Improving Cap Water Quality in An Oil Sands End Pit Lake with Microbial Applications

by

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Abstract

The oil sands industry in Alberta, Canada has been thriving for over five decades, and this multi-million-year-old resource has fueled rapid economic growth in Alberta. However, the oil sands industry has left behind tons of oil sands tailings and oil sands process-affected water (OSPW), and the surface mining process has greatly disturbed the landscape. Oil sands end pit lakes (EPLs) are proposed to reclaim the impacted land and remediate the large quantities of accumulated oil sands tailings and OSPW. A typical EPL is commissioned within a depleted mine pit, with oil sands tailings stored below a water cap. Using materials from Base Mine Lake (BML, the first full scale EPL), this thesis investigates the applicability of microbes for improving cap water quality. The objectives of this thesis were: (1) biological treatment of the residual bitumen; (2) model NAs degradation by the microalgae, the BML cap water microbes, and the co-culture of the two; and (3) cap water turbidity mitigation using microbial addition.

Recovery of the bitumen from the oil sands is not 100% effective, and the unextracted bitumen will remain in the oil sands tailings. In an EPL, the residual bitumen in the tailings can potentially cause environmental concerns if proper action is not taken. As bitumen migrates from the tailings to float on top of the water cap, it might change the surrounding water chemistry by releasing hydrocarbons and biodegradation by-products. Firstly, a biological amendment was used to treat three different types of oil sands tailings to remove bitumen content. Secondly, biostimulation treatment with acetate of the indigenous tailings microbial community was used to treat the bitumen. The presence of bitumen was found to increase the water toxicity. Analysis of the indigenous tailings microbial community profile combined with monitoring of CO_2 (complete mineralization), dissolved organic carbon (indirect parameter) and petroleum hydrocarbons revealed that four genera (*Rhodoferax*, *Acidovorax*, *Pseudoxanthomonas* and *Pseudomonas*) were potential bitumen-degraders.

Chlorella kessleri and *Botryococcus braunii* were tested for their capability to tolerate and biodegrade three model NAs (cyclohexanecarboxylic acid (CHCA), cyclohexaneacetic acid (CHAA), and cyclohexanebutyric acid (CHBA)): *C. kessleri* showed better tolerance and more effective removal of the tested NAs than *B. braunii*. BML cap water was also used as inoculum alone and co-cultured with *C. kessleri* to biodegrade the CHBA and CHCA. All tested cultures used β-oxidation pathway to biodegrade model NAs. The co-culture of BML inoculum and *C. kessleri* had a higher biodegradation rate of CHBA than BML inoculum and *C. kessleri* alone, and removed CHCA 25 d faster than *C. kessleri* alone (70 d). *C. kessleri* greatly increased the bacterial diversity of the BML inoculum, and this more diverse community was thought to lead to the more rapid and complete degradation of model NAs.

Sporosarcina pasteurii, *C. kessleri* and *B. braunii* were tested to remove the cap water turbidity. *C. kessleri* addition can remove the cap water turbidity effectively in the bench scale experiment, and nutrient addition can remove a comparable level of turbidity possibly by stimulating the growth of the indigenous algal community. *B. braunii* didn't achieve any turbidity removal alone. *S. pasteurii*, a urea-hydrolyzing bacterium, can carry out microbial induced calcite precipitation (MICP) process leading to biocementation. MICP requires several conditions to occur: alkaline pH, Ca^{2+}, $CO_3{}^{2-}$ and nucleation site availability. With the addition of *S. pasteurii* providing the nucleation sites, MICP has the potential to occur in BML cap water. Results in this study showed that with *S. pasteurii* alone, MICP might not be fully carried out in the BML cap water. However, the addition of *S. pasteurii* with calcium or urea could achieve effective turbidity removal with the formation of particles of increased size.

Preface

This thesis is an original work by Xiaoxuan Yu under supervision of Dr. Ania Ulrich.

A version of Chapter 2 has been published as Yu, X., Cao, Y., Sampaga, R., Rybiak, S., Burns, T. and Ulrich, A.C., 2018. Accelerated dewatering and detoxification of oil sands tailings using a biological amendment. Journal of Environmental Engineering, 144(9), p.04018091. The conributions of the coauthors are as follows: Ms. Cao, Mr. Sampaga, Mr. Rybiak helped with sample analysis, and Ms. Cao designed the column test. Mr. Burns contributed to research meetings.

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Chapter 4 presents the results for a journal article, which has been submitted to Chemosphere. The conributions of the coauthor are as follows: Mr. Lee did the sample preparation for the next generation sequencing and contributed to the manuscript edits.

Chapter 5 presents the results for a journal article, which will be submitted to Journal of Environmental Engineering.

Dedication

To my dear parents: Yu, Yun−Tong and Pan, Yu−Rong

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List of Abbreviations

1-CHCA 1-cyclohexene-1-carboxylic acid

AEO Acid extractable organic

AER Alberta Energy Regulator

BBM Bold's Basal Medium

BCR Beaver Creek Reservoir

BML Base Mine Lake

BOD Biological oxygen demand

BTEX Benzene, toluene, ethybenzene and xylene isomers

CCME Canadian Council of Ministers of the Environment

CEMA Cumulative Environmental Management Association

CHAA Cyclohexaneacetic acid

CHBA Cyclohexanebutyric acid

CHCA Cyclohexanecarboxylic acid

Chl a Chlorophyll a

DIC Dissolved inorganic carbon

DO Dissolved oxygen

DOC Dissolved organic carbon

EPL Oil sands end pit lake

FFT Fluid fine tailings

GC-FID Gas chromatography equipped with a flame ionization detector

GC-TCD Gas chromatography equipped with a thermal conductivity detector

HAA (1-hydroxycyclohexyl)-acetic acid

HPLC High performance liquid chromatography

IC Ion chromatography

IC_{20} Inhibition concentration 20%

IC_{50} Inhibition concentration 50%

ICP-MS Inductively coupled plasma-mass spectrometry

ICP-OES Inductively coupled plasma-optical emission spectrometry

LC-MS Liquid chromatography-mass spectrometry

MFT Mature fine tailings

MICP Microbial induced calcite precipitation

MLSB Mildred Lake Settling Basin

MTBSTFA N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide

NA Naphthenic acid

NTU Nephelometric turbidity unit

OD Optical density

OSPW Oil sands process-affected water

OSTP Oil sands tailings pond

OTU Operational taxonomic unit

PAH Polycyclic aromatic hydrocarbon

PCB Polychlorinated biphenyl

PHC Petroleum hydrocarbon

TDS Total dissolved solids

TMF Tailings Management Framework

TPW Tailings pond water

TU Toxicity unit

WIP West-In Pit

Chapter 1

Introduction

1.1 Research rationale

The objective of my proposed PhD research is to understand the promising, but not fully tested reclamation technique of end pit lakes (EPLs) in the oil sands industry, and how to best improve the water quality using microbial treatments. Through microbial processes, degradation of contaminants can lead to detoxification, while biomass growth can help mitigate turbidity issues in the cap water. Base Mine Lake (BML) is the first full-scale oil sands industry EPL and was commissioned by Syncrude Canada Ltd. (henceforth Syncrude) in 2013. All research was conducted with materials obtained directly from BML. A summary of current research literature, research questions, hypotheses, methodologies and results are presented in this thesis.

1.2 Background

1.2.1 Alberta oil sands

Albertas oil sands are the third largest oil reserve in the world after Venezuela and Saudi Arabia (Percy, 2012). Oil sands production has been growing greatly (thousand cubic meters per day): 20.4 (1980), 50.1 (2000), 104 (2008) and 163.8 (2017) (CAPP, 2018). Athabasca, Cold Lake and Peace River are three major oil sands mining areas covering 142,200 km^2 of land in the northern Alberta shown in Fig. 1.1 (Alberta Government, 2014). Recovery of bitumen from the oil sands ore occurs through two techniques: open pit mining (20% of the reserve, 3% of the total oil sands surface area) and in situ recovery (80% of the reserve, 97% of the surface area) (Percy, 2012; Alberta Government, 2014). Only the Athabasca oil sands area contains surface mineable oil sands (less than 75 m below the surface) (Alberta Government, 2014). The surface mineable area equals 4,800 km^2 (Jordaan, 2012; Alberta Government, 2014). Oil sands have been recovered only by open pit mining until 1980s, and since then in situ technique was also used for bitumen extraction (Oil Sands Magazine, 2018). Due to the limitations of the in situ recovery efficiency, although 80% of the reserve is only feasible for in situ recovery, surface mining has been the predominant technique used, creating a large footprint and a complex task for land/water reclamation and management (Chalaturnyk et al., 2002; Jordaan, 2012; Mikula, 2013; Oil Sands Magazine, 2018). In this thesis, the surface mined oil sands are the concerning process.

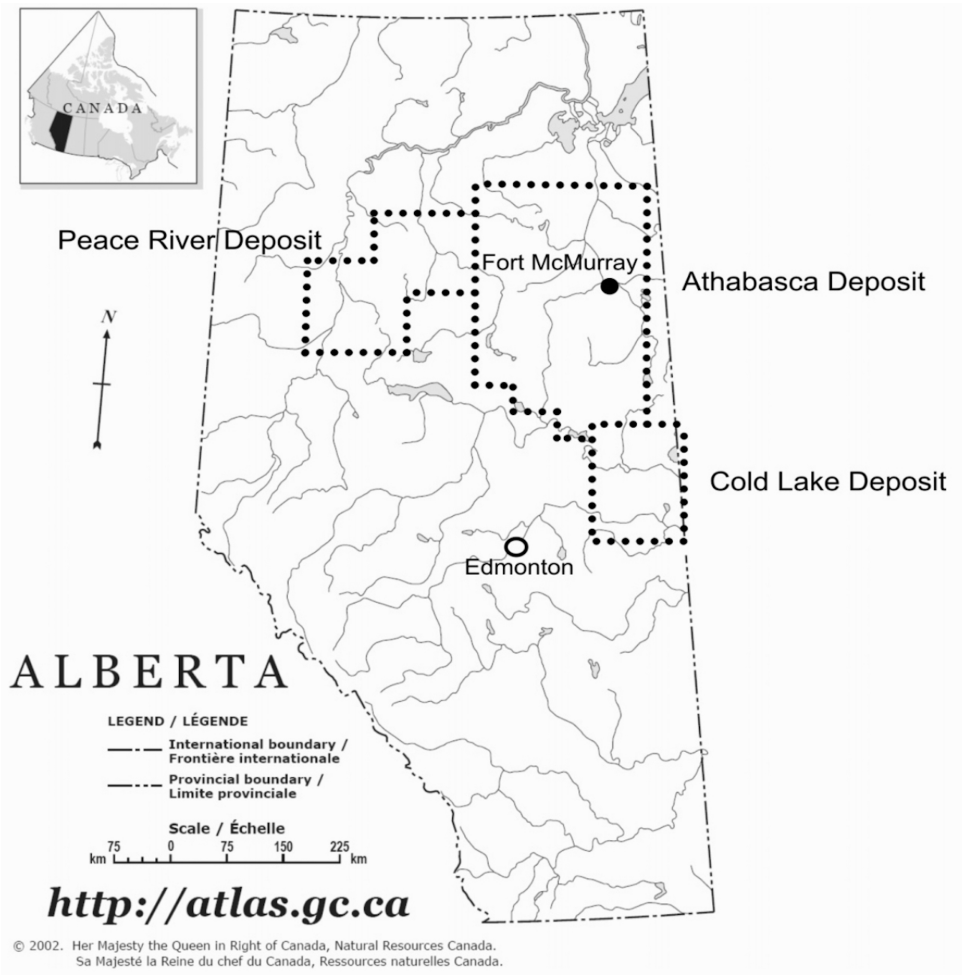

Figure 1.1: Major oil sands deposit location in Alberta, Canada (Allen, 2008a).

The mined ores excavated by surface mining can be then extracted by the Clark Hot Water Extraction process, which occurs by adding hot water (50−80 °C) and caustic soda to the sand to generate a slurry and allow bitumen to separate from the sands and clay (Chalaturnyk et al., 2002). After the sediments settle, bitumen froth is collected at the surface of the slurry (Clark and Pasternack, 1932). The recovered bitumen is then subjected to an upgrading process to transform it into synthetic crude oil. For each barrel

of bitumen, about 3.0−4.5 barrels of water are consumed, and 6 m^3 of sand and 1.5 m^3 of tailings are produced (Allen, 2008a; Natual Resources Canada, 2009). Oil sands projects have strict limits on water usage, and to minimize the use of fresh water, 80−95% of the water is recycled (Alberta Government, 2014; Alberta Environment and Parks, 2015). After collecting bitumen froth, oil sands tailings is produced and accumulated in large quantities (Chalaturnyk et al., 2002; Jordaan, 2012). Tailings are a mixture of water (60−70 wt%), solids (sand, clay, silts, etc.: 30 wt%), residual bitumen (1−4 wt%), other hydrocarbons, salts and trace metals (Chalaturnyk et al., 2002; Alberta Environment and Parks, 2015; Pennetta de Oliveira et al., 2018). The total volume of the accumulated tailings in the oil sands tailings ponds (OSTPs) until 2017 was estimated at 1 billion m^3, and OSTPs occupied an area of 176 km^2, more than a quarter of the area of the City of Edmonton (Foght et al., 2017; Vajihinejad et al., 2017). Tailings management has been challenging due to the huge existing volume and the slow dewatering of the tailings (Chalaturnyk et al., 2002). Alberta Energy Regulator (AER) released Directive 074 in 2009 in order to reduce the growing volumes of tailing. In 2017, AER replaced Directive 074 with Directive 085 to enforce the Tailings Management Framework (TMF) to furthermore minimize oil sands tailings accumulation (Natual Resources Canada, 2009; Alberta Environment and Parks, 2015). Directive 085 sets out requirement to manage tailings by measuring the tailings reduction using the total volume of the tailings and ensures that the tailings are treated and reclaimed during the lifespan of a project, while Directive 074 was introduced to set the criteria for development of policy direction of tailings management from oil sands mining projects. Directive 085 is closely aligned with TMF, and both documents are important for the stakeholders to achieve the goal that all tailings from a project will be ready to claim 10 years after the mining life of a project (Alberta Environment and Parks, 2015). Research has focused on fluid fine tailings (FFT) dewatering and consolidation, and much effort has been put into finding ways to

accelerate the solid-liquid separation process. However, with the enormous volume of tailings produced, failures in tailings pond infrastructure and perceived risk of seepage, a variety of environmental concerns have been raised and current techniques are inadequate to fully solve this issue (Jordaan, 2012; Wang et al., 2014). In the TMF and Directive 085, EPLs are a wet-based landscape reclamation and water-capping technique with potential for reclaiming the mine closure landscape (Chalaturnyk et al., 2002; Jordaan, 2012; Mikula, 2013; Dompierre and Barbour, 2016). EPLs are the most economical route for disposing of tailings, as such EPLs research has grown rapidly at both the bench/pilot and field demonstration scale (WWF-Canada, 2010).

1.2.2 Oil sands end pit lakes

The Cumulative Environmental Management Association (CEMA) defines an oil sands EPL as: "*an engineered water body, located below grade in an oil sands post-mining pit. It may contain oil sands by-product material and will receive surface and groundwater from surrounding reclaimed and undisturbed landscapes. EPLs will be permanent features in the final reclaimed landscape, discharging water to the downstream environment*" (Westcott and Watson, 2007). In EPLs, tailings waste (if included) will be placed in the bottom of mined out pits and fresh water from natural lakes or other healthy water bodies (referred to as cap water) will be placed on the top of the tailings, as shown in Fig. 1.2 (Charette et al., 2010). As described in the definition, an EPL will develop into a biologically sustaining ecosystem, which connects to downstream aquatic environments in the future (Westcott and Watson, 2007; Charette et al., 2010). Tailings will dewater and consolidate over time and cap water chemistry will be altered due to the microbial activities and potential physicochemical reactions occurring in situ in an EPL (Allen, 2008a,b; Abed et al., 2002).

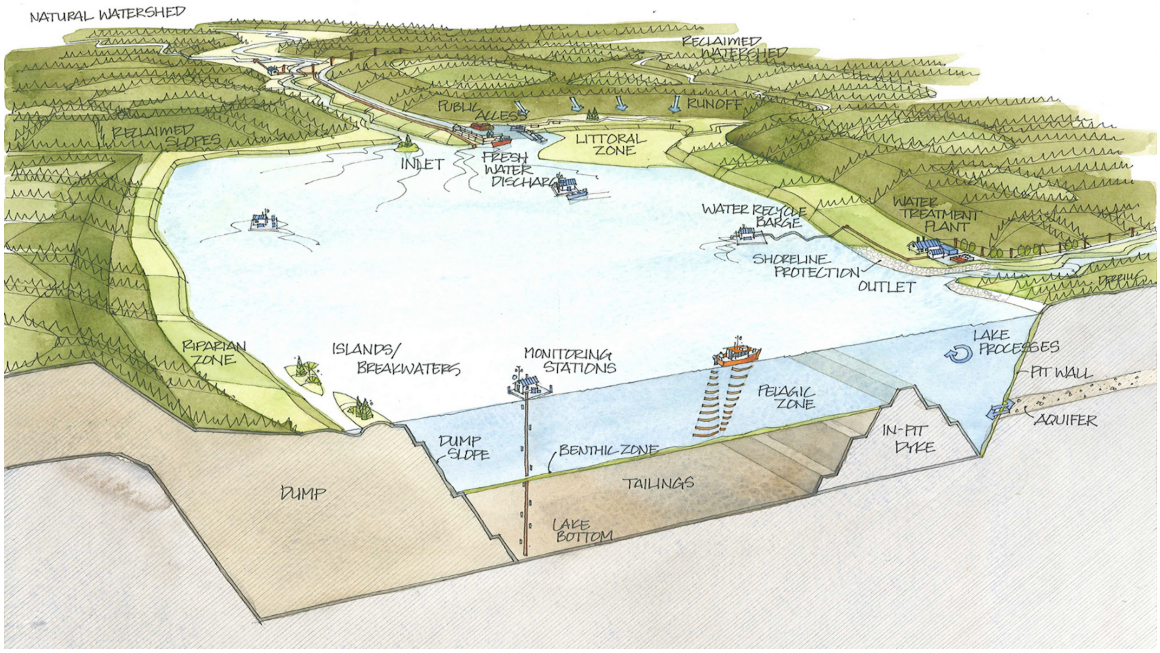

Figure 1.2: A conceptual model for an EPL with tailings: mature stage (Charette et al., 2010)

EPLs are not a new concept to the land reclamation (Gammons et al., 2009). Other mining industries have constructed end pit lakes in depleted mining pits to fulfill different purposes and objectives successfully (Westcott and Watson, 2007; Gammons et al., 2009). East Pit Lake, an example of successful reclaimed mine pit, was commissioned in an old coal mining pit and has been successfully reclaimed locally in Parkland County, Alberta (Gammons et al., 2009). Just like end pit lakes in other mining industries, oil sands tailings characteristics and performance of an EPL tend to be site-specific. Due to the development of the bitumen extraction technique, oil sands tailings can be quite different, such as the labile component and the tailings microbial community (Chalaturnyk et al., 2002).

In tailings containing EPLs, EPL cap water consists of oil sands process-affected water (OSPW) and/or fresh water from a reservoir, river or other healthy water bodies, or surface and groundwater from reclaimed watersheds (Chalaturnyk et al., 2002; Westcott and Watson, 2007; Lawrence et al., 2016). Therefore, initially, the chemical profile of cap water is similar to diluted OSPW. Usually, OSPW with a pH of 8.0−8.8 has a moderate hardness (15−25 mg L^{-1} Ca^{2+} and 5−10 mg L^{-1} Mg^{2+}) and alkalinity of 800−1000 mg L^{-1} $CaCO_3$ (Nix and Martin, 1992; Siwik et al., 2000; Farrell et al., 2004; Allen, 2008a; Charette et al., 2010; Zhang, 2016). Dissolved oxygen (DO) is low at less than 1 mg L^{-1} (Nix and Martin, 1992; Charette et al., 2010). Turbidity is relatively high (100−120 Nephelometric Turbidity Units (NTU)) (Wang et al., 2015a). Total dissolved solids (TDS) are also high in OSPW (1500−2500 mg L^{-1}) and high conductivity is also high, ranging from 500 µS cm^{-1} to 2500 µS cm^{-1} (Allen, 2008a). The main constituents of TDS are Na^+ (100−700 mg L^{-1}), HCO_3^- (800−1000 mg L^{-1}), Cl^- (50−600 mg L^{-1}) and SO_4^{2-} (100−300 mg L^{-1}) (Nix and Martin, 1992; Allen, 2008a; Farrell et al., 2004; Siwik et al., 2000; Abolfazlzadehdoshanbehbazari et al., 2013; Wang et al., 2015a; Zhang, 2016). NaCl concentrations are high enough to show chronic toxicity to phytoplankton (Harmon et al., 2003). For OSPW from settling basins from Syncrude and Suncor, some of these constituents also show a tendency to increase over time due to water reuse and recycling (Allen, 2008a). Except for the major cations and anions, trace metal (Al, Cd, Fe, Pb, Ti, V, Cr, Mn, Co, Ni, Cu, Zn, As, Sr, Mo, Ba) concentrations also have exceeded the Canadian Council of Ministers of the Environment (CCME) surface water quality guideline for protection of aquatic life and therefore may require effective removal (Allen, 2008a; Mahdavi et al., 2013; Wang et al., 2015a). Organic compounds identified in OSPW include bitumen, naphthenic acids (NAs), benzene, toluene, ethylbenzene, xylene, creosols, phenols, phthalates and polycyclic aromatic hydrocarbons (PAHs) (Strosher and Peake, 1978; Madill et al., 2001; Rogers et al., 2002; Allen, 2008a; Brown and Ulrich, 2015; Wang

et al., 2015a). Dissolved organic carbon (DOC) can reach up to 100 mg L^{-1}, 80% of which are NAs (Allen, 2008a; Brown and Ulrich, 2015; Morandi et al., 2015; Nelson et al., 1993). Due to the limitations of the hot water extraction technique, bitumen cannot be fully recovered from oil sands. The residual bitumen can therefore pose challenges to the treatment of OSPW and tailings management, which makes consolidating/flocculating/drying tailings challenging and also contributes to the toxicity to aquatic biota (Harmon et al., 2003; Quagraine et al., 2005a; Allen, 2008a; Sobkowicz, 2013). Other organics of concern, such as benzene, toluene, phenols and PAHs, which are more readily degradable or highly volatile, have appeared to decline below the CCME surface water quality guidelines over time in the tailings pond surface water (Nelson et al., 1993; Rogers et al., 2002; Allen, 2008a). This makes them less of a remediation concern to oil sands operators.

The EPL water cap is designed to contain fresh water, which contains less toxicants and higher DO concentration, and the tailings placed in the bottom might serve as a source of microbial inoculums and substrates (Charette et al., 2010). Under this scenario, natural attenuation can be an effective option for reducing chemical loads and toxicity to the surrounding environment. Not all EPLs will have tailings, but the ones with tailings will have a lake bottom that declines in elevation due to the dewatering of the tailings (Charette et al., 2010). The tailings pore water (interstitial water) has a chemistry profile similar to OSPW but with elevated concentrations of most constituents and generally more toxic (Siwik et al., 2000; Sobkowicz, 2013). As tailings dewater over time, the pore water will be expressed into the cap water and the constituents of concern might decrease by dilution, biodegradation and photodegradation. Ideally in an EPL context, the expressed contaminants will be bioremediated in the water cap if an appropriate community of contaminant-degrading microorganisms have been established.

In the 1980s, Syncrude established small-scale test ponds with different water compositions (fresh water only, OSPW only or tailings pond water (TPW), and a fresh wa-

ter/OSPW mixture) overlying tailings (Siwik et al., 2000; Allen, 2008a). These small-scale test ponds provided information on the evolution of water quality based on initial FFT:water ratio and total volume ((Siwik et al., 2000; Westcott and Watson, 2007; Oil Sands Tailings Consortium, 2012; Dompierre and Barbour, 2016). Tables 1.1, 1.2 and 1.3 summarized the test pond information (Tables 1.1 and 1.2) and a brief comparison of BML and the test ponds (Table 1.3). Fathead minnows only had lower survival rates in test pits 7 (MFT capped with expressed water and melt water) and 10 (MFT capped with TPW) (Siwik et al., 2000). Based on the preliminary results from these test ponds, it was thought the exposure to the tested materials would not impede the fish growth significantly (Siwik et al., 2000).

In 2013, the first full-scale demonstration EPL (BML) was established by Syncrude. Since EPLs require further study in the areas of geochemistry, hydrogeology, limnology, microbiology, and environmental remediation (Charette et al., 2010; Miall, 2013), BML will provide invaluable information in understanding how EPLs perform as a reclamation tool and will bridge the gap between theory and practical application (Charette et al., 2010; Dompierre and Barbour, 2016).

Table 1.1: Summary of materials used in test ponds from Siwik et al.'s study (2000) (modified from Siwik et al. 2000).

Site	Date of pond construction	Volume for MFT and water cap	Pond area (ha)
Deep wetland	1993	None	4
South bison	early 1980s	None runoff from mine site	6
Test pit 3	1989	1,000 m^3 MFT 1,000 m^3 unprocessed water cap	0.16
Demonstration pond	1993	70,000 m^3 MFT 80,000 m^3 unprocessed water cap	4
Test pit 5	1989	1,000 m^3 MFT 1,000 m^3 TPW water cap	0.16
Test pit 7	1989	2,000 m^3 MFT water cap comprised of expressed and melt water	0.16
Tailings pond water pond	1993	No MFT 50,000 m^3 of TPW	4
Storage cell 10	1993	29,000 m^3 MFT 6,300 m^3 TPW water cap	4

Table 1.2: Water chemistry (only average is shown here) of waters used in Siwik et al.'s study of bioassays from the test ponds established by Syncrude. Parameters are expressed in mg L^{-1} unless otherwise noted. Blank cell indicates no data. T Phenol to total phenol, TN to total nitrogen, and TP to total phosphorus (table modified from Siwik et al. 2000).

Parameter	Test pit 3	Demonstration pond	Test pit 5	Test pit 7	Tailings pond water pond	Storage cell 10
pH	8.5	8.48	8.71	8.25	8.80	8.80
Conductivity (µS cm^{-1})	486	720	1,621	603	1977	2,283
TDS	400	580	1,592	1042	1,542	1,792
NA	3.3	6.8	28.6	22.3	45.6	59.4
DOC	25.9	33.1	44.1	50.9	51.4	57.8
T Phenol (µg L^{-1})	2	1	2	2	3	1.5
NH_3	0.16	0.10	0.15	0.15	0.06	0.03
NO_2 + NO_3	0.026	0.028	0.041	0.025	0.041	0.12
TN	1.16	2.07	2.12	1.66	1.30	1.52
TP	0.03	0.074	0.030	0.045	0.049	0.030
Na^+	98.9	147.4	495.4	347.8	45.6	608.0
K^+	1.86	4.22	8.44	6.10	5.16	6.46
Mg^{2+}	15.1	16.5	22.8	10.7	10.1	9.4
Ca^{2+}	26.1	41.4	26.1	24.0	19.3	15.2
Cl^-	19.2	39.6	124.3	85.6	226.2	258.0
$SO_4{}^{2-}$	69.8	104.3	512.8	86.9	224.3	233.3
$HCO_3{}^-$	219	322	535	667		
Al (µg L^{-1})	20	63	87	583	397	395
Sb (µg L^{-1})	< 0.2	< 0.2	< 0.2	< 0.2	< 0.2	
As (µg L^{-1})	1	1	3	2	3	6
Ba (µg L^{-1})	30	35	37	63	39	40
Be (µg L^{-1})	1.5	2.3	2	< 1	2	
B	0.35	0.51	2.34	1.42	2.05	2.46
Cd (µg L^{-1})	< 3	< 3	< 3	< 3	< 3	< 3
Cr (µg L^{-1})	< 2	4	< 2	< 2	2	3
Co (µg L^{-1})	< 3	4	< 3	3	3	< 3
Cu (µg L^{-1})	2	2	2	5	3	2
Fe (µg L^{-1})	87	41	97	119	334	400

Table 1.3: Comparison of test ponds and BML (modified from OSTC 2012).

Variable	Test Ponds	Base Mine Lake
Surface area (ha)	0.05−4	$\sim$ 800
Initial depth of water cap (m)	0.5−2.8	$\geq$ 5
Volume of water cap (m^3)	(1−80) $\times 10^3$	(35−40) $\times 10^6$
Volume of MFT (m^3)	(1−80) $\times 10^3$	$>$ 175 $\times 10^6$
Volume ratio (MFT:water)	1	$>$ 4
Maximum fetch (km)	0.04−0.25	$>$ 3
Fill time (y)	$<$ 0.1 (all)	17 (MFT)//1−5 (water)
Hydrology	Closed (no surface flow-through)	Open (flow-through) potential
Residence time (y)	$>$ 15	$>$ 19
MFT source	Mildred Lake Settling Basin North	Mildred Lake Settling Basin South
Water cap source	Natural surface water and/or OSPW	Mixed OSPW and natural water

1.2.3 Base Mine Lake

Base Mine was the original mine area where mining began by Syncrude and decommissioned in 1994. A portion of the decommissioned pit was utilized as a tailings pond named West In-Pit (WIP) (WIP is the precursor of BML), receiving FFT and OSPW from Mildred Lake Mine from 1994 to 2012 (Dompierre and Barbour, 2016; Dompierre et al., 2016). In 2012, after filling of the lake with FFT and OSPW was complete, the pond was commissioned as an operational scale demonstration oil sands EPL containing FFT (Fig. 1.3). The maximum depth of FFT was 45 m with approximately 1.9 $\times$ 10^8 m^3 of FFT covering approximately 8 km^2 (Dompierre, 2016). In 2013, to establish a water cap, fresh water from Beaver Creek Reservoir (BCR) was pumped into BML

(Dompierre and Barbour, 2016; Dompierre et al., 2016). Water was also recycled for bitumen extraction use from 2013 to 2015 to maintain the lake surface elevation and establish flow-through, while the average depth of the water cap increased by 1 m from 2013 to 2015 due to the dewatering of the FFT (Lawrence et al., 2016).

It is anticipated that in the BML, microbial activities can remove the constituents of concern (e.g., NAs, metals, residual bitumen) and mitigate turbidity, DO and other related issues to the point where flora and fauna can colonize BML and form a healthy ecosystem (Charette et al., 2010). Lab scale research will be utilized to conduct proof-of-concept for treatment plans.

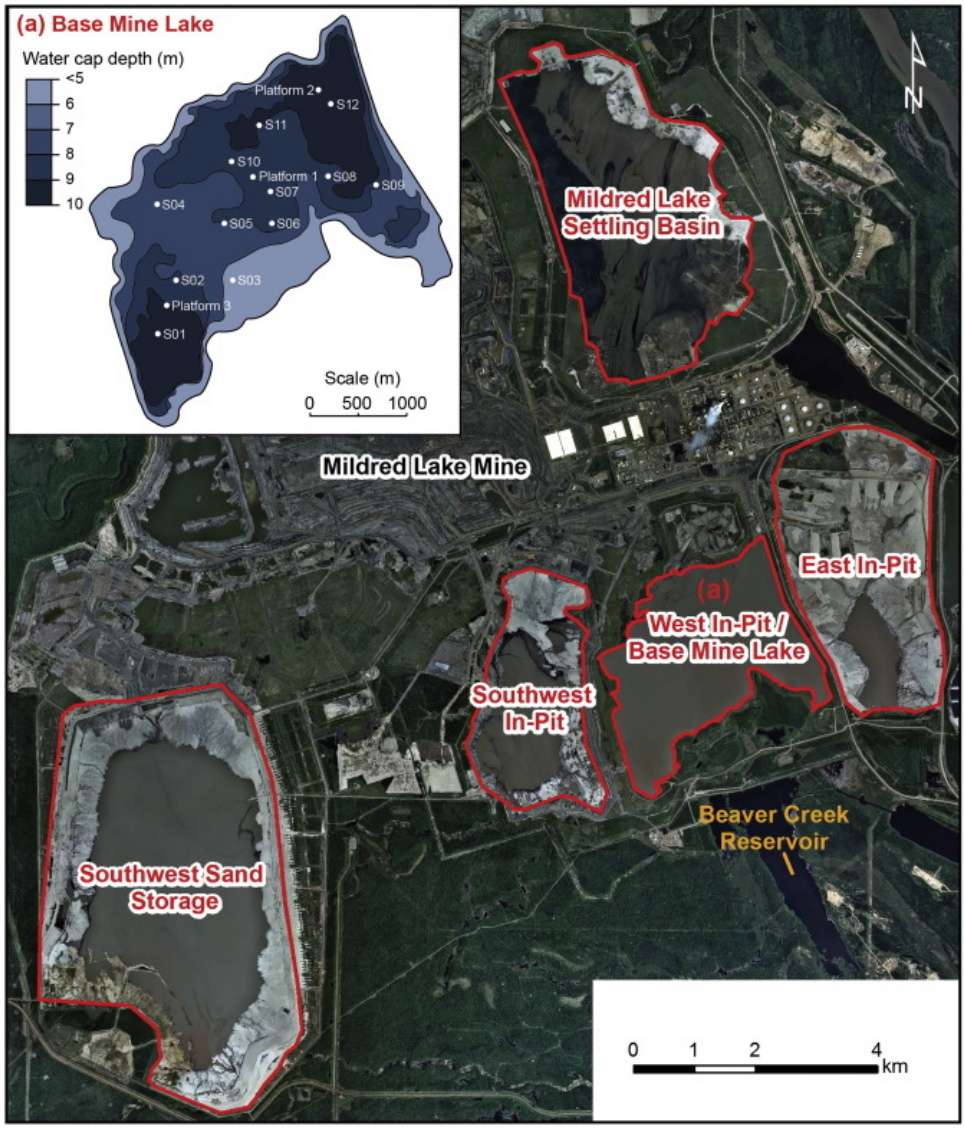

Figure 1.3: Oil sands tailings ponds and BML (WIP, the precursor tailings pond) are shown in the satellite image. Inset (a) shows a detailed image of the BML monitoring stations for the lakes monitoring program and water cap depth profile in 2016 (Dompierre and Barbour, 2016).

1.3 Microbial treatment applications

1.3.1 Microorganisms in EPLs

The underlying tailings in an EPL have long been known to harbour complex microbial communities (Herman et al., 1993; Johnson et al., 2011; Siddique et al., 2012; Mahdavi et al., 2013; Brown, 2014; Siddique et al., 2014a,b; Arkell et al., 2015; Collins et al., 2016; Foght et al., 2017). Metagenomics analysis of the Athabasca deep oil sands region revealed a rich community of aerobes, facultative anaerobes and anaerobes, including hydrocarbon-degrading bacteria and methanogens (An et al., 2013). These Athabasca indigenous microbial communities are thought to be the crucial inoculum for the fresh oil sands tailings (Foght et al., 2017). With improvements in the bitumen extraction technique, the working extraction temperature has been greatly reduced from 80 °C to 50 °C, and even as low as 35−50 °C at the Aurora Mine of Syncrude (BGC Engineering Inc., 2010; Foght et al., 2017; Chalaturnyk et al., 2002). The reduced temperature poses less pressure on the microbial communities. A diluent, which is usually a light hydrocarbon, such as C5−C16 alkanes or mono-aromatics (BTEX: benzene, toluene, ethylbenzene and xylene isomers), is typically added during froth treatment to enhance the bitumen recovery from the slurry (BGC Engineering Inc., 2010). The fugitive diluent subsequently ends up in the tailings stream (Small et al., 2015; Foght et al., 2017). Although the diluent is a small portion, due to the massive volumes of tailings, these alkanes and BTEX compounds become the carbon source to support the adapted microbial communities continuously thrive (Foght et al., 2017). However, due to the different techniques adopted by the oil sands operators, tailings microbial communities can be significantly different and diverse.

Besides the tailings, fresh water and recycled OSPW that are an integral part of an EPL cap water can also be a source of microorganisms. OSPW has also been used as microbial

inoculum to degrade model NAs and organics in the OSPW itself (Herman et al., 1993, 1994; Clemente et al., 2004; Del Rio et al., 2006; Han et al., 2009; Brown et al., 2013; Demeter et al., 2014, 2015). The surface water in WIP was sampled in 2011, where DO was approximately 0.38 mg L^{-1} (Saidi-Mehrabad et al., 2013), and the next-generation sequencing proved the presence of a relatively diverse microbial eukaryotic community including Chlorophyta (green algae) and Chrysophyceae (golden algae) (Aguilar et al., 2016). Chlorophyta and Chrysophyceae mostly contain O_2^- generating phototrophs that may support methanotroph metabolism and decrease the DO consumption of the methanotrophs, and in the long term, increase the DO in the cap water (Mahdavi et al., 2013; Saidi-Mehrabad et al., 2013; Aguilar et al., 2016). With the development of the BML, enhancement in the cap water DO levels and reduced turbidity levels will help the cap water community, especially the eukaryotic members, grow and be more active. According to Syncrude Canada Ltd.'s monitoring program of BML, the eukaryotic community (including microalgae) has thrived, and both enhanced DO and reduced turbidity have been observed in BML (Syncrude Canada Ltd., 2017).

Therefore, the rich and relatively diverse microbial community indigenous to an EPL makes the microbial application a feasible and promising approach to the environmental remediation and land reclamation plan. In this thesis, both indigenous and exogenous microorganisms have been investigated for their possibility for the microbial application. In the later part of this sub-section, bioremediation applications relevant to EPLs and tailing/OSPW will be reviewed, and algal application will be highlighted and discussed. The last sub-section will discuss the bacterial-algal consortia application and its advantages.

1.3.2 Bioremediation overview

Biological treatment of domestic wastewater has been applied since the mid-1800s, and in situ bioremediation by aerobic and anaerobic biodegradation has been studied and applied in petroleum-contaminated sites since the 1970s (Atlas and Raymond, 1977; Thomas and Ward, 1989; Leahy and Colwell, 1990; U.S. EPA, 2013). Later findings about the bacteria capable of completely dechlorinating trichloroethylene and the related chloroethenes stimulated the passion from the researchers and industry (Maymó-Gatell et al., 1997; U.S. EPA, 2013; Seshadri et al., 2005). After several demonstration applications went full scale, bioremediation for treatment of halogenated compounds in the soil and groundwater is now well know and widely accepted (Stroo, 2010; U.S. EPA, 2013). Crude oil spills, on the other hand, also pushed the bioremediation field further by calling for urgent remediation tasks worldwide (Leahy and Colwell, 1990). Bacteria are commonly used in bioremediation along with archaea, fungi and protists, and organic removal can be achieved by biodegrading organics to intermediate products or completely mineralize the organics into water and CO_2/CH_4 (Leahy and Colwell, 1990; Clemente and Fedorak, 2005).

As introduced in Section 1.3.1, active and diverse microbial activities have been observed in EPLs and OSTPs, and microbial application showed great potential for, but not limited to OSPW detoxification, biodegradation of the organics (including NAs, residual diluent, and un-extracted bitumen), heavy metal removal, accelerated tailings dewatering and turbidity removal (Wyndham and Costerton, 1981; Nelson et al., 1993; Allen, 2008a; Johnson et al., 2011; Mahdavi et al., 2012; Woodworth et al., 2012; Siddique et al., 2014a,b; Brown and Ulrich, 2015; Kasiri et al., 2015b; Mahdavi et al., 2015; Morandi et al., 2015; Foght et al., 2017; Song et al., 2018). Potential microbial remediation of OSPW and tailings by native microbial communities has also been investigated by researchers (Nix and Martin, 1992; Herman et al., 1994; Yergeau et al., 2012; Siddique

et al., 2012; Mahdavi et al., 2015). In order to enhance the degradation ability of the native microbial community, pretreatment of OSPW (such as ozonation to oxidize organics and gypsum addition to enhance dewatering of tailings), biostimulation (addition of nutrients), bioaugmentation (addition of enriched indigenous contaminant-degrading cultures), and application of surfactants (addition of surfactants or biosurfactant-producing bacteria to enhance the availability of hydrophobic contaminants) can be utilized.

DOCs, most of which are NAs, dominate water quality issues in OSPW, and NAs are also the most significant acute toxicity contributor to OSPW (Nelson et al., 1993; Allen, 2008b; Brown and Ulrich, 2015; Morandi et al., 2015). NAs are a complex mixture of alkyl-substituted acyclic and cycloaliphatic carboxylic acids that have been the focus of oil sands related remediation studies shown in Fig. 1.4 (Quagraine et al., 2005b; Brown and Ulrich, 2015). NAs have a general chemical formula $C_nH_{2n+Z}O_2$, where n indicates the carbon number, Z describes the degree of cyclization (Holowenko et al., 2001). The value of Z/2 indicates the number of ring structure: e.g., one-ring NA belongs to the Z = -2 family, and three-ring NA belongs to the Z = -6 family.

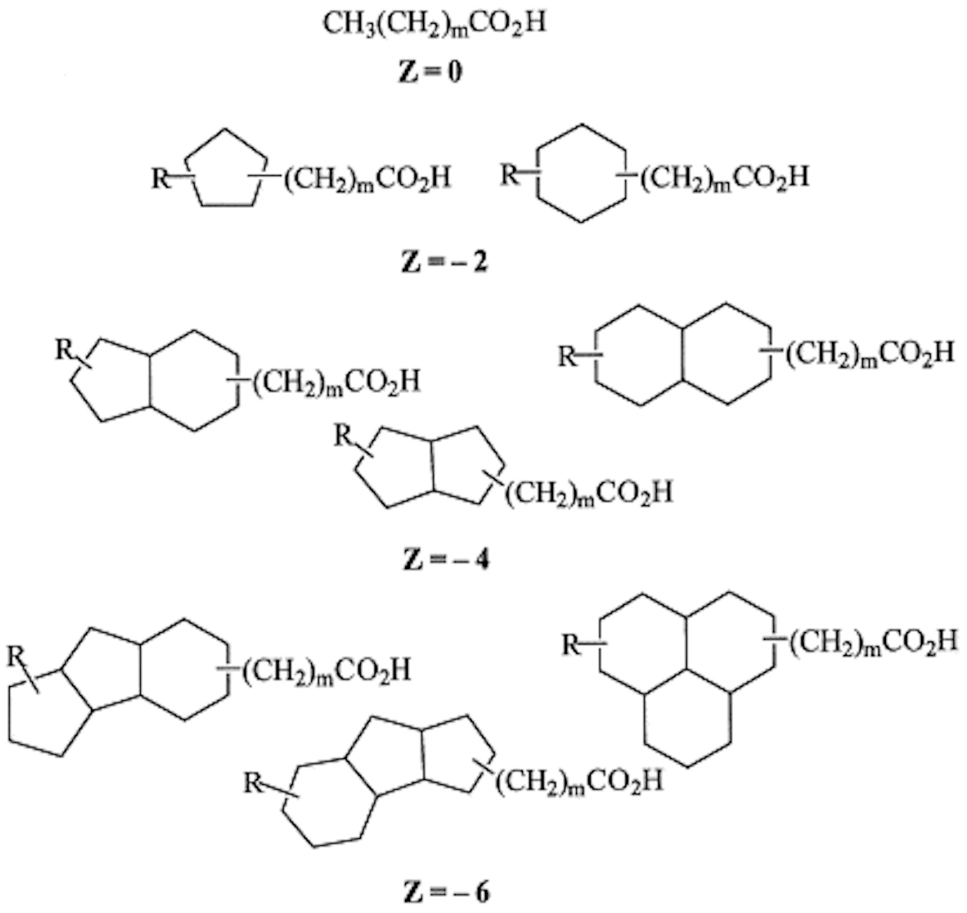

Figure 1.4: Typical NA structures in the Z = 0, -2, -4, -6 families are shown. R represents an alkyl chain, m indicates number of CH_2 units (modified from Holowenko et al., 2001).

The lack of knowledge of the inherent complexity of the NAs and the limitation of the analytical methods for NAs have long been the bottleneck of NA biodegradation research. To overcome these limitations, both model and commercially available NAs have been used as surrogates for the NA biodegradation studies and helped isolate some NA-degrading microbes. Cyclohexanecarboxylic acid (CHCA, a simple model NA) aerobic degradation has been reported by *Corynebacterium cyclohexanicum*, *Trichosporon*

cutaneum KUY-6A, *Acinetobacter anitratum*, *Alcaligenes faecalis*, *Pseudomonas putida* and oil sands tailings microcosms and fresh water enriched culture, and its anaerobic degradation has been reported by *Rhodopseudomonas palustris* and *Geobacter metallireducens* (Tokuyama and Kaneda, 1973; Rho and Evans, 1975; Blakley, 1978; Blakley and Papish, 1982; Hasegawa et al., 1987; Herman et al., 1993, 1994; Meckenstock et al., 2000; Whitby, 2010; Kung et al., 2014). *Pseudomonas putida* KT2440 was reported to degrade two model NAs: (4-t-butylphenyl)-4-butanoic acid and (4-n-butylphenyl)-4-butanoic acid aerobically (Johnson et al., 2013). Oil sands tailings microcosms have also reported to degrade many other model NAs, such as 2-methylcyclohexane carboxylic acid, *trans*-4-Pentyl-cyclohexanecarboxylic acid, decahydro-2-naphthoic acid and cyclohexanepropanoic acid (Herman et al., 1994; Lai et al., 1996; Holowenko et al., 2001; Clemente et al., 2004). Kodak commercial NA mixture was found to be removed by $>$ 95% by a mixed consortium of two species of bacteria, *Pseudomonas putida* and *Pseudomonas fluorescens* (either of the two isolates can only remove 15%) (Del Rio et al., 2006). Various types of bioreactors have been used for model NAs, commercial NAs and extracted oil sands NAs and OSPW NAs for the treatment efficiency (Xue et al., 2018). Incorporation of biolms in membrane bioreactor is considered promising for NA treatment after intensively reviewing research focusing on the bioreactor application in OSPW treatment (Xue et al., 2018). More examples of NA-degrading microorganisms and enriched cultures using model NAs in biodegradation studies are listed in Table 1.4. While these studies above mostly exemplified the biodegradation capacity of bacteria, phytoremediation should be involved in OSPW bioremediation, especially in an EPL where DO of the cap water is a crucial factor for a healthy ecosystem establishment.

Table 1.4: Summary of NA-tolerating or degrading bacteria

Aerobic biodegradation	
Substrate NA	Organism or culture (reference)
Cyclohexanecarboxylic acid	*Corynebacterium cyclohexanicum* (Tokuyama and Kaneda, 1973)
	Acinetobacter anitratum (Rho and Evans, 1975)
	Alcaligenes faecalis (Blakley, 1978)
	Pseudomonas putida (Blakley and Papish, 1982)
	Trichosporon cutaneum KUY-6A (Hasegawa et al., 1987)
	Oil sands tailings microcosms (Herman et al., 1993, 1994)
	Alcanivorax sp. strain MBIC 4326 (Dutta and Harayama, 2001)
	Rhodococcus sp. NDKK48 (Koma et al., 2004)
	Sediment microorganisms (Del Rio et al., 2006)
	OSPW community (Demeter et al., 2014, 2015)
	Cupriavidus gilardii CR3 (Wang et al., 2015b)
	Biofilm-biochar complex (Frankel et al., 2016)
	Activated sludge (Wang et al., 2016)
	Rhodococcus aetherivorans BCP1 (Presentato et al., 2018)
	Indigenous microbial community in the shallow subsurface near tailings ponds (Ahad et al., 2018)
	A low-lying wetland microbial community near oil sands deposit (Ahad et al., 2018)
	Aerobic granular sludge (Tiwari et al., 2019)
Cyclohexaneacetic acid	*Arthrobacter* sp. strain CA1 (Ougham and Trudgill, 1982)
	OSPW community (Demeter et al., 2015, 2014)
	Biofilm-biochar complex (Frankel et al., 2016)
	Aerobic granular sludge (Tiwari et al., 2019)
Cyclohexanebutyric acid	*Arthrobacter* sp. strain CA1 (Ougham and Trudgill, 1982)
	Biofilm-biochar complex (Frankel et al., 2016)
Cyclopentanecarboxylic acid	*Rhodococcus aetherivorans* BCP1 (Presentato et al., 2018)
4-methyl-1-cyclohexane carboxylic acid	Microbial fuel cells (Labrada and Nemati, 2018)
1,2-cyclohexanedicarboxylic acid	Indigenous microbial community in the shallow subsurface near tailings ponds (Ahad et al., 2018)
	A low-lying wetland microbial community near oil sands deposit (Ahad et al., 2018)
Decahydro-2-naphthoic acid	Sediment microorganisms (Del Rio et al., 2006)
Anaerobic biodegradation	
Substrate NA	Organism or culture
Cyclohexanecarboxylic acid	Fresh water enrichment culture (Meckenstock et al., 2000)
	Rhodopseudomonas palustris (Kung et al., 2014)
	Geobacter metallireducens (Kung et al., 2014)
	Indigenous oil sands tailings microorganisms (Clothier and Gieg, 2016)
Cyclohexaneacetic acid	Indigenous oil sands tailings microorganisms (Clothier and Gieg, 2016)

Microalgae are known for its ability to remove contaminants such as metals, organics, nutrients, and pathogens in polluted environments (Muñoz et al., 2006). Microalgae are photosynthetic: they can utilize energy from light to generate chemical energy, produce sugars from inorganic carbon (CO_2), and release oxygen into the environment (Kirk, 1994). The main mechanism for contaminant removal involves biosorption, bioaccumulation, uptake and biotransformation, and biodegradation (Mallick, 2002; Davis, 2003; Lei et al., 2007; Haritash and Kaushik, 2009; Mahdavi et al., 2013).

Algae uptake nutrients directly for protein, nucleic acid and lipid synthesis, and also remove nutrients indirectly by ammonia-nitrogen volatilization and orthophosphate precipitation (Olgun, 2003). Algal biomass is easy to grow and harvest, and this feature creates opportunities for later reuse (feedstock, biofuel, etc.) and low-cost operation. Thus algal based technologies in wastewater treatment draw great attention, and some of these technologies have been commercially available (Laliberte et al., 1994; Olgun, 2003; Oswald, 2003; Muñoz et al., 2006; Kesaano and Sims, 2014). Advanced Integrated Wastewater Pond System (AIWPS®) is an example commercialized algal-based technology for metal/nutrient and biological oxygen demand (BOD) removal by Oswald and Green, LLC in the United States (Oswald, 1990; Green et al., 1996).

Biological fixation of CO_2 is a sustainable and feasible option because of the photosynthetic process (Kirk, 1994; Banerjee et al., 2008). The burning of algal biomass is not a net increase to the atmospheric CO_2 level (Banerjee et al., 2008). For example, if a 100-MW thermal power plant replaces coal to algae-derived fuel (*B. braunii*), 150,000 tons of CO_2 emission can be reduced per year (Sawayama et al., 1999). It is very advantageous to combine this nutrient removal feature with CO_2 fixation, and this helps reduce the cost of the required medium for the algal growth (Nakamura and Senior, 2005; Muñoz et al., 2006). Besides the potential capacity varies with the algal species, light, nutrients, pH, temperature, operation condition, etc., will all influence the CO_2 fixation process

(Kasiri et al., 2015a,b).

Heavy metals are non-biodegradable, and the clean-up of the heavy metals are challenging since cost is a significant limitation factor (He and Chen, 2014). Compared with the traditional technologies such as chemical precipitation, adsorption, coagulation, membrane separation, etc., algal removal of heavy metals shows great advantages: low cost, high efficiency when the metal concentrations are low ($10-100$ mg L^{-1}) and less treatment by-product (Veglio' and Beolchini, 1997; Mehta and Gaur, 2005; Mahdavi et al., 2013; He and Chen, 2014). Removal of metals involves two processes: an initial rapid process that is passive, and followed by a slower but active uptake (Dönmez et al., 1999; Mehta and Gaur, 2005). The rapid passive process includes adsorption, ion exchange, complexation, chelation and microprecipitation, and the active process includes covalent bonding, redox reaction, crystallization, and other extracellular/intracellular binding processes (Dönmez et al., 1999; Mehta and Gaur, 2005; Mahdavi et al., 2012; He and Chen, 2014).

Microalgae cells have a variety of functional groups: carboxyl, hydroxyl, sulfate, phosphate, and amine groups, and the cell walls are surrounded by large amounts of exopolysaccharides, which are negatively charged (Mehta and Gaur, 2005; Mahdavi et al., 2013). These functional groups and exopolysaccharides provide abundant binding sites for metal. Different algal cells have different types and amounts of functional groups, and the more free sites are available, the more effective metal binding can be achieved (Mahdavi et al., 2013). After metals binding on the surface of the cells, the permeability of the cell membrane may increase, and metal diffusion into the cell may occur (Mehta and Gaur, 2005). Intracellular accumulation of metals has also been reported vastly. By digesting the cells with EDTA (Bates et al., 1982), the contribution of intracellular meal accumulation can be measured. The relative contribution of surface adsorption and intracellular uptake varies with algal species and metal ions, however, a greater contribution of surface adsorption is more frequently observed (Mehta and Gaur, 2001a,b,c, 2005;

Mehta et al., 2002; Mahdavi et al., 2013). The main mechanism may change over time: Cu removal by *Kirchneriella aperta* is dominated by surface adsorption initially, and over time, the surface-bond Cu declines with a concurrent increase concentration within the cells (Lombardi et al., 2002). The surface adsorption might also be a mechanism invoked in algae to be more tolerable to the elevated heavy metal concentrations (Lombardi et al., 2002).

While nutrient removal, CO_2 fixation and metal sorption are more commonly observed among many algal species, degradation and metabolism of the organics are more species-specific and toxicant-dependent (Lei et al., 2007; Haritash and Kaushik, 2009; Chakravarty et al., 2015). Both polychlorinated biphenyls (PCBs) and PAHs remediation by algae have been reported. Aromatic hydrocarbon oxidation is potentially widely overserved with most microalgal species *Chlorella vulgaris*, *Scenedesmus platydiscus*, *Scenedesmus quadricauda*, and *Selenastrum capricornutum* all showed capability to remove fluoranthene, pyrene and a mixture of both PAH compounds effectively (Lei et al., 2007). *Skeletonema costatum* and *Nitzschia* sp. can remove two PAHs (phenanthrene and fluoranthene) with an initial removal of passive sorption, and then active absorption and biodegradation (Hong et al., 2008). PCBs removal by algae can overcome the bottleneck of inactivation of dehydroxybiphenyl oxygenase, which is a key enzyme of the PCB degradation pathway (Dai et al., 2002). The high affinity of PCBs for the lipid content (rich in algal cells), creates the pathway for PCB uptake and opportunities for algal remediation (Dai et al., 2002; Fitzgerald and Steuer, 2006; Chekroun et al., 2014; Cheney et al., 2014). Although less studies have reported petroleum-degrading algae, more and more species have been found capable of this task, especially combined with other microorganisms (Walker et al., 1975; Muñoz et al., 2006; Jain and Bajpai, 2012; Chekroun et al., 2014). However, compared to bacteria and fungi, hydrocarbon degradation by algal species is weaker (Leahy and Colwell, 1990). Petroleum-degrading algal species were first reported

in the 1970s: *Prototehca zopfii*, isolated from oil-contaminated sites, can degrade n- and iso-alkanes (Walker et al., 1975). *Selenastrum capricornutum* can remove benzene, toluene, naphthalene, phenanthrene and pyrene (Gavrilescu, 2010), and *Scenedesmus obliquus* GH2 was also reported to degrade a crude-oil mixture and able to enhance the effects when co-cultured with bacteria (Tang et al., 2010).

Algal tolerance in the presence of NAs and NAs removal (both oil sands NAs and the model NAs) ability has been reported recently (summarized in Table 1.5). Algal species belonging to Chlorophyta division were found to dominate the phytoplankton community in oil sands heavily-influenced sites (high NA levels) in the Athabasca oil sands region, and this study highlighted the importance of phytoplankton communities and their potential role in remediation of the wet landscape (Leung et al., 2003). *Naviculla* sp. was found capable of degrading a model naphthenic acid (4-methylcyclohexaneacetic acid), and *Selenastrum* sp. showed remediation potential for the oil sands NAs mixtures (extracted in lab) (Headley et al., 2008). *Dunaliella tertiolecta* can tolerate five model NAs at the concentration of 300 mg L^{-1}, namely cyclohexane carboxylic acid, cyclohexane acetic acid, cyclohexane propionic acid, cyclohexane butyric acid and 1,2,3,4-tetrahydro-2-naphthoic acid, and also biodegraded the first four model NAs through -oxidation (Quesnel et al., 2011). The algae *Chlamydomonas reinhardtii* showed rapid growth with oil sands NAs and other organics, and sorption to the cell wall was suspected to play a role to the changes in the organic composition (Goff et al., 2014). The high tolerance ability of *Chlorella vulgaris* to two model NAs ((4-n-butylphenyl)-4-butanoic acid and (4-tert-butylphenyl)-4-butanoic acid) and the oil sands NAs was reported (Beddow et al., 2016). In 2012, 21 phytoplankton species isolated from oil sands leases north of Fort McMurray, and 12 of the chlorophyte phytoplankton strains and all 6 cyanobacterial and euglenoid strains showed no inhibition when oil sands NAs were at or below 100 mg L^{-1} (Woodworth et al., 2012). Among the same 21 phytoplankton species, *Stichococcus* sp.

was proved capable of removing acid extracted acids at all tested concentrations (10, 30, 100 mg L^{-1}; Ruffell et al., 2016). All these studies highlighted the feasibility and great potential for using microalgae for oil sands remediation in the industry.

Table 1.5: Summary of NA-tolerating or degrading alage

NA	Alga and its capability (reference)
Extracted NA mixtures	*Selenastrum* sp.: possible degradation (Headley et al., 2008)
or oil sands NAs	*Scenedesmaceae* (strain: ChlA01, ChlA02): tolerance (Woodworth et al., 2012)
	Chlorellaceae (strain: ChlA05): tolerance (Woodworth et al., 2012)
	Oocystaceae (strain: ChlA08): tolerance (Woodworth et al., 2012)
	Selenastraceae (ChlA13, ChlA14, ChlA15): tolerance (Woodworth et al., 2012)
	Chlamydomonas reinhardtii: tolerance and possible degradation (Goff et al., 2014)
	Indigenous algae-bacteria consortium: tolerance and possible degradation (Mahdavi et al., 2015)
	Chlorella vulgaris: tolerance (Beddow et al., 2016)
	Stichococcus sp.: degradation (Ruffell et al., 2016)
(4-n-butylphenyl)-4-butanoic acid	*Chlorella vulgaris*: tolerance (Beddow et al., 2016)
(4-tert-butylphenyl)-4-butanoic acid	*Chlorella vulgaris*: tolerance (Beddow et al., 2016)
Cyclohexanepropionic acid	*Dunaliella tertiolecta*: degradation (Quesnel et al., 2011)
1,2,3,4-tetrahydro-2-naphthoic acid	*Dunaliella tertiolecta*: degradation (Quesnel et al., 2011)
Cyclohexanecarboxylic acid	*Dunaliella tertiolecta*: degradation (Quesnel et al., 2011)
Cyclohexaneacetic acid	*Dunaliella tertiolecta*: degradation (Quesnel et al., 2011)
Cyclohexanebutyric acid	*Dunaliella tertiolecta*: degradation (Quesnel et al., 2011)
4-methylcyclohexaneacetic acid	*Naviculla* sp.: degradation (Headley et al., 2008)

1.3.3 Bioremediation using bacterial-algal consortia

Microalgae coexist with bacteria naturally, and laboratory cultures have also been utilized to maintain a synergistic relationship with bacteria (Muñoz et al., 2004; Subashchandrabose et al., 2011; Zhou et al., 2013; Mahdavi et al., 2015; Ramanan et al., 2016). Bacteria can benefit algal growth by providing algae substances such as CO_2, vitamins, organic chelating agents and assimilable nitrogen derivatives, and also limit algal growth by se-

creting algaecides, degrade algal polysaccharides and cells and competing for the nutrients (Haines and Guillard, 1974; Reim et al., 1974; Fallon and Brock, 1979; Saks and Kahn, 1979; Muñoz et al., 2004; Banerjee et al., 2008; Zhou et al., 2013). Algae can produce O_2 through photosynthesis and organic matter to support the bacterial growth (Muñoz et al., 2004; Guo and Tong, 2014; Mahdavi et al., 2015). The consortia of algae and bacteria adaptation ability is usually stronger than algae or bacteria alone, and through their activities, a hostile, contaminated environment can be altered and remediated(Bender and Phillips, 2004). Thus algal-bacterial consortia can achieve better remediation and more effective targeted contaminants degradation (Bender et al., 1995; Muñoz et al., 2004, 2006; Zhou et al., 2013). Algal-bacterial consortia have been reported to degrade organics, remove metal and nutrients (Muñoz et al., 2004; Bender and Phillips, 2004; Muñoz et al., 2006; Zhou et al., 2013; Mahdavi et al., 2015).

The great potential of bioremediation for the oil sands industry has been discussed in Section 1.3.2, and many studies highlighted that an algae-bacteria consortia can be more effective than either algae or bacteria alone (Muñoz et al., 2006; Bruckner et al., 2008; Mahdavi et al., 2015). In EPLs, besides the possible direct benefits of algae for improving water quality in EPLs, algae-produced oxygen and metabolites can also stimulate the growth of bacteria. This is important because bacteria are the main contributors to the biodegradation of organics in OSPW and tailings (Mahdavi et al., 2015; Muñoz et al., 2006). The overall contaminant-degrading rate by aerobic biodegradation is often limited by oxygen concentration, and therefore, algae can support the bacterial-algal consortia better to realize detoxification and contaminant removal.

In addition, sedimentation with an algal-bacterial consortium occurs faster than sedimentation with algae alone (Muñoz et al., 2004). Biofilm structure and the exopolysaccharides surrounding the biofilm will help microbes adhere to the clay and fine particles, which will promote the particle aggregation process (Bordenave et al., 2010). Microorganisms

indigenous to the tailings pond, such as *Pseudomonas*, *Thauera*, *Hydrogenophaga*, *Rhodoferax*, and *Acidovorax*, can all grow in a biofilm structure, and readily attach to the mineral matter and particles suspended in the cap water (Foght et al., 2017). Therefore, faster sedimentation of the particles might also occur in EPLs and help mitigate EPL cap water turbidity issues (Golby et al., 2012; An et al., 2013).

A microbial mat is a special form of consortia of bacteria and cyanobacteria occurring naturally, and is a laminated and multi-layered microbial community (Bender and Phillips, 2004). In this research, this concept was extended: microalgae are also involved. Since microalgae are capable of releasing exopolysaccharides, mat forming feature can also be achieved. Establishing a microbial mat consortium with the integration of contaminant-degrading microorganism species can help degrade organic contaminants and mitigate cap water turbidity in the context of EPLs, and also alter the microenvironment for better support of a healthy lake ecosystem.

Microbial mats have also been used in other mining pit lakes and industries. Algal-bacterial consortia can be grown with proper substrates to upgrade the water quality to an acceptable level in acid mine drainage-affected lakes and ponds, with effective removal of Pb, Cd, Cu, Zn, Co, Cr and Fe (Sheoran and Bhandari, 2005). Highland Valley Copper mine is the largest open pit copper mine in BC, Canada, and the mining operation has created copper mine tailings ponds and pit lakes that require proper reclamation and healthy aquatic ecosystem establishment (Larratt et al., 2007). Reclamation has been achieved through the successful colonization of microflora (bacteria, algae, fungi, yeasts), aquatic plants, and animals (zooplankton, invertebrates and fish) (Larratt et al., 2007). Once proper inorganic nutrients exist (macro-nutrients, like N, P, C, Si, K, and micro-nutrients, like Fe, Mn, Cu, S), bacteria colonized naturally and secondary microflora (fungi and algae) also began to expand (Larratt et al., 2007). The algae delivered adsorbed metals to the substrate, accelerating metal binding reactions. Filamentous algae

formed mats in the system and provided substantial amounts of organic carbon within this ecosystem (Larratt et al., 2007). However, algae production was limited by the lack of some unknown growth factor (suspected to be B vitamins). Overall, a self-sustaining microbial system has been established in this copper pit lake, and desired water quality has been achieved (Larratt et al., 2007). The copper mine pit lake development demonstrates proper introduction of foreign microbes, as well as the proper provision of necessary macro and micro-nutrients.

1.3.4 Introduction to microorganisms used in this thesis research

Several microorganisms will be tested in this work, namely BML cap water/tailings indigenous microbial community, *Chlorella kessleri* (microalga), *Botryococcus braunii* (microalga) and *Sporosarcina pasteurii* (bacterium). The reasons for choosing these microorganisms are discussed below.

Both tailings and cap water are important inocula for an oil sands end pit lake. Tailings microbial community can be very distinct in different operators tailings ponds (Foght et al., 2017). Metagenomic studies revealed that bacterial and archaeal communities are very rich and diverse in the tailings, and eukaryotic groups including fungi and algae are also detected in the tailings (Penner and Foght, 2010; Siddique et al., 2012; An et al., 2013; Stasik et al., 2015; Aguilar et al., 2016; Foght et al., 2017). Cap water has a different redox level (more aerobic), therefore, cap water tends to harbor completely different microbial communities, and aerobes and facultative anaerobes play more significant roles (An et al., 2013; Ramos-Padrón et al., 2010; Foght et al., 2017). More discussion can be found in the Section 1.3.1. *C. kessleri* has been isolated and identified from Syncrude

cyclone overflow water by Dr. Hamed Mahdavi in Dr. Ulrichs lab (Mahdavi et al., 2012, 2013). This strain has been proved to be capable of metal removal (^{53}Cr, Mn, Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, As, ^{88}Sr, ^{95}Mo and Ba) from tailings pond water (Mahdavi et al., 2012, 2013). Intracellular bioaccumulation dominated the mechanism for metal removal with the help of carboxylic functional groups on the surface of *C. kessleri* (Mahdavi et al., 2012, 2013). *C. kessleri* has also been proven to uptake CO_2 effectively (Kasiri et al., 2015b). The growth of *C. kessleri* can be stimulated by the addition of nitrogen and phosphorous, and when ammonia-nitrogen is not available, nitrate-nitrogen will be utilized instead (Kasiri et al., 2015a).

B. braunii is a unicellular green microalga and the strain used in this study was obtained from UTEX Culture Collection of Algae at the University of Texas at Austin (UTEX 2441, USA). Botryococcane ($C_{34}H_{70}$), first discovered in Sumatran crude oils, and then in a variety of coastal bitumen sites in Australia, was a biological marker derived from *B. braunii* (McKirdy et al., 1986; Banerjee et al., 2008). *B. braunii* is considered a promising renewable biofuel source because of its hydrocarbon-rich feature (Banerjee et al., 2008; Tasić et al., 2016). Cells of *B. braunii* can also synthesize exopolysaccharides dominated by galactose (Banerjee et al., 2008). Due to the rapid growth and exopolysaccharides synthesis capacity, the ability to fix CO_2 by *B. braunii* in OSPW was observed (Kasiri et al., 2015a). In tailings pond water, *B. braunii* could still flourish and support high biomass growth in the presence of high levels of NA (Leung et al., 2003). In this study, the same strain was used in the BML cap water remediation application again.

S. pasteurii (ATCC® 11859) is a urea-hydrolyzing bacterium purchased from Cedarlane (ON, Canada), and this bacterium is able to carry out effective microbially induced calcite precipitation (MICP) processes in sands and clay and has been utilized in biocementation processes in civil engineering, reservoir engineering and restoration engineering (Ferris et al., 1996; DeJong et al., 2010; Bhaduri et al., 2014; Liang et al., 2015; Moravej

et al., 2018). The mechanism involved in the MICP process is shown in Fig. 1.5 (DeJong et al., 2010). If urea is added, the urea hydrolysis process will occur: NH_3 and CO_2 are generated, and pH increases through the reaction of ammonia and water. *S. pasteurii* contains an enzyme (urease) that can break down urea in a one-step process and makes urea hydrolyze more rapidly (Strope et al., 2011). The bacterial cells attract the calcium ions and bridge the particles and clays to form precipitation with carbonate ions (DeJong et al., 2010).

Several conditions are required for the MICP process to occur: alkaline pH, Ca^{2+}, ${CO_3}^{2-}/{HCO_3}^{-}$ ions, and availability of nucleation sites (DeJong et al., 2010). *S. pasteurii* has been shown to provide nucleation sites for calcite precipitation and act as the effective catalyst bacteria for MICP in the tailings (Ferris et al., 1996; DeJong et al., 2010; Liang et al., 2015). Also, MICP has been proven to increase the shear strength of the tailings samples (Liang et al., 2015). Attachment of organics to the oil sands tailings particles increases the negative surface potentials of the particles, which inhibits flocculation and aggregation, lengthening the tailings dewatering process (Chandrakanth et al., 1996; Adegoroye et al., 2010; Liang et al., 2015). If MICP occurs, calcite crystals will bridge particles and clays, which provides opportunities for more rapid aggregation and flocculation of particles in EPL cap water to mitigate turbidity issues. The required conditions for MICP can be partially found in the EPL cap water as well: pH of cap water is expected to be about 7.5−9.0, Ca^{2+} levels are expected to be moderate (10−30 mg L^{-1}), and moderate ${CO_3}^{2-}/{HCO_3}^{-}$ levels are expected as well ($\sim$700 mg L^{-1}). If *S. pasteurii* cells are supplied along with other required conditions, the turbidity mitigation can potentially be achieved.

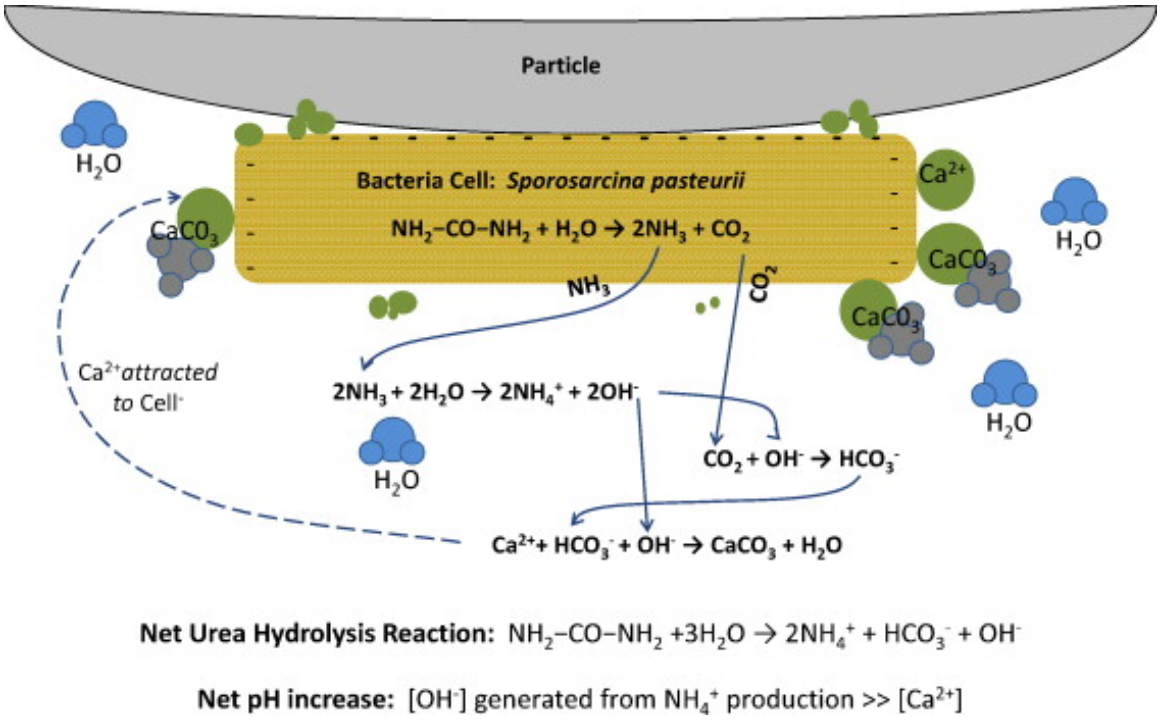

Figure 1.5: The mechanism involved in the MICP process is shown (DeJong et al., 2010).

1.4 Objectives

The purpose of this thesis research was to use microbial applications to improve cap water quality in an oil sands end pit lake. All materials were obtained directly from BML. To facilitate the establishment of a healthy ecosystem in BML, it is crucial to improve cap water quality. This involves: removing residual bitumen, mitigating turbidity, and biodegrading the constituents of concern (model NA in this thesis). This thesis will investigate the potential of BML indigenous microbes to remove bitumen with/without biostimulatory amendment and the potential of microbial consortia, generated by microalgae and bacteria (both indigenous and foreign) to mitigate turbidity and remove model NA compounds through biodegradation. These data may be instrumental in the

validation of EPLs as an oil sands tailings-remediation concept.

The specific objectives are highlighted, followed by the novelty of this work:

1. *Explore the biological treatment of the residual bitumen content using the bitumen extracted from the oil sands in lab and the bitumen obtained from the Base Mine Lake.*

   Research question: Will bitumen be a source of toxicity in bitumen-containing water? Can the residual bitumen found in BML be biodegraded by indigenous microorganisms?

   Hypothesis: We hypothesized that the indigenous microorganisms would be capable of biodegrading the residual bitumen in BML cap water. It has been reported in the literature that many indigenous microbes to oil sands tailings and OSPW, such as *Pseudomonas*, *Acinetobacter* etc., potentially have the capacity to degrade bitumen (Traxler, 1966; Dashti et al., 2015). However, biodegradation is challenging due to the reduced bioavailability of the bitumen. Therefore, supplying more easily degradable carbon source was hypothesized to stimulate the biodegradation process. The microbial community in BML tailings would also experience a shift in the population or species composition in response to the high concentration of bitumen. Analysis of the microbial community over the whole course of the experiment could provide information and guidance for future work. For the first time, the indigenous tailings microbial community over the course of exposure to the bitumen content and the water chemistry were assessed, and the information would provide insights for the potential bitumen degraders and bitumens remediation strategy.

2. *Investigate the model NAs degradation by the microalgae, the BML cap water microbes, and the co-culture of the two, and propose the potential pathway.*

   Research question: Will the tested algae species and BML cap water microbes

be able to biodegrade or absorb model NA? Will the co-culture of the two have a more effective NA degradation?

Hypothesis: Both *C. kessleri* and *B. braunii* showed growth in the OSPW (Kasiri et al., 2015a). *C. kessleri* was isolated from cyclone overflow water from a bitumen extraction facility, and this strain showed a strong metal removal capacity in the earlier study (Mahdavi et al., 2012). We hypothesized that both algae would tolerate model NAs and possibly biodegrade model NAs. BML cap water microbes have been well adapted to the OSPW environment. It was hypothesized that BML cap water microbes would biodegrade model NAs. If co-cultured with alga (*C. kessleri*), BML cap water microbes would develop a synergistic relationship with algae: the mixed consortia would have a more effective degradation of the model NAs by enhancing the growth and diversity of BML cap water microbes. Model NA degradation pathways were identified with a liquid chromatography-mass spectrometry (LC-MS), and the bacterial community analysis was assessed in the presence of the algal biomass. The improvement of model NA removal by co-culturing bacteria and algae was proven in a well-controlled lab environment.

3. *Determine if the turbidity mitigation strategies using microbial additions will be effective.*

   Research question: Can MICP processes help reduce turbidity in cap water? What conditions will MICP require to occur in BML cap water? Will algal additions help mitigate turbidity issues in BML cap water?

   Hypothesis: We hypothesized that both the MICP bacterium (*S. pasteurii*) and algal addition would help mitigate the turbidity in the cap water effectively. Additionally, it was hypothesized that urea addition would be required to maximize the effectiveness of the MICP process. The calcite precipitation resulting from this process would settle to the tailings:cap water interface. During the turn-over pe-

riod in the lake, turbidity was expected to decrease to the previous level within days with the help of MICP process. For the first time, microbially induced calcite precipitation process was introduced to the BML cap water for its potential application, and algal addition was tested for turbidity mitigation.

1.5 Thesis organization

This thesis contains six chapters. Chapter 1 provides a brief introduction to the thesis. The first chapter also reviews the relevant literature about Alberta oil sands, oil sands end pit lakes, BML, and related research in microbial applications. This chapter focuses more on the research and studies that are not covered in the introduction sections in Chapters 2, 3, 4, and 5. Chapters 2 and 3 investigate the biodegradation of bitumen by using a commercial biological amendment and BML indigenous microbial community with/without acetate treatment, respectively. Chapter 4 presents the results of model naphthenic acids degradation by algae and BML indigenous microbial community, and also proposed potential degradation pathways of the tested model naphthenic acids. Chapter 5 explores how microbial culture additions can mitigate cap water turbidity by using both indigenous and exogenous microbial communities. Chapter 6 summarizes the contributions of this work and also makes recommendations for future research. A bibliography is presented after the main chapters. Appendices are in the end of the thesis and include data, figures, tables and methodologies that are helpful to understand the thesis.

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Chapter 2

A biological treatment reduces bitumen contents in oil sands tailings

2.1 Introduction

Oil sands bitumen is recovered through the Clark Hot Water Extraction process, which involves adding hot water and caustic soda to the oil sands, generating a slurry and allowing the froth containing bitumen to separate from the sands and clay (Clark and Pasternack, 1932). Oil sands consists of 6−13 wt% of bitumen, and the extraction efficiency is usually about 90%. The produced tailings consequently contains 1−5 wt% bitumen content remaining uncovered (Foght et al., 2017). The bitumen content in the tailings is characterized by Dean Stark extraction by industry and academia along with the other two phases: water content and solids content (Dean and Stark, 1920).

The residual bitumen in the tailings has long been observed in tailings ponds and must

be removed or sequestered before the tailings ponds can be reclaimed (Nix and Martin, 1992; Allen, 2008; Quagraine et al., 2005; Sobkowicz, 2013; Alam and Shang, 2017). The unrecovered bitumen in the tailings is often overlooked as a possible source of additional NAs (Quagraine et al., 2005). During the microbial degradation of the residual bitumen, carboxylic acids can be formed as metabolic intermediates, which might include NAs (Nascimento et al., 1999; Quagraine et al., 2005). The chemical composition of bitumen suggests that it will persist in the environment; however, many studies have demonstrated that bitumen is susceptible to microbial degradation under various conditions (Wyndham and Costerton, 1981a,b; Lin et al., 1989; Ait-Langomazino et al., 1991; Wolf and Bachofen, 1991; Potter and Duval, 2001). The presence of the residual bitumen might contribute to the toxicity in the surrounding aquatic environment.

UltraZyme is a proprietary blend of microorganisms, enzymes and organic carrier developed by Cypher Environmental Ltd., and is designed to remediate various organic contaminants. This chapter investigated the potential for UltraZyme as a biological amendment to aid in reclamation of oil sands tailings. Firstly, this biological amendment was amended to tailings: organics in tailings and pore water quality were monitored to understand the interaction between a biological amendment and the tailings. Secondly, bitumen was cultured with the biological amendment with/without the tailings to understand the role of the biological amendment and tailings indigenous microbial community in biodegrading the bitumen.

2.2 Materials and methods

2.2.1 Materials

Three different mature fine tailings (MFTs), one type of FFT, and OSPW were used in this research. MFT-Mix is a mixture of tailings from several companies. MFT-D1 and MFT-D2 come from the same tailings pond at 11.2m and 12 m below the tailings/water interface, and can be considered replicates. FFT-S is from just below the tailings/water interface. MFT-D1, MFT-D2, FFT-S and OSPW are from the same tailings pond.
MFT material was used in the dewatering experiments, as MFT has reached a stable solids content of ∼30 wt%, and further dewatering of MFT will require hundreds of years without additional treatment. FFT was used in the biodegradation experiments since the interface region of the tailings pond is the most biologically active. Mineral Medium (see Appendix B) served as the nutrient source for the degradation studies. To extract bitumen from oil sands, a toluene-based extraction process was used (see Appendix A). Bitumen mixing was aided by 3 mm glass beads (Fisher Scientific, Canada).

2.2.2 Chemical analysis

Pore water was extracted from the MFT by centrifugation at 10000 × g for 20 min, and preserved at 4 °C in glass vials for further chemical analysis. The water released during the experiment was sampled directly for comparison to the initially extracted pore water. CO_2 was measured using an Agilent 7890A gas chromatography equipped with a thermal conductivity detector (GC-TCD; Agilent HP-PLOT/Q column: 30 m × 320 µm × 0.2 µm) (details in Appendix A). NAs were extracted from pore water (Lu et al., 2013; Brown, 2014), derivatized by N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA), and analyzed using a gas chromatography equipped with a flame ioniza-

tion detector (GC-FID; Agilent 7890A; Agilent DB-1HT column: 30 m × 320 µm × 0.10 µm) (details in Appendix A). DOC was measured using a Shimadzu Model TOC-L_{CPH}. Samples for DOC analysis were filtered with a 0.45 µm membrane filter and diluted to a working range (5−100 mg L^{-1}) (details in Appendix A).

The toxicity of aquatic samples was analyzed using Microtox® bioassay with the 81.9% Basic Test protocol and an incubation time of 5 min (Microtox® 500 Analyzer, Azur Environmental). The Microtox® bioassay utilizes the bioluminescent bacterium, *Vibrio fischeri* (Blaise and Férard, 2005; Anderson et al., 2011). The light emission from the bioluminescent bacterium was measured and analyzed with MicrotoxOmni software. The percentage of the sample needed to inhibit 20% or 50% light emission of this bioluminescent bacterium corresponds to the inhibitory concentration 20% (IC_{20}) or inhibitory concentration 50% (IC_{50}) value (Blaise and Férard, 2005). Phenol (1 g L^{-1}) is usually considered as the reference toxicant, which has a IC_{50} of 10−30. IC units can be converted to toxicity unit (TU) to better view trends in toxicity. The equation for TU is shown below:

$$TU = 100 \div IC_{50} \tag{2.1}$$

The composition of the tailings was determined using the Dean Stark extraction process (Dean and Stark, 1920), which has long been the industrial standard procedure for analysis of solids, water, and bitumen content of oil sands and tailings. The Dean Stark extraction procedure can be found in the Appendix A. The whole bitumen content separated by Dean Stark extraction was classified into petroleum hydrocarbon fractions (F2: C6−C10, F3: C16−C34) and bitumen content.

The bitumen content in the biodegradation experiment without tailings samples was determined via a simplified gravimetric method. After filtration with 8 µm filter paper, the residue was dissolved with toluene so that there was no discernible color in the solvent. The mixture was collected into a clean, bake-dried beaker, and the mass of the mixture

was determined after evaporation of toluene and baking at 105 °C to remove residual water.

F2 (C10−C16) and F3 (C16−C34) fractions were determined by analyzing the hydrocarbon fraction from the samples collected from the Dean Stark extraction on an Agilent 7890A GC-FID (Agilent DB-1HT column: 30 m × 320 µm × 0.10 µm). O-terphenyl was used as the internal standard to recalibrate the result by using internal standard recovery (details in Appendix A).

2.2.3 Column experiments

The column experiments were conducted with MFT-Mix, MFT-D1, and MFT-D2 under different conditions to better understand the biological treatment process. Bitumen content and petroleum hydrocarbons (F2 and F3 fractions) were measured in the solid phase; and pH, NAs, DOC, and toxicity were measured in the expressed water at their respective prescribed frequencies. Three sets of tests are summarized in Table 2.1. All experiments were conducted in pre-autoclaved 1 L columns containing 800 mL tailings. OSPW (20 mL), with/without UltraZyme or nitrogen, was added on top of the tailings (setup shown in Fig. C.1). Test 1 was conducted at 22 °C in triplicate for 60 d, Test 2 was conducted at 20 °C in duplicate for 55 d, and Test 3 was conducted in duplicate at 10 °C, 20 °C and 50 °C for 140 d, 56 d and 28 d, respectively.

Table 2.1: Treatment groups for column experiments.

Test 1: Influence of nitrogen using MFT-Mix		
Group	UlratZyme[4]	Nitrogen
T[1]	-	-
U[2]T	+	-
N[3]T	-	+
UNT	+	+
Test 2: Influence of UltraZyme dosage using MFT-D1		
Group	UltraZyme	
T	-	
0.5UT[5]	+	
1.0UT	+	
1.5UT	+	
2.5UT	+	
5.0UT	+	
Test 3: Influence of temperature and autoclaving pretreatment using MFT-D2		
Group	UltraZyme[7]	
T-10 °C	-	
UT-10 °C	+	
T-20 °C	-	
UT-20 °C	+	
U(-)T[6]-20 °C	+(sterile)	
T-50 °C	-	
UT-50 °C	+	

[1]T for MFT

[2]U for UltraZyme

[3]N for nitrogen (ammonium nitrate) at the concentration of 150 mg L^{-1}

[4]1.5 g UltraZyme L^{-1} of MFT applied to + treatments

[5]Number+UT represents the UltraZyme dosage, e.g., 0.5 UT means 0.5 g of UltraZyme per L of MFT

[6](-) for sterilization of the symbol preceding the sign, e.g., U(-)T-20 °C means the sterilization of UltraZyme (sterilization achieved by addition of sodium azide and autoclaving) and nonsterile tailings

[7]1.5 g UltraZyme L^{-1} of MFT applied to + treatments

2.2.4 Biodegradation experiments

Aerobic biodegradation of hydrocarbons occurs at a higher rate than anaerobic biodegradation (Ait-Langomazino et al., 1991; Wolf and Bachofen, 1991); therefore, experiments were carried out under aerobic conditions in this study. Bitumen biodegradation was carried out in 500 mL Fisherbrand™ reusable glass media bottles with septum-customized caps, allowing sample withdrawal via needle penetration (setup shown in Fig. C.2). All bottles were placed on a horizontal shaker at 150 rpm and 20 °C with air renewals (0.22 µm filtered-sterile air). The frequency of air renewal was every 10 d for the active groups and every month for control groups. Bottles, caps, and glass beads were autoclaved (121 °C, 100 kPa) before use to eliminate microbial contamination. Three replicates were set up for each treatment, and details are shown in Table 2.2. Mineral Medium (250 mL, recipe in Appendix B) and glass beads (20 g) were added in all groups. When indicated, the following were also added: 0.25 g UltraZyme, 1 g bitumen, and 20 g FFT. Tailings were sterilized by autoclaving and the addition of sodium azide (2 wt%), while bitumen was sterilized by autoclaving only.

Table 2.2: Treatment groups for bitumen biodegradation experiments.

Group	UlratZyme	Bitumen	FFT
U[1]B[2]	+	+	-
UB(-)[3]	+	+ (sterile)	-
U	+	-	1
BT[4]	-	+	+
BT(-)	-	+	+ (sterile)
Control	-	-	-

[1]U for UltraZyme

[2]B for bitumen

[3](-) for sterilization of the content before the sign, e.g., UB(-) means UltraZyme and sterilized bitumen

[4]T for FFT

Microbial biodegradation is quantified by measuring CO_2 production and DOC (Dibble and Bartha, 1979; Ait-Langomazino et al., 1991; Wolf and Bachofen, 1991; Margesin and Schinner, 2001; Clemente et al., 2004; Decesaro et al., 2016). To evaluate the bitumen biodegradation process, the following parameters were measured: 1) CO_2 production was monitored and quantified in the headspace to show mineralization of the hydrocarbons, 2) DOC concentration was monitored as a nonspecific parameter to indicate soluble metabolic intermediates in the liquid fraction, 3) liquid phase toxicity was measured using the Microtox® bioassay, 4) bitumen was recovered with toluene extraction and measured gravimetrically at the end of the experiment. All experimental groups except the Group U were monitored for 100 d. Monitoring of Group U was discontinued after 45 d when headspace CO_2 plateaued and reached a level equivalent to Group UB.

2.3 Results and discussion

2.3.1 Column experiments

Organics in tailings

Hydrocarbons likely served as a carbon and energy source for the microbial consortium in UltraZyme and the indigenous microorganisms in the tailings. Therefore, the hydrocarbon composition was compared before and after incubation to determine the preferred substrate in the tailings. To determine if UltraZyme influenced the released water chemistry, pH, DOC, NAs, and toxicity were measured during the test period.

F2 and F3 hydrocarbons, and bitumen content were measured in the tailings for all treatment groups (MFT-Mix, MFT-D1, MFT-D2) before and after incubation (Fig. 2.1; Table D.1). The MFT-Mix had the lowest concentrations of organics, while MFT-D1 had the highest. MFT-D2 had a similar profile to MFT-D1 (these samples are from the

same depth and pond).

As shown in Fig. 2.1a (MFT-Mix), Group T and Group NT did not exhibit a significant change in F2, F3 or bitumen content, and Group UT and UNT had decreased in F2, F3 and bitumen. The greatest change was observed in Group UNT where bitumen content decreased from 16.1 mg g^{-1} to 12.5 mg g^{-1}. In Figs. 2.1b and 2.1c (MFT-D1 and MFT-D2), no significant change was observed in F2 or F3 fraction, while bitumen content decreased greatly by 30.0−46.2% for all UltraZyme amended columns, regardless of dosage and temperature. For the different MFTs, differences in carbon removal indicate different carbon source preferences in the presence of UltraZyme. As discussed earlier, UltraZyme contains microbes, enzymes and hydrocarbons. The indigenous microbial communities may interact with the UltraZyme consortium and compete for the preferred carbon sources. Also, nutrients, such as nitrogen and phosphorous, could be very different in these MFTs, also possibly affecting metabolic processes and substrate preference (Leahy and Colwell, 1990; Pandey et al., 2009).

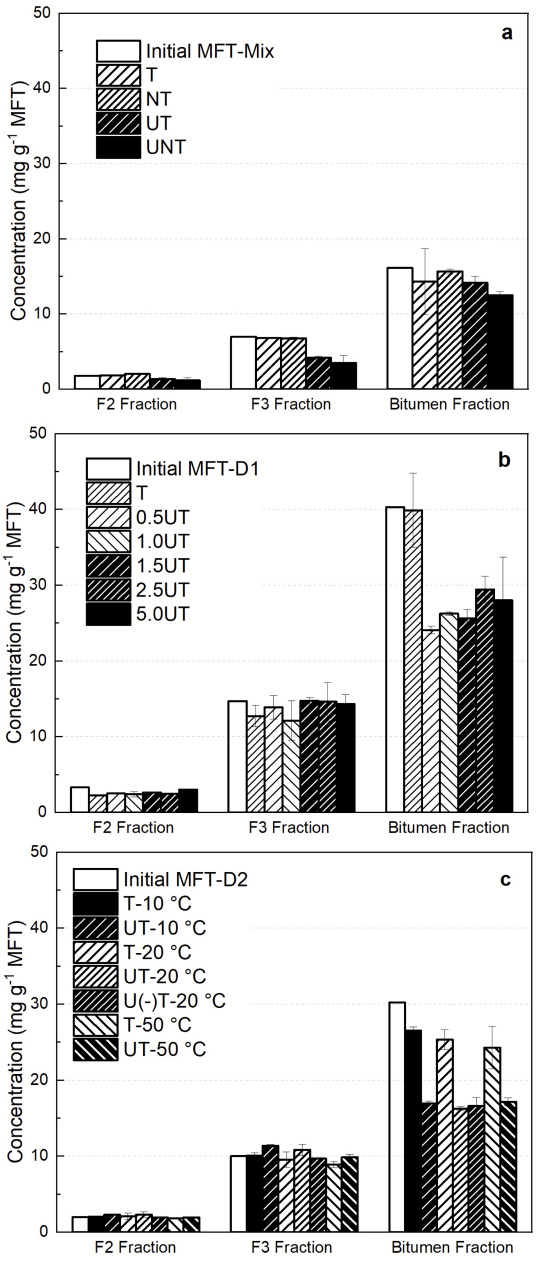

Figure 2.1: Change in hydrocarbon content over the course of the experiment in a) MFT-Mix (triplicate columns at 22 °C); b) MFT-D1 (duplicate columns at 20 °C) and c) MFT-D2 (duplicate columns at 10 °C, 20 °C and 50 °C). Results are presented as an average (n = 3 for MFT-Mix, n = 2 for MFT-D1 and MFT-D2) ± one standard deviation.

Pore water analysis

The released pore water from CT amended with gypsum is high in calcium (Matthews et al., 2002), as is pore water released from tailings amended with other calcium-containing flocculants. In contrast, A biological amendment (UltraZyme as an example) has the potential to improve the quality of released pore water, allowing water to be recycled for hot water extraction of bitumen. To determine whether UltraZyme improves water quality, pH, NAs, DOC, and toxicity were measured at the beginning and the end of the experiment.

For all groups, pH remained relatively constant during the test period (MFT-Mix: 8.5−8.7, MFT-D1: 8.5−8.9, MFT-D2: 8.7−9.0; shown in Table D.2). For MFT-Mix, DOC levels also remained relatively constant (shown in Table D.3). MFT-D1 had a much higher initial DOC ($\sim$200 mg L^{-1}). Increasing UltraZyme dosages resulted in greater DOC removal, as shown in Fig. C.3, however, limited enhancement in DOC removal was observed at the highest dosages. The dosage of 2.5 g L^{-1} resulted in the highest DOC removal (27.7 $\pm$ 10.8%). MFT-D2 had a lower initial DOC concentration ($\sim$70 mg L^{-1}, shown in Table D.3), and DOC was not significantly reduced except for in Group UT-50 °C, where the DOC decreased by 16.4 $\pm$ 1.3% after 28 d. Because a higher reduction was seen at 50 °C, the columns at lower temperatures may have exhibited a similar level of DOC removal if time permitted.

MFT-Mix pore water had an initial NA concentration of 69.7 mg L^{-1}. In Group T and Group NT, NA concentrations did not decrease. In Group UT and UNT, NA concentration decreased by 46.4 $\pm$ 13.8% and 38.7 $\pm$ 3.3%, respectively. NAs concentration (initially 35.8 mg L^{-1}) did not significantly change in MFT-D1. NA was not measured in MFT-D2.

Acute toxicity by Microtox® bioassay was measured at the beginning and the end of the experiment, as shown in Fig. 2.2. MFT-Mix did not exhibit any reduction in tox-

icity in any of the treatments (Fig. 2.2a). In contrast, reduced toxicity was observed for MFT-D1 amended with UltraZyme. The initial toxicity was around 2 TU, and after 55 d, all UltraZyme amended groups had toxicity reduced by 46.9−53.0% (final toxicity: 1.0−1.1 TU). The unamended treatment exhibited a 13.8% toxicity reduction. MFT-D2 had an initial toxicity of 1.5 TU. At 10 °C and 20 °C, UltraZyme amended groups had toxicity reduced by 26.6−33.3% (final toxicity: 1.0−1.1 TU). Autoclaved UltraZyme amended treatments had a comparable decrease in toxicity (33.3%) to the non-autoclaved UltraZyme amended treatments at 20 °C. However, columns incubated at 50 °C were not statistically-significantly reduced in toxicity. This may be because enhanced microbial activity could break down larger hydrocarbons and produce smaller, more toxic molecules (Klamerth et al., 2015).

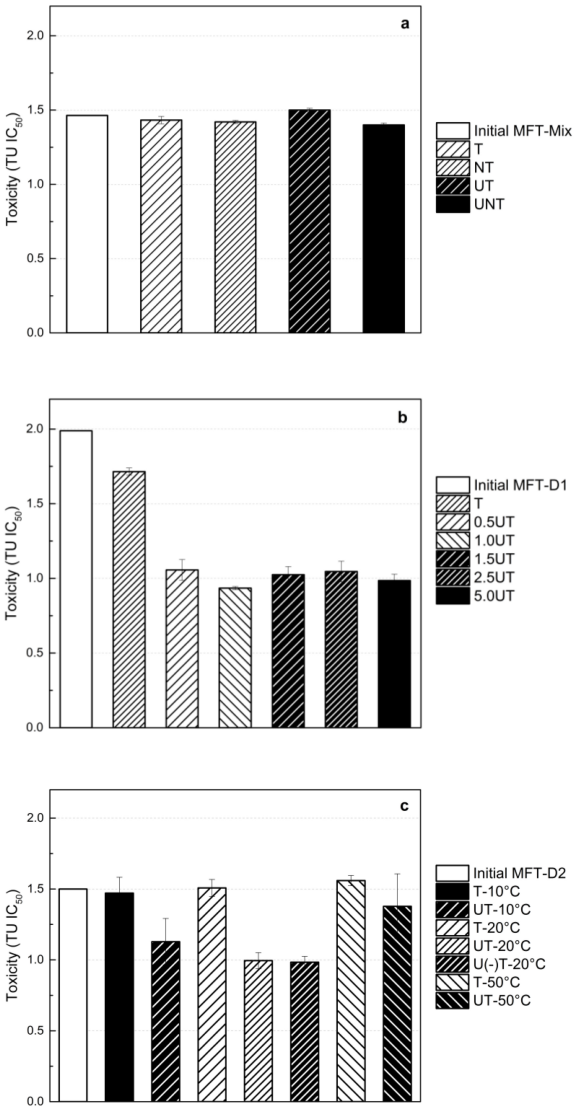

Figure 2.2: Released pore water toxicity before and after treatment from a) MFT-Mix (triplicate columns at 22 °C), b) MFT-D1 (duplicate columns at 20 °C), and c) MFT-D2 (duplicate columns at 10 °C, 20 °C and 50 °C). Results are presented as an average (n = 3 for MFT-Mix, n = 2 for MFT-D1 and MFT-D2) ± one standard deviation. TU resultes are converted from the Microtox IC_{50} test results.

Experiments using MFT-D1 and MFT-D2 did not show a significant reduction in NA and DOC in released pore water, however the released pore water toxicity was decreased. The analytical techniques chosen in this study were unable to track the detailed changes in the composition of organic compounds. Previous research has confirmed that classical NA (O_2^-) are the most predominant compounds contributing to acute toxicity (MacKinnon and Boerger, 1986; Morandi et al., 2015; Verbeek et al., 1993, 1994), but nonacidic species (O^+, O_2^+, SO^+, NO^+) also can contribute to the acute toxicity (Morandi et al., 2015). Therefore, it is likely that the composition of organics has changed with Ultra-Zyme treatment: complex organics may have been metabolized into simpler and less toxic molecules, which helps mitigate the toxicity of the released pore water (Leahy and Colwell, 1990).

Microtox® bioassays have been widely used to study acute toxicity in OSPW. In most studies, 1.0 TU (or lower) is considered completely detoxified (Scott et al., 2008; Toor et al., 2013; Dong et al., 2015). Adsorption with petroleum-coke effectively reduced OSPW acute toxicity from 4.3 TU to 1.1 TU (74.4% reduction) (El-Din et al., 2011). A laboratory scale photocatalysis system (UV_{254} florescent lamps) in the presence of TiO_2 catalyst realized a 71.2% reduction in acute toxicity when treating the OSPW NA extract in deionized water (Mishra et al., 2010). In a study by Yue et al. (2016), a continuous flow biofilm reactor demonstrated 40% degradation of acid extractable organics and 73.3% reduction in toxicity (untreated OSPW: 41.97 TU, and treated OSPW: 11.21 TU) (Yue et al., 2016). Toor et al. (2013) investigated a simulated wetland microcosm system with a short (40 d) and long (400 d) hydraulic retention time (HRT) and nutrient addition to detoxify Syncrude OSPW (untreated: 2.0 TU) and Suncor OSPW (untreated: 1.3 TU). A simulated wetland treatment with a long HRT, both with and without nutrients did not statistically-significantly reduce toxicity. However, treatment with a short HRT without nutrients exhibited toxicity reduction from IC_{20} 20 to IC_{20}

38. Klamerth et al. (2015) used ozonation to treat OSPW and demonstrated higher toxicity after ozonation, indicating that degradation products might have contributed to the enhanced toxicity. Martin et al. also used ozonation to treat OSPW and observed slightly reduced toxicity from IC_{20} 5 to IC_{20} 12 (Martin et al., 2010). Treatment with UltraZyme achieves complete detoxification (1.0−1.1 TU) for the MFT sources used in this study, which is equivalent or better than previously tested detoxification methods. The column experiments showed that UltraZyme can detoxify the released pore water. However, it is not known why UltraZyme interacts differently with MFT from different sources. A comparison of PHC fraction data and toxicity data suggest that when bitumen content decreased in tailings (MFT-D1 and MFT-D2), the released pore water toxicity also decreased. The opposite is also true, as MFT-Mix did not show a substantial change in bitumen content and there was no corresponding improvement in released pore water toxicity. Therefore, bitumen reduction in the tailings phase might have contributed to a different composition of organics expressed into the pore water, leading to the difference in toxicity. The analytical techniques used in this experiment did not allow for a detailed analysis of organic composition, therefore this theory cannot be confirmed. To determine whether UltraZyme can degrade bitumen, and to determine the contribution of bitumen to toxicity, bitumen biodegradation experiments were designed and conducted.

2.3.2 Biodegradation experiments

CO_2 and DOC

Biodegradation experiments were set up using sealed bottles with customized caps allowing for sample withdrawal. Headspace pressure was measured while injecting a gas sample (200 µL) into a GC-TCD to accurately determine CO_2 concentration. Air renewal

introduced a negligible amount of CO_2 into the system. Headspace CO_2 concentration and DOC data are shown in Fig. 2.3. Table 2.3 summarizes CO_2 production rates during the rapid growth periods.

CO_2 production rates in UltraZyme-amended groups (UB, UB(-) and U) were higher than Group BT (Table 2.3). UltraZyme contains a mixture of microorganisms, enzymes and organic carrier, and these microorganisms can produce CO_2 by degradation of UltraZymes organic carrier. In Group UB and Group UB(-), both bitumen and UltraZyme were the available carbon sources, while in Group U, the only carbon source was UltraZyme. Group U produced 220 mg L^{-1} of CO_2, and Group UB and UB(-) produced a similar final amount of CO_2 (230 mg L^{-1} and 200 mg L^{-1}, respectively). Therefore, it is likely that a limited amount of bitumen was removed.

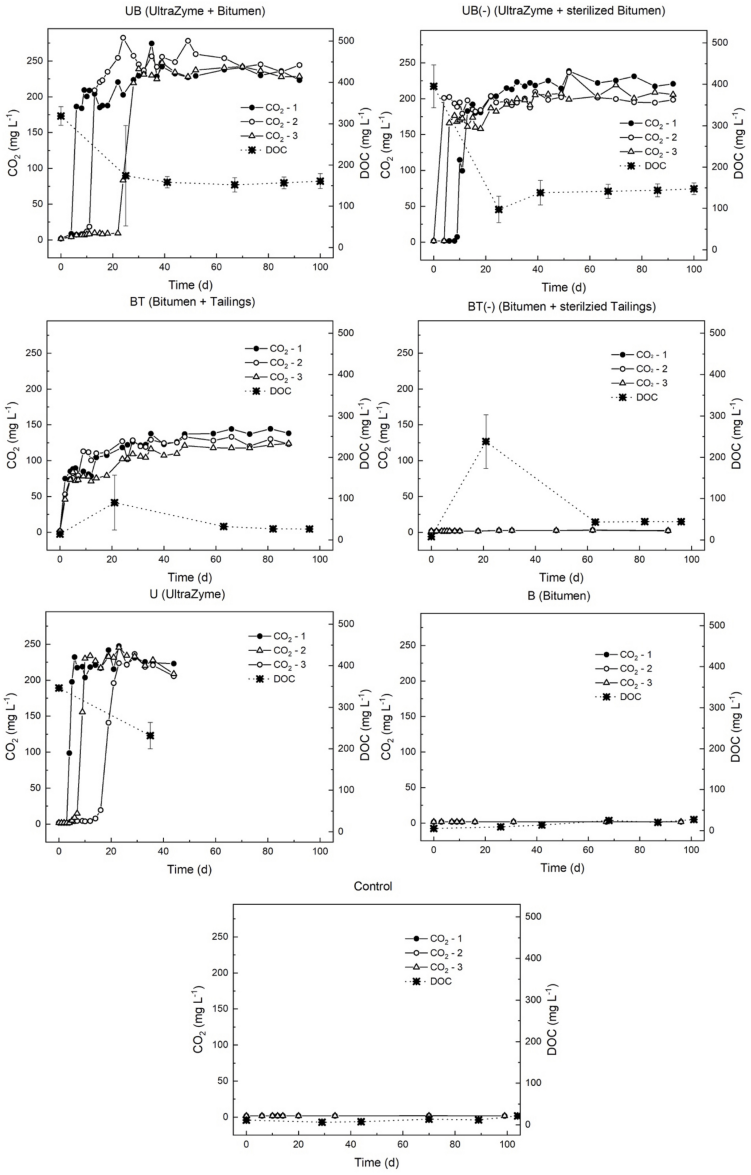

Figure 2.3: CO_2 in the headspace and DOC in the aqueous phase, were measured over a period of 100 d in bitumen-amended biodegradation experiments. DOC data are presented as an average (n = 3) ± one standard deviation, and all triplicate CO_2 data are shown separately due to different lag times in degradation. The black circle, hollow circle, and hollow triangle represent CO_2, and the star symbol represents DOC. Group U was discontinued after the CO_2 production plateaued.

Table 2.3: CO_2 production rates (mmol L^{-1} d^{-1}) in the headspace were calculated during individual rapid production periods. Group BT(-), Group B and Control CO_2 production rates were not significant, and are not presented in this table. Linear regression parameters are presented as r^2.

Group	Replicate 1		Replicate 2		Replicate 3	
	rate (mM L^{-1} d^{-1})	r^2	rate (mM L^{-1} d^{-1})	r^2	rate (mM L^{-1} d^{-1})	r^2
UB	0.9	0.992	1.2	0.908	0.8	1.000
UB(-)	0.9	0.973	2.6	1.000	1.9	1.000
U	1.8	0.963	1.6	0.999	0.7	0.958
BT	0.5	0.840	0.4	0.946	0.4	0.982

Group U had an initial DOC of about 350 mg L^{-1}, indicating the organic content in UltraZyme are soluble and easily degradable. In UltraZyme-amended groups (UB, UB(-) and U), the DOC decrease corresponded well to CO_2 increase. In the presence of bitumen and UltraZymes organic carrier, the microorganisms would preferentially degrade the simpler organics in UltraZyme over the more complex bitumen. Group UB had a 49.5% removal of the initial DOC of about 320 mg L^{-1}, and Group UB(-) had 62.7% removal of the initial DOC of about 400 mg L^{-1}.

In Group BT, significant CO_2 generation was observed without any lag phase, and CO_2 production rates ranged from 0.4−0.5 mM $L^{-1}d^{-1}$ for all replicates during the first 5 d (Table 2.3). CO_2 production plateaued after 35 d with a final CO_2 concentration of ∼130 mg L^{-1}, which was much lower than in the UltraZyme-amended groups. In contrast, when the tailings were autoclaved (Group BT(-)), there was no significant CO_2 production, indicating that the indigenous microorganisms in the tailings contributed to CO_2 production. The carbon source for the CO_2 production in the Group BT may be the bitumen hydrocarbons, but is more likely the residual solvent from the tailings. More

data is needed to confirm the carbon source.

Trends in DOC concentrations in the biodegradation experiments were similar: both experienced an initial increase, followed by a decrease. However the sterilized tailings in Group BT(-) had a higher final DOC of 44 mg L^{-1}, compared to 26 mg L^{-1} in Group BT. Since the tailings were sterilized by autoclaving and addition of sodium azide, the higher DOC concentration in Group BT(-) could be due to the high temperature and pressure pre-treatment. The tailings contain high concentrations of organics, which may have desorbed from the solid tailings phase into the mineral medium during continuous shaking, resulting in higher DOC concentrations. In Group B and Group Control, the CO_2 level remained below the detection limit for the whole test period. DOC in these two groups exhibited a slow increase over time, from 5 mg L^{-1} to 27 mg L^{-1} for Group B, and 11 mg L^{-1} to 21 mg L^{-1} for Group Control. These results suggest that bitumen made little contribution to DOC increase in the liquid phase.

Bitumen weight

Due to the complexity of the sample, bitumen weight in Group BT was measured by Dean Stark extraction, while bitumen weights in UB and B were measured by the simplified gravimetric method. Bitumen weights are shown in Fig. C.4. Bitumen in Group UB decreased slightly over the experiment, but Group B (the control) exhibited a similar decrease. It is not known if the decrease in these groups is from measurement error, or the physical weathering of the bitumen (Wang et al., 1998). Group BT contained tailings and mineral medium together, making the Dean Stark extraction difficult. The apparent increase in bitumen in Group BT may have resulted from solids remaining in the solvent in the reaction flask of the Dean Stark apparatus, or may have been extracted from the tailings (which also contain a small amount of bitumen). Due to the limited data, it is not possible to determine if bitumen was removed or not.

The bitumen used in this study was extracted directly from oil sands ore in the lab, sacrificing the volatile and lighter ends of the bitumen mixture. Therefore, in this study, extracted bitumen consisted of mainly high-molecular weight hydrocarbons, which require more energy for the initial microbial attack during biodegradation. By comparing bitumen weight measurement data with CO_2 data, it is likely that the UltraZyme organic molecules were preferentially degraded over bitumen in the UltraZyme-amended bottles.

Toxicity

The toxicity of the liquid phase of the biodegradation experiments was measured on Day 0, 40, 80, and 100 (Fig. 2.4). In Group Control, toxicity remained low over the experiment period (< 0.1 TU). In Group B, toxicity increased in the aqueous phase over the course of 100 d, where bitumen was the predominant and probably the single contributor to toxicity. In the presence of UltraZyme, toxicity decreased slightly over 100 d in Group UB and Group UB(-). Group UB(-) had extremely high toxicity on Day 0, which might have been due to the autoclaving process, possibly causing small molecules to leach into the liquid phase. These leached molecules may have contributed to the toxicity, and either were either degraded or resorbed to the bitumen afterwards. As discussed above, bitumen was not substantially degraded in Group UB and Group UB(-), however, detoxification was still observed. This may indicate that the addition of easily degradable organics (UltraZyme) could help to detoxify bitumen-polluted aqueous environments.

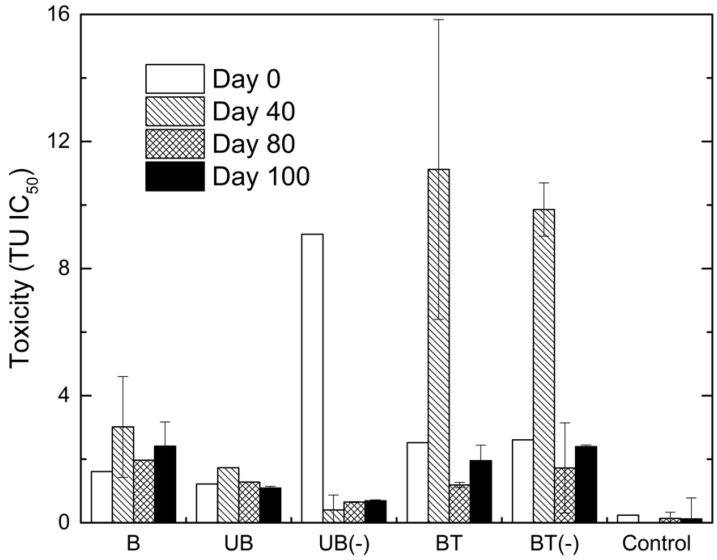

Figure 2.4: Aqueous toxicity over a period of 100 d (day 0, day 40, day 80, and day 100). Results are presented as an average (n = 3) ± one standard deviation.

Toxicity also decreased by 0.5 TU in Group BT and BT(-) after 100 d (Fig. 2.4). On Day 40, both groups experienced an increase in toxicity, corresponding to an increase in DOC. This increase in DOC might be a result of desorption of organic compounds from tailings particles, after which these organics were resorbed or degraded, decreasing the toxicity again.

In the column experiment, UltraZyme was shown to detoxify the released pore water when amended to MFT-D1 and MFT-D2. Reduced toxicity was accompanied by reduced bitumen content in the MFT. Comparing the dewatering and biodegradation results suggest that bitumen may be a potential source of toxic compounds, increasing the toxicity in the aqueous phase. Mitigation of the aqueous toxicity by degradation was observed, indicating that by the addition of more readily degradable organics, co-metabolism or biostimulation could remove toxic hydrocarbons originating from bitumen.

2.4 Conclusions

The application of UltraZyme (Cypher Environmental) for treating tailings was investigated. Tailings characteristics and expressed pore water quality were monitored during the MFT treatment process. A separate series of experiments were established to monitor non-specific indicators (CO_2 and DOC) to determine the effects of the UltraZyme consortium and indigenous tailings microorganisms on bitumen biodegradation. NA concentration decreased on average by 36% in UltraZyme-amended MFT-Mix after treatment. In MFT-D1 and MFT-D2, bitumen content decreased by 30−46% for all UltraZyme-amended columns, regardless of dosage and temperature. UltraZyme amendment also reduced the toxicity of the released pore water from MFT-D1 and MFT-D2, and similar detoxification was observed in the biodegradation experiments. Although the results from the biodegradation experiment do not directly explain why bitumen concentration decreased in the MFT decreased during the column experiments, the biodegradation experiments still demonstrate that the indigenous tailings microbes can remove organics effectively. The biodegradation experiments demonstrate that the indigenous tailings microbes can remove organics effectively, and UltraZyme might have acted as a source of easily degradable organics to biostimulate the native microbes and co-metabolize the heavier organic compounds.

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Chapter 3

Indigenous microorganisms residing in oil sands tailings biodegrade residual bitumen

3.1 Introduction

BML is the first commercial-scale demonstration EPL, and was commissioned by Syncrude in 2013 to support the development of this water capping technique for oil sands reclamation. BML contained FFT and OSPW from Mildred Lake Mine and received fresh water from Beaver Creek Reservoir (BCR). BML will provide the industry, academia and stakeholders invaluable data and experience for future plan and construction for EPLs. Efforts have been made to bioremediate organic compounds in OSPW and oil sands tailings, such as BTEX and NAs. The biodegradation of BTEX, n-alkanes (C14−C18) and naphtha (C3−C14) has been confirmed under methanogenic conditions by oil sands tailings microorganisms (Siddique et al., 2007, 2011; Mohamad Shahimin and Siddique,

2017). NAs were known to be the major toxicity contributor in OSPW (Morandi et al., 2015), and its biodegradation tends to be more difficult where the utilization of chemical pre-treatment, such as advanced oxidation, has shown some success (Brown et al., 2013; Brown and Ulrich, 2015; Zhang, 2016; Zhang et al., 2018). Gamma irradiation treatment was also reported to stimulate the hydrocarbon degraders from the tailings microbial community (VanMensel et al., 2017).

A less-studied aspect of EPLs is the bitumen in the tailings, left behind by the successive incomplete extraction processes. During the comprehensive monitoring program of BML, bitumen was observed to float on top of the lake, especially along the shoreline. The active biogenic gas production created the pathway for the residual bitumen in the tailings to be released into the water cap, and the bitumen started to spread over the water cap (Darling, 2011). This bitumen acts as a hydrocarbon source: as it migrates through the water cap, the hydrocarbons are released and subsequently biodegraded, a process which consumes dissolved oxygen and prevents the establishment of a healthy lake ecosystem. If the bitumen cannot be further degraded or mineralized in situ, the hydrocarbons can contaminate the aqueous environment and the nearby littoral zone.

Hydrocarbon-degrading microbial isolates from sediments of the Athabasca River have been shown to grow on the lighter components of Athabasca bitumen (not on the recalcitrant asphaltene fraction) (Wyndham and Costerton, 1981). Microbial degradation of bitumen (up to 40% removal at 37 °C) was also reported in similarly polluted environments in other regions of the world (Wyndham and Costerton, 1981; Potter and Duval, 2001; Das and Chandran, 2011). Bitumen biodegradation can be enhanced by nutrient addition (nitrogen or phosphorus) and stimulated by addition of more easily biodegradable carbons (Das and Chandran, 2011). The most effective hydrocarbon degradation is usually accomplished under aerobic conditions, while nutrients and temperature are often the most important limiting factors of the process (Das and Chandran, 2011). Because

bitumen is complex, and its biodegradation has been demonstrated to occur under various conditions, site-specific factors are important to the feasibility of in situ remediation. Chapter 2 showed that the addition of a proprietary blend of microbes, enzymes and organics to tailings resulted in reduction in the petroleum hydrocarbon fractions and tailings pore water toxicity. It was unclear whether these changes were caused by the indigenous microbial community or by the added microbes and organic carrier in the commercial product. Therefore, the focus of this chapter was to investigate the ability of the microbial communities in BML to degrade bitumen, and the effectiveness of biostimulation with acetate. The changes in community composition and community responses were assessed by comparing 16S rRNA gene sequence profiles to identify the potential bitumen-tolerant species and bitumen degraders.

3.2 Materials and methods

3.2.1 Materials

All samples were transported to the laboratory in sealed buckets and stored at 4 °C prior to use (bitumen samples were stored for 6 months; water and tailings samples were stored for less than one month). Fluid fine tailings (FFT) were provided by Syncrude Canada Ltd. FFT was sampled at the depth of 12 m below the sediment:water interface at Platform 1 at BML. Extraction technique limitations cause unrecovered bitumen to end up in tailings; this residual bitumen can be observed upon commission of the BML (in this paper, bitumen refers to the residual bitumen in the BML). BML bitumen used in this research was sampled directly from the BML surface. To eliminate moisture content, the bitumen was oven-dried at 105 °C overnight. However, this drying process sacrificed any volatile and semi-volatile hydrocarbon that may have resided within the bitumen.

Clays, sands and other small particles were retained, but vegetation and large stones were manually removed. BML cap water used in this research was sampled from the surface of BML at Platform 1.

3.2.2 Chemical analysis

CO_2 was measured by a gas chromatography thermal conductivity detector. DOC was measured with a Shimadzu Model TOC-L_{CPH}. Acetate was measured by ion chromatography (IC). NAs were measured by GC-FID or by reversed-phase chromatography paired with a linear ion trap-Orbitrap mass spectrometer. Detailed procedures and machine conditions for all methods above can be found in the Appendix A.
Petroleum hydrocarbons (PHC) are grouped into these fractions by using a Canada-Wide Standard: F1 (C6−C10), F2 (C10−C16), F3 (C16−C34), F4 (C34−C50), and F4G-SG (> C50) (CWS, 2003). F4 and F4G-SG fractions are classified into bitumen content in the Chapter 2. F1 fractions were measured prior to submission to Maxxam Analytics and were non-detectable in all samples. All samples were mixed with organic solvent (toluene) and sonicated for greater homogeneity prior to submission to Maxxam. Maxxam then further homogenized the samples. One duplicate was submitted for analysis due to the sample size limitations.

3.2.3 Microbial analysis

Toxicity bioassay

The toxicity of aqueous samples was analyzed using the Microtox® bioassay. The 81.9% Basic Test protocol was followed (Microtox® 500 Analyzer, Azur Environmental) with

an incubation time of 5 min (Anderson et al., 2011). Light emission was measured with MicrotoxOmni software to determine inhibitory concentration 20% (IC_{20}) or inhibitory concentration 50% (IC_{50}) value. Toxicity units, derived from IC_{50} ($TU = 100 \div IC_{50}$), was used to visualize high-level toxicity trends (details in Section 2.2.2).

DNA extraction

DNA was isolated from the tailings phase using the FastDNA™ SPIN Kit for Soil (MP Biomedicals). Up to 500 mg of tailings were used per extraction, following the DNA isolation protocol suggested by the manufacturer.

Bacterial population by qPCR assay

The bacterial population was determined by the qPCR amplification of the RNA polymerase beta subunit (*rpoB*) gene, utilizing rpoB 1698f (5'-AACATCGGTTTGCTCAAC-3') and *rpoB* 2041r (5'-CGTTGCATGTTGGTACCCAT-3') primers (Brown et al., 2013; Nava et al., 2011). The qPCR assay was performed using a Bio-Rad CFX96 optical reaction module conversion of the C1000 Touch thermal cycler. All samples and standards were completed in triplicate, and the amplification data was analyzed using Bio-Rad CFX Manager™ 3.0 software. The reaction followed the protocol suggested by the manufacturer (details in Appendix A). The qPCR assay was performed on DNA samples extracted from the tailings/solid phase at the start and end of the experiment. Each biological duplicate was measured three times (n = 6) for the end of the experiment, and the original tailings samples were measured six times (n = 6) to determine the initial bacterial population density for all groups.

Microbial communities

DNA samples were used for PCR amplification of the V4 hypervariable regions of bacterial 16S rRNA genes, using the primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACVSGGGTATCTAAT-3') (Caporaso et al., 2011). The PCR conditions were 94 °C for 2 min; 30 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min of extension; 72 °C for 6 min. The PCR products were purified and combined in equimolar ratios with the quantitative DNA binding method to create a DNA pool that was further used for sequencing from the adapter. The 16S rRNA gene fragments were sequenced using the Illumina MiSeq platform. The sequences were deposited in Sequence Read Archive (SRA) within the study with accession number SRP131750. Operational taxonomic units (OTUs) were constructed using an identity threshold of 97% and assigned to taxa using the UPARSE pipeline (Edgar, 2013). The community matrix was normalized with the DESeq package (Love et al., 2014). The bacterial communities were ordinated with non-metric multidimensional scaling based on the Bray-Curtis distance matrix using phyloseq (McMurdie and Holmes, 2013).

3.2.4 Biodegradation experiments

Aerobic conditions were used for the biodegradation experiments since the BML water cap is aerobic during the summer and fall season. A second benefit is because oxidative biodegradation is also considered more effective and occurs at higher rates than anaerobic biodegradation (Ait-Langomazino et al., 1991; Wolf and Bachofen, 1991). Experiments were set up in 500 mL Fisherbrand™ reusable glass media bottles with customized septum caps for sample withdrawal. All bottles were placed on a horizontal shaker at 150 rpm at room temperature (20 °C). Aerobic conditions were maintained by a sequential renewal of air (0.22 µm filtered-sterile, every 10 d for the active groups and every month for control

groups). Bottles, caps, and glass beads were sterilized by autoclave (121 °C, 100 kPa). Filtered BML water (500 mL), and glass beads (40 g) were used for all groups. When indicated, the following was also added: sodium acetate (250 mg measured as carbon), 10 g bitumen, and 40 g FFT. FFT was used as the indigenous inoculation used in this study. The four test groups were BML-B, BML-BT, BML-BTC and BML-T (BML: 0.22 µm-filtered BML water, B: bitumen addition, T: tailings addition and C: sodium acetate addition). Two replicates were set up for each group.

3.3 Results and discussion

3.3.1 CO_2 and DOC

Microbial degradation of an undefined and complex substrate (i.e. BML bitumen) can be quantified by monitoring CO_2 production (Wolf and Bachofen, 1991). Biodegradation experiments were set up in sealed bottles with customized caps allowing for sample withdrawal. The pressure in the headspace was also measured to convert the CO_2 concentration within the sealed bottle to the concentration under the ambient atmosphere pressure. Air renewal introduced low amounts of CO_2 into the system but did not significantly contribute to the CO_2 concentration within the bottle. Headspace CO_2 concentration and DOC are shown in Fig. 3.1.

The data indicate that CO_2 production rates are 5.3−58 times greater in the other three groups relative to the control (Fig. 3.1). BML-B and BML-T groups both had linear CO_2 production with rates of 0.17 mg L^{-1} d^{-1} ($R^2 = 0.97$) and 0.91 mg L^{-1} d^{-1} ($R^2 = 0.97$), respectively. BML-BTC and BML-BT had linear CO_2 production during the first 10 d with rates of 9.97 mg L^{-1} d^{-1} ($R^2 = 0.99$) and 6.19 mg L^{-1} d^{-1} ($R^2 = 0.99$), respectively. BML-BTC and BML-BT rates decreased rapidly and concentrations plateaued at 40 d

with final concentrations of approximately 130 mg L^{-1} and 90 mg L^{-1}, respectively.

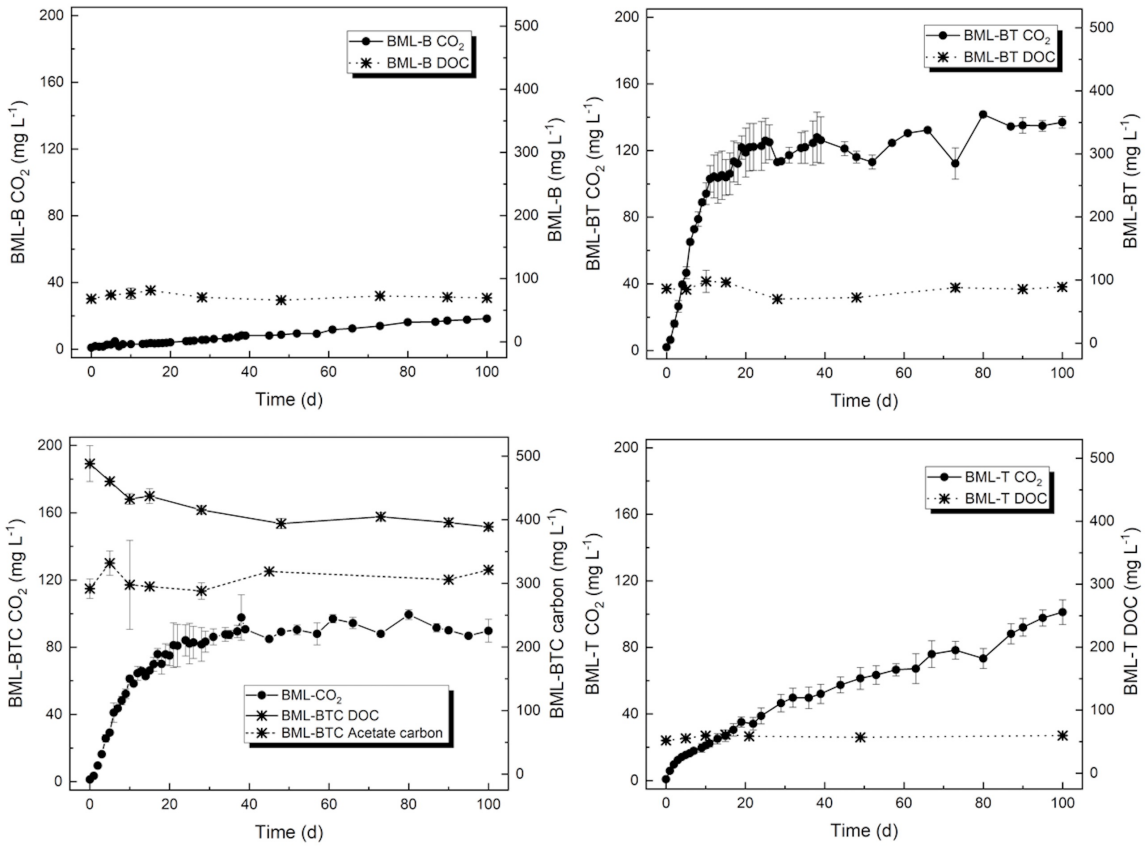

Figure 3.1: Microbial degradation of bitumen measured by CO_2 production in the headspace and DOC concentration in the aqueous phase over a period of 100 d. For BML-BTC group, acetate carbon was also included. Results are presented as an average ± one standard deviation (n = 2). Black circles represent CO_2, and star symbols represent DOC.

3.3.2 Non-aqueous phase organics

Insoluble or slightly soluble hydrocarbons might also be microbially degraded or altered during incubation. Therefore, tailings samples were analysed for petroleum hydrocarbon composition. F2, F3, F4, and F4G-SG data are shown in Fig. 3.2.

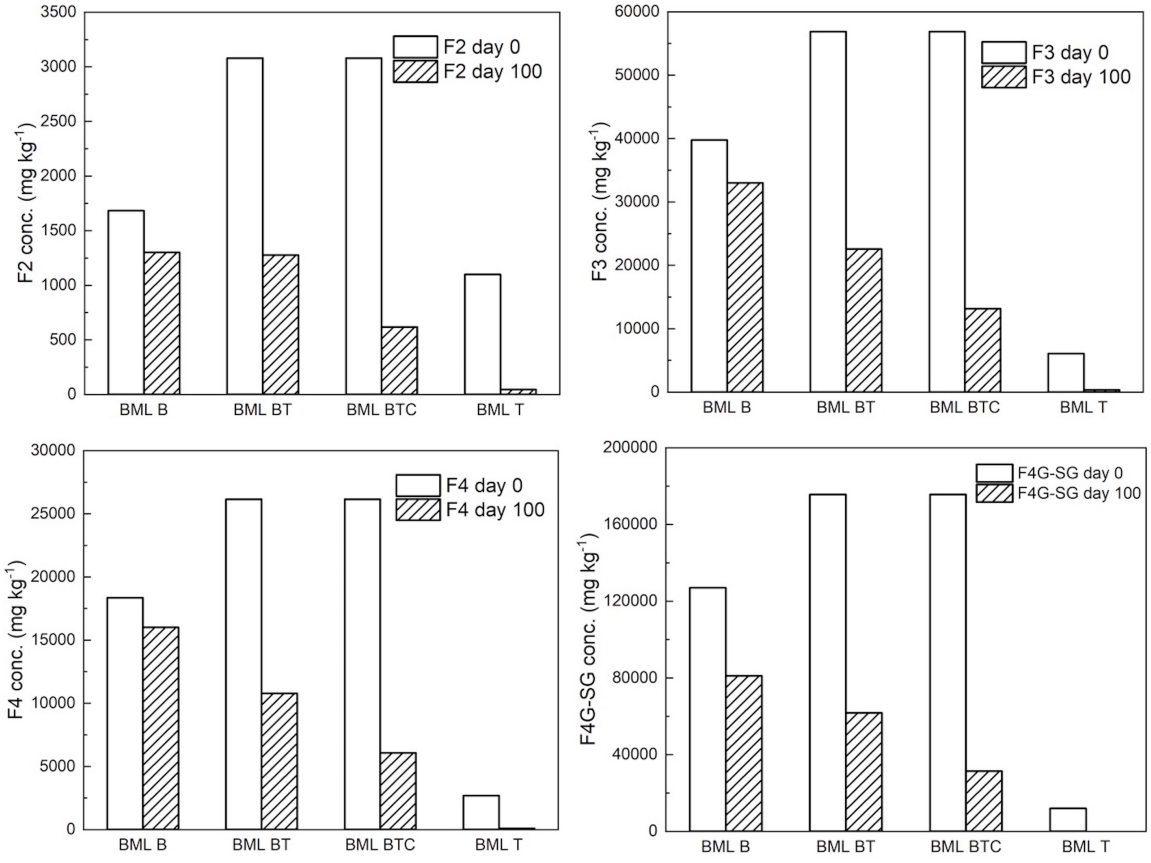

Figure 3.2: Petroleum hydrocarbon contents (F2, F3, F4 and F4G-SG) in all groups on day 0 and day 100. Different y axis scales were used. The white columns represent day 0 data, and shadow columns represent day 100 data. Results were based on one duplicate and the error bars represented the measurement uncertainty.

The BML-T group had the lowest concentrations of all hydrocarbon fractions. Bitumen was added to the other three groups. Heavier hydrocarbons were present in the bitumen than in the tailings. Bitumen addition greatly influenced the petroleum hydrocarbon distribution: BML-B, BML-BT and BML-BTC had a similar distribution of these four classifications: F2: 1%, F3: 21−22%, F4: 10% and F4G-SG: 67−68%, while BML-T had a unique distribution of: F2: 5%, F3: 28%, F4: 12% and F4G-SG: 55%.

Concentrations of all hydrocarbon fractions on day 100 followed a similar trend: BML-B > BML-BT, BML-BTC > BML-T. The decrease in hydrocarbon concentration in the BML-B group represents any baseline abiotic (desorption) and biotic (microorganisms could be attached to the bitumen surface) processes occurring in this fraction. The BML-T group displayed a > 90% removal of all hydrocarbon fractions. This is likely due to microbial degradation, since FFT is a known source of microorganisms. Greater reduction in hydrocarbon fractions was seen in the BML-BTC group (reductions: F2: 64%, F3: 58%, F4: 58% and F4G-SG: 68%) when compared to the BML-BT group (reductions: F2: 23%, F3: 26%, F4: 24% and F4G-SG: 35%). Due to the intrinsic complexity of the analysis, PHC change was more statistically significant for BML-BTC group, not in BML-B and BML-BT groups. The PHC results indicates that the addition of acetate may have triggered co-metabolic processes and that more hydrocarbons were catabolized in the presence of acetate.

Removal of dissolved organics was not improved in the presence of acetate. Therefore, although acetate may have stimulated the degradation of heavier non-aqueous organic compounds from residual bitumen, it did not substantially affect the removal of dissolved organics as shown by DOC in Section 3.3.1. Previous studies have shown that acetate addition to oil sands tailings resulted in reduced anaerobic degradation of lower end PHCs (Stasik et al., 2015). The delay in biodegradation may be linked to pH reduction as a result of acetate accumulation and competition for limited nutrients and electron

acceptors (Stasik et al., 2015). However, this inhibition of acetate was not seen in this research, potentially because of the different redox level or because of different metabolic pathways of the various PHCs.

3.3.3 AEOs and O_2^- compounds

Acid extractable organics (AEOs) were measured by GC-FID, which comprises a broad class of organic compounds (e.g., O_2^- compounds, nitrogencontaining species (NO_n and N_2O_n), and sulfurcontaining species (O_nS and O_nS_2)), and AEOs include NAs as defined by O_2^- compounds, which were more specifically measured with an Orbitrap mass spectrometer as described in Appendix A (Headley et al., 2011). Start and end data were shown in Fig. 3.3. NA's solubility is influenced by pH (Headley et al., 2002), so pH was also tracked. On day 0, pH was about 8.3 for all groups. After 100 d, pH was 8.15 $\pm$ 0.22, 7.53 $\pm$ 0.25, 7.70 $\pm$ 0.01, and 8.04 $\pm$ 0.03 for BML-B, BML-BT, BML-BTC, and BML-T respectively. This pH change is not significant enough to greatly influence NA solubility (Headley et al., 2002). Therefore, the main influence on NAs concentration changes should be physiochemical and biological processes.

Unextracted bitumen has long been suspected a source of petroleum acids including NAs (Quagraine et al., 2005a). BML-B group demonstrated that bitumen was a source of AEOs but not of O_2^- compounds. 20$-$40% removal of O_2^- compounds was observed in groups containing tailings (BML-BT, BML-BTC, BML-T), demonstrating the ability of indigenous microbes to remove both NAs and bitumen-sourced organic acids.

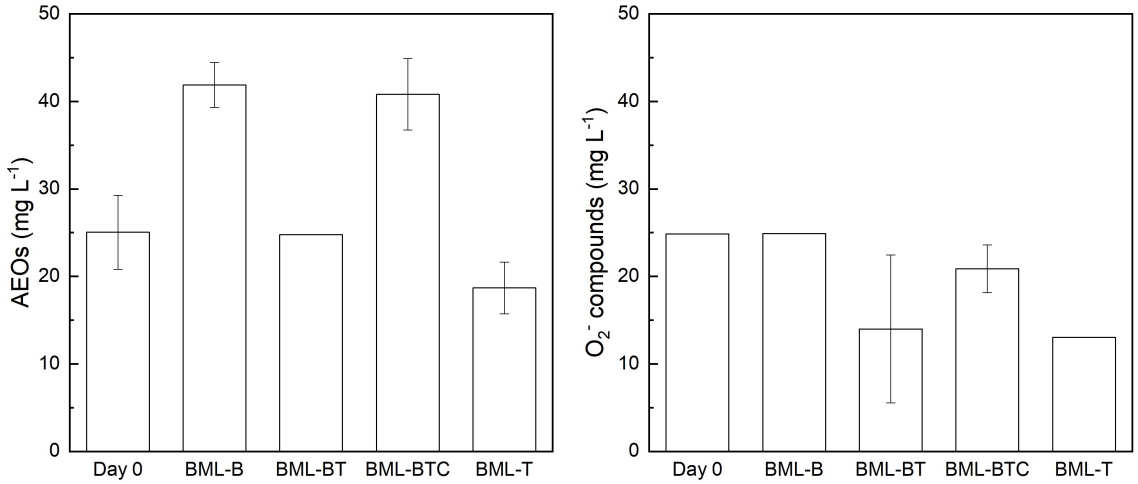

Figure 3.3: NAs in the liquid phase measured as AEOs (left) and O_2^- compounds (right) on day 0, and after 100 d in all groups respectively. Results are presented as an average one standard deviation (n = 4 for all AEOs, n = 2 for BML-BT and BML-BTC O_2^- compounds, n = 1 for Day 0, BML-BTC and BML-T O_2^- compounds due to the limited volume).

3.3.4 Toxicity

Liquid phase toxicity was measured on day 0, 48, and 100, as shown in Fig. 3.4. Day 0 samples were taken within 3 h of setting up the bottles. On day 0, differences could be observed: BML-B had the highest toxicity tested by Microtox® indicating that bitumen may significantly contribute to toxicity. In the BML-T group, aqueous toxicity was reduced over 100 d from 1.0 TU to about 0.2 TU, which indicates the aqueous phase could be detoxified by exposure to the native microbial activities in cap water. In other groups, bitumen was likely the primary cause of the increased toxicity over time.

In BML-B and BML-BT groups, toxicity increased 3.5 times and 25 times, respectively. The higher final toxicity in the sample containing the tailings may have been caused by

toxic metabolic intermediates produced by the microbial degradation of organics in the tailings. In the BML-BTC group, a different trend was observed: after 48 d, toxicity increased 8.3 times (10 TU, similar to BML-B and BML-BT) but did not significantly increase further after 100 d (7.9 ± 4.2 TU). Different microbial degradation pathways may have resulted from the addition of acetate, which resulted in different metabolic intermediates, indicating that the addition of acetate could help detoxify bitumen-polluted aqueous environments. In Chapter 2, the addition of a proprietary blend of microbes and organics allowed the detoxification of bitumen-containing cultures, suggesting that the addition of readily-degradable organic compounds could help detoxify bitumen-polluted aqueous environments in the presence of proper microbial communities.

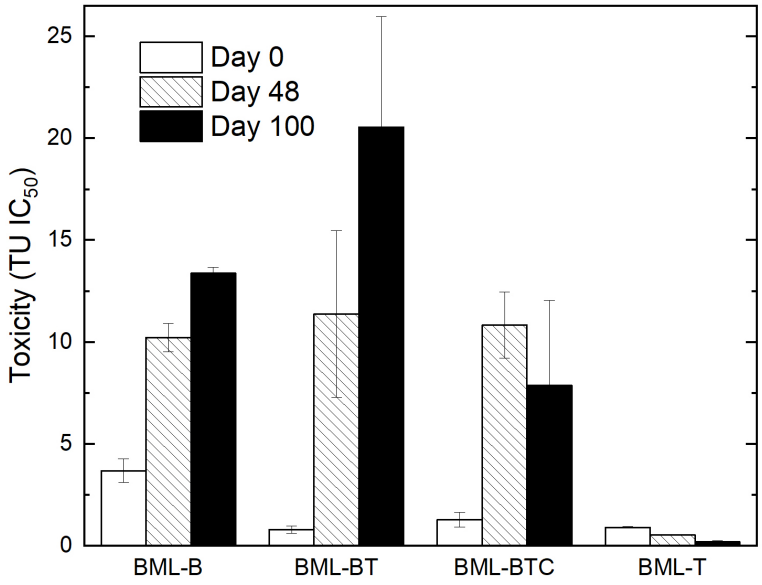

Figure 3.4: Aqueous toxicity over a period of 100 d (day 0, day 48 and day 100). Results are presented as an average one standard deviation (n = 2). The open bars, shadow bars, and black bars represent day 0, day 48, and day 100, respectively.

3.3.5 qPCR

DNA extraction from the BML-B group was not successful, suggesting low bacterial populations. DNA from other groups was extracted and rpoB gene copy numbers were measured by qPCR (Fig. 3.5). BML-BT had a 70% reduction in the bacterial density, while this group had the highest CO_2 production. No DNA samples were tested between day 0 and day 100, so it is unknown how the bacterial population changed over time. As shown in Fig. 3.4, the toxicity increased significantly over time, which might have caused the decrease in bacterial density (Fig. 3.5). Bacterial growth showed a reduction in the bacterial density in the presence of the bitumen. BML-BTC had a 3.8 times bacterial density increase, which was stimulated by the addition of acetate. Although complete mineralization of hydrocarbons (observed as CO_2 generation) was less effective in the BML-BTC group, more effective removal of heavier hydrocarbons (Fig. 3.2) and more rapid population growth of bacteria was observed. BML-T groups bacterial density remained relatively constant (1.3 times denser). BML-T group had the lowest available hydrocarbons, but also the lowest toxicity levels due to the lack of bitumen.

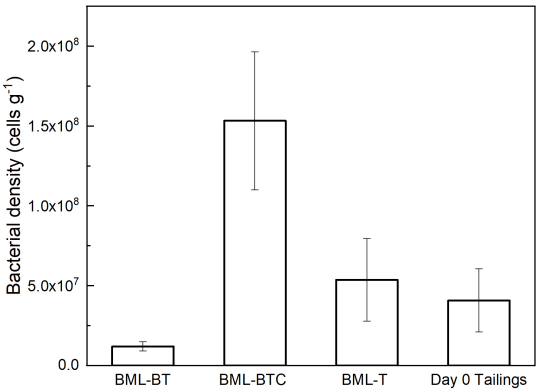

Figure 3.5: qPCR results targeting at *rpoB* gene at time 0, and after 100 d in other three groups respectively. Results are presented as an average ± one standard deviation (n = 6).

3.3.6 Microbial community analysis

Oxidative culture conditions were used in this study. Therefore, archaeal species, which have been reported to be mostly methanogens in oil sands tailings (Penner and Foght, 2010; Siddique et al., 2012) were rarely detected in this set of experiments (e.g., relative abundance about 0.1% in BML-T group). This discussion focuses on bacterial communities.

Microbial community composition profiles, shown in relative abundance (%), of BML-T, BML-BT, BML-BTC groups and the original tailings microbial community (labeled as Day 0 Tailings) are shown in Fig. 3.6. The microbial community composition profile in the Day 0 Tailings sample represents the indigenous tailings microbial community, used as a reference for the other three groups. The change in the microbial community composition profile during the experiment in groups BML-T, BML-BT and BML-BTC compared to Day 0 Tailings represents the response of the indigenous microbial community under the conditions described in Section 3.2.4. Microbial communities in these three groups have changed significantly from the original tailings microbial community (Day 0 Tailings).

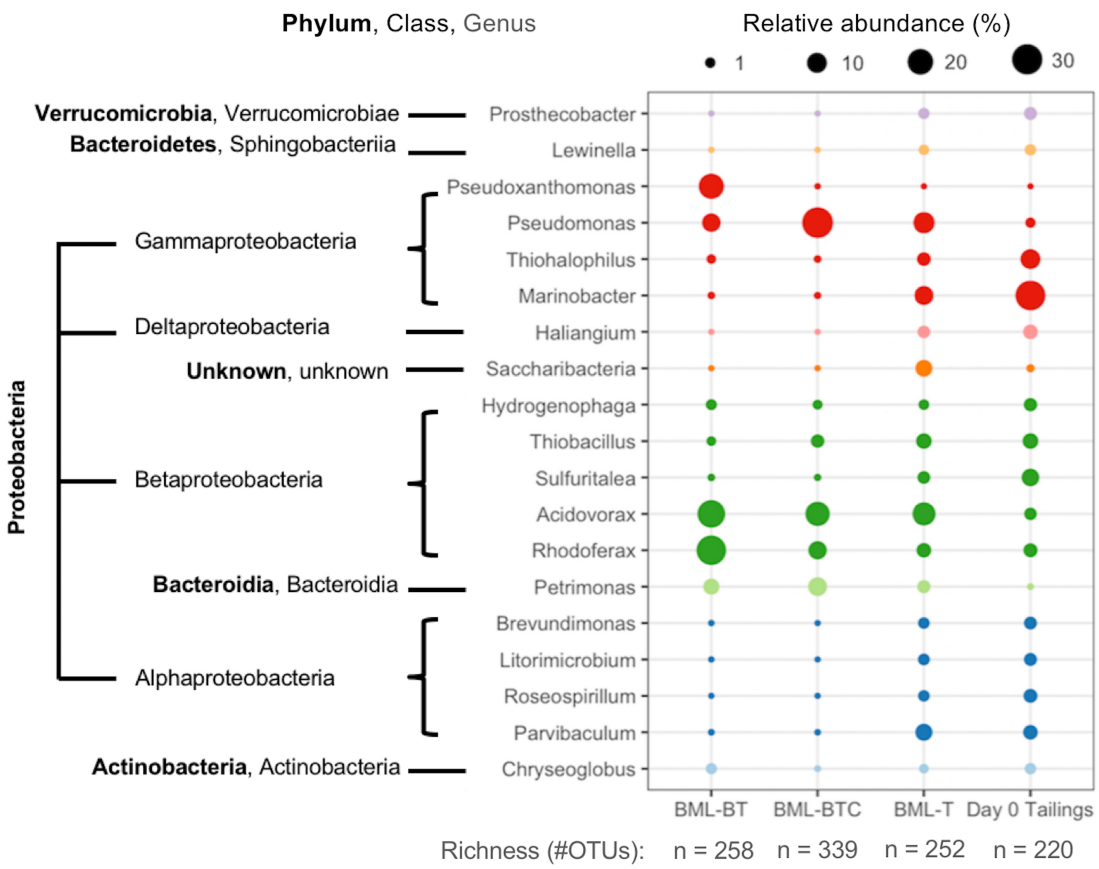

Figure 3.6: Microbial community profiles of the original tailings microbial community (Day 0 Tailings), and BML-BT, BML-BTC and BML-T microbial communities after 100 d incubation. Phylum, class and genus information is shown in bold black, black and grey text, respectively. The size of the bubble represents the relative abundance (%). The microbial community richness (n = observed OTUs) is shown.

Not surprisingly, the relatively more abundant species found in this study are well-known oil/hydrocarbon degraders which have been reported in a variety of oil-contaminated environments (Sanchez et al., 2006; Yakimov et al., 2007; Gray et al., 2011; Kostka et al.,

2011; Siddique et al., 2012; Bartram et al., 2011; Yergeau et al., 2012). These bacteria have also been reported to exist in oil sand tailings ponds and in the Athabasca River and its tributaries (Penner and Foght, 2010; Ramos-Padrón et al., 2010; Siddique et al., 2012; Yergeau et al., 2012; Chávez, 2014; Foght et al., 2017). Many of these species are facultative anaerobes. However, nitrate and sulphate levels were constant for these two electron acceptors, and methane in the headspace was below detection limit (< 1 mg L^{-1}) during the incubation period.

Marinobacter was the most abundant genus found in the Time 0 Tailings ($> 29\%$). *Marinobacter* has been found in many studies to be an effective oil degrader, and is recognized to play a role in the degradation of hydrocarbons from oil polluted marine waters (Sanchez et al., 2006; Yakimov et al., 2007; Gray et al., 2011; Kostka et al., 2011). However, the abundance of *Marinobacter* decreased in all three groups after 100 d, especially in the cultures with bitumen.

In the BML-T group, the most abundant genera were *Acidovorax* ($> 15\%$), *Pseudomonas* ($> 12\%$), *Marinobacter* ($> 8\%$) and *Parvibaculum* ($> 6\%$). The BML-T group showed a similar trend to those seen in previous investigations of WIP tailings and Mildred Lake Settling Basin (MLSB) tailings (Penner and Foght, 2010). WIP was a previous tailings impoundment at the Mildred Lake Mine site, and was later commissioned as BML. It contains FFT mainly transferred from MLSB and water transferred from BCR (Dompierre and Barbour, 2016). *Acidovorax* spp. and *Pseudomonas* spp. are frequently detected in hydrocarbon-contaminated environments (Penner and Foght, 2010; Eriksson et al., 2003). *Acidovorax* is a denitrifier and facultative lithoautotroph, which can use molecular hydrogen, and has been found in anaerobic sites contaminated with toluene (Aburto and Peimbert, 2011). This genus has also been found in mineral oil hydrocarbon-contaminated soil (Popp et al., 2006). *Pseudomonas* spp. are found ubiquitously in natural soil environments as well as hydrocarbon-contaminated sites, and certain species

are capable of degrading model and commercial NAs (Lai et al., 1996; Kato et al., 2001; Quagraine et al., 2005b; Del Rio et al., 2006; Popp et al., 2006; Whitby, 2010). *Pseudomonas* is also involved in biofilm formation, which provides advantages for growth in extreme environments (Golby et al., 2012).

In the BML-BT group, *Rhodoferax* (> 28%), *Acidovorax* (> 23%), *Pseudoxanthomonas* (> 18%), and *Pseudomonas* (> 7%) were detected at the highest abundance. *Rhodoferax* spp. have been found to be abundant in oil sands tailings (Penner and Foght, 2010; Golby et al., 2012; Yergeau et al., 2012). *Rhodoferax* has been identified as effective hydrocarbon degraders, and identified in aerobic benzene degrading community as a dominant genus (Fahy et al., 2006; Aburto and Peimbert, 2011). *Pseudoxanthomonas* spp. have been found in oil contaminated sites, and identified as BTEX degraders. Members of this genus can also produce biosurfactants and degrade crude oil (Sanchez et al., 2006; Kim et al., 2008; Nayak et al., 2009; Mortazavi et al., 2013; Nopcharoenkul et al., 2013). There are no publications regarding *Pseudoxanthomonas* spp. in the context of oil sands tailings. The presence of this genus might be correlated with the high dose of the bitumen added in this group.

In the BML-BTC group, *Pseudomonas* (> 31%), *Acidovorax* (> 17%), *Petrimonas* (> 8%), and *Rhodoferax* (> 7%) were detected at the highest abundance. Acetate addition likely stimulated *Pseudomonas* spp., which dominated this group, and the growth of *Pseudomonas* may have contributed to the significant bacterial growth (qPCR results shown in Fig. 3.5) and the highest rate of removal of PHC in the BML-BTC group (shown in Fig. 3.2). *Petrimonas* has not been reported in environmental samples, however, this genus has been reported in previous bioreactor studies (Sun et al., 2015; Li et al., 2016). Intermittent anoxic conditions might have occurred in this group because of the rapid bacterial growth and effective removal of hydrocarbons.

A recent study using metatranscriptomics correlated highly expressed genes with energy

metabolism and hydrocarbon degradation from samples collected along the Athabasca River freshwater tributaries, and indicated that the expression of *alkB* (alkane monooxygenase) could potentially serve as a bioindicator gene for active hydrocarbon degradation potential (Reid et al., 2018). The *alkB* is responsible for aerobic hydrocarbon degradation in the oil-polluted sites and abundantly distributed among bacteria belonging to Alpha-, Beta- and Gammaproteobacteria (Nie et al., 2014). Alpha- (> 7% for BML-T), Beta- (> 28% for BML-BTC, > 54% for BML-BT, > 26% for BML-T) and Gammaproteobacteria (> 31% for BML-BTC, > 27% for BML-BT, > 39% for BML-T) were also the three most abundant classes found in this study.

3.4 Conclusions

Bitumen in the BML would greatly contribute to the PHC level, especially in the presence of tailings. Bitumen in this study increased the aquatic toxicity (measured by Microtox®) by four times when mixed with the BML water, and by 20 times when mixed with the BML water and tailings. Through the on-site monitoring program carried by Syncrude, the acute toxicity of BML has been decreasing every year indicating that in situ remediation occurring (Syncrude Canada Ltd., 2017). Acetate addition mitigated this toxicity and effectively removed the PHC compounds. The quantitative increases in bacterial populations and the increase of the relative abundances of known oil-degrading bacteria indicated a strong selective response of indigenous microbial communities in the presence of the bitumen obtained from BML. *Rhodoferax*, *Acidovorax*, *Pseudomonas* and *Pseudoxanthomonas* were genera that were best able to tolerate bitumen-derived toxicity. *Rhodoferax*, *Acidovorax* and *Pseudomonas* spp. showed more potential for biostimulation treatment with acetate to remove PHC/bitumen. *Pseudomonas* spp. were the most stimulated species by acetate and might serve as the biggest contributor to bitumen removal

and toxicity mitigation.

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Chapter 4

Model naphthenic acids removal by microalgae and Base Mine Lake cap water inoculum

4.1 Introduction

In an EPL, the initial water chemistry is dominated by the OSPW. The quality of the water cap will improve over time as oxygen concentrations increase and the microbes (inoculated from the freshwater sources) start to degrade toxic organic constituents (Charette et al., 2010). NAs are one of the main contributors to acute toxicity in OSPW, and have been the focus of many oil sands-related remediation studies (Quagraine et al., 2005; Brown and Ulrich, 2015; Morandi et al., 2015). In an EPL, NA removal is one of the reclamation goal. Microbial NA detoxification and degradation has been widely reported, indicating that in situ bioremediation could help to achieve reclamation goals in the Athabasca regions (Quagraine et al., 2005; Johnson et al., 2011; Brown and Ulrich,

2015). Model NAs are simpler NAs that are often used in research and biodegradation studies. Although oil sands NAs are less biodegradable than model NAs, using model compounds instead of naturally occurring NAs will help to identify potential NA degraders and elucidate possible degradation pathways (Headley et al., 2008; Quesnel et al., 2011; Ruffell et al., 2016). Summary of model NA biodegradation studies were listed in Chapter 1 (Tables 1.4 and 1.5).

Algae can enhance bacterial degradation of organics (Reim et al., 1974; Fallon and Brock, 1979; Saks and Kahn, 1979; Bender and Phillips, 2004; Muñoz et al., 2004, 2006; Banerjee et al., 2008; Zhou et al., 2013; Guo and Tong, 2014), including NAs (Mahdavi et al., 2015). This enhancement may be because the bacteria benefit from the O_2-producing photosynthetic processes, and also can use algal polysaccharides and dead algal cells as an additional carbon source (Muñoz et al., 2006). In return, bacteria can remove the toxicants in the environment and release CO_2, vitamins and other micronutrients to benefit algal growth (Haines and Guillard, 1974; Fallon and Brock, 1979; Saks and Kahn, 1979; Banerjee et al., 2008; Zhou et al., 2013). Therefore, a co-culture of BML cap water inoculum and one alga was also tested to degrade NA in this study.

In this chapter, the algal species *C. kessleri* and *B. braunii* were investigated for their abilities to remove three model NAs (Sigma Aldrich, Canada): CHCA, cyclohexaneacetic acid (CHAA) and cyclohexanebutyric acid (CHBA). BML cap water was used alone as an indigenous inoculum, and also co-cultured with *C. kessleri* to remove the same three model NAs. Autoclaved controls were used to distinguish any passive adsorption processes. The degradation pathways used by algal species and BML inoculum were also elucidated. The bacterial community in the BML inoculum was sequenced targeting at 16S rRNA gene using Illumina MiSeq platform with or without the addition of *C. kessleri*.

4.2 Materials and methods

4.2.1 Algal cultures and BML inoculum preparation

C. kessleri was isolated from cyclone over-flow water collected from Syncrude's bitumen extraction facility (Fort McMurray, AB; Mahdavi et al., 2012). *B. braunii* is a unicellular green microalga, and the strain used in this study was obtained from UTEX Culture Collection of Algae at the University of Texas at Austin (UTEX 2441, USA). *B. braunii* is well-known for its ability to effectively take up CO_2 and for its potential use in biofuel production because of its hydrocarbon-rich structures (Banerjee et al., 2008; Tasić et al., 2016). Although previous studies showed limited growth of this strain in OSPW (Kasiri et al., 2015), in this study it was tested for its ability to remove model NAs when grown in Bold's Basal Medium (BBM, recipe in Appendix B).

BML cap water was sampled (surface water depth) at Platform 1 in BML on August 8, 2017. All water samples were transported to the laboratory in sealed buckets and stored at 4 °C for four months prior to use. BML cap water was used as inoculum to represent the microbial communities present in the BML cap water. It must be noted that this inoculum was representative of the time of sampling, and the microbial community will change after the incubation. Twenty-five mL of BML cap water was mixed with 100 mL LB media, and then incubated at 20 °C on a continuous horizontal shaker at 150 rpm until the optical density at 600 nm (OD_{600}) reached about 0.10−0.11. The BML inoculum was prepared in this way prior to every experiment.

4.2.2 Algal tolerance bioassay

A model NA tolerance assay was set up following Quesnel et al. (2011). Two algal cultures were spiked with stock solutions (5 g L^{-1} in 0.1 M NaOH solution) of CHCA,

CHAA, or CHBA to a final concentration of 0 mg L^{-1}, ∼100 mg L^{-1} or ∼300 mg L^{-1} in a 250 mL Erlenmeyer Flask. Ten mL of pure algal culture with OD680 of about 1.0 were added and topped up with BBM to 150 mL. Cultures were incubated at 20 °C with an 18 h light: 6 h dark cycle on a continuous horizontal shaker at 150 rpm for 80 d. Growth of cultures were monitored via OD680 and chlorophyll a (Chl a) concentration (Appendix A). Nutrient (nitrogen and phosphorus) uptakes, model NAs concentrations and pH were measured during the experiment.

4.2.3 Biodegradation experiments

Algal pure culture (OD_{680} of about 1.0) was centrifuged at 9500 × g for 5 min and washed with 0.9% NaCl three times. The algal biomass was re-suspended into 0.9% NaCl and used as inoculation. Similarly, BML inoculum (preparation method shown in Section 4.2.1) was centrifuged at 6000 × g for 3 min and washed with 0.9% NaCl three times. The pellet was re-suspended into 0.9% NaCl and used for inoculation.

Algal pure cultures of *C. kessleri*, *B. braunii*, BML inoculum, and a co-culture of *C. kessleri* and BML inoculum, were cultured with approximately 100 mg L^{-1} of one model NA (CHCA, CHAA or CHBA) at 20 °C on a continuous horizontal shaker at 150 rpm. About 2 g L^{-1} of algal biomass, or the pellet harvested from 50 mL of BML inoculum (Section 4.2.1), was inoculated and topped up with 0.9% NaCl to a final volume of 100 mL. In the biodegradation experiments, no nutrient was supplied with the high density of algal biomass to limit algal growth. Autoclaved cultures of the two algae and BML inoculum were also set up to show if any passive adsorption of model NAs occurred. Bottles that only contained the model NA and 0.9% NaCl were also set up to serve as a control. Groups with BML inoculum were incubated for 60 d, and algal groups were incubated until NAs were completely removed.

Model NAs concentrations were monitored by high performance liquid chromatogra-

phy (HPLC, Appendix A). Samples were also submitted for LC-MS analysis to identify possible intermediates and to investigate the potential degradation pathway of the model NAs by respective cultures. DNA was extracted at the end of the experiment (from the microcosms containing BML inoculum and the co-culture of *C. kessleri* and BML inoculum) using the FastDNA™ SPIN Kit for Soil (MP Biomedicals, Canada) following manufacturers recommended isolation protocol. The V4 hypervariable regions of bacterial 16S rRNA genes were amplified by PCR using the primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACVSGGGTATCTAAT-3'; Caporaso et al., 2011), and sequenced using Illumina MiSeq platform (accessible in Sequence Read Archive (SRA) with accession number PRJNA499087). The PCR conditions and analysis methodologies were described previously (Section 3.2.3).

4.3 Results and discussion

4.3.1 Tolerance to model NAs

Various algal species have shown high tolerance to the model NAs and OSPW NAs (Quesnel et al., 2011; Woodworth et al., 2012; Ruffell et al., 2016), and the oil sands tailings ponds have been shown to have relatively diverse algal communities (Aguilar et al., 2016). OD_{680} and Chl a concentrations indicated algae were growing with 0 mg L^{-1}, 100 mg L^{-1} and 300 mg L^{-1} model NAs (Fig. 4.1). The addition of 100 mg L^{-1} CHCA, CHBA or CHAA did not have a significant negative influence on *C. kessleri*, but adding 100 mg L^{-1} CHBA and CHAA did slow the growth of *B. braunii*.

However, adding 300 mg L^{-1} of CHCA, CHBA or CHAA inhibited both algae by introducing an approximate 15−30 d lag phase on algal growth. Phosphate uptake by both algae was not significantly affected by the addition of model NAs (Fig. C.5). Ad-

dition of 300 mg L^{-1} of CHBA, CHAA and CHCA all decreased the nitrogen uptake by *C. kessleri* and *B. braunii*. Addition of 100 mg L^{-1} of model NAs did not influence the nitrogen uptake by either alga except the scenario: 100 mg L^{-1} CHCA on *C. kessleri*.

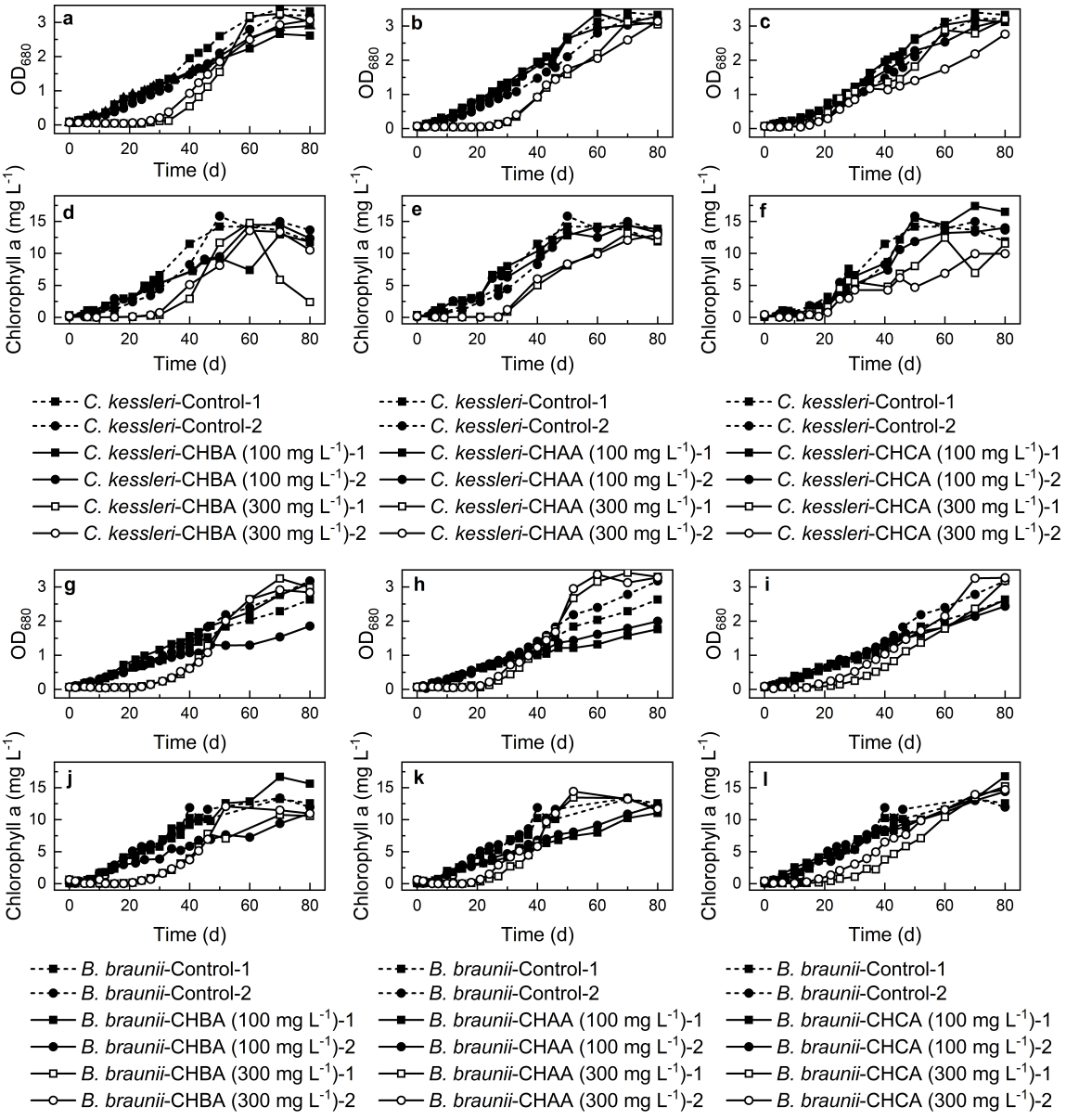

Figure 4.1: Algal growth was monitored by OD_{680} and Chl a concentration. Panels a−f show the growth of *C. kessleri*, and panels g−l show the growth of *B. braunii*. Duplicate data are presented separately. Black lines represented the algal culture growing in the normal media, and colored lines represented the algal culture growing in the presence of 100 mg L^{-1} (solid dot) or 300 mg L^{-1} (blank dot) of model NAs.

4.3.2 Model NA removal by individual culture

CHBA and CHAA removal

CHBA is readily transformed via β-oxidation to CHAA in many organisms (Davis and Raymond, 1961). However, the β-oxidation of CHAA is often blocked due to the ring structure in the β-carbon position (Davis and Raymond, 1961; Beam and Perry, 1974; Ougham and Trudgill, 1982; Demeter et al., 2014). Metabolism of both CHBA and CHAA via β-oxidation was first reported in *Arthrobacter* sp. strain CA1 (Ougham and Trudgill, 1982). CHAA degradation was later reported in a few other bacterial strains such as *Cupriavidus metallidurans* strain KUA-1, a *Rhodococcus* sp., and a *Dietzia* sp. Among these strains, *Cupriavidus metallidurans* strain KUA-1 was the first Gram-positive bacterium to show degradation with a novel intermediate 1-cyclohexenylacetic acid (Iwaki et al., 2008). In 2011, CHBA and CHAA degradation by *D. tertiolecta*, a marine alga, was reported; CHBA was transformed to CHAA via β-oxidation (Quesnel et al., 2011). In Quesnel et al.s study, the CHAA degradation pathway was suspected to be similar to *Arthrobacter* sp. strain CA1 (β-oxidation), but also could be similar to *Cupriavidus metallidurans* strain KUA-1 with the intermediate 1-cyclohexenylacetic acid (Quesnel et al., 2011).

In this research, β-oxidation of CHBA was also observed during biodegradation by *B. braunii*, *C. kessleri* and BML inoculum. CHBA and CHAA (as a metabolite) were both monitored by HPLC during the experiment for 60 d (Fig. 4.2, control data in Fig. C.8). Autoclaved algal groups did not show significant reduction in CHBA (less than 5%), indicating that the removal of CHBA was by biodegradation.

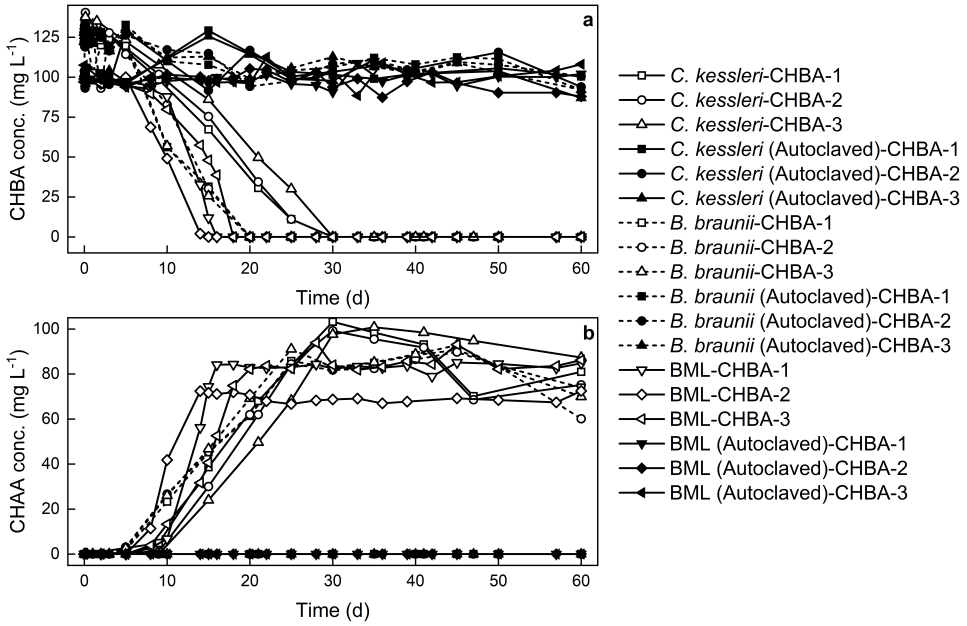

Figure 4.2: CHBA concentrations in the biodegradation experiment by *C. kessleri*, *B. braunii*, BML inoculum and their autoclaved cultures in panel a. The transiently generated/consumed CHAA levels in all groups are shown in panel b. Triplicate data are presented separately.

The LC-MS method detects the molecular mass of intermediates in the cultures, and different chemical structures can have identical molecular masses. By comparing the chemicals found with the metabolism pathways in the literature, the most possible pathway was proposed. Chemicals that matched exact mass detected by LC-MS are as follows: $C_{10}H_{18}O_3$, $C_8H_{14}O_2$, $C_8H_{12}O_2$, $C_8H_{14}O_3$ and $C_6H_{10}O_3$ in the samples of CHBA degradation by *C. kessleri*; $C_{10}H_{16}O_2$, $C_{10}H_{18}O_3$, $C_8H_{14}O_2$ and $C_8H_{14}O_3$ in the samples of CHBA degradation by *B. braunii*; and $C_8H_{14}O_2$ and $C_6H_{10}O_4$ in the samples of CHBA degradation by BML inoculum. $C_8H_{14}O_2$ was identified and quantified as CHAA with the authentic standard, and $C_8H_{14}O_3$ was identified as (1-hydroxycyclohexyl)-acetic acid

(HAA) with the authentic standard.

With the help of LC-MS analysis and the authentic standards of CHAA and HAA, the degradation pathway of CHBA was integrated into the CHAA degradation pathway via β-oxidation, and the CHAA metabolism was shown to be similar to that of *Arthrobacter* sp. strain CA1 (Fig. 4.5a). In the *C. kessleri* culture, *B. braunii* culture and BML inoculum culture, one or more of the intermediate metabolites of CHAA were found, which indicates that CHAA was biodegraded in all groups.

About 100 mg L^{-1} (roughly equivalent to 580 mM) of CHBA was added to all cultures, and about 80 mg L^{-1} (roughly equivalent to 580 mM) of CHAA was generated, indicating that CHBA was stoichiometrically transformed into CHAA. In the *B. braunii* and *C. kessleri* groups, 10−20% of CHAA was removed by day 60, however, in the BML inoculum group, no significant reduction of the generated CHAA was seen (Fig. 4.2). In order to know more about CHAA degradation, CHAA was added to new cultures of both algae, and a complete removal of approximately 100 mg L^{-1} CHAA was achieved by both algae (Fig. 4.3, control data in Fig. C.8). BML inoculum group was not used to monitor CHAA degradation because without another carbon source, the bacterial inoculum may not be able to survive the lag phase.

Chemicals that matched exact mass detected by a LC-MS in the CHAA degradation cultures are as follows: $C_8H_{14}O_3$ in the samples of CHAA degradation by *C. kessleri*, $C_8H_{12}O_2$ and C8H14O$_3$ in the samples of CHAA degradation by *B. braunii*. As expected, the degradation of CHAA was slower when compared to the degradation of CHBA and a lag phase for CHAA degradation was observed (60-d lag for *B. braunii* and 40-d lag for *C. kessleri*). In a study by Quesnel et al. (2011), CHAA degradation occurred much faster and without a lag phase by *D. tertiolecta* than by *C. kessleri* and *B. braunii* in this experiment. The difference in the lag phase might indicate that the proposed transformation pathway of CHAA into 1-cyclohexenylacetic acid followed by α-oxidation would

be more efficient than the β-oxidation of CHAA into cyclohexylideneacetic acid.

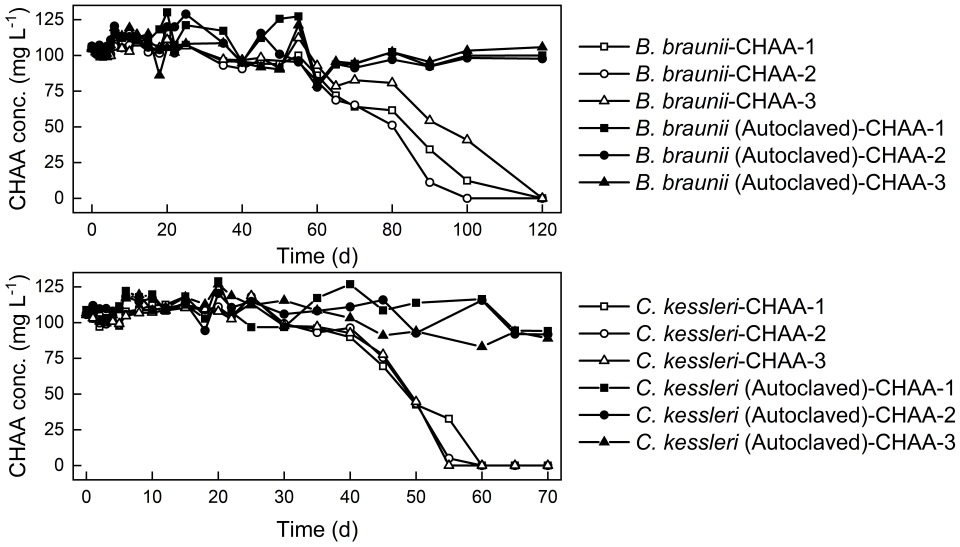

Figure 4.3: CHAA concentrations in the biodegradation experiment by *C. kessleri*, *B. braunii* and their autoclaved cultures. Triplicate data are presented separately.

CHCA removal

CHCA biodegradation has been observed both aerobically and anaerobically, each using different enzymatic mechanisms (Kung et al., 2014). CHCA is also an intermediate product of anaerobic benzoate metabolism (Perrotta and Harwood, 1994). Two different microbial CHCA degradation pathways have been proposed: 1) β-oxidation with the intermediate 1-cyclohexene-1-carboxylic acid (1-CHCA); 2) aromatization to generate p-hydroxybenzoic acid (Blakley, 1974, 1978; Perrotta and Harwood, 1994; Kung et al., 2014). The proposed biodegradation pathway of CHCA by *D. tertiolecta* was suspected to occur via β-oxidation because of the identification of 1-CHCA, which has also been observed during bacterial metabolism (Quesnel et al., 2011).

In this research, CHCA was monitored by HPLC until it dropped below the detection

limit (5 mg L^{-1}) or the degradation plateaued (Fig. 4.4, control data in Fig. C.8). In all groups (*C. kessleri*, *B. braunii* and BML inoculum), 100 mg L^{-1} CHCA was almost completely removed while the autoclaved controls did not show any significant removal. The CHCA degradation by BML inoculum occurred without a lag phase, while *C. kessleri* and *B. braunii* had 40-d and 60-d lag phases, respectively. The data in this study also suggested that CHCA was more susceptible to biodegradation than CHAA (Iwaki et al., 2008; Quesnel et al., 2011). *C. kessleri* and *B. braunii* showed similar ability to degrade CHCA and CHAA, and both algal strains had longer lag phases than *D. tertiolecta* (Quesnel et al., 2011).

Chemicals that matched the exact mass detected by a LC-MS are as followed: $C_7H_{10}O_2$, $C_7H_{12}O_3$ and $C_7H_{12}O_4$ in the samples of CHCA degradation by *C. kessleri*, $C_7H_{12}O_3$ and $C_7H_{10}O_3$ in the samples of CHCA degradation by *B. braunii*, $C_7H_{10}O_2$, $C_7H_{12}O_3$ and $C_7H_{12}O_5$ in the samples of CHCA degradation by BML inoculum. $C_7H_{10}O_2$ was identified as 1-CHCA with the authentic standard and semi-quantified by peak area. $C_7H_{10}O_3$ and was identified as 2-oxocyclohexnaecarboxylic acid with the authentic standard. The peak area of 1-CHCA was used to indicate the semi-quantitative change of 1-CHCA (Fig. C.6). Based on these identified intermediates, the β-oxidation pathway (Fig. 4.5b) with the intermediate 1-CHCA was proposed for the CHCA degradation pathway for *C. kessleri*, *B. braunii* and BML inoculum.

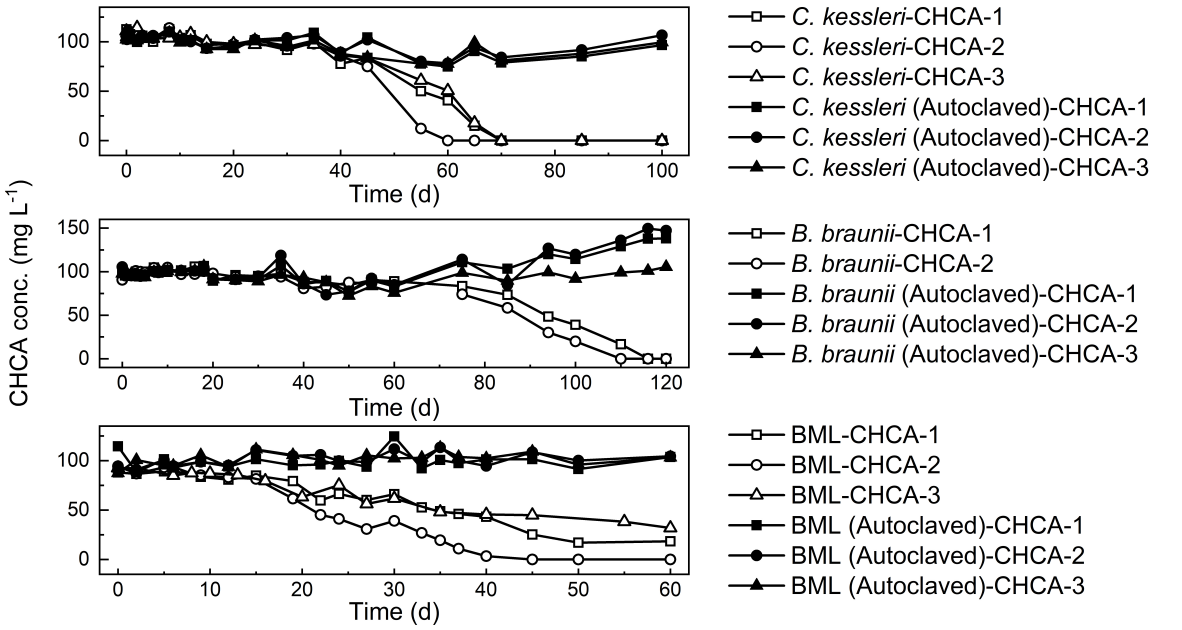

Figure 4.4: CHCA concentrations for the biodegradation experiments with *C. kessleri*, *B. braunii*, BML inoculum and their autoclaved controls. *B. braunii* groups were monitored for 120 d, *C. kessleri* groups were monitored for 100 d, and BML group were monitored for 60 d.

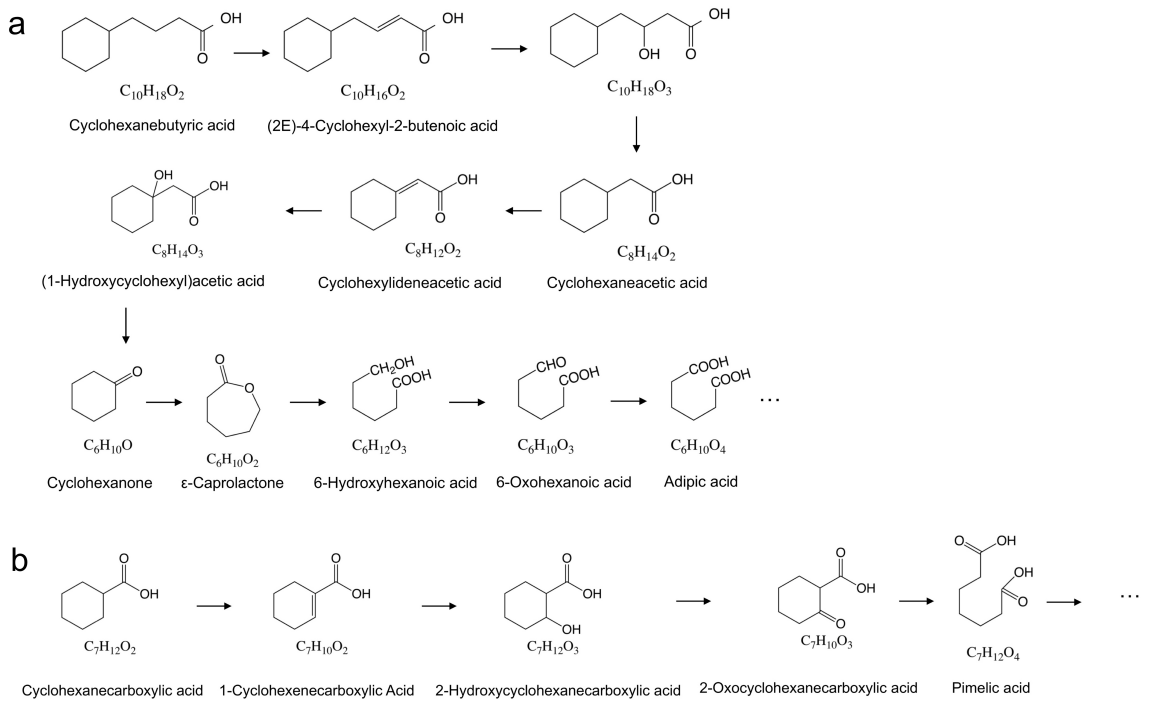

Figure 4.5: Proposed CHBA (CHAA) degradation pathway by all cultures in this research is shown in panel a, and proposed CHCA degradation pathway is shown in panel b.

4.3.3 Model NA removal by co-culture

CHBA degradation rates by BML inoculum, *C. kessleri* culture, and the co-culture of *C. kessleri* and BML inoculum were 8.04 mg L^{-1} d^{-1} (R^2: 0.982, lag phase: ∼5 d), 6.10 mg L^{-1} d^{-1} (R^2: 0.987, lag phase: 8−9 d) and 12.83 mg L^{-1} d^{-1} (R^2: 0.968, lag phase: 1−2 d), respectively (Fig. 4.6). The CHAA intermediate was also metabolized in the co-culture of *C. kessleri* and BML inoculum group; the CHAA generation rate was 11.3 mg L^{-1} d^{-1} (R^2: 0.944), and consumption rates were 3.32 mg L^{-1} d^{-1} (R^2: 0.970), 4.46 mg L^{-1} d^{-1} (R^2: 0.987) and 9.00 mg L^{-1} d^{-1} (R^2: 0.991) for the three replicates, respectively. The consumption of CHAA intermediate was observed in the *C. kessleri* culture group, but the rate was much lower (∼0.54 mg L^{-1} d^{-1}, R^2: 0.996). The

consumption of CHAA intermediate by BML inoculum was not significant. Therefore, the co-culture of *C. kessleri* and BML inoculum showed faster removal of both CHBA and the generated CHAA with shorter lag phases when compared to algal culture or BML inoculum alone. A more complete metabolism of CHBA and its intermediate products by the co-culture would be expected, because the microbial community is more diverse. The enhanced degradation of CHAA as an intermediate product also indicates that CHAA is more biodegradable by the mixed cultures than algal culture or bacterial cultures alone. CHCA degradation rates by BML inoculum, *C. kessleri* culture, and the co-culture of *C. kessleri* and BML inoculum were 1.62−2.64 mg L^{-1} d^{-1} (R^2: 0.942−0.962, lag phase: ∼5 d), 2.90 mg L^{-1} d^{-1} (R^2: 0.987, lag phase: ∼35 d) and 3.01 mg L^{-1} d^{-1} (R^2: 0.974, lag phase: ∼5 d), respectively (Fig. C.7). Similar to the results for CHBA, CHCA was biodegraded more completely by the co-culture. The peak area of the intermediate 1-CHCA showed that the 1-CHCA was completely consumed (not detectable) after 40 d in the co-culture group, while in the BML inoculum group and *C. kessleri* culture group, 1-CHCA was not completely removed or was removed more slowly than in the co-culture group (Fig. C.6).

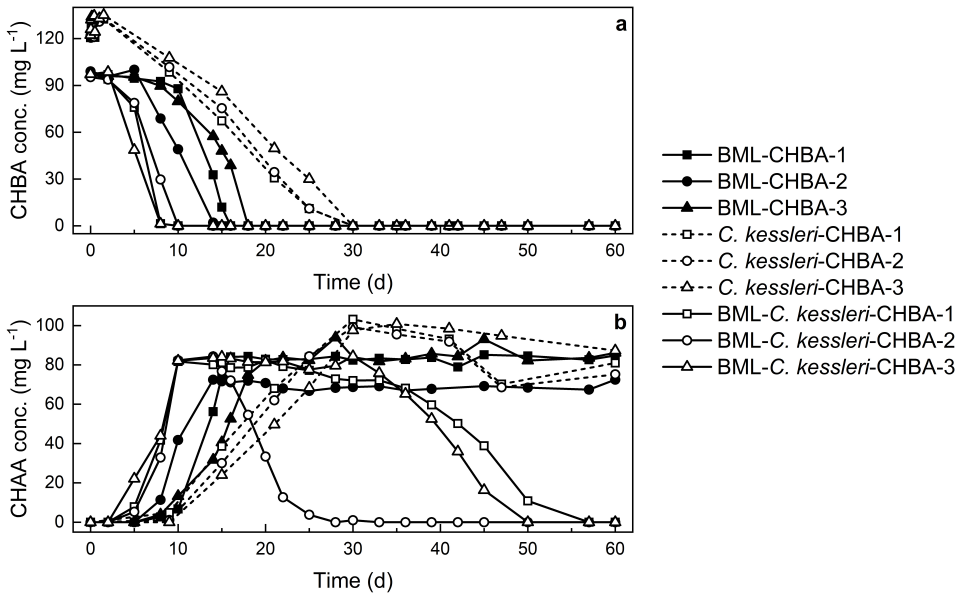

Figure 4.6: CHBA concentrations in the biodegradation experiment by *C. kessleri*, BML inoculum and the co-culture in panel a. The transiently generated/consumed CHAA levels in all groups are shown in panel b. Triplicate data were presented separately.

Analysis of 16S rRNA genes amplified from the DNA extracted from different cultures showed the influence of *C. kessleri* on the BML inoculum microbial community. Many studies show better contaminant removal and bioremediation by algal-bacterial consortia, because microalgae can develop synergistic relationships with bacteria (Muñoz et al., 2006; Bruckner et al., 2008; Zhou et al., 2013; Mahdavi et al., 2015). In this research, improved degradation of model NAs (CHBA and CHCA) was also achieved by co-culturing *C. kessleri* and BML inoculum. Fig. 4.7 shows the BML microbial community composition with, and without, *C. kessleri* in the presence of CHBA or CHCA.

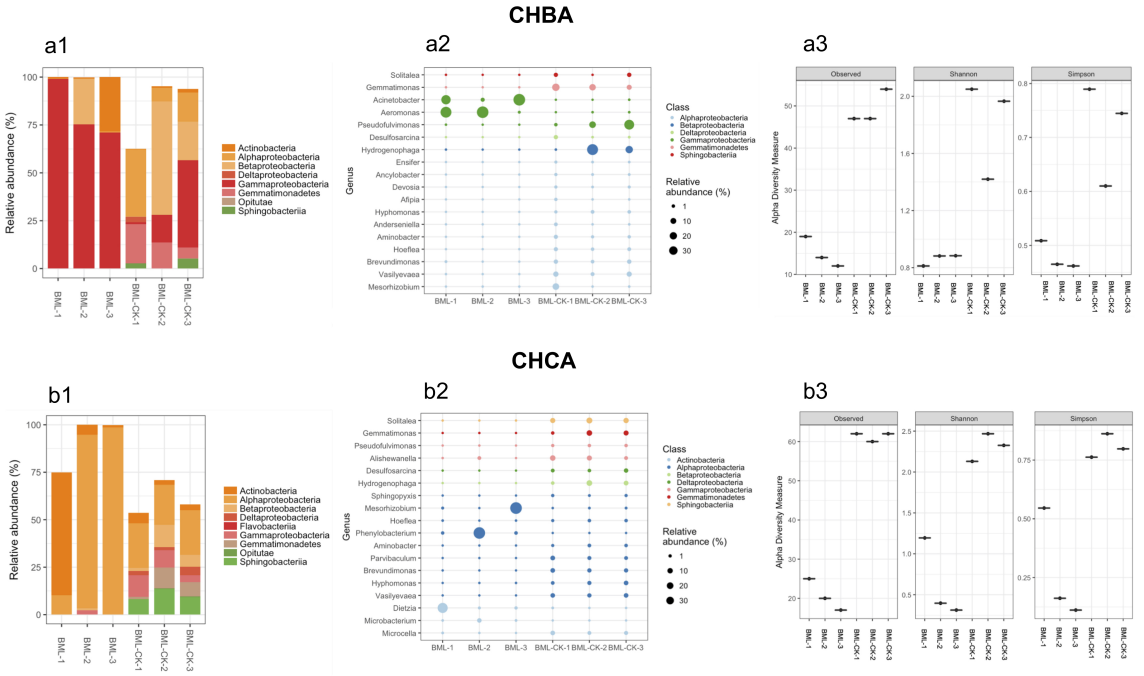

Figure 4.7: Microbial community profiles (at Class level) of the microbial community of the BML inoculum and co-culture with *C. kessleri* after 60 d incubation with CHBA or CHCA in panels a1 and b1. Abundance of selected genera is shown in panels a2 and b2. The microbial community diversity is shown in panels a3 and b3.

After incubating with model NAs for 60 d, the microbial community in BML inoculum was dominated by very few genera: *Acinetobacter* and *Aeromonas* in the presence of CHBA, *Dietzia*, *Phenylobacterium* and *Mesorhizobium* in the presence of CHCA. *Acinetobacter* spp. have been reported to exist and dominate in oil sands tailings ponds depending on depth, and *Acinetobacter* sp. has been isolated and identified as NA degraders including carboxylic acid, CHCA and CHAA (Herman et al., 1993; Del Rio et al., 2006; Whitby, 2010; Golby et al., 2012). *Aeromonas* spp. are ubiquitous in fresh water and blackwater but have not been reported in oil sands-related environments. *Dietzia*

spp. have been isolated and identified as CHAA degrading-strains and strong hydrocarbon degraders in the presence of high salinity and high alkalinity environments (Iwaki et al., 2008; Wang et al., 2011; Dashti et al., 2015; Chen et al., 2017; Song et al., 2018). *Phenylobacterium* spp. are polycyclic aromatic hydrocarbon degraders and have been reported to flourish in oil-contaminated sites (Yang et al., 2014; Nazina et al., 2017; Song et al., 2018). *Mesorhizobium* spp. have been found in the indigenous microbial communities in the Athabasca River area, and were able to degrade compounds of concern in oil sands tailings environments (Yergeau et al., 2012; Auffret et al., 2015). In the present study, it was confirmed again that Acinetobacter spp. are probably involved in degrading model NA compounds through β-oxidation. The other three genera might also have the potential to degrade model NA compounds.

When BML inoculum were co-cultured with *C. kessleri*, the diversity of the microbial community greatly increased, measured by the observed richness, Shannon index and Simpson index (Fig. 4.7a3 and Fig. 4.7b3). When CHBA was the main substrate, the following genera showed higher relative abundances: *Brevundimonas* (1.3−3.2%), *Vasilyevaea* (1.9−6.7%), *Hydrogenophaga* (up to 59%), *Gemmatimonas* (5.7−20.4%), *Pseudofulvimonas* (1−45%) and *Mesorhizobium* (up to 14.3%). When CHCA was the main substrate, the following genera showed higher relative abundances: *Brevundimonas* (3.7−5.4%), *Vasilyevaea* (3.8−5.3%), *Hydrogenophaga* (1.5−11.6%), *Gemmatimonas* (1.2−10.8%), *Hyphomonas* (1.9−5.2%), *Alishewanella* (2.4−10.8%), *Solitalea* (8−13.4%), *Parvibaculum* (2.3−6%).

All the genera mentioned above were not detected in the BML inoculum alone. Many of the genera have been previously detected in similar oil sands environments like OSPW and tailings. Brevundimonas has been found to be dominant in OSPW microbial communities, and has also shown potential for aerobic hydrocarbon degradation (An et al., 2013; Rochman et al., 2018; Yu et al., 2018). *Brevundimonas* sp. was isolated with

extracted NAs from OSPW as the sole carbon source, and showed potential to degrade polycyclic aromatic model NAs (Phillips et al., 2010; Yue et al., 2015). *Alishewanella* and *Hydrogenophaga* have been reported to be dominant in OSPW communities and biofilm communities developed from oil sands tailings ponds (Golby et al., 2012; Rochman et al., 2018). Cultures capable of degrading diluted bitumen and toluene/benzene had abundant *Hydrogenophaga* (Aburto and Peimbert, 2011; Deshpande et al., 2018; Song et al., 2018). *Hyphomonas* and *Pseudofulvimonas* were found in asphaltene-degrading cultures (Song et al., 2018). *Parvibaculum* is a known hydrocarbon-degrader and has also been found in oil sands tailings environments and cultures degrading crude oil (Rodriguez-r et al., 2015; Song et al., 2018; Yu et al., 2018; Deshpande et al., 2018).

In this study, *C. kessleri* greatly increased the diversity of the BML inoculum, and the co-culture was able to degrade the model NAs more completely and rapidly. The degradation of model NAs was faster in the co-cultures because the diverse community contained many species capable of hydrocarbon and NA degradation. Overall, the results showed that adding the algal culture to the BML inoculum has great potential for bioremediation in the BML by enhancing the diversity of the indigenous microbial community, and allowing it to biodegrade NAs more effectively.

4.4 Conclusion

In this study, degradation of CHCA, CHAA and CHBA by *C. kessleri*, *B. braunii* and BML indigenous microbes were investigated, and the same potential degradation pathways were proposed for all tested cultures in this study. CHBA was transformed into CHAA through β-oxidation. CHAA was metabolized through β-oxidation with the identified HAA. CHCA was degraded through β-oxidation pathway to 1-CHCA. Degradation of CHBA and CHCA was enhanced in the co-culture of *C. kessleri* and BML inoculum.

Adding algae greatly increased the diversity of the microbial community in the BML inoculum; many of the identified genera were known hydrocarbon and NA degraders (e.g., *Brevundimonas*, *Hydrogenophaga*, *Parvibaculum*, *Pseudofulvimonas* and *Hyphomonas*). *C. kessleri*, belonging to the Chlorophyta, was isolated earlier by our lab from the cyclone over-flow water at Syncrudes bitumen extraction facility. In WIP (precursor of BML), sequences belonging to Chlorophyta have also been identified among the existing eukaryotic community (Aguilar et al., 2016). As BML develops, the algal and microbial communities are expected to play a more important role in remediation.

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Chapter 5

Removing turbidity from Base Mine Lake cap water using microbial additions

5.1 Introduction

In previous chapters, biodegradation of organics (bitumen and model NAs) has been investigated, and the indigenous microbial communitys contribution to organic removal was highlighted. Due to the limitation of time, part of the results in this chapter remained preliminary (without replication) but showed valuable proof-of-concept methods for remediation. In this chapter, turbidity removal using microbial applications was investigated.

Through the comprehensive monitoring program of BML conducted by Syncrude, high turbidity during the lake turnover was identified (Syncrude Canada Ltd., 2017). High turbidity limits light penetration, which in turn will affect rates of primary production.

A cloudy lake also poses challenges for stakeholder acceptance in term of lake aesthetics. Suspended tailings particles cause the elevated turbidity in the ice-off season due to the lake turnover activity, and the increase in turbidity was consistently observed in 2013−2016 (Lawrence et al., 2016; Syncrude Canada Ltd., 2017). The clarity of the cap water is vital for the light to penetrate and support photosynthetic activities, and to establish the primary producers in the lake. The littoral zone requires 2−3 m light penetration to maintain a healthy level of biodiversity (Charette et al., 2010).

To mitigate the elevated turbidity, experience from the wastewater industry was explored. Inorganic and organic coagulants and flocculants have been used to both dewater oil sands tailings and improve the clarity of release water (Sworska et al., 2000; Wang et al., 2015; Botha et al., 2017; Gumfekar et al., 2017; Vajihinejad et al., 2017; Pennetta de Oliveira et al., 2018). A recent study also showed that pH adjustment at a proper ionic strength can potentially reduce turbidity (Poon et al., 2018). In September 2016, alum was added to the lake to reduce the cap water turbidity (Syncrude Canada Ltd., 2017). Cap water turbidity remained low (5−10 NTU) after the alum addition even during fall turnover (Syncrude Canada Ltd., 2017). This alum addition has shown promising clarity enhancement in the cap water, and metagenomic studies of the biota in the water cap showed that the eukaryotic community has increased along with the improved light penetration (Aguilar et al., 2016; Syncrude Canada Ltd., 2017).

Biological strategies to enhance the dewatering of the tailings have also been explored (Siddique et al., 2014; Yang et al., 2016; Yu et al., 2018), which could also be used to reduce cap water turbidity in an EPL by settling the tailings particles. This study investigates a turbidity mitigation strategy using microbial addition. The role of algae cannot be neglected because algae have the potential to remove organics and metals and develop synergistic relationships with the bacterial community (García-Meza et al., 2005; Muñoz et al., 2006; Mahdavi et al., 2012, 2015). Algal-bacterial consortia are found to

cause faster sedimentation than algae alone (Muñoz et al., 2004). Additionally, biofilm structure and high biomass concentration will also help microbes adhere to the clay and fine particles, which will accelerate the particle aggregation process (Bordenave et al., 2010; Golby et al., 2012). As the algal-bacterial consortium develops and matures, the aged algal-bacterial detritus will settle on the bottom of the lake and provide carbohydrates, proteins and lipids to feed the indigenous microbial communities (Hamilton et al., 1992). To explore the use of algae to mitigate turbidity, two microalgae were chosen: *C. kessleri*, which was isolated from Syncrude cyclone overflow water, and *B. braunii* which represented an exogenous species.

Liang et al. (2015) tested MICP process on the oil sands tailings materials and found that it increased the shear strength of the tailings. MICP can be carried out by *S. pasteurii*, a urea-hydrolyzing bacterium: in the presence of calcium ions, calcite precipitates on the surface of the bacterial cells leading to biocementation within the matrix (DeJong et al., 2010; Liang et al., 2015). MICP has been used effectively in the civil engineering industry to solidify loose soil and stabilize disperse soils (DeJong et al., 2010; Moravej et al., 2018). Liang et al. (2015) applied *S. pasteurii* and urea to the tailings, and the resulting shear strength of the tailings (initial solids content 35 wt%) was 27 times higher than the un-treated tailings (Liang et al., 2015). Based on the results of these previous studies, turbid EPL cap water can also be treated by MICP. Several conditions are required in the MICP process: alkaline pH, Ca^{2+}, $CO_3{}^{2-}$, and available nucleation sites (DeJong et al., 2010). *S. pasteurii* can provide the nucleation sites for calcite precipitation, while the first three conditions are readily or potentially readily available in the EPL cap water (Allen, 2008; DeJong et al., 2010). Of particular interest is whether MICP can occur in an oil sands EPL scenario, and what conditions are required in this situation. If effective MICP occurs, calcite crystals will bridge particles, providing opportunities for rapid aggregation and flocculation of the suspended particles, and will reduce the turbidity in

the EPL cap water. This research will fill the existing knowledge gaps and will test if *S. pasteurii* can be used as a microbial turbidity mitigation strategy in BML cap water.

5.2 Materials and methods

5.2.1 Strains and materials

S. pasteurii was purchased from Cedarlane (ON, Canada) and cultured in NH4-YE medium (Yeast extract: 20 g L^{-1}, $(NH_4)_2SO_4$: 10 g L^{-1}, Tris buffer: 0.13 mM, pH = 9.0). *C. kessleri* was previously isolated and identified from Syncrude cyclone overflow water (Mahdavi et al., 2012). *B. braunii* is a unicellular green microalga; the stain used in this study was obtained from UTEX Culture Collection of Algae at the University of Texas at Austin (UTEX 2441, USA). Algae were cultured in 0.22 µm-filtered BML cap water. The water and oil sands tailings used in this study were collected from BML and shipped in sealed buckets to the lab. All materials were stored at 4 °C cold room prior to use.

5.2.2 Water characterization

Water turbidity was measured with a turbidimeter (Orbeco-Hellige Digital Direct-Reading Turbidimeter; Orbeco Analytical System Inc., USA; or 2100Q Portable Turbidimeter Hach, Canada). Aqueous pH was measured using an Accumet® Research Dual Channel pH/Ion/Conductivity Meter (Fisher Scientific, USA). Optical density (OD) was measured by a NanoDrop ND-2000C UV-Vis Spectrophotometer (Thermo Scientific, USA). DOC and dissolved inorganic carbon (DIC)were measured with a Shimadzu Model TOC-L_{CPH}. Samples for DOC and DIC analysis were filtered through a 0.45 µm membrane

filter and diluted to a working range (5−100 mg L^{-1}) before analysis. Ca^{2+} was measured by PerkinElmer-SCIEX ELAN 9000 inductively coupled plasma mass spectrometry (ICP-MS) and by inductively coupled plasma optical emission spectrometry (ICP-OES) at the Natural Resources Analytical Laboratory at the University of Alberta. Ammonia was measured with a Nitrogen-Ammonia Reagent Set using Hach Method 10031 (details in Appendix A). Particle size was measured using a Malvern Zetasizer Nano ZSP instrument at 25 °C through electrophoretic light scattering. The water sample was diluted 1:50 with ultrapure water and sonicated for 30 min before particle size analysis to ensure that the particles were scattered enough to be measured individually. The mean value particle size measurement was reported (Zhang et al., 2018).

5.2.3 Experimental design

Turbidity removal by *S. pasteurii*

In MICP test 1, 12 Erlenmeyer flasks with different treatments were set up (Table 5.1). BML cap water was filtered through a 0.22 µm membrane filter, and 1 mL FFT from BML was added to increase the initial turbidity in all treatments. The bottles each contained approximately 500 mL of the mixed solution. Urea (1 mM, 5 mM, 10 mM, 20 mM, 50 mM or 333 mM), Ca^{2+} (100 mM or 500 mM $CaCl_2$), and 3 mL of *S. pasteurii* culture (grown in NH4-YE medium to an $OD_{600} = \sim 1$) were added as indicated in Table 5.1. Bacterial medium (NH4-YE medium) was added with the bacterial cells in test 1. This trial was monitored for 12−15 d, and on day 8, the bottles were shaken vigorously by hand and stirred by pipette to resuspend the settled particles. A sample was taken from water surface.

Table 5.1: MICP test 1 treatments. (U: urea, S: *S. pasteurii* , C: calcium, BML: filtered BML cap water mixed with FFT). The number in the bracket represents the concentration (mM) of the material indicated before the bracket, e.g., SU(333)C(100) represents 333 mM of Urea and *S. pasteurii* and 100 mM of calcium.

Treatment	*S. pasteurii*	Urea	Ca^{2+} (mM)
S	+	-	-
SU(1)	+	+ (1 mM)	-
SU(5)	+	+ (5 mM)	-
SU(10)	+	+ (10 mM)	-
SU(20)	+	+ (20 mM)	-
SU(50)	+	+ (50 mM)	-
SU(333)	+	+ (333 mM)	-
SU(333)C(100)	+	+ (333 mM)	+ (100)
SU(333)C(500)	+	+ (333 mM)	+ (500)
U(333)C(500)	-	+ (333 mM)	+ (500)
U(50)	-	+ (50 mM)	-
BML	-	-	-

MICP test 2 was set up in duplicate. Calcium was added at low or high concentration (Low: 0.175 mM, High: 1.35 mM) alone or in combination with *S. pasteurii* cells at low or high concentration (OD_{600} ∼1; Low: 3 mL, High: 30 mL). The *S. pasteurii* cells were washed by centrifuging at 6000 ×g for 3 min and washed three times by 0.9% NaCl solution before adding to the BML water. Ca^{2+} and *S. pasteurii* were added as indicated in Table 5.2. Unfiltered BML cap water was added to a final volume of 500 mL. Particles were re-suspended by shaking at 150 rpm for three hours. A sample was taken from water surface.

Table 5.2: MICP test 2 treatments (SL: 3 mL of *S. pasteurii* culture, SH: 30 mL of *S. pasteurii* culture, CL: ∼0.175 mM Ca^{2+}, CH: ∼1.35 mM Ca^{2+}, BML: BML cap water).

Treatment	*S. pasteurii*	Ca^{2+} (mM)
BML	-	-
BML-SL	+	-
BML-SH	+	-
BML-SH-CL	+	+ (0.175)
BML-SH-CH	+	+ (1.35)
BML-CL	-	+ (0.175)
BML-CH	-	+ (1.35)

There were some important differences between MICP tests 1 and 2. (1) Bacterial medium (NH4-YE medium) was added with the bacterial cells in test 1, while only the bacterial cells were added in test 2. This meant that further bacterial growth occurred during test 1. Adding the culture with the bacterial cells is a common practice in achieving MICP process in other situations (Liang et al., 2015; Moravej et al., 2018). (2) The calcium dosages in test 2 were significantly lower than the ones in test 1, and no urea was used. These optimized calcium dosages were determined through a series of preliminary tests. (3) The source of the turbidity was FFT particles in test 1 and BML cap water particles in test 2. (4) The resuspension was conducted in different manners: test 2 was considered more complete (3 hours shaking at 150 rpm), while test 1 resuspension was more arbitrary (manual shaking).

MICP test 3 was conducted in 2 L column in duplicate (setup shown in Fig. C.9). *S. pasteurii* was cultured in NH4-YE medium before addition. Twenty-five mL *S. pasteurii* culture (OD_{600} ∼0.65) was amended with 5 g of urea (final concentration in the column was ∼40 mM)), and BML cap water was added to a final volume of 2 L. This solution

was mixed in a sterile 4 L flask and then transferred into the column. Two litres of BML cap water were used as a control. All columns were placed on the bench with natural light conditions at 20 °C. During the experiment, the columns were sealed with Parafilm to help slow water evaporation. The sampling point was from the mid-point of the column (the 1 L mark).

Turbidity reduction by adding nutrients and algae

In the same 2 L column set up as MICP test 3, *C. kessleri* and *B. braunii* cultures were added to the BML cap water. Two litres of BML cap water was used as the control (shared control column in MICP test 3). Both algae were cultured in 0.22 µm-filtered BML cap water before addition. One hundred millilitres of algal culture (OD_{680} = 0.46−0.48) and 1900 mL of BML cap water were mixed in a sterile 4 L flask and transferred into the column. All columns were placed on the bench with natural light conditions at 20 °C. The columns were sealed with Parafilm to help slow water evaporation during the experiment.

The sampling point was the mid-point of the column (the 1 L mark). Nutrients (ammonium and phosphate as K_2HPO_4, $NH_4H_2PO_4$ or $(NH_4)_2HPO_4$) were spiked into one of the duplicates on day 49 (0.1 mM PO_4^{3-}), day 63 (0.15 mM PO_4^{3-} and 0.1 mM NH_4^+) and day 91 (12 mM PO_4^{3-} and 1 mM NH_4^+). For each nutrient spike, ammonium or phosphate salts were dissolved in 15 mL of BML cap water and then pumped into the column surface with a peristaltic pump (Cole Parmer, USA). The lowest pump speed was used to minimize disturbance. For the control column, 15 mL of BML was added with the same method to reflect any disturbance due to the addition. A summary of the experimental design is in Table 5.3.

Table 5.3: Experimental design for 2 L column test

Treatment	BML cap water	Algae	Nutrients	Description
BML	+	-	-	BML cap water (control)
BML-nutrients	+	-	+	Nutrient spike on day 49, 63 and 91
B. braunii	+	+	-	*B. braunii*
B. braunii-nutrients	+	+	+	Nutrient spike on day 49, 63 and 91
C. kessleri	+	+	-	*C. kessleri*
C. kessleri-nutrient	+	+	+	Nutrient spike on day 49, 63 and 91

5.3 Results and discussion

5.3.1 Turbidity removal by *S. pasteurii*

Turbidity

The mechanism of MICP is as follows: urea hydrolysis is catalyzed by an enzyme (urease) in *S. pasteurii*, generating NH_3 and CO_2 and increasing the pH through the reaction of ammonia and water. Increased pH causes the carbonate acid to dissociate into carbonate ions, and calcium ions will precipitate with carbonate ions to form calcite on the surface of microbial cells (DeJong et al., 2010; Strope et al., 2011; Liang et al., 2015). Three tests were conducted to understand if *S. pasteurii* can mitigate turbidity issue in an EPL scenario. A summary of test data is listed in Table 5.4. The following three subsections present the data and discuss the results of the individual tests.

(1) Urea and calcium influence on turbidity

The ability of *S. pasteurii* to mitigate turbidity in an EPL scenario was tested under different calcium and urea conditions. In test 1, the water turbidity was monitored daily (Fig. 5.1).). A positive control, U(333)C(500), was included because Ca^{2+} is used as

a coagulant in wastewater treatment to reduce turbidity (Lee et al., 2014). Turbidity was rapidly removed in the control U(333)C(500) treatment, and after resuspension, the turbidity dropped to the same level within only 1 d. The treatments S, SU(1), SU(5), SU(10), SU(20), SU(50) and SU(333) had higher turbidity removal with increasing concentrations of urea (0−333 mM). The SU(333) treatment had the highest turbidity removal (> 98% removal) among all the treatments without calcium addition. After resuspension, the SU(333) treatment returned to the previous turbidity level within 1 d. For other treatments, the turbidity returned to the previous turbidity levels within 3−5 d.

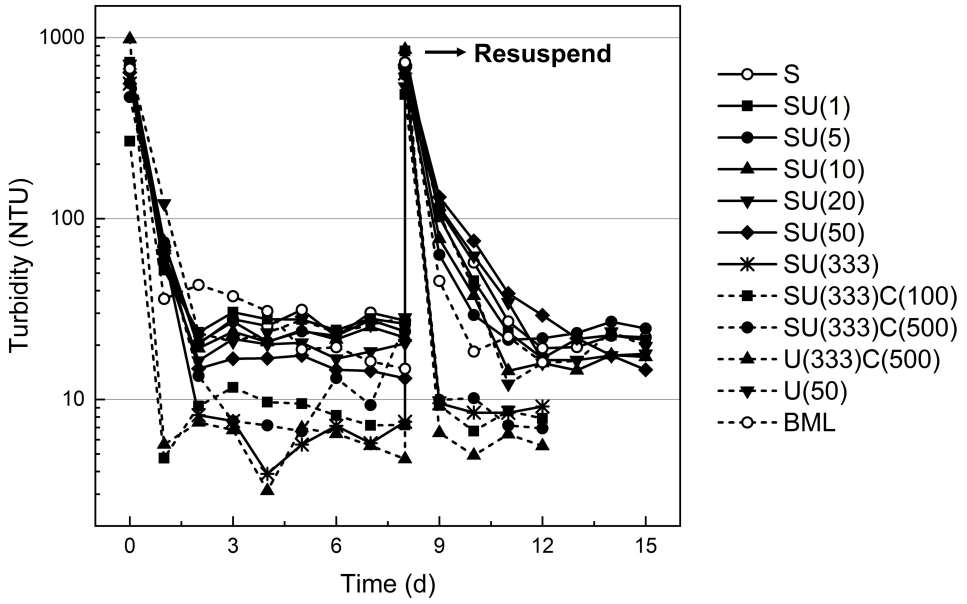

Figure 5.1: The effect of calcium and urea concentration on the MICP test 1 (y axis: log-10 scale). U: urea (concentration in mM), S: *S. pasteurii* addition, C: $CaCl_2$ (concentration in mM), BML: filtered cap water with FFT addition.

The treatments SU(333)C(100) and SU(333)C(500) treatments had similar turbidity re-

duction compared to the SU(333) treatment. Urea addition provides an optimal condition for the MICP process to remove turbidity, but adding additional calcium did not increase turbidity reduction. This indicates that the BML cap water (∼0.4−0.5 mM Ca^{2+}) had enough calcium ions for MICP to occur. However, difference between the particle appearance was observed (Fig. 5.2). Characterization of the particles was not conducted, but it was hypothesized that calcium and bacterial cells helped form larger particles, resulting in a more stable precipitation through the MICP process.

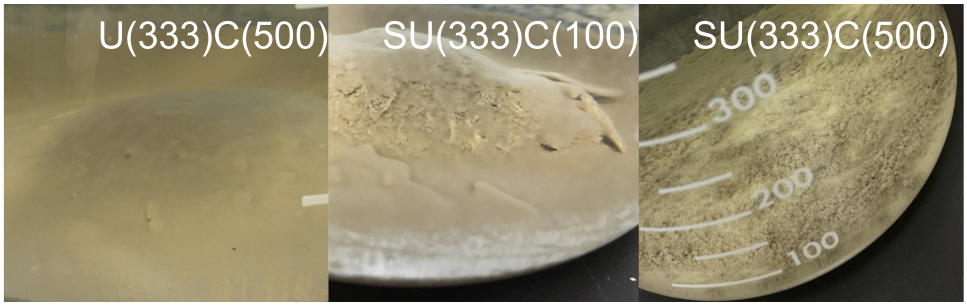

Figure 5.2: Photos of the settled particles in different treatment flasks (after 12 d). U: urea addition (concentration in mM), S: *S. pasteurii* addition, C: $CaCl_2$ addition (concentration in mM).

(2) Bacterial cell addition with lower calcium dosage

In test 2, lower doses of calcium (0.175 mM and 1.35 mM) were added, and washed bacterial cells were added instead of the bacterial culture (Fig. 5.3). The first step in MICP is urea hydrolysis, but the second step of calcite precipitation is what aggregates the particles and remove turbidity. Test 2 had no added urea to bypass the urea hydrolysis step and achieve only the calcite precipitation step.

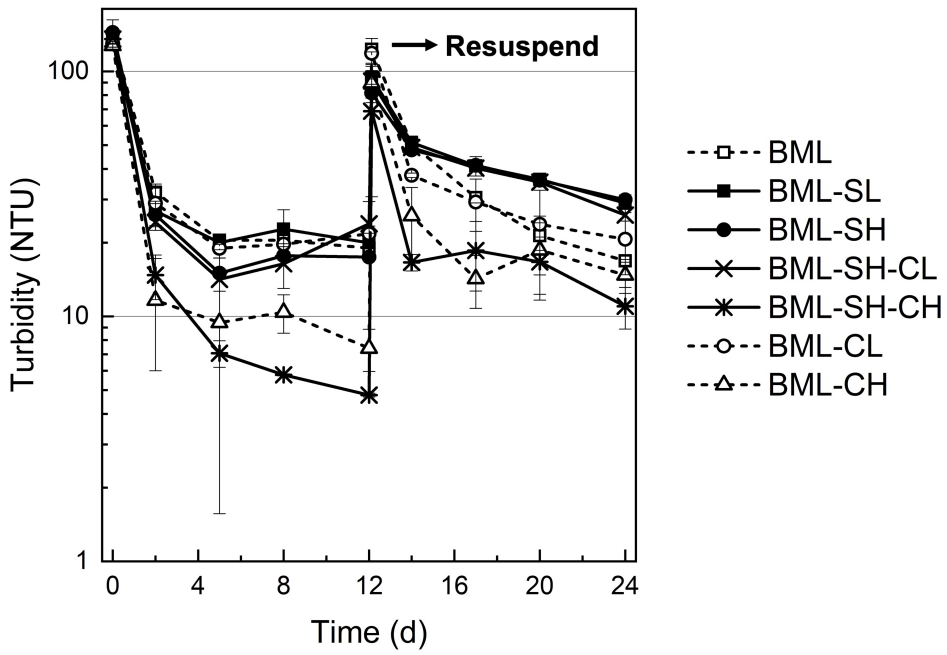

Figure 5.3: The effect of calcium concentration and bacterial added amount on the MICP test 2 (y axis: log-10 scale). SL and SH represent the 3 mL and 30 mL of *S. pasteurii* bacterial cells addition, respectively. CL and CH represent 0.175 mM and 1.35 mM $CaCl_2$ addition, respectively. BML is the cap water from BML. Results are presented as an average ± one standard deviation (n = 2).

BML cap water was used as a control with an initial turbidity of ∼130−140 NTU and no FFT was added to increase the turbidity. Treatments with only bacterial cell addition (BML-SL and BML-SH) and 0.175 mM calcium addition (BML-SH-CL and BML-CL) showed no significant enhancement of turbidity removal compared to the control (Table 5.4). The bacterial cell addition alone showed no effective turbidity removal because the added cells were unable to grow in BML cap water, and the interaction with calcium ions was limited by the low cell density and low available calcium ions.

In BML-SH-CH, with more calcium (1.35 mM) added, ∼92% of the turbidity was removed, while 1.35 mM calcium addition (BML-CH) alone had ∼88% turbidity removal. The relatively higher calcium ion concentration provided more opportunities for the calcium and bacterial cells to interact and form calcite on the bacterial surface. The particles formed with *S. pasteurii* settled faster after resuspension on day 12. The treatment BML-SH-CH (∼10 NTU) achieved a 40% lower turbidity than BML-CH.

The particle size was measured before and after all treatments using a Malvern Zetasizer Nano ZSP instrument (Fig. 5.4). The particle sizes increased slightly by 6−18% in BML-CL and BML-CH (summarized in Table D.4). In the *S. pasteurii*-amended treatments (BML-SL, BML-SH, BML-SH-CL and BML-SH-CH), particle sizes increased by at least 64%. In BML-SL and BML-SH, although the particle size increased, no enhancement in turbidity was achieved. The bacterial cells tended to adhere to and grow on the particle surface, which was the reason particle size increased with bacterial addition (Bordenave et al., 2010; Golby et al., 2012). When calcium was added, the particles were able to coagulate. Calcium is a divalent cation and is not as strong as polymer coagulants or alum (trivalent cation). The coagulation was limited at this low dosage when there were a relative high number of particles to be treated. When calcium was added at a higher dosage (1.35 mM) along with bacterial cells, calcite precipitation occurred using bacterial cells as nucleation sites in the presence of BML cap water existing alkalinity (DeJong et al., 2010), and a more stable structure was formed. The density of the formed particles was potentially increased.

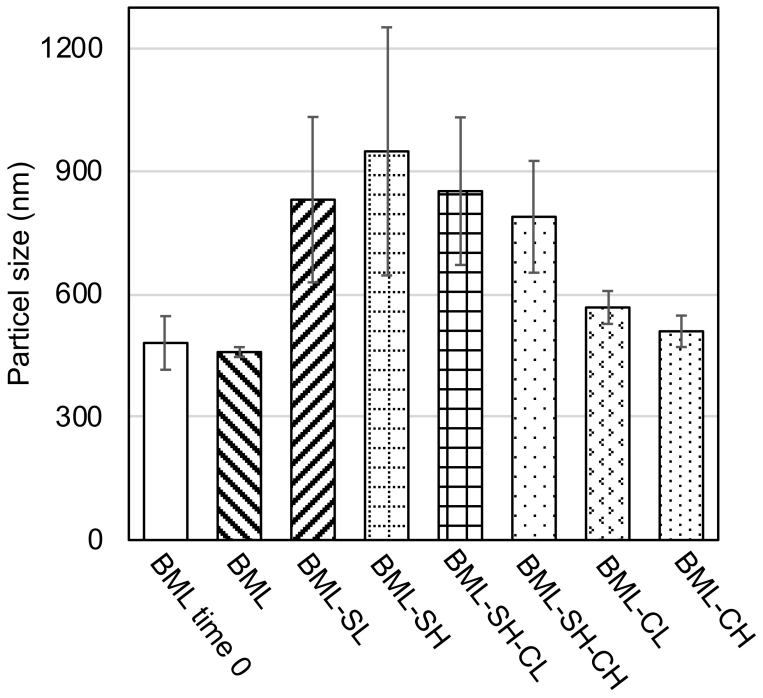

Figure 5.4: Particle sizes of the particles in MICP test 2. BML time 0 represents the original particle size in the BML cap water before any treatment. The other 7 columns represent the particle size after 24 d in each treatment. Results are presented as an average ± one standard deviation (for BML time 0: n = 3; for other treatments: n = 6).

(3) Column test

In MICP test 3, a larger scale experiment was set up in 2 L columns. More than 86% of turbidity was removed in the columns amended with *S. pasteurii* (Fig. 5.5). In test 3, the two duplicates had 23% and 49% more turbidity reduction after 160 d when compared to the test 3 control. BML cap water was used as a control in tests 2 and 3, however the two controls behaved differently. In test 2, 86% of the turbidity was removed in the BML control after 12 d, while in test 3 only 15% turbidity was removed in test 3 BML control after 13 d. This discrepancy might be due to the different vessels used for these

two tests. Test 2 was conducted in 500 mL flask with surface water sampled for analysis, and test 3 was conducted in 2 L columns with a narrower diameter, and were sampled at the mid-point (the 1 L mark). Removing turbidity in water further below the surface is more difficult since particles need to settle a longer distance. Treatment of BML cap water at a larger scale may be more difficult if the water turbidity needs to be reduced at depth as well as at the surface.

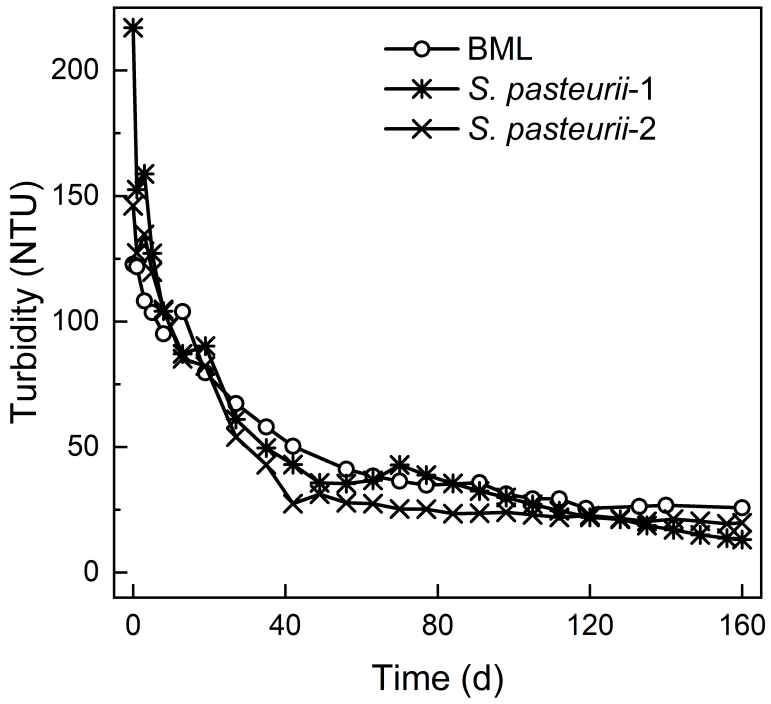

Figure 5.5: Turbidity reduction by MICP in test 3 (2 L column experiment). *S. pasteurii*-1 and *S. pasteurii*-2 are duplicates, and BML is BML cap water.

Table 5.4: Turbidity removal (%) and enhancement (%) in MICP tests over the whole experiment. In test 1, U: urea, S: *S. pasteurii*, C: calcium, BML: filtered BML cap water and FFT mixed solution. The number in the bracket represents the concentration (mM) of the content before the bracket, e.g., SU(333)C(100) represents 100 mM of calcium, 333 mM of urea and *S. pasteurii* addition. In test 2, SL: 3 mL of *S. pasteurii* culture, SH: 30 mL of *S. pasteurii* culture, CL: 0.175 mM Ca^{2+}, CH: 1.35 mM Ca^{2+}, BML: BML cap water. In test 3, BML: BML cap water column, *S. pasteurii*-1 and *S. pasteurii*-2: two duplicate columns.

Test 1	Turbidity removal[1]	Enhancement[2]
BML	96.0%	Control
S	96.8%	-
SU(1)	97.0%	-
SU(5)	95.6%	-
SU(10)	97.6%	21.5%
SU(20)	97.5%	18.3%
SU(50)	97.7%	33.3%
SU(333)	98.7%	68.0%
SU(333)C(100)	97.0%	60.7%
SU(333)C(500)	98.3%	62.8%
U(333)C(500)	99.2%	67.8%
U(50)	97.2%	10.0%
Test 2	Turbidity removal	Enhancement
BML	87.8%	Control
BML-SL	78.3%	-
BML-SH	79.2%	-
BML-SH-CL	80.9%	-
BML-SH-CH	91.9%	34.7%
BML-CL	84.1%	-
BML-CH	88.4%	12.5%
Test 3	Turbidity removal	Enhancement
BML	79.0%	Control
S. pasteurii-1	94.0%	49.0%
S. pasteurii-2	86.4%	23.0%

[1]Turbidity removal was calculated by: (turbidity end-turbidity beginning)/turbidity beginning × 100%.

[2]Enhancement was calculated by: (turbidity end of the treatment-turbidity end in control)/turbidity end in control × 100%.

The role of pH, calcium and carbonate ions in MICP

As introduced earlier, during the MICP process, four major reactions occur (Equations 5.1−5.4), and carbonate/bicarbonate, calcium and ammonia/ammonium are the dominant ions. The MICP process is initiated by the urea hydrolysis, accelerated by the urease enzyme from *S. pasteurii*. The generated CO_2 will react with water to form carbonic acid. The pH-dependent dissociations of ammonia and carbonic acid will occur to generate aqueous ammonia/ammonium and bicarbonate/carbonate in the system. Since ammonia is a strong base and carbonic acid is a relatively weak acid, urea hydrolysis will increase the pH of the system. The carbonate ions will precipitate with the cells that attract the calcium to form calcite. Theoretically, 1 mol of urea will generate a maximum of 2 mol of ammonia and 1 mol of CO_2. However, the system in this study is open to the air, therefore, loss of ammonia and CO_2 into the gas phase is also possible.

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{Urease}} 2\,NH_3 + CO_2 \tag{5.1}$$

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \rightleftharpoons CO_3^{2-} + 2\,H^+ \tag{5.2}$$

$$\text{Cell} + Ca^{2+} \longrightarrow \text{Cell}{\cdot}Ca^{2+} \tag{5.3}$$

$$\text{Cell}{\cdot}Ca^{2+} + CO_3^{2-} \longrightarrow \text{Cell}{\cdot}CaCO_3\downarrow \tag{5.4}$$

In test 1, different concentrations of urea (1 mM, 5 mM, 10 mM, 20 mM, 50 m and 333 mM) and calcium (100 mM and 500 mM) were added. Theoretically, urea will increase pH by generating ammonia during hydrolysis, and calcium as a Lewis acid will decrease the pH by liberating the hydrogen. The rate of urea hydrolysis is closely related to the bacterial enzyme urease, while the reaction of calcium with water occurs immediately. For ammonium, the pK_a is 9.25, and for carbonic acid, pK_{a1} is 6.35 and pK_{a2} is 10.32. If the pH is higher than 9.25, ammonia (aq) will dominate over ammonium, and when pH is greater than 10.3, 90% will be present as ammonia (aq). If pH is between 6.5 and 10.1, more than 60% of total carbonic species be present as bicarbonate.

The pH increased as expected when more urea was added in the presence of *S. pas-*

teurii (Fig. C.10). For SU(333), pH increased to 10, the highest in all treatments. The pH remained relatively stable with the higher calcium addition in SU(333)C(500) and U(333)C(500). The DIC (carbonic acid, bicarbonate and carbonate) and NH_3-N (ammonia (aq) and ammonium) concentrations in test 1 are shown in Fig. 5.6 and Fig. 5.7. The calcium concentrations from test 1 are shown in Fig. C.11. Based on the pH, the compositions of DIC (dissolved carbon dioxide, carbonate or bicarbonate) and NH_3-N (ammonia or ammonium) can be calculated. The BML water alone had a moderate concentration of DIC (approximately 12 mM with bicarbonate $> 95\%$), low calcium ($0.2-0.5$ mM) and non-detectable NH_3-N. Adding only *S. pasteurii* did not significantly change the DIC, DIC, calcium and NH_3-N concentrations. When higher concentrations of urea were added with *S. pasteurii*, more DIC and NH_3-N were generated, and more calcium was consumed indicating a more effective MICP process. Based on a linear approximation of the urea dosage and calcium removal within 10 d ($R^2 = 0.96$, Fig. 5.3), 65 mM of urea can fully react with the existing calcium to precipitate as calcite, and achieve a reasonale turbidity removal.

Without *S. pasteurii*, urea hydrolysis was not effective. In U(333)C(500), less than 10 mM of NH_3-N was generated, and DIC was mostly consumed by reacting with calcium. The treatments SU(333)C(100) and SU(333)C(500) had the same concentration of added urea, but SU(333)C(100) had generated more than 3 times the NH_3-N compared to SU(333)C(500). The low pH or high calcium concentration in SU(333)C(500) hindered the urea hydrolysis process. Treatment SU(333)C(100) had an immediate increase in DIC and NH_3-N levels after resuspension; resuspension might have resulted in better mixing of bacteria and the urea, which facilitated the MICP process. Also, in SU(333)C(100) and SU(333)C(500), a similar amount of calcium ($50-60$ mM) was consumed after 4 d indicating that the additional calcium could not be fully utilized.

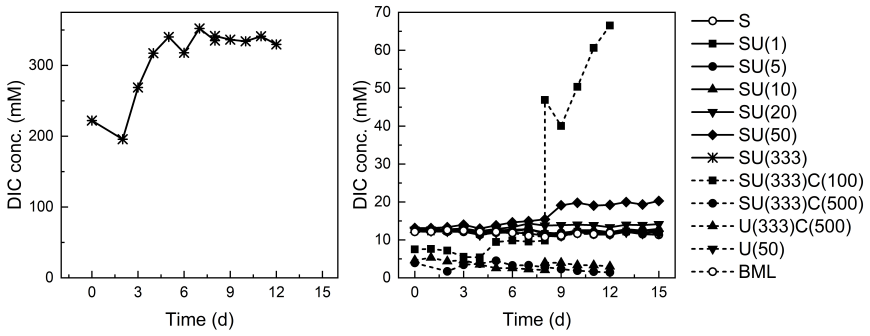

Figure 5.6: DIC concentrations in all treatments of test 1 are presented. Treatment SU(333) is in a different scale due to its higher concentration.

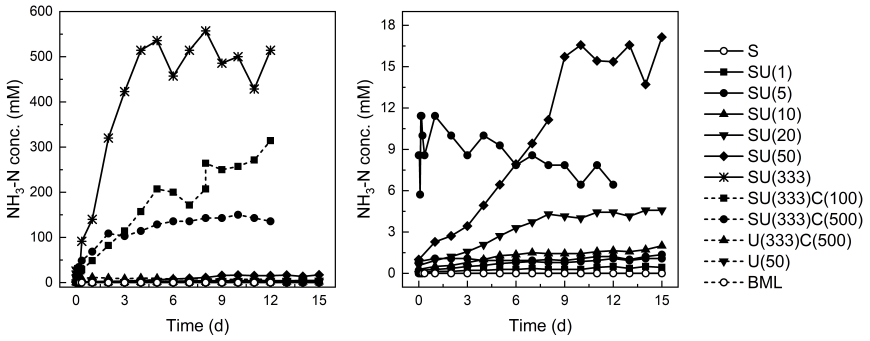

Figure 5.7: Nitrogen concentrations in the form of ammonium and ammonia in all treatments of test 1 are presented (two different y scales are used).

Fig. 5.8 shows the test 3 nitrogen concentrations in different forms. About 40 mM of urea was added to both *S. pasteurii* columns, however, only 14% of urea in *S. pasteurii*-1 column and 5% of urea in *S. pasteurii*-2 column was hydrolyzed. *S. pasteurii*-1 had more urea hydrolysis and also had a 30% lower turbidity than *S. pasteurii*-2 column. In test 1, further hydrolysis of SU(333)C(100) was observed after resuspension, indicating that resuspension might benefit MICP process.

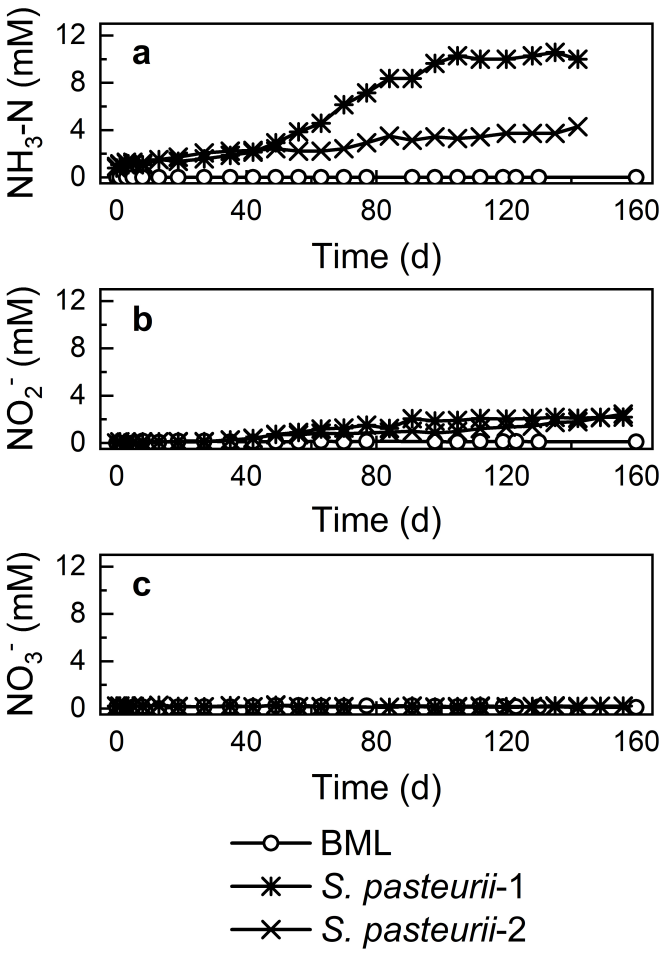

Figure 5.8: Nitrogen concentrations (mM) in different forms (a) NH_3/NH_4^+; b) NO_2^-; c) NO_3^-) are shown. Duplicate treatments of test 3 are presented separately.

5.3.2 Turbidity removal using nutrient and algal additions

According to previous data, turbidity of 23 NTU corresponded to having 1 m light penetration (Secchi depth) and indicated sufficient water clarity (Brandon, 2016). Levels of turbidity (Fig. 5.9), phosphate (Fig. C.13) and nitrogen in different forms (NH_3/NH_4^+, NO_2^-, NO_3^-; Fig. 5.10) were monitored over the 160-day experiment. Although BML

column had an obvious turbidity reduction (88%) without any intervention after 160 d, the turbidity level of 25 NTU was still insufficient for 1 m light penetration (23 NTU). A more effective turbidity removal ($> 90\%$) is required to have at least 1 m light penetration. No enhancement in turbidity removal was observed in any column before the second nutrient spike. Nutrient addition did not enhance the turbidity removal in *C. kessleri*-nutrients column; both *C. kessleri* and *C. kessleri*-nutrients columns achieved a 92−95% turbidity removal after 140 d. However, the *B. braunii*-nutrients column had a lower turbidity (92% reduction) than the *B. braunii* column (74% reduction) after 140 d (BML column: 88%).

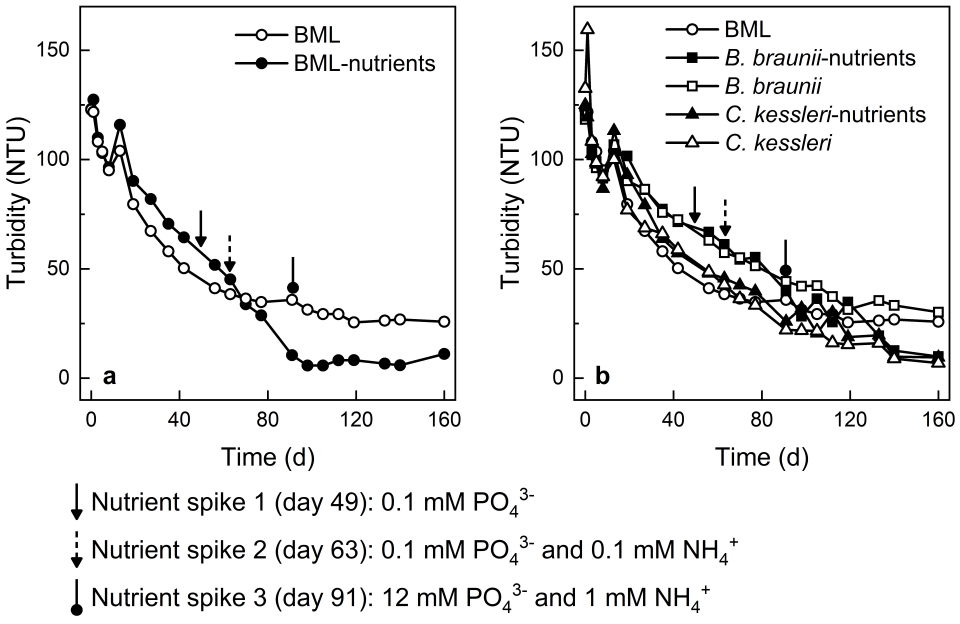

Figure 5.9: Panel a shows the nutrient addition influence on turbidity, and panel b shows the effect of two algal species with or without nutrient addition on the turbidity of BML cap water in 2 L columns. Nutrient spikes on day 49, 63 and 91 are marked with arrows as shown.

The BML-nutrients column began to show enhanced turbidity reduction two weeks after the second nutrient spike, and the turbidity was reduced by 91% before the third nutrient spike (BML column: 71%). The third addition did not decrease the turbidity further. The BML-nutrients column turbidity remained low (6−10 NTU) until the end of the experiment. The water in the BML-nutrients column also became green when the turbidity was reduced, indicating that native algal growth was stimulated by the nutrient addition.

The added PO_4^{3-} was partially consumed (∼20% reduction in the BML-nutrients and *B. braunii*-nutrients columns, and ∼50 % reduction by *C. kessleri*-nutrients column). The added NH_4^+ was consumed completely in all columns with nutrient addition. In *B. braunii*-nutrients column, no transformation of NH_4^+ into NO_2^- or NO_3^- was observed, indicating that *B. braunii* assimilated NH_4^+ directly. In BML-nutrients and *C. kessleri*-nutrients columns, NO_2^- was measured, possibly generated by the ubiquitous ammonia-oxidizing bacteria residing in the BML water. The generated NO_2^- was consumed by the algae in BML-nutrients and *C. kessleri*-nutrients columns by the end of the experiment.

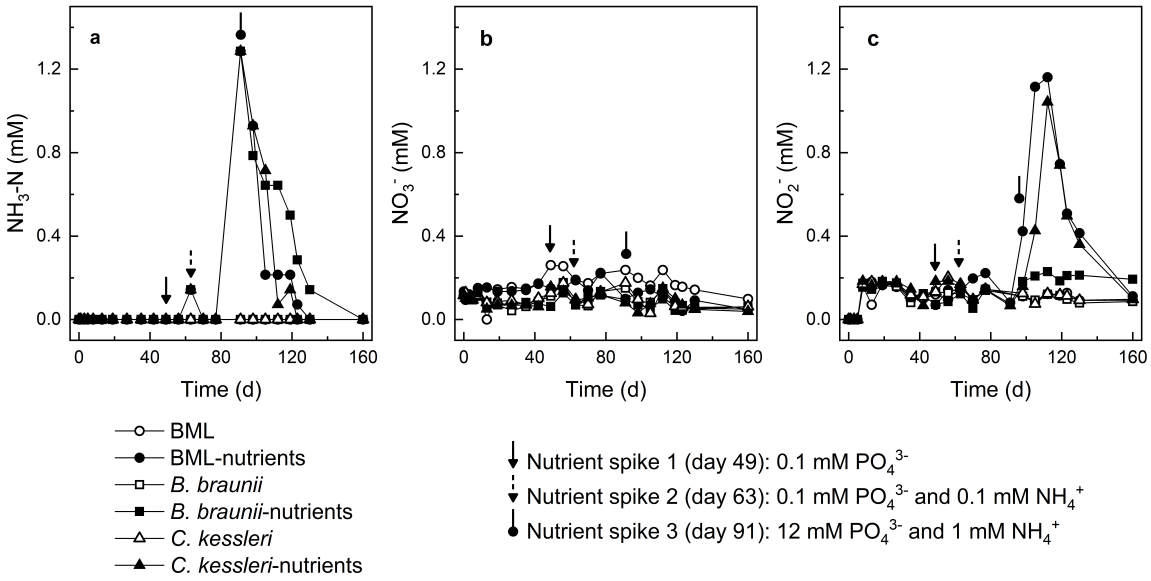

Figure 5.10: Nitrogen concentrations (mM) in different forms (a)NH_3/NH_4^+; b) NO_2^-; c) NO_3^- in 2 L column experiment exploring nutrient addition influence and the effect of two algal species with or without nutrient addition on the turbidity. Nutrient spikes on day 49, 63 and 91 are marked with arrows as shown.

5.3.3 Effectiveness of microbial application in reducing turbidity in an EPL

Although this trial study lacked replication and did not include characterization of the particles or microbial analysis, the data indicate that turbidity mitigation strategy using microbial additions is possible. Oil sands EPL cap water contains some key components required in the MICP process: alkaline pH (usually 8.0−8.8), alkalinity of 8−10 mM $CaCO_3$ and calcium (0.3−0.7 mM) (Siwik et al., 2000; Farrell et al., 2004; Allen, 2008). The role of urea in the MICP process is to increase the alkalinity and provide an optimal pH. Ammonia uptake by *S. pasteurii* was limited, but nitrite generation was observed

after ammonia was consumed, possibly facilitated by the native ammonia-oxidizing bacteria in BML cap water. Therefore, this urea addition can be replaced with any action that increases alkalinity and pH, such as adding more carbonate salts or adding a strong base. *S. pasteurii* has not been identified as a member of the microbial community indigenous to BML, however if used at field scale, *S. pasteurii* cells would become part of the flocculated solids and would not proliferate in the long term. The preliminary results showed that *S. pasteurii* with urea addition could reduce turbidity as well as flocculation with calcium, and larger particles were formed during MICP process compared to the ones formed during flocculation process. When a low dosage of calcium was used (1.35 mM), the addition of *S. pasteurii* enhanced the turbidity removal by 75% by generating larger particles. The MICP process potentially helps generate flocs that are more resistant to resuspension. The MICP process can also potentially be used to pre-treat OSPW before placing in an EPL to reduce fine particles, which contribute to high turbidity during spring and fall turnover.

The addition of nutrients and algae highlighted the significance of the indigenous algaes role in the reclamation of an EPL. Algae have been previously shown to be effective in removing organics and metals, and the algal community could develop synergistic relationships with bacteria to improve the microbial remediation process (García-Meza et al., 2005; Muñoz et al., 2006; Mahdavi et al., 2012). The algal-bacterial consortia can adhere to the clay and fine particles to accelerate the particle aggregation process and achieve a faster sedimentation (Muñoz et al., 2004; Bordenave et al., 2010; Golby et al., 2012). In the real-world application, added nutrients might not be necessary. A relatively diverse microalgal community was discovered in the samples from MLSB (tailings source for BML) and WIP (precursor of BML) tailings and water (Aguilar et al., 2016). In addition, Syncrude's BML monitoring program has detected a thriving eukaryotic community (including microalgae), as well as increased DO and reduced turbidity especially after the

addition of alum to the lake in 2016 (Syncrude Canada Ltd., 2017). BML will provide important information on the role of algal communities in the reclamation process.

5.4 Conclusion

Two tested algal species (*C. kessleri* and *S. pasteurii*) were shown to effectively remove turbidity from BML cap water. *S. pasteurii* can help increase the size of suspended particles in the BML cap water, and if calcium or urea are also added, the bacteria can remove turbidity in the BML cap water. The larger particles could have more stable structure and settle faster, and potentially reduce turbidity during the lake turnover events. The preliminary results in this study also showed the potential of the MICP process to pre-treat OSPW. In the column tests, nutrient addition stimulated the growth of indigenous microbes, and further reduced turbidity. The indigenous algal community showed potential to facilitate the particle settling process while establishing a community of primary producers in an EPL. The reduced turbidity will increase light penetration and further benefit the growth of the indigenous algal community. When constructing a new EPL, microbial turbidity mitigation processes can be taken into consideration to lower the cost of the entire reclamation process.

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Chapter 6

Conclusions and further work

6.1 Summary of findings

The purpose of this research was to explore microbial remediation solutions for cap water quality issues in an EPL context. Syncrude's BML is the first field-scale EPL demonstration, as such the majority of research conducted to date on oil sands EPLs, concentrates on BML. Many studies have been focusing on tailings, since it is the tailings presence, depth, and properties that drive most lake biogeochemical processes (Dompierre and Barbour, 2016; Dompierre et al., 2016; Foght et al., 2017; White et al., 2017). As BML develops, microorganisms will play a significant role in cap water quality improvement and the water quality can be used as an indicator of the health of the lake ecosystem (Yergeau et al., 2012). The research in this thesis specifically investigated the ability of the indigenous tailings/cap water microbial community as well as individual microbial strains to remove residual bitumen, model NAs and cap water turbidity. Lab research in this thesis conducted proof-of-concept ideas for the EPL strategy of oil sands impacted land reclamation. A summary of key research findings from each chapter in this thesis

are provided below.

Chapter 2:

Research question: Will bitumen be a source of toxicity in bitumen-containing water? Can the residual bitumen found in BML be biodegraded by indigenous microorganisms?

1. MFT-D1 and MFT-D2 were oil sands tailings sampled from the same tailings pond at two depths (11.2 m and 12 m), and MFT-Mix is a mixture of tailings from several companies. These MFTs had different concentrations of F2 (C10−C16), F3 (C16−C34) and bitumen content (> C35), but bitumen content (60−70 wt%) usually dominated in the concentrations of the organics in the tailings.

2. In MFT-D1 (initial bitumen content: ∼40 mg g^{-1}) and MFT-D2 (initial bitumen content: ∼30 mg g^{-1}), bitumen contents decrease by 30−46% with UltraZyme amended, regardless of dosage (0.5−5.0 g L^{-1}) and temperature. No significant removal of bitumen was observed in the MFT-Mix.

3. The released pore water from MFT-D1 was detoxified (∼1 TU) after 55 d (50% Microtox® toxicity reduction regardless of UltraZyme dosage). The released pore water from MFT-D1 had a lower initial toxicity (∼1.5 TU), and in Group UT-20 °C, detoxification was also achieved. NA concentration in the pore water decreased on average by 36% from ∼70 mg L^{-1} in UltraZyme-amended MFT-Mix after treatment. In MFT-D1, increasing UltraZyme dosages resulted in greater DOC removal (up to 28%), however, NA removal was not found in MFT-D1. No DOC removal occurred in MFT-D2. The released pore water from MFT-Mix didn't show any toxicity reduction. This detoxification was suspected to be due to the bitumen removal instead of NA or DOC.

4. Groups UT-20 °C and U(-)T-20 °C contained non-autoclaved and autoclaved UltraZyme, and both had a similar level of bitumen removal and released water toxicity

reduction. This indicated that the easily degradable hydrocarbons in UltraZyme played a role instead of the microorganisms.

5. The presence of bitumen in water with/without tailings led to a toxicity increase (1.5−5.5 times, initial toxicity: 2−3 TU). The addition of easily degradable hydrocarbons (UltraZyme) could help to detoxify bitumen-polluted aqueous environments (< 1 TU).

6. The indigenous tailings microbes were shown to be active in removing organics effectively. Bitumen reduction observed in MFT-D1 and MFT-D2 could be caused by the stimulation of indigenous tailings microbes using UltraZyme as a carbon source, potentially co-metabolizing the heavier organic compounds.

Chapter 3:
Research question (same as Chapter 2): Will bitumen be a source of toxicity in bitumen-containing water? Can the residual bitumen found in BML be biodegraded by indigenous microorganisms?

7. Bitumen addition greatly influenced the petroleum hydrocarbon distribution. BML-B, BML-BT and BML-BTC had a similar distribution of these four classifications (B represents bitumen, T represents tailings and C represents acetate): F2: 1%, F3: 21−22%, F4: 10% and F4G-SG: 67−68%, while BML-T had a unique distribution of: F2: 5%, F3: 28%, F4: 12% and F4G-SG: 55%.

8. Acetate addition (BML-BTC group) mitigated toxicity from the organics present and effectively removed the PHC compounds (parentheses indicate initial concentrations; reductions: F2 (∼3000 mg kg^{-1}): 64%, F3 (∼56000 mg kg^{-1}): 58%, F4 (∼26000 mg kg^{-1}): 58% and F4G-SG (180000 mg kg^{-1}): 68%), when compared to the BML-BT group (F2 (∼3000 mg kg^{-1}): 23%, F3(∼56000 mg kg^{-1}): 26%, F4

(∼26000 mg kg^{-1}): 24% and F4G-SG (∼180000 mg kg^{-1}): 35%). The PHC results indicates that the addition of acetate may have triggered co-metabolic processes and that more hydrocarbons were catabolized in the presence of acetate. Along with the findings 4 and 6 in Chapter 2, biostimulation using readily degradable carbon is considered one of the potential remediation methods for heavy hydrocarbon removal.

9. Bitumen addition increased the aquatic toxicity by approximately three times when mixed with the BML water (from ∼4 TU to ∼13 TU), and by 20 times when mixed with the BML water and tailings (from ∼1 TU to ∼20 TU). The toxicity data showed the bitumen could increase acute toxicity if present in water. This finding was consistent with finding 5 in Chapter 2.

10. BML-B group demonstrated that bitumen was a source of AEOs but not of O_2^- compounds. 20−40% removal of O_2^- compounds was observed in groups containing tailings (BML-BT, BML-BTC, BML-T), demonstrating the ability of indigenous microbes to remove both NAs and bitumen-sourced organic acids.

11. The quantitative increases in bacterial populations and the increase of the relative abundances of known oil-degrading bacteria indicated a strong selective response of indigenous microbial communities in the presence of the bitumen obtained from BML. BML-T groups bacterial density remained relatively constant (1.3 times denser after 100 d). BML-BT had a 70% reduction in bacterial density, while BML-BTC had a 3.8 times bacterial density increase with the help of acetate stimulation treatment.

12. Among the bacteria that were sequenced in different treatment groups, *Rhodoferax*, *Acidovorax* and *Pseudomonas* showed more potential for biostimulation treatment with acetate to remove PHC/bitumen. *Pseudomonas* spp. were the most signifi-

cantly stimulated genus by acetate and might serve as the biggest contributor to bitumen removal and toxicity mitigation.

Chapter 4:

Research question: Will the tested algae species and BML cap water microbes be able to biodegrade or absorb model NA? Will the co-culture of the two have a more effective NA degradation?

13. Two algae species (*C. kessleri* and *B. braunii*) were tested for their tolerance to three model NAs (CHCA, CHAA and CHBA). The addition of 100 mg L^{-1} CHCA, CHBA or CHAA did not have a significant negative influence on the growth of *C. kessleri* or its nitrogen or phosphorus uptake rate except the following scenario: 100 mg L^{-1} CHCA on *C. kessleri* lowered the nitrogen uptake. *B. braunii*, however, was less tolerant to 100 mg L^{-1} of CHBA and CHAA. Also, adding 300 mg L^{-1} of CHCA, CHBA or CHAA inhibited both algae by introducing an approximate 15−30 d lag phase on algal growth and decreasing the nitrogen uptake.

14. *B. braunii*, *C. kessleri* and BML inoculum metabolized CHBA into CHAA via β-oxidation. The degradation pathway of CHBA was integrated into the CHAA degradation pathway. With the help of LC-MS, $C_8H_{14}O_3$ was identified as (1-hydroxycyclohexyl)-acetic acid (HAA) with the authentic standard. The CHAA metabolism was shown to be similar to that of *Arthrobacter* sp. strain CA1.

15. The CHCA degradation by BML inoculum occurred without a lag phase, while *C. kessleri* and *B. braunii* had 40-d and 60-d lag phases, respectively. Bacterial degradation of CHCA is more effective than the algal degradation.

16. 1-cyclohexene-1-carboxylic acid (1-CHCA, $C_7H_{10}O_2$) and 2-oxocyclohexnaecarboxylic acid ($C_7H_{10}O_3$) were identified with the authentic standard, and β-oxidation path-

way with the intermediate 1-CHCA was proposed for the CHCA degradation pathway for *C. kessleri*, *B. braunii* and BML inoculum.

17. A co-culture of BML inoculum and *C. kessleri* was used to degrade CHBA. The co-culture degraded CHBA 60% and 110% faster than BML inoculum and *C. kessleri* alone, respectively. The generated CHAA was also completely degraded in the co-culture, while less than 20% of the CHAA was removed by BML inoculum and *C. kessleri* alone.

18. A co-culture of BML inoculum and *C. kessleri* was used to degrade CHCA. The co-culture degraded CHCA 14% and 4% faster than BML inoculum and *C. kessleri* alone, respectively. Similar to the results for CHBA, CHCA was biodegraded more completely by the co-culture. The peak area of the intermediate 1-CHCA showed that the 1-CHCA was completely consumed (not detectable) after 40 d in the co-culture group, while in the BML inoculum group and *C. kessleri* culture group, 1-CHCA was not completely removed or was removed more slowly than in the co-culture group.

19. The microbial community results revealed that in the co-culture, *C. kessleri* greatly increased the diversity of the BML inoculum. The degradation of model NAs was faster in the co-cultures due to the more diverse community containing many species (e.g., *Brevundimonas*, *Hydrogenophaga*, *Parvibaculum*, *Pseudofulvimonas* and *Hyphomonas*) capable of hydrocarbon and NA degradation.

Chapter 5:

Research question: Can MICP processes help reduce turbidity in cap water? What conditions will MICP require to occur in BML cap water? Will algal additions help mitigate turbidity issues in BML cap water?

20. The *S. pasteurii* cell addition could increase the particle size in the BML cap water.

21. Preliminary tests indicated that with *S. pasteurii* alone, MICP might not be fully carried out in the BML cap water. However, the addition of *S. pasteurii* with calcium or urea could achieve effective turbidity removal in the BML cap water, and after resuspension, the treated water can return to previous turbidity levels.

22. The particles that were formed during the MICP process might have a more stable structure and show potential to be more resistant to the lake turnover.

23. *C. kessleri* addition can remove the $> 90\%$ cap water turbidity in a 2 L column set up after 160 d. Nutrient addition can also achieve 90% turbidity removal in the same set up by stimulating the growth of the indigenous algal community.

6.2 Future research and recommendations

As monitoring of BML progresses, more information and experience can be obtained from the BML field-scale experiment. After nearly 6 years of development, bitumen mats can be seen floating on the lake surface, bringing the issue of residual bitumen to the forefront (Syncrude Canada Ltd., 2017). The transport mechanism of this bitumen mat remains unknown, and although biogenic gas was thought to play a role, the link between biogenic gas production and floating bitumen was not confirmed, indicating that there could be many other mechanisms involved in the bitumen transport process. If the bitumen transport mechanism can be better understood, a better tailings management approach can be developed i.e. if any pre-treatment of the tailings is needed, or if a good indicator for the bitumen in the tailings can be developed. In Chapters 2 and 3, we found that bitumen significantly changed the petroleum hydrocarbon profile in the tailings phase and Microtox® toxicity of the cap water. Similar questions about bitumen influence on

the water quality have been asked in the past, such as whether bitumen biodegradation is a source of the NAs (Quagraine et al., 2005). Biostimulation treatment of the indigenous tailings community remains the strongest candidate for bitumen removal. However, biodegradation does not always correlate with toxicity reduction, and a more in depth understanding of residual bitumen fate is needed for a more comprehensive reclamation plan.

Next-generation sequencing results revealed a relatively diverse eukaryotic community in the MLSB and WIP, both of which contain materials similar to BML. In the long term, algal community in the BML will serve as primary producers and stimulate a healthy lake ecosystem (Aguilar et al., 2016). *C. kessleri* and *B. braunii* were tested for their capability to degrade three simple model NAs. The indigenous BML cap water was co-cultured with *C. kessleri* to degrade the same model NAs and showed more effective and complete removal. To continue the research, a co-culture can be used to degrade oil sands NAs. Although efforts in analyzing oil sands NA were continuously made, no universal technique has been applied. To overcome this difficulty, a mixture of model NAs, including the ones with more complex structure, may be tested. In this research, only the bacterial community targeting 16S rRNA was sequenced. Unfortunately, the algal community profile was missing due to technique limitations. Future research can investigate the indigenous algal community response after adding *C. kessleri*.

The cause of cap water turbidity should also be investigated. After adding alum in 2016, the water turbidity was temporarily maintained lower than pre-alum cap water (Syncrude Canada Ltd., 2017). However, it is still difficult to predict the future turbidity level in the lake without knowing what drives the turbidity in the cap water. While the results for removing turbidity using microbial application remained preliminary, *S. pasteurii* and indigenous algae (including *C. kessleri*) showed their potential to remove the cap water turbidity in a lab scale study. *S. pasteurii* achieved turbidity removal by carrying out

the MICP process and interacting with the cap water particles. Larger particles were also formed when adding *S. pasteurii*. More lab scale experiments can be conducted to optimize conditions for utilizing *S. pasteurii* for long-term turbidity removal and to characterize the *S. pasteurii*-formed particles. Using *S. pasteurii* to pre-treat the cap water also shows potential if the fine particles in the cap water can be confirmed as a significant turbidity contributor. In the 2 L column test in Chapter 5, adding only nutrients to the cap water could help remove the turbidity, and the water showed a green color indicating algal growth. Algae are ubiquitous in natural lakes and known for causing algal bloom issues if excess nutrients exist. In a lake like BML, nutrient level can be actively monitored, and nutrients are usually at a limited level (relative to the amount needed for optimal algal growth) in OSPW and oil sands tailings. The algae are unlikely to cause algal bloom, but instead, grow on the clay particle surface, settle to the bottom as a detrital layer and benefit the bacterial community at the same time. It will be of particular interest to investigate the capabilities of *C. kessleri* and similar indigenous algal species.

The validation of EPL is important for the oil sands industry. With more in-depth laboratory studies and BML as a full-scale experiment with a comprehensive research and monitoring program, there is a greater understanding of the viability of EPLs for tailings reclamation. Water quality issues, such as residual bitumen and turbidity, are challenging when the causes remain poorly understood. I would recommend continuing to understand microbial activities in tailings reclamation, including pit lakes and consider using microbial applications in EPL adaptive management. As BML reveals more mysteries about the interaction between the dewatering FFT and water cap, efforts should be made to better utilize the strength of microorganisms, especially those of the indigenous community.

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Appendices

Appendix A

Analytical method

A.1 CO_2

CO_2 measurement was performed using an Agilent 7890A gas chromatography equipped with a thermal conductivity detector (GC-TCD) (Agilent HP-PLOT/Q column: 30 m $\times$ 320 µm $\times$ 0.2 µm). The oven temperature gradient was as follows: 50 °C for 2 min, then at a rate of 30 °C min^{-1} increased to 150 °C which was then maintained for 2 min. Helium was used as carrier gas with the following flow program: 8.83 mL min^{-1} for 2 min, decreasing to 5.67 mL min^{-1} until the end of the separation. Total run time was 7.33 min. The detector was maintained at 200 °C, and the injection port at 300 °C. The makeup gas (helium) was set to 5 mL min^{-1}. The injector split ratio was set to 5:1 (no gas saver), with a column flow of 8.89 mL min^{-1}, split vent flow of 44.4 mL min^{-1}, and a septum purge flow of 58.3 mL min^{-1} under a pressure of 30 psi. A typical injection volume was 1 µL.

A.2 DOC

Dissolved organic carbon (DOC) measurement was performed using a Shimadzu Model TOC-L_{CPH}. Samples for DOC analysis were filtered with a 0.45 µm membrane filter and diluted to a working range (5−100 mg L^{-1}). Non-Purgeable Organic Carbon (NPOC) method was used for DOC analysis, which only measures the nonvolatile DOC content during the purging process. DOC was considered to be a nonspecific parameter to track soluble metabolites during any degradation process. Native microorganisms may also catabolize high-molecular hydrocarbons into smaller molecules that may increase DOC concentration.

A.3 Acetate

Acetate was measured by Ion Chromatography (Dionex ICS 2100, Dionex™ IonPac™ AS18 IC columns). Eluent conditions were as follows: 10 mM KOH from zero to 7 min, 10−32 mM from 7 to 9 min, 32 mM from 9 to 20 min, 32−10 mM from 20 to 22 min, 10 mM from 22−23 min. Eluent flow rate was at 0.25 mL min^{-1}. Oven temperature was maintained at 30 °C. Conductivity detector temperature was maintained at 35 °C. Detection was achieved with a suppressed conductivity-using anion self-regeneration suppresser (ASRS 2 mm, AutoSuppression mode, 20 mA current). Background conductance was lower than 1 µS, and system backpressure was approximately 2000 psi. Samples were diluted into appropriate concentrations for detection (1−20 mg L^{-1}, $R^2 > 0.995$).

A.4 Nitrogen-ammonia

Nitrogen-ammonia range is 0.4−50.0 mg L^{-1}. Set the DR900 Multiparameter Portable Colorimeter program at 343 N, Ammonia HR TNT. Prepare the blank using 0.1 mL of ammonia-free water into the AmVer™ Diluent Reagent Test 'N Tube for High Range Ammonia Nitrogen. Measure 0.1 mL of the sample into the test tube. Add one Ammonia Salicylate Reagent Powder Pillow and one Ammonia Cyanurate Reagent Powder Pillow into each tube. Close the caps and shake the tubes thoroughly to dissolve the power. Insert the blank tube after 20 min and push ZERO in the instrument. Measure the sample by pushing READ. The result is shown in mg L^{-1} NH_3-N.

A.5 NA

Two methods were used for naphthenic acids (NAs) determination. NAs measured by the first method were defined as acid extractable organics (AEOs). NAs measured by the second method were separated into different species, which is discussed below.

The first method was performed through extraction and then detection by a gas chromatograph: AEOs were extracted from 0.22 µm-filtered BML water (Brown and Ulrich, 2015a), derivatized by N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA), and analyzed using an Agilent 7890A gas chromatography equipped with a flame ionization detector (GC-FID) (J&W 122-5512 column: 15 m × 250 µm × 0.25 µm). The temperature gradient was as follows: 50 °C for 2 min, then at a rate of 30 °C min^{-1} increasing to 280 °C which was then maintained for 8 min. Helium was used as carrier gas with the following flow program: 0.15 mL min^{-1} for 2 min, then decreased to 0.063 mL min^{-1} which was then maintained until the end of the separation. Total run time was 17.7 min. The detector was maintained at 300 °C. Column flow plus makeup gas

(helium) was set to 32.1 mL min^{-1}, air flow 450 mL min^{-1} and H_2 flow 40 mL min^{-1}. A typical injection volume was 1 µL. Merichem NAs were used as standards for quantification, and fluorene-9-carboxylic acid (FCA) was used as an internal standard to determine extraction efficiency.

The second method was performed as follows: reversed-phase chromatography paired with a linear ion trap-Orbitrap mass spectrometer (Orbitrap XL, Thermo Fisher Scientific, San Jose) was used (Pereira and Martin, 2015). Matrix effects were reduced by operating using an atmospheric pressure chemical ionization source positive (+) and negative (-). For each sample, suspended solids were removed by syringe filtration using a 0.45 µm nylon membrane filter. Prior to injection, 1 mL aliquots of sample were spiked with 100 µL of a combined internal standard solution with a final concentration of 1 mg L^{-1} D23-lauric acid and 0.01 mg L^{-1} for D3-progesterone. Chromatographic separation was performed on a Hypersil Gold C18 Selectivity Column (Thermo Scientific, Edmonton, AB) (50 × 2.1 mm, particle size 1.9 µm), using the High Performance Liquid Chromatography (HPLC) Accela system (Thermo Scientific, San Jose, CA) at 40 °C with a flow rate of 0.5 mL min^{-1}. Xcalibur software was used to acquire the output of HPLC-MS data. Species were binned into heteroatomic classes: $O^{\pm}$, $O^{2\pm}$, $O^{3\pm}$, $O^{4\pm}$, $O^{5\pm}$, $O^{6\pm}$, $SO^{\pm}$, $SO^{2\pm}$, $SO^{3\pm}$, $SO^{4\pm}$, $NO^{\pm}$, $NO^{2\pm}$, $NO^{3\pm}$. Only those species with a response at least three times greater than that of the response in the procedural blank were included in analyses. A 100 mg L^{-1} Merichem stock solution was prepared in isopropanol and subsequently diluted in methanol to produce a standard curve with a range of 0.5 to 50 mg L^{-1}.

A.6 Dean Stark extraction

Dean Stark extraction procedure: About 300 mL toluene was added to a round-bottom flask. After boiling the solvent, toluene vapor contacted the samples, and vapors containing water and toluene entered the condenser and cooled, to be collected into the glass trap. Immiscible liquids formed two phases, with water on the bottom, thereby allowing easy separation and collection by the trap. During refluxing, toluene vapor dissolved the bitumen, permeated the thimble wall, and then returned to the round-bottom flask. Each extraction required at least two hours to complete, confirmed by a stable water level in the glass trap. The volume of water collected from the glass trap was measured to determine water content. The thimble was vented of toluene in the fume hood and then baked at 105 °C overnight to remove residual water. The weight difference of the thimble before and after the extraction represents the solids content. The bitumen-containing solution in the round-bottom flask was transferred into a 250 mL volumetric flask, and toluene was added to the 250 mL mark. Twenty-five mL of the liquid was poured onto a weighed filter paper and allowed to dry overnight in the fume hood, followed by oven drying at 105 °C overnight. The mass increase in the filter paper was multiplied by the 10× dilution factor to determine bitumen content. If PHC fraction (F2−4) data were available, PHC fractions were subtracted from bitumen content, and the calculated bitumen content was used instead.

A.7 Model NA

Model NA compound determination was performed by High Performance Liquid Chromatography (HPLC) with an Acclaim OA column (4 × 250 mm, 5 mm particle size). One mL of the culture was sampled and filtered through a 0.2 µm membrane filter prior

to analysis on Agilent 1260 Infinity series HPLC. To quantify CHAA, CHBA and CHCA, the mobile phase consisted of 60% (vol/vol) KH_2PO_4 (2 mM, pH 2.5) and 40% (vol/vol) acetonitrile (Optima grade, Fisher, Canada). The UV absorbance detector was set at 210 nm for all molecules. The method was modified from Mouttaki et al. (2007) and Clothier et al. (2016). Calibration curves were prepared using authentic standards of the model NAs ($R^2 > 0.995$).

A.8 Bitumen extraction procedure

Bitumen was extracted from oil sands by toluene extraction. This procedure ensures the purity of bitumen but sacrifices the volatile and semi-volatile content in the bitumen. The bitumen extraction procedure is as follows: 1 L toluene is added to 80 g oil sands and left overnight to solubilize bitumen and allow the settling of insoluble particles. The supernatant is transferred into another container and placed in a fume hood overnight to evaporate the toluene. The remaining material is dried at 105 °C for 2 hours and transferred into a desiccator to cool to room temperature. Extracted bitumen is covered with foil for storage. The first extraction of 80 g of oil sands can yield 4−5 g of bitumen, and the extraction can be repeated twice for greater yield. Up to 8 g of bitumen can be obtained from 80 g of oil sands.

A.9 PHC

Petroleum hydrocarbon fractions were sent to Maxxam Analytics for F2, F3, F4, and F4G-SG analysis. The Canada-wide Standard for Petroleum Hydrocarbons in soil (PHC CWS) was used as the method. The PHC CWS groups petroleum hydrocarbons into these practical fractions: F1 (C6−C10), F2 (C10−C16), F3 (C16−C34), F4 (C34−C50),

and F4G-SG (> C50). Tailings samples were preserved at 4 °C before testing. F1 fractions were measured prior to submission to Maxxam Analytics, which showed that F1 fractions were not present in the samples.

A.10 qPCR

The rpoB gene exists as one copy in bacterial cells, and can more accurately describe bacterial density (Dahllöf et al., 2000). The qPCR assay was performed using a Bio-Rad CFX96 optical reaction module conversion of the C1000 Touch thermal cycler. All samples and standards were in triplicate, and the amplification data were analyzed using Bio-Rad CFX Manager™ 3.0 software. The 20 µL qPCR reaction solution contained 1 L extracted DNA template, 10 µL Thermo Scientific™ DyNAmo™ ColorFlash SYBR™ Green qPCR supermix 2× 2 µL of each primer (final concentration: 0.5 µM), in sterile Ultrapure water. The cycle conditions were as follows: 95 °C for 7 minutes, 40 cycles of 95 °C for 10 seconds, 50 °C for 20 seconds, and 72 °C for 15 seconds. The qPCR assay was performed on DNA samples extracted from the tailings/solid phase in the beginning and end of the experiment. Each biological duplicate was measured three times (n = 6) for the end of the experiment, and the original tailings samples were measured six times (n = 6) for the initial bacterial population density for all groups.

A.11 Chlorophyll a extraction and measurement

A volume of 1.9 mL of sample was transferred to a microcentrifuge tube for centrifugation at 9200 × g for 5 min. Supernatant was discarded and 1.1 mL 99% methanol was added.

Tubes were incubated in the dark at 45 °C for 24 h. Optical density was measured at 665 nm and 652 nm. Chl a concentration (mg L^{-1}) = 16.72 × OD_{665} - 9.16 × OD_{652}

Appendix B

Mediuam recipe

B.1 Mineral Medium

Mineral Medium was used for all degradation experiments in this experiment. One L Mineral Medium contains 428.5 mg K_2HPO_4, 209.6 mg KH_2PO_4, 535 mg NH_4Cl, 70 mg $CaCl_2{\cdot}6H_2O$, 20 mg $FeCl_2{\cdot}4H_2O$, 0.6 mg H_3BO_3, 0.2 mg $ZnCl_2$, 0.146 mg $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 1.5 mg $NiCl_2{\cdot}6H_2O$, 2 mg $MnCl_2{\cdot}4H_2O$, 0.2 mg $CuCl_2{\cdot}2H_2O$, 3 mg $CoCl_2{\cdot}6H_2O$, 0.04 mg Na_2SeO_3, 0.2 mg $Al_2(SO_4)_3{\cdot}18H_2O$, 3.6 g H_2SO_4, 101.6 mg $MgCl_2{\cdot}6H_2O$, 690 mg $NaHCO_3$, 0.2 mg Biotin, 0.2 mg Folic acid, 1 mg Pyridoxine HCl, 0.5 mg Riboflavin, 0.5 mg Thiamine, 0.5 mg Nicotinic acid, 0.5 mg Pantothenic, 0.5 mg PABA, 0.5 mg Cyanocobalamin (Vitamin B12), 0.5 mg Thioctic (lipoic) acid, 10 mg Coenzyme M and the rest is Milli-Q® water (Millipore, Billerica, MA).

B.2 Bold's Basal Medium

250 mg L^{-1} $NaNO_3$, 25 mg L^{-1} $CaCl_2{\cdot}2H_2O$, 75 mg L^{-1} $MgSO_4{\cdot}7H_2O$, 75 mg L^{-1} K_2HPO4, 175 mg L^{-1} KH_2PO_4, 25 g L^{-1} NaCl, 50 mg L^{-1} EDTA, 31 mg L^{-1} KOH, 4.98 mg L^{-1} $FeSO_4{\cdot}7H_2O$, 1 µL L^{-1} H_2SO_4, 11.42 mg L^{-1} H_3BO_3, 8.82 mg L^{-1} $ZnSO_4{\cdot}7H_2O$, 1.44 mg L^{-1} $MnCl_2{\cdot}4H_2O$, 0.71 mg L^{-1} MoO_3, 1.57 mg L^{-1} $CuSO_4{\cdot}5H_2O$, 0.49 mg L^{-1} $Cu(NO_3)_2{\cdot}6H_2O$.

Appendix C

Figures

Figure C.1: Experimental setup for the column test in Chapter 2.

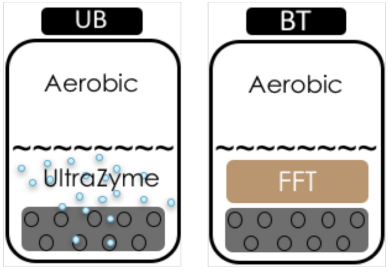

Figure C.2: Example experimental setup for the biodegradation test in Chapter 2.

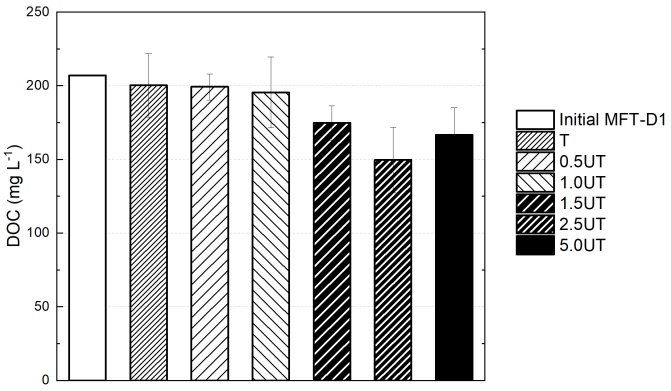

Figure C.3: DOC concentrations in the expressed pore water from MFT-D1 before and after treatment with various dosages of UltraZyme. Duplicate columns with MFT-D1 were incubated at 20 °C. Results are presented as an average (n = 2) ± one standard deviation.

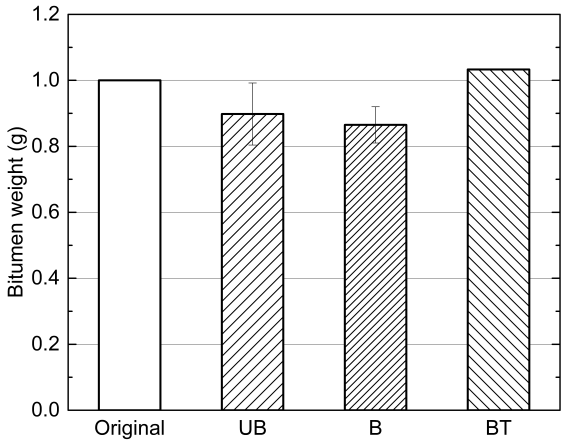

Figure C.4: Weight of bitumen added (original), and bitumen remaining after biodegradation after 100 d in Group UB, B, and BT. Results are presented as an average ± one standard deviation.

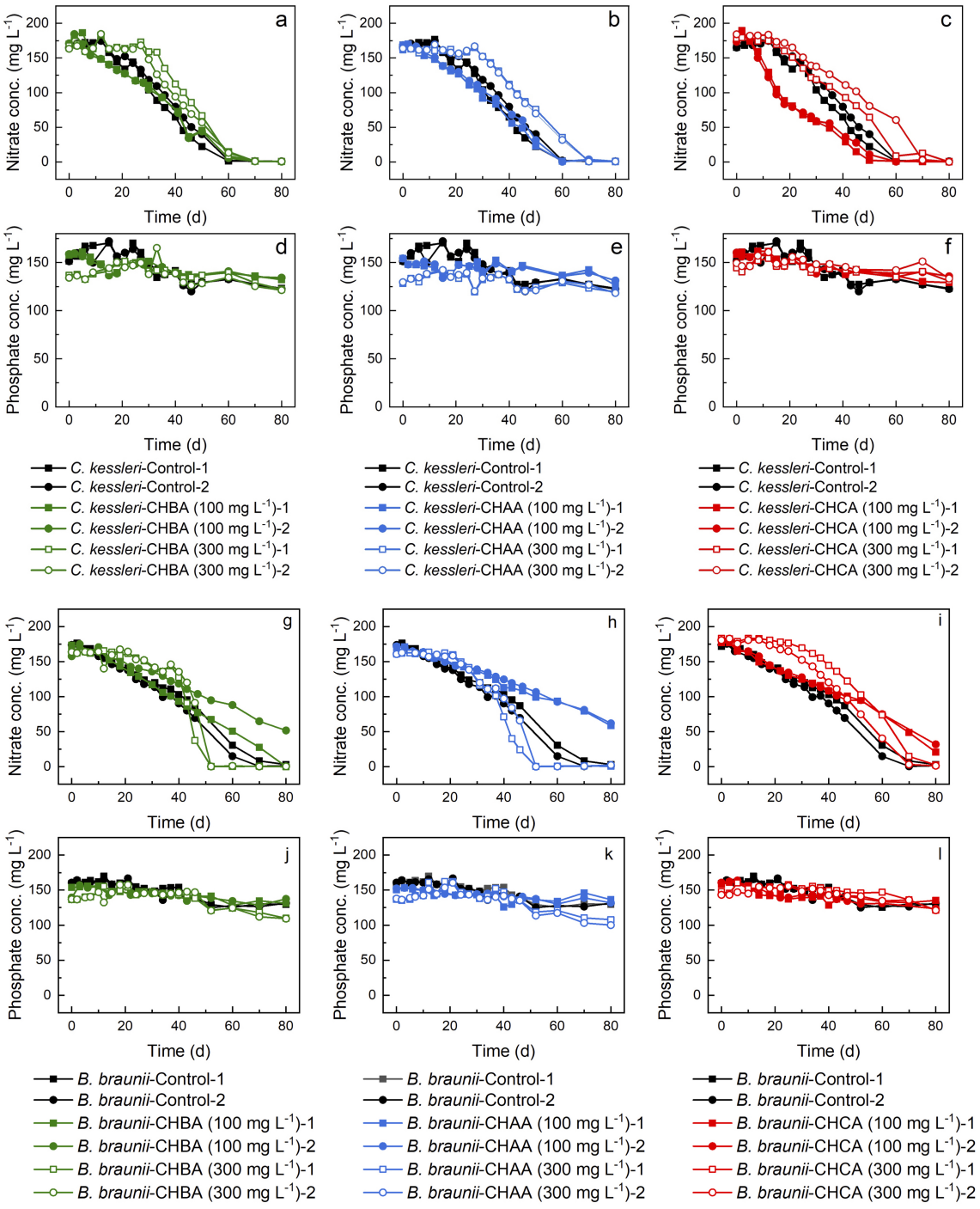

Figure C.5: Nutrient uptake (phosphate and nitrate) in *C. kessleri* and *B. braunii* tolerance bioassay. Nitrite and ammonium were also measured but both were below the detection limit.

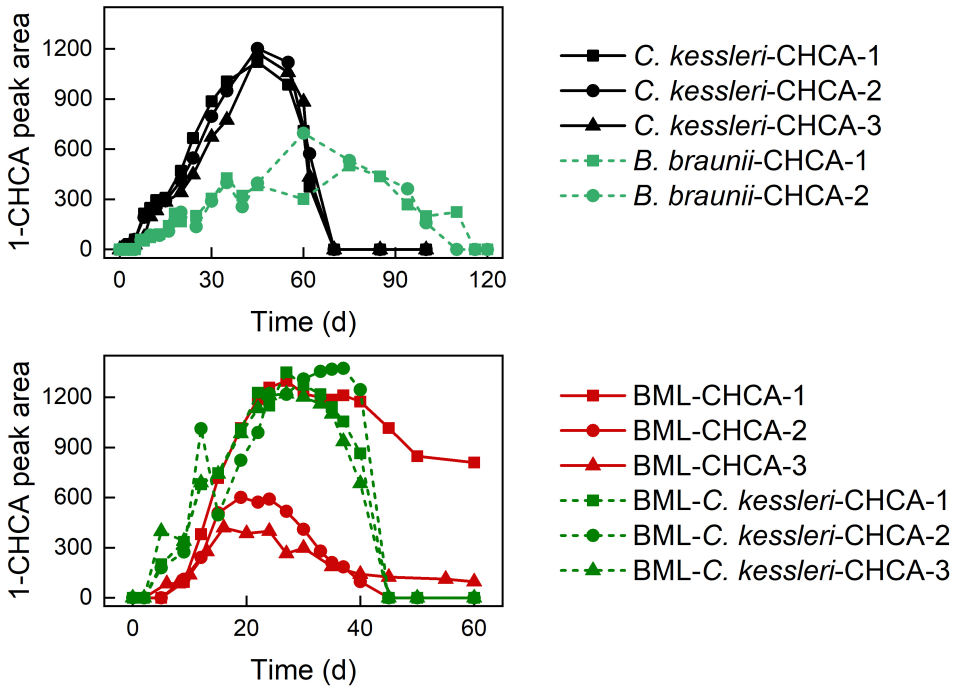

Figure C.6: 1-CHCA peak area summary in all groups that biodegraded CHCA.

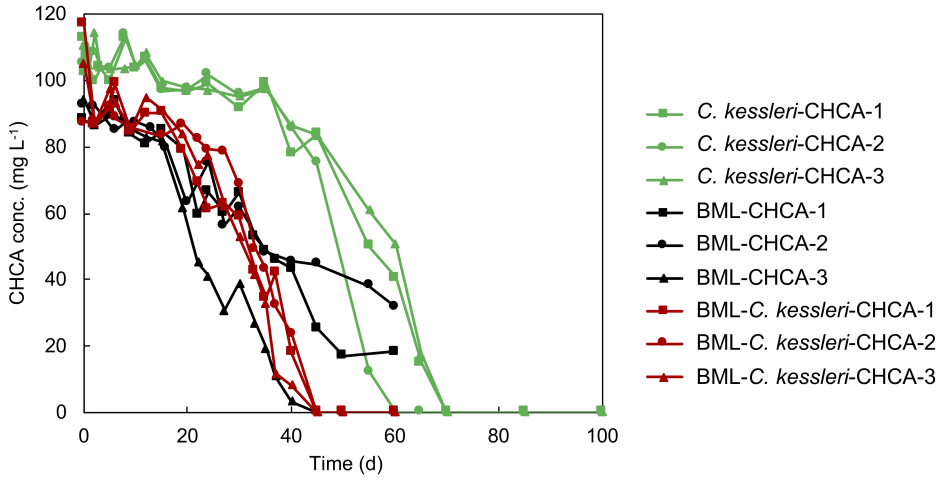

Figure C.7: CHCA concentrations in the biodegradation experiment by *C. kessleri*, BML inoculum and the co-culture. Triplicate data were presented separately.

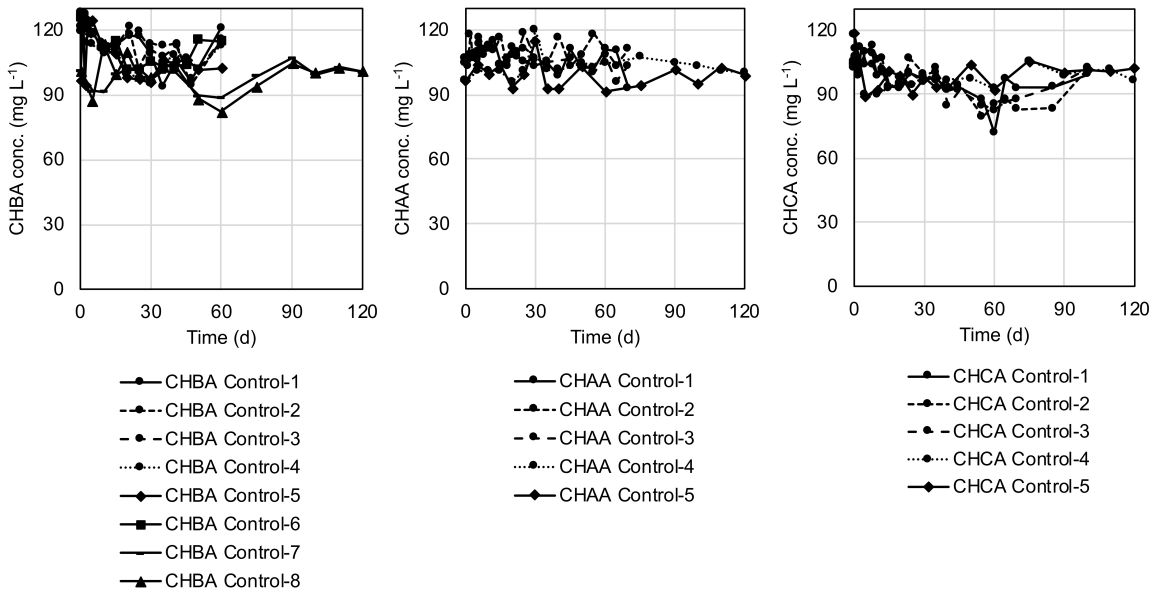

Figure C.8: All model NA control group data are presented.

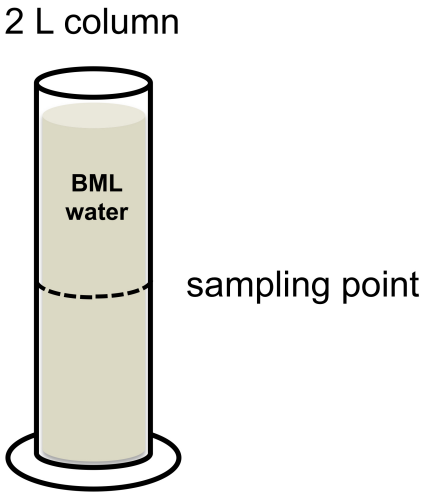

Figure C.9: Example experimental setup for the 2 L column test in Chapter 5.

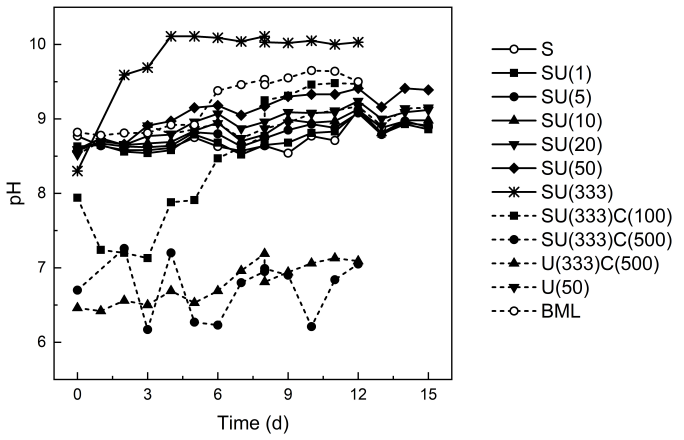

Figure C.10: pH data for all groups in the MICP test 1. U: urea (concentration in mM), S: *S. pasteurii* addition, C: $CaCl_2$ addition (concentration in mM), BML: filtered cap water with FFT addition.

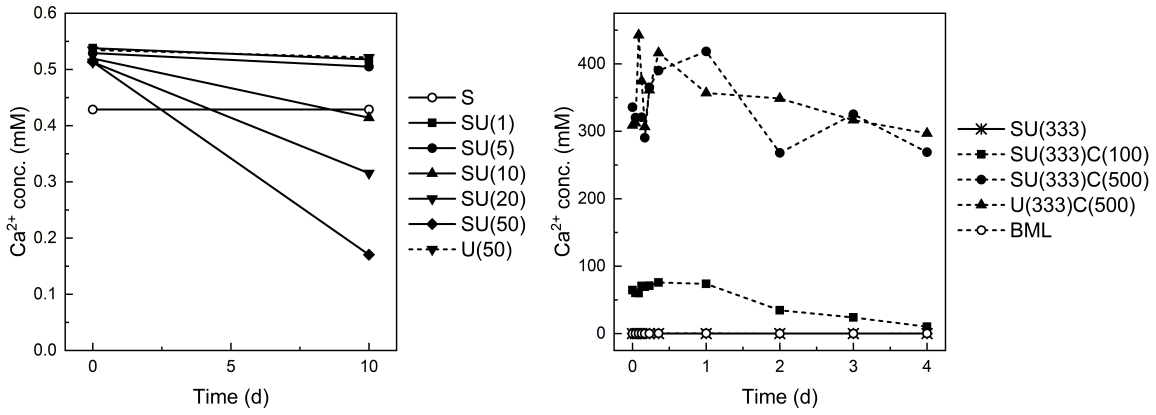

Figure C.11: Calcium concentrations for all groups in the test 1 are presented (two different y scales are used). U: urea (concentration in mM), S: *S. pasteurii* addition, C: $CaCl_2$ addition (concentration in mM), BML: filtered cap water with FFT addition.

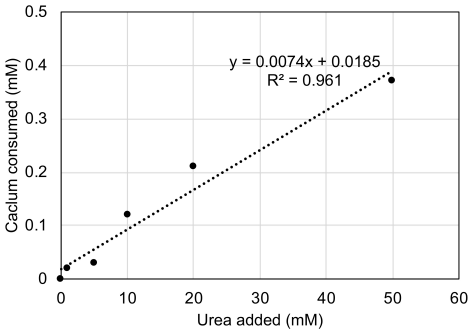

Figure C.12: A linear approximation of the urea dosage and calcium removal within 10 d based on data in MICP test 1.

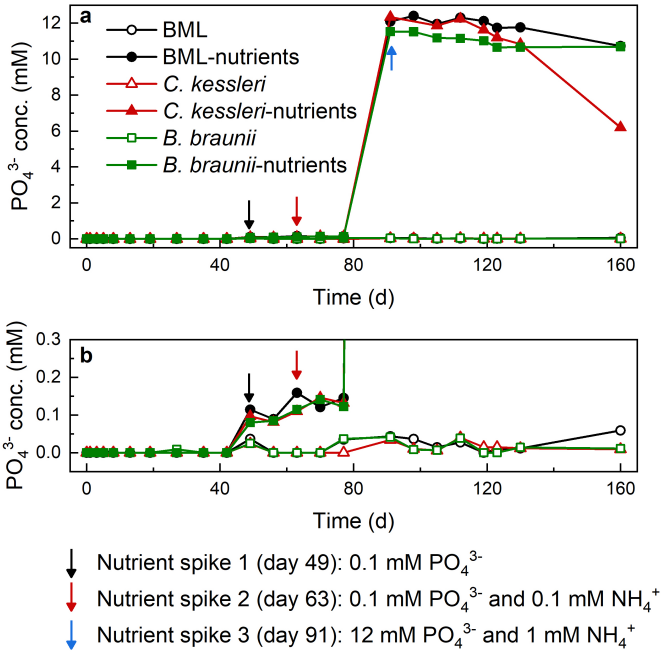

Figure C.13: Phosphate concentrations (mM) in 2 L experiment using nutrient and algal additions. Panel b has a different scale than panel a to show the first two nutrient spikes more clearly. Nutrient addition occurred in BML and algae-amended columns on day 49 (black arrow), 63 (red arrow) and 91 (blue arrow).

Appendix D

Tables

Table D.1: Initial F2, F3 and bitumen content values of different MFTs.

MFT type	MFT-Mix	MFT-D1	MFT-D2
F2 (mg g^{-1})	1.8	3.3	2.0
F3 (mg g^{-1})	7.0	14.7	10.0
Bitumen content (mg g^{-1})	16.1	40.3	30.2

Table D.2: pH in the pore water of MFT-Mix, MFT-D1 and MFT-D2. Before dewatering tests, samples were taken from the original bulk MFT, Initial pH of pore water was measured. After dewatering tests were completed, pH was measured in both control and treated columns. Triplicate columns were studied for MFT-Mix, and duplicate columns were studied for MFT-D1 and MFT-D2.

Test 1: Influence of nitrogen using MFT-Mix		
Group	pH	SD
Initial	8.75	
T	8.57	0.05
UT	8.63	0.01
NT	8.66	0.18
UNT	8.61	0.04
Test 2: Influence of UltraZyme dosage using MFT-D1		
Group	pH	SD
Initial	8.56	
T	8.65	0.08
0.5UT	8.82	0.02
1.0UT	8.82	0.07
1.5UT	8.80	0.02
2.5UT	8.58	0.09
5.0UT	8.42	0.01
Test 3: Influence of temperature and autoclaving pre-treatment using MFT-D2		
Group	pH	SD
Initial	8.94	
T-10 °C	8.92	0.11
UT-10 °C	8.82	0.03
T-20 °C	8.94	0.22
U(-)T-20 °C	8.75	0.15
UT-20 °C	8.79	0.11
T-50 °C	8.74	0.04
UT-50 °C	9.04	0.02

Table D.3: DOC in pore water of MFT-Mix and MFT-D2. Before the experiment, samples were taken from the original bulk MFT, Initial DOC of pore water was measured. After dewatering tests were completed, DOC was measured in both control and treated columns. Triplicate columns were studied for MFT-Mix, and duplicate columns were studied for MFT-D2.

Test 1: Influence of nitrogen using MFT-Mix		
Group	DOC (mg L^{-1})	SD (mg L^{-1})
Initial	118.3	
T	110.5	3.50
UT	123.5	4.30
NT	117.4	1.00
UNT	103.9	4.70
Test 2: Influence of temperature and autoclaving pre-treatment using MFT-D2		
Group	DOC (mg L^{-1})	SD (mg L^{-1})
Initial	71.9	
T-10 °C	67.1	0.5
UT-10 °C	61.7	0.4
T-20 °C	61.4	1.2
U(-)T-20 °C	79.3	0.3
UT-20 °C	79.1	0.4
T-50 °C	75.6	1.4
UT-50 °C	60.1	0.9

Table D.4: Change in particle size after each treatment in MICP test 2.

Group	Paricle size change
BML	-4.6 ± 2.5%
BML-SL	72.7 ± 42.0%
BML-SH	97.2 ± 62.9%
BML-SH-CL	77.0 ± 37.4%
BML-SH-CH	64.0 ± 28.4%
BML-CL	18.0 ± 8.3%
BML-CH	5.9 ± 8.0%