in the fume hood for two hours and reflux for 30 minutes without boiling (about 20 mL of solution was remained in the beaker).

The sample was allowed to cool and was transferred to a 50 mL volumetric flask and then water was added to bring the volume to 50 mL. Then the sample was allowed to settle overnight, or was centrifuged until clear. Any possible particulates in the solution were removed by filtration through Whatman No. 41 filter paper. In order to adjust the chloride concentration 10 mL of the prepared solution were pipetted into a 50 mL volumetric flask diluted to volume with deionized water and mixed (EPA 2007). The prepared sample solution was analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent 7500ce).

3.5.2.4 Heavy metal, rhamnolipid and hydrocarbon removal by Ultrafiltration System

The concept of MEUF is that heavy metals are attached to the surface of the micelle in the process water. The QuixStand BenchTop System (Figure 3-2) (M series from A/G Technology Corporation) was used for separation of Cu from the liquid. The system included a feed reservoir, peristaltic recirculation pump, inlet pressure gauge, hollow fiber cartridge (Xampler cartridge), retentate outlet, outlet pressure gauge, sampling valve, and backpressure valve. The peristaltic pump that was included in the ultrafiltration system to pump the fluid was purchased from Watson-Marlow Company (313 S) (Abbasi-Garravand 2012).

The hollow fiber cartridge which was used in a QuixStand BenchTop (Ultrafiltration System) was purchased from A/G Technology Corporation. A bundle of polysulfone fibers which are parallel inside a plastic housing forms the cartridge. Molecular Weight Cut-Off (MWCO) is an important parameter in classification of ultrafiltration membranes. Ultrafiltration membranes are classified based on molecular weight cut-off which is the molecular weight of a dissolved particle when its rejection coefficient is 90% (Abbasi-Garravand 2012). Typically molecular weights of dissolved particle or micelles range from 1000 to 100000 Da (Abbasi-Garravand 2012) . The MWCO that was used in the experiments was 10,000 MWCO (Abbasi-Garravand 2012) as in the solution there are biosurfactant (with molecular weights in the range of 500 - 1500 Dalton) and high molecular weight microbial organics.

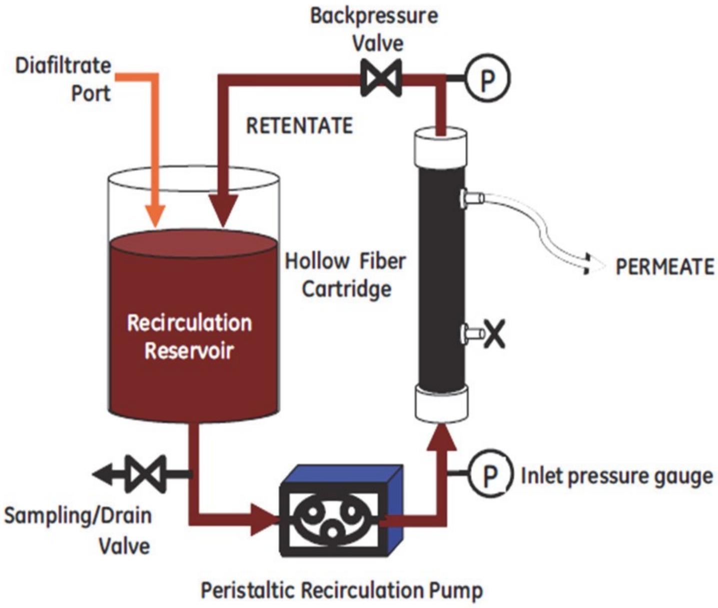


Figure 3-2. QuixStand BenchTop System flow diagram (GE Healthcare, 2004)

The feed solution was the process water from sedimentation experiments at 15 ºC ± 2 ºC. The feed solution in the reservoir was pumped by a peristaltic pump into the ultrafiltration membrane and the retentate solution was returned to the feed reservoir after exiting the cartridge. Samples were gathered from the permeate, retentate and feed for measuring the concentration of heavy metals by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and oil content by gas chromatography (GC). All experiments were done in 23 ºC ± 2 ºC for 1 hour at outlet pressure 1-2 psi (6.89-13.79 kPa), inlet pressure 11-12 psi (75.84-82.74 kPa) (and pump speed about 35%. The control solutions were process water from the control sedimentation experiment (no rhamnolipid and microbial culture). The flow loop was flushed by passing the distilled water through the system after each experiment. Each test was repeated three times and the average was used as the final result.

4. RESULTS AND DISCUSSION

4.1 Tailing Characteristics

4.1.1 Particle size distribution

Using the particle size analyzer, the particle size of dried settling oil sand tailings samples dispersed in deionized water was measured. The measured particle diameters based on cumulative% (90%) and the mean diameter, were respectively 4.36 µm and 1.10 µm. This result means that the tailing samples contain a majority of the fines clay particles which take longer to settle.

In another study it has been mention that the fine tailings of Syncrude's caustic extraction process consists of 94% fines, 76% clay minerals and 24% rock forming minerals (Islam 2014). Generally, for the CHWE process, about half of the fine tails are in the clay-size range (<2 µm) with more than 90% of the material being silt or clay-size particles (Jeeravipoolvarn 2005). Jeeravipoolvarn, (2005) reported that the mature fine tailings are generally composed of 5% sands, 45% of silt size particles and 50% of clay size particles.

4.1.2 Zeta potential measurement

Using the zeta meter, the zeta potential of dried settling tailings resuspended in deionized water was determined as -42.1 mV at the pH range of 7.3-7.8. It has been reported that the zeta potential of oil sand tailings from disposal pond in Fort McMurray was in the range of -25 mV to -27 mV between pH 6 and pH 8 (Guo 2012). The difference might be due to the difference in composition of tailings.

4.1.3 Organic matter content

The organic matter content determination of the tailings using hydrogen peroxide digestion method and ignition method was determined to be 1 wt% and 2.3 wt% respectively. These values contain the natural organic matter content and the residual hydrocarbon remained in the tailings.

There is a difference between these two values as the majority of the mass loss in the range of 250-550^{o}C temperature is due to the decomposition of all residual organics (in the form of hydrocarbons, carbon dioxide, carbon monoxide and water) and possibly tightly bound water

(Jalali 2007; Kaminsky 2008). This temperature range is consistent with the dehydroxylation of kaolinite but overlaps with the decomposition of the residual organics and the decomposition of carbonates (such as siderite ($FeCO_3$)). Hence, it was not possible to exactly determine the amount of mass loss correlating to each possible source (Kaminsky 2008). It might be possible to determine the nature of the organic content of tailings sample by doing incremental LOI in the future work (Brickner 2013). Incremental LOI could be done with measurement of the amount of material lost taken at 110ºC, 440ºC and 550ºC as the destruction of any inorganic carbonates (such as siderite ($FeCO_3$) and calcite ($CaCO_3$)), that may be present in the sample (Brickner 2013; Kaminsky 2008), at the temperature below 440ºC is avoided (Schumacher 2002).

4.2 Results of Sedimentation Experiments

4.2.1 Role of rhamnolipid, microbial cultures, and mixture of rhamnolipid and microbial cultures in tailings sedimentation (at 23 ºC± 2 ºC)

The potential of rhamnolipid to increase sedimentation was analyzed by comparing sedimentation of diluted tailing pond samples amended with different concentrations of rhamnolipids (0.5%, 1%, and 2%). The presence of rhamnolipid increased the sedimentation compared to the control under the experimental conditions (Fig. 4.1). Increasing the rhamnolipid concentrations can increase sedimentation and according to the student's t-test there is a significant increase in sedimentation at $p < 0.05$ for the 2% rhamnolipid (The calculated t exceeds the critical value ($2.5681>2.228$)). However after a longer time, rhamnolipid concentrations of 1% have shown approximately the same amount of sedimentation compared with 2%.

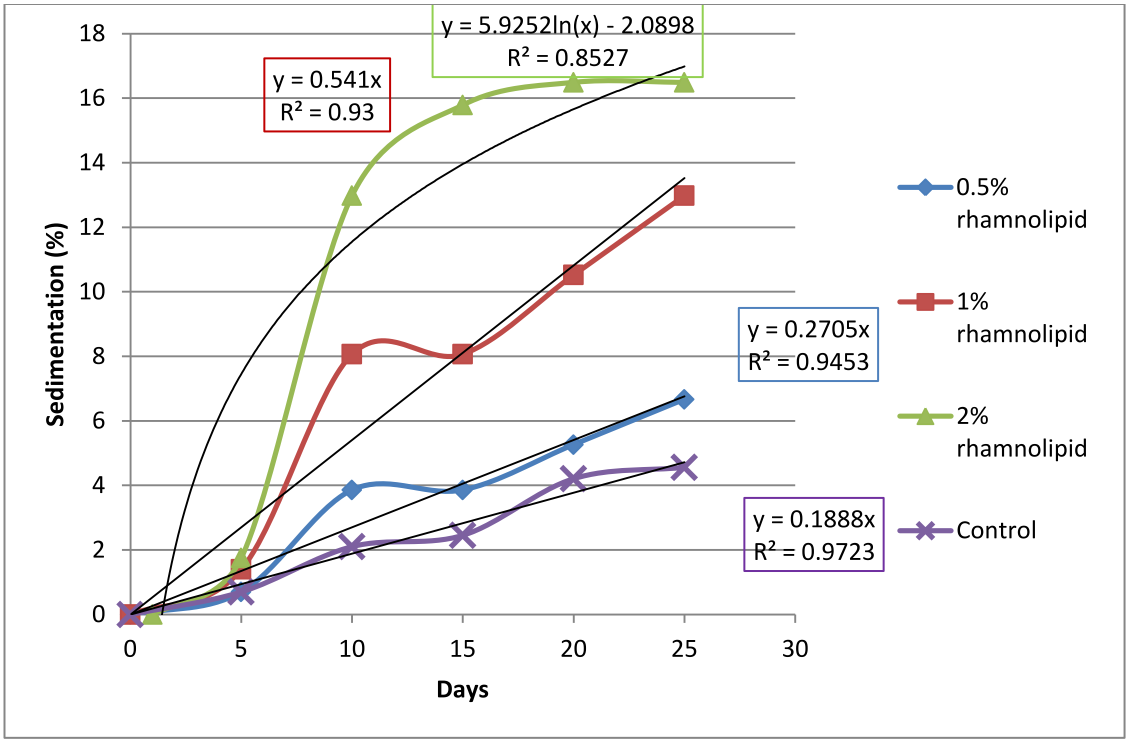


Figure 4-1. Sedimentation of oil sand tailings at different concentrations of rhamnolipid over time and kinetic model for sedimentation

As it is seen in Figure 4-1 the sedimentation rate is faster at first and then it became slower. First rapid sedimentation rate, primarily is governed by gravitational and frictional forces (Carstensen and Su 1970b). At a particular, well-reproducible point, the rate changes abruptly, and further sedimentation appears to be governed by forces over and above those just mentioned (Carstensen and Su 1970b). (Michaels and Bolger 1962) have reported a linear pattern for sedimentation rates for dilute suspensions (< 1 %). (Haines and Martin 1961) also show that very concentrated suspensions follow yet another pattern. Suspensions of "intermediate" concentration, however, have a downward curvature, i.e., the sedimentation boundary moves downward with greater and greater velocity until a certain critical height, H_a, is reached, at which time (t_o) the rate decreases abruptly (Carstensen and Su 1970a). Results for kinetics of sedimentation suggest a linear kinetics for samples amended with 1% rhamnolipid, 0.5% rhamnolipid and control and logarithm kinetic for samples amended with 2% rhamnolipid.

The role of microbial cultures to increase sedimentation was analyzed by comparing sedimentation of diluted tailing pond samples inoculated with cultures of the biosurfactant producer *Bacillus subtilis* strain, and a mixed culture of two microbial strains isolated from weathered oil (Fig. 4-2). The presence of a mixed culture of two microbial strains isolated from weathered oil increased the sedimentation compared to the control but according to the student's t- test this increase is not at $p < 0.05$ as the absolute calculated t value is smaller than critical value (0.5394<2.228). However, the sedimentation tubes inoculated with the *Bacillus subtilis* strain gave almost the same sedimentation amount as the control.

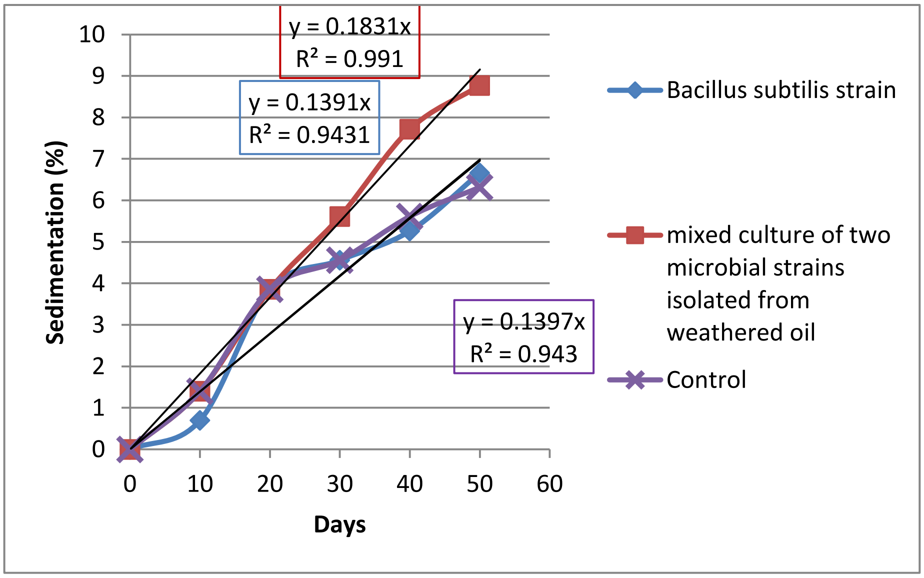


Figure 4-2. Sedimentation of oil sand tailings inoculated with cultures of biosurfactant producer *Bacillus subtilis* strain, and a mixed culture of two microbial strains isolated from weathered oil over time and kinetic model

Results for kinetics of sedimentation suggest a linear kinetics for sedimentation of oil sand tailings inoculated with cultures of biosurfactant producer *Bacillus subtilis* strain, and a mixed culture of two microbial strains isolated from weathered oil (Figure 4-2).

The results of the cell surface hydrophobicity (CSH) in the control showed that the isolated bacteria recovered from the biodiesel had hydrophilic properties (negative CSH, tendency to interact with the hydrophilic compounds), while the isolated bacteria recovered from the diesel and light crude oils had hydrophobic properties (positive CSH, tendency to interact with the hydrophobic compounds). For example, the hydrophobicity values of -50%, 16% and 2% were obtained following 1 h of incubation of bacterial cells on the biodiesel, diesel and light crude oil, respectively (Saborimanesh and Mulligan 2015). This result and other studies on the effect of hydrocarbons on the bacterial cell surface properties bacteria show that they are capable of modifying their cell surface structures based on the availability and the compositions of hydrocarbons (Bouchez Naïtali et al. 1999; Kaczorek et al. 2008; Krasowska and Sigler 2014; Prabhu and Phale 2003) The cell surface hydrophobicity is modified in order to help the microorganisms avoid contact with toxic compounds (Bouchez Naïtali et al. 1999; Kaczorek et al. 2008; Krasowska and Sigler 2014; Prabhu and Phale 2003; Torres et al. 2011) or to uptake food (e.g., hydrocarbons) Kaczorek et al. 2008, Krasowska and Sigler 2014). For example, some bacteria release vesicles (which have an intercellular structure and an outer membrane of a lipid bilayer) from the outer membrane (Baumgarten et al. 2012; Bouchez Naïtali et al. 1999; Kaczorek et al. 2008; Krasowska and Sigler 2014; Prabhu and Phale 2003), some release lipopolysaccharide (LPS) to change the cell surface hydrophobicity (Al-Tahhan et al. 2000), and some form an exopolysaccharide (EPS) matrix to create a stable environment and optimal conditions for growth (exopolymer microdomains as a structural agent for heterogeneity within microbial biofilms) (Rehm 2009).

In one study, the adhesion to a sandy soil and a clay loam soil of a series of *Lactobacillus* strains with various cell surface characteristics were investigated (Huysman and Verstraete 1993). Bacterial cell surface hydrophobicity, as determined by the bacterial adherence to octane and polystyrene, was the major parameter influencing the adhesion to the sandy soil. The cell surface charge of the bacteria was of minor importance in the adhesion to the sandy soil (Huysman and Verstraete 1993). It has been reported that the attachment to the benthos is facilitated by the common action of both coflocculation and hydrophobic interactions. EPS also can help the bacteria to adhere to the surface and can serve as flocculants to bind small clay particles (Rehm 2009).

Sedimentation of tailings amended with different concentrations of rhamnolipid (0.5% and 1%) and two microbial strains isolated from weathered oil were compared in order to evaluate the role of adding microbial cultures in sedimentation of tailing pond samples (Fig. 4-3). All of these show an increase in sedimentation compared to the control. According to the student's t-test this increase is significant at $p < 0.05$ except for samples amended with 0.5% rhamnolipid. Microbial cultures together with rhamnolipid can increase the sedimentation of tailings compared to the amount of sedimentation of tailings amended only with rhamnolipid. Adding microbial cultures results in more sedimentation at a lower concentration of rhamnolipid (0.5% compared to 1%). Results for kinetics of sedimentation suggest linear kinetics sedimentation (Figure 4-3).

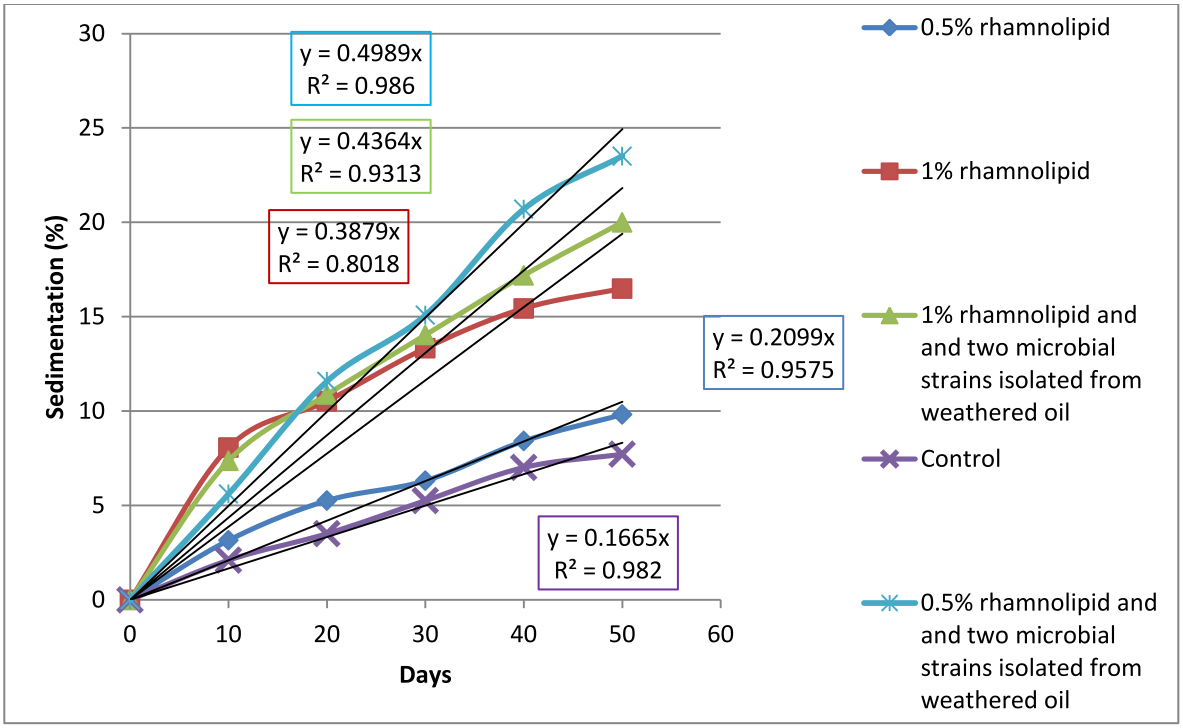


Figure 4-3. Sedimentation of oil sand tailings at different concentrations of rhamnolipid (0.5% and 1%) and two microbial strains isolated from weathered oil over time and kinetic model for sedimentation

4.2.2 Role of mixture of rhamnolipid and microbial cultures in tailings sedimentation (at 15 ºC ± 2 ºC)

Sedimentation of tailings amended with the rhamnolipid (0.5%) and two microbial strains isolated from weathered oil were compared to the control at 15 ºC ± 2 ºC in order to evaluate the role of lower temperature in sedimentation of tailing pond samples (Fig. 4-4). These results show an increase in sedimentation compared to the control and according to the student's t- test this increase is significant at $p < 0.05$ (the absolute calculated t value is greater than critical value). It means that microbial cultures together with rhamnolipid can significantly increase the sedimentation of tailings even at lower temperature. Results for kinetics of sedimentation suggest a logarithmic kinetics for sedimentation (Figure 4-4). Table 4-1 summarizes the results of student's t- test at $p < 0.05$ and n=6 (number of sampling points) for sedimentation in different samples. Table 4-2 summarizes the results of kinetic rates for sedimentation for different samples. The analytical calculated results are very close to the experimental data.

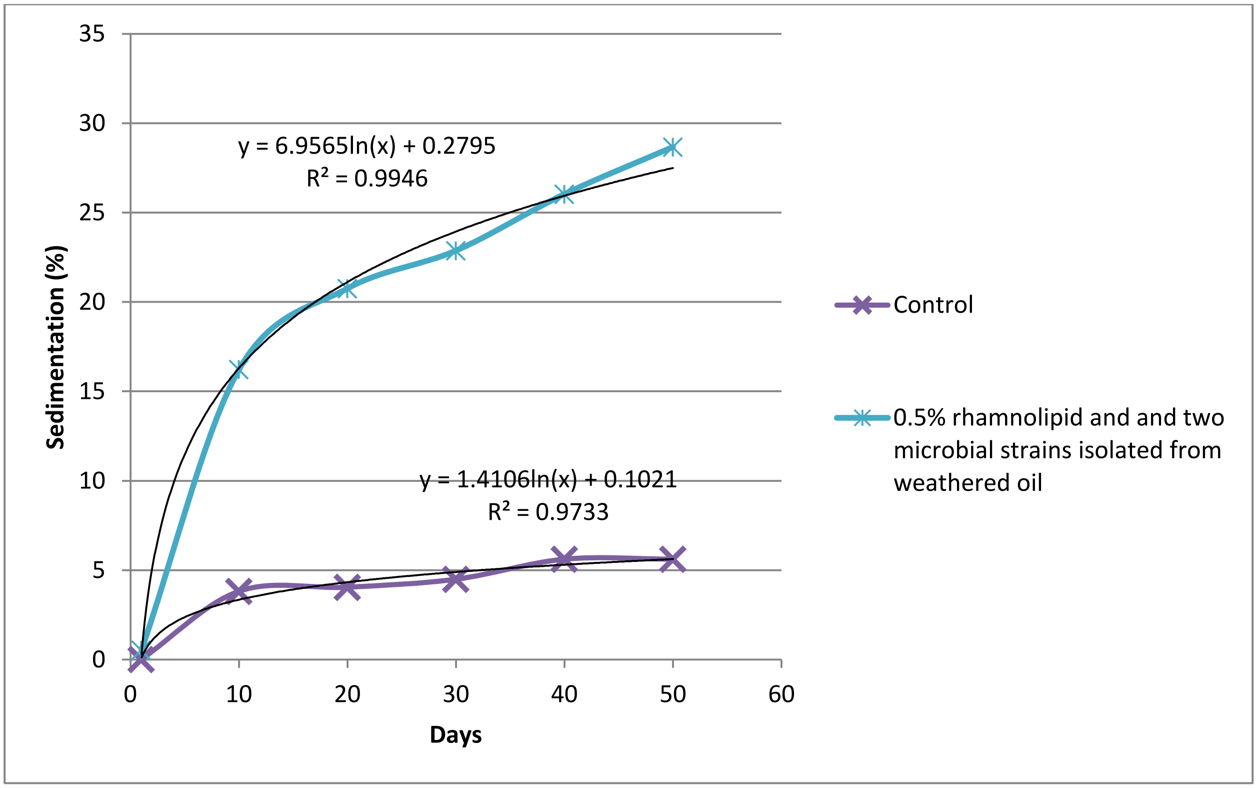


Figure 4-4. Sedimentation of oil sand tailings at 15 ºC ± 2 ºC using rhamnolipid (0.5%) and two microbial strains isolated from weathered oil over time and kinetic model for sedimentation

Table 4-1 Results of student's t- test at $p < 0.05$and $n=6$ (number of sampling points) for sedimentation in different samples

Samples		**Critical value**	**t**
25 days at 23 ºC ± 2 ºC	0.5% rhamnolipid	2.228	0.8148
	1% rhamnolipid	2.12	1.5104
	2% rhamnolipid	2.228	2.5681
50 days at 23 ºC ± 2 ºC	*Bacillus subtilis* strain	2.228	0.0797

	a mixed culture of two microbial strains isolated from weathered oil	2.228	-0.5394
	0.5% rhamnolipid	1.943	0.466
	1% rhamnolipid	1.943	2.3108
	0.5% rhamnolipid and two strains isolated from weathered oil	1.943	-2.206
	1% rhamnolipid and two strains isolated from weathered oil	1.943	2.2937
50 days at 15 ºC ± 2 ºC	0.5% rhamnolipid and two strains isolated from weathered oil	2.015	-3.6237

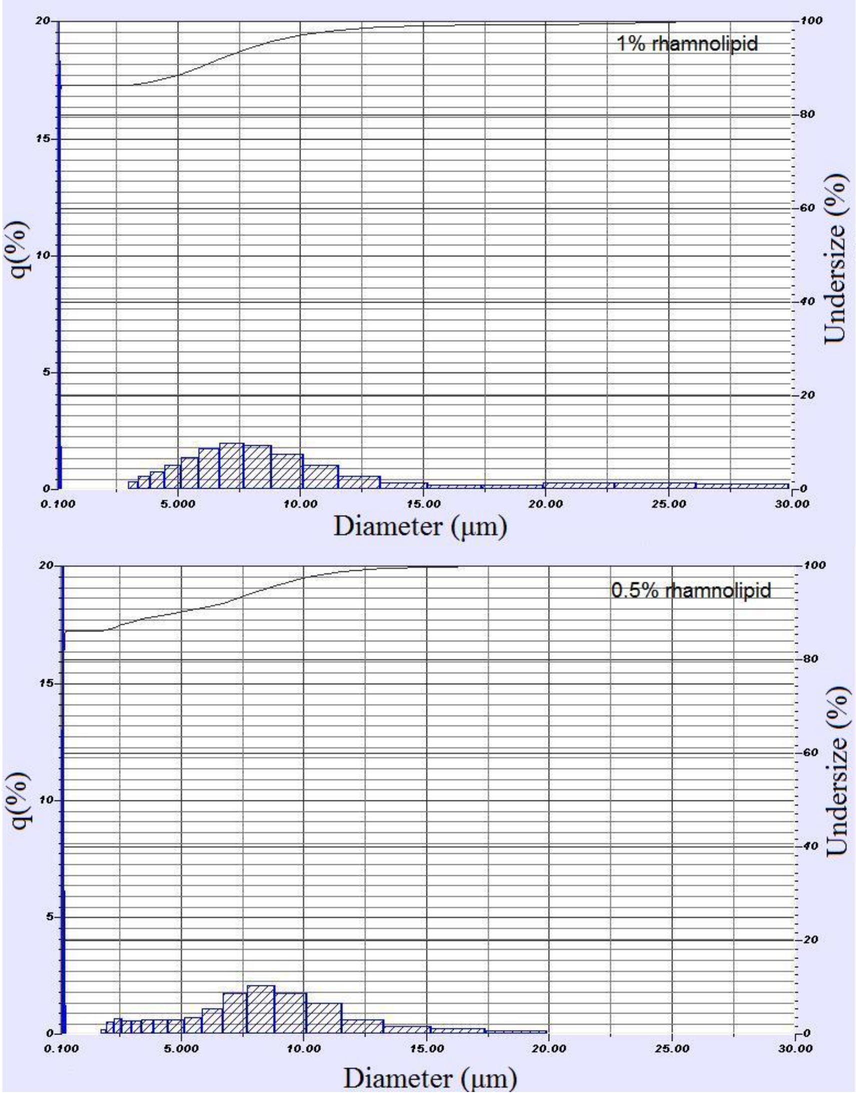
Table 4-2 Results of kinetic model for sedimentation for different samples

Samples		**Kinetic rate (sedimentation%/day)**	**Final sedimentation (%)**	
			Experimental	**Analytical**
25 days at 23 ºC ± 2 ºC	0.5% rhamnolipid	0.2705	6.67	6.76
	1% rhamnolipid	0.541	12.98	13.52
	2% rhamnolipid	5.9252	16.49	16.98
50 days at 23 ºC ± 2 ºC	*Bacillus subtilis* strain	0.1391	6.67	6.96
	a mixed culture of two microbial strains isolated from weathered oil	0.1831	8.77	9.15
	0.5% rhamnolipid	0.2099	9.82	10.94
	1% rhamnolipid	0.3879	16.50	19.39
	0.5% rhamnolipid and two strains isolated from weathered oil	0.4989	23.51	24.95
	1% rhamnolipid and two strains isolated from weathered oil	0.4364	20	21.82
50 days at 15 ºC ± 2 ºC	0.5% rhamnolipid and two strains isolated from weathered oil	6.9565	28.67	26.94

4.2.3 Particle size distribution

a) at room temperature (23 ºC ± 2 ºC)

Using the particle size analyzer, the particle aggregation and flocculation were evaluated. Dried settling tailings samples from sedimentation experiments using microbial cultures and different concentrations of rhamnolipid were dispersed in deionized water. Figure 4-5 shows the particle size distribution using four different sedimentation agents: 1% rhamnolipid, 0.5% rhamnolipid, 1% rhamnolipid and microbial cultures, and 0.5% rhamnolipid and microbial cultures. The measured particle diameter based on cumulative% (90%) and the mean diameter are respectively 5.83, 4.85, 7.20, 8.30 μm and 1.31, 1.16, 1.81, 2.32 μm for the four different sedimentation agents. These values are actually the diameters of the flocculation of tailings particles.


1% rhamnolipid
q(%)
20
15
10
5
0
Undersize (%)
100
80
60
40
20
0
0.100
5.000
10.00
15.00
20.00
25.00
30.00
Diameter (μm)
0.5% rhamnolipid
q(%)
20
15
10
5
0
Undersize (%)
100
80
60
40
20
0
0.100
5.000
10.00
15.00
20.00
25.00
30.00
Diameter (μm)

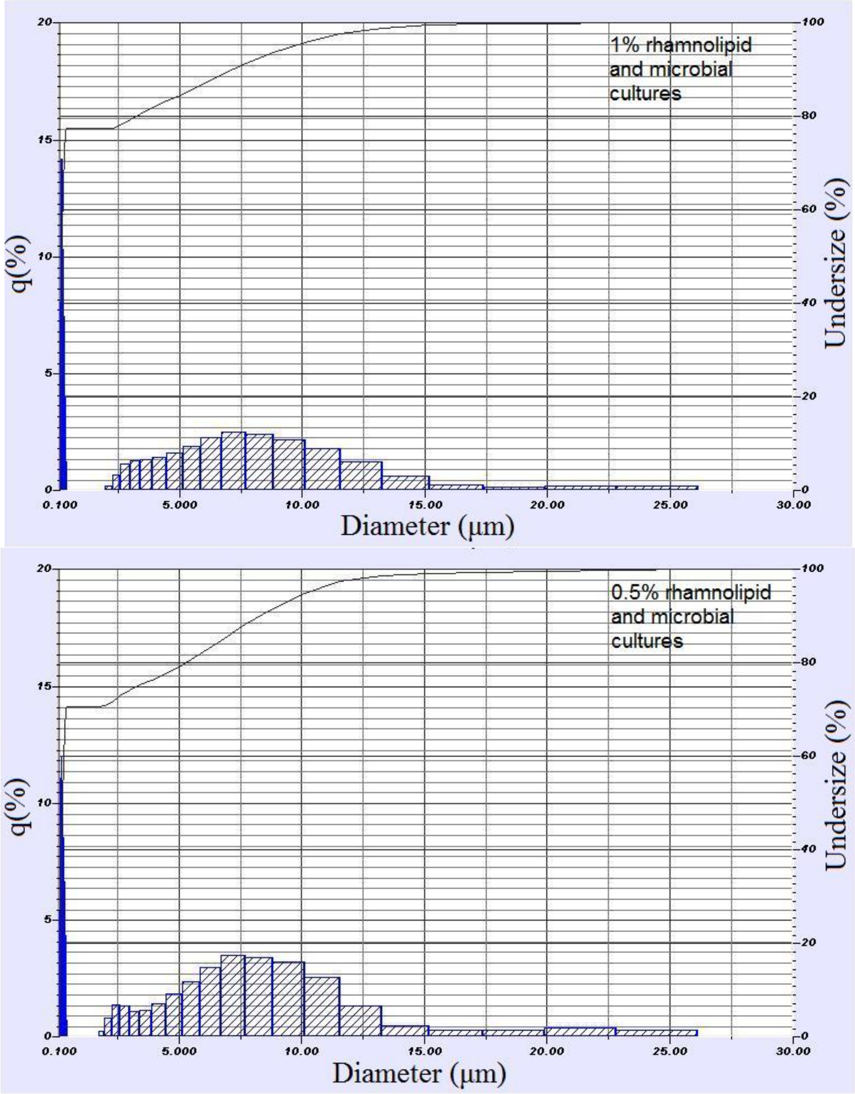


Figure 4-5. Particle size distribution of the settled oil sand tailings from sedimentation experiments using microbial cultures and different concentrations of rhamnolipid dispersed in deionized water

Compared to the measured particle diameter based on cumulative% (90%) (4.36 μm) and particle mean diameter (1.10 μm) of control experiments which were simply composed of deionized water and tailings without any sedimentation agents, one can conclude that both rhamnolipid and microbial cultures mixed with oil sand tailings can improve effectively the aggregation and flocculation of tailings particles. The tailings are comprised of a high concentration of smaller and lower concentrations of larger particles before the flocculation process and a relatively high concentration of large flocculates after the flocculation process.

Comparing the size distribution and mean diameter of particles obtained from tailings samples and rhamnolipid inoculated with microbial cultures and tailing samples with non inoculated rhamnolipid, shows that the microbial culture can improve particle aggregation and flocculation and relatively increase the concentration of larger particles when mixed with rhamnolipid.

It seems that microbial cultures can work better at the lower rhamnolipid concentration as mixing tailings with microbial cultures and 0.5% rhamnolipid was more effective for particle flocculation and aggregation than those of 1% rhamnolipid probably due to antimicrobial effect of rhamnolipid which inhibits the microbial growth and EPS production.

*a) at the lower temperature (*15 ºC ± 2 ºC)

Figure 4-6 shows the particle size distribution using 0.5% rhamnolipid and microbial cultures as the sedimentation agents at 15 ºC ± 2 ºC. The measured particle diameter based on cumulative% (90%) and the mean diameter are respectively 7.03μm and 2.11μm. These values are actually the diameters of the flocculation of tailings particles.

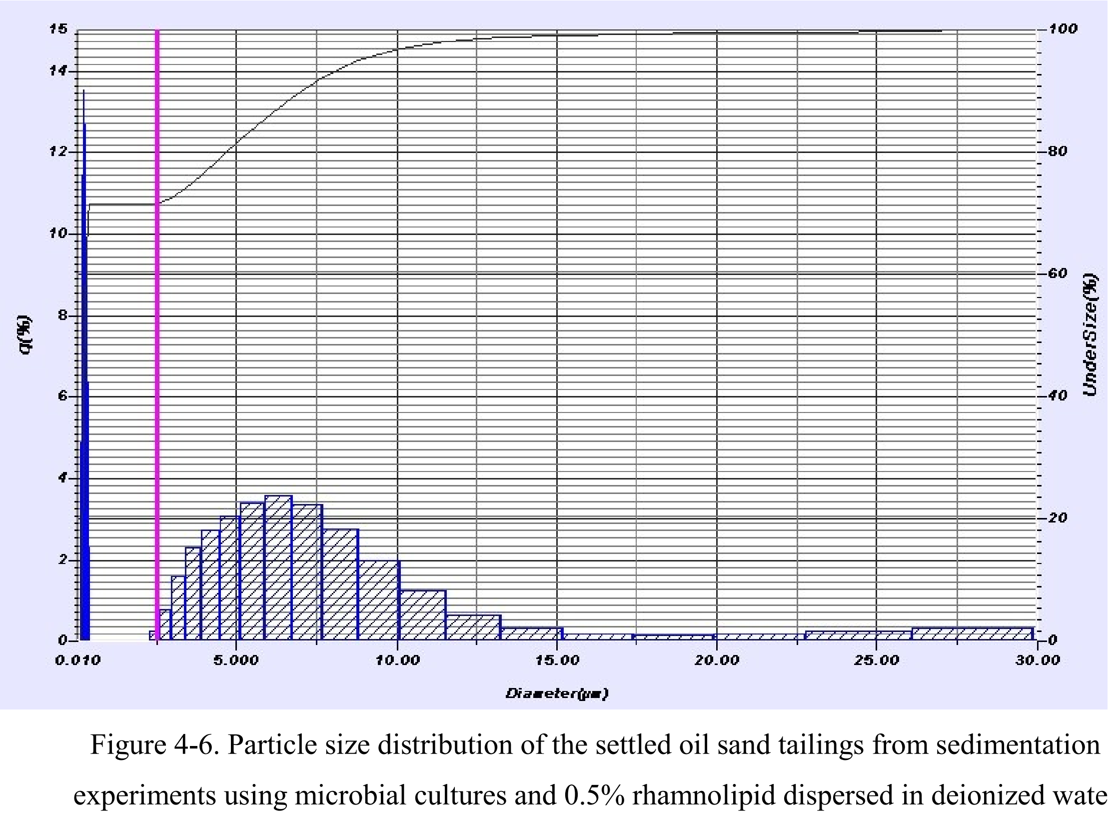


Figure 4-6. Particle size distribution of the settled oil sand tailings from sedimentation experiments using microbial cultures and 0.5% rhamnolipid dispersed in deionized water

Compared to the measured particle diameter based on cumulative% (90%) (2.67 μm) and particle mean diameter (0.78 μm) of control experiments which were simply composed of deionized water and tailings without any sedimentation agents, one can conclude that rhamnolipid and microbial cultures mixed with oil sand tailings can improve effectively the aggregation and flocculation of tailings particles in this case. It seems that lowering the temperature did not result in a large change in measured particle diameter based on cumulative% (90%) and particle mean diameter and flocculation compared to the result of particle size distribution at room temperature. The results at lower temperature showed the strong potential of using rhamnolipid and microbial culture according to the site condition temperature.

4.2.4 Zeta potential measurement

a) at room temperature (23 ºC ± 2 ºC)

The results of zeta potential measurement of dried settling tailings samples (gained from sedimentation experiments using microbial cultures and different concentrations of rhamnolipid) resuspended in deionized water are presented in Figure 4-7. The zeta potential remained negative and decreased after adding rhamnolipid and decreased more by increasing the concentrations of anionic rhamnolipid. However adding microbial culture and rhamnolipid together slightly increased the zeta potential but still remained negative.

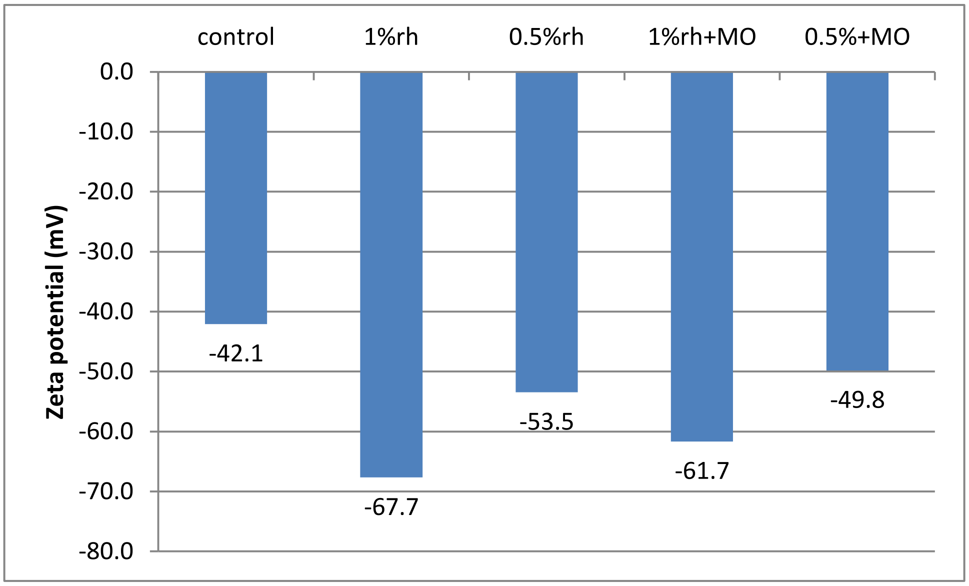


Figure 4-7. Zeta potentials of different dried settling tailings samples suspended in deionized water (23 ºC ± 2 ºC) (MO is used for defining the microbial cultured amended samples)

b) at lower temperature (15 ºC± 2 ºC)

The results of zeta potential measurement of dried settling tailings samples at 15 ºC ± 2ºC (gained from sedimentation experiments using microbial cultures and 0.5% rhamnolipid) resuspended in deionized water are presented in Figure 4-8.

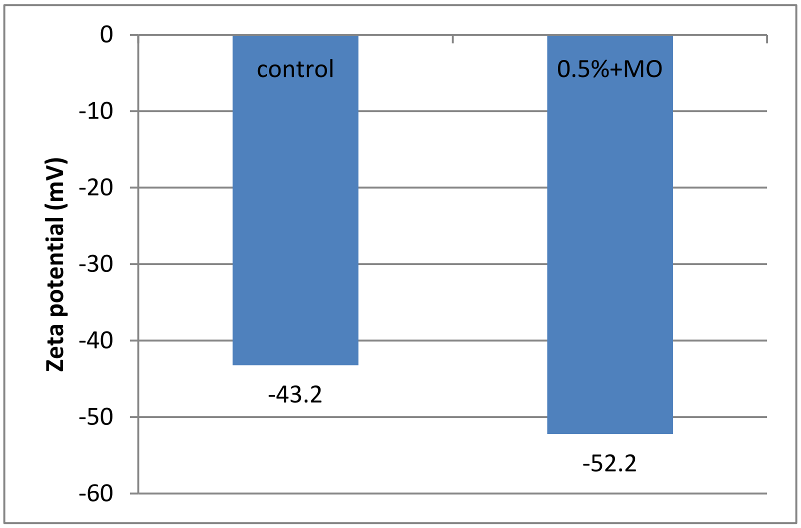


Figure 4-8. Zeta potential of dried settling tailings samples resuspended in deionized water (15 ºC ± 2 ºC) (MO is used for defining the microbial cultured amended samples)

The results of zeta potential and particle size distribution (at 23 ºC ± 2 ºC and 15 ºC ± 2ºC) supported the idea that rhamnolipid has potential to be used as flocculating agents for oil sand tailings sedimentation. It is well known that the particle hydrophobicity has a significant effect on flocculation (Song et al. 2000; Song et al. 2001; Ucbeyiay Sahinkaya and Ozkan 2011; Warren 1992). Increased surface hydrophobicity, which is dependent on increasing the concentration of surfactant could increase flocculation of clay particles (Ucbeyiay Sahinkaya and Ozkan 2011). The rhamnolipid anions adsorb on the oil sand tailings surfaces, rendering the surfaces hydrophobic and resulting in the flocculation of oil sand clay particles due to the hydrocarbon chain association (Ucbeyiay Sahinkaya and Ozkan 2011) when the rhamnolipid adsorption layers on particles contact each other.

The increase in negative zeta potential should give rise to the increase of the energy barrier, preventing the particle aggregation. However, the increase in the surface charge by the rhamnolipid adsorption on the particle surfaces did not lead to a decrease in the flocculation of tailing particles and even led to improving their flocculation which means that the rhamnolipid adsorption onto the tailing particle surfaces improved the hydrophobic interaction between the particles much more strongly than the electrical double layer repulsion.

These results also show that the rhamnolipid together with microbial culture had a stronger activity than rhamnolipid by itself. Rhamnolipid (which is a biosurfactant produced by *Pseudomonas aeruginosa*) mixed with microbial culture showed strong flocculating activity, while zeta potential still remained negative. It means that the mechanism of flocculation is not charge neutralization.

Microbial activity can increase MFT by microbial cells and/or EPS secreted by microbial cells (Bordenave et al. 2010) and/or biogenic gas production (Bressler et al. 2010; Fedorak et al. 2003). Macromolecules (such as EPS) could be viewed as naturally produced flocculants (Tenney and Stumm 1965). Addition of macromolecules to stabilize inorganic dispersions (kaolinite, silica, or alumina) could increase flocculation (Chen 2007). Yu et al. (2010) showed that EPS causes aggregation of particles through a bridging mechanism which can be viewed as the result of the interaction of naturally produced, high molecular weight, and long chain organics with kaolin clay particles in the way that the macromolecules bridge the individual clay particles into an aggregate.

It is possible that the strong flocculating activity of rhamnolipid mixed with microbial culture was probably due to the biosurfactant and high molecular weight microbial organics. Bioflocculants with high molecular weights involved more adsorption sites, stronger bridging, and higher flocculating activity (Yu et al. 2009).

Another possible reason for the strong flocculating activity of rhamnolipid mixed with microbial culture could be due to the change in chemistry of pore water as a result of microbial metabolism. Siddique et al. (2014) showed that microbial metabolism could alter the chemistry of pore water that in turn influences the consolidation of clay particle suspensions (Siddique et al. 2014). They showed that dissolution of MFT carbonate minerals (presumably calcite/dolomite) increased Ca^{2+} and Mg^{2+} concentrations in pore water. They also observed a relatively higher concentration of HCO_3^- in the pore water of amended MFT, presumably due to dissolution of biogenic CO_2 in pore water and/or dissolution of carbonate minerals (Morse et al. 2007). The dissolution of entrapped CO_2 reduced pore water pH, thereby dissolving carbonate minerals and releasing divalent cations (Figure 4-9).

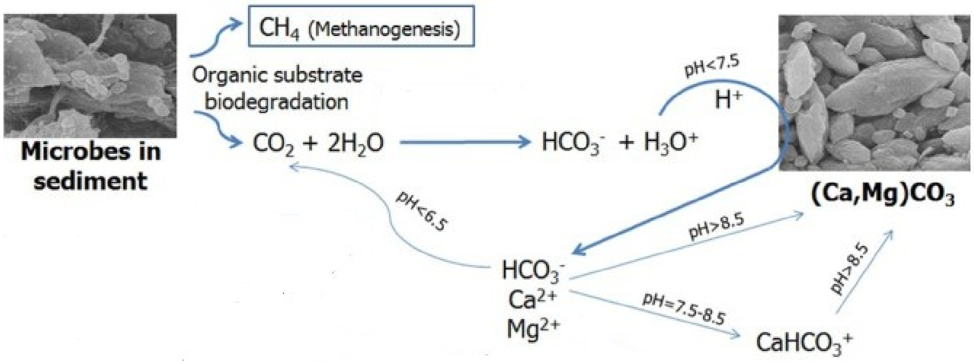


Figure 4-9. Dissolution of biogenic CO_2 in tailings decreases pore water pH by producing hydronium ion (H_3O^+). Increased H_3O^+ dissolves carbonate minerals such as $CaMg(CO_3)_2$ and releases calcium (Ca^{2+}) and magnesium (Mg^{2+}) (Siddique et al. 2014)

The effect of benthic bacterial activity on dissolution of carbonate mineral in marine sediments has been described (Moulin E. 1985). It also has been described that increased atmospheric CO_2 is responsible for acidification and consequently dissolution of marine carbonate minerals (Morse et al., 2007).

Siddique et al. (2014) calculated the ionic strength (I) and thickness of the diffuse double layer (DDL) of clay particles and find that increased (I) of the MFT decreased DDL of clay particles, lower MFT pH dissolved carbonate minerals and changed pH-dependent charges on clays, leading to consolidation of MFT. If the ionic strength (I) was not high enough to reduce the thickness of the DDL of clay particles and cause flocculation as a result of double layer compression. The other mechanism which can be considered for the flocculation is bridging. Cations such as Ca^{2+} stimulate flocculating activity by neutralizing and stabilizing the negative charge of functional groups and by forming bridges between particles (Yu et al. 2009). Therefore, the negatively charged rhamnolipid could flocculate suspended clay particles by absorbing onto the surfaces of neighboring negatively charged clay particles via cation bridging and also it could be by hydrophobic interactions.

4.2.5 pH measurement

In order to understand better the role of the microbial culture in this work, the pH of samples was measured. The results show slight reduction in pH for all samples amended with a mixture of rhamnolipid and microbial culture (~7.5 for 1% rhamnolipid and microbial culture, and ~7.6 for 0.5% rhamnolipid and microbial culture) at room 23 ºC ± 2 ºC. This range of pH means that dissolution of biogenic CO_2 and H_3O^+ concentration is not significant to support the possibility of dissolution of MFT carbonate minerals and releasing divalent cations.

Figure 4-10 shows the pH change during the time for samples at 15 ºC ± 2 ºC. The pH slightly increased with time. Methanogenesis in MFT might be responsible for the observed pH increase as CO_2 was consumed (Siddique et al. 2014). Gas production resulted in ebullition of bubbles dominated by CH_4 (due to the poor solubility of CH_4 in water), creating transient channels for escape of pressurized pore water, particularly in the MFT near the mud line (Siddique et al. 2014).

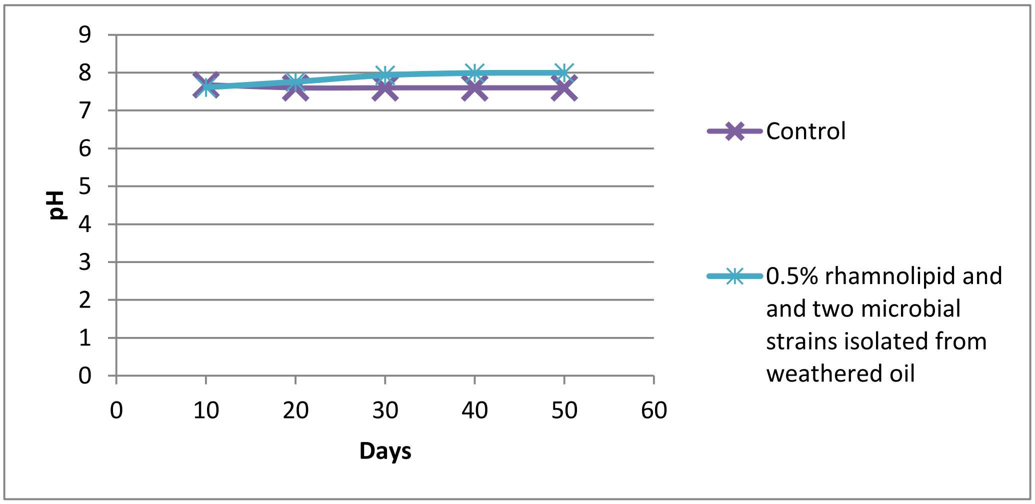


Figure 4-10. pH change over time for samples at 15 ºC ± 2 ºC

All these findings support that during this experiment's time (50 days) at 23 ºC ± 2 ºC and 15 ºC ± 2 ºC there could not be any significant change in ionic strength (I) of the pore water and the thickness of diffuse double layer (DDL) of clay particles due to dissolving carbonate minerals and releasing divalent cations. So flocculation is not a result of double layer compression or cation (such as (Ca^{2+})) bridging between particles. However there is the possibility of an increase in the consolidation due to small amounts of CH_4 production at 15 ºC

± 2 ºC as the higher column provides a thicker layer of mud compared to the 15 cm glass tubes used at 23 ºC ± 2 ºC. However in this case rhamnolipid mixed with the microbial culture could improve the hydrophobic interactions and increase the flocculation.

4.2.6 Total petroleum hydrocarbon and gas chromatography results

Total petroleum hydrocarbon in the tailings process water gained from the sedimentation experiments using microbial cultures and different concentrations of rhamnolipid at 23 ºC ± 2 ºC were evaluated through GC analysis and expressed as total petroleum hydrocarbon relative to the control (Table 4-3).

Table 4-3. Relative total petroleum hydrocarbons (wt/wt) to the control in the tailings process water gained from sedimentation experiments using microbial cultures and different concentrations of rhamnolipid

	Control	1% rhamnolipid	0.5% rhamnolipid	1% rhamnolipid and two microbial strains isolated from weathered oil	0.5% rhamnolipid and two microbial strains isolated from weathered oil
Total petroleum hydrocarbon relative to the control (wt/wt)	1 RSD: 23.8%	1.43 RSD: 21.2%	1.12 RSD: 23.2%	1.32 RSD: 25.2%	1.07 RSD: 20.4%

In the presence of rhamnolipid the concentrations of hydrocarbon in the supernatant water is high which means that the remaining settled sediment contains lower concentrations of hydrocarbons. Higher concentrations of rhamnolipid could extract more hydrocarbons from sediments. It means that the remaining oil was extracted by rhamnolipid from the tailings sediment into the water. The presence of bacterial culture did not show any large changes in hydrocarbon concentration compared to the samples which contains only rhamnolipid without bacterial cultures.

4.2.7 Heavy metal contents and ICP-MS analysis in settled tailings and tailing process water

a) at room temperature (23 ºC± 2 ºC)

The metal contents of the settled tailings (gained from the sedimentation experiments using microbial cultures and different concentrations of rhamnolipid) and tailing process water (supernatant) were evaluated through ICP-MS analysis. Al, Si, Fe are naturally structural soluble metals. Initial concentrations of vanadium, copper, zinc, arsenic, selenium, silver, cadmium, and lead in the oil sand tailings are shown in Figure 4-11. The concentrations of these heavy metals in the remaining dry sediment and supernatant are shown in Figures 4-12 and 4-13.

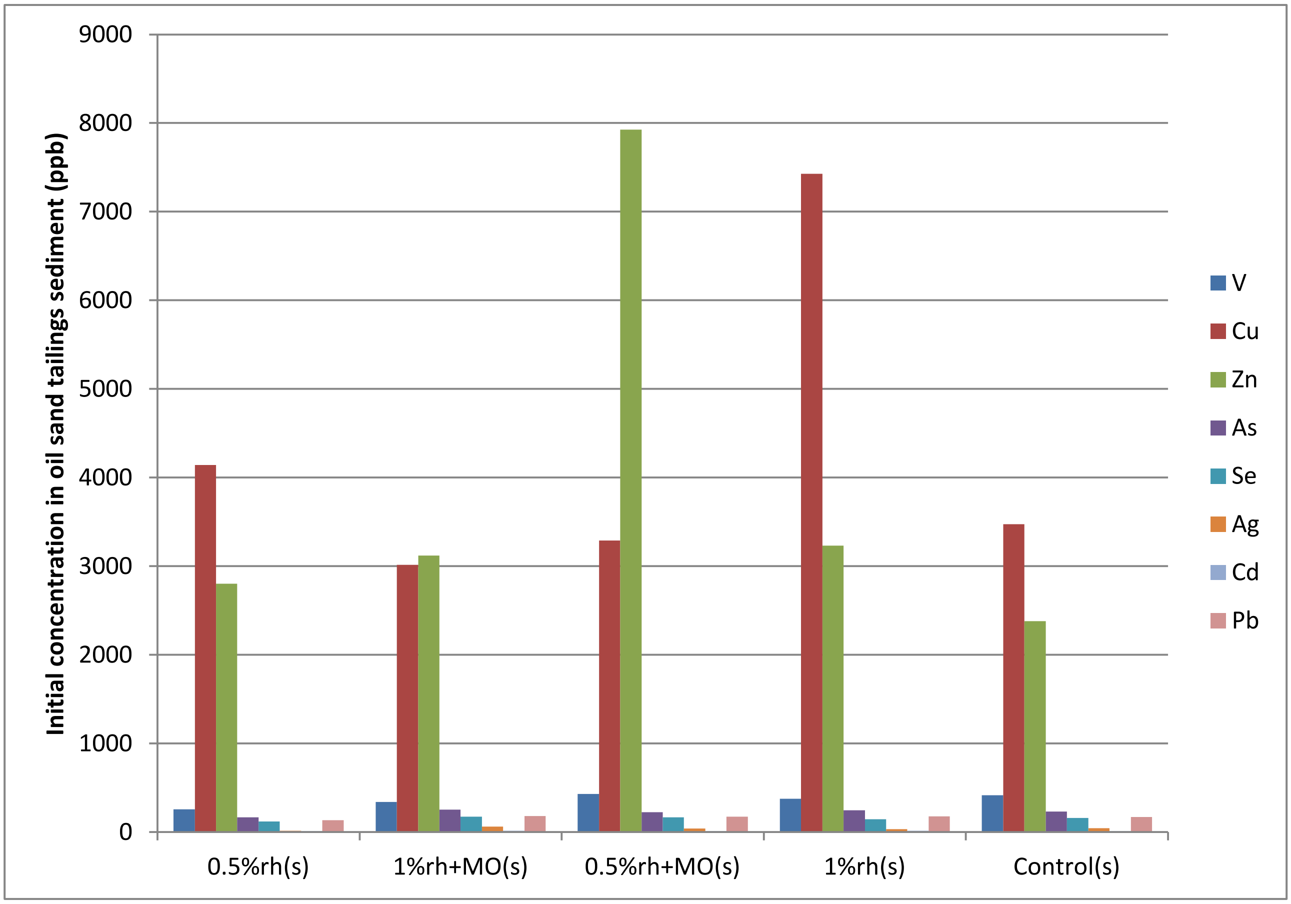


Figure 4-11. Initial concentration of heavy metals in the oil sand tailings sediment for each sample before sedimentation process

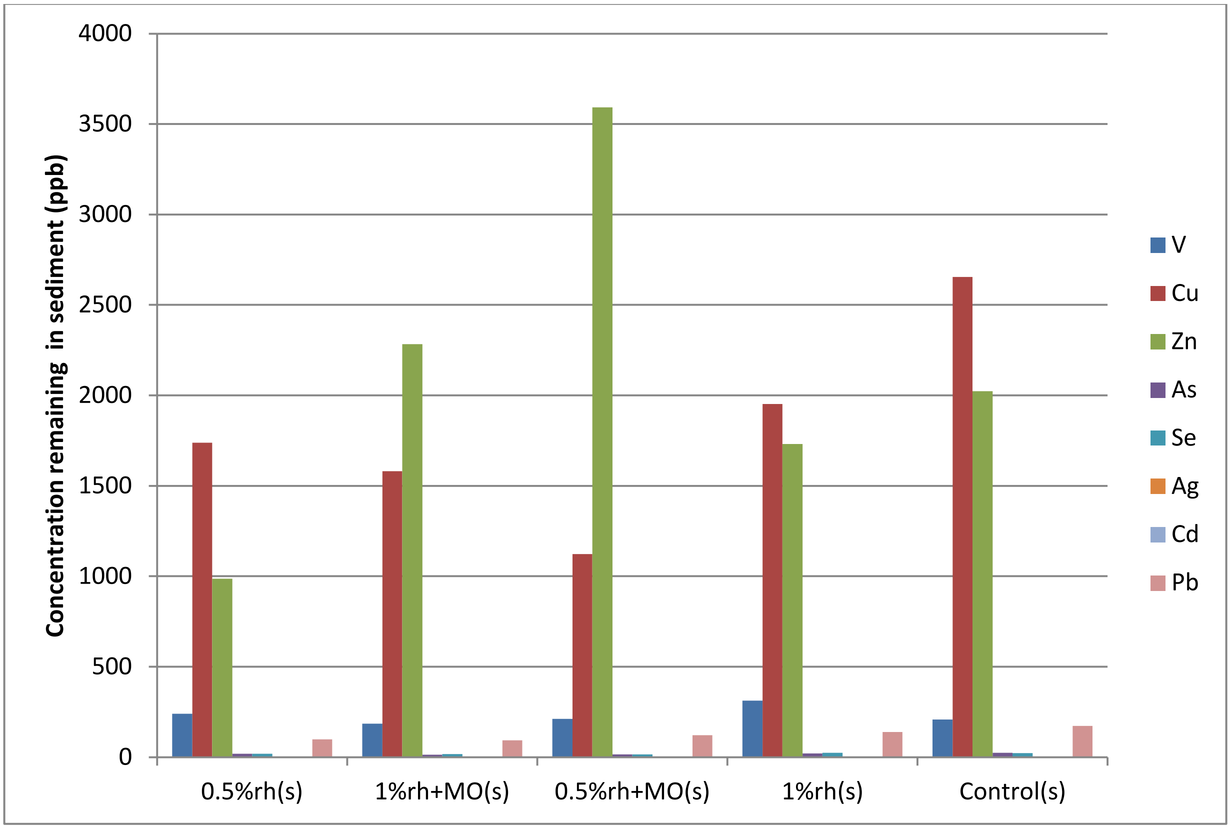


Figure 4-12. Concentration of heavy metals in the remaining dry sediment in the experiments using microbial cultures and different concentrations of rhamnolipid

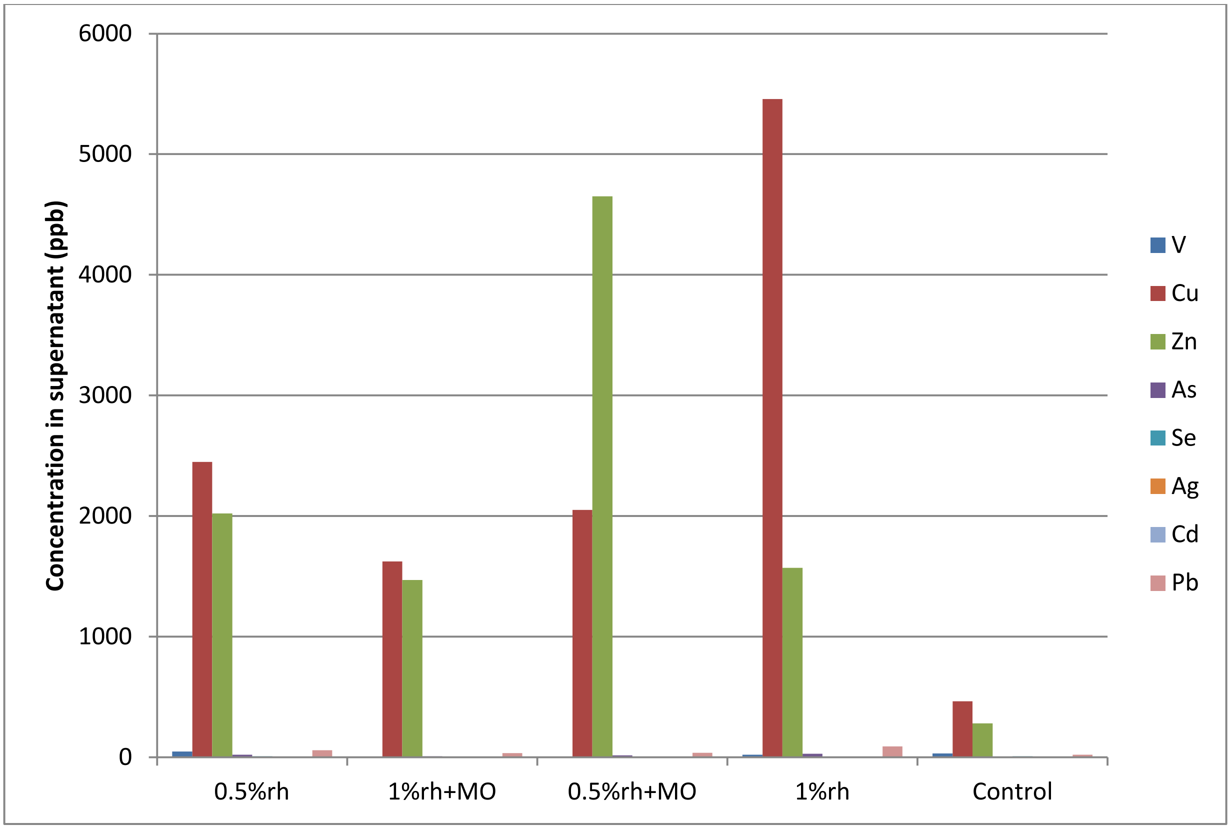


Figure 4-13. Concentration of heavy metals in the supernatant in the experiments using microbial cultures and different concentrations of rhamnolipid

In order to understand the distribution of heavy metals in the supernatant (liquid phase) and remaining dry sediment we can define a K value as K= heavy metal concentration in the liquid phase (ppb_l)/ heavy metal concentration in the sediment (ppb_s). The values for each heavy metal in the experiments using microbial cultures and different concentrations of rhamnolipid are shown in Figure 4-14.

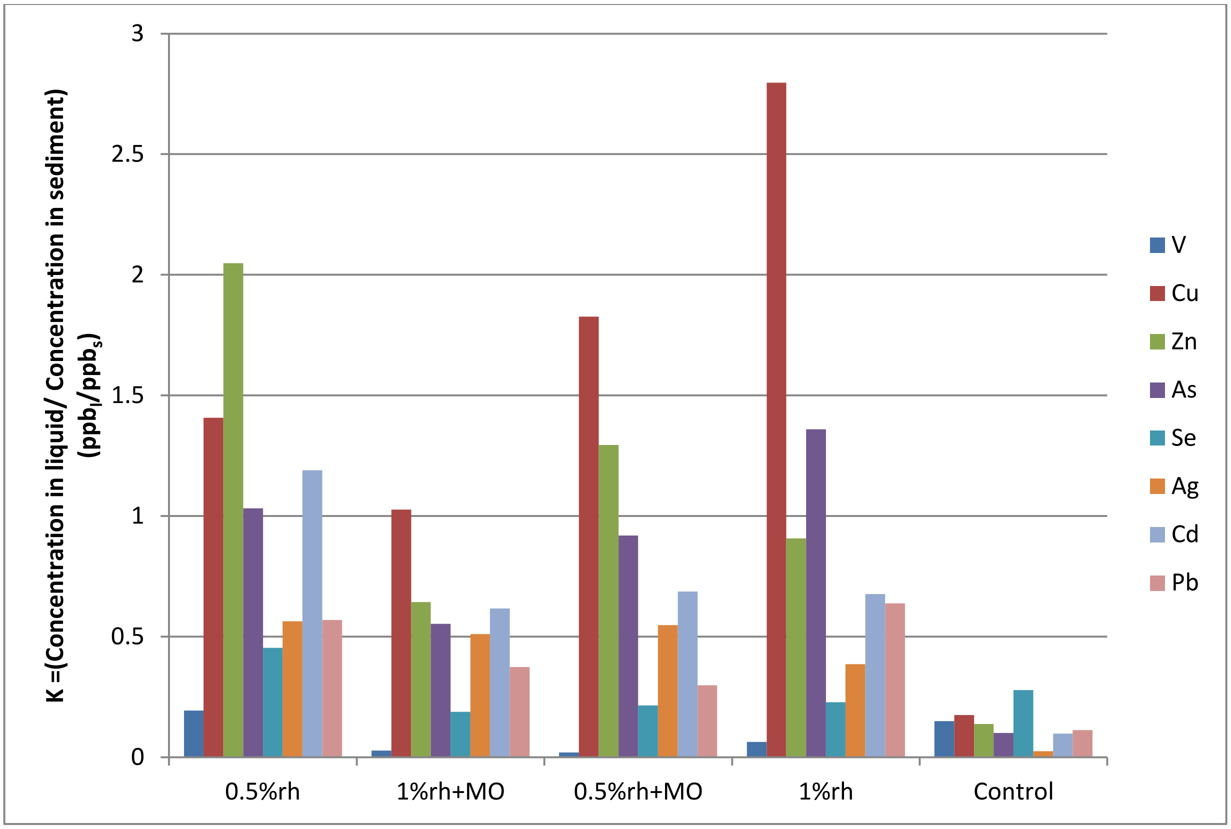


Figure 4-14. K values (heavy metal concentration at liquid phase (ppb$_l$)/ heavy metal concentration in the sediment (ppb$_s$) for each heavy metal in the experiments using microbial cultures and different concentrations of rhamnolipid

Except for vanadium and selenium, higher concentrations of heavy metals in the supernatant (higher K values) were achieved in the presence of rhamnolipid compared to the control. Except for copper which shows higher concentrations in the liquid phase for all experiments, the concentration of heavy metals in recycled water at the top of the sediment is lower than the concentration of heavy metals in the settled tailings. It means that rhamnolipid can bring small amounts of insoluble metals from the sediments to the supernatant and the remaining dry sediment still has relatively high concentrations of harmful heavy metals which are problematic.

b) at lower temperature (15 ºC ± 2 ºC)

The concentrations of vanadium, copper, zinc, arsenic, selenium, silver, cadmium, and lead in the supernatant at 15 ºC ± 2 ºC are shown in Figures 4-15. This supernatant is used as the feed for the ultrafiltration system.

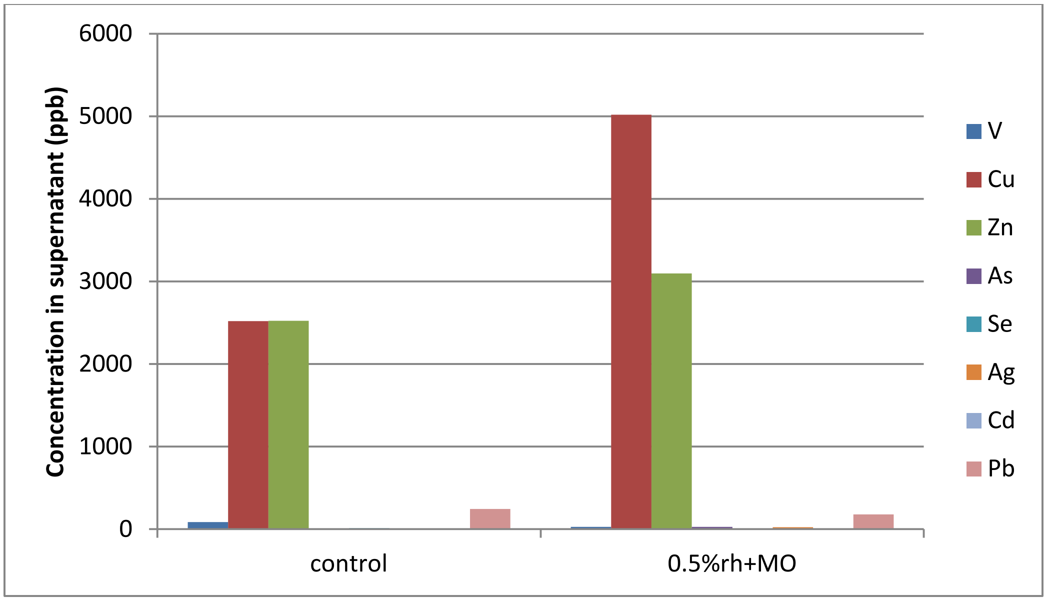


Figure 4-15. Concentration of heavy metals in the supernatant at in the experiments using microbial cultures and 0.5% of rhamnolipid at 15 ºC ± 2 ºC

The concentrations of heavy metals and rhamnolipid in the permeate were measured after ultrafiltration. R is defined as the rejection percent and is calculated according to the following relation (Abbasi-Garravand 2012):

R (rejection %)= (1-Cp/Cs)*100%

Where Cs is the concentration in the supernatant (feed) and Cp is the concentration in the permeate. This rejection percent can be determined for heavy metals and for rhamnolipid. The concentrations of rhamnolipid were determined according to its relation with surface tension from Figure 3-1 for the supernatant (feed) solution and the permeate solution. The concentrations of rhamnolipid in the supernatant (feed) and permeate were around 800 mg/l and 20 mg/l respectively which gives a 97.5% of rejection for rhamnolipid. The ratio of total petroleum hydrocarbon in the permeate to total petroleum hydrocarbon in the supernatant (feed) is 0.57 which gives a 43% of rejection for hydrocarbon. Figure 4-16 shows the heavy metal rejection for supernatant (feed) amended with 0.5% rhamnolipid and microbial culture after doing ultrafiltration. The concentrations of heavy metals, hydrocarbon and rhamnolipid are low in the permeate after ultrafiltration while they are high in the supernatant (feed). It means that micelles form and just a few free monomers of rhamnolipid and heavy metals can pass through the membrane to the permeate. Hydrocarbon molecules (composed mostly of naphthenic acids molecules) which are not soluble in water will also align in the micelle with the hydrophobic part inside and polar part outside. This enables more efficient solubilization of hydrocarbon in the biosurfactant micelles resulting in higher retention of hydrocarbon (Sirshendu and Sourav 2012)

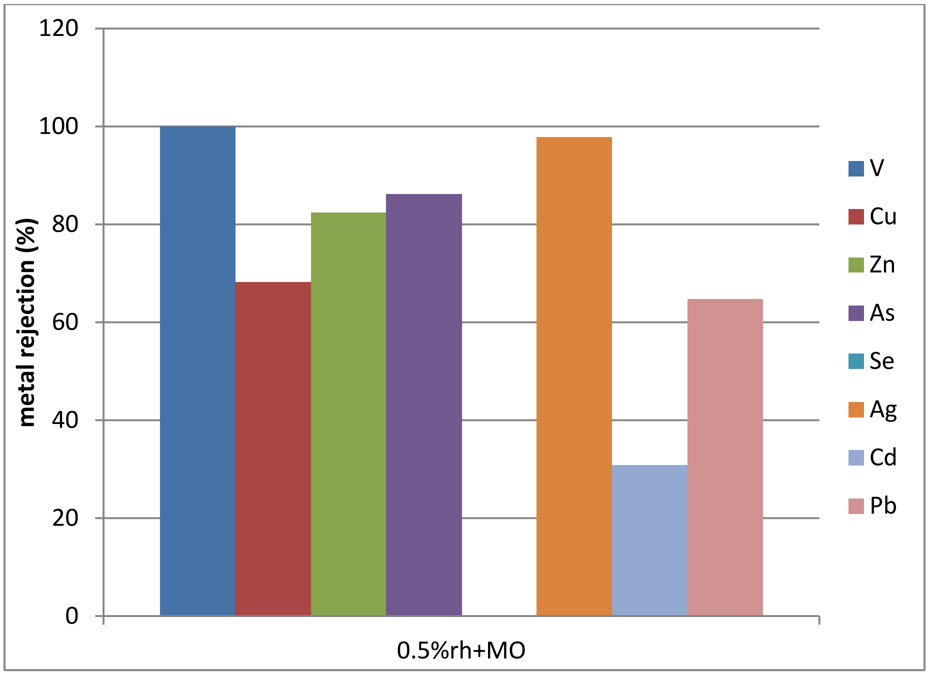


Figure 4-16 Heavy metal rejection after the ultrafiltration of the supernatant in the experiments using microbial cultures and 0.5% of rhamnolipid at 15 ºC ± 2 ºC

4.3 Results of In Situ Biosurfactant Production Using Indigenous Microorganisms of Oil Sand Tailings Pond, *Bacillus subtilis* Strain, and Two Microbial Strains Isolated From Weathered Oil

4.3.1 Surface tension measurement

Surface tension measurement can be applied to determine the biosurfactant concentration indirectly as there is a relation between biosurfactant concentration below the CMC and the surface tension of sample. In order to demonstrate biosurfactant production, the surface tension of filtered supernatant from each set of batch experiments was measured. Figure 4.17 shows the results for surface tension for each batch type over time.

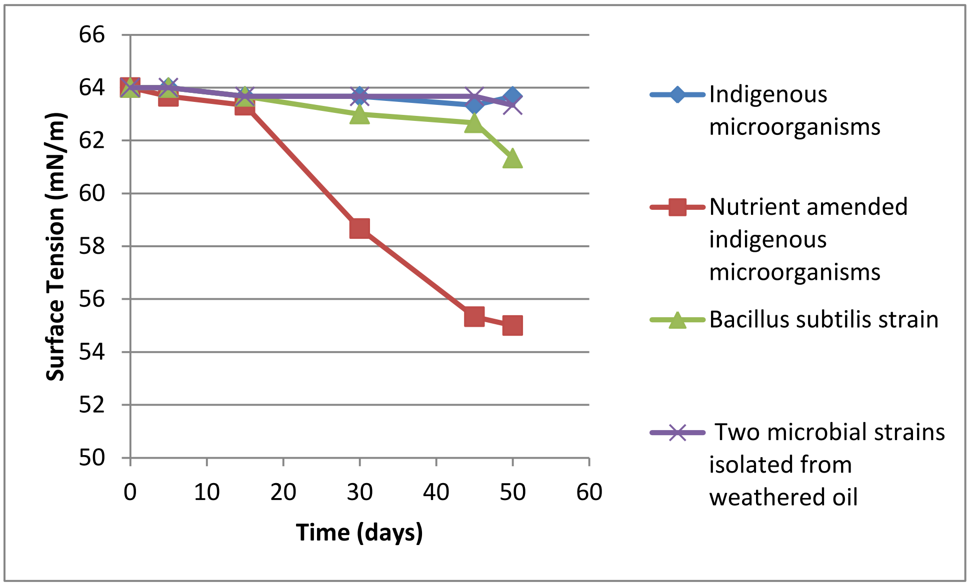


Figure 4-17. Surface tension values of filtered supernatant over time for each batch

The initial surface tension for all samples was about 64 mN/m. This value reduced to about 63 mN/m for indigenous microorganisms of oil sand tailings pond in the control experiment and was lowered to 55 mN/m after 50 days for indigenous microorganisms of oil sand tailings pond in the presence of nutrients which means that the amount of produced biosurfactant by indigenous microorganisms is very low. *Bacillus subtilis* strain and two microbial strains isolated from weathered oil reduced the surface tension of supernatant to the value of 61 mN/m and 63 mN/m respectively after 50 days which means a very low amount of biosurfactant could be produced by them. However with a longer time it is possible to achieve more biosurfactant production.

4.4 Mechanism of Sedimentation Using Rhamnolipid and Microbial Culture

Using rhamnolipid by itself can increase the sedimentation as the rhamnolipid anions adsorb on the oil sand tailings surfaces, rendering the surfaces hydrophobic and resulting in the flocculation of oil sand clay particles due to the hydrocarbon chain association when the rhamnolipid adsorption layers on particles contact each other (Figure 4-18).

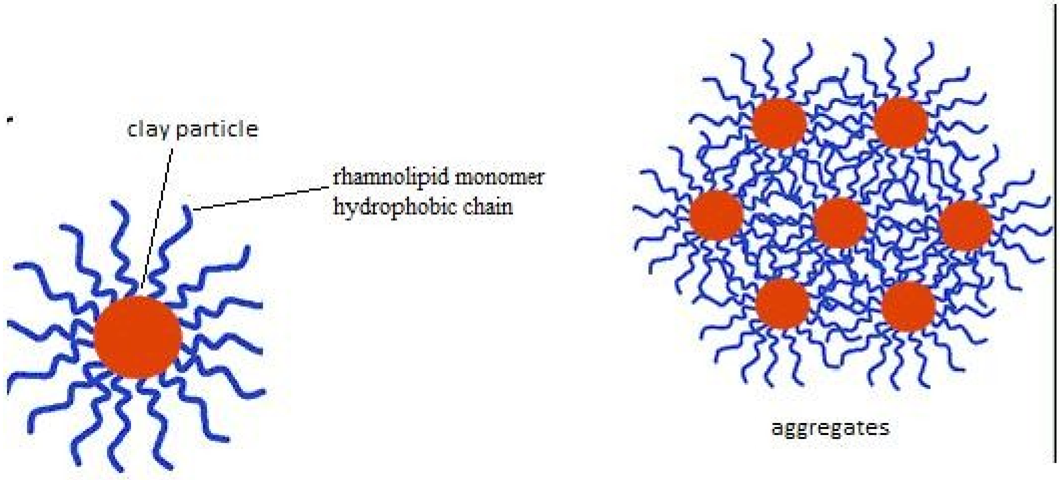


Figure 4-18. Aggregation of clay particles by rhamnolipid adsorption and increase in hydrophobicity

Microbial activity can increase sedimentation by microbial cells and/or EPS secreted by microbial cells. Exopolysaccharides or extracellular polymeric substances (EPS) are compounds secreted by microorganisms into their environment (Lünsdorf et al. 2000). Formation of exopolysaccharide (EPS) matrix help them to create a stable environment and optimal conditions for growth (exopolymer microdomains as a structural agent for heterogeneity within microbial biofilms) (Rehm 2009) as they can modify their cell surface hydrophobicity according to the availability and the composition of hydrocarbons. In this way their attachment to the clay particle is facilitated by the common action of both coflocculation and hydrophobic interactions. EPS can help the bacteria to adhere to the surface and causes aggregation of particles through a bridging mechanism in the way that the macromolecules bridge the individual clay particles into an aggregate (Figure 4-19).

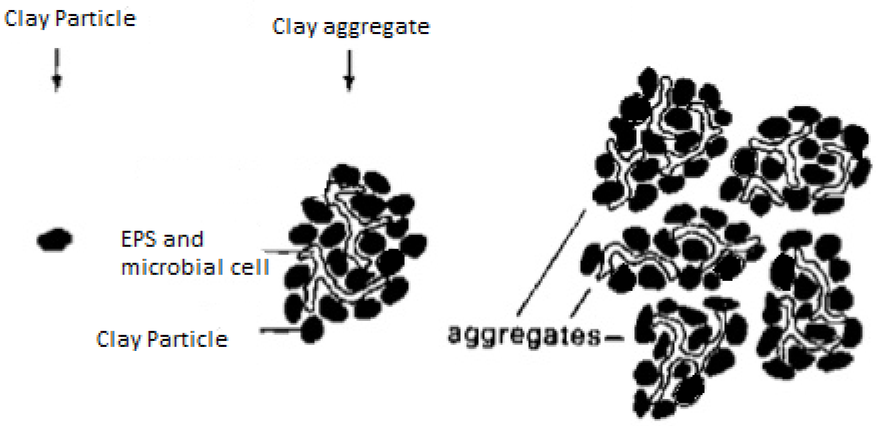


Figure 4-19. Aggregation of clay particles by microbial cells and/or EPS secreted by microbial cells (http://www.slideshare.net/vikkis/rhs-year-2-week-2-presentation-2012)

Using rhamnolipid together with microbial culture had a stronger activity than rhamnolipid by itself. This was mainly due to the improvement of hydrophobic interactions by microbial culture and by rhamnolipid adsorption on the clay particle and high molecular weight microbial organics interaction with clay particles as bioflocculants with high molecular weights involved more adsorption sites, stronger bridging, and higher flocculating activity (Figure 4-20). Besides these, there is also the possibility of a small increase in consolidation due to small amounts of CH_4 production.

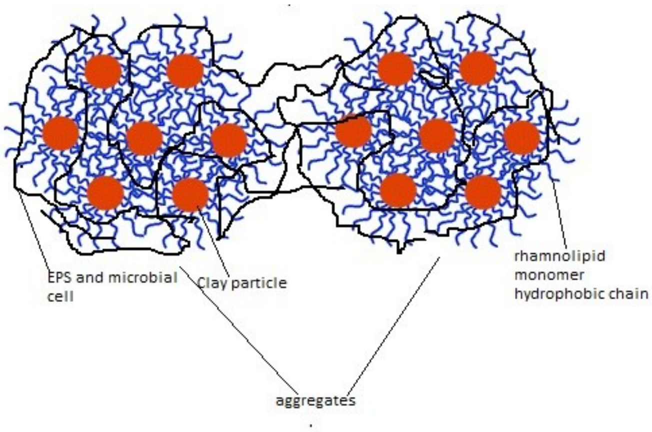


Figure 4-20. Aggregation of clay particles rhamnolipid together with microbial cell and/or EPS secreted by microbial cells

4.5 Using Rhamnolipid and Microbial Cultures Compared to the Other Sedimentation Approaches

Compared to the tailings coagulation technique and flocculation technique using polymeric materials as flocculating agents (such as Thickened Tailings (TT) and flocculation technique), this method is more environmental friendly. This method does not require a large containment area (containment area is expensive) or large amounts of sand (in contrast to the Consolidated Tailings (CT) and drying Tailings Reduction Operations techniques (TRO)). This method will not produce high level of H_2S (in contrast to using gypsum as coagulant which emit H_2S due to the anaerobic reduction of SO_4^{-2} with the residual bitumen in the tailings) and CH_4 and CO_2

(compared to the use of methanogenic anaerobic microbes) as the mechanism of sedimentation in this case is increasing the particle hydrophobicity.

Based on the clay content (between 30-60w%), it can be considered that there is an average of 45wt% clay in each sample. Based on the results from the sedimentation experiment by this approach at about 46% sedimentation it would become completely dry and the kinetics for sedimentation rate suggest that it will take at about 720 days (or about 2 years). It means that this approach is not as fast as the TRO approach (which takes few weeks are required (Mamer 2007)) but it is faster than CT (which takes around 30 years (Mamer 2007)) and the TT approach (which takes a few years (BGC Engineering Inc 2010; Shell 2012)).

Regarding the recycled water quality, this method could reduce the water quality concerns compared to the other methods. However biosurfactant will bring the remaining oil and heavy metals from the sediment to the water but in the longer period the microbial strain can improve the further biodegradation of remaining oil in the recycled water while biosurfactant can improve their efficiency. After sedimentation also rhamnolipid could help removing the heavy metals and oils through ultrafiltration method which could reduce the recycled water treatment cost and environmental impact. In this way the remaining sediment also will have less heavy metal and oil contents.

5. CONCLUSIONS

The results obtained from sedimentation tests and particle size distribution analysis indicate that presence of rhamnolipid at different concentrations (0.5%, 1% and 2%) at 23 ºC ± 2 ºC could increase the sedimentation and the sedimentation would be increased by increasing the rhamnolipid concentrations. A mixed culture of two microbial strains isolated from weathered oil increased the sedimentation while the *Bacillus subtilis* strain at 23 ºC ± 2 ºC gave almost the same sedimentation amount as the control. Different concentrations of rhamnolipid (0.5% and 1%) together with these two microbial strains could lead to significant increases in sedimentation at 23 ºC ± 2 ºC (by a factor of 3.04 and 2.59 for 0.5% and 1% rhamnolipid respectively), increase the concentration of larger particles (by a factor of 1.9 and 1.65 for 0.5% and 1% rhamnolipid respectively), particle mean diameter (by a factor of 2.11 and 1,65 for 0.5% and 1% rhamnolipid respectively) and flocculation in the tailings samples amended with them compared to the control.

Microbial cultures can work better in the lower rhamnolipid concentration (0.5%) probably due to antimicrobial effect of rhamnolipid which inhibits the microbial growth and EPS production. However it is not proven yet. The experiments performed at 15 ºC± 2 ºC using rhamnolipid (0.5%) together with these two microbial strains shows significant increases in sedimentation (by a factor of 5.1) the concentration of larger particles (by a factor of 2.63) particle mean diameter (by a factor of 2.70) and flocculation in the tailings samples amended with them compared to the control.

The results of zeta potential and particle size distribution at 23 ºC ± 2 ºC and 15 ºC ± 2 ºC supported the idea that rhamnolipid have potential to be used as flocculating agents for oil sand tailings sedimentation. According to the results of zeta potential measurement, rhamnolipid adsorption on the particle surfaces increase the negative surface charge while it improved the hydrophobic interaction between the particles much more strongly than the electrical double layer repulsion. Mixing microbial cultures with rhamnolipid slightly increased the zeta potential which still remained negative. A higher activity than rhamnolipid by itself was shown. It means that the mechanism of flocculation is not charge neutralization and probably it is due to the interaction of the biosurfactant and high molecular weight microbial organics through a bridging mechanism with clay particles in the way that the macromolecules bridge the individual clay particles into an aggregate. According to the pH measurements there are not enough change in chemistry of pore water as a result of microbial metabolism which could lead to increase the ionic strength (I) of the pore water and reduce the thickness of the DDL of clay particles during the 50 days. Strong flocculating activity of rhamnolipid mixed with microbial culture could not be as a result of double layer compression or by cation (such as Ca^{2+}) bridging but there might be small amount of CH_4 production at 15 ºC ± 2 ^{o}C in the deeper layer of mud which could create transient channels for escape of pressurized pore water and increase the consolidation of tailings. However rhamnolipid mixed with microbial cultures could improve the hydrophobic interactions and increase the flocculation in this way.

According to the heavy metal analyses, rhamnolipid as a flocculating agent could bring higher amount of insoluble heavy metals (except for vanadium and selenium) from the sediments to the supernatant compared to the control. However (except for copper) these amounts are small and the remaining dry sediment still have the relatively high concentrations of harmful heavy metals. Ultrafiltration was applied to the supernatant of settled tailing samples which lead to significant removal of heavy metals and rhamnolipid (between of 30% for Cd and 100% for V, and 97.5% for rhamnolipid).

According to the hydrocarbon analyses, rhamnolipid also could extract the remaining oil from the tailing sediment into the water. However after 50 days the remaining treated sediment still has a high level of oil content. In situ biosurfatctant production was investigated and surface tension measurements shows that indigenous microorganisms (even in the presence of nutrients), *Bacillus subtilis* strain and two microbial strains isolated from weathered oil could produce a very low amount of biosurfactant.

These results show the potential of using rhamnolipid and microbial culture in order to increase the oil sand sedimentation through flocculation and microbial activity without producing large amounts of CH_4 while taking advantage of the biosurfactants for remaining water and sediment bioremediation as the significant contribution of the bacteria which were used in this study in the biodegradation was reported (Saborimanesh and Mulligan 2015). Using a micellar ultrafiltration system would reduce amount of heavy metals and oil in the remaining water significantly. This work shows the potential of using rhamnolipid together with mixed microbial culture to develop a more environmentally friendly and economical oil sands tailings densification method without having the limitations of other methods.

6. FUTURE STUDIES

-Investigation of the possibility of in situ biosurfactant production by these microbial strains over a longer period.

- Investigation of oil biodegradation in recycled water over a longer period by these microbial strains and rhamnolipid.

- Investigation of increasing the heavy metal and oil removal efficiency from the recycled water by Micellar Enhanced Ultrafiltration.

- Investigation of the effect of other biosurfactants and their combination with other microbial strains in sedimentation and recycle water quality.

7. CONTRIBUTION TO KNOWLEDGE

-The results of this work show the potential of using rhamnolipid and microbial culture isolated from weathered oil in order to increase the oil sand sedimentation through flocculation and microbial activity at 23 ºC ± 2 ºC and 15 ºC ± 2 ºC (which is close to the condition of oil sand tailing ponds) without producing large amounts of CH_4.

-Experimental data for different concentrations of rhamnolipid and microbial activity proposed kinetic models which could analytically calculate the rate of the sedimentation through this method in oil sand tailing ponds.

-The mechanism of their effect which could lead to development of the method for application in oil sands tailing ponds is proposed.

-The potential of rhamnolipid for sediment bioremediation by extracting the remaining oil and heavy metals from the sediment has been demonstrated.

-It also shows the potential of using biosurfactants for recycled water treatment through Micellar Enhanced Ultrafiltration (a surfactant based ultrafiltration method) for separation of heavy metals and rhamnolipid from the recycled water.

-It suggested the possibility of in situ biosurfactant production for oil and sedimentation.

REFERENCES

Abbasi-Garravand, E. (2012). *Removal of Cr (VI) and Cr (III) From Water by Reduction and Micellar Enhanced Ultrafiltration Techniques* MSc Thesis, Concordia University, Montreal, Quebec, Canada.

Abbasi-Garravand, E., and Mulligan, C. N. (2014). "Using micellar enhanced ultrafiltration and reduction techniques for removal of Cr(VI) and Cr(III) from water." *Separation and Purification Technology*, 132(0), 505-512.

Al-Tahhan, R. A., Sandrin, T. R., Bodour, A. A., and Maier, R. M. (2000). "Rhamnolipid-Induced Removal of Lipopolysaccharide from Pseudomonas aeruginosa: Effect on Cell Surface Properties and Interaction with Hydrophobic Substrates." *Applied and Environmental Microbiology*, 66(8), 3262-3268.

Alamgir, A., Harbottle, D., Masliyah, J., and Xu, Z. (2012). "Al-PAM assisted filtration system for abatement of mature fine tailings." *Chemical Engineering Science*, 80(0), 91-99.

Alberta Chamber of Resources. (2004). *Oil Sands Technology Roadmap: Unlocking the Potential*. Edmonton, Ablerta, Canada. http://www.strategywest.com/downloads/ACR200401.pdf.

Alberta Energy. (2010). "Enhancing Assurance – The First 90 Days", Government of Alberta, (ed.). City: Edmonton, Alberta, Canada. http://www.energy.gov.ab.ca/Initiatives/1872.asp, "Access date:16-08-2014.

Alberta Energy. (2015). "Oil sands". City: Edmonton, Alberta, Canada. http://www.energy.gov.ab.ca/ourbusiness/oilsands.asp, "Access date: 19-04-2016".

American Public Health Association (APHA), A. W. W. A. A., and Water Pollution Control Federation (WPCF). (1998). "Standard Methods for the Examination of water and Wasewater". City: New York.

ASTM. (2006). *Annual Book of ASTM Standards*, West Conshohocken, PA: ASTM International.

Bade, R., and Lee, S. H. (2011). "A Review of Studies on Micellar Enhanced Ultrafiltration for Heavy Metals Removal from Wastewater." *Journal of Water Sustainability*, 1(1), 85–102.

Baker, S., and Chen, C.-Y. (2010). "Enrichment and Purification of Lipopeptide Biosurfactants", in R. Sen, (ed.), *Biosurfactants*. Springer New York, pp. 281-288.

Banat, I. M., Makkar, R. S., and Cameotra, S. S. (2000). "Potential commercial applications of microbial surfactants." *Applied Microbiology and Biotechnology*, 53(5), 495-508.

Barker, J., Rudolph, D., Tompkins, T., Oiffer, A., Gervais, F., and Ferguson, G. (2007). "Attenuation of contaminants in groundwater impacted by surface mining of oil sands, Alberta, Canada[monograph on the internet]"*14th International Petroleum Environmental Conference*. City: Houston, TX.

Baumgarten, T., Sperling, S., Seifert, J., von Bergen, M., Steiniger, F., Wick, L. Y., and Heipieper, H. J. (2012). "Membrane vesicle formation as a multiple-stress response mechanism enhances Pseudomonas putida DOT-T1E cell surface hydrophobicity and biofilm formation." *Applied Environmental Microbiology*, 78(17), 6217-24.

Besra, L., Sengupta, D. K., Roy, S. K., and Ay, P. (2003). "Influence of surfactants on flocculation and dewatering of kaolin suspensions by cationic polyacrylamide (PAM-C) flocculant." *Separation and Purification Technology*, 30(3), 251-264.

BGC Engineering Inc. (2010). *Oil Sands Tailings Technology Review*. Oil Sands Research and Information Network (OSRIN) Report, University of Alberta, Edmonton, Alberta, Canada.

Boag, D. A., and Lewin, V. (1980). "Effectiveness of three waterfowl deterrents on natural and polluted ponds." *Journal of Wildlife Management* 44, 145–154.

Bordenave, S., Kostenko, V., Dutkoski, M., Grigoryan, A., Martinuzzi, R. J., and Voordouw, G. (2010). "Relation between the activity of anaerobic microbial populations in oil sands tailings ponds and the sedimentation of tailings." *Chemosphere*, 81(5), 663-668.

Bouchez Naïtali, M., Rakatozafy, H., Marchal, R., Leveau, J. Y., and Vandecasteele, J. P. (1999). "Diversity of bacterial strains degrading hexadecane in relation to the mode of substrate uptake." *Journal of Applied Microbiology*, 86(3), 421-428.

Bressler, D., Cardenas M., Fedorak P. M., Guigard S., Gupta R., and Siddique T., e. a. (2010). "Microorganisms in oil sand tailings ponds influence the properties and behaviour of mature fine tailings"*2nd International Oil Sands Tailings Conference*. City: Edmonton, Alberta, Canada, pp. 44.

Brickner, H. A. (2013). *THE MOBILITY OF PETROLEUM HYDROCARBONS IN ATHABASCA OIL SANDS TAILINGS*, MSc Thesis, University of Saskatchewan, Saskatoon.

Brown, D., Ramos-Padrón, E., Gieg, L., and Voordouw, G. (2013). "Effect of calcium ions and anaerobic microbial activity on sedimentation of oil sands tailings." *International Biodeterioration & Biodegradation*, 81(0), 9-16.

Cabrera-Valladares, N., Richardson, A.-P., Olvera, C., Treviño, L., Déziel, E., Lépine, F., and Soberón-Chávez, G. (2006). "Monorhamnolipids and 3-(3-hydroxyalkanoyloxy)alkanoic acids (HAAs) production using Escherichia coli as a heterologous host." *Applied Microbiology and Biotechnology*, 73(1), 187-194.

Cameotra, S. S., and Singh, P. (2008). "Bioremediation of oil sludge using crude biosurfactants." *International Biodeterioration & Biodegradation*, 62(3), 274-280.

CAPP. (2009). *Crude Oil: Forecast, Markets, and Pipeline Expansions.* . Canadian Association of Petroleum Producers.

Carstensen, J. T., and Su, K. S. E. (1970a). "Sedimentation kinetics of flocculated suspensions I: Initial sedimentation region." *Journal of Pharmaceutical Sciences*, 59(5), 666-670.

Carstensen, J. T., and Su, K. S. E. (1970b). "Sedimentation kinetics of flocculated suspensions II: Sedimentation below the critical height." *Journal of Pharmaceutical Sciences*, 59(5), 671-676.

Caughill, D. L., Morgenstern, N. R., and Scott, J. D. (1993). "Geotechnics of nonsegregating oil sand tailings." *Canadian Geotechnical Journal*, 30(5), 801-811.

Chalaturnyk, R. J., Scott, J. D., and Ozum, B. (2002). "Management of oil sands tailings." *Petroleum Science and Technology* 20(9), 1025-1046.

Chaudhari, R. R., and Marathe, K. V. (2010). "Separation of Dissolved Phenolics from Aqueous Waste Stream using Micellar Enhanced Ultrafiltration." *Separation Science and Technology*, 45(8), 1033-1041.

Chen, F. (2007). *Bacterial Auto-Aggregation and C-Aggragation in activated sludge*, PhD Thesis, Clemson University, South Carolina.

Chen, M., Walshe, G., Chi Fru, E., Ciborowski, J. J. H., and Weisener, C. G. (2013). "Microcosm assessment of the biogeochemical development of sulfur and oxygen in oil sands fluid fine tailings." *Applied Geochemistry*, 37(0), 1-11.

Chen, Y. (2009). *Cancer incidence in Fort Chipewyan, Alberta 1995-2006*. Alberta Cancer Board Edmonton.

Chhatre, S., Purohit, H., Shanker, R., and Khanna, P. (1996). "Bacterial consortia for crude oil spill remediation." *Water Science and Technology*, 34(10), 187-193.

Chow, D. L., T.N. Nasr, R.S. Chow, and Sawatzky, R. P. (2008). "Recovery Techniques for Canada's Heavy Oil and Bitumen Resources." *Journal of Canadian Petroleum Technology*, 47(5), 12-17.

Chuang, Y.-H., Liu, C.-H., Tzou, Y.-M., Chang, J.-S., Chiang, P.-N., and Wang, M.-K. (2010). "Comparison and characterization of chemical surfactants and bio-surfactants intercalated with layered double hydroxides (LDHs) for removing naphthalene from contaminated aqueous solutions." *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 366(1–3), 170-177.

Clifford, J. S., Ioannidis, M. A., and Legge, R. L. (2007). "Enhanced aqueous solubilization of tetrachloroethylene by a rhamnolipid biosurfactant." *Journal of Colloid and Interface Science*, 305(2), 361-365.

Colavecchia, M. V., Backus, S. M., Hodson, P. V., and Parrott, J. L. (2004). "Toxicity of oil sands to early life stages of fathead minnows (Pimephales promelas)." *Environ Toxicol Chem*, 23(7), 1709-18.

Colavecchia, M. V., Hodson, P. V., and Parrott, J. L. (2006). "CYP1A induction and blue sac disease in early life stages of white suckers (Catostomus commersoni) exposed to oil sands." *J Toxicol Environ Health A*, 69(10), 967-94.

Colavecchia, M. V., Hodson, P. V., and Parrott, J. L. (2007). "The relationships among CYP1A induction, toxicity, and eye pathology in early life stages of fish exposed to oil sands." *J Toxicol Environ Health A*, 70(18), 1542-55.

Cookson, J. (1995). *Bioremediation Engineering: Design and Applications*, New York, NY, USA: McGraw-Hill Professional.

CRBSCF. (1999). *(Colorado River Basin Salinity Control Forum). Water Quality Standards for Salinity, Colorado River System.* . Supplemental Report, Bountiful (Utah).

Crittenden, J. C. T., R.R.; Hand, D.W.; Tchobanoglous, G. . (2005). *Water Treatment: Principals and Design*. Hoboken, New Jersey, USA: John Wiley & Sons.

Desai, J. D., and Banat, I. M. (1997). "Microbial production of surfactants and their commercial potential " *Microbiology and Molecular Biology reviews*, 61 (1), 47–64.

Devenny, D. a. R. N. (2009). *Economic screening of tailings options for oil sands plants.* . University of Alberta, Department of Civil & Environmental Engineering, Edmonton, Alberta., Banff, Alberta.

Devenny, D. W. (2010). *A screening study of oil sand tailings technologies and practices*. Two part report, Alberta Energy Research Institute, Edmonton, Alberta.

Dyke, G. R., Birdsall, D. A., and L., S. P. (1976). *Test of a bird deterrent device at a tailings pond, Athabasca Oil Sands, 1974*. Syncrude Canada Ltd, Ft. McMurray.

Dyke, M. I. V., Couture, P., Brauer, M., Lee, H., and Trevors, J. T. (1993). "Pseudomonas aeruginosa UG2 rhamnolipid biosurfactants: structural characterization and their use in removing hydrophobic compounds from soil." *Canadian Journal of Microbiology*, 39(11), 1071-1078.

Eckert, W. F., Masliyah, J. H., Gray, M. R., and Fedorak, P. M. (1996). "Prediction of sedimentation and consolidation of fine tails." *AIChE Journal*, 42(4), 960-972.

El Zeftawy, M. A. M., and Mulligan, C. N. (2011). "Use of rhamnolipid to remove heavy metals from wastewater by micellar-enhanced ultrafiltration (MEUF)." *Separation and Purification Technology*, 77(1), 120-127.

EPA. (1996). "Method 3050b: Acid digestion of sediments, sludges, and soils, vision 2", E. P. A. U. EPA), (ed.). City: Washington, DC.

EPA. (2007). "METHOD 200.8: DETERMINATION OF TRACE ELEMENTS IN WATERS AND WASTES BY INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY, Revision 5.4", O. O. R. A. D. ENVIRONMENTAL MONITORING SYSTEMS LABORATORY, U.S. ENVIRONMENTAL PROTECTION AGENCY, (ed.). City: CINCINNATI, OHIO 5268.

EPA. (2010). "Method 1664, Revision B:n-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated n-Hexane Extractable Material (SGT-HEM; Non-polar Material) by Extraction and Gravimetry", E. P. A. O. o. W. (4303T), (ed.). City: Washington,DC.

ERCB. (2009). *Directive 074: Tailings performance criteria and requirements for oil sands mining schemes*. Energy Resources Conservation Board, Calgary, Alberta.

ERCB. (2010). E. R. C. Board, (ed.). City: Edmonton, alberta, Canada.

Fair, A. (2008). "The Past, Present and Future of Tailings at Syncrude"*International Oil Sands Tailings Conference*. City: Edmonton, Alberta, Canada.

Fedorak, P. M., Coy, D. L., Dudas, M. J., Simpson, M. J., Renneberg, A. J., and MacKinnon, M. D. (2003). "Microbially-mediated fugitive gas production from oil sands tailings and increased tailings densification rates." *Journal of Environmental Engineering and Science*, 2(3), 199-211.

Fischer, J. M., Robbins, S. B., Al-Zoughool, M., Kannamkumarath, S. S., Stringer, S. L., Larson, J. S., Caruso, J. A., Talaska, G., Stambrook, P. J., and Stringer, J. R. (2005). "Co-mutagenic activity of arsenic and benzo[a]pyrene in mouse skin." *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 588(1), 35-46.

Foght, J., and Dunfield, P. (2013). "The Impact of Genomics Sciences on the Oil and Gas Industry- Part 1: The Good, the Bad and the Hungry: Micro-organisms and Their Effects on Oil Sands Tailings Ponds "*Petroleum Technology alliance of Canada (PTAC)* City: Calgary, Alberta,Canada.

Fuerstenau, D. W. F., M.C.; Lovell, V.M.; King, R.P.; Allison, S.A.; Harris, P.J. . (1982). "Principles of Flotation". Johannesburg, South Africa: South African Institute of Mining and Metallurgy.

García-Junco, M., De Olmedo, E., and Ortega-Calvo, J.-J. (2001). "Bioavailability of solid and non-aqueous phase liquid (NAPL)-dissolved phenanthrene to the biosurfactant-producing bacterium Pseudomonas aeruginosa 19SJ." *Environmental Microbiology*, 3(9), 561-569.

Ghosh, G., and Bhattacharya, P. K. (2006). "Hexavalent chromium ion removal through micellar enhanced ultrafiltration." *Chemical Engineering Journal*, 119(1), 45-53.

Golby, S., Ceri, H., Gieg, L. M., Chatterjee, I., Marques, L. L. R., and Turner, R. J. (2012). "Evaluation of microbial biofilm communities from an Alberta oil sands tailings pond." *FEMS Microbiology Ecology*, 79(1), 240-250.

Golder-Associates-Ltd. (2000). *Report on oil sands tailings pond bird deterrent systems–a review of research and current practices*. Suncor Energy Inc. (Oil Sands), Syncrude Canada Ltd., and Albian Sands Energy Inc, Calgary, Alberta, Canada.

Government of Alberta. (2006). "Investing in our Future: Responding to the Rapid Growth of Oil Sands Development. Final report ", G. o. Alberta, (ed.). City: Edmonton, Alberta, Canada. http://www.energy.gov.ab.ca/pdf/OSSRadkeReportInvesting2006.pdf.

Government of Alberta. (2008). "Alberta’s Oil Sands – Opportunity. Balance", G. o. Alberta, (ed.). City: Edmonton, Alberta, Canada. http://www.environment.alberta.ca/documents/oil_sands_opportunity_balance.pdf.

Government of Alberta. (2010). "Fact Sheet: Alberta’s Oil Sands – The Resource", G. o. Alberta, (ed.). City: Edmonton, Alberta, Canada. http://www.oilsands.alberta.ca/FactSheets/About_Albertas_oil_sands.pdf.

Government of Alberta. (2013). "Oil sand tailings". City: Edmonton, Alberta, Canada.

Gulley, J. R. (1980). *Factors influencing the efficacy of human effigies in deterring waterfowl from polluted ponds.*, MSc Thesis, University of Alberta.

Guo, C. (2009). *Rapid densification of the oil sands mature fine tailings (MFT) by microbial activity*, PhD Thesis, University of Alberta, Edmonton.

Guo, H.-R. (2003). "The lack of a specific association between arsenic in drinking water and hepatocellular carcinoma." *Journal of Hepatology*, 39(3), 383-388.

Guo, Y. (2012). *ELECTROKINETIC DEWATERING OF OIL SANDS TAILINGS* MSc Thesis, The University of Western Ontario, London, Ontario, Canada

Guo, Y., and Mulligan, C. N. (2006). "Combined treatment of styrene contaminated soil by rhamnolipid washing followed by anaerobic biodegradation", in R. C. Hudson, (ed.), *Hazardous materials in* *the soil and atmosphere: treatment, removal, and analysis*. New York: Nova Science Publisher, Inc., pp. 1-38.

Gzara, L., and Dhahbi, M. (2001). "Removal of chromate anions by micellar-enhanced ultrafiltration using cationic surfactants." *Desalination*, 137(1–3), 241-250.

Haines, B. A., and Martin, A. N. (1961). "Interfacial properties of powdered material; Caking in liquid dispersions I. Caking and flocculation studies." *Journal of Pharmaceutical Sciences*, 50(3), 228-232.

Hamza, H. A. D., Stanonil, J., and Kessick, M. A. (1996). "Flocculation of Lime Treated Oil SandsTailings." *Fuel*, 75(3), .

Harvey, S., Elashvili, I., Valdes, J. J., Kamely, D., and Chakrabarty, A. M. (1990). "Enhanced Removal of Exxon Valdez Spilled Oil from Alaskan Gravel by a Microbial Surfactant." *Nature Biotechnology*, 8(3), 228-230.

Herman, D. C., Artiola, J. F., and Miller, R. M. (1995). "Removal of Cadmium, Lead, and Zinc from Soil by a Rhamnolipid Biosurfactant." *Environmental Science & Technology*, 29(9), 2280-2285.

Holakoo, L., and Mulligan, C. N. "On the capability of rhamnolipids for oil spill control of surface water." *Presented at Annual Conference of the Canadian Society for Civil Engineering*, Montreal, Canada.

Holowenko, F. M., MacKinnon, M. D., and Fedorak, P. M. (2000). "Methanogens and sulfate-reducing bacteria in oil sands fine tailings waste." *Can J Microbiol*, 46(10), 927-37.

Houlihan, R., and Haneef, M. (2008). "Oil Sands Tailings: Regulatory Perspective"*International Oil Sands Tailings Conference*. City: Edmonton, Alberta, Canada.

http://www.anms.ca/products/tailing-ponds-of-oil-sands. "Access date: 16-08-2014."

http://www.horiba.com. "Access date:16-08-2014."

http://www.slideshare.net/vikkis/rhs-year-2-week-2-presentation-2012. "Access date: 28-01-2016."

http://www.somatco.com/ZM3-U-G_D390-2.pdf. "Access date:16-08-2014."

https://www.cosia.ca. "Access date: 28-01-2016."

Humphries, M. (2008). *North American Oil Sands: History of Development, Prospects for the Future CRS*. Report for Congress.

Hunter, G. (2001a). *Investigation of groundwater flow within an oil sand tailings impoundment and environmental implications*, MSc Thesis, University of Waterloo.

Hunter, R. J. (2001b). *Foundations of Colloid Science*. New York, NY, USA.: Oxford University Press

Huysman, F., and Verstraete, W. (1993). "Effect of cell surface characteristics on the adhesion of bacteria to soil particles." *Biology and Fertility of Soils*, 16(1), 21-26.

Hyndman, A. W. L., R. W, and Luhning, R. W. (1991). "Recovery And Upgrading Of Bitumen And Heavy Oil In Canada." *Journal of Canadian Petroleum Technology*, 30(2), 61-71.

Irwin Wislesky, Sue Longo, and Chloe Crossley. (2013). "Oil sands tailings: addressing the challenge"*Paste and Thickened Tailings*. City: Australian Centre for Geomechanics: Belo Horizonte, Minas Gerais, Brazil

Isaacs, E. (2007). "The Canadian Oil Sands in the Context of the Global Energy Demand"*17th Convocation of the International Council of Academies of engineering and Technological Sciences (CAETS)*. City: Tokyo, Japan.

Islam, S. (2014). *Thickening of Mature Fine Oil Sands Tailings*, The University of Western Ontario, London.

Jalali, F. (2007). *Enhancement Bioremediation of a Soil Contaminated With Both Petroleum Hydrocarbons and Heavy Metals With In-Soil Biosurfactant Production*, MSc Thesis, Concordia University, Montreal, Quebec.

Jeeravipoolvarn, S. (2005). *compression Behaviour of Thixotropic Oil Sands Tailings*, University of Alberta, Edmonton.

Ju, Z., Jianbing, L., Lailin, C., and Thring, R. W. "Remediation of refinery oily sludge using isolated strain and biosurfactant." *Presented at Water Resource and Environmental Protection (ISWREP), 2011 International Symposium on*.

Kaczorek, E., Chrzanowski, Ł., Pijanowska, A., and Olszanowski, A. (2008). "Yeast and bacteria cell hydrophobicity and hydrocarbon biodegradation in the presence of natural surfactants: Rhamnolipides and saponins." *Bioresource Technology*, 99(10), 4285-4291.

Kaminsky, H. A. W. (2008). *Characterization of an Athabasca oil sands ore and process streams* PhD Thesis, University of Alberta, Edmonton, Alberta.

Komex. (2005). *Ecological risk assessment problem formulation and screening assessment, Ponds 1/1A operations node.* . Suncor Energy Inc., Ft. McMurray.

Kotsyurbenko, O. R., Chin, K. J., Glagolev, M. V., Stubner, S., Simankova, M. V., Nozhevnikova, A. N., and Conrad, R. (2004). "Acetoclastic and hydrogenotrophic methane production and methanogenic populations in an acidic West-Siberian peat bog." *Environ Microbiol*, 6(11), 1159-73.

Krasowska, A., and Sigler, K. (2014). "How microorganisms use hydrophobicity and what does this mean for human needs?" *Frontiers in Cellular and Infection Microbiology*, 4.

Lai, C.-C., Huang, Y.-C., Wei, Y.-H., and Chang, J.-S. (2009). "Biosurfactant-enhanced removal of total petroleum hydrocarbons from contaminated soil." *Journal of Hazardous Materials*, 167(1–3), 609-614.

Landaburu-Aguirre, J., Pongrácz, E., Perämäki, P., and Keiski, R. L. (2010). "Micellar-enhanced ultrafiltration for the removal of cadmium and zinc: Use of response surface methodology to improve understanding of process performance and optimisation." *Journal of Hazardous Materials*, 180(1–3), 524-534.

Lang, S., and Wagner, G. (1987). "Structure and properties of biosurfactants", in N. Kosaric, W. L. Cairns, and N. C. C. Gray, (eds.), *Biosurfactants and biotechnology*. New York: Marcel Dekker, Inc., pp. 21-47.

Lang, S., and Wullbrandt, D. (1999). "Rhamnose lipids--biosynthesis, microbial production and application potential." *Applied Microbiology and Biotechnology*, 51(1), 22-32.

Li, X. M., Yang, Q., Huang, K., Zeng, G. M., Liao, D. X., Liu, J. J., and Long, W. F. (2007). "Screening and characterization of bioflocculants produced by *Aeromonas sp*. ." *Biomedical and Environmental Science* 20, 274–278.

Liang, L., Tan, J., Li, Z., Peng, Y., and Xie, G. (2016). "Coal Flotation Improvement Through Hydrophobic Flocculation Induced by Polyethylene Oxide." *International Journal of Coal Preparation and Utilization*, 36(3), 139-150.

Lünsdorf, Erb, Abraham, and Timmis. (2000). "'Clay hutches': a novel interaction between bacteria and clay minerals." *Environmental Microbiology*, 2(2), 161-168.

MacKinnon, M. D. (1989). "Development of the tailings pond at Syncrude's oil sands plant; 1978-1987." *AOSTRA J. Res*, 5(2), 109-133.

Maier, A., Schumann, B. L., Chang, X., Talaska, G., and Puga, A. (2002). "Arsenic co-exposure potentiates benzo[a]pyrene genotoxicity." *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, 517(1–2), 101-111.

Mamer, M. (2007). *Oil Sands Tailings Technology: Understanding the Impact to Reclamation*. Suncor Energy Inc.

Masliyah, J. H., and Bhattacharjee, S. (2005). "Electric Double Layer", *Electrokinetic and Colloid Transport Phenomena*. John Wiley & Sons, Inc., pp. 105-178.

Masliyah, J. M. G., M.R. (2007). "Extracting and Upgrading of Oilsands Bitumen. Course Pack. ". City: University of Alberta.

Matsufuji, M., Nakata, K., and Yoshimoto, A. (1997). "High production of rhamnolipids by Pseudomonas aeruginosagrowing on ethanol." *Biotechnology Letters*, 19(12), 1213-1215.

McInerney, M., Javaheri, M., and Nagle, D., Jr. (1990). "Properties of the biosurfactant produced byBacillus licheniformis strain JF-2." *Journal of Industrial Microbiology*, 5(2-3), 95-101.

Mehdi, H., and Giti, E. (2008). "Investigation of alkane biodegradation using the microtiter plate method and correlation between biofilm formation, biosurfactant production and crude oil biodegradation." *International Biodeterioration & Biodegradation*, 62(2), 170-178.

Michaels, A. S., and Bolger, J. C. (1962). "Settling Rates and Sediment Volumes of Flocculated Kaolin Suspensions." *Industrial & Engineering Chemistry Fundamentals*, 1(1), 24-33.

Mikula, R. J., Munoz, V. A., and Omotoso, O. (2009). "Centrifugation Options for Production of Dry Stackable Tailings in Surface- Mined Oil Sands Tailings Management." *Journal of Canadian Petroleum Technology*.

Morse, J. W., Arvidson, R. S., and Lüttge, A. (2007). "Calcium Carbonate Formation and Dissolution." *Chemical Reviews*, 107(2), 342-381.

Moss, N., and Dymond, B. (1978). *Flocculation:Theory & Application*. Ciba Specialty Chemicals, Bradford, West Yorkshire, United Kingdom.

Moulin E., J. A., Wollast R. (1985). "Influence of the aerobic bacterial respiration on the early dissolution of carbonates in coastal sediments", in W. R. Van Grieken R., (ed.), *Progress in Belgian Oceanographic Research*. Brusells: University of Antwerpen pp. 196–208.

Mulligan, C. N. (2005). "Environmental applications for biosurfactants." *Environmental Pollution*, 133(2), 183-198.

Mulligan, C. N. (2014). "Enhancement of Remediation Technologies with Biosurfactants", in C. N. Mulligan, S. K. Sharma, and A. Mudhoo, (eds.), *Biosurfactants: Research Trends and Applications*. Boca Raton, Florida, USA: CRC Press, Taylor & Francis Group, pp. 239-254.

Mulligan, C. N., and Gibbs, B. F. (1990). "Recovery of biosurfactants by ultrafiltration." *J Chem Technol Biotechnol*, 47(1), 23-9.

Mulligan, C. N., Yong, R. N., and Gibbs, B. F. (2001). "Heavy metal removal from sediments by biosurfactants." *Journal of Hazardous Materials*, 85(1–2), 111-125.

Nakata, K., and Ishigami, Y. (1999). "A facile procedure of remediation for oily waste with rhamnolipid biosurfactant." *Journal of Environmental Science and Health, Part A*, 34(5), 1129-1142.

Nasim, T., and Bandyopadhyay, A. (2012). "Introducing different poly (vinyl alcohol)s as new flocculant for kaolinated waste water." *Separation and Purification Technology*, 88(0), 87-94.

Nguyen, T. T., Youssef, N. H., McInerney, M. J., and Sabatini, D. A. (2008). "Rhamnolipid biosurfactant mixtures for environmental remediation." *Water Research*, 42(6–7), 1735-1743.

Nitschke, M., and Costa, S. G. V. A. O. (2007). "Biosurfactants in food industry." *Trends in Food Science & Technology*, 18(5), 252-259.

Ochoa Loza, F. J. (1998). "Physico-chemical factors affecting rhamnolipid (biosurfactant) application for removal of metal contaminants from soil". City: The University of Arizona.

Ochsner, U. A., Fiechter, A., and Reiser, J. (1994). "Isolation, characterization, and expression in Escherichia coli of the *Pseudomonas aeruginosa* rhlAB genes encoding a rhamnosyltransferase involved in rhamnolipid biosurfactant synthesis." *Journal of Biological Chemistry*, 269(31), 19787-95.

OSRIN. (2013). *Glossary of Terms and Acronyms used in Oil Sands Mining, Processing and Environmental Management – December 2013 Update.* . Oil Sands Research and Information Network (OSRIN) Report

Pasquali, M. (2010). "Gelation: Grow with the flow." *Nat Mater*, 9(5), 381-382.

Patterson, J. (2012). "Exploitation of unconventional fossil fuel: enhanced greenhouse gas emission", in G. Liu, (ed.), *greenhouse gases- emission, measurment and management*. InTech. www.intechopen.com, pp. 147-170.

Penner, T. J., and Foght, J. M. (2010). "Mature fine tailings from oil sands processing harbour diverse methanogenic communities." *Canadian Journal of Microbiology*, 56(6), 459-470.

Prabhu, Y., and Phale, P. S. (2003). "Biodegradation of phenanthrene by Pseudomonas sp. strain PP2: novel metabolic pathway, role of biosurfactant and cell surface hydrophobicity in hydrocarbon assimilation." *Applied Microbiology and Biotechnology*, 61(4), 342-351.

Quagraine, E. K., Peterson, H. G., and Headley, J. V. (2005). "In Situ Bioremediation of Naphthenic Acids Contaminated Tailing Pond Waters in the Athabasca Oil Sands Region—Demonstrated Field Studies and Plausible Options: A Review." *Journal of Environmental Science and Health, Part A*, 40(3), 685-722.

Rahim, R., Ochsner, U. A., Olvera, C., Graninger, M., Messner, P., Lam, J. S., and Soberón-Chávez, G. (2001). "Cloning and functional characterization of the Pseudomonas aeruginosa rhlC gene that encodes rhamnosyltransferase 2, an enzyme responsible for di-rhamnolipid biosynthesis." *Molecular Microbiology*, 40(3), 708-718.

Rahman, K. S. M., Rahman, T. J., McClean, S., Marchant, R., and Banat, I. M. (2002a). "Rhamnolipid Biosurfactant Production by Strains of *Pseudomonas aeruginosa* Using Low-Cost Raw Materials." *Biotechnology Progress*, 18(6), 1277-1281.

Rahman, P. K. S. M., Banat, I. M., Rahman, T. J., Thayumanavan, T., and Lakshmanaperumalsamy, P. (2002b). "Bioremediation of gasoline contaminated soil by a bacterial consortium amended with poultry litter, coir pith and rhamnolipid biosurfactant " *Bioresource Technology*, 81(1), 25-32.

Rahmanian, B., Pakizeh, M., and Maskooki, A. (2010). "Micellar-enhanced ultrafiltration of zinc in synthetic wastewater using spiral-wound membrane." *Journal of Hazardous Materials*, 184(1–3), 261-267.

Ramos-Padrón, E., Bordenave, S., Lin, S., Bhaskar, I. M., Dong, X., Sensen, C. W., Fournier, J., Voordouw, G., and Gieg, L. M. (2010). "Carbon and Sulfur Cycling by Microbial Communities in a Gypsum-Treated Oil Sands Tailings Pond." *Environmental Science & Technology*, 45(2), 439-446.

RAMP. (2001). *(Regional Aquatics Monitoring Program), Oil Sands Regional Aquatics Monitoring Program, volume I:Chemical and Biological Monitoring*. Ft McMurray.

RAMP. (2006). *(Regional Aquatics Monitoring Program). Regional Aquatics Monitoring Program*. Technical Report, Ft. McMurray: RAMP 2006.

Rehm, B. (2009). *Microbial Production of Biopolymers and Polymer Precursors: Applications and Perspectives*: Caister Academic.

Ridha, and Mulligan, C. N. (2011). "Simultanous removal of Benzene and Copper From Contaminated Water Using Micellar-Enhanced Ultrafiltration"*Annual Conference of the Canadian Society for Civil Engineering*. City: Ottawa, Ontario, Canada.

Ridha, Z. A. M. (2010). *Simultaneous removal of benzene and copper from water and watewater using micellar enhanced ultrafiltration*, MSc Thesis, Concordia University, Montreal, Canada.

Rodrigues, L., Banat, I. M., Teixeira, J., and Oliveira, R. (2006). "Biosurfactants: potential applications in medicine." *The Journal of Antimicrobial Chemotherapy*, 57(4), 609-618.

Ronconi, R. A. (2006). "Predicting bird oiling events at oil sands tailings ponds and assessing the importance of alternate water bodies for waterfowl: a preliminary assessment." *The Canadian Field-Naturalist*, 120 (1).

Ronconi, R. A., and Cassady St. Clair, C. (2006). "Efficacy of a radar-activated on-demand system for deterring waterfowl from oil sands tailings ponds." *Journal of Applied Ecology*, 43(1), 111-119.

Rowell, D. E. (1994). "Soil science: Methods & applications D. L. Rowell, Longman Scientific & Technical, Longman Group UK Ltd, Harlow, Essex, UK (co-published in the USA with John Wiley & Sons Inc. New York), 1994, SBN 0-592-087848." *Journal of the Science of Food and Agriculture*, 66(4), 573-574.

Saborimanesh, N., and Mulligan, C. N. (2015). "Effect of sophorolipid biosurfactnat on oil biodegradation by the natural oil degrading bacteria on the weathered biodiesel, diesel and light crude oil." *Bioremediation and Biodegradation*, 6(314).

Salloum, M. J., Dudas, M. J., and Fedorak, P. M. (2002). "Microbial reduction of amended sulfate in anaerobic mature fine tailings from oil sand." *Waste Management Research*, 20, 162-171.

Samper, E., Rodríguez, M., De la Rubia, M. A., and Prats, D. (2009). "Removal of metal ions at low concentration by micellar-enhanced ultrafiltration (MEUF) using sodium dodecyl sulfate (SDS) and linear alkylbenzene sulfonate (LAS)." *Separation and Purification Technology*, 65(3), 337-342.

Santa Anna, L. M., Soriano, A. U., Gomes, A. C., Menezes, E. P., Gutarra, M. L. E., Freire, D. M. G., and Pereira, N. (2007). "Use of biosurfactant in the removal of oil from contaminated sandy soil." *Journal of Chemical Technology & Biotechnology*, 82(7), 687-691.

Scheibenbogen, K., Zytner, R. G., Lee, H., and Trevors, J. T. (1994). "Enhanced removal of selected hydrocarbons from soil by *Pseudomonas aeruginosa* UG2 biosurfactants and some chemical surfactants." *Journal of Chemical Technology & Biotechnology*, 59(1), 53-59.

Schick, C. D., and Ambrock, K. R. (1974). *Waterfowl investigations in the Athabasca Tar Sands Area*. Canadian Wildlife Service, Ottawa.

Schumacher, B. A. (2002). "METHODS FOR THE DETERMINATION OF TOTAL ORGANIC CARBON (TOC) IN SOILS AND SEDIMENTS ", E. R. L. Environmental Sciences Division National, (ed.). City: US Environmental Protection Agency: Las Vegas, NV.

Sharp, P. L., Birdsall, D. A., and Richardson, W. J. (1975). *Inventory studies of birds on and near crown lease number 17, Athabasca Tar Sands, 1974.*, Syncrude Canada Ltd, Ft. McMurray.

Shell. (2012). "SHELL ALBIAN SANDS: OVERVIEW OF TAILINGS AND SHELL TECHONOLOGIES.".

Shell Canada. (2016). "http://www.shell.ca/en/aboutshell/our-business-tpkg/downstream/oil-sands/albian-sands.html, "Access date: 19-04-2016"."

Siddique, T., Fedorak, P. M., MacKinnon, M. D., and Foght, J. M. (2007). "Metabolism of BTEX and Naphtha Compounds to Methane in Oil Sands Tailings." *Environmental Science & Technology*, 41(7), 2350-2356.

Siddique, T., Kuznetsov, P., Kuznetsova, A., Arkell, N., Young, R., Li, C., Guigard, S., Underwood, E., and Foght, J. M. (2014). "Microbially-accelerated consolidation of oil sands tailings. Pathway I: changes in porewater chemistry." *Front Microbiol*, 5(106).

Singh, B. P., Besra, L., Reddy, P. S. R., and Sengupta, D. K. (1998). "Use of surfactants to aid the dewatering of fine clean coal." *Fuel*, 77(12), 1349-1356.

Sirshendu, D., and Sourav, M. (2012). *Micellar Enhanced Ultrafiltration: Fundamentals & Applications*: CRC Press.

Soberón-Chávez, G., Aguirre-Ramírez, M., and Sánchez, R. (2005). "The Pseudomonas aeruginosa RhlA enzyme is involved in rhamnolipid and polyhydroxyalkanoate production." *Journal of Industrial Microbiology and Biotechnology*, 32(11-12), 675-677.

Sobkowicz, J. (2012). *Oil sands tailings technology deployment roadmaps.* . Project summary report, Alberta Innovates - Energy and Environment Solutions, Edmonton, Alberta.

Song, S., Lopez-Valdivieso, A., Reyes-Bahena, J. L., Bermejo-Perez, H. I., and Trass, O. (2000). "Hydrophobic Flocculation of Galena Fines in Aqueous Suspensions." *Journal of Colloid and Interface Science*, 227(2), 272-281.

Song, S., Lopez-Valdivieso, A., Reyes-Bahena, J. L., and Lara-Valenzuela, C. (2001). "Floc Flotation of Galena and Sphalerite Fines." *Minerals Engineering*, 14(1), 87-98.

Straube, W. L., Nestler, C. C., Hansen, L. D., Ringleberg, D., Pritchard, P. H., and Jones-Meehan, J. (2003). "Remediation of Polyaromatic Hydrocarbons (PAHs) through Landfarming with Biostimulation and Bioaugmentation." *Acta Biotechnologica*, 23(2-3), 179-196.

Suncor Energy. (2011). *Report on Sustainability*. Suncor Energy Inc., Calgary, Alberta, .

Sworska, A., Laskowski, J. S., and Cymerman, G. (2000a). "Flocculation of the Syncrude fine tailings: Part I. Effect of pH, polymer dosage and Mg2+ and Ca2+ cations." *International Journal of Mineral Processing*, 60(2), 143-152.

Sworska, A., Laskowski, J. S., and Cymerman, G. (2000b). "Flocculation of the Syncrude fine tailings: Part II. Effect of hydrodynamic conditions." *International Journal of Mineral Processing*, 60(2), 153-161.

Syldatk, C., Lang, S., and Wagner, F. Z. (1985). *Naturforsch*, 40, 51-60.

Syldatk, C., and Wagner, F. (1987). "Production of biosurfactants", in N. Kosaric, W. L. Cairns, and W. L. Gray, (eds.), *Biosurfactants and Biotechnology* New York, Basel Marcel Dekker, pp. 21–45.

Syncrude. "Tailings Management". City: Calgary, Alberta.

Tan, H., Champion, J. T., Artiola, J. F., Brusseau, M. L., and Miller, R. M. (1994). "Complexation of Cadmium by a Rhamnolipid Biosurfactant." *Environmental Science & Technology*, 28(13), 2402-2406.

Tenney, M. W., and Stumm, W. (1965). "chemical flocculation of microorganisms in biological waste treatment." *Journal of the Water Pollutant Control Federation*, 37, 1370-1388.

Tetreault, G. R., McMaster, M. E., Dixon, D. G., and Parrott, J. L. (2003). "Physiological and biochemical responses of Ontario slimy sculpin (Cottus cognatus) to sediment from the Athabasca oil sands area." *Water Quality Research Journal of Canada* 38, 361-77.

Timoney, K. P., and Lee, P. (2009). "Does the Alberta Tar Sands Industry Pollute? The Scientific Evidence." *Open Conservation Biology Journal* 3 65-81.

Timoney, K. P., and Ronconi, R. A. (2010). "Annual Bird Mortality in the Bitumen Tailings Ponds in Northeastern Alberta, Canada." *The Wilson Journal of Ornithology*, 122(3), 569-576.

Torres, S., Pandey, A., and Castro, G. R. (2011). "Organic solvent adaptation of Gram positive bacteria: Applications and biotechnological potentials." *Biotechnology Advances*, 29(4), 442-452.

Tripathy, T., and Ranjan De, B. (2006). "Flocculation : A New Way to Treat the Waste Water." *Journal of Physical Sciences*, 10, 93 – 127.

Troy, D. B. (2005). *Remington : the science and practice of pharmacy*, Philadelphia, PA: Lippincott, Williams & Wilkins.

Ucbeyiay Sahinkaya, H., and Ozkan, A. (2011). "Investigation of shear flocculation behaviors of colemanite with some anionic surfactants and inorganic salts." *Separation and Purification Technology*, 80(1), 131-139.

Urum, K., and Pekdemir, T. (2004). "Evaluation of biosurfactants for crude oil contaminated soil washing." *Chemosphere*, 57(9), 1139-1150.

Urum, K., Pekdemir, T., and Gopur, M. (2003). "Optimum Conditions for Washing of Crude Oil-Contaminated Soil with Biosurfactant Solutions." *Process Safety and Environmental Protection*, 81(3), 203-209.

Vasefy, F., and Mulligan, C. N. (2008). "Bioremediation of oil in a marine environment using rhamnolipid biosurfactant"*International Conference on Waste Engineering and Management*. City: Hong Kong, .

Voordouw, G. (2013). "Interaction of oil sands tailings particles with polymers and microbial cells: First steps toward reclamation to soil." *Biopolymers*, 99(4), 257-262.

Walworth, J. L., and Reynolds, C. M. (1995). "Bioremediation of a petroleum-contaminated cryic soil: Effects of phosphorus, nitrogen, and temperature." *Journal of Soil Contamination*, 4(3), 299-310.

Wang, S., and Mulligan, C. N. (2009). "Rhamnolipid biosurfactant-enhanced soil flushing for the removal of arsenic and heavy metals from mine tailings." *Process Biochemistry*, 44(3), 296-301.

Wang, X., Feng, X., Xu, Z., and Masliyah, J. H. (2010). "Polymer aids for settling and filtration of oil sands tailings." *The Canadian Journal of Chemical Engineering*, 88(3), 403-410.

Warren, L. J. (1992). in J.S. Laskowski and J. Ralston, (eds.), *Colloid Chemistry in Mineral Processing*. New York, NY, USA: Elsevier, pp. 309–329.

Whang, L.-M., Liu, P.-W. G., Ma, C.-C., and Cheng, S.-S. (2008). "Application of biosurfactants, rhamnolipid, and surfactin, for enhanced biodegradation of diesel-contaminated water and soil." *Journal of Hazardous Materials*, 151(1), 155-163.

Witek-Krowiak, A., Witek, J., Gruszczyńska, A., Szafran, R., Koźlecki, T., and Modelski, S. (2011). "Ultrafiltrative separation of rhamnolipid from culture medium." *World Journal of Microbiology and Biotechnology*, 27(8), 1961-1964.

WWF. (2010). *Tailings, a lasting oil sands legacy. World Wildlife Fund's final report*. Final report.

Xu, Q., Nakajima, M., Liu, Z., and Shiina, T. (2011). "Biosurfactants for microbubble preparation and application." *International Journal of Molecular Sciences*, 12(1), 462-475.

Yan, P., Lu, M., Guan, Y., Zhang, W., and Zhang, Z. (2011). "Remediation of oil-based drill cuttings through a biosurfactant-based washing followed by a biodegradation treatment." *Bioresource Technology*, 102(22), 10252-10259.

Yang, B., and Song, S. (2014). "HYDROPHOBIC AGGLOMERATION OF MINERAL FINES IN AQUEOUS SUSPENSIONS AND ITS APPLICATION IN FLOTATION: A REVIEW." *Surface Review and Letters*, 21(03), 1430003.

Yang, W. Y., Qian, J. W., and Shen, Z. Q. (2004). "A novel flocculant of Al(OH)3–polyacrylamide ionic hybrid." *Journal of Colloid and Interface Science*, 273(2), 400-405.

Yin, H., Qiang, J., Jia, Y., Ye, J., Peng, H., Qin, H., Zhang, N., and He, B. (2009). "Characteristics of biosurfactant produced by *Pseudomonas aeruginosa* S6 isolated from oil-containing wastewater." *Process Biochemistry*, 44(3), 302-308.

Youssef, N. (2006). *Bacillus Lipopeptide Biosurfactants: Purification, Structure, Activity Relationship and In-situ Production in Oil Reservoirs*, PhD Thesis, UNIVERSITY OF OKLAHOMA, Norman, Oklahoma, USA.

Yu, G.-H., He, P.-J., and Shao, L.-M. (2009). "Characteristics of extracellular polymeric substances (EPS) fractions from excess sludges and their effects on bioflocculability." *Bioresource Technology*, 100(13), 3193-3198.

Yuan, S., and Lahaie, R. (2013). "Thickened tailings (paste) technology and its applicability in oil sand tailings management"*13th International Conference on Tailings and Mine Waste*. City: University of Alberta: Banff, Alberta, pp. 829–845.

Zhu, S., Chen, Y., Li, G., and Liang, S. (2011). "Effect of Biosurfactant on Desorption of Sediment-Sorbed Phenanthrene"*5th International Conference on Bioinformatics and Biomedical Engineering, (iCBBE) 2011* City: Wuhan, China, pp. 1-4.