Applications of Lime and Enzymes for Oil Sands Tailings Management: Dewatering and Mitigation of Methane Emissions

by

Nesma Mohamed Elhosseny Eltoukhy Allam

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Department of Civil and Environmental Engineering
University of Alberta

ABSTRACT

The remediation of oil sands tailings is a crucial environmental aspect of the Canadian oil sands industry. Oil sands tailings management involves the endless storage of massive volumes of fluid fine tailings (FFT). The slow gravity settling of tailings, the release of toxic compounds, and the release of greenhouse gases (GHGs) emissions are all synergistic reasons for the comprehensive tailings environmental concerns and challenges. These mandates finding a cost-effective tailings reclamation strategy. This thesis focused on the engineered non-natural novel strategies to improve the consolidation of tailings and the inhibition of GHGs emissions. This thesis was divided into three research components.

First, the effect of lime treatment on FFT dewatering and improving cap water quality under the simulated End pit lake (EPL) scenario was investigated. EPLs are being examined as one potential approach to reduce FFT inventories for the oil sands industry. Experiments were conducted with various doses of lime (650 to 4000 ppm). The results illustrated that a high lime dose of 3500 ppm achieved the highest FFT water recovery% (WR), decreased the cap water alkalinity after 90 days, and increased the possibility of cation exchange (at day 0). In contrast, the degradation of petroleum hydrocarbons was slightly enhanced at a low lime dose of 650 ppm. In addition, the 650 ppm dose resulted in minimal change in the microbial cell counts at day 90, compared with high lime doses that significantly reduced the cell counts. FFT pore water exhibited higher toxic effects for lime dosages >1600 ppm. Nevertheless, at all lime doses, low cap water toxicity (i.e., <1.0 Toxicity Unit) at day 90 was attained. The low water

toxicity for the cap water can be ascribed to the reduction of cap water pH over time due to the dissolution of atmospheric carbon dioxide into cap water.

Second, the feasibility of enzymatic treatment (cellulase, protease, and lysozyme) was investigated for the first time to accelerate the dewatering of FFT. The findings illustrated that lysozyme (0.5% and 1%) significantly improved FFT dewatering by increasing the WR up to 20% compared with the other enzymes (up to 12%) or the control (2%). Moreover, lysozyme treatment resulted in the highest increase in ionic strength (0.038 to 0.1 mol/L), decrease in diffuse double layer (DDL) thickness (1.54×10^{-7} to 9.40×10^{-8} cm), and increase in zeta potential (−34.7 to −14.8 mV). Increased methane production was observed for cellulase, lysozyme, and protease (0.5%). The enhanced dewatering could be linked to the ebullition of methane gas resulting from the methanogenic activity, which created pathways for the trapped water release. In addition, the dissolution of carbonate minerals during the release of methane gas increased ionic strength and decreased the DDL of the FFT. Lysozyme 1% treatment was also the most effective in reducing naphthenic acid fractions (1934.6 to 243 ng/mL); however, the released water had high toxicity toward *Vibrio fischeri* and had a slight decrease in microbial populations.

The final investigation focused on the engineering strategies to reduce GHGs emissions through methanogenesis inhibition in tailings (methane inhibition) by chemical treatment (lime) and biological treatment using enzymes (lysozyme and protease). Overall, treatment with protease 3%, lysozyme 3%, and lime 5000 ppm inhibited CH_4 production (by 52%, 28%, and 25%, respectively) and were weakly associated with the archaeal abundance. Enzyme treatment resulted in a higher

reduction in CH_4 production compared with lime treatment. A 3% protease suppressed CH_4 production throughout the experiment (the change in methane was 0.78 mM), which could be attributed to the pH reduction to pH 4.9 at week 23 resulting from the formation of volatile fatty acids. The toxicity effect was greater with protease 3% and lysozyme 3% treatment than with lime treatment. Lime treatment resulted in the highest reduction in 16S rRNA gene copies.

In summary, the significant implications for the use of lime to improve water quality in EPLs and benefit the long-term success of FFT remediation within EPLs were highlighted in the first part of the thesis. The second and third parts of the study introduced a novel technique based on the enzymatic treatment that was applied for the first time in oil sand tailings. These parts provide fundamental insights into the dewatering and inhibition of GHGs emissions by biological treatment using enzymes.

PREFACE

All research completed on this thesis is an original work in which I, Nesma Allam, proposed, designed, and conducted all the sets experiments as well as the interpretation, the analysis of the data and the preparation of the manuscripts, under full revision and the supervision of Dr. Ania Ulrich and Dr. Bipro Dhar. Some researchers and lab mates also contributed to chemical preparation, or sample analysis, manuscript edits, and some of them were co-authors of the manuscripts submitted for publication. Other analyses were done in other departments of the University of Alberta, as specified below.

Chapter 3: A version of this chapter has been published as Allam, N.E.; Romaniuk, N.; Tate, M.; Meshref, M.N.A.; Dhar, B.R.; Ulrich, A. (2021): "Impact of lime treatment on tailings dewatering and cap water quality under an oil sands end pit lake scenario", Science of the Total Environment Journal, 781, 146699. I conducted all the experimental plan, experimental set-up, sampling, data collection, writing the manuscript and analysis including anions, alkalinity, DOC, toxicity, DNA extraction and qPCR analysis at Dr. Ulrich's research lab (Environmental Engineering at University of Alberta). Mr. Nikolas Romaniuk and Mr. Mike Tate, all from Graymont Western Canada provided the tailings samples and contributed with ideas for the experimental set-up. Dr. Mohamed Meshref in Dr. Dhar's research group contributed to the statistical analysis and manuscript edits. Dr. Ania Ulrich and Dr. Bipro Dhar were responsible for the review, editing, and supervising this work.

Chapter 4: A version of this chapter has been published as "Allam, N.E.; Anwar, M. N.; Kuznetsov, P.V.; Ulrich, A. C.; Dhar, B. R. (2022): "Enzyme-assisted

dewatering of oil sands tailings: Significance of water chemistry and biological activity" Chemical Engineering Journal, 437, 135162. I was responsible for all the experimental plan, experimental set-up, data collection, analysis and writing the manuscript. Dr. Mian Nabeel Anwar in Dr. Ulrich's research group contributed to microbial community analysis and manuscript edits, while the data processing was done by me. Dr. Petr Kuznetsov in Dr. Ulrich's research group contributed to the experimental procedure and the manuscript edits. Dr. Ania Ulrich and Dr. Bipro Dhar were responsible for the review, editing, and supervising this work.

Chapter 5: A version of this chapter has been published as: Allam, N.E.; Zakaria, B.S.; Kuznetsov, P.V.; Dhar, B. R., & Ulrich, A. C. (2023): "Mitigating Methane Emission from Oil Sands Tailings Using Enzymatic and Lime Treatments" Chemosphere, 313, 137455. I was responsible for all the experimental set-up, sampling, analysis and writing the manuscript. Dr. Basem Zakaria in Dr. Dhar's research group contributed to microbial community analysis, and he helped in the data processing. Dr. Petr Kuznetsov in Dr. Ulrich's research group contributed in the help of the setup of the experiments and manuscript edits. Dr. Ania Ulrich and Dr. Bipro Dhar were responsible for the review, editing, and supervising this work.

DEDICATION

This work is dedicated:

To my beloved parents who have been my source of inspiration and gave me strength, support and encouragement. Words can never tell how much I am grateful to you for your care, love and support.

To my beloved husband Mohamed who I am truly blessed to have him in my life.

To my superheroes Ahmed and Salma who surrounded me with all love and care during my PhD journey. You are an endless joy, support, and inspiration to me.

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NOMENCLATURE

OSPW	Oil sands process-affected water
FFT	Fluid fine tailings
MFT	Mature fine tailings
EPL	End pit lake
BML	Base Mine Lake
GHGs	Greenhouse gases
PHCs	Petroleum hydrocarbons
COPCs	Constituents of potential concern
$Ca[OH]_2$	Hydrated lime
Na^+	Sodium
Ca^{2+}	Calcium
HCO_3^-	Bicarbonate
CH_4	Methane
TU	Toxicity unit
DDL	Diffuse Double Layer
I	Ionic strength
NAs	Naphthenic acids
NAFCs	Naphthenic acid fractions compounds
CT	Consolidated tailings
AEF	Acid-extractable organic fractions
O_2-NAs	Classical naphthenic acids
O_x-NAs	Sum of classical NAs and oxidized NAs
PCA	Principal component analysis
DOC	Dissolved organic carbon
sCOD	Soluble chemical oxygen demand
DLVO	Derjaguin-Landau-Verwey-Overbeek

1 GENERAL INTRODUCTION AND RESEARCH OBJECTIVES

1.1 Background and Motivation

1.1.1 Oil sands tailings management

Alberta's oil sands are considered the third largest oil deposits in the world and contribute to Canada's economic growth. However, mitigating the adverse environmental impact of oil sands operations has been challenging. In surface mining operations and during the bitumen extraction using the Clark hot water extraction process, large volumes of fluid fine tailings (FFT) and oil sands process-affected water (OSPW) are continuously generated. To comply with the zero-discharge practice and the provincial environmental legislation, FFT and OSPW need to be stored in engineering ponds called tailings ponds (Allen 2008).

Oil sands tailings management and treatment warrant further research to deal with such endless storage of massive volumes of FFT. Due to the poor settleability of tailings, other non-natural strategies are warranted to solve the issue of the suspended particle and to accelerate the settling successfully. Oil sands operators have used several techniques such as composite tailings, freeze and thaw, centrifugation and atmospheric drying; however, these applications have been hindered due to multiple drawbacks. Thus, more economical and effective solutions are being investigated. Of these potential strategies, demonstrated end pit lakes (EPLs) have been hypothesized and emerged as one of the options to reduce FFT inventories within tailings ponds.

EPLs approach, previously introduced in 2012 by Syncrude, is water-capped tailings technologies in which FFT is incorporated into oil sands mine closure landscapes. These potential attempts aim to reduce FFT inventories in tailings ponds for subsequent reclamation (Kabwe et al. 2018; Zubot 2010). EPL is created in old mined out pits by pumping fresh water and OSPW to cap the FFT. This water cap will facilitate FFT integration into mine closure landscapes (Dompierre and Barbour 2016). In Base Mine Lake (BML-the first field-scale demonstration of pit lakes by Syncrude), FFT was settled naturally without any treatment through gravity settling (i.e., self-weight consolidation) (Dompierre 2016).

The environmental development of EPLs encounters many key concerns and potential challenges (COSIA 2012). One of these concerns is the released pore water contributes OSPW- derived constituents to the EPL cap water (i.e., chemical flux of constituents of potential concern (COPCs)) (Dompierre 2016) during the slow settling of FFT. The presence of COPCs possesses high toxicity to the aquatic microorganisms (Dompierre 2016; McQueen et al. 2017; Samadi 2019; White and Liber 2018).

The release of greenhouse gases (GHGs) from tailings ponds is another key environmental concern facing the oil sands industries. The gaseous emissions from Mildred Lake Settling Basin (MLSB) are currently uncontrolled and are input into atmospheric GHGs (Siddique and Kuznetsova 2020). In 2000, the estimated methane production from MLSB was up to 43 million L/day (Holowenko et al. 2000). The release of CH_4 from tailings ponds is over 21 times that of CO_2 (Small

et al. 2015). The methanogenic activity can be attributed to the organic components in tailings ponds such as residual bitumen, organic diluents, unrecovered naphtha during the extraction process and citrate. The organic components are metabolized by the indigenous microorganisms in tailings ponds and then converted into GHGs under methanogenic conditions (Siddique and Kuznetsova 2020; Small et al. 2015; Small et al. 2012). The solvents usage during oil sands operation (i.e., bitumen extraction) contributes to the organic components such as naphtha (a mixture of *n*-alkanes and monoaromatics BTEX) and paraffinic solvents (Siddique and Kuznetsova 2020). Previous studies reported the methanogenic biodegradation of short-chain *n*-alkanes (C_6–C_{10}) in MLSB that include a significant amount of unrecovered naphtha (Siddique et al. 2006). The presence of different microorganisms in tailings and their activity depends on multiple factors such as tailings types (FFT, MFT, CT, TT), ponds age, depth, and the additives used during the bitumen extraction (Penner and Foght 2010).

1.2 Problem Statement

The problem statement of the thesis can be summarized as follows:

I. Slow consolidation of fine tailings solid.
   - The storage of oil sands tailings faces many challenges due to the very slow consolidation of fine tailings solids which segregate from the sand component after deposition in tailings ponds. These fine tailings need decades to settle down (Chalaturnyk et al. 2002).

II. Presence of inorganic and organic contaminants

- The elevated concentrations of inorganic compounds in both FFT and OSPW contribute to the toxicity (Allen 2008).
- The presence of organic contaminants such as petroleum hydrocarbons (PAHs) and naphthenic acids (NAs) in OSPW contribute to toxicity (Leishman et al. 2013; Sabyasachi et al. 2010). NAs are considered the major contributor to the toxicity of OSPW (Headley and McMartin 2004).

III. The movement of COPCs in EPL

- Although the development of EPLs would decrease the volumes of FFT stored in tailings, the practicability of establishing an aquatic ecosystem in the lake has not been fully evaluated yet due to the movement of COPCs from the FFT into the overlying water cap. The main constituents of the FFT pore water are high concentrations of dissolved COPCs, NAs, petroleum hydrocarbons, and unrecovered bitumen (Allen, 2008). The control of the transport of these constituents is still a key role in developing EPLs.

IV. Uncontrolled GHGs emissions

- The methane emission from tailings ponds is considered as input to atmospheric GHGs and it is currently uncontrolled (Siddique and Kuznetsova 2020).
- The remediation of oil sands tailings faces environmental problems with the increase of the GHGs emissions from the ponds, such as CO_2 and CH_4, due to the methanogenesis activity in the tailings.

- A goal to reduce methane emissions from the oil and gas sector by 40–45 % by 2025 was established by the Government of Canada with having regulations in place to assist in the mitigation of methane emissions from the oil and gas sector (Government of Canada 2018).

1.3 Research Scope and Objectives

This thesis focuses on the reclamation of oil sands tailings by enhancing the dewatering of FFT using chemical treatment (i.e., lime) and biological treatment using enzymes. Additionally, it focuses on mitigating GHGs emitted from tailings ponds which can be translated into new treatment technologies for oil sands tailings remediation. The specific objectives for the thesis can be summarized as follows:

I. To study the impact of adding different dosages of lime on the quality of cap water and FFT porewater in EPLs and dewatering of FFT;

- Evaluate the influence of adding different dosages of lime on the quality of cap water and FFT porewater in EPLs.
- Elucidate the role of cation exchange on the reduction and removal of organic fractions in FFT (DOC and PHCs).
- Examine the variation of water chemistry (alkalinity, pH, and cations), bacterial cell counts, and toxicity toward *Vibro fischeri*.

II. To evaluate Enzyme-assisted treatment of oil sands tailings for dewatering;

- Examine the technical feasibility of enzymatic treatment to enhance FFT dewatering.

- Investigate the corresponding mechanism for the dewatering after Enzymatic treatment.
- Study the influence of enzymatic treatment on reducing or removing organic fractions such as naphthenic acid fractions (NAFCs).
- Determine the shifts in microbial community diversity and toxicity toward *V. fischeri.*

III. To assess the impact of enzyme and lime treatment on the mitigation of methane emissions from oil sands tailings ponds:

- Investigate the feasibility of enzymatic and lime treatment to inhibit the methane emission from tailings ponds.
- Study the effect of enzymatic and lime treatment on the reduction of hydrocarbons.
- Determine the changes in microbial community diversity and toxicity toward *V. fischeri.*

1.4 Thesis Organization

This thesis consists of six chapters. Chapter 1 presents a general introduction to the research background, objectives, and significance. Specifically, it encompasses a brief review of the oil sands operation, tailings management, and the motivation for the current research, objectives, and thesis organization. The detailed experimental procedures, methodologies, results, and discussions are presented separately in each chapter (Chapters 3-5).

Chapter 2 provides background information about tailings management and challenges faced by oil sand operators, a problem statement, and a comprehensive overview of the related research conducted in this area.

Chapter 3 explores the outlook of adding hydrated lime ($Ca[OH]_2$) in the FFT dewatering and improving cap water quality under the simulated EPL scenario. The viability of lime treatment was investigated by evaluating the FFT water recovery and the EPL simulation cap water performance in terms of water chemistry and toxicity. The efficacy of the EPL simulation in accelerating the FFT dewatering and improving the cap water quality was tested under a range of lime dosages to optimize the optimum dose.

Chapter 4 introduces the investigation of enzymatic treatment (using cellulase, protease, and lysozyme) to accelerate the dewatering of fluid fine tailings (FFT). This study assessed the visibility of using 3 different enzymes to accelerate the dewatering of FFT and identifying the different proposed mechanisms for the dewatering. Also, it examined the changes in the microbial community and toxicity after enzymatic treatment.

Chapter 5 examines the inhibition of methane in tailings ponds by using chemical treatment (i.e., lime) and biological treatment using two different enzymes (lysozyme and protease). This project identifies the significance of inhibition potential of the methane emissions. This study assessed the treatment effectiveness on the inhibition of methane emissions, the degradation of hydrocarbons, the changes in the microbial community, and toxicity after treatment.

Chapter 6 illustrates the major conclusion of the research presented in Chapters 2-5. Additionally, future recommendations for further research are encompassed in this last chapter. Finally, the Appendix section presents some experimental methodologies, with supplementary tables and figures in the main chapters.

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2 LITERATURE REVIEW[1]

2.1 Water and tailings management in oil sands operation

Surface-mined oil sands ore in north-eastern Alberta produces almost two-thirds of the total bitumen production in Alberta (ERCB 2012; 2009). In 2017, while supplying 2.5% of the global oil consumption, 4 million barrels of bitumen was produced per day from the Alberta oil sands (Choudhary et al. 2019; Alberta 2019; Government of Alberta 2015). In 2035, there is an expected increase in the oil sands production in Canada to reach around 5,500 thousand barrels per day (Choudhary et al. 2019; Government of Alberta 2019). The Clark Hot-Water Bitumen Extraction occurs by adding hot water and caustic soda to the sand to generate a slurry and allow bitumen to separate from the sand and clay. 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 the process of upgrading in order to transform it into synthetic crude oil. This process currently demands large volumes of water in which 2 to 2.5 m^3 of water is required for each m^3 of crude oil produced (AER 2019; Zhu et al. 2017; Zubot et al. 2012). Thus, water management challenges are considered a fundamental part of oil sands operation.

[1] A version of this chapter will be submitted to Science of the Total Environment Journal as: Allam, N.E.; Dhar, B.R.; Ulrich, A. (2023): "A critical review on oil sands dewatering and Greenhouse Gas emissions".

The surface mining operation reuses the water to a certain extent, where a large number of slurries are generated after the bitumen extraction process and stored in engineered tailings ponds. The hot water extraction process generates massive volumes of oil sands process-affected water (OSPW) as well as fluid tailings, which consists of water, dissolved salts, an aqueous suspension of clay, silt, organics and bitumen (Allen 2008a). OSPW is usually recycled back to the extraction process a few times until it reached specific water quality that can deteriorate the bitumen quality (Allen 2008b). The produced water cannot be directly discharged into the environment due to Directive 074 discharge practice following the environmental legislation (ERCB 2012; McQueen et al. 2017b). Therefore, oil sands operators store them in tailings ponds until further treatment.

To date, the tailing ponds cover 170–180 km^2 while the surface area is continuously increasing as new ponds are being developed (Vedoy and Soares 2015; Young et al. 2020; Zhu et al. 2017). A schematic diagram for the traditional management of oil sands tailings ponds is shown in Figure 2.1. The suspension of the fine clay and silt particles hinders the reclamation of tailings ponds due to the slow consolidation rate of the colloidal particles. The colloidal particles need decades to settle down by self-weight consolidation (Jeeravipoolvarn et al. 2009). Hence, the growing inventory compels operators to seek alternative tailings management strategies (Allen 2008b). The presence of dissolved organics, salts, and trace metals within OSPW and FFT have been identified as compounds of potential concerns (COPCs) that possess toxicity effects on the aquatic organisms in many studies (Anderson et al. 2012; Kavanagh et al. 2011; Li et al. 2017;

McQueen et al. 2017a). To reduce the environmental risks of FFT and OSPW on the landscape, oil sands operators are seeking different tailings reclamation strategies to manage the accumulated volumes of tailings.

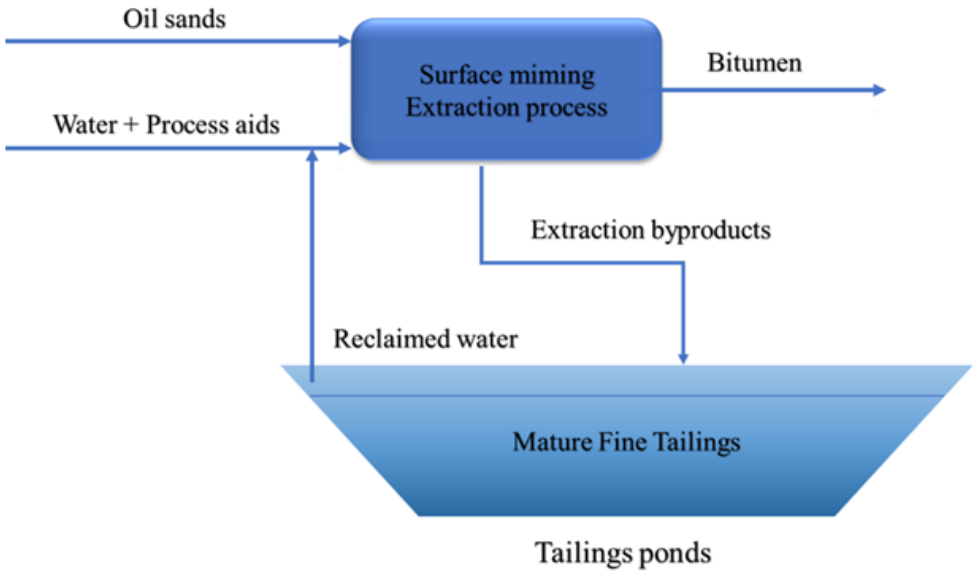

Figure 2.1. Schematic diagram for the oil sands extraction process and traditional tailings management adapted from (Beier and Sego 2008).

2.1.1 FFT characteristics

The composition of the oil sands tailings is 20–30 wt.% of solids (i.e., sand, silt, clays, dissolved salts, organics, and minerals) as well as 70–80 wt.% water, and 1–3 wt.% as residual bitumen. In the tailings ponds, the coarse sands quickly settle when deposited, while much of the fine solids and residual bitumen remain suspended, leading to the formation of mature fine tailings (MFT). The settling of tailings usually results in three layers in which the course sands occurs at the bottom, the fine tailings in the middle (i.e., fluid fine tailing (FFT) or MFT), and an aqueous layer at the top also known as oil sands process-affected water (OSPW)

(Mackinnon and Zubot 2013; Wang et al. 2015). Fine tailings can settle to 20 wt% solids within a few weeks; however, to reach 30–35 wt% of solids, several years are required. When this solids content is reached, the consolidation becomes considerably slow, and the sludge is designated as MFT (Allen 2008a).

The composition and water chemistry of the tailings make them hard to dewater. The coarse particles settle quickly; however, the fine particles remain in suspension, with no further dewatering for many decades (Gumfekar et al. 2017). The particle size (i.e., radius) plays an essential role in settling according to Stokes settling velocity equation as shown in Equation 1 (Yang et al. 2015). This equation has been derived based on the balance of buoyancy force, drag force and gravity force which governs the particle settling velocity in a uniform stagnant fluid (Yang et al. 2015). The overall particle size distribution in the typical MFT samples are approximately 57% in the range of 1–44 μm, 33% are less than 1 μm and 10% are greater than 44 μm (Dompierre et al. 2016a). In particular, the clay particles that are 0.2–2 μm (Kaminsky et al. 2009) are highly stable in suspensions and slow to settle (Siddique et al. 2014a; Zhu et al. 2011).

$$W_s = \frac{2}{9}\frac{g' r_p^2}{v} \quad (1)$$

Where, Ws = settling velocity (cm/s)

g’ = reduced gravity = $(g(\rho_p-\rho_w)/\rho_w)$

ρ_p = particle density (g/cm^3)

ρ_w = water density (g/cm^3)

r_p = particle radium (cm)

v = kinematic viscosity of water (cm^2/s)

Due to the surface charge of these fine particles coagulation or aggregation is difficult, so their settling is hindered. The electrical double layer (EDL) thickness surrounding the clay particle is considered the main factor that controls the flocculation mechanism (Brandon 2016; Schofield and Samson 1954; Siddique et al. 2014a). In brief, the diffuse double layer (DDL) is formed in aqueous solution due to the surrounding of the clay particle with the negatively charged ions (co-ions) and the excess positively charged ions (counter-ions) as shown in Figure 2.2.

To evaluate the stability of clay in aqueous media, the zeta potential is one of the electrokinetic parameters that can be used in DLVO (Derjaguin-Landau-Verwey-Overbeek) mathematical model by balancing the attractive force (Van der Waals) and repulsive force (electrostatic) (Benitez et al. 2007; Grasso et al. 2002; Missana and Adell 2000). DLVO theory was applied to understand the stability of FFT particles by understanding the distribution of forces between two particles (i.e., repulsive and attractive forces) (Derjaguin and Landau, 1993; Li et al. 2008). The shear plane serves as a boundary to separate the fixed (stern plane) and more mobile diffuse layers, as well as a region at which the electrical potential (zeta potential) can be measured (Masliyah and Bhattacharjee 2006; Sawyer et al. 1994). Therefore, the high stability of the clay particles in suspension is due to their high zeta potential (Brandon 2016).

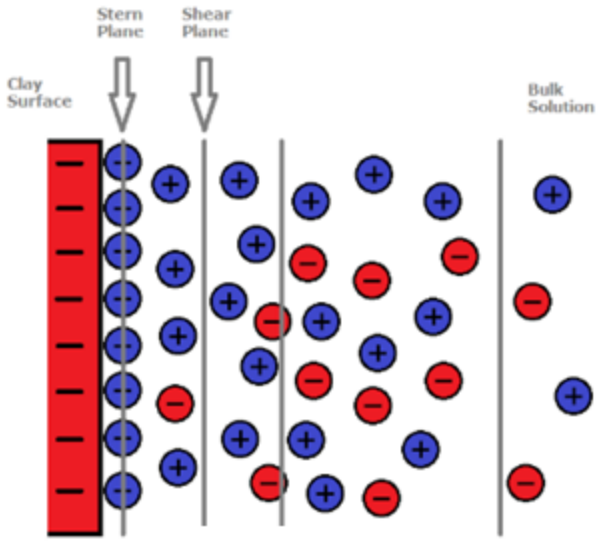

Figure 2.2. A schematic diagram for the Stern models (left) of an interaction between negatively charged clay surface and anions and cations in the bulk solution as well as diffuse double layer surrounding a clay particle (right). Adapted from Masliyah and Bhattacharjee (2006).

2.1.2 OSPW characteristics

OSPW contains suspended solids, salts, organic compounds, inorganic ions, and trace elements (Allen 2008b; El-Din et al. 2011). After the addition of OSPW in the tailing ponds, some solids, and the rapidly settled particles are separated from the OSPW. The fine particles with ~20% (wt.) as well as a low amount of solids form a surface water layer (Allen 2008a; Pourrezaei 2013). The presence of NAs and PAHs compounds in OSPW contribute to toxicity (Leishman et al. 2013; Sabyasachi et al. 2010). NAs has the major contribution to the toxicity of OSPW (Headley and McMartin 2004). OSPW is identified to have chronic and acute toxicity to a variety of organisms such as algae, bacteria and fish (Gosselin et al. 2010). The high toxicity of OSPW possesses an extreme risk when release into the

environment. Therefore, it is necessary to be stored in tailing ponds in order to comply with the zero-discharge practice (ERCB 2012; Kannel and Gan 2012). Treating OSPW is essential to reduce fresh water quantity consumption and protect the extracted bitumen quality by using a recycled treated water with good quality (Allen 2008a).

2.1.3 Inorganic compounds

The elevated concentrations of inorganic compounds in both FFT and OSPW, compared to the natural surface waters of the Athabasca region could contribute to the toxicity (Allen 2008a). OSPW is usually alkaline (~ 800–1000 mg L^{-1} HCO_3^-) with pH in the range of 7.9–8.4 (Allen 2008a). Since tailings water contains high concentrations of total dissolved solids (TDS, 2000–2500 mg/L), it has been classified as brackish (Allen 2008a). It contains high concentrations of dissolved solids, such as sodium, sulfate, ammonia, and chloride (Choi et al. 2014; Pourrezaei et al. 2011). During the extraction process, trace metals such as aluminum, nickel, arsenic, copper, zinc, and chromium that presents in the ores are released into OSPW (Allen 2008a; Baker et al. 2012).

2.1.4 Organic compounds

OSPW comprised a mixture of a wide variety of organic compounds, such as bitumen (measured as oil and grease), asphaltenes, naphthenic acids (NAs), phthalates and polycyclic aromatic hydrocarbons (PAHs), BTEX (benzene, toluene, ethylbenzene, xylene), phenols, and other organic acids (humic and fulvic acids) (Allen 2008a; Madill et al. 2001; Strosher and Peake 1978). Bitumen

concentration in OSPW may range from 9 to 92 mg/L in Suncor tailings ponds (Gulley 1992; Nix 1983). In Mildred Lake Settling Basin (MLSB; Syncrude tailings pond) the concentration of oil and grease was reported as 25 mg/L (MacKinnon and Boerger 1986; Pourrezaei 2013). The concentration of PAH combined with BTEX concentration is typically less than 0.01 mg/L (Allen 2008a; Rogers et al. 2002).

NAs is considered the major organic COPC in the OSPW. During the bitumen extraction process, NAs are solubilized from bitumen (Morandi et al. 2017), it is mainly alkyl-substituted cyclic and aliphatic carboxylic acids (Allen 2008a). NAs are considered the primary contributor of OSPW toxicity (Meshref et al. 2017; Scott et al. 2005). In tailings ponds, the concentrations of NAs differ from 8.9 to 39.2 mg/L and it can reach 130 mg/L in some cases (Grewer et al. 2010). The general formula of NAs is $C_nH_{2n+Z}O_x$, where n is the number of carbon atoms ($7 \leq n \leq 26$), Z ($0 \leq -Z \leq 18$) represents the hydrogen deficiency due to the formation of the ring or double bond structure, and x shows the number of oxygen atoms present in the NAs (Balaberda and Ulrich 2021). The classical NAs are represented by O_2 species at x= 2 and the oxidized NAs species are the O_3, O_4, O_5, O_6 at ($3 \leq x \leq 6$) (Meshref et al. 2017). The background concentrations of NAs in surface water such as Athabasca River is typically <1 mg/L (Allen 2008a; Headley and McMartin 2004). The natural degradation processes can result in the decrease of NAs concentrations over time. For example, NAs concentrations reduced from 73 mg/L to 33 mg/L in eight years (1995–2003) in MFT pore water at depth 10–15 m (Allen 2008a; MacKinnon 2004).

2.2 Toxicity of OSPW/FFT

Continuous accumulation and storage of oil sand tailings present significant challenges, with a high risk similar to any release of OSPW or FFT into the environment (Allen, 2008a). The primary toxicological effects of OSPW are typically related to oil sands organic acid fractions (i.e., NAs in the acid extractable organic fraction - AEO) in which NAs are major contributors and the main source in oil sands byproducts (OSPW, FFT) for the chronic (Gosselin et al. 2010) and acute toxicity towards aquatic organisms (Clemente and Fedorak 2005; Grewer et al. 2010; Li et al. 2017; Scott et al. 2005; Zubot et al. 2021). The toxicity effect attributed to NAs can vary based on the NAs composition, source, and age and the relative concentrations as low concentrations of NAs can have a relatively high toxicity. For instance, at a low NAs concentration of 16 mg/L, a Microtox IC_{50} value of 32 % for MLSB water (Holowenko et al. 2002). The complex mixture of the acid extractable organic fraction in OSPW contains O_2^- NA species and other species (Balaberda and Ulrich 2021). This mixture requires a significant decrease of the absolute bulk concentrations of the O_2^- NAs and O_x^- NAs and the removal of specific NAs structures (Meshref et al. 2017). Previous studies reported the correlation between the lower molecular weight constituents and the toxicity of the NAs toward *Vibrio fischeri* (Frank et al. 2008). The toxicity of NAs also could be related to their classification as surfactants with hydrophobic ends that can penetrate the organisms' cells membrane wall and cause toxicity (Beltrán-Heredia and Sánchez-Martín 2009; Crittenden and Trussell 2005). Previous studies reported the toxicity of NAs to a variety of organisms such as amphibians, plants, mammals

and fish (Beltrán-Heredia and Sánchez-Martín 2009; Crittenden and Trussell 2005; Preez and S Preston 1992; Wagner et al. 1982). Furthermore, NAs had been reported to have an adverse effect on mice's immune system (Shen et al. 2008). NAs could be introduced to the fresh surface and underground water resources through leaching Fields (Mohamed et al. 2011; Seyedy Niasar 2017). Given the above-mentioned facts, the benefits of developing passive low-cost and low energy treatment technologies for both OSPW and tailings are crucial (White et al. 2018). The oil sands industry could incorporate to ensure sustainable management of treated OSPW and the protection of the environment (Brown and Ulrich 2015; Zubot et al. 2021).

2.3 MFT remediation/dewatering technologies

To date, different remediation/dewatering technologies have been explored to tackle the increased volume of tailings. The main targets of these studies were to enhance the settling of fine particles and recover the trapped water. The following subsections will discuss the fundamental and applied aspects of these approaches and their limitations and key research gaps.

2.3.1 Physicochemical, natural, and mechanical technologies

Composite/Consolidated tailings (CT) is considered one of the first technologies developed for the tailings management (Caughill et al. 1993). For CT, gypsum is added to MFT at a sand to fines ratio of 4:1 (Beier and Sego 2008; Caughill et al. 1993). After mixing, a non-segregating slurry is formed, achieving faster consolidation and water recovery when deposited in the tailings pond (Beier

and Sego 2008). One of the drawbacks of CT is the production of aqueous and gaseous H_2S as a result of gypsum addition (Reid and Warren 2016). In addition, elevated ion concentrations (e.g., NA^+, Ca^{2+}) were reported in the CT porewater, which can posture challenges to the growth of vegetation (Huc 2011; Wu et al. 2011).

Another technology is the freeze-thawing process, where CT or MFT is loaded in multiple thin layers before the winter season to freeze the layers and then thaw in the summer season. Beier et al. (2009) reported increasing the MFT solid contents from 35% to 56% after this treatment. Several studies that used the freeze-thawing technology reported a considerable amount of released water when the thickness of layers is 5 cm to 15cm (Dawson and Sego ; 1993; Dawson et al. 1999; Johnson et al. 1993). Paste or thickened tailing technology is another reclamation technology where flocculants, such as polyacrylamides (PAM), starch derivatives as natural flocculants and other minerals (e.g., silica, bentonite, alum and ferrichydroxide), are used to promote the settling of suspended fines particles (Farkish 2013; Wells and Riley 2007; Yuan 2009). The flocculated fines allow settling quickly and forming a stable solids bed that can be deposited in tailings ponds for land reclamation. Also, the released water can be recycled back to the bitumen extraction process (Gumfekar et al. 2019). Since the paste technology can only thicken low solids effluent, it is still not considered an effective method (Gumfekar et al. 2019). Furthermore, to improve the settling rates of tailings and release water, solid-liquid separation (mechanical treatment) processes, such as filtration, centrifuge (Allen 2008b; Gumfekar et al. 2019; Mikula et al. 2008; Wang

et al. 2014) or filtration combined with polymer additives (Farkish and Fall 2014) will still be required. Centrifugation requires a high initial cost and also polymer addition requires proper control for the polymer dosage concerning the variation in the contents of the clay in the FFT (Brandon 2016; Mikula et al. 2008). Overall, all the above-mentioned remediation methods may provide a partial solution but they cannot solve the challenges of FFT and OSPW in oil sands tailings alone (Brandon 2016).

Flocculants and coagulants are widely used for solid-liquid separation, they can reduce the suspension of fine particles suspended in water, reduce the time of settlement and achieve a high solid content (Jeeravipoolvarn 2010; Yuan 2009). Previous studies found that the repulsion between clay particles and coagulating colloidal suspensions was reduced more by using salts containing multivalent ions compared with salts containing monovalent ions (Hardy 1900; Schulze 1882). Many coagulation/flocculation agents have been commonly used in drinking water treatment and wastewater treatment to enhance the settling of fine particles such as ferric chloride, calcium sulfate, and aluminum sulfate (White et al. 2011). In the same sense, in the oil sands industry, multivalent cations have been used to improve the tailings dewatering (Chalaturnyk et al. 2002; Long et al. 2006; Sworska et al. 2000). Alum and gypsum are considered two of the more successful coagulant aids that have been used for oil sands tailings reclamation Fields (MacKinnon et al. 2001; Matthews et al. 2002). Alum ($Al_2(SO_4)_3 \cdot 14.3\ H_2O$) is a trivalent cation Al^{3+} that has been used as a coagulant aid in the oil sands industry to accelerate the dewatering of FFT. In the CT treatment process, FFT, sand fractions, and alum are

mixed to rapidly dewater the FFT (Matthews et al. 2002). In CT, a lower amount of alum is required compared to gypsum which can illustrate the role of the trivalent cation Al^{3+} ion versus the divalent cation Ca^{2+} (MacKinnon et al. 2000; Redfield et al. 2003).

Coagulation/flocculation by the addition of divalent cations can enhance the settling rate or densification of clay particles in an aqueous media (Chalaturnyk et al. 2002; Chen et al. 2013b; Zhu et al. 2011). Previous studies investigated using lime as a sole coagulant or in combination with gypsum or polymers to treat tailings such as non-segregating tailings and rim ditching drying (Baillie and Malmberg 1969; Beaty and Lane 1983; Caughill et al. 1993; Chalaturnyk et al. 2002; Ewin et al. 1981; Hamza et al. 1996; Lorentz et al. 2014). Caughill et al. (1993) reported that the addition of Ca^{2+} as a flocculating agent converts the tailings into a non-segregating mix, where the fines aggregate and clasp sand particles. The formation of non-segregating tailings is a function of tailings solid fines content (17% (wt%) of <44 mm particles of the total solids) and total fines ratio (wt% of water and fines together). Using different hydrated lime $Ca[OH]_2$ and gypsum $CaSO_4$ as flocculants improve the formation of the non-segregating tailings (i.e., the lower solids concentrations) (Chalaturnyk et al. 2002). Chalaturnyk et al. (2002) investigated the effectiveness of combining $Ca[OH]_2$ and CO_2 followed by a suitable thickener in accelerating the FFT released water. They reported that the FFT could dewater by 70% in 50 hours compared with 60% without CO_2 addition. In addition, using 2000 ppm of CaO at 70 °C, accelerated the dewatering of FFT by half in two hours. Proskin et al. (2010) reported an increase in the hydraulic conductivity of FFT that

results in faster consolidation after the addition of quicklime before freeze-thawing. Tate et al. (2016) reported that adding $Ca[OH]_2$ alone as a coagulant to a diluted FFT sample at a dose of 1250 ppm resulted in the settling of FFT. In general, adding $Ca[OH]_2$ causes an increase in pH and provides Ca^{2+} cations, which is required for improving the FFT coagulation. Cation exchange improved at pH > 11, as calcium ions become soluble and react with the negative charge at the surface of clay particles (Tate et al. 2016). Romaniuk et al. (2015) investigated the influence of CaO on the recovery of residual bitumen from FFT. They reported that using doses of 750 to 1500 mg-CaO/kg-MFT could facilitate the floatation of residual bitumen and hence improve the dewatering of MFT.

Due to the high efficiency in dewatering oil sands tailings, flocculation with polymers has been widely explored and implemented by the oil sands industries over the last two decades (Farkish and Fall 2013; Vajihinejad et al. 2021; Vedoy and Soares 2015). In brief, polymers (as flocculants) are generally employed to overcome the potential energy barrier between the negatively charged solid particles in the tailings (Lu 2016; Lu et al. 2016). The polymers can prompt bringing the particles into the effective range of attractive Van der Waals force and polymer bridging attraction to trigger the destabilization and flocculation of suspended particles (i.e., forming large flocs/or sediments) (Ji et al. 2013). Different mechanisms of destabilization could be involved in the flocculation process based on the composition of tailings samples and the properties of employed polymer flocculants. These mechanisms include charge patching, bridging, and charge neutralization (O'Shea et al. 2011). Different polymers have been used in the

industrial flocculation of oil sands tailings. For instance, Polyacrylamide (PAM) has been widely used in the industrial flocculation of oil sands tailings Fields (Amoako 2021; Haveroen et al. 2005). PAM could be found in different forms, and it is varied due to different parameters such as charge densities, molecular weights, and charges (anionic, cationic, or non-ionic) (Cossey et al. 2021; Haveroen et al. 2005). Ji et al. (2013) investigated the effect of three polymers: PAM, Al $(OH)_3$-polyacrylamide (AL-PAM), and Magnafloc 1011 (MF), on enhancing the settling of tailings minerals in freshwater and saline solution. MF resulted in the best performance for tailings flocculation in both cases saline and freshwater. Farkish and Fall (2013) examined the superabsorbent polymer (SAP) to produce dense MFT. SAP is a hydrophilic gel that consists of a network of polymer chains, relative to their mass it is capable to adsorb and retain massive amounts of water (Staples and Chatterjee 2002). The results illustrated a significant improvement in the tailings dewatering, density and an increase in the undrained shear strength of MFT (Farkish and Fall 2013). Polymeric flocculants not only have been used alone in the oil sands industry, but also combination with other materials in other applications. In CT, polymers are combined with electrolyte coagulants (Matthews et al. 2002). During tailing reduction operation (TRO), MFT is mixed with polymer anionic PAM (Mamer 2010).

2.3.2 Biological processes

Biological treatment has also been investigated to treat or remediate oil sands tailings and densify high fine content in tailings by bacterial actions (Fedorak et al. 2003; Guo 2009). Biological treatment is a well-known bioremediation method

where bacteria, archaea, fungi, and protists can biodegrade organics to intermediate products and mineralize the organics completely into water and biogenic gases (CO_2 and CH_4) and achieve high organics removal (Clemente and Fedorak 2005; Leahy and Colwell 1990). Microbial activity exhibited great potential for OSPW detoxification, turbidity removal, heavy metals, and biodegradation of the organics, such as NAs, residual bitumen, and diluent. It can also accelerate the tailings dewatering (Allen 2008a; Brown and Ulrich 2015; Mahdavi et al. 2012; Nelson et al. 1993; Siddique et al. 2014b). Previous studies have examined the role of native microbial communities in OSPW and tailings microbial remediation (Herman et al. 1994; Mahdavi et al. 2015; Nix and Martin 1992; Siddique et al. 2012).

Previous research identified the presence of harbour indigenous microbial communities in tailings ponds that can anaerobically biodegrade the organic compounds such as BTEX and hydrocarbons to biogenic gases CH_4 and CO_2 (Penner and Foght 2010; Siddique et al. 2011) (Figure 2.3). In the MLSB tailings pond, diverse aerobic and anaerobic microorganisms have been identified, including methanogens, denitrifies, iron and sulfate reducing bacteria (Holowenko et al. 2000; Penner and Foght 2010). Tailings ponds provide highly anaerobic environments due to the high depth of FFT and tailings material properties that prevent light and oxygen from entering the ponds. Thus, methanogenic archaea can be enriched in FFT. Both acetoclastic and hydrogenotrophic methanogens were identified in the oil sands tailings (Foght et al. 2017; Penner and Foght 2010; Samadi 2019; Siddique and Kuznetsova 2020; Siddique et al. 2015). However, there is a syntrophic relationship between bacteria and archaea in methanogenesis.

Bacteria can initially biodegrade complex organic substrates into simple byproducts (i.e., acetate, hydrogen, and carbon dioxide). Then, methanogens use these byproducts as precursors to produce methane by either acetoclastic methanogens (utilize acetate to produce methane) or hydrogenotrophic methanogens (consume H_2 and CO_2) to produce methane (Siddique et al. 2012; Siddique et al. 2018). Figure 2.3 shows BTEX and hydrocarbons biodegradation pathways to CH_4 and CO_2.

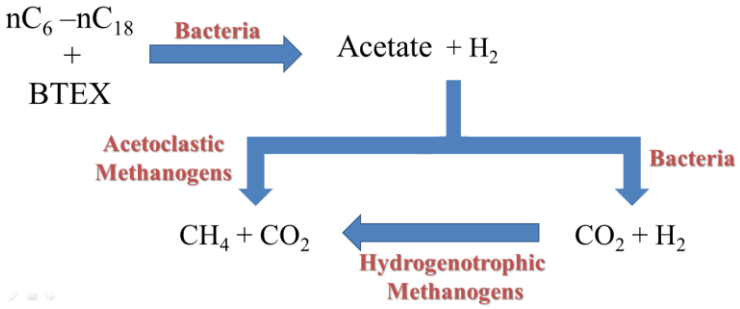

Figure 2.3. The proposed pathway of the anaerobic hydrocarbon degradation in oil sands tailings (adapted from Siddique et al. (2011)).

2.3.3 Application of enzymes for dewatering

Enzymes are proteins with a catalytic activity that have been previously used as conditioners for enhancing sludge dewaterability in the conventional wastewater treatment (Ayol and Dentel 2005; Dursun et al. 2006; Thomas et al. 1993). Many studies have examined the possibility of using enzymes to enhance the dewatering of wastewater sludge. Thomas et al. (1993) reported improvement of digested sludge dewaterability by using an enzymatic product consisting of carbohydrase, lipase and protease. Enzymes improved the dewatering of anaerobically digested sludge at the lab scale (27% increase in the cake solid content); however, the centrifuge pilot-scale did at the Wilmington, Delaware (US)

wastewater treatment plan not show the same improvement in the dewatering (only 20% increase in the cake solid content-field; Dursun et al. 2006). The reason for the lack of the improvement in the solid content in the pilot-scale is due to the high shear applied during the centrifuge which leads to floc deterioration. Recently, the dewaterability of deinking sludge (DS) after enzymatic treatment using cellulase enzymes was enhanced (i.e., 10–14% increase in cake solid content) (Steffen et al. 2018). In another study by Wu et al. (2016), a reduction in capillary suction time (CST) by 48.8% and enhancement in waste-activated sludge (WAS) dewatering were observed after the treatment with composite hydrolysis enzymes. Additionally, cellulase and protease were used to enhance the dewaterability of WAS from the paper mill (Wu et al. 2014). The authors reported high solid content at a dose 0.5% compared with other doses (0.25, 0.75 and 1%) for both cellulase and protease Fields (Wu et al. 2014). Lu et al. (2011) investigated the use of cellulase enzymes to increase the cake solids content and improve the dewatering of wood pulp fibers with a belt filter press simulator.

Bonilla et al. (2015) revealed the mechanisms of enzymatic treatment and their effect on the physical and chemical properties of pulp and paper mills sludge. In their study, five different enzymes (cellulase, amylase, protease from Bacillus licheniformis, protease from Bacillus sp. and lysozyme) were examined; however, only lysozyme could improve the dewaterability of the sludge at an optimum dose of 0.5% (wt.%) (i.e., increase in the dry solids content of the cake from 5.8% to 8.9%). In terms of the effectiveness of enzymatic activity (active or thermo-inactive), it has been reported that doses in the range of 0.2–0.6% (wt.%), showed

a similar enhancement of the dewaterability. The mechanism of dewatering was thought to be a physicochemical interaction between lysozyme and the sludge particles. Lysozyme was reported to act as a cationic polymer (Bonilla et al. 2015). At a pH of approximately 7.4, a lysozyme that carries a positive charge on its surface can interact with the negative charge on the sludge particles. Thus, the repulsion force will be reduced, and aggregation of particles will improve (i.e., larger particles size range). Consequently, the sludge dewaterability can be improved (Bonilla et al. 2015).

Despite the positive reports on the effectiveness of enzymes in dewatering, enzymatic treatment has not yet been thoroughly explored for accelerating the dewatering of oil sands tailings. Only one study used a proprietary biological amendment of microbes, enzymes, and organic carrier called UltraZyme, to accelerate the dewatering of oil sands tailings (Yu et al. 2018). They reported that using 1000 ppm of UltraZyme could increase the MFT solids content from 34 wt.% to 38 wt.% in 50 days. The authors reported that UltraZyme could trigger the microbially induced accelerated dewatering of MFT through the methane flux, which created channels to pressurize pore water to release. Also, they reported an increase in the concentration of divalent ions such as Ca^{2+} and Mg^{2+} due to the dissolution of minerals resulting in an increase in clay flocculation by increasing the ionic strength and decrease the DDL. To date the feasibility of using enzymatic treatment (such as: cellulase, protease and lysozyme) to accelerate the dewatering of oil sands tailings has not been investigated yet.

2.3.4 End pit lakes

Due to the slow gravity settling of tailings, other non-natural strategies are warranted to effectively accelerate the settling of suspended solids. Furthermore, the growing inventory compels oil sand mine operators to seek alternative tailings management strategies to integrate FFT into reclaimed landscapes (ERCB 2012). Of these potential strategies, demonstrated end pit lakes (EPLs) have been hypothesized and emerged as one of the options to reduce FFT inventories within tailings ponds. EPLs are created in old mined-out pits by pumping in fresh water and OSPW to cap the FFT (Figure 2.4). The water cover is initially dominated by OSPW (Dompierre et al. 2016b). The water cover quality is expected to be improved over time by the in situ biogeochemical processes and the continuous freshwater inputs (e.g., precipitation and runoff from the nearby reclaimed areas). This water cap will facilitate FFT integration into mine closure landscapes (Dompierre and Barbour 2016a).

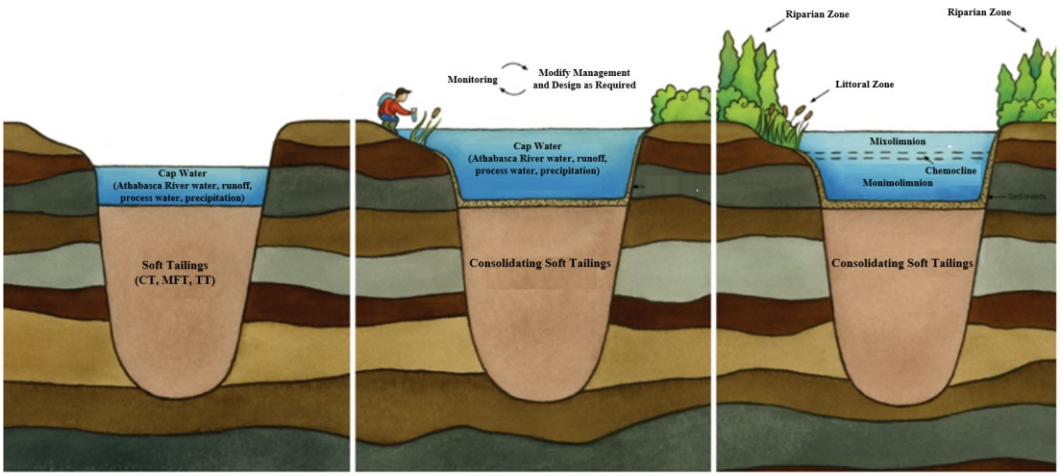

Figure 2.4. EPL content and performance over time starting from the early stage (on the left), intermediate stage and future stage (on the right). Adapted from (Westcott and Watson 2005).

Base Mine Lake (BML) is the first full-scale Athabasca region EPL commissioned in 2013 and operated by Syncrude Canada Ltd. BML was filled with 186 million m^3 FFT (45 m depth) and capped with 90% OSPW and 10% freshwater. The initial solids content of the FFT placed in BML was approximately 35% (wt/wt). The increase of FFT solids content in BML with depth can be attributed to consolidation by self-weight (Dompierre and Barbour 2016b). Although the development of EPLs would decrease the volumes of FFT stored in tailings, the practicability of establishing an aquatic ecosystem in the lake has not been fully evaluated yet due to the movement of constituents of potential concerns (COPCs) from the FFT into the overlying water cap. The main constituents of the FFT pore water are high concentrations of dissolved COPCs, NAs, petroleum hydrocarbons, and unrecovered bitumen (Allen, 2008). The control of the transport of these constituents is still a key role in the development of the EPLs (Dompierre et al. 2016b).

Previous studies investigated an improvement in FFT dewatering and settlement of fine particles due to the methanogenesis process within the FFT layer where hydrocarbons (i.e., n-alkanes and monoaromatics) are degraded and produce CH_4 and CO_2 (Fedorak et al. 2003; Siddique et al. 2006; 2007; 2014b; 2011). Siddique et al.(2014b) illustrated the associated mechanism, where the CO_2 dissolved in the MFT endorses the dissolution of carbonate minerals. Subsequently, the divalent cations such as Ca^{2+}, Mg^{2+} are released to FFT pore water and exchanged with Na^{+} at the surfaces of clay minerals. Therefore, enhancement in the FFT dewatering and settlement were reported due to the reduction in the electrical double layer (EDL) thickness (Brown et al. 2013; Siddique et al. 2014b).

Furthermore, in FFT deposits Fe and microbial SO_4^{2-} reduction are considered very essential anaerobic respiration processes (Stasik et al. 2014; Stasik and Wendt-Potthoff 2014). During these processes, H_2S and Fe^{2+} are released as a result of the reduction of SO_4^{2-} and Fe^{3+} associated with the organic carbon oxidation (Siddique et al. 2014c; Stasik et al. 2014). The secondary Fe^{2+} sulfide precipitate and the dissolved Fe^{2+} promote the precipitation of siderite ($FeCO_3$) within the FFT (Chen et al. 2013a; Dompierre et al. 2016b; Siddique et al. 2014c). The surface charge neutralization between these secondary phases and clay particles will promote the aggregation (Siddique et al. 2014c).

2.4 Tailings Emission

The release of CH_4 from tailings ponds is over 21 times that of CO_2; thus, it represents an environmental concern as a greenhouse gas (GHG) (Small et al.

2015). Many components influence the emissions from tailings ponds, such as organic diluents, citrate, and residual bitumen. These components serve as substrates for tailings indigenous microorganisms and metabolize them into GHG under methanogenic conditions (Siddique and Kuznetsova 2020; Small et al. 2015; Small et al. 2012). Unrecovered bitumen is considered the key organics in the MFT, which is hard to biodegrade (Holowenko et al. 2000).

The bitumen represents around 2–5% w/w of the bitumen extraction process tailings (MacKinnon 1989). During the bitumen extraction process, oil sands operators use different solvents. Some of them use naphtha such as Syncrude Canada Ltd., Suncor, and Canadian Natural Resource (CNRL), and some use paraffinic solvents, such as Canadian Natural Upgrading Limited (CNUL), Imperial Oil, and Suncor at Fort Hills (Siddique and Kuznetsova 2020). Naphtha consists of a mixture of n-alkanes (i.e., heptane, octane, nonane), monoaromatics BTEX (i.e., benzene, toluene, ethylbenzene, xylenes), iso-alkanes and cycloalkanes (Siddique et al. 2007; Siddique and Kuznetsova 2020). The composition of paraffinic solvent is relatively simpler than naphtha (e.g., C_5–C_6 *n*-and iso-alkanes). A very small amount of naphtha is unrecovered during the extraction process (<1%), which escapes into tailings ponds and serves as a substrate for methanogens in FFT (Siddique et al. 2007). Siddique et al. (2006) reported the methanogenic biodegradation of short-chain *n*-alkanes (C_6–C_{10}) in MLSB that include a significant amount of unrecovered naphtha. Siddique et al. (2007) reported metabolism of only 15-23% of whole naphtha, especially *n*-alkanes (nonane > octane > heptane) and BTEX compounds (toluene > *o*-xylene > *m*-xylene).

However, no changes were observed for other naphtha constituents, such as (i.e., iso-alkanes and cycloalkanes) (Siddique et al. 2007).

Siddique et al. (2012) reported the dominance of Firmicutes (*Peptococcaceae*) and Proteobacteria (*Syntrophus/Smithella*) in Syncrude MFT as well as acetoclastic methanogens *(Methanosaetaceae)* during the biodegradation of short-chain *n*-alkanes (C_6–C_{10}) and monoaromatics (BTEX) compounds. The biodegradation of long-chain *n*-alkanes (C_{14}, C_{16}, and C_{18}) was reported under methanogenic conditions after a lag phase of 180 days (Siddique et al. 2011). Another report was published on the methanogenic degradation of major iso-alkanes (C_7–C_9) and cycloalkanes in the MLSB MFT (Siddique et al. 2020). The report indicated biodegradation of iso-alkanes in the MFT after a lag phase of ~630 days, while complete degradation took 1700 days; however, the cycloalkanes remained undegraded. The time required for biodegradation and the lag phase of short-chain *n*-alkanes (C_6–C_{10}) < longer-chain *n*-alkanes (C_{14}–C_{18}) < iso-alkanes (C_7–C_9) (Siddique and Kuznetsova 2020). Siddique et al. (2015) evaluated the methanogenic biodegradation of the n-and iso-alkanes (C_5–C_6) for MFT collected from tailings ponds (e.g., Muskeg River Mine) operated by CNUL and uses the paraffinic solvent as a diluent. During 6 years of incubation, biodegradation happened between 900 to 1800 days. Such longer lag phases could be attributed to the young age of the CNUL MFT compared with MLSB. The differences in MFTs from different operators (Albian and CNRL) may result in differences in the diversity of bacterial communities during the biodegradation of longer chain *n*-alkanes (C_6–C_{18}) (Shahimin et al. 2016; Shahimin and Siddique 2017). Both

acetoclastic (e.g., *Methanosaetaceae*) and hydrogenotrophic methanogens (e.g., *Candidatus* Methanoregula) were co-dominant in the CNRL MFT containing naphtha, BTEX, long-chain *n*-alkanes (C_{14}–C_{18}), and some iso-alkanes (Shahimin et al. 2016; Shahimin and Siddique 2017). During biodegradation of the two-alkane and four-alkane in the CNRL amended MFT, *Methanosaetaceae* was most dominant (~87–93%). However, further microcosm study showed the dominance of *Candidatus* Methanoregula (~74%) over the *Methanosaetaceae* (Shahimin et al. 2016).

2.4.1 Greenhouse Gases

Due to methanogenic activity, the remediation of oil sands tailings might face a potential challenge of GHGs emissions from the ponds. Many factors affect the presence and activity of different microorganisms in tailings, such as tailings types (FFT, MFT, CT, TT), depth, the tailings ponds age and the additives used during the bitumen extraction (Penner and Foght 2010; Ramos-Padrón et al. 2010). The methane production in 2000 from MLSB was estimated at up to 43 million L/day (Holowenko et al. 2000). Furthermore, toxic compounds such NAs, and H_2S gas might transfer from the FFT into the overlying water cap during the methane flux (Holowenko et al. 2000). The emission of GHGs from tailings ponds raises environmental concerns. The gaseous emissions from MLSB are currently uncontrolled and are input into atmospheric greenhouse gases (Siddique and Kuznetsova 2020).

Siddique et al. (2008) investigated the kinetics of GHG emissions from tailings. They used the data obtained from the literature (Siddique et al. 2006; Siddique et al. 2007), where Syncrude MFT from MLSB was spiked with naphtha, monoaromatics, and short-chain *n*-alkanes (C_6–C_{10}) and incubated for 365 days (Siddique et al. 2008). Only *n*-alkanes (C_6–C_{10}), toluene, and isomers of xylenes were used to predict the emissions of CH_4 for the kinetic analysis. Other naphtha components such as iso-alkanes, cycloalkanes, benzene, and ethylbenzene, were excluded from the analysis due to their trivial degradation (Siddique et al. 2008). Siddique et al. (2008) reported that degradation of naphtha (0.01–1.0 wt%) would follow zero and first-order kinetic models, CH_4 production from naphtha degradation in MLSB was estimated at 8.9–400 million L day^{-1}.

Kong et al. (2019) developed a kinetic model including microbial growth and death rates, an extended range of biodegradable hydrocarbons, and nitrogen (N) as a growth-limiting nutrient. The predicted CH_4 emissions using the model were compared with the reported CH_4 emissions from tailings ponds. The model predicted 50%–55% and 77%–95% of the reported emissions from Syncrude MLSB and CNUL tailings ponds measured emissions. These results illustrated that other endogenous organic compounds (e.g., additional labile diluent hydrocarbons, recalcitrant hydrocarbons, slowly-degradable metabolites produced, and labile organic matter associated with clays in oil sands ores) exist in oil sands tailings ponds and support the CH_4 production rather than the compounds considered in the model. Moreover, several studies focused on predicting CH_4 production under longer incubation time (> 4 years) for different types of MFTs fields (Shahimin and

Siddique 2017; Siddique et al. 2020). Shahimin and Siddique (2017) illustrated the role of microbial metabolism in the degradation of carbon from hydrocarbons to CH_4. GC-FID (purge and trap) was used to quantify the mass of individual alkanes consumed during the incubation (i.e., maximum theoretical CH_4 production). Then the theoretical production was compared with the measured CH_4 in microcosms during the experiment. The results illustrated a variation of 68 and 88% of the predicted theoretical maximum CH_4 in Albian and CNRL MFT amended with paraffinic solvent during the 1600 d incubation period. Siddique et al. (2020), compared the methanogenic biodegradation of iso- and cycloalkanes by incubating them for 1700 d with MFT with the measurements of CH_4 in live amended cultures after subtracting the CH_4 productonproduction from endogenous substrates (i.e., measured in parallel live). The amended cultures (i.e., with MFT) produced around 56–73% CH_4 compared to the theoretical CH_4 at 1700 d; however, in the No-MFT-amended cultures, less biodegradation of hydrocarbons to CH_4 was observed at 43% of the theoretical maximum. These results illustrated the feasibility of using kinetic models for predicting in situ methane from different tailings ponds.

2.5 Key research gaps

The enzyme applications in oil sands dewatering were rarely explored. In contrast, municipal sludge, such as waste-activated sludge (WAS), was widely examined for enzymatic treatment to improve the sludge dewaterability. The summary of enzyme applications for municipal and pulp and paper mill sludge dewatering is illustrated in Table 2.1.

The first attempt of the enzymatic treatment was reported by Thomas et al. (1993), where hydrolytic enzymes were utilized to improve the dewatering of sludge. The study examined the hydrolytic enzymes in both labs and full-scale belt press. The study highlighted the benefit of combining belt-filter presses preceding the enzymatic treatment with flocculation aid to improve the dewatering. However, despite improved dewatering, the need for both mechanical dewatering and flocculant aid presents economic concerns. Using commercial Enviro-Zyme 216 (enzyme mixture of protease and lipidase and anaerobic bacteria, Aspergillus), Wu et al. (2016) showed the enhanced dewatering of WAS by composite hydrolysis enzymes. The study referred to the sensitivity of the reaction temperature in which the preferential temperature range of the culture of Aspergillus oryzae is 25–35 °C. The dewatering of WAS by Enviro-Zyme 216 could be limited at high temperatures> 35 ºC, due to the negative effect on enzyme activity at high temperatures. Recently, Lin et al. (2019) reported a positive impact on the combination of lysozyme and freeze-thaw conditioning effect on sludge dewaterability. The work suggested optimum conditions for freeze-thaw conditioning following the enzymatic treatment at a freezing temperature of -26.56°C and a freezing time of 5.78 h. The cell walls of bacteria have been broken down by lysozyme conditioning and the bound water has been released consequently. To date, enzymatic treatment has not yet been thoroughly investigated on the oil sands remediation field. Yu et al. (2018) reported an enhancement in the dewatering of tailings after using a proprietary biological amendment (UltraZyme). Further research still necessary to the explore the

practicability of using different enzymes such as cellulase, protease and lysozyme to accelerate the dewatering of oil sands tailings.

In contrast to the enzymatic treatment, the application of lime or calcium hydroxide ($Ca[OH]_2$) for oil sands tailings dewatering was previously considered in previous studies (see Table 2.2). The initial concept of using lime in oil sands was to expedite the rate of tailings settling and provide a coagulant and flocculant aid (Chalaturnyk et al. 2002; Hamza et al. 1996; Lorentz et al. 2014; Romaniuk et al. 2015; Tate et al. 2017). For instance, Hamza et al., (1996) demonstrated the positive impact of co-flocculation of tailings with the slaked lime treatment and a low dose of a high molecular weight anionic PAM. In their study, lime as a secondary flocculant on tailings treatment followed by a polymer flocculant showed an improvement in the dewatering rate of tailings.

Previous research highlighted the benefits of lime addition and stabilization on enhancing the mineral properties in both soil (Locat et al., 1990) and oil sands tailings (Chalaturnyk et al., 2002; Farkish, 2013; Hamza et al., 1996; Lane, 1983; Tate et al., 2017). Lime was endorsed as an approach for tailings in which a reasonable improvement of soil properties and high-water separation efficiency can be attained (Chalaturnyk et al., 2002; Lane; 1983; Tate et al., 2017). Lane (1983) suggested coagulation process as a good description of the lime stabilization in soil and tailings. Lime addition can modify particle surface charge and lead to agglomeration and interparticle bonding.

A few studies also examined the ability of lime as a sole coagulant (Lorentz et al. 2014; Tate et al. 2017). Chalaturnyk et al. (2002) found that lime could not improve the water release from tailings; however, the combination of lime and CO_2 followed by thickening resulted in a reduction in the time for the water to release without any increase in the concentrations of ions. Similarly, Tate et al. (2017) investigated the lime alone for the tailings settling. The study reported that the increase of pH above 11 could increase the solubility of Ca^{2+}. At this pH level cation exchange start to occur where Ca^{2+} combines with the clay particle. Therefore, an increase in the particle size of the tailings and enhancement of tailings settling were reported after lime addition. Tate et al. (2017) highlighted the effect of samples dilution as one of the limitations of settling improvement. Nevertheless, a similar impact of lime addition on FFT coagulation was reported in the literature due to the increase of pH and providing the divalent Ca^{2+} ions (Chalaturnyk et al. 2002; Tate et al. 2017). Based on literature, the max pH can be attained after lime treatment is 12.5, any addition to extra lime will not increase the pH above this range (Burnham et al. 1992; Christy et al. 1990). Burnham et al. (1992) reported that the solubility constant (Ksp) of the lime at 25°C is 1.4×10^{-6}. Additionally, Lane (1983) reported the occurrence pozzolanic reaction after lime addition at pH above 11, in which a stabilization process can occur. The pozzolanic reactions can be described as the soluble Ca^{2+} reacts with alumina and silica of the clay forming hydrated minerals of calcium aluminate and silicate. In the same sense, the study elucidated the stabilization reactions as the pozzolanic reactions and carbonation reactions (Lane, 1983).

Table 2.1. Application of various enzymes for dewatering of municipal, pulp and paper mill sludge.

Enzyme	Application	Key focus	Key findings	Research gaps/further research needs	Reference
Hydrolytic enzymes	Sludge dewatering	Improving the dewaterability of sludge in both lab and full-scale belt press tests.	Using enzymes with polymer flocculant can improve the mechanical dewatering of belt-filter presses.	Using enzymes alone was not able to improve the sludge dewatering.	(Thomas et al. 1993)
Enviro-Zyme 216	Anaerobic digested biosolids	Examining the effect of biosolids pre-treatment with enzyme on the amount of polymer conditioning.	The combination of enzyme and polymer enhanced the dewatering of biosolid samples, and increase the solid contents using CST,	The sole effect of enzyme was not assessed.	(Ayol and Dentel 2005)
Enviro-Zyme 216	Anaerobically digested sludge	Assessing the impact of enzyme pre-treatment on sludge dewaterability at both laboratory and pilot scale.	27% increase in cake solid content after enzyme pre-treatment for the lab experiment and only 20% increase in the pilot scale.	Failure to achieve high solid content for the pilot scale compared with lab scale.	(Dursun et al. 2006)

Enzyme	Application	Key focus	Key findings	Research gaps/further research needs	Reference
Cellulase	Wood pulp fibers or (cellulosic sludge)	Examining the dewatering of fibers using a belt press simulator.	An increase in cake solids (3%–6%) was achieved after enzyme treatment.	Only a little increase in the cake solids was achieved.	(Lu et al. 2011)
Lysozyme, Cellulase Protease and a-Amylase	Pulp and paper mill biosludge	Investigating the effect of enzymes treatment in the dewaterability of biosludge.	5.6% to 8.9% increase in the Dry solids content after lysozyme treatment.	Longer incubation period was required instead of CST to assess the behavior of enzyme in the long run.	(Bonilla et al. 2015)
Enviro-Zyme 216	Waste-activated sludge (WAS)	Improving the dewatering of WAS by hydrolysis enzymes using CST.	48.8 % reduction in CST was achieved with enzyme dosage of 1 mg/g DS.	The effect of enzymes treatment needs to be addressed for long time experiment.	(Wu et al. 2016)
Cellulase	Deinking sludge (DS)	Investigating the effect of four cellulase on the DS conditioning under neutral pH and without pH adjustment (the pH of DS).	14.4% increase in cake solids was achieved by cellulase. 20% reduction on the annually generated	Limited to the applications at neutral pH.	(Steffen et al. 2018)

Enzyme	Application	Key focus	Key findings	Research gaps/further research needs	Reference
			amount of DS at neutral pH.		
Alpha amylase + and protease	Activated sludge	Examining the combination of different enzymatic lysis with chemical flocculant ($FeCl_3$ and PACl) on the dewatering of activated sludge.	An improvement in the cake solid content of sludge and filtration rate after ά-amylase treatment compared with protease.	Enzymes were used as a pre-treatment step only.	(Chen et al. 2015)
Lysozyme (LZM)	Activated sludge	Examining the combination of LZM and freeze-thaw (26.56°C and 5.78 h) conditioning effect on sludge dewaterability.	19.84% decrease in the water content was achieved by lysozyme treatment.	Freeze-thaw conditioning was implemented for the enhancement of dewatering.	(Lin et al. 2019)
UltraZyme	Oil Sands Tailings	Assessing the effect of UltraZyme on the dewatering of three tailings sources after treatment.	30% increase of the solids content in 112 days was achieved using 1.0 g/L of UltraZyme in all three tailings sources without physical mixing.	The effect of a commercial enzyme needs to be addressed instead of the proprietary biological amendment (UltraZyme).	(Yu et al. 2018)

Table 2.2. Summary of previous studies that used lime for oil sands tailings processing and treatment.

Key focus	Key findings	Research gaps/further research needs	Reference
Using lime as a secondary flocculant on tailings treatment followed by polymer flocculant.	Oil sands tailings can be co-flocculated after slaked lime treatment followed by a low dose of a high molecular weight anionic PAM.	The sole impact of lime treatment on the tailings dewatering was not assessed.	(Hamza et al. 1996)
Studying the impact of pH adjustment (12.0 to 11.6) by injecting CO_2 and adding lime as a flocculant agent on the consolidation of tailings.	The combination of using lime and CO_2 and then thickening using thickener resulted in a reduction in the retention time for the recovered water to release without any increase in the concentrations of ions such as Ca^{2+}.	Using lime alone could not improve the water release from tailings samples compared with lime and CO_2.	(Chalaturnyk et al. 2002)
Understanding the effects of lime coagulation on the reclamation properties of FFT	At pH >11, Ca^{2+} becomes soluble and combines with clay through cation exchange, resulting in increased particle size and enhanced settling. At pH >12, pozzolanic chemical reactions between Ca^{2+} and clay improve FFT dewatering.	FFT samples without dilution showed marginal improvement in settling.	(Romaniuk et al. 2015)

Key focus	Key findings	Research gaps/further research needs	Reference
Application of rim ditching to dewater the oil sands tailings and additives (lime, gypsum and polymer) to produce a trafficable deposit for reclamation.	The tailings properties (solids fraction) were improved after using additives and the tailings strength have been developed to yield a non-segregating deposit for reclamation. The consolidation and dewatering of tailings have been accelerated due to the control of release water and rainfall or runoff.	Limited information found on the released water and dewatering performance.	(Lorentz et al. 2014)
Examining the effect of using lime as a coagulant to enhance the treatment of oil sands tailings	Lime addition increases pH >12, it provides divalent Ca^{2+} for coagulation of FFT. This increases particle size and enhances settling.	In this study diluted FFT samples was to improve the settling.	(Tate et al. 2017)

2.6 References

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3 IMPACT OF LIME TREATMENT ON TAILINGS DEWATERING AND CAP WATER QUALITY UNDER AN OIL SANDS END PIT LAKE SCENARIO[2]

3.1 Introduction

Large volumes of fluid fine tailings (FFT) and oil sands process-affected water (OSPW) are generated from surface mining of oil sands in northern Alberta. The continuous storage of the massive quantities of FFT in engineered tailing ponds (Allen, 2008b; Clemente and Fedorak, 2005; Siddique et al., 2014) requires oil sands operators to use various reclamation strategies to achieve reclamation obligations. Several remediation approaches (e.g., composite tailings, atmospheric drying, etc.) have been studied, however these methods have drawbacks that hinder their applications. Most of the proposed alternatives are either energy-consuming and costly (e.g., centrifugation; (Wang et al., 2014) or need a large amount of land (e.g., drying requiring freeze-thaw cycles (Proskin et al., 2012) (Allen, 2008a; Board, 2012 ; Hyndman and Sobkowicz, 2010; Vajihinejad and Soares, 2018; Wells and Riley, 2007). Thus, more economical and effective solutions are being investigated to incorporate FFT into oil sands mine closure landscapes (Famakinwa et al., 2018). Water-capped tailings technologies such as end pit lakes (EPLs) are one of those

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potential approaches to reduce FFT inventories in tailings ponds for subsequent reclamation (Kabwe et al., 2018; Zubot, 2010).

EPLs are engineered water bodies created in the post-mining pit by pumping freshwater and OSPW on top of the FFT (Zubot, 2010). EPLs are predicted to slowly develop into self-sustaining aquatic ecosystems (Hrynyshyn, 2012; Zubot, 2010) and act as permanent features in the final reclaimed landscape (Kabwe et al., 2018). In the first field scale demonstration of pit lakes, Base Mine Lake (BML), the FFT was not treated before being deposited into the lake, and the FFT is settling by self-weight consolidation (natural dewatering through gravity settling; (Dompierre, 2016). The FFT (with initial solids content around 10% solids (wt./wt.) as first deposited into tailing ponds) comprises fine clays suspended in water, and the clay particles slowly settle over decades (MacKinnon, 1989).

There are key concerns and potential challenges relevant to the environmental development of EPLs (COSIA, 2012). For instance, during the slow settling of FFT, the released pore water contributes OSPW-derived constituents to the EPL cap water (Dompierre et al., 2016a), including the chemical flux of constituents of potential concern (COPCs) (Dompierre and Barbour, 2016; Lawrence et al., 2015). The presence of COPCs (e.g., major inorganic salts, dissolved organic compounds [DOC], naphthenic acids [NAs], and petroleum hydrocarbons [PHCs]) are potentially toxic to aquatic microorganisms (Dompierre et al., 2016a; McQueen et al., 2017; Samadi, 2019; White, 2017; White and Liber, 2018).

Earlier studies investigated the influence of vertical mixing (Lawrence et al., 2015) and have used a numerical modeling approach to simulate the movement of COPCs (Dompierre and Barbour, 2016) and identify the key mechanisms controlling mass transport in FFT layers within EPLs. Similarly, White and Liber (2018) monitored the concentrations of inorganic salts in the cap water of BML over three consecutive years and assessed the corresponding toxicological risk of elevated concentrations of inorganic constituents such as sodium (Na^+) and bicarbonate (HCO_3^-) on EPL cap water toxicity (White, 2017; White and Liber, 2018; 2020). The studies reported a sufficient level of Daphnia's populations within the BML surface water with a promising improvement trend of BML surface water quality over time (White and Liber, 2018; 2020). However, this was constrained with the less sensitive toxicity endpoints samples used in the study (White and Liber, 2018) that could necessitate further investigations to claim the development into a robust EPL aquatic ecosystem. Several research groups have looked at the ability of the indigenous microbial community in BML to remediate COPCs (Bowman et al., 2016; Bradford et al., 2017; Richardson et al., 2020; Yu et al., 2019). While investigating methanogenic activity, Samadi et al. (2019) reported an enhancement in FFT dewatering with the addition of hydrocarbons and nutrients due to biogenic gas production. Also, Poon et al. (2018) reported a decrease in the turbidity of the EPL cap water through pH reduction by inducing CO_2. However, that study was focused on improving cap water clarity by enhancing the clay flocculation in BML.

Because FFT takes a long time (decades) to settle (Masliyah et al., 2011), coagulation and flocculation were implemented to enhance the dewatering process

(Famakinwa et al., 2018; Wang et al., 2015; Wang et al., 2014). Promising results in the acceleration of tailings dewatering were reported using lime or calcium hydroxide ($Ca[OH]_2$) as a stand-alone coagulant or in combination with gypsum ($CaSO_4 \cdot 2H_2O$) or polymers (Baillie and Malmberg, 1969; Beaty and Lane, 1983; Caughill et al., 1993; Chalaturnyk et al., 2002; Ewin et al., 1981; Hamza et al., 1996; Lorentz et al., 2014; Matthews et al., 2002). Lime addition caused an increase in pH and provided divalent calcium ions (Ca^{2+}) that improved FFT coagulation (Chalaturnyk et al., 2002; Tate et al., 2017). To date, chemical treatment, particularly lime addition, has not been thoroughly and comprehensively explored in EPL scenarios. Moreover, the effect of the slow dewatering of FFT on the development of EPLs is still unclear (Risacher et al., 2018) and should be investigated further.

Based on previous studies, lime addition can be hypothesized to accelerate the dewatering of FFT through cation exchange. The increase in pH resulting from lime addition can reduce methanogenesis in the FFT layer, and therefore reduce the chemical flux of ions and organic constituents to the water cap and enhance the cap water quality in EPLs.

Consequently, the key goal of this study is to examine the impact of the addition of various lime doses on FFT dewatering and cap water quality in EPL configurations. The specific objectives are as follows: a) to investigate the feasibility of lime treatment to enhance FFT dewatering in EPLs; b) to evaluate the influence of adding different dosages of lime on the quality of cap water and FFT pore water in EPLs; c) to elucidate the role of cation exchange on the reduction and removal of organic fractions in FFT (DOC and PHCs); and d) to examine the variation of water

chemistry (alkalinity, pH, and cations), bacterial cell counts, and toxicity toward *Vibrio fischeri*.

3.2 Materials and methods

3.2.1 Materials

FFT was collected from an active oil sands tailings pond in northern Alberta. Hydrated lime ($Ca[OH]_2$) was provided by Graymont Inc. (Calgary, Alberta, Canada). FFT and lime were stored in sealed buckets at 4 °C until use. Raw synthetic OSPW was prepared in 15 L deionized water with: 10.80 g $NaHCO_3$, 4.35 g Na_2SO_4, 8.70 g NaCl, 0.75 g KCl, 2.10 g $MgCl_2 \cdot 6H_2O$, and 0.75 g $CaCl_2 \cdot 2H_2O$. The specifications for Raw synthetic OSPW were provided by Graymont Inc., and mimics the solute concentrations in Albian OSPW (Mahdavi, 2014; Poon, 2019). The Raw synthetic OSPW didn't contain any organics to better understand the movement of COPCs from the FFT layer to the cap water.

3.2.2 Experiment

Four different lime dosages (650, 1600, 3500, and 4000 ppm on a wet weight basis) were used. Lime slurry was prepared by adding hydrated lime to Millipore ultrapure water (18.2 MΩ cm) to achieve a 5% solids slurry (wt./wt.). Each lime dose was added to 1 kg FFT in a glass beaker and mixed with a mechanical agitator at moderate speed (1200 rpm). The initial solids content of the raw FFT was 32.8%. To achieve 50% solids content, both control (no lime) and lime-treated FFT were

compressed in an air pressure filter (OFITE; Bench-Mount Filter Press with Hose and Regulator; #140-31) at 100 psi.

Experiments were conducted in 1 L glass columns, where 500 ml FFT (50% solids content [wt./wt.]) was placed at the bottom of the column and covered with 500 ml Raw synthetic OSPW. All experiments were conducted at ambient conditions (23ºC) using uncovered columns in triplicate, except for the 4000 ppm treatment which had both covered and uncovered conditions. This step was conducted in order to determine the effect of atmospheric CO_2 on the cap water quality and toxicity and to highlight any discrepancies between covered and uncovered conditions. This can be also attributed to the plateau reached at pH 12.5 regardless the increase of lime dose (> 3500 ppm) that requires better understanding about the potential influence of any adverse impact of overdosing.

To better understand the effectiveness of lime addition on FFT dewatering, % water recovery (WR) was used as a comparable index in our assessment. The WR was calculated as follows:

$$WR\% = \frac{\text{Volume of pore water released at day 90}}{\text{total initial volume of FFT}} \times 100$$

The released water from FFT (FFT pore water) at day 0 was collected using the pressure filter, while FFT pore water at day 90 was collected from the FFT samples by centrifugation at 8,000 *g* for 40 min. FFT pore water was collected in 50 ml plastic tubes and stored at 4 °C before further analyses. For cap water analysis, samples were collected directly from the columns every two weeks. The initial FFT and final treated

FFT samples at day 90 were collected directly from the columns, centrifuged at 8000g for 5 mins and stored at −20°C in 50 ml plastic vials for DNA extraction. For PHC analysis, FFT samples were stored at 4 °C until further analysis.

3.2.3 Chemical analysis

Water samples were analyzed for cations including Ca^{2+}, Na^{+}, K^{+}, and Mg^{2+} using a Thermo iCAP 6000 series Inductively Coupled Plasma–Optical Emission Spectrometer (ICP-OES, ThermoFisher). A Mettler Toledo DL53 (Mississauga, Canada) was used to measure alkalinity with 0.02 N H_2SO_4 as a titrant, and pH values were measured using an Accumet® Research AR50 (Fisher Scientific, Lenexa, Kansas). DOC was measured using a TOC analyzer (Shimadzu TOC-LCPH, Japan) and a non-purgeable organic carbon method (Brown et al., 2013). Prior to any analysis and unless otherwise specified, liquid samples were filtered with polytetrafluoroethylene (PTFE) syringe filters (Fisher Scientific; 0.22 μm for cation analysis; 0.45 μm for DOC analysis). For DOC analysis, all water samples were diluted 10× using ultrapure water.

PHCs were analyzed based on the Canada-Wide Standard for Petroleum Hydrocarbons in soil (CCME, 2008). Fractions were grouped as follows: F1 (C6–C10), F2 (C10–C16), F3 (C16–C34), F4 (C34–C50), and F4G-SG (>C50; (CCME, 2008). According to the Dean Stark extraction method (the industry-accepted method) the F4 and F4G-SG fractions were categorized with respect to bitumen content (Dean and Stark, 1920). F1 fractions were non-detectable in all samples and were measured by purge and trap gas chromatography (Column: DB1 0.25 mm ID; 1.0 μm film; 60

m length). Other fractions (F2, F3, F4, and F4G-SG) were analyzed by Maxxam Analytics (Edmonton, Alberta, Canada). Further details on PHC fractions analysis can be found in the Canada-Wide Standard for Petroleum Hydrocarbons in Soil (CCME, 2008) and elsewhere (Yu et al., 2018a). To determine the statistical differences between the treatment conditions, p-values were calculated using a two tailed t-test in Microsoft Excel. The F-test was initially used to determine the variance among sample pools whether it is equal or unequal. The difference between the treatment conditions is considered significant if p-value < 0.05.

3.2.4 Toxicity Bioassay

The toxicity of the aqueous samples toward *V. fischeri* was measured with the Microtox® bioassay test (Osprey Scientific, Edmonton, Alberta, Canada). Briefly, the 81.9% Basic Test protocol was followed by 5 min and 15 min incubation in the Microtox® 500 Analyzer (Azur Environmental time, (Anderson et al., 2011). The light emission was measured and recorded with MicrotoxOmni software to determine the inhibitory concentration resulting in 20% less light emission (IC_{20}) or inhibitory concentration resulting in 50% less light emission (IC_{50}) values. There were no significant differences in the toxicity measured between 5 min and 15 min tests, therefore only the 5 min data is described (Miles et al., 2019). Toxicity units, derived from IC_{50} ($TU = 100 \div IC_{50}$), were used to represent high-level toxicity trends (Yu et al., 2018a). In previous studies, 1.0 toxicity unit (TU) or lower is considered completely detoxified (Scott et al., 2008; Yu et al., 2018a).

Both principal component analysis (PCA) and cluster analysis were employed using Minitab 19 in order to evaluate and highlight any variations between the lime treated conditions and control. Implementing the PCA and cluster analysis in addition to Tukey Pairwise comparisons also supported the determination of clustering, similarity, and significant differences between the treatment conditions (i.e., p-values <0.05 is considered significant different).

3.2.5 DNA Extraction and qPCR Analysis

DNA was extracted from the FFT samples (up to 500 mg of FFT used per sample), using the FastDNA™ SPIN Kit of Soil (MP Biomedicals) according to the isolation protocol provided by the supplier. The extraction was conducted at the start and end of the experiment to examine the initial and final microbial population density for all conditions. Microbial cell counts were determined by 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; Mahdavi et al., 2015; Yu et al., 2018b). Using a Bio-Rad CFX96 optical reaction module conversion for the C1000 Touch thermal cycler, the qPCR assay was performed on DNA extracted samples from the lime treated/ untreated tailings samples. The amplification data were analyzed using Bio-Rad CFX Manager™ 3.0 software. All standards and samples were analyzed in triplicate. More details can be found elsewhere (Yu et al., 2018b).

3.3. Results and discussion

3.3.1 Influence of lime treatment on FFT dewatering

The effectiveness of lime addition on the FFT dewatering was assessed based on released water volumes, water recovery (WR), and solids content. The released pore water volumes were 21.7, 30.7, 37.7, and 30 ml for the 650, 1600, 3500, and 4000 ppm lime dosages, respectively. Therefore, an increase in the released volume of FFT pore water was observed after lime treatment.

To better compare our study with others, the WR was also used as a comparable index. In general, WR increased at day 90 for the lime-treated FFT samples, as illustrated in Table 3.1. At the 3500 ppm dose, the maximum WR was 7.9%. However, increasing the lime dose to 4000 ppm decreased the WR to 6.4%, implying that there is an adverse effect from lime overdosing. This can be attributed to the overdose effect (i.e., 4000 ppm), which might create an extra strength on the solids and resist the self-weight settling. Tate et al., (2017) reported the same notion that increasing lime dosages could lead to an increase in the yield stress of the FFT (i.e., developing strength by lime addition) and hence can enhance the geotechnical properties of FFT. Another explanation could be due to the premature cracking as a result of the reduction of solids percentage happened after lime overdosing (Tate et al., 2017).

Comparing our results with previous studies, the 3500 ppm lime treatment achieved a similar WR (7.9%) to Yu et al. (2018a), who reported 8% WR after 100 d using a biological amendment (Ultrazyme). In contrast, Siddique et al. (2014) reported

a higher WR of 26% after 90 d using hydrolyzed canola meal (i.e., as an organic substrate). For solids content in our study, the highest increase was also observed at a dose of 3500 ppm (the final solids content of the FFT increased to 51.6% at day 90; Table 3.1). Despite the increase in the solid content, it can be considered marginal. It is worth to note that the initial solids content had a significant impact on the final WR (or the final volume of released water) where the lowest initial solids content led to a high WR (Yu et al., 2018a). The initial solids content in our study was considerably higher (50%) than those used by Siddique et al. (2014); 25%) and Yu et al. (2018a); 34.6%).

Table 3.1. The FFT pore water recovery % (WR%) at the end of the experiment (day 90) as well as FFT solids content at day 90. The solids content for all columns at day 0 was 50%. All columns consisted of 500 ml lime treated or untreated FFT covered with 500 ml Raw synthetic OSPW. All columns were open to the atmosphere. The untreated FFT represents a 0 ppm treatment and acts as the control experiment. Results illustrated are an average (n = 2 for 0 ppm and n = **3** for 650, 1600, and 3500 ppm) ± one standard deviation.

Lime Dose (ppm)	**WR%**	**Solid content% Day 90**	**Volume of FFT Porewater Released (ml)**
0	2.5**%** ± 0.4**%**	50.2% ± 0.04%	11.5 ± 2.1
650	4.7**%** ±0.5**%**	50.7% ± 0.3%	21.7 ± 2.8
1600	6.3**%**± 0.1**%**	50.9% ± 0.1%	30.7 ± 1.2
3500	7.9**%** ± 0.8**%**	51.6% ± 2.1%	37.7 ± 2.5
4000	6.4**%**	50.8%	30.0

3.3.2 Variations in water chemistry of FFT pore water and cap water

3.3.2.1 Alkalinity

As shown in Figure 3.1a, pore water alkalinity immediately increased after the addition of high lime doses (day 0; Figure 3.1a). For example, after the addition of 1600 or 3500 ppm lime (day 0), the alkalinity increased to 2390 mg/l and 5720 mg/l (measured as $CaCO_3$), respectively, compared with the control alkalinity of 450 mg/l (measured as $CaCO_3$). The increased alkalinity at day 0 with the higher lime dose could be attributed to the increase of the hydroxide (OH^-) concentrations, which agrees with Caughill et al. (1993).

In contrast, at day 90, the alkalinity sharply decreased at all lime doses, with only a slight change in the control. This could be argued as the pH of the FFT pore water decreased in all the lime-treated columns at day 90 (Figure 3.2a). As a result of the dissolution of carbon dioxide CO_2 into the cap water; the pH of the pore water started to decrease, and the formation of carbonate minerals, such as $CaCO_3$, also reduces the alkalinity (Hrynyshyn, 2012).

The alkalinity level of the cap water continuously decreased throughout all the lime experiments as well as in the control (Figure 3.1b). However, compared with the control after 90 d (315 mg/l measured as $CaCO_3$), the alkalinity level of the samples treated with 1600 ppm and 3500 ppm lime significantly decreased to 170 and 200 mg/l (measured as $CaCO_3$), respectively. The slight change in cap water alkalinity in the control experiment (20% decrease) could be attributed to methanogenesis in the FFT layer (Dompierre et al., 2016b; Siddique et al., 2014) that can increase and

maintain a stable alkalinity level in cap water. Similar observations were reported in BML (White and Liber, 2018) where methanogenesis resulted in elevated and high cap water alkalinity. Based on Figure 3.2b, the pH of the control FFT decreased below pH 7.5 at day 90. This can be attributed to CO_2 production during methanogenesis (Samadi, 2019; Siddique et al., 2014) or the contribution of atmospheric CO_2 to decrease the pH. Dissolved CO_2 is converted to carbonic acid through hydration and then dissociates to HCO_3^- (Li, 2010), which is transported to the cap water through any movement of gas (including CH_4). To confirm such prospect, further investigations are warranted about monitoring of the redox potential for the FFT layer throughout the experiments.

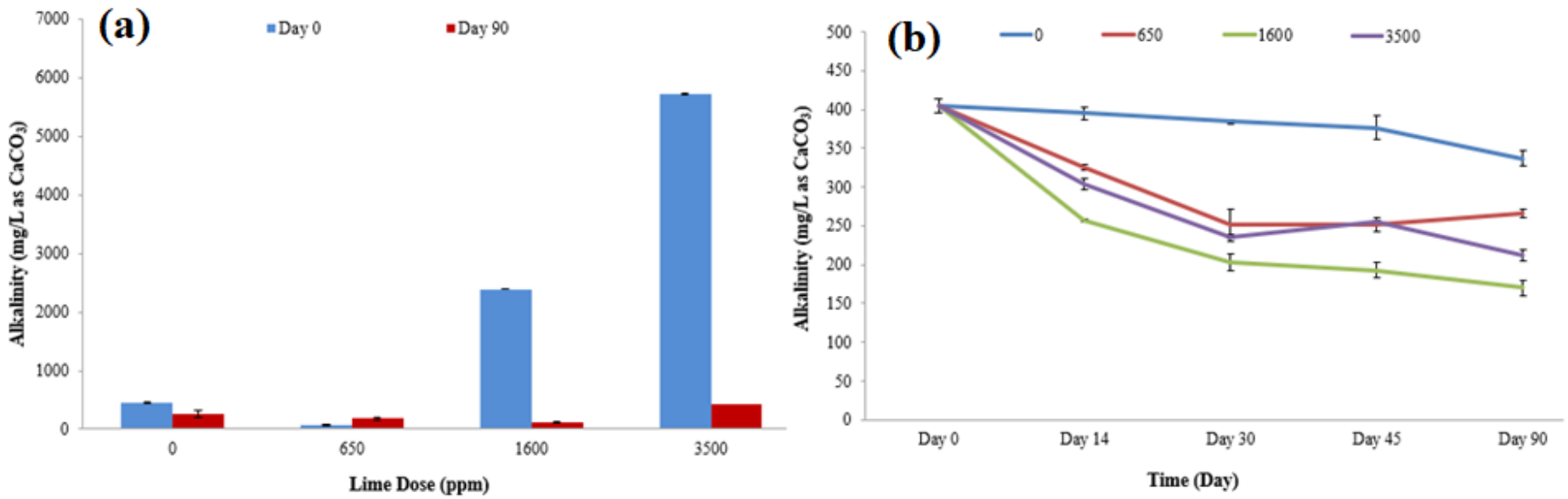

Figure 3.1. Change in alkalinity of (a) FFT pore-water at day 0 and at the end of the experiment (day 90); and (b) cap water at day 0, day 14, day 45, and day 90. Results illustrated are an average (n = 2 for 0 ppm and n = 3 for 650, 1600, and 3500 ppm) ± one standard deviation. All columns consisted of 500 ml treated or untreated FFT covered with 500 ml Raw synthetic OSPW. All the columns were open to the atmosphere. The untreated FFT represents a 0 ppm treatment and acts as the control experiment.

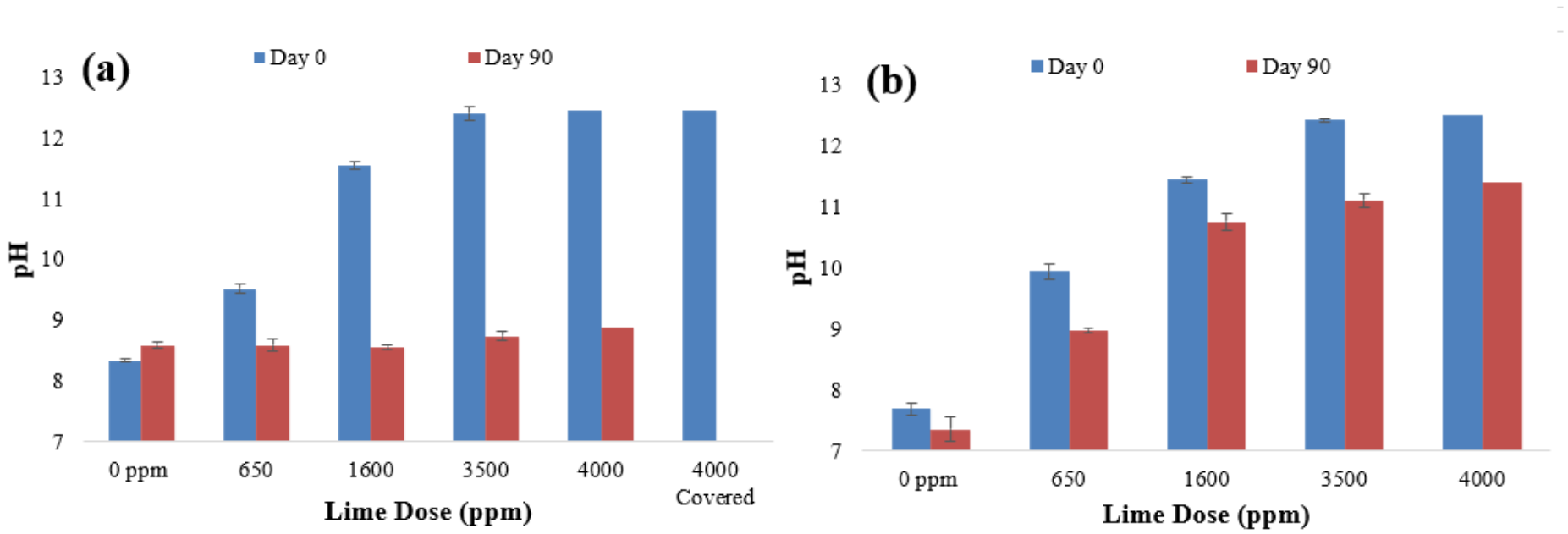

Figure 3.2. (a) FFT Pore-water pH at day 0 and at the end of the experiment (day 90). (b) FFT pH at day 0 and at the end of the experiment (day 90). All the columns consisted of 500 ml of treated or untreated FFT covered with 500 ml Raw synthetic OSPW. All the columns were open to the atmosphere except the 4000 ppm treatment, which has two conditions: uncovered and covered with parafilm. Results illustrated are an average (n = 2 for 0 ppm and n = 3 for 650, 1600, and 3500 ppm) ± one standard deviation. The untreated FFT represents a 0 ppm treatment and acts as the control experiment.

An overall decrease in cap water alkalinity in the lime-treated conditions was observed after 90 d, with the highest magnitude of the decrease for the 1600 ppm. For example, the alkalinity decreased after 90 d by 36 %, 58%, and 51% at lime doses of 650, 1600, and 3500 ppm, respectively. The decrease was significantly rapid and sharp in the first 30 d in all lime-treated FFT. A similar trend can be observed for HCO_3^- concentrations (Figure 3.3). There are two explanations for this decrease. The methanogenesis process may have been negatively influenced by lime addition which inhibited the methanogens (Nyberg et al., 2011). The changes in the microbial community are discussed later (section 3.3). Alternatively, the change in pH after lime addition could have led to the predominance of CO_3^{2-} species (MacKinnon et al., 2001). Although further studies are needed to confirm the underlying mechanisms, these results reveal the positive role of lime addition to maintain an appropriate cap water quality for the aquatic life (White, 2017; White and Liber, 2018). The changing pH is correlated with lime dosage, and Figure 3.4 displays the corresponding effect of lime treatment on the pH of cap water with time.

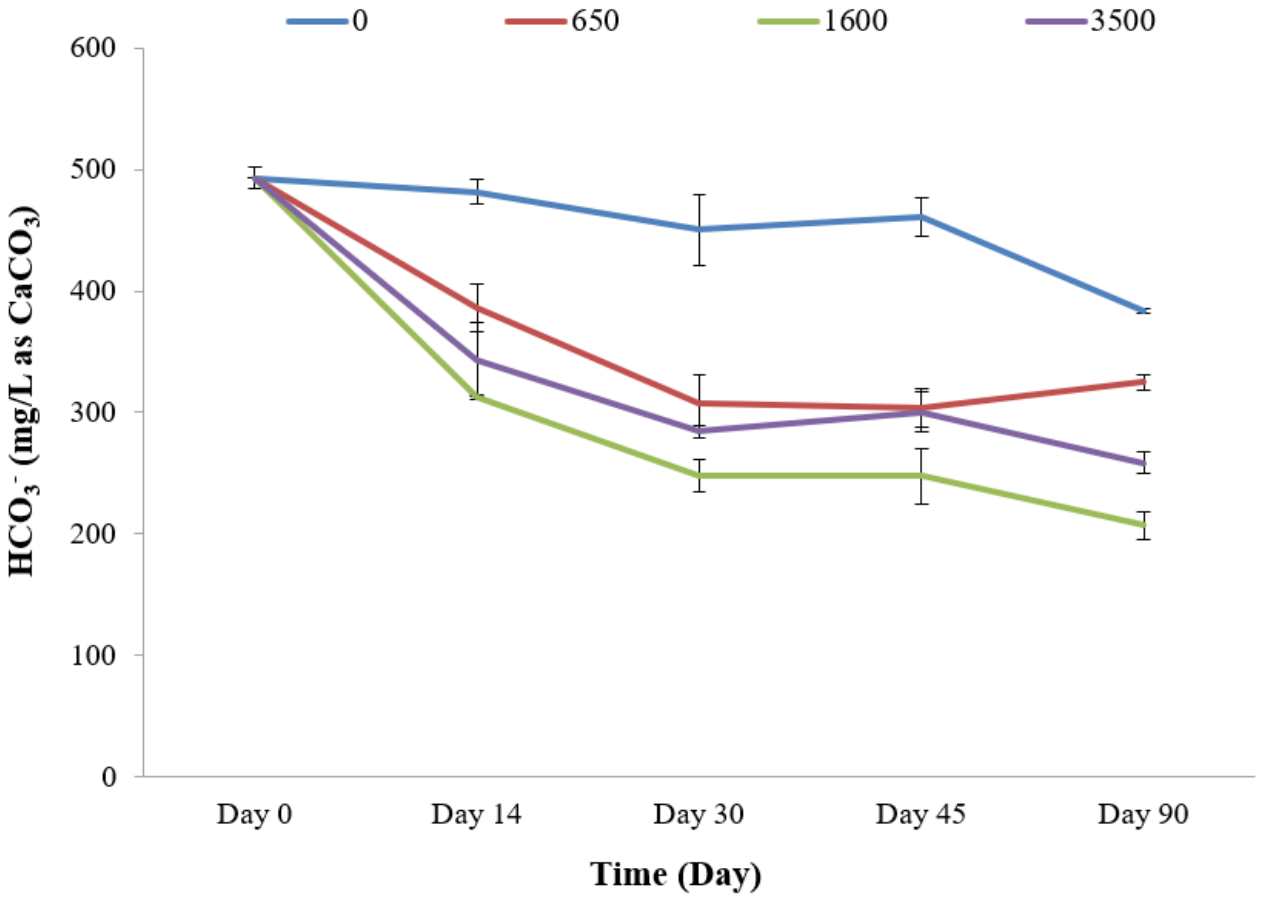

Figure 3.3. Change in bicarbonate (HCO_3^-) concentration measured as (mg/l $CaCO_3$) in cap water at day 0, day 14, day 45, and day 90. Results illustrated are an average (n = 2 for 0 ppm and n = 3 for 650, 1600, and 3500 ppm) ± one standard deviation. All columns consisted of 500 ml treated or untreated FFT covered with 500 ml Raw synthetic OSPW. All the columns were open to the atmosphere. The untreated FFT represents a 0 ppm treatment and acts as the control experiment.

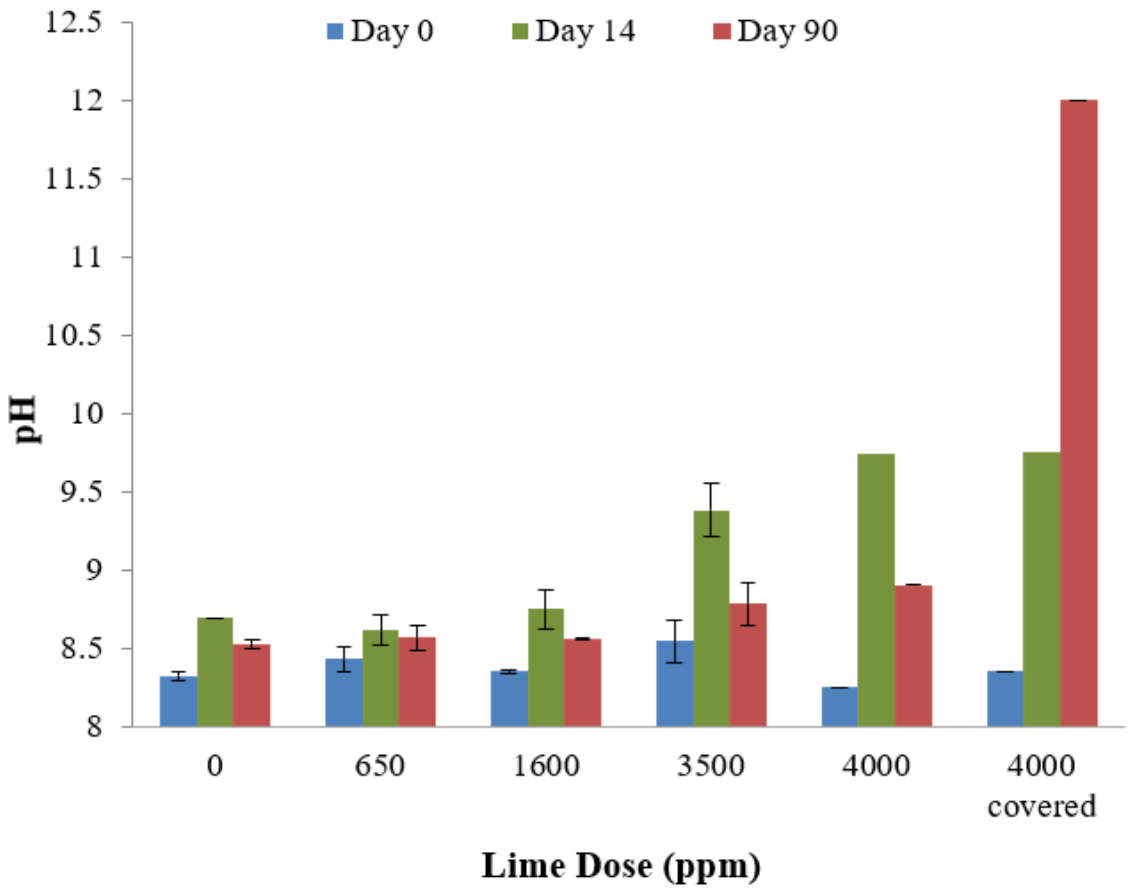

Figure 3.4. Cap-water pH at day 0, day 14, and at the end of the experiment (day 90). All the columns consisted of 500 ml treated or untreated FFT covered with 500 ml Raw synthetic OSPW. All the columns were open to the atmosphere except the 4000 ppm treatment which has two conditions: uncovered and covered with parafilm. Results illustrated are an average (n = 2 for 0 ppm and n = 3 for 650, 1600, and 3500 ppm) ± one standard deviation. The untreated FFT represents a 0 ppm treatment and acts as the control experiment.

3.3.2.2 Cations

Figure 3.5 depicts the change in cation concentrations (Ca^{2+} and Na^{+}) in the FFT pore water with different lime doses. For the low lime dose (650 ppm; pH < 10), the Ca^{2+} concentration was slightly lower than the control (0 ppm lime) at day 0. Similarly, Ca^{2+} concentrations were slightly higher in the 1600 lime dose at day 0 compared to the control. An immediate sharp increase in the Ca^{2+} concentration at day 0 was observed immediately after the addition of high lime dosages 3500 and 4000 ppm (pH > 11.5). At day 90, similar Ca^{2+} concentrations were observed for all lime doses as well as the control. For lime doses 650 and 1600 ppm, there was no significant change in cation concentration ($p > 0.05$), while a significant reduction in cation concentration was observed at high lime doses ($p < 0.05$). The summary of the cation results is presented in Table 3.2.

Elucidating the influence of pH on changes in cations concentrations at days 0 and 90 can lead to a better understanding to propose the mechanisms involved. Briefly, there are two main phases of Ca^{2+} availability based on the consumption of carbonate species. In the first phase, as lime is initially added at day 0, (e.g. pH 10, lime dose 650 ppm), the equilibrium of the bicarbonate-carbonate reaction will be shifted toward CO_3^{2-}. Lime reacts with sodium bicarbonate ($NaHCO_3$) through water softening reaction to produce sodium hydroxide (NaOH) and insoluble calcium carbonate that precipitate as calcite ($CaCO_3$; (Hamza et al., 1996; MacKinnon et al., 2001). This mechanism explained the lower Ca^{2+} concentrations at day 0 for the 650 ppm lime compared with the control at this pH range (Figure

3.5a). This is the dominant reaction pathway up to a pH of approximately 11.5 (lime dose <1600 ppm), at which the carbonate species are effectively removed. Accordingly, at this phase, no soluble Ca^{2+} is available for cation exchange reactions. It is worth noting that the softening reactions are fast to occur before ion exchange mechanisms. The NaOH produced from water softening reacts with the edges of kaolinite clays, removing hydrogen ions which create new exchange sites that will be occupied by Na^{+}. This proposed mechanism can explain the significant reduction in Na^{+} concentration at day 0 in the FFT pore water for the 650 ppm compared to control (Figure 3.5b). With regards to the second phase (i.e., pH > 11.5, lime dose >1600 ppm), the immediate increase in the Ca^{2+} concentration at day 0 can be attributed to increased solubility of Ca^{2+} from the dissolution of $Ca[OH]_2$(MacKinnon et al., 2001; Tate et al., 2016). This provides Ca^{2+} ions that can exchange on mineral surfaces. This can be demonstrated where the soluble Ca^{2+} exchanges and displaces the Na^{+} increasing its concentration in the pore water (i.e., Na^{+} concentrations are 210, 300, and 315 mg/L at 1600, 3500, and 4000 ppm lime dosage respectively).

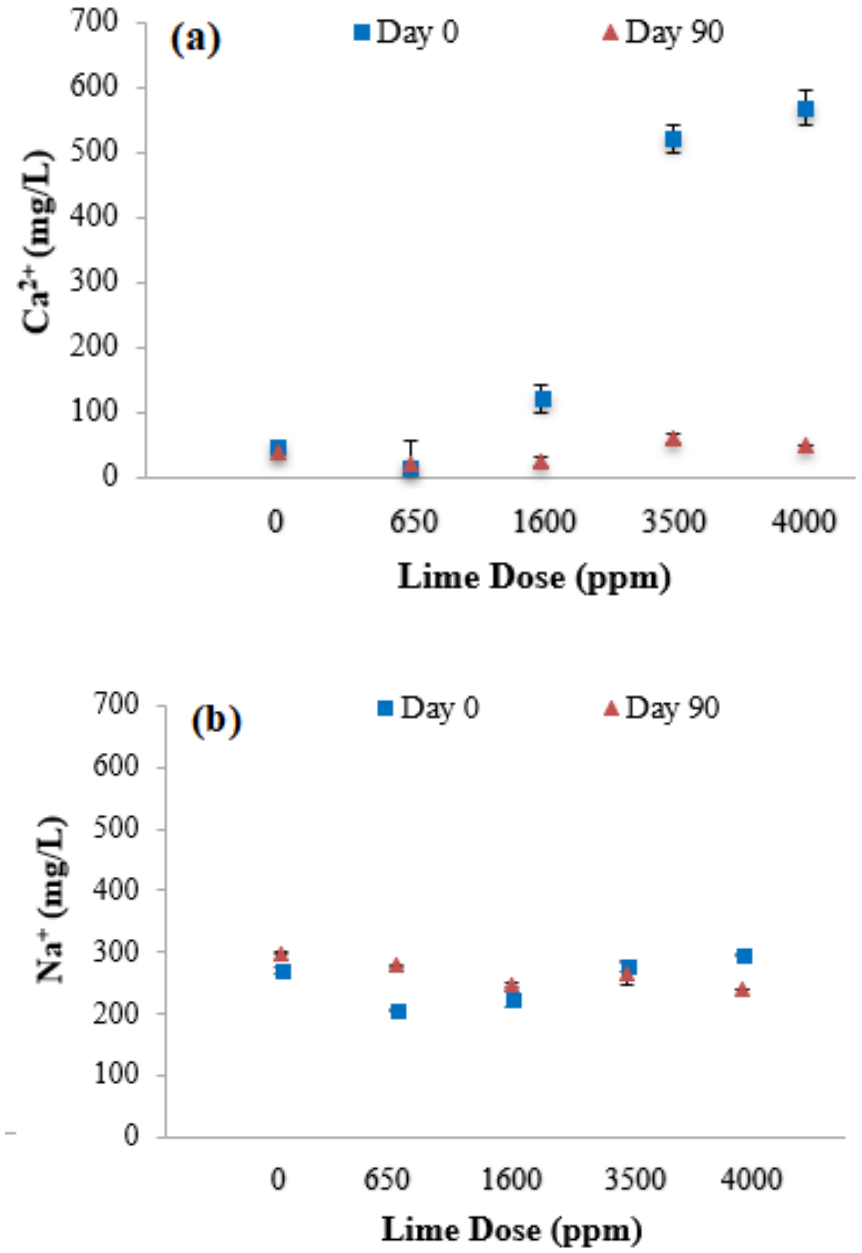

Figure 3.5. Concentrations of cations for treated and untreated FFT pore water at day 0 and at the end of the experiment (day 90) measured by ICP-OES. a) Ca^{2+} and b) Na^{+}. Results illustrated are an average (n = 2 for 0 ppm and n = 3 for 650, 1600, and 3500 ppm) ± one standard deviation. All columns consisted of 500 ml treated or untreated FFT covered with 500 ml Raw synthetic OSPW. All the columns were open to the atmosphere. The untreated FFT represents a 0 ppm treatment and acts as the control experiment.

Table 3.2. Concentrations of cations Ca^{2+} and Na^{+} for treated and untreated FFT pore water and cap water at day 0 and the end of the experiment (day 90) measured by ICP-OES. Results illustrated are an average (n = 2 for 0 ppm and n = 3 for 650, 1600, and 3500 ppm) ± one standard deviation. All columns consisted of 500 ml treated or untreated FFT covered with 500 ml Raw synthetic OSPW. All the columns were open to the atmosphere. The untreated FFT represents a 0 ppm treatment and acts as the control experiment.

Sample ID	Ca^{2+}			
	Pore water		Cap water	
	Day 0	Day 90	Day 0	Day 90
0 ppm	48.3 ± 1.3	38.7 ± 0.7	14.1 ± 0.4	15.7 ± 0.6
650 ppm	15.2 ± 3.9	21.4 ± 35.2	13.6 ± 0.2	20.2 ± 0.4
1600 ppm	120.8 ± 20.9	24.6 ± 6.5	14.5 ± 1.3	6.8 ± 1.6
3500 ppm	557.4 ± 73.4	60.5 ± 7.4	14.4 ± 0.8	4.0 ± 1.6
4000 ppm	544.6 ± 60.9	49.8 ± 0.0	12.60	2.2
Sample ID	Na^{+}			
	Pore water		Cap water	
	Day 0	Day 90	Day 0	Day 90
0 ppm	272.1 ± 5.7	298.9 ± 3.0	294.3 ± 1.0	299.4 ± 3.2
650 ppm	204.9 ± 1.2	279.6 ±1.2	303.7 ± 3.7	303.3 ± 1.0
1600 ppm	223.6 ± 12.5	247.4 ± 4.7	298.8 ± 7.7	281.4 ± 4.3
3500 ppm	277.9 ± 9.3	266.4 ± 17.4	298.8 ± 5.9	303.6 ± 6.8
4000 ppm	295.4 ± 1.3	240.4 ± 0	283.7	294.5

With regards to day 90, we can hypothesize that increasing the pH > 12 (i.e., 3500 ppm and 4000 ppm lime) promote the pozzolanic reaction where soluble Ca^{2+} started to react with dissolved silica and alumina from the clay forming hydrated minerals of calcium aluminate and silicate (Tate et al., 2017; Wang, 2017). This can elucidate the reduction of Ca^{2+} concentration at day 90 for the pore water. Lane (1983) suggested that the pozzolanic reaction can occur with lime addition at high pH in which a stabilization process can occur through lime addition (i.e., the authors suggested coagulation process as a good description of the lime stabilization in soil and tailings). Furthermore, the lime addition can modify particle surface charge and lead to agglomeration and interparticle bonding. In the same sense, the study elucidated the stabilization reactions as the pozzolanic reactions and carbonation reactions.

In terms of the cap water, Na^{+} concentration was almost stable throughout the experiment at different lime doses (Figure 3.6b). In contrast, Ca^{2+} levels decreased in the cap water with increasing lime dose (Figure 3.6a). The change in Ca^{2+}concentrations confirms that CO_2 is being absorbed by the system to react with alkaline Ca^{2+}. The Student's t-test results suggested significant differences in Ca^{2+} concentrations at day 90 at high lime doses (>1600 ppm) compared with lower lime doses ($p < 0.05$). Our investigations generally depicted a marginal drop in K^{+} concentration for all lime doses throughout the experiment (Figure 3.7) that slightly varied with the control. Although this could partially correspond with the findings of White and Liber (2018), who reported a slight change in K^{+} levels in

the surface water of BML over three consecutive years, further investigations are warranted to confirm this phenomenon. In summary, confirming cation exchange and pozzolanic reactions occurrence would require a more focused study.

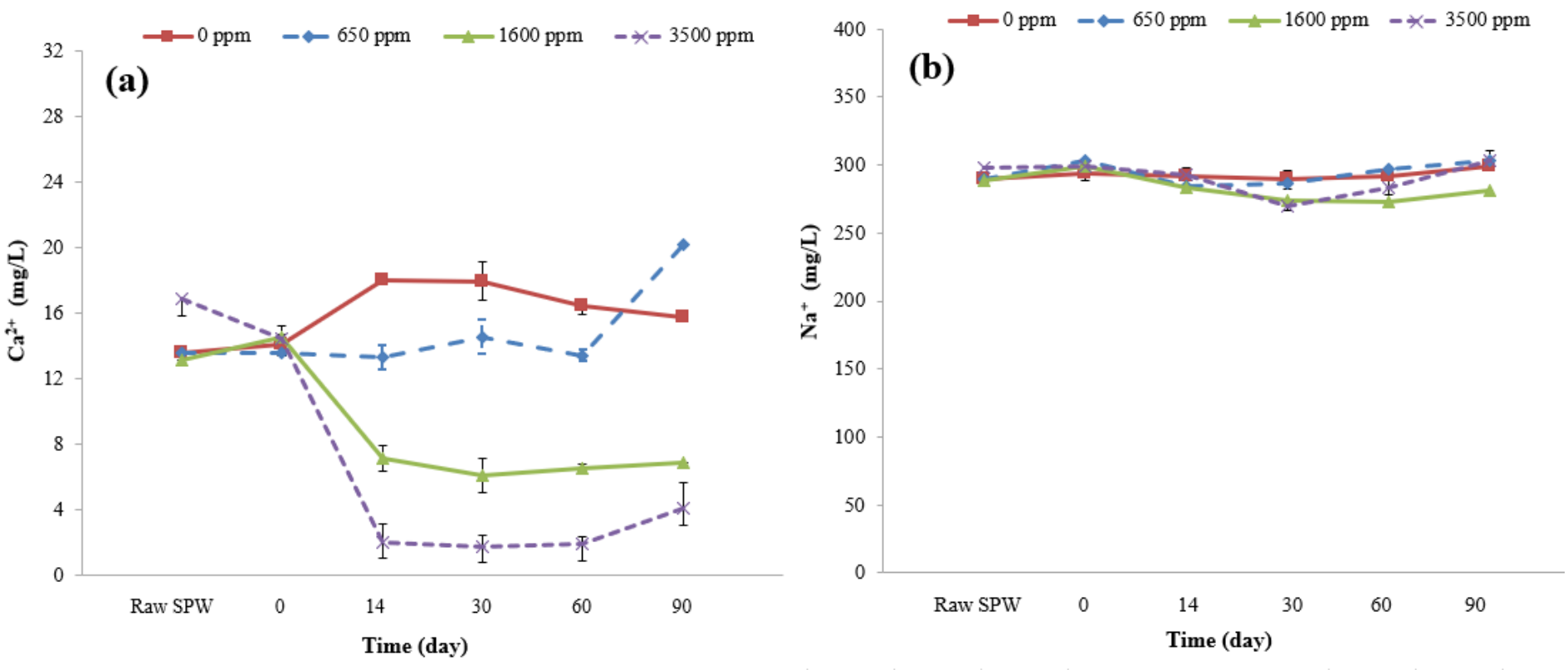

Figure 3.6. Variation of cation (Ca^{2+} and Na^{+}) concentrations in cap water at day 0 and at the end of the experiment (day 90) measured by ICP-OES. Illustrated results are an average (n = 2 for 0 ppm and n = 3 for 650, 1600, and 3500 ppm) ± one standard deviation. All columns consisted of 500 ml treated or untreated FFT covered with 500 ml Raw synthetic OSPW (Raw SPW). All the columns were open to the atmosphere. The untreated FFT represents a 0 ppm treatment and acts as the control experiment.

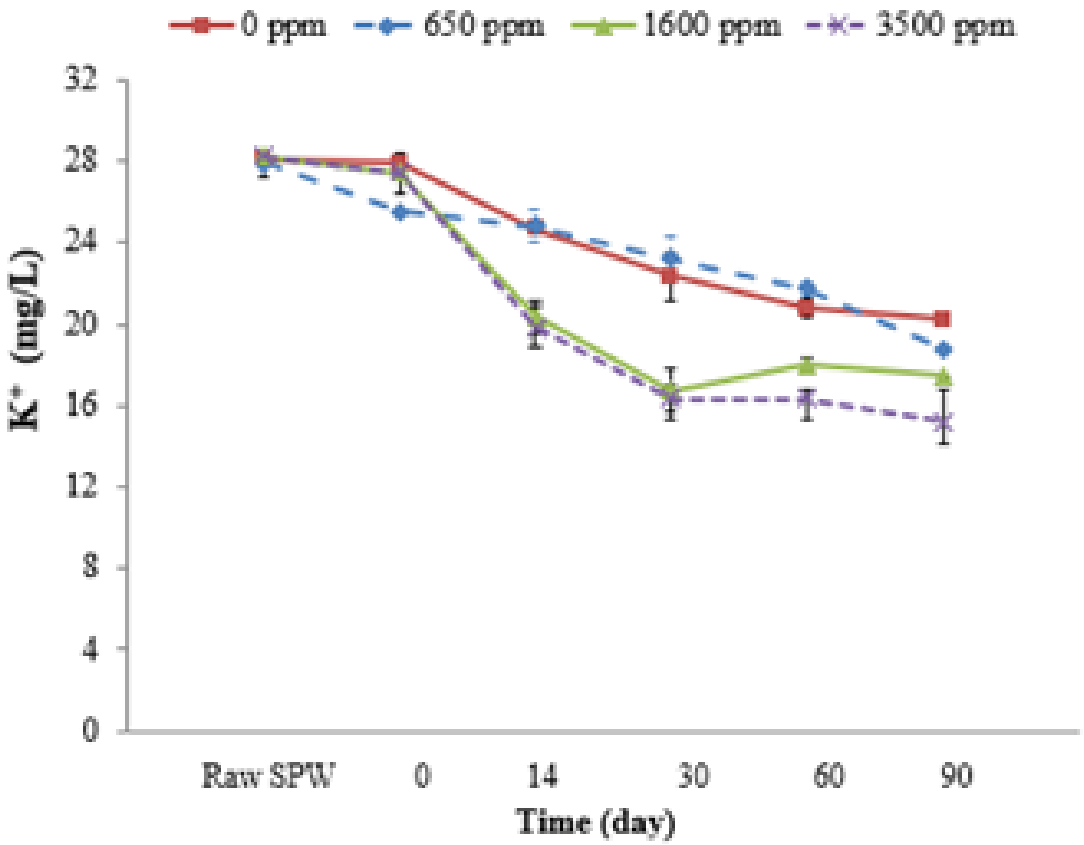

Figure 3.7. Variation of cation K^+ concentrations in cap water at day 0 and the end of the experiment (day 90) measured by ICP-OES. Illustrated results are an average (n = 2 for 0 ppm and n = 3 for 650, 1600, and 3500 ppm) ± one standard deviation. All columns consisted of 500 ml treated or untreated FFT covered with 500 ml Raw synthetic OSPW (Raw SPW). All the columns were open to the atmosphere. The untreated FFT represents a 0 ppm treatment and acts as the control experiment.

3.3.3 Variations in bacterial cell counts

The initial indigenous microbial cell counts are represented by the number found in the control sample on day 0 (8.15×10^{10} cells/µl), and is used to compare the effect of lime treatment on the microorganisms. The addition of lime resulted in an immediate adverse impact on the bacterial cell counts at day 0 after lime addition, and the microbial count was considerably reduced for all lime conditions (Figure 3.8). On day 90, the bacterial cell counts were significantly decreased at high lime doses (pH > 11.5), with the greatest reduction of cell counts occurring at 4000 ppm lime under covered conditions (4.41×10^4 cells/µl; 98% reduction) resulting from the sharp pH increase. In contrast, at a lime dose of 650 ppm (pH < 10) there was a minimal reduction in the microbial cell counts after 90 d, highlighting the adverse impact of increased pH, particularly pH > 11.5, on the cell counts. Overall, there is significant difference in the microbial cell counts for most of the lime-treated FFT (i.e., lime doses > 1600 ppm; $p < 0.05$), but no significant difference in the control or low lime-treated FFT (i.e., $p > 0.05$ for both control and 650 ppm lime).

Our findings correspond with previous studies that showed a similar adverse impact of a rapid increase in pH (pH > 12) on bacterial cell membranes, which inhibited their growth (Burns and Gremminger, 1994; Nyberg et al., 2011; Wong et al., 2001; Wong and Selvam, 2009). Furthermore, the addition of lime (pH 11) was demonstrated to effectively inactivate pathogens in municipal biosolids (Bennett et al., 2003).

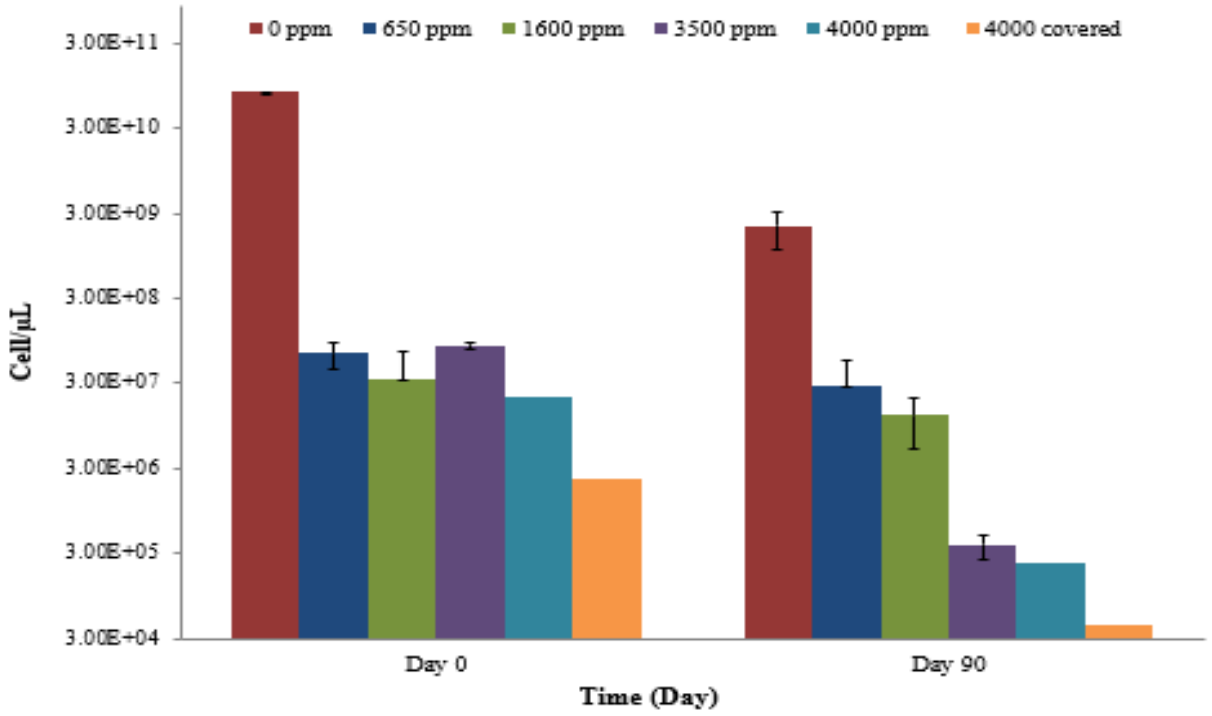

Figure 3.8. Bacterial cell counts for treated and untreated FFT at day 0 and at the end of the experiment (day 90) measured by qPCR of the rpoB gene. Results illustrated an average (n = 2 for control and n = 3 for 650, 1600, and 3500 ppm) ± one standard deviation. All columns consisted of 500 ml treated or untreated FFT covered with 500 ml Raw synthetic OSPW. All the columns were open to the atmosphere except the 4000 ppm which has two conditions: uncovered and covered with parafilm. The untreated FFT represents a 0 ppm treatment and acts as the control experiment.

3.3.4 Impact of lime treatment on organic fractions

To determine the impact of lime treatment on organic fractions, we characterized both PHCs and DOC. Figure 3.9a shows the concentrations of PHC fractions after different lime treatments at day 90 compared with the initial concentration in untreated FFT (control) at day 0 and day 90. The greatest reduction in PHC concentrations is observed at 650 ppm lime, with removal efficiencies of 54%, 57%, 60%, and 66% for F2, F3, F4, and bitumen, respectively. However, for lime doses of 1600, 3500, and 4000 ppm, the PHC reductions were minimal compared with the reduction in the control sample after 90 d. The high bacterial cell counts in the

sample treated with 650 ppm lime could explain the better degradation of PHCs compared with the degradation in the high lime doses (>1600 ppm).

As stated earlier, at a lime dose of 650 ppm, Ca^{2+} reacts with carbonate and precipitates as $CaCO_3$ (Hamza et al., 1996; MacKinnon et al., 2001). In comparison to control, both F4 and bitumen fractions (i.e., the heaviest fractions of PHCs) might complex with calcite precipitates ($CaCO_3$) and then settle down. Nevertheless, the F2 fraction (i.e., demonstrated slight reduction, Figure 3.9a), which is the highly volatile fraction of PHC (Brickner, 2013), seems to solubilize from the bitumen and dissolve in the FFT pore water.

Our findings highlight the negative influence of high doses of lime on microbial cell counts that subsequently affect the degradation of the PHCs. Various studies have reported the effectiveness of various microbial communities such as hydrocarbon-degrading microorganisms, sulfate-reducing bacteria, and iron-reducing bacteria on the degradation of PHCs (Allen et al., 2007; Pandey et al., 2009). Similarly, Yu et al. (2018a) suggested that the hydrocarbons present in tailings serve as a carbon source for the indigenous microorganisms. These findings can explain the reduction of the PHC fractions in the control columns after 90 d.

The variations in DOC concentrations in the pore water throughout the experiment are illustrated in Fig. 3.9b. No significant change was observed between day 0 and day 90 at 650 ppm; however, at lime doses of 1600, 3500, and 4000 ppm, a significant increase in DOC was observed. The changes in DOC agree with the minimal decrease in the PHC fraction; in which at high lime doses, bacterial cell numbers were decreased and were not able to degrade either the PHC or DOC. Additionally, during cation exchange when pH > 11.5, the F2 fraction might dissolve in the FFT pore water and increase the DOC concentration. This mechanism is similar

to one proposed by Fine et al. (1997) that highly volatile fractions of PHCs can dissolve into soil pore water or groundwater. The increase in DOC may also be ascribed to the release of organic compounds into the cap water during FFT dewatering (Yu et al., 2018a).

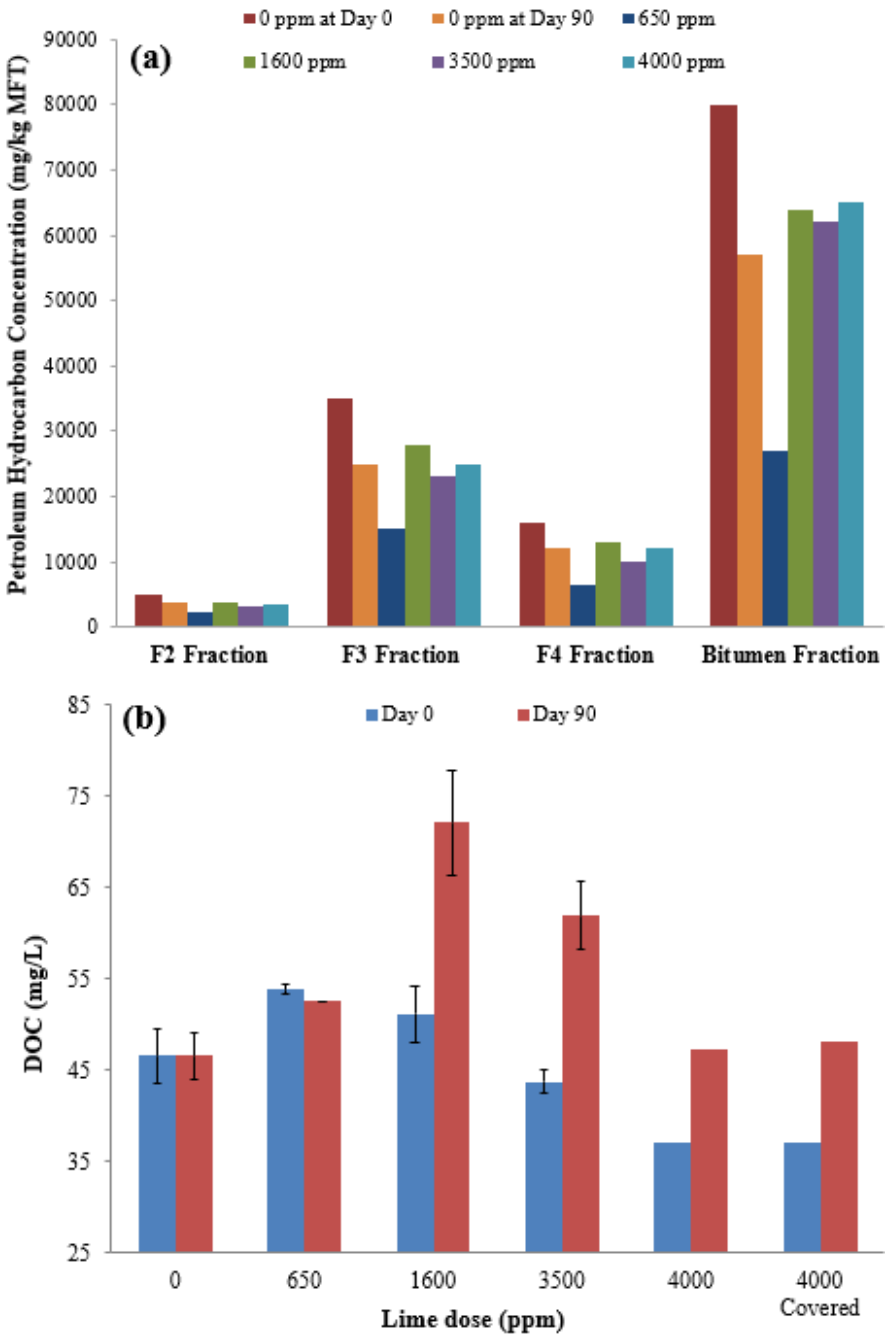

Figure 3.9. (a) Petroleum hydrocarbons (PHC) concentrations for lime-treated FFT at the end of experiment (day 90) compared with the untreated/0 ppm FFT at day 0 and day 90. (b) Dissolved organic carbon (DOC) concentrations for pore water at day 0 and at the end of the experiment (day 90). All columns consisted of 500 ml treated or untreated FFT covered with 500 ml of Raw synthetic OSPW. All the columns were open to the atmosphere except the 4000 ppm treatment, which was covered with parafilm. Results illustrated are an average (n = 2 for 0 ppm and n = 3 for 650, 1600, and 3500 ppm) ± one standard deviation. The untreated FFT represents a 0 ppm treatment and acts as the control experiment.

3.3.5 Toxicity and implications of pH influence

Table 3.3 summarizes the impact of lime addition on the toxicity to *V. fischeri* of the FFT pore water (days 0 and 90) and cap water (day 14 and day 90). High lime doses (i.e., > 1600 ppm) caused higher toxicity of the FFT pore water (above instrument detection limit) compared with the lower toxicity in the control and in the 650 ppm lime dose (0.1 and 1.2 TU, respectively). These results are consistent with the negative impact of lime addition (i.e., > 1600 ppm) on bacterial cell counts measured in the FFT.

With respect to the acute toxicity effect of the cap water to *V. fischeri* over time (Table 3.3), for lime doses ≤1600 ppm (pH ≤ 11.5), the cap water at day 14 was non-toxic after lime addition. At high lime doses (3500 and 4000 ppm; pH > 11.5), the toxicity at day 14 slightly increased to 0.8 and 1.1 TU at 3500 and 4000 ppm, respectively, then decreased at day 90 to 0.6 and 0.4 TU for 3500 and 4000 ppm, respectively.

Even though the lime treated samples (uncovered conditions after 90 days) in comparison to the control (Table 3.3), showed a slight increase in toxicity; the toxicity of the cap water for all lime doses did not exceed 1 TU (Table 3.3). This translates as a nontoxic effect toward *V. Fisheri*. To confirm our observations, principal component analysis (PCA, Figure 3.10) and cluster analysis (Figure 3.11) were assessed to pinpoint the influence of lime treatment on cap water toxicity after 14 and 90 days. The PCA data support very minimal variations and high similarity levels (i.e., statistically confirmed as insignificant different at 95% confidence interval and significance level of 0.05) between the toxicity effect of lime treated samples and control samples in cap water.

It is worth noting that our rationale was to monitor the toxicity of the cap water after FFT lime treatment which necessitate using the synthetic OSPW in the experiment without any organics to better understand the movement of COPCs. According to our earlier observations about the adverse effects on WR occurred due to overdosing (i.e., 4000 ppm), we have decided to elucidate the potential influence of any adverse impact of overdosing or discrepancies between covered and uncovered conditions with regards to toxicity. For the 4000 ppm covered condition, a significant increase in toxicity occurred at both days 14 and 90 (14.83 and 16.13 TU, respectively). This can be initially attributed to the significant effect on pH at high lime doses, as shown in Figure 3.4. Although at day 14, both of the 4000 ppm conditions (covered and uncovered) had a similar pH (≈ 9.8), the TUs were quite different (1.1 TU for the uncovered condition; 14.8 TU for the covered condition). Therefore, high toxicity is not only related to the pH increase in the covered condition, but also possibly to COPCs moving from the FFT into the cap water. These results highlight the significant impact of atmospheric CO_2 to reduce cap water pH and generate a carbonate buffer in the uncovered conditions.

In summary, the slight increase in toxicity of cap water samples can be related to the movements of COPCs from FFT to the released pore water and cap water. This would necessitate more toxicological investigations to examine the critical concentrations, species, and the environmental impact of lime treatment on live-dead viability assays. This would develop better understanding of the influence of end pit lake surface water on acute toxicity and in vitro assays (e.g. RAW 264.7 mouse macrophage cell line and the response of bone marrow-derived macrophages).

Table 3.3. Toxicity of FFT pore water presented in Toxicity Units (TU) at day 0 and the end of the experiment (day 90) and cap water toxicity at day 14 and the end of the experiment (day 90) assessed using Microtox. TU < 1.0 indicates complete detoxification (Scott et al., 2008). All columns consisted of 500 ml treated or untreated FFT covered with 500 ml Raw synthetic OSPW. All the columns were open to the atmosphere except the 4000 ppm treatment, which was covered with parafilm. The untreated FFT represents a 0 ppm treatment and acts as the control experiment.

Sample ID	**Toxicity Unit (TU)**			
	Pore water		**Cap water**	
	Day 0	**Day 90**	**Day 14**	**Day 90**
0 ppm	Non-toxic*	0.1	0	0.1
650 ppm	1.3	1.2	0	0.4
1600 ppm	High level	4.3	0	0.3
3500 ppm	High level	High level	0.8	0.6
4000 ppm	High level**	High level	1.1	0.4
4000 ppm Covered	High level	High level	14.9	16.2

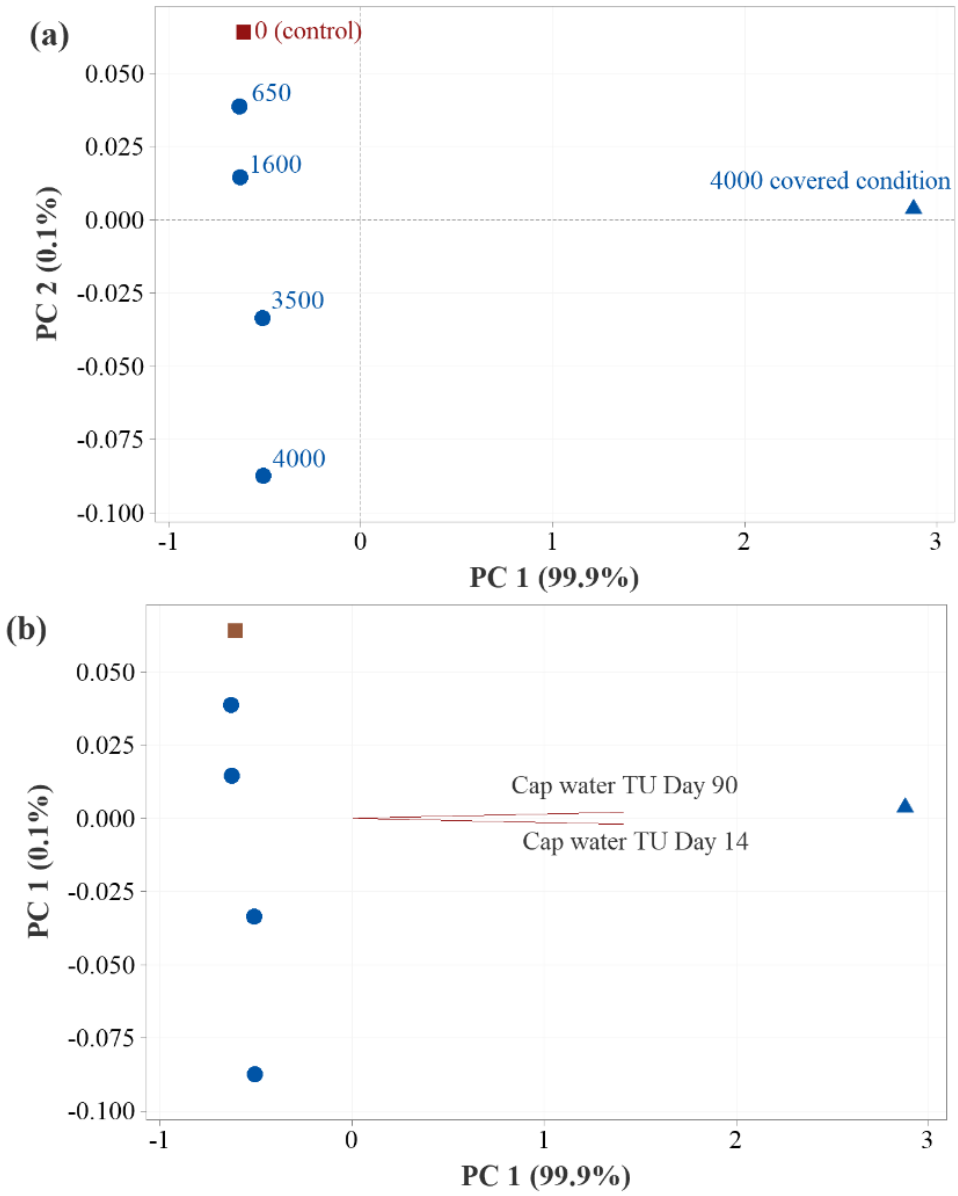

Figure 3.10. (a) Score plot for PCA analysis of all lime treated samples and control, PC 1 (99.9%) and PC 2 (0.1%), (b) Biplot and loading plot of PC1 and PC2 with project lines of all samples. The vectors radiating from the origin represent the sample loadings. Samples that are chemically similar are plotted near to each other (clustered together), while sample scores are color-coded based on treatment conditions and symbols indicated by square or circle.

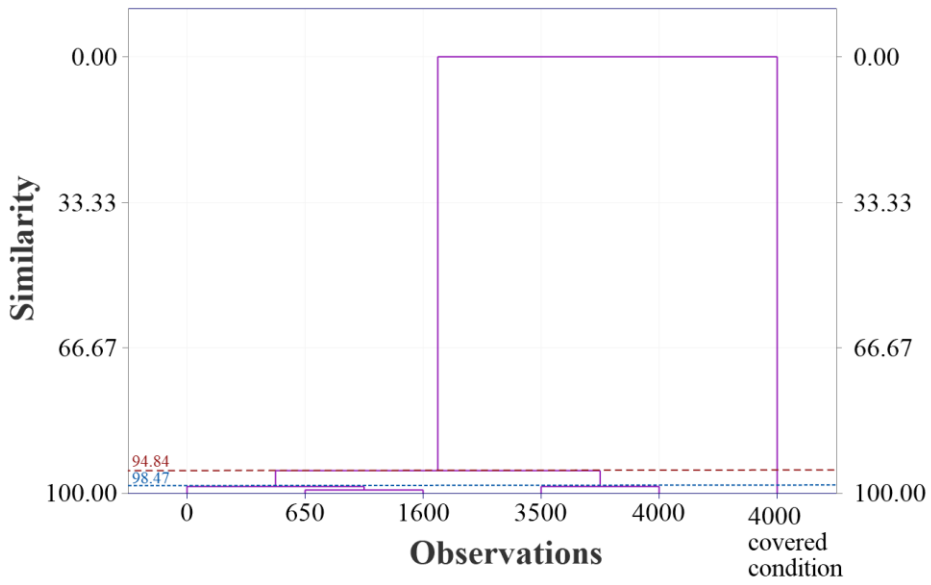

Figure 3.11. Dendrogram of the cluster analysis for toxicity effects of cap water.

3.4 Conclusions

While mimicking the EPL approach, this study investigated lime application to control the water quality in EPLs and aid the dewatering of FFT from oil sands operations. In terms of the better performance of WR, alkalinity of cap water, and cation exchange, lime dose of 3500 ppm achieved 7.9% WR despite the relatively high initial solids content of FFT (50% wt./wt.). The alkalinity of the cap water decreased after adding lime dose of 3500 ppm, compared with the untreated FFT, promoting and maintaining an appropriate, low alkalinity environment for an aquatic ecosystem in EPLs. Furthermore, the cation concentrations indicate that cation exchange occurs at high lime doses (pH > 11.5), which aids in clay settling. However, high lime doses (1600, 3500, and 4000 ppm; pH > 10) had a negative impact on microbial cell numbers in the underlying FFT, resulting in minimal reduction of organics (PHC) at high lime doses compared with moderate reduction

at a low lime dose (650 ppm). Assessing the toxicity of the FFT pore water after lime treatment demonstrated high toxicity effects to *V. fischeri* because of the increased pH. Nevertheless, the toxicity of the cap water was not affected by the high toxicity of the FFT, except under the covered conditions. This highlights a considerable influence of atmospheric CO_2 on regenerating the carbonate buffer and reducing pH of the cap water over time. As such any pH transport from the FFT will rapidly be neutralized due to the associated impact of dissolution of atmospheric CO_2 into the cap water. Overall, the performance metrics and indicative parameters (e.g. increase the WR% from the FFT, increase the possibility of the cation exchange of Ca^{2+} with the clay particles, and decrease the alkalinity of the cap water) revealed the role of lime addition towards the reclamations benefits within EPLs. Future work is still required to build upon the current findings in order to further optimize the lime treatment and the occurrence of cation exchange. The movement of COPCs into the cap water should be further investigated with a focus on chemical characterization of the FFT organic fractions and their associated potential risks toward the aquatic life in the EPLs. This better understanding will infer how to proceed to larger-scale and successful applications of lime treatment in EPLs.

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4 ENZYME-ASSISTED DEWATERING OF OIL SANDS TAILINGS: SIGNIFICANCE OF WATER CHEMISTRY AND BIOLOGICAL ACTIVITY[3]

4.1 Introduction

The oil sands reserves in Alberta are the third largest reserves of crude oil worldwide. Surface-mined oil sands ore in north-eastern Alberta produces almost two-thirds of the total bitumen production in Alberta (Kaminsky et al. 2009). Hot-water bitumen extraction currently demands large volumes of water, ranging from 2 to 2.5 m^3 per m^3 of crude oil produced (Zhu et al. 2017; Zubot et al. 2012). The mining companies reuse the water to a certain extent based on water quality; however, a large amount of slurry (known as tailings) is continuously generated from the process and stored in engineered tailings ponds (Government of Alberta 2015; Congressional Research Service 2008). These tailings and process water cannot be directly discharged into the environment due to a zero-discharge practice following environmental legislation (ERCB 2012; McQueen et al. 2017). Until 2017, the total area occupied by oil sands tailings ponds was approximately 176 km^2 (with ≃ 1.25 billion m^3 of accumulated tailings (AER 2019; Foght et al. 2017; Miles et al. 2020; Vajihinejad et al. 2017). Oil sands tailings typically contain 20–

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30 wt% solids (i.e., sand, silt, clays, dissolved salts, organics, and minerals) and 1–3 wt% residual bitumen. In the tailings ponds, the coarse sands can quickly settle when deposited, while fine solids and residual bitumen remain suspended. Mackinnon and Zubot (2013) suggested that the tailings will settle into three layers, with the course sands at the bottom, tailings in the middle (known as fluid fine tailings [FFT] or mature fine tailings [MFT]), and water on top. Within a few weeks, the fine tailings can settle up to 20 wt% solids; however, several years are required to reach 30–35 wt% solids (Allen 2008b). In order to achieve regulatory guidelines, oil sands operators need advanced reclamation strategies to manage such massive volumes of tailings (Allen 2008a; Allen 2008b; Clemente and Fedorak 2005; Siddique et al. 2014).

A number of engineering strategies, including biological, chemical, physical, and/or their combinations, have been examined during the last decades to achieve sustainable reclamation, emphasizing dewatering of tailings. Although these methods developed the tailings stream and enhanced the solids content to 50–60% (Devenny 2010), subsequent dewatering of tailings is still required for eventual reclamation and safe deposition as a soil (Owolagba and Azam 2015). The dewatering processes based only on chemical methods could treat much larger volumes with less energy; however, some chemical methods could introduce toxic and/or recalcitrant species into the water that are difficult to remove. For example, despite the NA reduction after ozonation treatment of OSPW reported by Anderson et al. (2011), there was increasing toxicity due to the formation of toxic by-products post ozonation. Additionally, traditional methods can be applied to dewater the

FFT, including consolidated tailings (CT), centrifugation, thin lift dewatering, filtering, rim ditching, and the freeze and thaw method, but the cost of these methods is high (Kasperski and Mikula 2011; Masliyah et al. 2011). Therefore, several studies have addressed the combined effect of natural processes (e.g., evaporation) with chemical process (e.g., coagulation and flocculation) to increase the FFT strength (Yuan and Shaw 2007). Combinations of mechanical and chemical dewatering techniques, such as flocculation using polymers followed by filtration or centrifugation, have also been investigated. However, such energy-intensive solutions are beneficial only if the recovered sediments have little water (Gumfekar et al. 2017).

In comparison with physical and chemical treatment, biological treatment is considered more cost-effective and associated with better water quality (Yu et al. 2018a). However, limited research studies have investigated biological treatment for accelerating the dewatering of tailings. Siddique et al. (2014) investigated the effectiveness of hydrolyzed canola meal as an organic substrate to improve microbial activity and improve the dewatering of tailings. They reported an 8% increase in the water recovery compared with the untreated condition. Recently, Young et al. (2020) reported an increase in solids content from 23.2 wt% to 39.4 wt% for FFT amended with hydrolyzed canola meal and acclimatized in an anaerobic environment. Yu et al. (2018a) achieved 8% water recovery from FFT using Ultrazyme, a proprietary digestive enzyme. These recent studies primarily observed cracks in the tailings during the dewatering process. These cracks could be attributed to biogenic gas, facilitating pathways for pore water release. Previous

studies reported the presence of methanogens in oil sands tailings ponds that can slowly biodegrade BTEX and residual hydrocarbons to biomethane (Penner and Foght 2010; Siddique et al. 2011). Consistent with the report by Yu et al. (2018a) (i.e., biogenic gas production due to enzymatic treatment of tailings), several studies previously reported enhanced methane production due to improved hydrolysis via enzymes amendment in conventional anaerobic bioreactor studies (Ehimen et al. 2013; Kim et al. 2019; Odnell et al. 2016; Parawira 2012; Passos et al. 2016).

In conventional wastewater treatment, many studies have examined the feasibility of enzymatic treatment to improve the dewaterability of wastewater sludge. Parmar et al. (2001) investigated the influence of composite hydrolysis enzymes (cellulase, protease, and lipase) to enhance the dewatering of waste activated sludge. Ayol and Dentel (2005) investigated the use of a commercial product called Enviro-Zyme 216 which contains protease, lipidase, anaerobic bacteria, *Aspergillus oryzae,* and a mixture of enzyme complex and polymer to increase the dewaterability of anaerobically digested biosolid samples. Their study demonstrated enhanced final solid contents, filtrate turbidity, and suspended solids based on the capillary suction test. Moreover, a study by Dursun et al. (2006) using enzymatic treatment showed a 27% increase in the cake solids content in simulated belt filter press dewatering anaerobically digested sludge at laboratory scale. In addition to sewage sludge, enzymatic treatment (cellulase, protease, and lysozyme) was also effective for treating pulp and paper mill sludge (Bonilla et al. 2015). Notably, lysozyme could increase the solids content in pulp and paper sludge from

5.6% to 8.9% (Bonilla et al. 2015). Despite these positive reports on the effectiveness of enzymes on dewatering, enzymatic treatment has not yet been thoroughly explored for accelerating the dewatering of oil sands tailings. Based on an extensive literature search, only one study (2018a) could be found examining the feasibility of a commercial enzyme (Ultrazyme) as an amendment for the dewatering of oil sands tailings. However, detailed insights on the effects of enzymes on water chemistry and biogenic activity are yet to be revealed to understand the subsequent impact on oil sands tailings dewatering.

Thus, this study investigated three different enzymes (cellulase, protease, and lysozyme) to aid in the reclamation of oil sands tailings and identify the significance of water chemistry and biogenic activity in dewatering. The detailed objectives of this study are a) to examine the technical feasibility of enzymatic treatment to enhance FFT dewatering; b) to investigate the corresponding mechanism for the dewatering; c) to study the influence of enzymatic treatment on the reduction or removal of organic fractions such as naphthenic acid fractions (NAFCs); and d) to determine shifts in microbial community diversity and toxicity toward *Vibrio fischeri*.

4.2 Materials and methods

4.2.1 Materials

FFT was collected from the oil sands region in northern Alberta and stored in sealed buckets at 4 °C until use. Three different enzymes were used in this study: cellulase from *Trichoderma reesei* (>700 U/g; Sigma-Aldrich, Canada);

Biomedicals™, a protease from *Bacillus* sp. (>16 U/g; Sigma-Aldrich, Canada); and MP Biomedicals™, a lysozyme from chicken egg white (20,000 U/mg; Fisher Scientific, Canada). Based on previous reports, these enzymes could effectively dewater sewage sludge and pulp and paper mill sludge (Bonilla et al. 2015; Lin et al. 2019; Liu et al. 2018); however, never been examined for oil sands tailings based on the authors' knowledge. Sterilized FFT was used as an abiotic control. Raw FFT was autoclaved for 1 h each day for 8 consecutive days to ensure the control was completely abiotic (i.e., no biological activity).

4.2.2 Experiment

Two dosages of each enzyme (0.5% and 1%) were used. Lysozyme slurry was prepared by adding lysozyme powder to Millipore Ultrapure water (18.2 MΩ cm) to achieve a 0.5% or 1% solids slurry (wt./wt.). Cellulase and protease were purchased in liquid form and were added directly to FFT. Each enzyme dose was added to 800 g of FFT in a glass beaker and mixed with a mechanical agitator at moderate speed (1200 rpm). The initial solids content of the raw FFT (30%) was measured. Experiments were conducted in 1 L glass columns, with 800 mL FFT placed in the column and covered with parafilm and aluminum foil to avoid evaporation of the FFT pore water. All experiments were conducted in triplicate at room temperature. Sterilized FFT was used as an abiotic control; FFT was autoclaved for 1 h per day for 8 successive days. In the abiotic control samples, only the 0.5% dose was examined for each enzyme. The same procedures were repeated for the abiotic control. The experiment period was 12 weeks for all the treatment conditions; this is when the volume of released FFT porewater began to

plateau for all the biotic conditions. The experiment was conducted in the dark by covering the columns with a black colored tarp to prevent light penetration. The tarp was temporarily removed during monitoring mudlines and sampling.

To test the effect of enzymes on microbial activities in the tailings, a parallel experiment was conducted with microcosms (serum bottles with 158 mL total volume and 100 mL working volume) to monitor biogenic methane gas (CH_4) production for each test condition. Each microcosm consisted of 100 mL FFT (treated or untreated FFT); bottles were sealed, crimped, and purged with 80% N_2/20% CO_2 to maintain methanogenic conditions.

4.2.3 Analysis and calculations

4.2.3.1 Water recovery and solids content

The changes in the FFT mudline were recorded over time for all columns. The mudline represents the interface between the FFT and FFT pore water. Water recovery % (WR) has been used to determine the amount of water recovered from FFT with time (Allam et al. 2021; Siddique et al. 2014; Yu et al. 2018a) after enzyme treatment. More details on the WR calculations can be found elsewhere (Allam et al. 2021; Yu et al. 2018a). For day 0 samples, the FFT samples were centrifuged at 8,000 *g* for 40 min, and the released porewater was collected and filtered using polytetrafluoroethylene (PFTE) filters (Fisher Scientific; 0.45 µm), then stored at 4 °C before further analyses. Chemical and physical parameters were measured, including pH, soluble cations and anions, zeta potential, and toxicity for

FFT porewater. The headspace composition of the serum bottles was analyzed to determine the methane production for each condition.

4.2.3.2 Chemical analysis

A Thermo iCAP 6000 series inductively coupled plasma–optical emission spectrometer (ICP-OES, ThermoFisher) was used to analyze FFT pore water for cations including Ca^{2+}, Na^{+}, K^{+}, and Mg^{2+}. Dionex ion chromatography (ICS-2100, ThermoFisher) using the AS18 32mM ISO method for Anions was used to analyze the anion concentrations in the FFT pore water (Cl^{-}, NO_2^{-}, SO_4^{2-}, Br^{-}, NO_3^{-}, PO_4^{3-}). Alkalinity and HCO_3^{-} (measured as $CaCO_3$ mg L^{-1}) were measured using a Mettler Toledo DL53 (Mississauga, Canada) with 0.02 N H_2SO_4 as a titrant. An Accumet® Research AR50 (Fisher Scientific, Lenexa, Kansas) was used to measure pH values. Prior to any analysis and unless otherwise specified, liquid samples were filtered with PFTE filters (Fisher Scientific; 0.22 μm for cation analysis; all water samples were diluted 10 × using Ultrapure water).

NAFCs comprise a broad class of organic compounds including O_2 compounds, nitrogen-containing species (NO_n and N_2O_n), and sulfur-containing species (O_nS and O_nS_2). O_2-NAFCs compounds were measured using Orbitrap high-resolution mass spectrometry and high-performance liquid chromatography at the University of Saskatchewan Toxicology Centre; the detailed protocols can be found elsewhere (Headley et al. 2011). Briefly, using the liquid-liquid extraction method as described by Ross et al. (2012) and Pereira et al. (2013a), the acidified sample (reduced to pH < 2 or 1 with sulfuric acid or formic acid) was extracted

twice into 200 mL of dichloromethane (DCM). The combined 400 mL of DCM extract was evaporated to 5 mL via rotary evaporation, reduced to dryness with nitrogen gas, then the dry extract was dissolved in 1 mL methanol for chemical analysis via Orbitrap mass spectrometry. The sample could also be reconstituted in 500 µL of 50:50 ACN/H_2O with 0.1% NH_4OH. Further details are described by Morandi et al. (2015). The methane content was measured with an Agilent 7890A gas chromatograph equipped with a flame ionization detector (GC-FID) and Agilent Technologies HP-5MS column (30 m × 0.25 mm × 0.25 µm). A typical injection volume was 100 µL.

4.2.3.3 Ionic strength (I) and Diffuse Double Layer (DDL)

The following formula was used to calculate the ionic strength of the FFT porewater (adapted from Essington, 2004):

$$I = 1/2\ \Sigma_i\ (M_i\ Z^2_i\ )$$

where M_i: the molar concentration of charged species (i); Z_i: charge in the porewater.

The following formula was used to calculate the thickness of the DDL (adapted from Essington, 2004):

$$k^{-1} = (3.042 \times 10^{-8}) / Z\ I^{0.5}$$

where k^{-1}: the thickness of the DDL (cm); Z: the average mean charge of the counterions (exchangeable cations); and *I*: the ionic strength of the porewater.

4.2.3.4 Zeta potential

The zeta potential was measured using a Zetasizer Nano ZSP (Malvern Instruments, Malvern, UK). Approximately 1 mL of the FFT pore water sample obtained from each column was injected into a folded capillary zeta cell (DTS 1070).

4.2.3.5 Toxicity Bioassay

The Microtox® bioassay test (Osprey Scientific, Edmonton, Alberta, Canada) was used to measure the toxicity of the FFT porewater samples to *V. fischeri*. The 81.9% Basic Test protocol was applied with 5 min incubation with the Microtox® 500 Analyzer (Azur Environmental time) (Anderson et al. 2011). More details are published elsewhere (Allam et al. 2021; Siddique et al. 2014; Yu et al. 2018a). Previous studies reported 1.0 toxicity unit (TU) or lower as detoxified (Scott et al. 2008; Yu et al. 2018a).

4.2.3.6 Microbial community and diversity

The FastDNA™ SPIN Kit for Soil (MP Biomedicals) was used to extract DNA from the untreated and enzyme-treated FFT samples (FFT sample weight ≃ 500 mg); more information can be found elsewhere (Allam et al. 2021). DNA extraction was conducted at the start and end of the experiment for the untreated/control sample: however, for the enzyme-treated samples only the final sample was analyzed (week 12). To determine the total microbial community, universal bacterial primers 341F: 5′ CCTACGGGNGGCWGCAG 3′ and 805R: 5′ GACTACHVGGGTATCTAATCC 3′ were used to target the 16S rRNA gene

(Zakaria and Dhar 2021a). To determine the methanogen gene copies after enzyme treatment, both archaeal-specific primers [517F: 5′ GCYTAAAGSRNCCGTAGC 3′ and 909R: 5′ TTTCAGYCTTGCGRCCGTAC 3′] and methyl coenzyme-M reductase gene (*mcrA*) primers (*mcrAf*: 5′ GGTGGTGTMGGATTCACACARTAYGCWACAGC 3′ and *mcrAr*: 5′ TTCATTGCRTAGTTWGGRTAGTT 3′) were used (Lin et al. 2020; Zakaria and Dhar 2021a). The quantitative polymerase chain reaction (qPCR) assay was performed on extracted DNA samples (1 µL) using a Bio-Rad CFX96 optical reaction module conversion for the C1000 Touch thermal cycler. Bio-Rad CFX Manager™ 3.0 software was used to analyze the amplification data. All standards and samples were analyzed in triplicate. Detailed protocols for preparing the qPCR mixtures can be found elsewhere (Lin et al. 2020).

To determine the microbial community diversity, 16S rRNA gene sequencing was performed by Microbiome Insights (Vancouver, BC, Canada) on the DNA samples. PCR amplification of the V4 region of the 16S rRNA gene in bacteria and archaea using the primers 515F (5′ GTGCCAGCMGCCGCGGTAA 3′) and 806R (5′ GGACTACHVGGGTWTCTAAT 3′) was performed. The Illumina PE250 platform (Illumina, CA, USA) was used for amplicon sequencing. More information can be found elsewhere (Cossey et al. 2021).

4.3 Results and discussion

4.3.1 Effects of enzymes on FFT dewatering

The WR and the volume of the released water from the FFT were used to assess the effectiveness of enzyme treatment for FFT dewatering (Fig. 4.1a and 4.1b). All three enzymes accelerated the amount of the released water after 12 weeks compared with the untreated control (WR: 12% and 11.5% for 0.5% and 1% cellulase; 8.5% and 6% for 0.5% and 1% protease; 19.8% and 20% for 0.5% and 1% lysozyme; control was 2%); (volume of pore water: 97 and 93 mL for cellulase 0.5% and 1%; 67 and 49 mL for protease 0.5% and 1%; 158 and 159.5 mL for lysozyme 0.5% and 1%; control was 15 mL). Both doses of lysozyme (0.5% and 1%) achieved the highest WR (~ 20%) and highest volume of released pore water (~ 159 mL) after 12 weeks. Despite the rapid initial improvement (i.e., first 2 weeks) for cellulase 0.5% and 1% (which achieved 12.5% and 12% WR, and 95 mL and 90 mL of released water), cellulase was relatively less effective than lysozyme at the end of the experiment. Among the three enzymes, the least dewatering was observed with protease 1% (6% WR and 49 mL of released water after 12 weeks).

As mentioned earlier, enzymes have rarely been investigated for oil sands tailings dewatering. Yu et al. (2018a) recently reported the effectiveness of a commercial enzyme (Ultrazyme) for enhanced dewatering of oil sands tailings. Nevertheless, our findings are consistent with studies by Bonilla et al. (2015) that reported enhanced dewaterability of pulp and paper mill sludge with lysozyme treatment compared with cellulase and protease. Their study suggested that

lysozyme could act as a cationic polymer rather than a bio-stimulant. Based on another report by Liu et al. (2018), lysozyme could degrade bacterial cells and extracellular polymeric substances (EPS) matrix in sludge, leading to the release of trapped intracellular water. This mechanism is relevant to our study as EPS and microbial cells were shown to be present in oil sands tailings under methanogenic and nitrate-reducing conditions (Bordenave et al. 2010). Our findings agreed with Wu et al. (2014) who found a 0.5% dose of cellulase or protease is more effective for dewatering paper mill sludge than 1% dose.

Interestingly, Yu et al. (2018a) found that Ultrazyme could stimulate microbial activity in tailings followed by biogenic gas production. The produced biogenic gases (e.g., CH_4, CO_2) provided trapped pore water release pathways, which were apparent from the visible cracks developed within the tailings. In our study, many cracks were also observed in the enzyme-treated tailings samples (see Figure 4.2). Therefore, further experiments were conducted with autoclaved tailings to acquire more insights into the underlying mechanisms. The biotic experiments provided no significant difference between the performance of 0.5% and 1% enzyme dosages, with only a slight increase in the performance of the 0.5% treatment compared with the 1% treatment in terms of WR and volume of pore water released. Therefore, for the abiotic control, only the 0.5% dose for each enzyme was examined. As shown in Figure 4.1c, all autoclaved abiotic samples showed considerably lower dewaterability (≤5% WR) than the biotic samples (see Figure 4.1a and 4.1b), suggesting the significance of microbial activity in FFT dewatering. Cellulase and protease treatment of the sterile samples resulted in

slightly higher WR than the lysozyme treated and control samples. Thus, these enzymes were still capable of slightly improving the dewatering by a physicochemical mechanism, but the results suggest that the enzymes tested here acted more as bio-stimulants and improved the dewaterability of tailings via enhanced microbial activity (i.e., biogenic methane production). Notably, a few studies previously reported that enzymes (e.g., lysozyme) could increase methane production in conventional anaerobic digestion (Bonilla 2017; Kim et al. 2019; Odnell et al. 2016). Therefore, further assessment of methanogenic activity in microcosms with enzyme-treated tailings was performed.

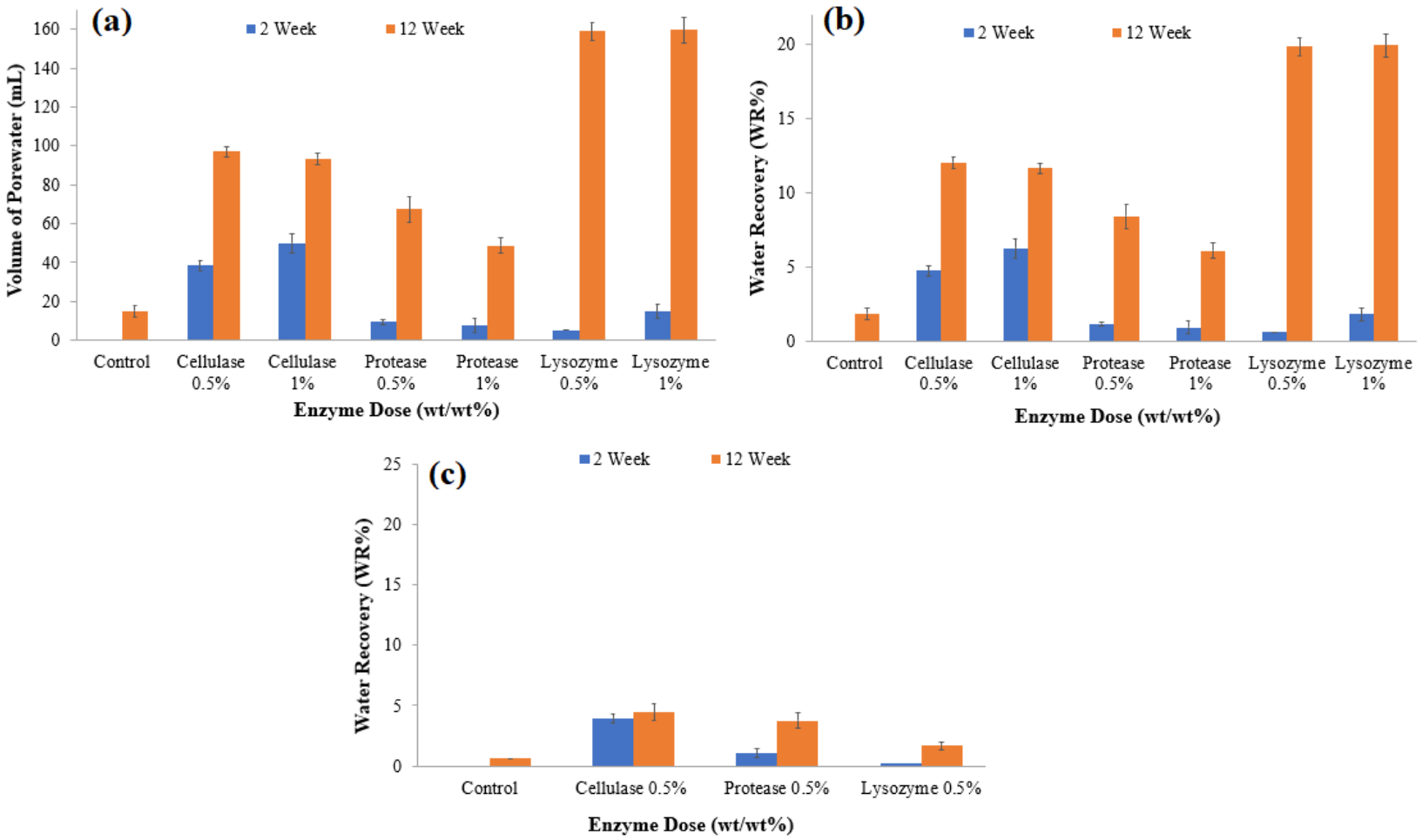

Figure 4.1. (a) Volume of FFT porewater released (mL); (b) The FFT pore water recovery % (WR) after 2 weeks and at the end of the experiment (12 weeks) for the biotic condition; and (c) The FFT pore water recovery % (WR) after 2 weeks and at the end of the experiment (12 weeks) for the abiotic condition. All columns consisted of 800 mL enzyme-treated or untreated FFT. All columns were sealed with parafilm and aluminum foil. The untreated FFT represents a 0 ppm treatment and acts as the control experiment. Results illustrated are an average (n = 3 for all treatment conditions) ± one standard deviation.

Figure 4.2. Different cracks appeared in the FFT columns treated with cellulase, protease, and lysozyme throughout the experiment compared to the control.

4.3.2 Effects on biogenic methane production

Figure 4.3 shows the effects of enzymes on methane production from FFT. The addition of enzymes (except for protease 1%) resulted in an increase in methane production from FFT. The addition of cellulase 0.5% and 1% led to higher methane production after 12 weeks (1.6–2.4 mmol) than the other two enzymes (i.e., lysozyme 1.2–1.5 mmol, and protease 1.8–0.06 mmol for each dose 0.5% and 1% respectively) and control (0.002 mmol). In contrast, methane production from lysozyme and protease-treated tailings had relatively lower methane production than cellulase. Although lysozyme exhibited the highest effectiveness in dewatering, methane production from lysozyme-treated tailings with either 0.5% and 1% doses

was slightly lower than for either dose of cellulase, (1.2–1.5 mmol vs. 1.6–2.4 mmol). Both doses of lysozyme resulted in similar methane production and similar dewatering. In contrast, different doses of cellulase and protease resulted in different methane production. Notably, 0.5% cellulase and protease treatments had considerably higher methane production than the 1% treatments, indicating the negative impact of higher enzyme doses on methanogenic activity. Also, the methane production results are similar to the WR, which slightly decreased after increasing the enzyme dose for both cellulase and protease. Nonetheless, it was apparent that methanogenesis might have occurred during the column dewatering tests. Previous reports also suggested the formation of anaerobic conditions in the settled tailings at the bottom of the columns during the dewatering tests (Yu et al. 2018a). Our finding agrees with the literature, showing enzymatic treatment with cellulase, protease, or lysozyme increases methanogenesis (Ehimen et al. 2013; Kim et al. 2019; Odnell et al. 2016; Parawira 2012; Passos et al. 2016). These studies suggested that enhanced methane production in anaerobic bioreactors could be attributed to improved hydrolysis due to enzymatic activity. Particularly, the EPS matrix in sludge flocs could be disintegrated by enzymes (Liu et al. 2018; Parawira 2012). A previous study also reported the presence of EPS in oil sands tailings, particularly under methanogenic and nitrate-reducing environments (Bordenave et al. 2010). As discussed later, enzymes could also increase the richness and diversity of bacterial communities, which might improve the degradation of complex organics in tailings to simple substrates that methanogens could utilize.

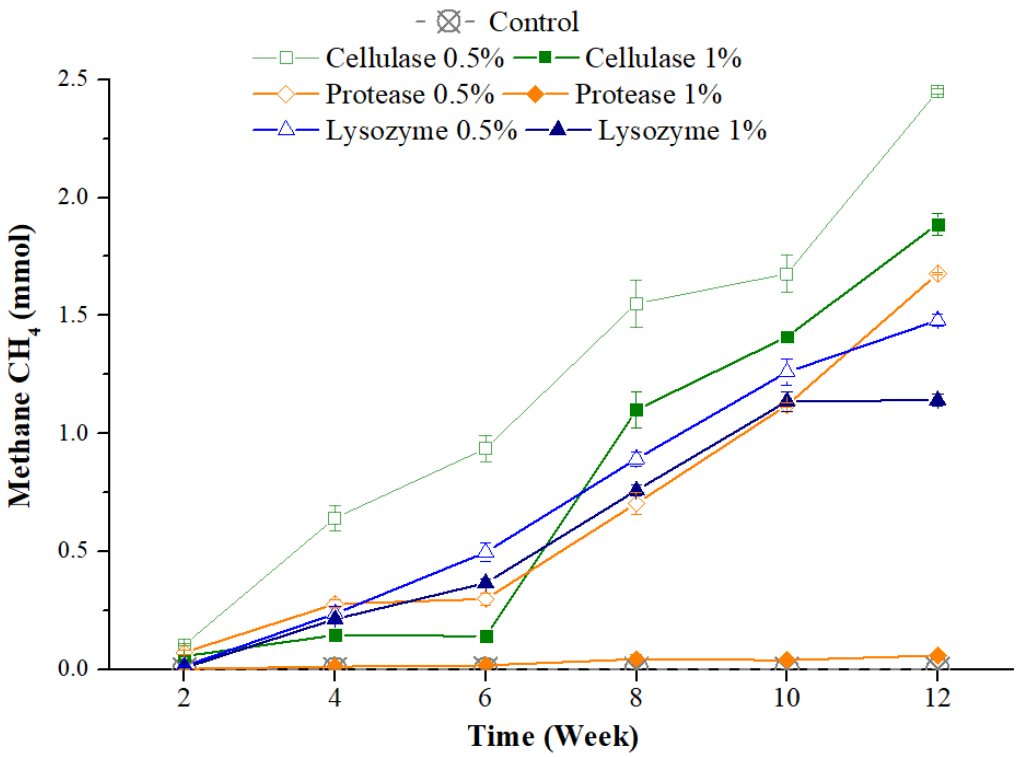

Figure 4.3. Temporal changes in methane production throughout the experiment for all enzyme-treated FFT compared with the control/untreated FFT. All bottles consisted of 75 mL enzyme-treated or untreated FFT and 25 mL anaerobic media. The untreated FFT represents a 0 ppm treatment and acts as the control experiment. Results illustrated are an average (n = 3 for all treatment conditions) ± one standard deviation.

4.3.3 Variations in water chemistry

4.3.3.1 Cations and anions

Figures. 4.4a, b, and c illustrate the changes in HCO_3^-, Mg^{2+}, and Ca^{2+} concentrations, respectively, in the FFT pore water for the control and enzyme-treated conditions after 4, 6, 9, and 12 weeks. In the control, the concentrations of the three ions were stable throughout the experiment. For enzyme-treated samples, the concentrations of ions increased until week 4. However, after week 4, the

changes in ions concentrations showed different trends for the different enzymes. For the FFT treated with cellulase 1%, after 4 weeks the Ca^{2+} concentration started to decrease until the end of the experiment. Similarly, the Mg^{2+} concentration reached a plateau from 4–9 weeks and then started to decrease up to week 12. This reduction in Ca^{2+} and Mg^{2+} concentrations could be related to the increase in the pH after 9 weeks to pH 7.6 at week 12. At higher pH, Ca^{2+} and Mg^{2+} can form calcium and magnesium bicarbonate (Siddique et al. 2014). However, the HCO_3^- concentration reached a plateau after 4 weeks. Such observations could be attributed to the reduction of bicarbonate to methane by methanogens, as suggested previously in the literature (Cervini-Silva et al. 2020; Yang et al. 2016). In the lysozyme-treated FFT, the concentrations of these ions increased up to week 12 (p-value < 0.05; significant difference).

For most of the enzyme-treated samples, the pH profiles showed declining trends. The methanogenic activity in the FFT might cause a pH reduction below 7.5 in FFT porewater after 4 weeks for the cellulase (0.5 and 1%) and protease (0.5%) treated samples (Figure 4.4d); CO_2 dissolve in FFT porewater and cause pH reduction (Samadi 2019; Siddique et al. 2014; Yu et al. 2018a). The slight increase in the pH after 4 weeks for the cellulase treatment comply with the reduction in Ca^{2+} concentrations in the porewater after 4 weeks. With lysozyme treatment, the pH took longer to reduce below 7.5 (6 weeks); this explains the sharp increase in HCO_3^-, Ca^{2+}, and Mg^{2+} concentrations in the porewater. As discussed earlier, the enzymatic treatment also enhanced methanogenic activity in the tailings in this study. During the ebullition of biogenic gases, the release of CO_2 caused a drop in

the FFT pH below 7.5 and the dissolution of carbonate minerals occurred which increase HCO_3^- and cation (Ca^{2+} and Mg^{2+}) concentrations in the FFT pore water (Siddique et al. 2014). The dissolution of carbonate minerals had a direct impact in the increase of ionic strength (will be discussed in the next section). Thus, methanogenic activities from the enzyme-treated samples agreed with the changes in pH and ions concentrations.

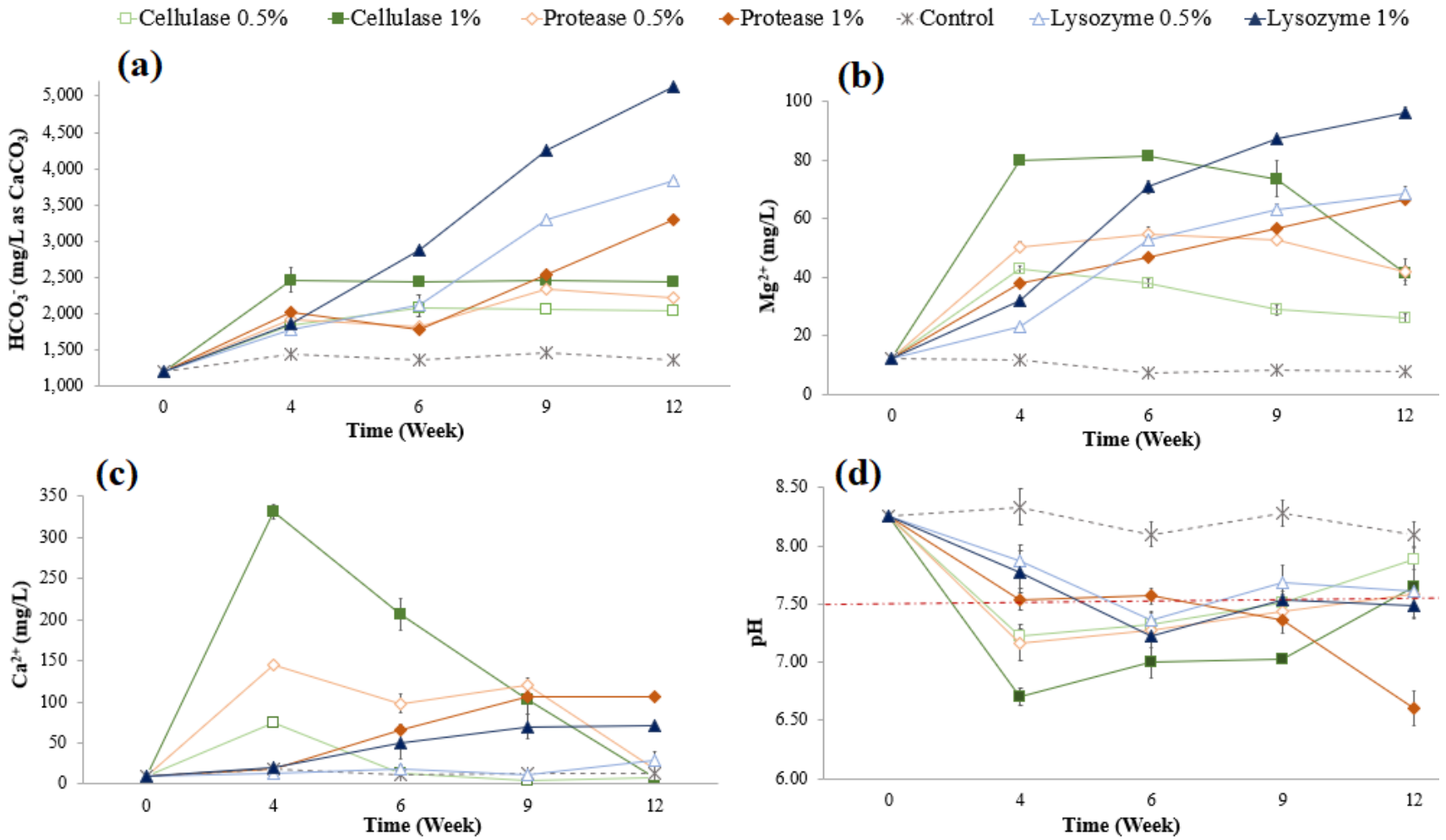

Figure 4.4. Change in (a) HCO_3^- , (b) Mg^{2+}, (c) Ca^{2+}, concentrations and (d) pH throughout the experiment for all enzyme-treated FFT compared with the control/ untreated FFT. All columns consisted of 800 mL enzyme-treated or untreated FFT. All columns were sealed with parafilm and aluminum foil. The untreated FFT represents a 0 ppm treatment and acts as the control experiment. Results illustrated are an average (n = 3 for all treatment conditions) ± one standard deviation.

4.3.3.2 Ionic strength, diffuse double layer, and zeta potential

Figure 4.5a shows the changes in ionic strength (*I*) of porewater and the diffuse double layer (DDL) of FFT over the 12 week dewatering test. Due to the increase in cation (Ca^{2+} and Mg^{2+}) and anion (HCO_3^-) concentrations throughout the experiment (Figure 4.4) in the enzyme treatment columns, an increase in the *I* values of the pore water and a decrease in the DDL were observed. In contrast, the control sample showed minimal changes. The highest *I* value was reported for the lysozyme 1% treatment (I = 0.1 mol L^{-1}) compared with the control (I = 0.038 mol L^{-1}); p-value < 0.05, (significant difference). Siddique et al. (2014) also previously reported an increase in *I* values due to the dissolution of carbonate minerals.

When comparing different doses of each enzyme, increasing the enzyme dose mostly increased the *I* value and DDL (Figure 4.5a). For instance, increasing the cellulase dose from 0.5% to 1% increased the *I* values (0.055 vs. 0.065 mol L^{-1}). Nevertheless, increasing the enzyme dose to 1% had an adverse impact on methane production and hence on WR from FFT (Figure 4.1). Protease treatment achieved the lowest *I* (0.055 and 0.045 mol L^{-1}) for the 0.5% and 1% doses, respectively. Additionally, the lysozyme 1% treatment resulted in the lowest DDL of clay particles (9.40×10^{-8} cm), while the DDL was as high as 1.54×10^{-7} cm for the control. These results agree with the *I* results, where *I* has a negative effect on the DDL: increasing the *I* strength of the solution decreases the DDL thickness of the clay particles (Siddique et al. 2014). Similarly, the protease 1% treatment had a higher DDL (1.39×10^{-7} cm) than the other two enzymes, but it was still lower than the control.

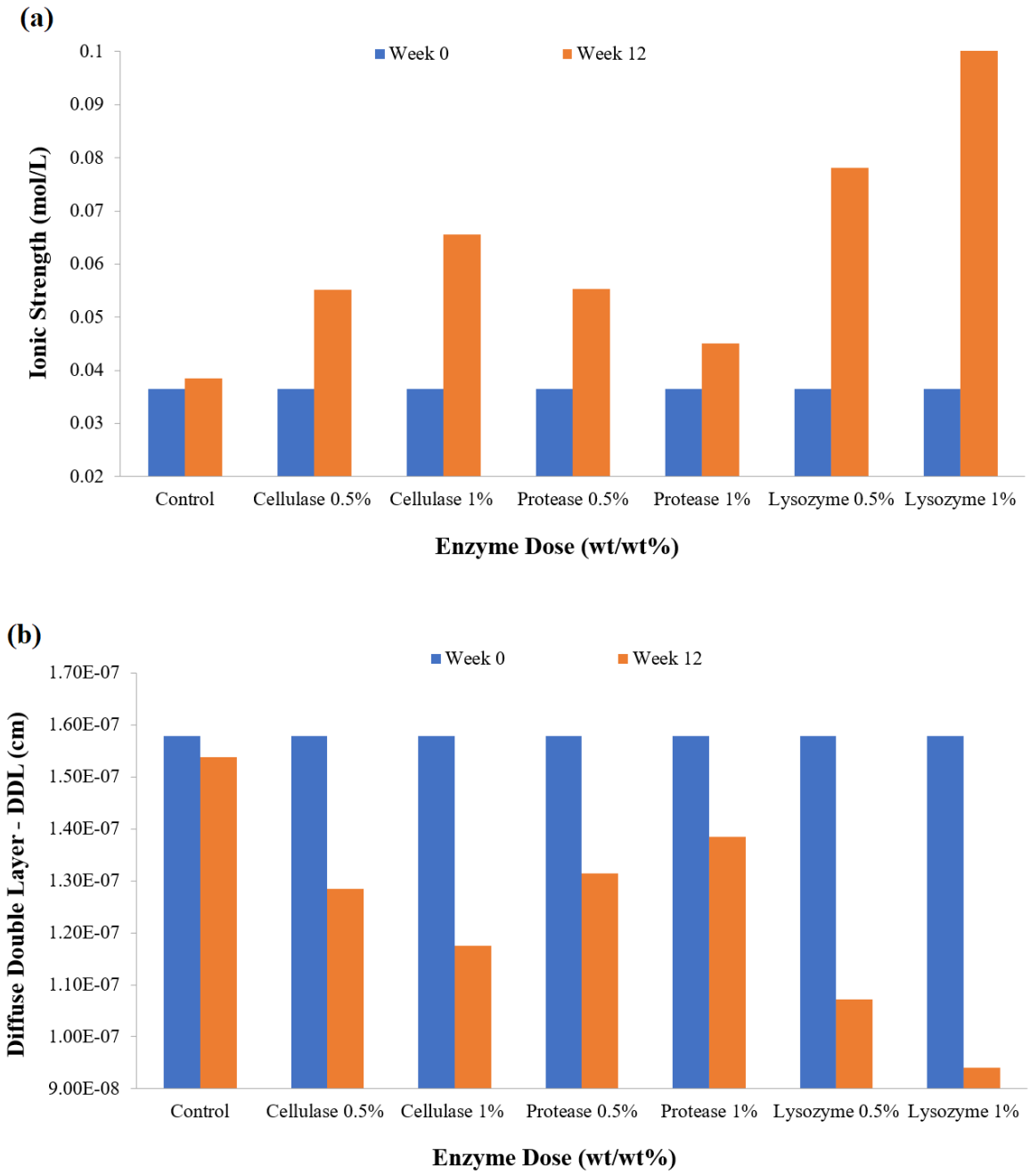

Figure 4.5. Changes of the a) ionic strength concentrations and b) the thickness of the diffuse double layer for all enzyme-treated FFT compared with the control/ untreated FFT at the beginning of the experiment (0 weeks) and at the end of the experiment (12 weeks). All columns consisted of 800 mL enzyme-treated or untreated FFT. All columns were sealed with parafilm and aluminum foil. The untreated FFT represents a 0 ppm treatment and acts as the control experiment. Results illustrated are an average (n = 3 for all treatment conditions) ± one standard deviation.

The enzymatic treatment resulted in lower DDL thickness and shifted zeta potential to less negative values at the end of the experiment (Figure 4.6). Lysozyme treatment significantly increased the zeta potential (−34.7 to −14.8 mV). However, there was a minor difference between the initial and final zeta potential values for the control (Figure 4.6). These findings agree with a previous study that suggested an increase in the Zeta potential of paper mill sludge from −44.2 mV to −26.1 mV due to lysozyme addition (Bonilla 2017). The changes in Zeta potential can be related to charge neutralization, facilitating effective flocculation. For instance, a shift of zeta potential to less negative values might be because the lysozyme carries a positive charge at pH ~ 7 (Bonilla 2017), neutralizing the negative charge surrounding the clay particles in FFT. Thus, the key reason for the decrease in the DDL thickness is the charge neutralization between the clay surface and positively charged divalent cations such as Ca^{2+} and Mg^{2+}. Also, the electrostatic repulsion between the clay particles decreased at a high *I* value, followed by a decrease in DDL thickness. The reduction in the DDL thickness for enzyme-treated samples suggested that enzymes facilitated clay flocculation and improved tailings consolidation.

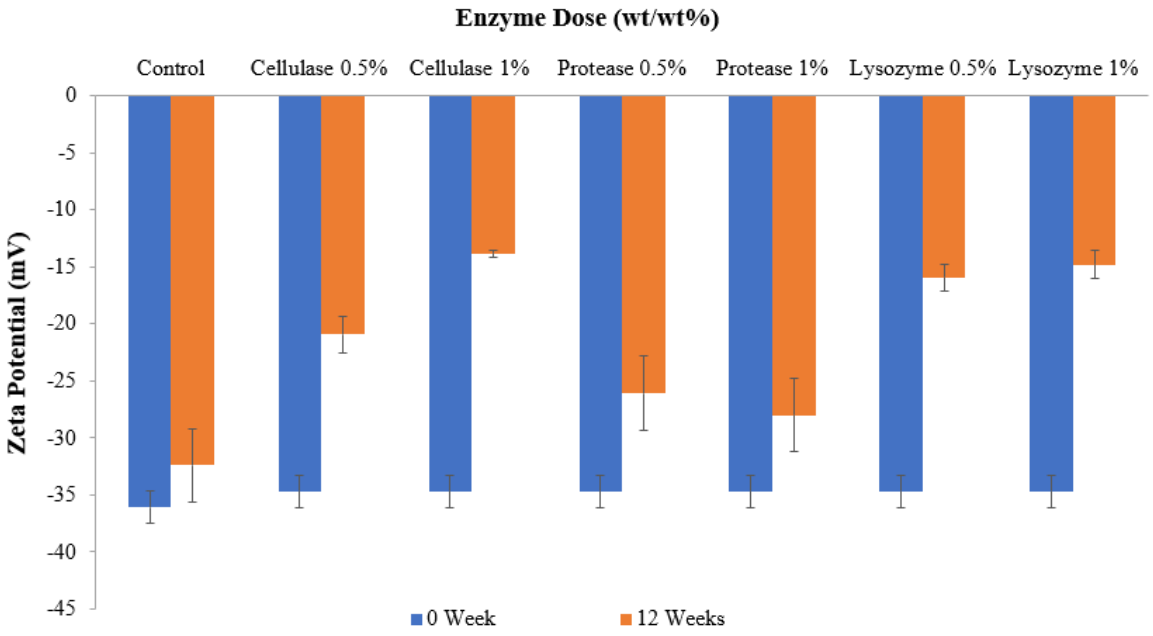

Figure 4.6 Variations of the zeta potential for all enzyme-treated FFT compared with the control/ untreated FFT at the beginning of the experiment (0 weeks) and at the end of the experiment (12 weeks). All columns consisted of 800 mL enzyme-treated or untreated FFT. All columns were sealed with parafilm and aluminum foil. The untreated FFT represents a 0 ppm treatment and acts as the control experiment. Results illustrated are an average (n = 3 for all treatment conditions) ± one standard deviation.

4.3.4 Naphthenic acid fractions

To determine the efficiency of enzymatic treatment of FFT for the degradation of naphthenic acids fractions (NAFCs), the concentration of the classical O_2 species in the released porewater were determined using Orbitrap–MS. NAFCs were measured in untreated columns at the beginning of the experiment, and in all treated and untreated columns at the end of experiment. In this study, we focused only on the classical O_2-NAFC because these are considered the main contributors to acute toxicity in OSPW (Balaberda and Ulrich 2021; Hughes et al. 2017; Meshref et al. 2017b) and FFT porewater. Figure 4.7a and 4.7b illustrate the concentrations of

total O_2-NAFC species in the control sample (untreated), indicating a slight decrease in the O_2-NAFC of approximately 5% within 90 d. This result agrees with the literature, which reports slow degradation of NAFCs by indigenous microbial communities (e.g., ~16% decrease in 5 years) (Allen 2008b; Schoof 2015; Wu et al. 2019). When comparing the two dosage levels (low versus high dose) for both cellulase and protease, the 1% dose resulted in higher O_2-NAFC residuals than lower doses (0.5%). For lysozyme, increasing the dose from 0.5% to 1% resulted in higher degradation of O_2-NAFC. Overall, the highest decreases in O_2-NAFC concentration were 344 and 243 ng mL^{-1} achieved by the lysozyme 0.5% and 1% treatments, respectively. The lowest reduction in the total O_2-NAFC species was observed with the protease 1% treatment after 12 weeks (1138 ng mL^{-1}).

One possible reason for the reduction in O_2-NAFC species in the lysozyme-treated FFT is the high amount of FFT WR after 12 weeks compared with the other two enzymes (Figure 4.1a). This might dilute the NAFC concentration in the FFT porewater compared with the control. Another reason could be the ability of lysozyme to degrade NAFCs; Guo and Xu (2011) reported previously that lysozyme could degrade organic molecules as well as lysing the bacterial cell wall. Additionally, the composition of NAFCs reported by Headley and McMartin, (2004) revealed that NAFCs have smaller amounts of acyclic aliphatic / paraffinic or fatty acids together with alkyl-substituted cycloaliphatic carboxylic acids. In the same sense, Peng et al. (2016) suggested that some NAFC structures are similar to fatty acids. Previous studies reported a reduction of volatile fatty acids after enzymatic treatment (Roman et al. 2006).

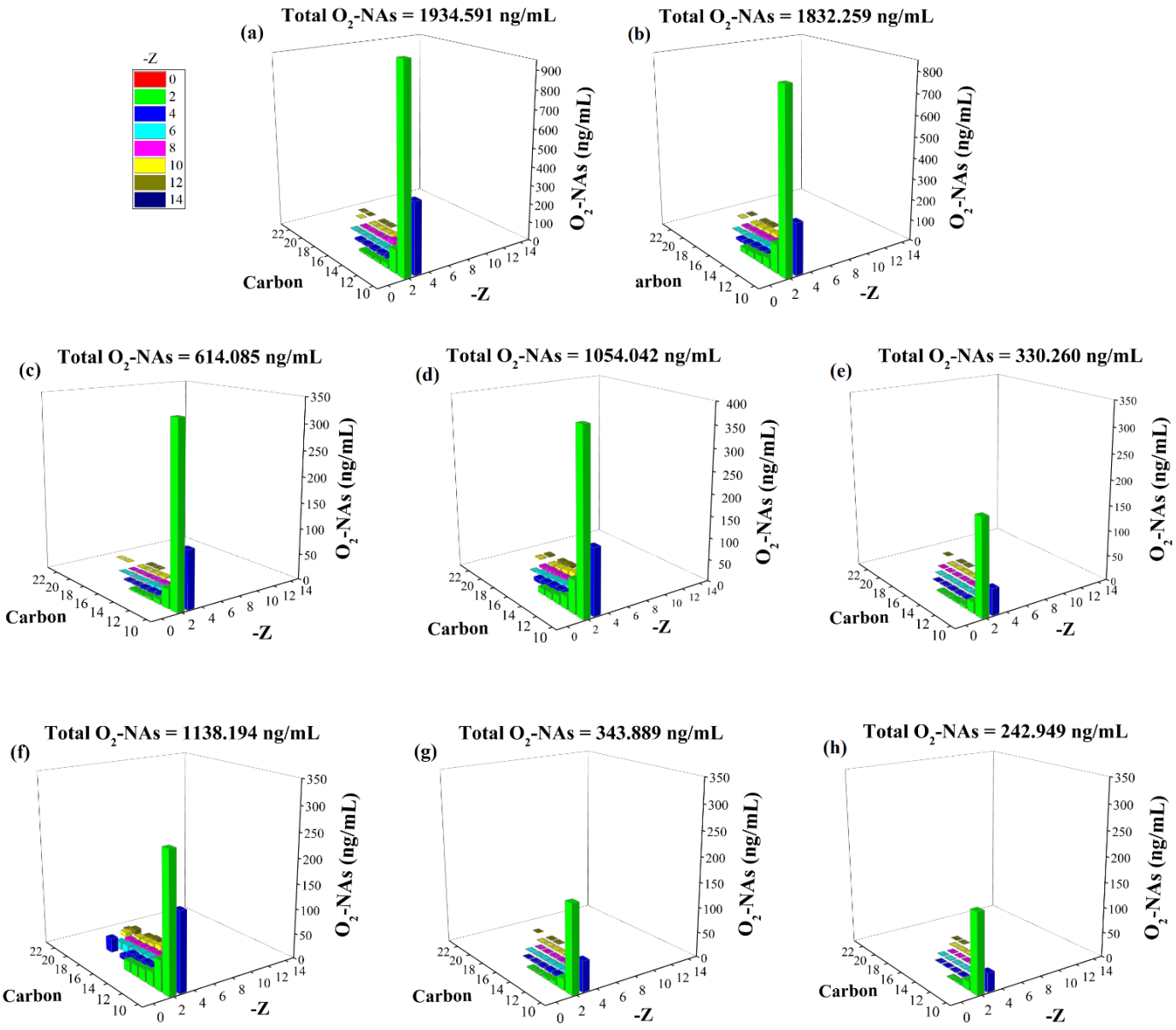

Figure 4.7. The concentrations of NAFCs for all enzyme-treated FFT compared with the control/untreated FFT. (a) Control at Day 0, (b) Control Day 90, (c) cellulase 0.5%, (d) cellulase 1%, (e) protease 0.5%, (f) protease 1%, (g) lysozyme 0.5% and (h) lysozyme 1%. All columns consisted of 800 mL enzyme-treated or untreated FFT. All columns were sealed with parafilm and aluminum foil. The untreated FFT represents a 0 ppm treatment and acts as the control experiment. Results illustrated are an average (n = 3 for all treatment conditions) ± one standard deviation.

4.3.5 Toxicity

Figure 4.8a summarizes the influence of enzyme treatments on the released FFT porewater toxicity to *V. fischeri* throughout the experiment at weeks 0, 4, 6, 9, and 12. Figure 4.8b summarizes the results from the abiotic control. Overall, cellulase 0.5% was the only treatment which resulted in a non-toxic effect for the released porewater after 12 weeks (0.25 TU) compared with other enzymes or the control (1.05 TU). However, treatment with lysozyme resulted in the highest toxic effect for the FFT porewater to *V. fischeri*: 27 TU and 32 TU for the 0.5% and 1% doses, respectively. For cellulase, increasing the dose to 1% increased the toxicity of the released water to 12 TU. For protease, although both doses resulted in porewater that was considered toxic (i.e., TU > 1), the 0.5% dose resulted in lower toxicity (2.5 TU) compared with the 1% dose (6.8 TU). For lysozyme, the toxicity increased sharply after 6 weeks, which could be ascribed to the release of organic fractions from the FFT. These findings are in agreement with Lin et al. (2019) who reported that lysozyme destroyed the microbial cell walls and released the substances inside the cells. In contrast, based on the O_2-NAFCs results, lysozyme-treated FFT had the lowest O_2-NAFCs after 12 weeks compared with the control and cellulase treatment. Many studies have reported that NAFC species, including classical and oxidized (Ox-NAFCs) species, are the main contributors to toxicity in OSPW (Grewer et al. 2010; Headley et al. 2009). In our study, only the classical NAFCs were measured. Other studies have reported that other organic compounds, such as alkylated phenols, are toxic compounds in OSPW (Hargesheimer et al. 1984). With cellulase 0.5% treatment, the toxicity reduction after 12 weeks might be attributed to the reduction in the lower molecular weight constituents compared with the control. This

observation is in agreement with Frank et al. (2008) who reported that the toxicity of the NAFCs toward *V. fischeri* correlated with the concentration of lower molecular weight constituents. However, for abiotic controls, all the enzyme treatments resulted in no toxicity to *V. fischeri* at the end of the experiment (Fig. 4.8b), indicating that the toxicity increase in the biotic columns is related to the distortion of microbial cell walls and release of the substances from inside the cells. Despite the fact that *V. fischeri* (Microtox assay®) is currently used as a standard method for measuring OSPW toxicity (Meshref et al. 2017a; Pourrezaei et al. 2014; Zubot et al. 2012), other organisms such as *Pseudomonas* sp., algae, invertebrates (e.g., *Daphnia magna)*, and fish can also be used (Barceló et al. 2020). The level of toxicity may be different for different organisms, leading to different sensitivities (Bartlett et al. 2017) and minor inconsistencies between the toxicity results and NAFC concentrations and CH_4 production. Moreover, this study focused on NAFCs (O_2^- species), which are often considered the primary source of toxicity in OSPW (Balaberda and Ulrich 2021; Meshref et al. 2017a). In contrast, a few studies reported that the non-naphthenic acids (O_2^+ species) could also be toxic (Pereira et al. 2013b). As discussed later, lysozyme could adversely impact the microbial population while resulting in better NAFC degradation. Thus, the release of non-naphthenic acids (O_2^+ species) in response to enzymatic treatment should be further investigated.

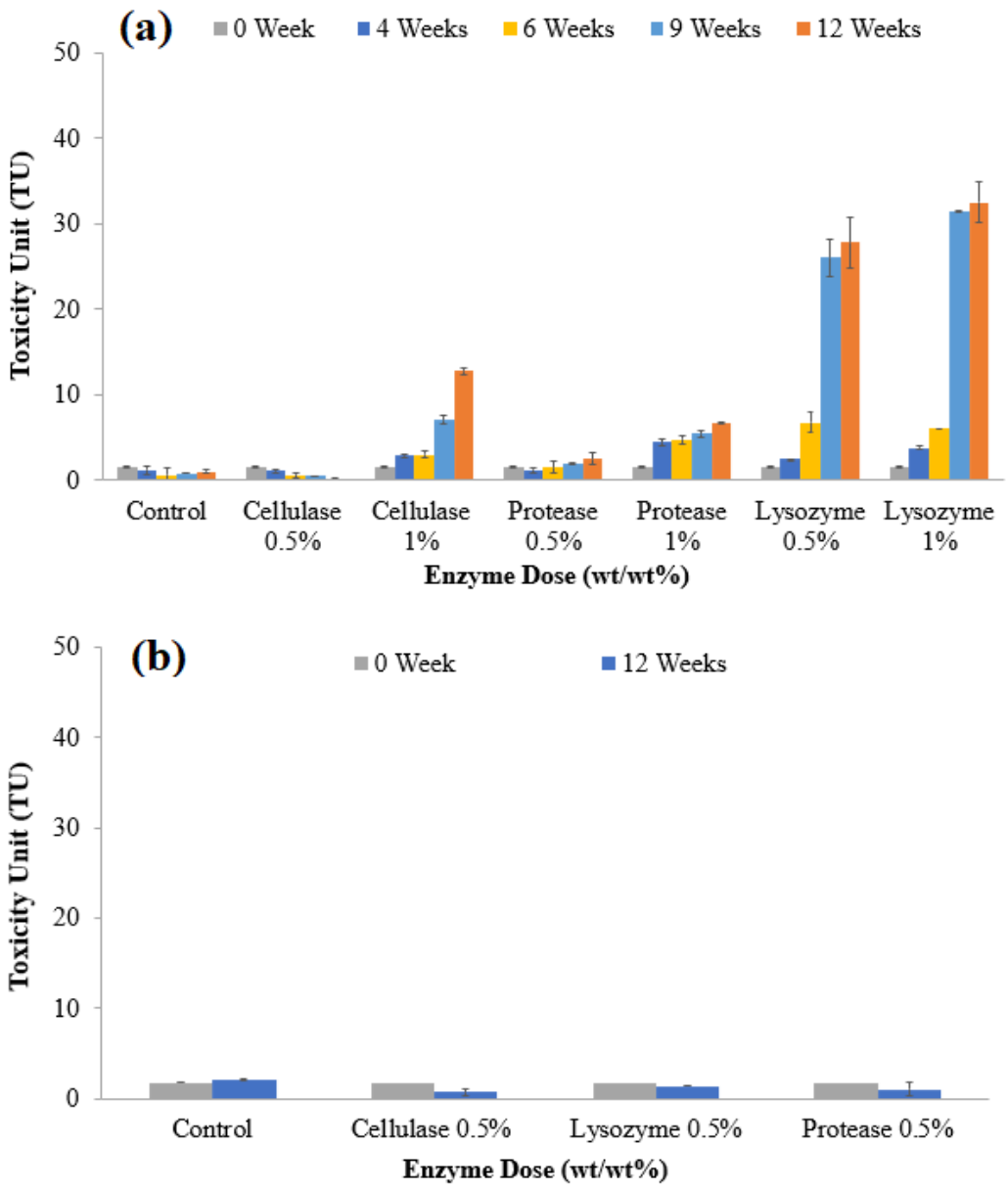

Figure 4.8. The effect of the released pore water from (a) biotic and (b) abiotic enzymatic treatment at various enzyme doses on the toxicity of *V. fisheri*. The impact of toxicity was monitored throughout the experiment for all enzyme-treated FFT compared with the control/untreated FFT. Results illustrated are an average (n = 3 for all treatment conditions) ± one standard deviation.

4.3.6 Microbial communities

In this study, the quantitative and qualitative characteristics of microbial communities were analyzed to evaluate the impact of enzymes on microbial communities. The untreated FFT sample from the beginning of the test served as the control (i.e., initial). After 12 weeks, FFT samples were collected from all columns, including untreated and enzyme-treated FFT samples. Figure 4.9 shows the qPCR results for total microorganisms using the 16S rRNA gene, archaeal gene copies, and *mcr*A. As reported in previous studies, *mcrA* gene copies are considered a biomarker for hydrogenotrophic methanogenesis (Wilkins et al. 2015; Zakaria and Dhar 2021b). The week 0 results for the untreated/control sample represent the indigenous microorganisms that existed in the raw tailings sample before treatment.

With the cellulase 0.5% and 1% treatments, the total microorganisms increased from 7.60×10^6 cell g^{-1} (measured at the control week 0) to 1.53×10^7 cell g^{-1} and 2.90×10^7 cell g^{-1}, respectively. The *mcr*A gene also increased significantly from 9.84×10^3 cell g^{-1} measured at the control week 0 to 3.72×10^4 cell g^{-1} and 6.84×10^4 cell g^{-1} for the cellulase 0.5% and 1% treatments, respectively. These results agree with the methane production results where high CH_4 was observed after 12 weeks for both cellulase doses. Also, as discussed earlier in the toxicity results, the cellulase 0.5% treatment resulted in no toxicity to *V. fischeri*, agreeing with the increase in the qPCR results observed from cellulase-treated FFT. In contrast, the cellulase 1% treatment resulted in high toxicity after 12 weeks (12 TU) despite the increase in the microbial community. Yu et al. (2018b) reported the same observation where the biogenic CO_2 increased and the bacterial density

decreased by 70%. For the lysozyme-treated FFT, a slight change in the microbial community was observed after 12 weeks; the total microorganisms decreased to 5.60 × 10^6 cell g^{-1} and 5.11 × 10^6 cell g^{-1} for the lysozyme 0.5% and 1% treatments, respectively, and to 5.00 × 10^3 cell g^{-1} and 3.05 × 10^3 cell g^{-1} for the methanogen *mcr*A gene, respectively. The protease 1% treatment had the highest reduction in the gene copies of 16S rRNA and *mcr*A. This agrees with the CH_4 production results where the protease 1% treatment resulted in the lowest CH_4 production. Nevertheless, despite low methane production, the control treatment did not have a substantial decrease in gene copies, suggesting that the differences in microbial community composition and diversity might contribute to the differences in methane production.

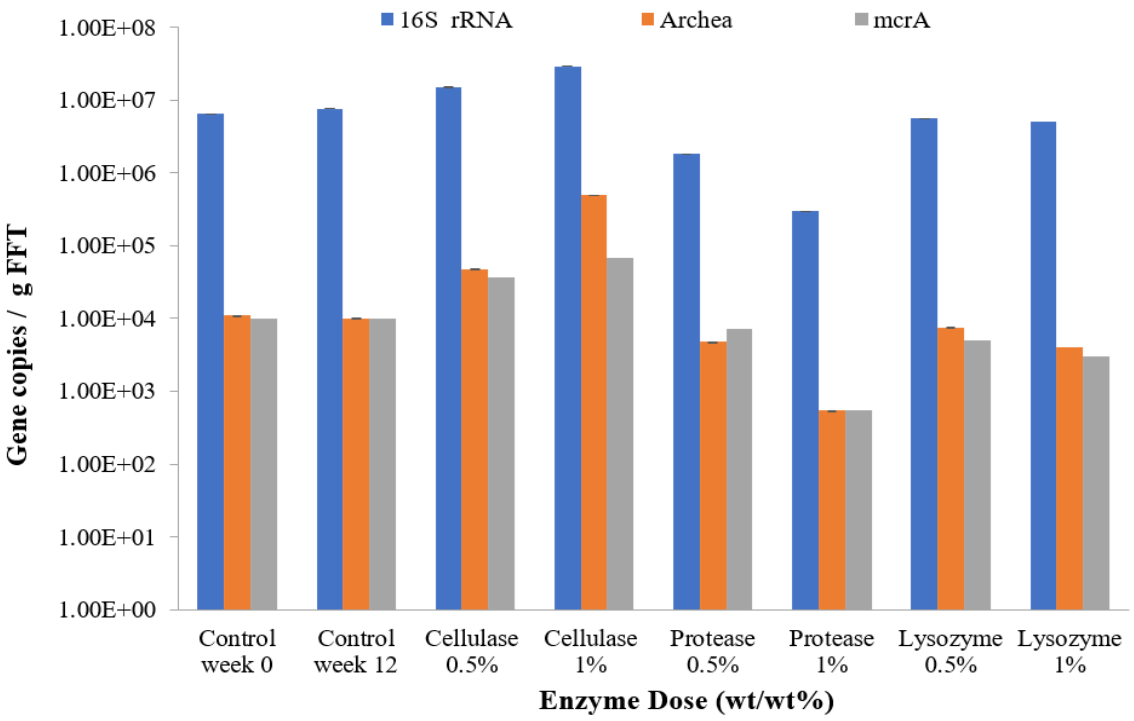

Figure 4.9. Change in gene copies g^{-1} FFT at the beginning and end of the experiment for the control conditions and at week 12 for all enzyme-treated conditions. The untreated FFT represents a 0 ppm treatment and acts as the control experiment. Results illustrated are an average (n = 3 for all treatment conditions) ± one standard deviation.

With regards to the microbial community diversity, it presents a diverse community of microbial species dominated by Firmicutes and Bacteroidetes at the phylum level (Figure 4.10). During the start-up (Week 0), Proteobacteria dominated in the control but their relative abundance significantly decreased after 12 weeks (3.9%). Firmicutes was observed as most dominant bacterial phylum at week 12 (66.9%) along with Bacteroidetes (20.3%). These bacterial phyla were previously identified in oil refinery activated sludge (Misiti et al. 2013) and OSPW (Wilson et al. 2016). For all the enzymes, increasing the enzyme dose from 0.5% to 1% directly impacted the relative abundance of the microbial community after 12 weeks (Figure 4.10). Proteobacteria and Actinobacteria have widely been observed in OSPW, despite variations in abundance (Johnson et al. 2012; Penner and Foght 2010); (Foght et al. 2017; Ramos-Padrón et al. 2011; Siddique et al. 2018). Some other important phyla, such as Acidobacteria, Bacteroidetes, and Firmicutes, are reported to be efficient NAFC-degrading microbes in bioreactors treating OSPW (Zhang et al. 2020).

At the class level (Figure 4.10 a), a complex and diverse microbial community can be seen more prominently. The control week 0 sample is mainly dominated by Betaproteobacteria (66.5%) and Deltaproteobacteria (17.2%) at the class level. In the control sample at week 12, three different classes pointedly appeared: Bacilli (44.4%), Clostridia (15.4%), and Bacteroidia (13.8%). A diverse microbial community facilitates synergistic relationships between species, as one species may be able to degrade metabolites more effectively or use substrates more efficiently (Demeter et al. 2015; Folwell et al. 2020; Yu et al. 2019). In the cellulase 0.5%

treatments, Bacteroidia (39.7%) and Clostridia (26.8%) were among the dominant classes. In the cellulase 1% treatment, the relative abundance of Bacteroidia dropped to 3.7%. However, Clostridia (28.3%) and Negativicutes (27%) prevailed. Through the use of cellulases, organized into cellulosomes, *Clostridium* is able to hydrolyze cellulose efficiently (Gold and Martin 2007; Zverlov et al. 2005). Gammaproteobacteria were also prominent in this sample, accounting for 2.66% for the cellulase 1% treatment. In aerobic cultures derived from froth treatment tailings, Gammaproteobacteria and other taxa played a critical role in hydrocarbon degradation (Foght et al. 2014). Among the well-known methanotrophic aerobic bacteria, Gammaproteobacteria are widespread (Danilova and Dedysh 2014; Op den Camp et al. 2009). Methane oxidation by these bacteria results in the release of methanol and formaldehyde, which are the main sources of nutrients for other organisms (Op den Camp et al. 2009; Saidi-Mehrabad et al. 2013). In the protease 0.5% and 1% treatments, the relative abundance of Clostridia decreased dramatically from 58.3% to 9.3%. Negativicutes (16.6% to 0.4%) were also considerably reduced in the protease 0.5% and 1% treatments. There was a noticeable increase in the relative abundance of Actinobacteria (3.5% to 30.8%) (Figure 4.10). With lysozyme 0.5% and 1% treatment, the microbial community remained consistent, with the relative abundance of Bacteroidia increasing (38% to 69.6%) and Clostridia decreasing (34.9% to 12.1%). Several studies reported that the presence of symbiotic *Clostridium* and *Bacteroides* consortia enhances methane production (Tukanghan et al. 2021). It is well known that both of these genera produce xylanase and cellulase (Robert et al. 2007; Thomas et al. 2014).

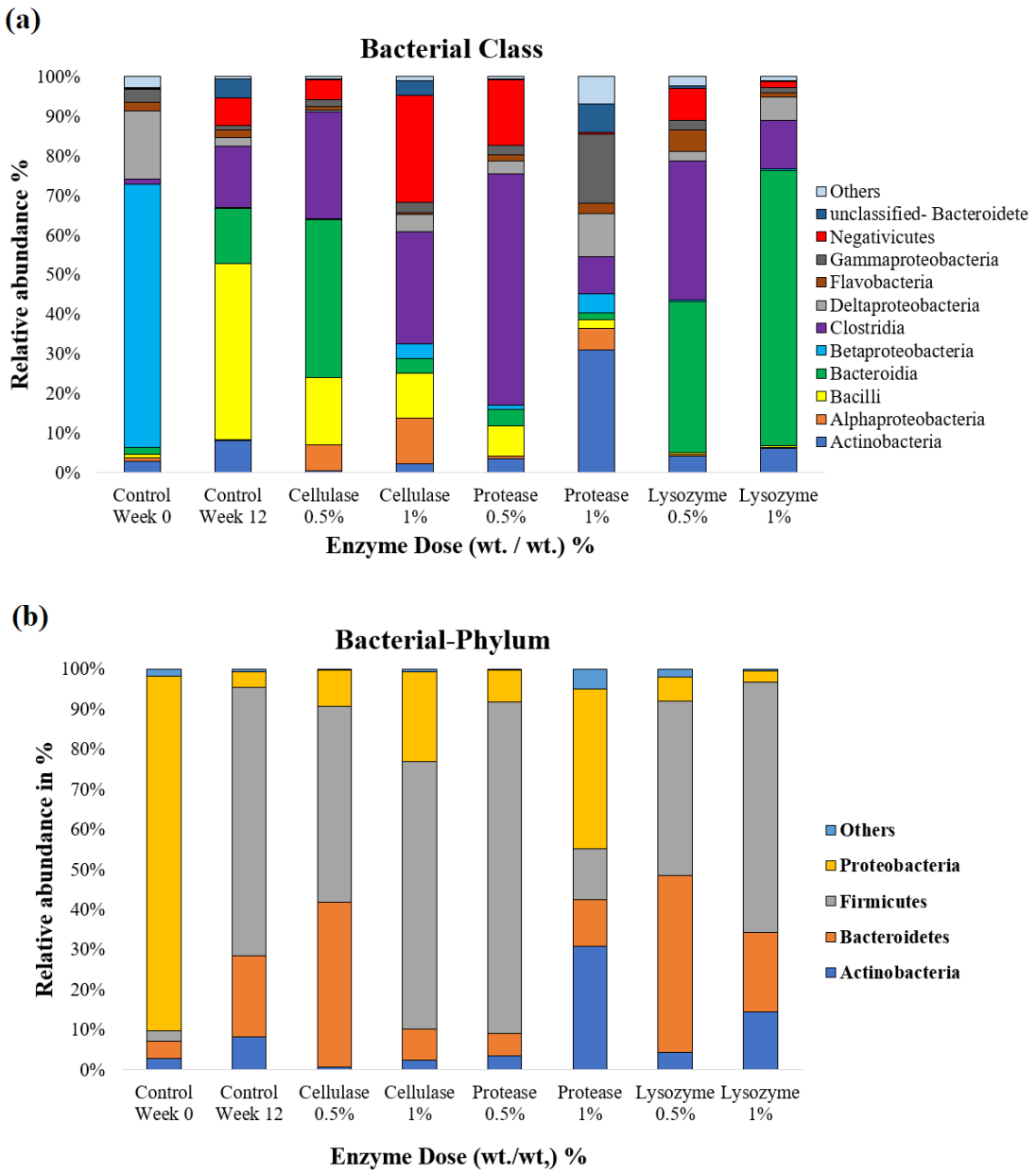

Figure 4.10. Change in bacterial-phylum and bacterial-class at the beginning and end of the experiment for the control conditions and at week 12 for all the enzyme-treated conditions. The untreated FFT represents a 0 ppm treatment and acts as the control experiment. Results illustrated are an average (n = 3 for all treatment conditions) ± one standard deviation.

In the archaeal community, Methanomicrobia was the most abundant enriched class (Figure 4.11 a). However, Methanobacteria (61.1%) and Methanomicrobia (36.8%) were found in the lysozyme 1% treatment. Both Methanobacteria and Methanomicrobia are methanogens from Euryarchaota and get their energy by converting specific substrates to methane gas (Choi et al. 2018; Matsuda and Ohtsuki 2016; Whitman et al. 2006). Methanomicrobia usually function as the main hydrogenotrophic/methylotrophic methanogens, according to several studies (Figure 4.11).

Among all samples, *Methanolinea* and *Methanosaeta* were significantly more abundant than any other archaeal genus. Protease treatments (0.5% and 1%) were mostly dominated by *Methanosaeta* (56.9% and 71.9%). The cellulase 0.5% treatment was dominated by *Methanolinea* (60.7%) and *Methanosaeta* (36.9%). In response to a shift in concentration, *Methanosaeta* (56.9%) became more abundant with the cellulase 1% treatment. The genus *Methanolinea* belongs to the newly identified Methanoregulaceae family and is hydrogenotrophic (Oren 2014). In the lysozyme 0.5% and 1% treatments, *Methanolinea* accounted for 45.7% and 61.6% of total methanogen abundance. Furthermore, *Methanosaeta* were found with an abundance of 44.6% and 33.8% in the same samples (Figure 4.11). The genus *Methanosaeta* belongs to the class Methanomicrobia and was previously known as *Methanothrix* (Anderson et al. 2003; Smith and Ingram-Smith 2007). *Methanosaeta* is one of the most active methanogens found in wetlands, and is responsible for large amounts of global methane production. In residual oil, palmitic acid, and hexadecane amended enrichments, the acetotrophic methanogen *Methanosaeta* was the most

abundant (Fowler et al. 2016; Liang et al. 2015). Clostridiales, Bacteroidia, Methanobacteria, and Methanomicrobia act synergistically in anaerobic digestion to produce hydrogen as well as methane (Vanwonterghem et al. 2014). Additionally, *Methanosaeta* and *Methanolinea* were abundant in communities that had syntrophic interactions with *Bacteroides* and *Clostridium* (Jaenicke et al. 2011; Wang et al. 2017).

We evaluated the alpha diversity of microbial communities (Table 4.1 and 4.2). The observed OTU, Chao1, and Shannon indices were relatively higher in the cellulase 1% and protease 0.5% and 1% treatments than in the control, reflecting their positive impact on the richness and diversity of these bacterial communities. As evidenced by the Chao1 index, methanogens were found in higher abundance in the cellulase 0.5%, protease 0.5%, and lysozyme 0.5% and 1% treatments, and coincided with high methane production under these conditions. Comparatively, in the enzymatically treated samples, the bacterial community was far more abundant and diverse than the archaeal community. In spite of this, their mutual interaction has vital benefits for accelerating oil sand tailings dewatering.

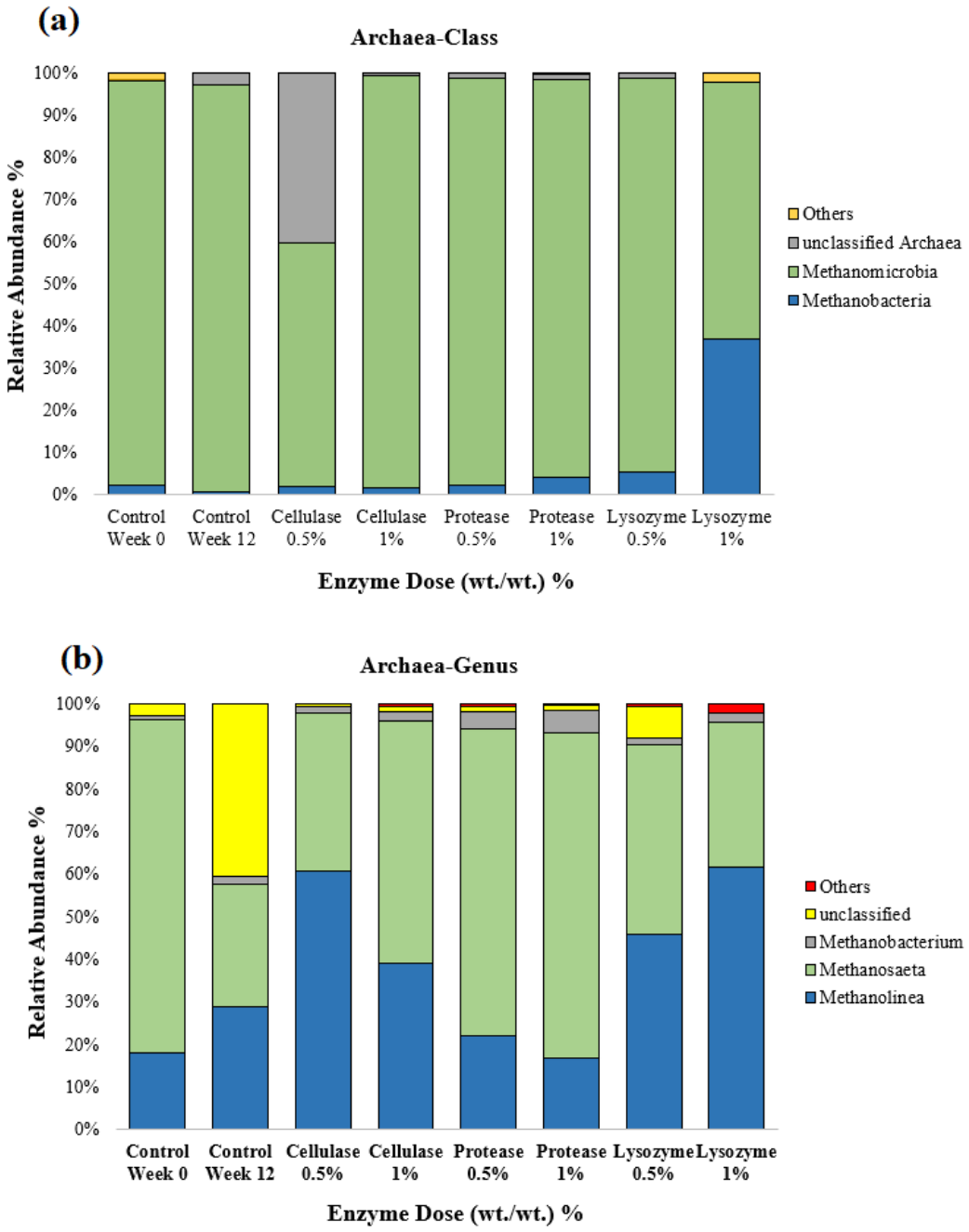

Figure 4.11. Change in a) Archaea-Class and b) Archaea-Genus at the beginning and end of the experiment for the control conditions and at week 12 for all enzyme-treated conditions. The untreated FFT represents a 0 ppm treatment and acts as the control experiment. Results illustrated are an average (n = 3 for all treatment conditions) ± one standard deviation.

Table 4.1. The bacterial diversity index for the control conditions at week 0 and week 12 for all enzymes treated conditions. The untreated FFT represents a 0 ppm treatment and acts as the control experiment.

Bacterial diversity Index					
Samples	**Observed OTU**	**Chao1**	**Shannon**	**Simpson**	**Read count**
Control Week 0	155	168.6	3.26	0.91	12984
Control Week 12	150	167.6	2.82	0.89	11552
Cellulase 0.5%	131	166.0	2.88	0.89	9211
Cellulase 1%	162	170.5	3.29	0.93	10848
Protease 0.5%	158	183.1	3.29	0.93	9126
Protease 1%	162	162.9	3.38	0.92	14338
Lysozyme 0.5%	156	165.6	2.92	0.90	14549
Lysozyme 1%	150	157.7	2.64	0.88	23465

Table 4.2. The archaeal diversity index for the control conditions at week 0 and week 12 for all enzyme-treated conditions. The untreated FFT represents a 0 ppm treatment and acts as the control experiment.

Archaeal diversity Index					
Samples	**Observed OTU**	**Chao1**	**Shannon**	**Simpson**	**Read count**
Control Week 0	7	7	0.63	0.35	2910
Control Week 12	8	8	1.16	0.67	2452
Cellulase 0.5%	10	10.5	0.76	0.49	5075
Cellulase 1%	6	6	0.82	0.52	369
Protease 0.5%	9	9.5	0.76	0.43	884
Protease 1%	7	7	0.71	0.38	1254
Lysozyme 0.5%	9	9	0.97	0.58	5619
Lysozyme 1%	9	9.5	0.75	0.48	1196

4.3.7 Dewatering mechanisms

This study demonstrated the feasibility of using enzymes for enhanced dewatering of oil sands tailings. Based on the results, improved FFT dewatering involved multiple mechanisms. First, enzymes could stimulate the biogenic CH_4 production, which provided release pathways for trapped pore water in tailings. Second, interactions between enzymes and clay particles enabled charge neutralization, facilitating flocculation.

Our results showed that the addition of enzymes increased CH_4 production (Figure 4.3). Moreover, columns amended with enzymes exhibited visible cracks developed within the tailings. Molecular biology analysis suggested that enzymes increased the richness and diversity of bacterial communities. Thus, enhanced bacterial activities could possibly improve biodegradation of tailings organics to simple substrates, which subsequently enhanced methanogenic activity. Nonetheless, as suggested in the previous literature, the disintegration of the EPS matrix by enzymes could also contribute to enhanced methanogenesis, which warrants further investigation.

During the release of the biogenic gases, CO_2 dissolved in the treated FFT pore water and the pH dropped below 7.5. This drop in the pH resulted in the dissolution of carbonate minerals. The dissolution of minerals can increase the ionic strength (Siddique et al. 2014); in which our results confirmed this notion. Thus, the addition of enzymes could increase ionic strength and decrease the DDL thickness (Figure 4.5). Increased ionic strength in the pore fluid can promote inter-particle attraction, leading to enhanced dewatering (Mishra et al. 2019). Zeta potential changes to less negative values (Figure 4.6) indicated that the shrinkage of the DDL was attributed to the charge neutralization of the clay surface. Negatively charged clay particles can attract ions of opposite charge. Based on the literature, enzymes like lysozyme can carry positive charges (Bonilla 2017), potentially interacting with negatively charged clay particles. A schematic representation of the above mechanisms associated with enhanced FFT dewatering aided by enzymes is provided in Figure 4.12.

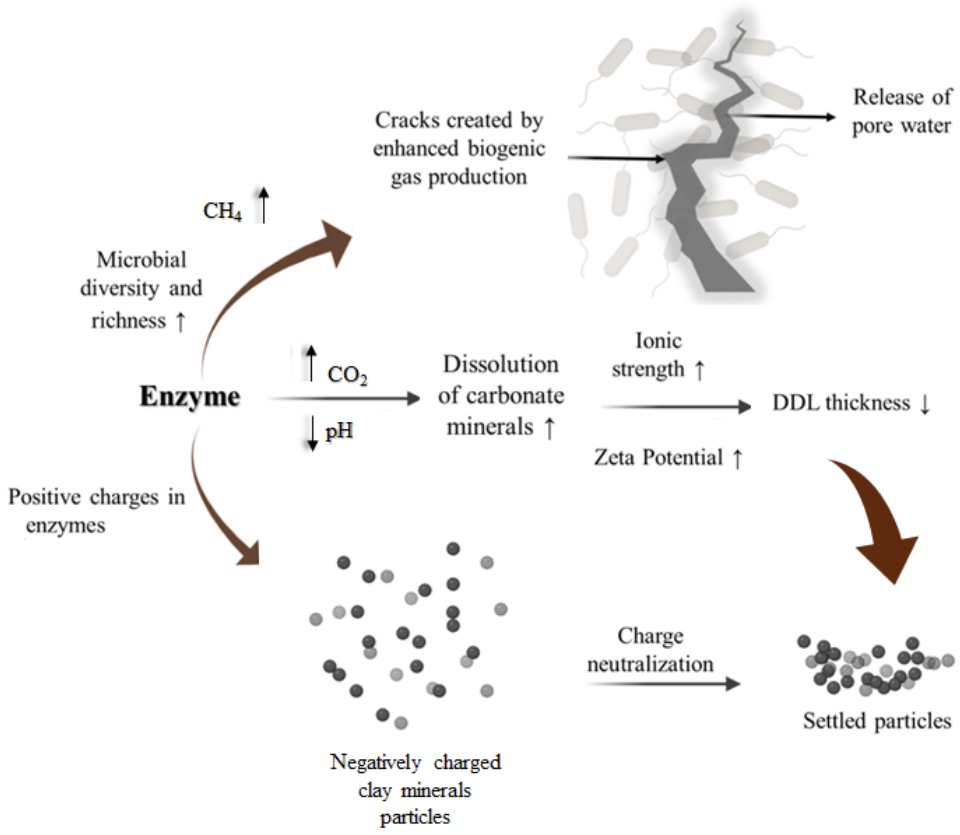

Figure 4.12. Proposed mechanisms of tailings dewatering aided by enzymes.

4.4 Conclusions

In summary, the results of this study demonstrated that enzyme addition could increase the methanogenic activity, which consequently influences the dewatering process. All enzymes (except for protease 1%) increased methane production. The ebullition of CH_4 caused cracks in the FFT, which created pathways for the trapped water to be released. Additionally, during the release of CH_4, the pH of the FFT decreased to <7.5, leading to the dissolution of carbonate minerals, which consequently increased ionic strength. Lysozyme was the best for improving FFT dewatering among the three enzymes. Lysozyme treatment provided the highest WR of 20%, highest increase in ionic strength and highest decrease in the DDL thickness,

following the highest shift of Zeta potential to less negative values (−34.7 to −14.8 mV). Thus, lysozyme was the most effective in charge neutralization of clay particles, which could be attributed to the positive charge carried by lysozyme under pH conditions of tailings. Both doses of lysozyme (0.5% and 1%) reduced the NAFC concentrations in pore water. However, the released pore water from the lysozyme treatment had high toxicity compared with the other enzyme treatments. Further investigation is still warranted to investigate the release of organic fractions from FFT and their associated potential risks.

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5 MITIGATING METHANE EMISSION FROM OIL SANDS TAILINGS USING ENZYMATIC AND LIME TREATMENTS [4]

5.1 Introduction

Surface-mined oil sands in north-eastern Alberta (Canada) represent the third largest production and reserves of global crude oil (Kaminsky et al. 2009). Regardless of the reuse of extraction water by the mining companies, large volumes of water are continuously produced during the hot-water bitumen extraction, and a massive quantity of tailings are stored in engineered tailings ponds (Government of Alberta 2015; Zhu et al. 2017). Environmental legislation mandating a zero-discharge practice prevents companies from directly discharging oil sands tailings and oil sands process water (ERCB 2012; McQueen et al. 2017). The surface area of tailings ponds is continuously increasing and new tailing ponds are being developed (Vedoy and Soares 2015; Young et al. 2020). The composition of oil sands tailings is 20–30 wt% solids (i.e., sand, silt, clays, dissolved salts, organics, and minerals), 70–80 wt% water, and 1–3 wt% residual bitumen and hydrocarbon diluent such as naphtha (Allen 2008). Naphtha is a mixture of low molecular weight aliphatic and monoaromatic hydrocarbons (C_6–C_{10}) and insoluble and complex asphaltenes (Holowenko et al.

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2000; Siddique et al. 2020). Previous studies have illustrated the existence of complex anaerobic microbial communities with the ability to biodegrade a portion of diluent hydrocarbons and produce greenhouse gases (GHGs) such as methane (CH_4) and carbon dioxide (CO_2) under methanogenic conditions (Siddique et al. 2007; Small et al. 2015).

The remediation of oil sands tailings is problematic because of the increase in GHG emissions from the ponds. Many factors affect the presence and activity of different microorganisms in tailings, such as age, type, depth of tailings, and the additives used during bitumen extraction (Penner and Foght 2010; Ramos-Padrón et al. 2010). Within a single milliliter of fluid fine tailings (FFT) from Syncrude's Mildred Lake Settling Basin (MLSB), 10^3 anaerobic heterotrophs, 10^4 sulfate-reducing prokaryotes, and methanogens (concentrations below detection limits) were detected using the conventional most probable number (MPN) method (Foght et al. 1985; Penner and Foght 2010; Ramos-Padrón et al. 2011). Many biogeochemical processes have been derived due to the methanogenesis processes in tailings ponds where hydrocarbon metabolism leads to GHGs emissions (Siddique et al., 2015). In the 1990s, methane bubbles started to appear at the surface of MLSB from methanogenic activity (Guo 2009; Sobolewski 1997), and methane production from MLSB in 2000 was estimated at up to 43 million L/day (Holowenko et al. 2000). The methane emissions from tailings ponds is currently uncontrolled and is considered an input to atmospheric GHGs (Siddique and Kuznetsova 2020).

Although natural detoxification of the ponds (i.e., degradation of contaminants) is beneficial, methane emissions pose significant environmental

concerns. The Government of Canada established regulations to assist the oil and gas sector reduce methane emissions by 40–45% by 2025 (Government of Canada 2018). Previous studies have contributed to the fundamental understanding of methanogenic biodegradation of hydrocarbon contaminants in tailings and the kinetics of methane emissions from tailing ponds (Liggio et al. 2019; Siddique et al. 2007; Simpson et al. 2010; You et al. 2021). However, engineered strategies to reduce GHGs emissions by methanogenic inhibition in tailings have rarely been examined. This information will allow the development of sustainable technologies for oil sands companies to achieve carbon emission reduction goals.

Methanogenic activity can be suppressed using various inhibitors such as 2-bromoethanesulfonate (BES), acetylene (C_2H_2), ethylene (C_2H_4), methyl fluoride (CH_3F), lime, and various enzymes (Biswas et al. 2016; Chan and Parkin 2000; Grawert et al. 2014; Liang et al. 2011; Magdalena et al. 2018; Park et al. 2019; Zinder et al. 1984). BES is a widely reported selective inhibitor for methanogens (Grawert et al. 2014; Zinder et al. 1984); however, BES failed to inhibit methanogenesis over a sustained period during a few *in vivo* trials (Henderson et al. 2016; Immig et al. 1996). Among various enzymes, protease pre-treatment of *Chlorella* biomass could inhibit methane production in anaerobic digestion due to the accumulation of volatile fatty acids (VFAs) and an increase in Actinobacteria species (Magdalena et al. 2018). Biswas et al. (2016) also reported that lysozyme addition could inhibit fermentation in an *in vitro* rumen and reduce methane production. This reduction was due to the increase in the amounts of acetic acid, propionic acid and total VFA after lysozyme treatment. Adding lime inhibited the dry digestion of smooth cordgrass (Liang et al. 2011). Lime treatment causes an increase in the pH and alkalinity which have an

adverse impact on the microbial activity resulting in an elimination of biogas production (Wong & Fang, 2000). Interestingly, treatment of oil sands tailings with various enzymes and lime is effective for dewatering oil sands tailings (Allam et al. 2022; Allam et al. 2021; Romaniuk et al. 2015; Tate et al. 2017; Tate et al. 2019). However, existing literature provides limited information on the effectiveness of enzymes and lime in inhibiting methane emissions from oil sands tailings.

This study focuses on investigating the effects of chemical treatment with lime and biological treatment with two different enzymes (lysozyme and protease) on biomethane emissions from oil sands tailings. The main objectives are as follows: 1) to investigate the feasibility of enzymatic and lime treatment to inhibit methane emissions from tailings ponds; 2) to investigate the corresponding mechanism for the inhibition; 3) to study the effect on the reduction of hydrocarbons; and, 4) to determine the changes in microbial community diversity and toxicity toward *Vibrio fischeri*. These objectives will provide insights to guide mitigation planning and evaluate any ongoing progress towards meeting the Canadian emission reduction goals.

5.2 Materials and methods

5.2.1 Materials

FFT was collected from the oil sands region in northern Alberta and stored in sealed buckets at 4 °C until use. Two different enzymes (protease and lysozyme) and lime were used as potential methanogenic inhibitors for tailings. Protease from *Bacillus* sp. (>16 U/g) was purchased from Sigma-Aldrich (Canada), MP

Biomedicals™ Lysozyme from Chicken Egg White (20,000 U/mg) was purchased from Fisher Scientific (Canada), and lime ($Ca[OH]_2$) was purchased from Fisher Scientific (Canada). Based on previous studies (Navarro et al. 2014; Zinder et al. 1984), BES was used as an additive to the negative control for methanogens. Notably, Zinder et al. (1984) reported complete inhibition of methanogens in sludge anaerobic digestion with the addition of 50 μM of BES. Sterilized FFT was used as an abiotic control condition; FFT was sterilized by autoclaving for 1 h per day for 8 successive days.

5.2.2 Experiment

The experiment was conducted in 158 mL microcosm bottles (Figure 5.1). Each bottle was sealed with a butyl rubber stopper and crimped, then purged with N_2:CO_2 (80:20%) to ensure anaerobic conditions. FFT (65 mL) was injected into each bottle using a 50 mL syringe and purged with N_2:CO_2 (80:20%). The bottles were then moved to an anaerobic chamber flushed with N_2 to perform the following steps. Anaerobic media (35 mL) was injected into each microcosm. The anaerobic media consisted of minerals, resazurin, phosphate solution, sodium bicarbonate, vitamin B solution, and sterilized sodium sulphide. Hexane (500 ppm) and toluene (150 ppm) were added as substrates. Further details regarding the media can be found in the literature (Holowenko et al. 2000). The abiotic control microcosms contained only 65 mL sterilized FFT and 35 mL anaerobic media purged with N_2:CO_2 (80:20%). The microcosms were incubated at 30 °C in the dark, and methane production was monitored. The lag phase for methane production was ~6 weeks. After the initiation of methane production, enzyme, lime, or BES were added to the microcosms. Three

dosages of protease (1%, 1.5%, and 3%), two doses of lysozyme (1.5% and 3%), three doses of lime (1600, 3500, and 5000 ppm), and one dose of BES (50 μM) were tested. To prepare lime and lysozyme slurry, the powder was dissolved in Millipore ultrapure water (18.2 MΩ cm) to achieve the required dose (wt/wt). Protease and BES were purchased in liquid form and were added directly to the inoculum.

Figure 5.1. Photographs of experimental setup (a) 158 mL microcosm consists of 65 mL FFT and 35 mL of anaerobic media control, treated with enzyme, lime or BES, (b) the microcosms incubated in dark conditions at 30 ºC.

5.2.3 Analysis

5.2.3.1 Chemical analysis

The methane content and hydrocarbons (i.e., hexane and toluene) concentrations were measured in the headspace of each microcosm with a typical injection volume of 100 µL. The gas chromatograph (Agilent 7890A) was equipped with an Agilent Technologies HP-5MS column (30 m × 0.25 mm × 0.25 µm) and a flame ionization detector (GC-FID). Soluble chemical oxygen demand (sCOD) was measured using HACH reagent kit (High range COD kit, 0–1500 ppm; HACH, Loveland, CO, USA). The sample was filtered through a 0.45 µm membrane filter before using the COD kit. pH was measured with a LAQUAtwin pH meter (HORIBA Scientific, USA).

5.2.3.2 Toxicity bioassay

The toxicity of the inoculum samples to *V. fischeri* was measured with the Microtox® bioassay test (Osprey Scientific, Edmonton, Alberta, Canada). *V. fischeri* is a luminescent bacterium used as a test organism in the Microtox bioassay. Microtox is a popular bioassay in the oil sands field to measure toxicity as the method is quick, easy, less expensive, and more reproducible than other toxicity bioassays (Brown & Ulrich, 2015). More details on the analytical method can be found elsewhere (Allam et al. 2021; Yu et al. 2018). According to the literature, 1.0 toxicity unit (TU) or lower is considered as not toxic (Scott et al. 2008; Yu et al. 2018).

5.2.3.3 Microbial community and diversity

Genomic DNA was extracted from the control and treated samples (sample weight ≈ 500 mg) with the FastDNA™ SPIN Kit for Soil (MP Biomedicals, USA); more information can be found elsewhere (Allam et al. 2021). The DNA extraction was conducted for the initial inoculum (at start up: week 0) and at the end of the experiment (week 23) for the control sample and treated samples. The extracted genomic DNA was sequenced using Illumina Miseq by the Research and Testing Laboratory (Lubbock, TX, USA). The universal primers 515F (5′ GTGCCAGCMGCCGCGGTAA 3′) and 806R (5′ GGACTACHVGGGTWTCTAAT 3′) were used to target the 16S rRNA gene (Zakaria and Dhar 2021b). The 16S rRNA and *mcr*A gene copies were quantified with quantitative polymerase chain reaction (qPCR) assay using universal bacterial primers, specific archaeal primers, and methyl coenzyme-M reductase gene (*mcr*A) primers; Wilkins et al. (2015) used *mcr*A gene copies as a biomarker for hydrogenotrophic methanogenesis. The primer sequences and detailed qPCR protocol can be found elsewhere (Zakaria and Dhar 2021a). To evaluate the microbial taxonomy and diversity, the sequenced raw data were processed using QIIME2 (Bolyen et al. 2019). The detailed methodology is available elsewhere (Zakaria and Dhar 2021a; 2021b).

The relative abundances of the microbial community revealed by 16S rRNA gene sequencing were statistically computed by principal component analysis (PCA) using weighted Unifrac metrics to evaluate the relationship between

microbial genera and treatment conditions. PCAs were computed and visualized using JMP software (v.11.0.0, SAS Institute Inc., US, https://www.jmp.com).

5.3 Results and discussion

5.3.1 Impact of enzymes and lime on biogenic methane production

Figure 5.2 illustrates the effects of enzymes (protease and lysozyme), lime, and BES on biogenic methane production compared with initial methane production after an incubation period of 23 weeks. The control microcosm had an increasing rate of methane production up to week 19, with a maximum change in methane production of ~1.3 mmol. After that, methane production started to decrease dramatically until the weekly change in methane production was 0.33 mmol at the end of the experiment (i.e., week 23). This reduction in the rate of methane production could be attributed to the lack of substrates (i.e., hexane and toluene) available for methanogens after week 19. BES (i.e., negative control) decreased the rate of methane production up to week 11 (0.25 mmol); after this, the effect of BES on the inoculum started to diminish, and the weekly methane production reached a maximum on week 21 with a 0.1 mmol change in methane production. A possible reason for this trend could be an insufficient dose of BES (Siddique et al. 2014).

The addition of 1.5% lysozyme led to higher change methane production throughout the experiment (2.3 mmol) than the lower dosages of protease, lime, and the control (0.9, 0.2, and 1.3 mmol change in methane, respectively). These results agree with our previous study where 1% lysozyme increased CH_4 production and

enhanced the water recovery from FFT (Allam et al. 2022). Kim et al. (2019) also reported an increase in CH_4 production from secondary sludge after lysozyme treatment. Lysozyme 1.5% increased the rate of CH_4 production up to week 17, then decreased the rate until the end of experiment at week 23 (0.55 mmol). The reduction in CH_4 production with lysozyme 1.5% treatment is similar to the control microcosm, where the CH_4 production rate reduction started at week 17 because of insufficient substrate. For lysozyme 3%, the change in CH_4 production decreased starting from 0.12 mmol at week 3 up to 0.48 mmol at week 23. Park et al. (2019) reported the significant inhibition of rumen protozoa with lysozyme but minimal or marginal inhibition with other peptidases. Another study by Biswas et al. (2016) reported the effect of lysozyme treatment on improving fermentation (i.e., increasing acetate, propionate, and volatile fatty acids (VFA) in an *in vitro* rumen and reducing CH_4 production. Lysozyme 3% had more of a negative impact on CH_4 production than BES after week 11. In contrast, protease 3% caused the highest reduction in CH_4 production throughout the experiment compared with lysozyme, lime, and BES. In the same sense, Allam et al. (2022) reported a low CH_4 production after protease treatment than other enzymes (i.e., lysozyme and cellulase). Increasing the treatment dose (i.e., lysozyme, protease, or lime) increased the inhibition of CH_4 production.

The protease 1% and 1.5% treatments had increased CH_4 production at the beginning of the experiment at weeks 3 and 5 (i.e., 0.375 and 0.4 for protease 1% and 0.86 and 0.89 for protease 1.5%, change in methane respectively). A slight decrease in CH_4 production was observed with both doses after week 5. As

illustrated in Figure 5.2, increasing the protease dose from 1% to 1.5% increases CH_4 production; however, increasing the dose to 3% has a negative influence on CH_4 production throughout the experiment. A decreasing trend was observed for protease 3%, with a maximum decrease in the change in CH_4 production at week 19 (0.72 mmol). A reduction in pH was observed from 6.8 at week 0 to 4.9 at week 23 with protease 3%. The reduction in pH might promote the accumulation of VFA and decrease CH_4 production, as previously reported by Magdalena et al. (2018) at pH 5.5. In addition, our results agree with Mahdy et al. (2015) who reported that protease pre-treatment leads to a high accumulation of VFA and inhibition of biogas production. The high nitrogen content produced after protease pre-treatment is toxic to the methanogenic microorganisms.

The addition of lime at all doses decreased the methane production from the inoculum. The highest reduction occurred with lime 5000 ppm at week 0 (pH $\backsimeq$ 12; Figure 5.3). Methane production with lime 1600 ppm (pH 8.5) increased up to week 4 then decreased to the end of the experiment. Lime addition and BES addition had different effects; up to week 9, BES has higher inhibition of CH_4 production, and after week 11, lime had more inhibition. This trend is similar to the pattern described by Georgiou et al. (2019) who reported substantial killing of pathogenic microorganisms at pH > 11.5 after lime pre-treatment of anaerobically digested animal manure. Liang et al. (2011) also reported an inhibition of anaerobic microorganisms with simultaneous lime treatment and dry anaerobic digestion of smooth cordgrass under high lime loadings.

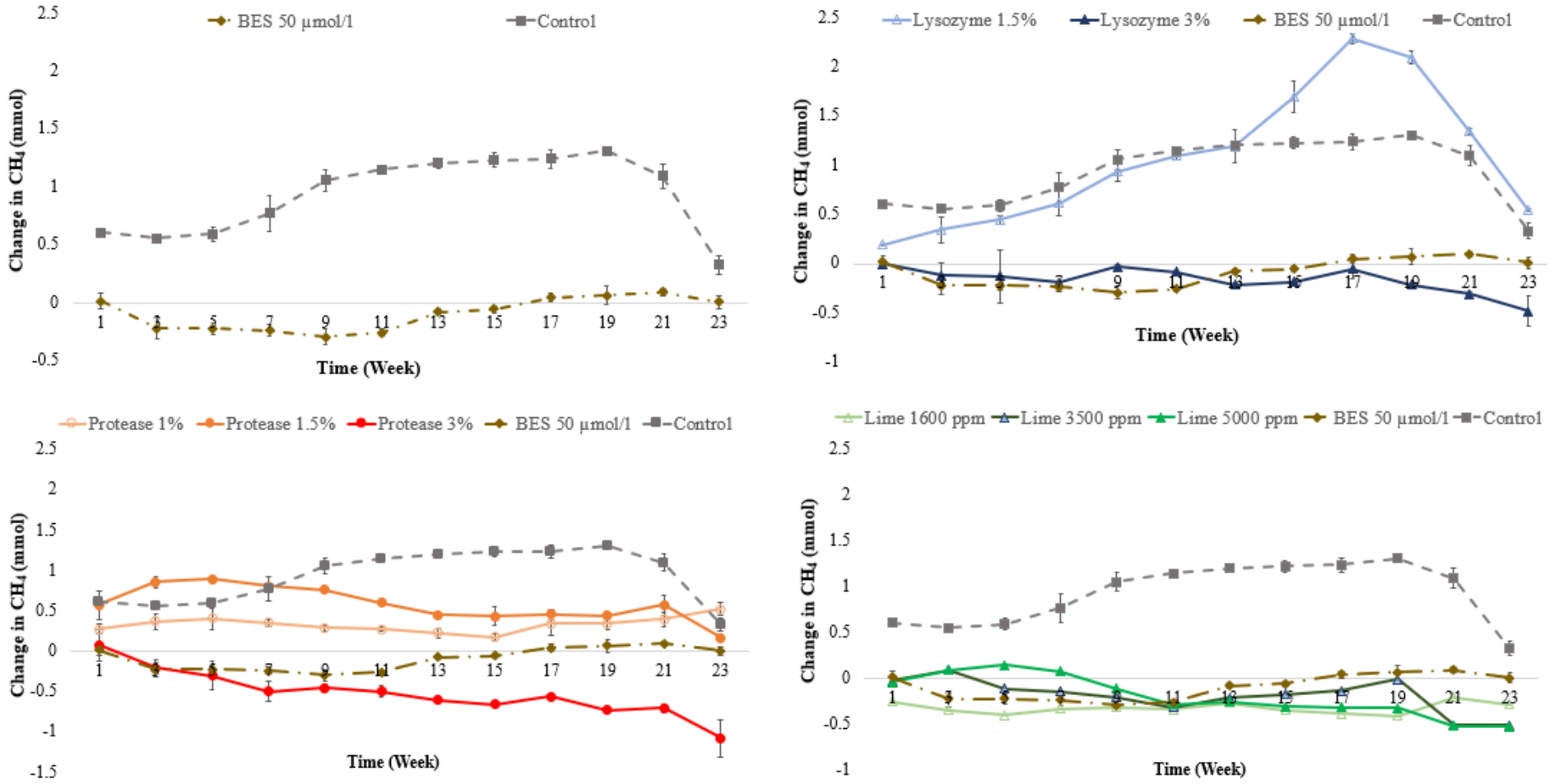

Figure 5.2. Temporal changes in methane production throughout the experiment for all treatment conditions, control/untreated FFT, and negative control 2-bromoethanesulfonate (BES) treatment. The temporal change represents the methane production at any week relative to the initial methane production for each microcosm. All microcosms consisted of 65 mL FFT and 35 mL anaerobic media. The control/untreated FFT represents a 0 ppm treatment and acts as the control treatment.

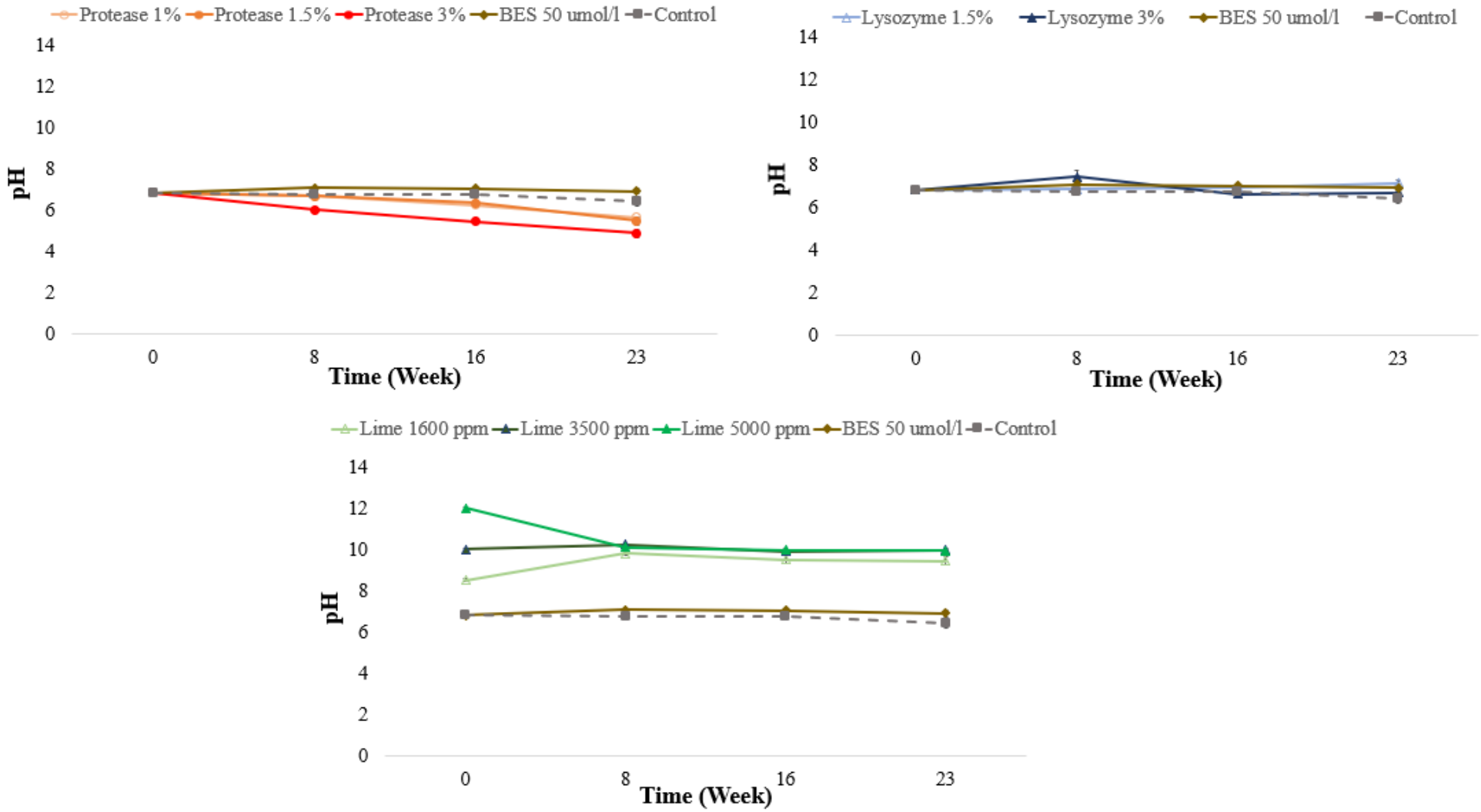

Figure 5.3. Change in pH throughout the experiment for all treatment conditions, the control/untreated FFT, and the negative control 2-bromoethanesulfonate (BES). All microcosms consisted of 65 mL FFT and 35 mL anaerobic media. The control/ untreated FFT represents a 0 ppm treatment and acts as the control experiment.

5.3.2 Organics and hydrocarbon degradation

The impact of different treatment conditions on organics biodegradation was characterized by changes in sCOD and hydrocarbon concentration. The increasing enzyme doses increased the sCOD in the enzyme-treated samples above the sCOD observed in the controls and lime treatment (Figure 5.4). Previous studies reported an increase in the sCOD concentrations of excess sludge hydrolysis using different enzymes, including lysozyme and protease (Liu et al. 2019). Notably, enzymes can hydrolyze complex insoluble organics.

Protease treatment increased the initial sCOD from 690 ppm to 30000, 18600, and 12500 ppm for the 3%, 1.5%, and 1% treatments, respectively, at week 16, followed by a slight decrease at week 23 for all protease doses (22000, 13000, and 7500 ppm for 3%, 1.5%, and 1% treatments) (Figure 5.4b). For lysozyme treatment, the 3% dose caused a rapid increase in sCOD to 28100 ppm at week 8, which then gradually decreased until the end of the experiment, reaching 22500 ppm at week 23. With 1.5% lysozyme, a significant decrease in sCOD occurred from 18000 ppm at week 16 to 5600 ppm at week 23. The substantial increase in sCOD for both lysozyme dosages could be a result of the release of large amounts of soluble organic matter produced from lysozyme activity. Lysozyme has been previously reported to directly disrupt cell walls and release cellular organics into solution (Chen et al. 2008; Xin et al. 2015). The reduction observed after week 8 and week 16 for 3% and 1.5% dosages, respectively, can also be explained by the consumption of organic matter by lysozyme, also previously reported by Wang et al. (2022); this explains the decrease in sCOD at the end of the experiment. These

results suggest that addition of enzymes initially increases sCOD in the inoculum and then degrades some of the organics by the end of experiment. In contrast, BES treatment resulted in a slight increase in sCOD after 8 weeks before reaching a plateau.

No significant change in sCOD concentration was observed with lime treatment (1290, 500, and 430 ppm sCOD for 1600, 3500, and 5000 ppm lime, respectively) compared with the control (192 ppm sCOD). This trend was opposite to the pattern described by Georgiou et al. (2019) who reported a 9% reduction in sCOD after lime treatment (pH = 12.1) of anaerobic digestate. However, the BES treatment resulted in a higher sCOD (5000 ppm) than the control and lime treatment.

Toluene was rapidly degraded in all treatment and control conditions. Siddique et al. (2007) previously reported that short chain *n*-alkanes (i.e., *n*-hexane) and BTEX compounds such as toluene can sustain methanogenesis; toluene is metabolized faster than other BTEX compounds. Lysozyme 1.5% had the highest hexane degradation at the end of the experiment compared with other treatment conditions (reduced to 45 ppm compared with 0 ppm in the control) (Figure 5.5). These results agree with the CH_4 production results where the lysozyme produced the highest CH_4 concentration after 23 weeks. Increasing the lysozyme dose to 3% reduced the hexane concentration to 157 ppm. The protease has the same effect on hexane degradation as CH_4 production, where increasing the protease dose from 1%, 1.5%, to 3% decreased the hexane degradation to 87, 171, and 194 ppm respectively. The negative control (BES) had higher degradation of hexane compared with other treatment conditions. These results suggested that the BES

dose used in this experiment was not sufficient to completely inhibit the methanogens, as observed in other studies (Cárdenas-Manríquez et al. 2020; Siddique et al. 2014). Lime treatment resulted in less degradation of hexane throughout the experiment with a maximum degradation of 380 ppm occurring in the lime 1600 ppm treatment. Lime treatment reduced the hydrocarbon utilization performance of methanogens in the microcosm.

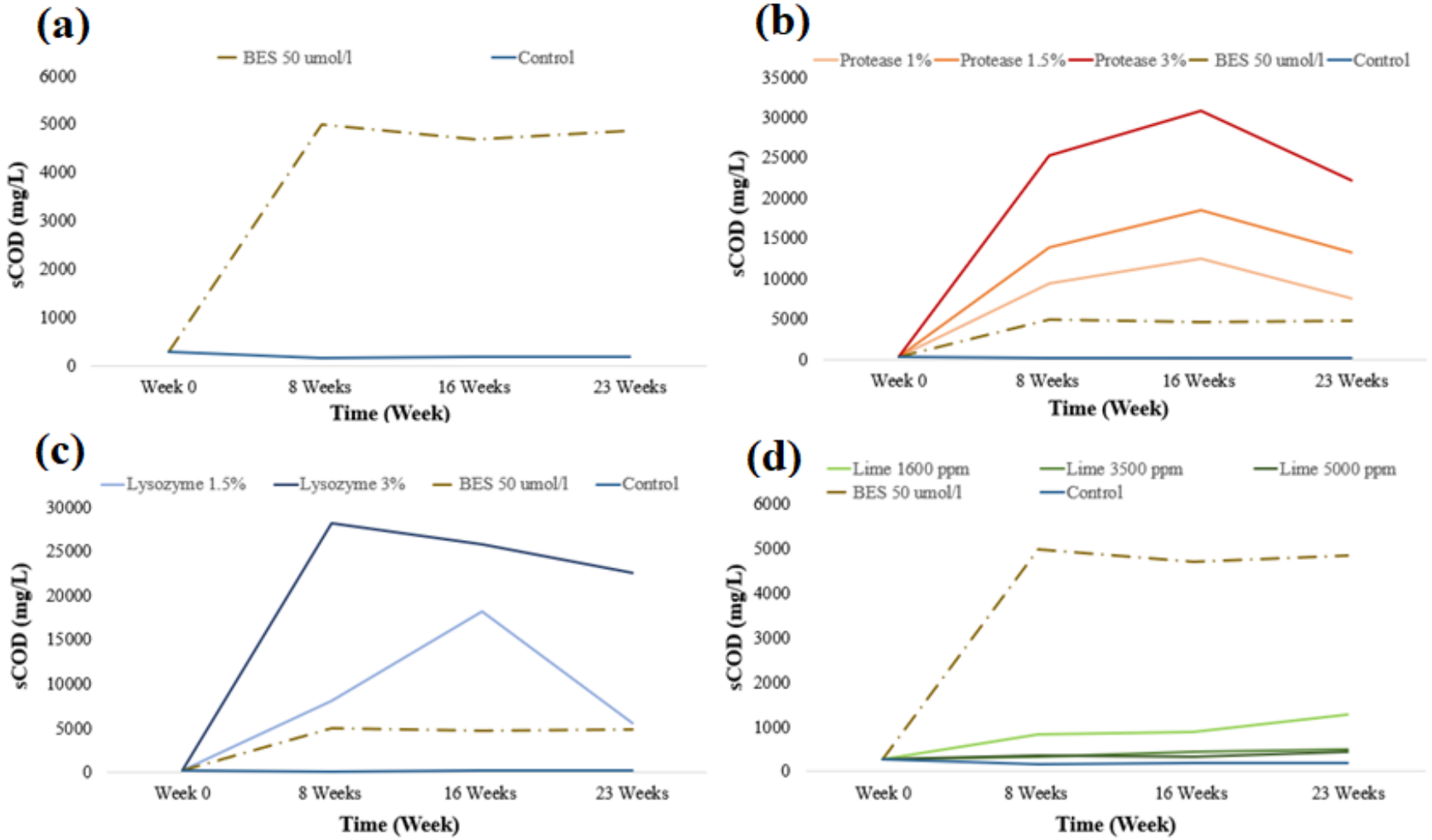

Figure 5.4. Changes in soluble chemical oxygen demand (sCOD) concentrations throughout the experiment for all treatment conditions, the control/untreated FFT, and the negative control 2-bromoethanesulfonate (BES). All microcosms consisted of 65 mL FFT and 35 mL anaerobic media. The control/ untreated FFT represents a 0 ppm treatment and acts as the control experiment.

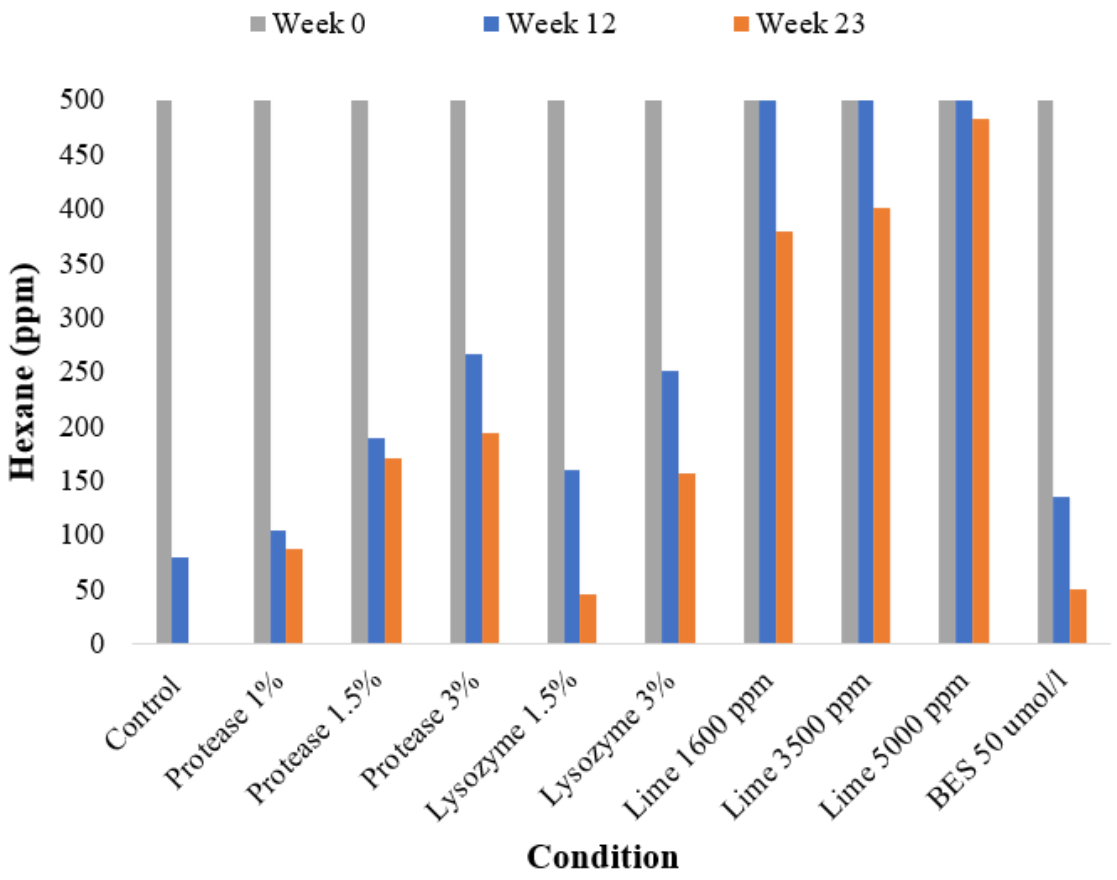

Figure 5.5. Changes in hexane concentrations throughout the experiment for all treatment conditions, the control/untreated FFT, and the negative control 2-bromoethanesulfonate (BES). All microcosms consisted of 65 mL FFT and 35 mL anaerobic media. The control/ untreated FFT represents a 0 ppm treatment and acts as the control experiment.

5.3.3 The effect of different treatment conditions on toxicity

Table 5.1 summarizes the influence of different treatment conditions on the toxicity to *V. fischeri*, as measured with the Microtox® bioassay. The control and BES microcosms had the lowest toxicity of all treatment conditions; toxicity decreased from 6.6 TU to 1.3 TU and 0.8 TU, respectively, at week 23. The lime treatment had relatively lower toxicity than the lysozyme and protease treatments. Nonetheless, increasing both enzyme and lime dosages consistently increased the

toxicity. For instance, increasing the protease dose from 1% to 3% increased the toxicity of the microcosm from 7.0 TU to 124 TU. These finding agrees with Allam et al. (2022), where increasing the enzyme dose (protease and lysozyme) from 0.5% to 1% increased the toxicity of FFT porewater. Lysozyme dosages (1.5% and 3%) demonstrated more toxic conditions after week 12 (21 and 76 TU, respectively) compared with other treatment conditions. For lime, although all doses were toxic (i.e., TU > 1), the 1600 ppm dose resulted in the lowest toxicity (6.3 TU) compared with the 3500 ppm and 5000 ppm doses (9.7 and 11.3 TU, respectively). These results are consistent with the negative impact of lime addition (i.e., pH >10) on bacterial cell counts in the FFT (Allam et al. 2021). Moreover, the toxicity of the enzyme and lime-treated conditions was consistent with the methane production and hydrocarbon degradation profiles. For instance, lysozyme 3% had lower methane production, lower hydrocarbon degradation, and higher toxicity than lysozyme 1.5%. Despite the inhibition of methanogenesis, BES had the lowest toxicity among all conditions, including the control. These results agree with methane production, where BES treatment produced less methane than the control. However, further investigation is still warranted regarding toxicity reduction after BES treatment.

Table 5.1. The effect of different treatment conditions on the toxicity to *Vibrio fisheri*. The impact of toxicity was monitored at week 0, week 12, and week 23 for all treatment conditions and compared with the control/untreated FFT and negative control BES.

Condition/ Time	**Toxicity (TU)**		
	Initial (Week 0)	**Week 12**	**Week 23**
Control	6.60 ± 0.5	2.2 ± 0.0	1.3 ± 0.4
Protease 1%		5.2 ± 0.4	7.0 ± 0.3
Protease 1.5%		6.3 ± 0.8	15.5 ± 0.4
Protease 3%		9.9 ± 0.9	124.8 ± 4.3
Lysozyme 1.5%		21.4 ± 0.7	41.1 ± 7.0
Lysozyme 3%		76.3 ± 1.3	81.3 ± 2.0
Lime 1600 ppm		1.6 ± 0.4	6.3 ± 0.1
Lime 3500 ppm		4.9 ± 0.1	9.7 ± 2.6
Lime 5000 ppm		7.6 ± 2.0	11.3 ± 3.9
BES 50 µmol/l		1.2 ± 0.2	0.8 ± 0.0

5.3.4 Microbial quantity

Figure 5.6 shows the quantitative analysis of microbial communities in the control and treated conditions using qPCR. For the control, the bacterial cell numbers were not significantly different between weeks 0 and 23 (5.7×10^6 vs. 5.4×10^6 cells g^{-1}). Lime doses resulted in the highest reduction in 16S rRNA gene copies compared with the control and other conditions (2.7×10^5, 1.71×10^5, and

1.4 × 10^5 cells g^{-1} for 1600, 3500, and 5000 ppm lime, respectively). As aforementioned, the changing pH during lime treatment (pH to 8.5, 10, and 12 for 1600, 3500, and 5000 ppm lime, respectively) caused a sharp decline in the bacterial cell numbers. The 16S rRNA gene copies were higher in the control than in the protease 1% (9.9 × 10^5 cells g^{-1}) and protease 1.5% treatments (1.0 × 10^6 cells g^{-1}), with a higher reduction in the protease 3% treatment (3.3 × 10^5 cells g^{-1}). Likewise, BES 50 μM reduced the bacterial cell numbers to 3.6 × 10^5 cells g^{-1}. In contrast, the lysozyme 1.5% and 3% treatments increased the bacterial cell numbers to 12.5 × 10^7 and 1.6 × 10^7 cells g^{-1}, respectively, which were higher than the control and other treatment conditions.

Similar to the bacterial cell number trends, lime resulted in the highest decrease in archaeal cell numbers. Using lime dose of 1600 ppm had the highest reduction in archaeal cell numbers (5.8 × 10^2 cells g^{-1}) compared with the control (3.76 × 10^5 cells g^{-1}) and other treatment conditions. The changing pH during lime treatment (pH 8.5, 10, and 12 for 1600, 3500, and 5000 ppm lime, respectively) caused a sharp decline in the archaeal cell numbers, which reduced the methane production. Protease 1% and 3% and BES 50 μM resulted in the second highest reduction in archaeal cell numbers, after that of the lime treatment condition. However, lysozyme treatment had almost no effect on the archaeal cell numbers when compared with the control. *mcr*A gene copies were quantified as a biomarker for hydrogenotrophic methanogenesis (Wilkins et al. 2015). Similar trends were observed for *mcr*A gene copies, with the lime 1600 ppm treatment having the highest decrease in *mcrA* gene copy numbers.

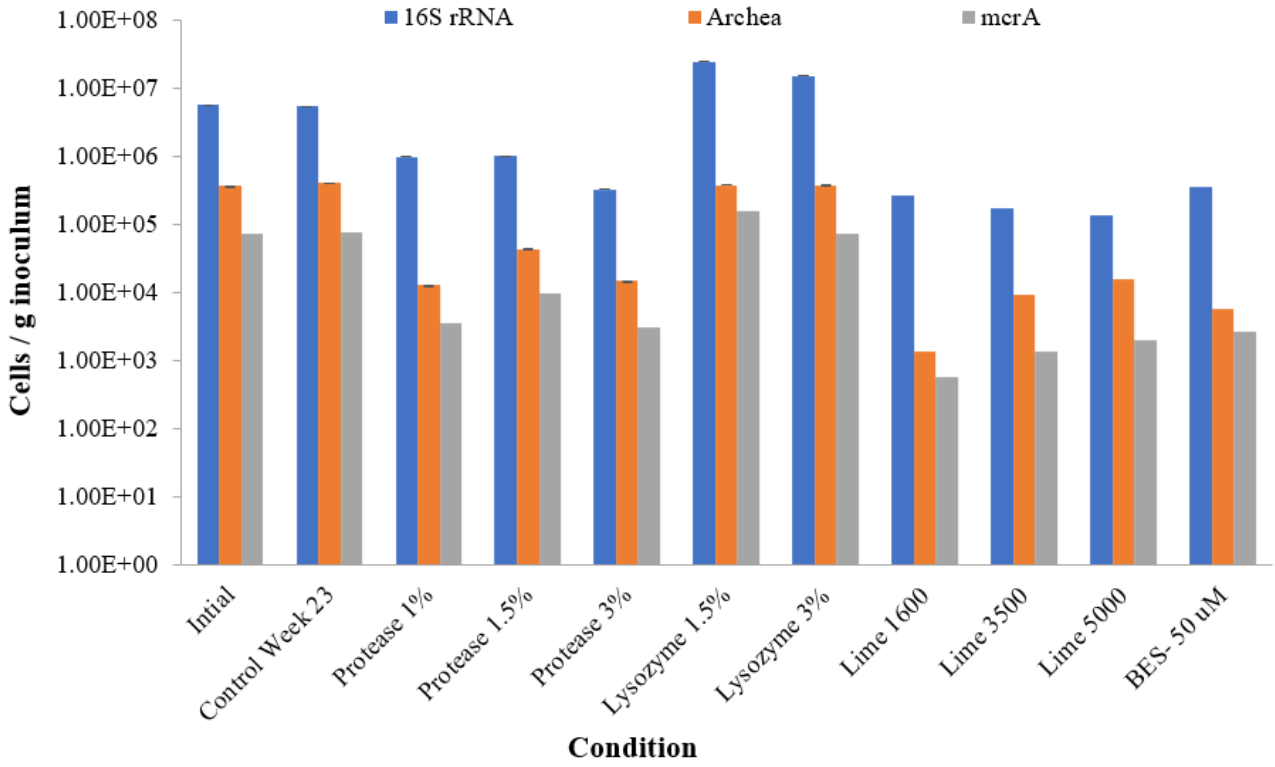

Figure 5.6. Changes in cell number or gene copy number per g inoculum at the beginning and end of the experiment for the control condition, and at week 23 for all treatment conditions and the negative control. All microcosms consisted of 65 mL of FFT and 35 mL anaerobic media. The control/ untreated FFT represents a 0 ppm treatment and acts as the control experiment.

Figure 5.7 represents the alpha diversity metrics of the microbial community computed using QIIME 2. The different treatments had an impact on the microbial diversity. The lowest richness of the microbial community (measured by Chao1 and observed taxonomic units; OTUs) was observed in the lysozyme 3% treatment, with a notable increase in diversity with other treatments such as protease 1% and 1.5%; lime 5000, 1600, and 3500; and BES 50 μM. The lime 3500 ppm treatment had the highest microbial community richness of all other treatment conditions. The highest microbial diversity (measured by Shannon index) was found in the BES 50 μM (6.6) and lime 1600 ppm treatments (6.6), followed by lime 5000 ppm (6.5) and lime 3500 ppm treatments (6.3); the lysozyme 1.5% treatment had a significantly decreased Shannon index (2.7), followed by lysozyme 3% (4.0) and protease 3% (4.8). These results indicate that the treatment conditions significantly shifted the microbial diversity.

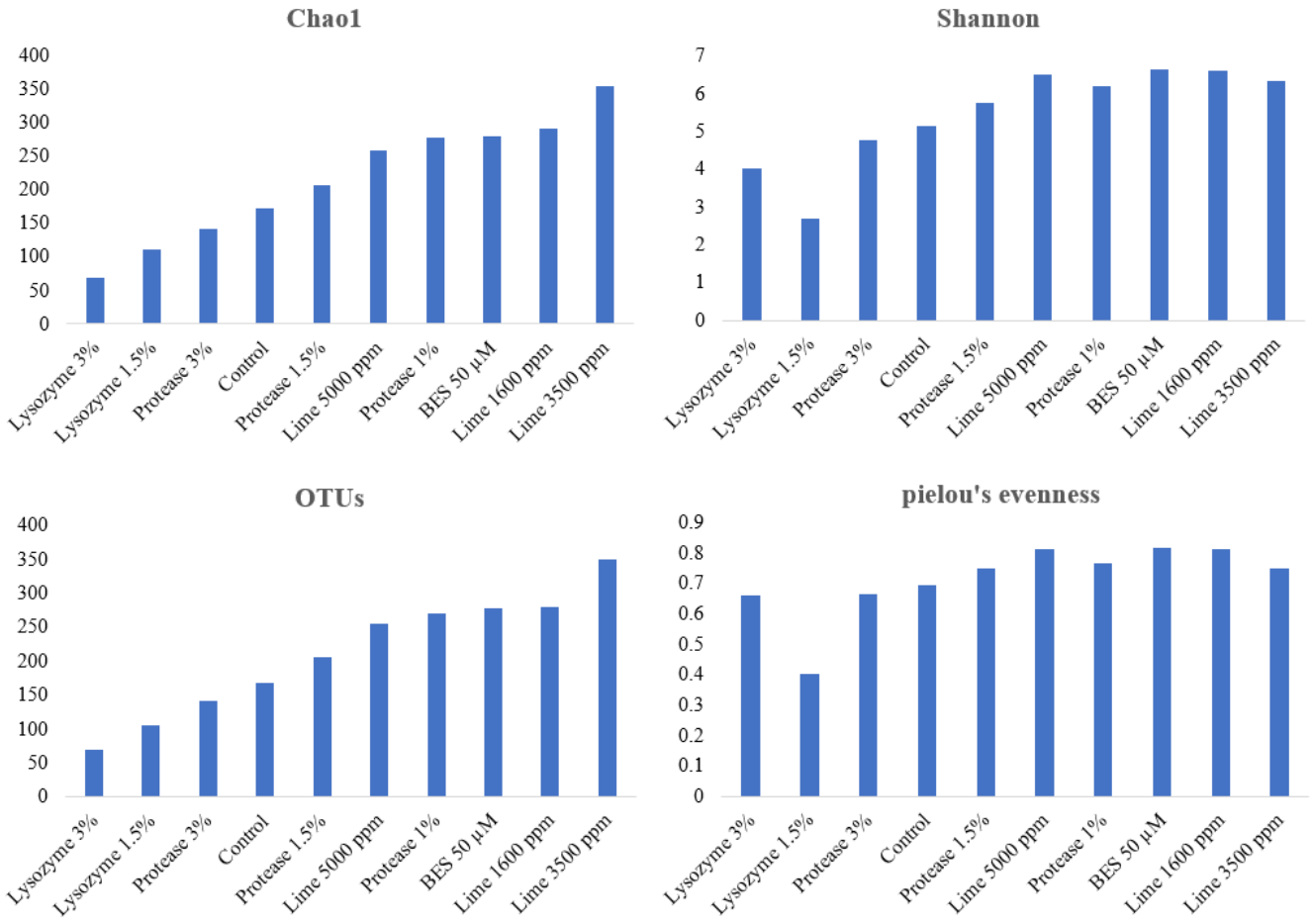

Figure 5.7. The alpha diversity metrics of the microbial community computed using QIIME 2 for all treatment conditions, the control/untreated FFT, and the negative control 2-bromoethanesulfonate (BES). All microcosms consisted of 65 mL FFT and 35 mL anaerobic media. The control/ untreated FFT represents a 0 ppm treatment and acts as the control experiment.

5.3.4.1 Microbial community

The microbial community composition of the control and treatment conditions are shown in Figure 5.8. The relative abundances of the microbial community were directly impacted by the treatment conditions after 23 weeks incubation. At the domain level, archaea decreased in the control microcosm after 23 weeks from 27.7% to 10.9%. All treatment conditions had lower relative abundance of archaea than in the control. The highest reduction in the relative abundance of archaea was 2.2% for the lime 5000 ppm treatment, followed by 3.3% for lysozyme 1.5% treatment and 4.9% for lysozyme 3% treatment.

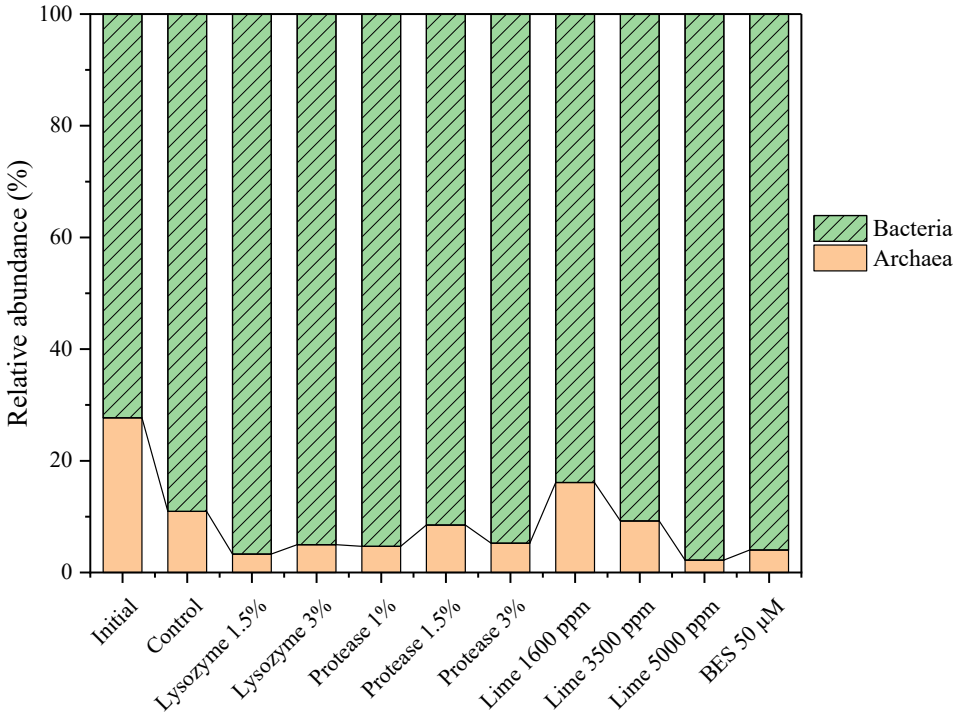

Figure 5.8. Change in the relative abundance at domain level for the control at the beginning of the experiment (Initial), the control at the end of the experiment (Control), and week 23 for all other treatment conditions.

The microbial community comprised diverse phyla mainly dominated by Firmicutes, Proteobacteria and Chloroflexi (Figure 5.9). In the control microcosm at week 0, Proteobacteria (40%) was the most abundant phylum, followed by Euryarchaeota (28%) and Firmicutes (16%). At week 23, the abundance of Proteobacteria increased to 67%, however, the Euryarchaeota and Firmicutes abundances decreased to 11% and 7%, respectively.

After the treatments, Firmicutes, Proteobacteria, and Chloroflexi remained the most abundant phyla. After protease treatment (1%, 1.5%, and 3%), Firmicutes increased and became the most dominant phylum with 62%, 66%, and 80% abundance, respectively. After lysozyme treatment, Bacteroidetes was enriched and became the most abundant phyla in lysozyme 1.5% with 79% abundance; however, increasing the dose to lysozyme 3% shifted the dominant phylum to Firmicutes (69% abundance). After lime treatment (1600, 3500, and 5000 ppm), Firmicutes (19%, 37%, and 31%) and Chloroflexi (19%, 21%, and 25%) were the most abundant phyla. After 50 μM BES treatment, Proteobacteria (40%) and Chloroflexi (27%) were the dominant phyla. These results agree with the increased CH_4 production with increased protease dose up to 1.5%. Previous studies reported the enrichment of the microbial community during methanogenic activity (Kuznetsova et al. 2021; Young et al. 2020). Chloroflexi was first reported by Ficker et al. (1999) to be related to toluene degradation in a toluene-degrading methanogenic consortium.

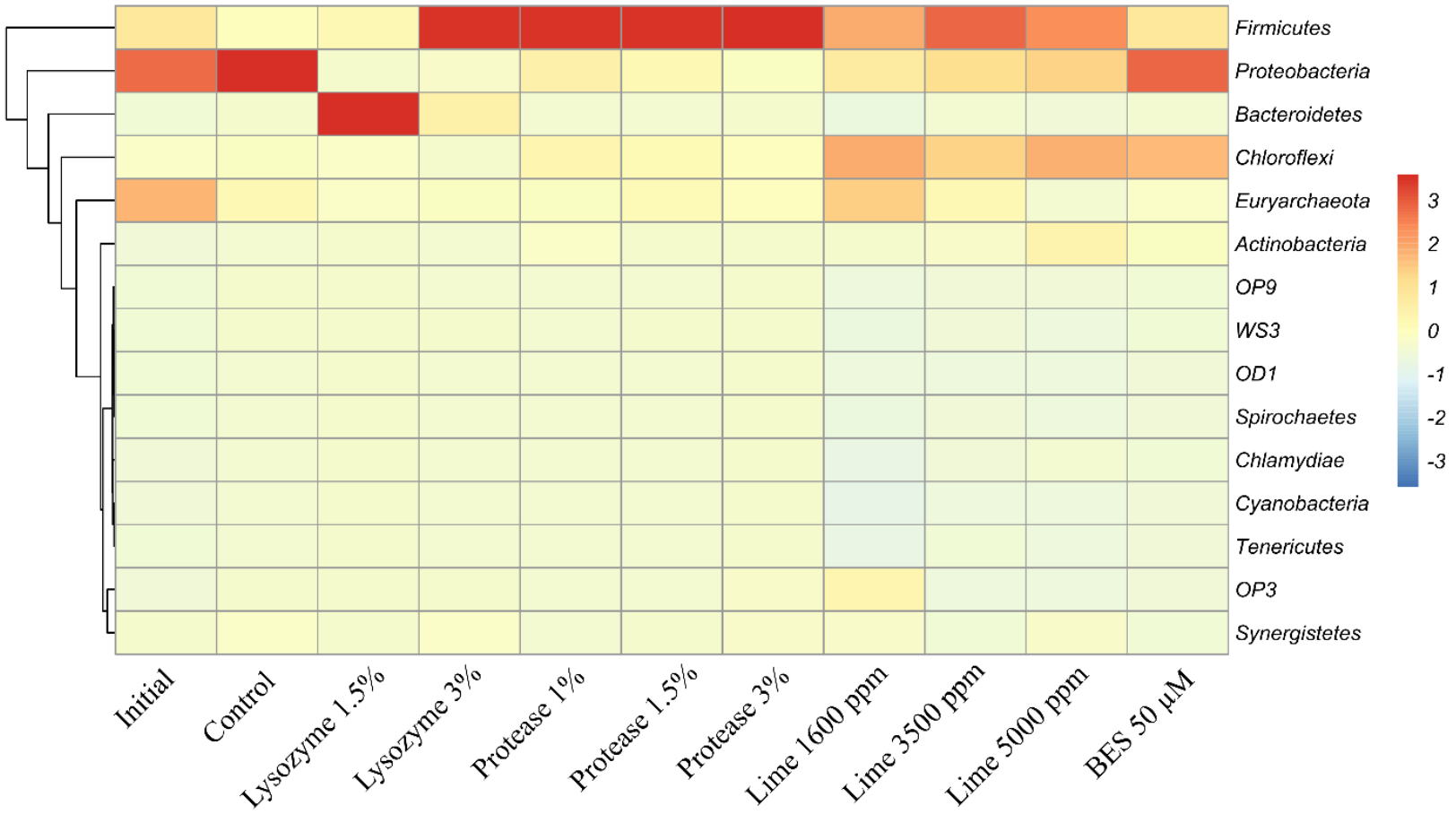

Figure 5.9. Changes in the relative abundance at the phylum level for the control at the beginning of the experiment (Initial), the control at the end of the experiment (Control), and week 23 for all other treatment conditions.

At the genus level (Figure 5.10a), a more diverse microbial community was observed. The control microcosm at week 0 was mainly dominated by *Desulfotomaculum* (16%); however, the abundance of this genus declined to 7% after 23 weeks of incubation. Another dominant genus was *Acidovorax* (14%) at week 0, which increased significantly to 53% after 23 weeks of incubation. *Acidovorax* was previously reported in Shell Albian sands tailings (Cárdenas-Manríquez et al. 2020).

Both *Desulfotomaculum* and *Acidovorax* significantly decreased in all other treatment conditions. In contrast, Siddique et al. (2020) reported a significant increase in the genus *Desulfotomaculum* after 120 d incubation after the degradation of isoalkanes and cycloalkanes. Toth and Gieg (2018) also reported an increase in *Desulfotomaculum* after methanogenic biodegradation of short chain *n*-alkanes, cyclohexane, and mono-aromatics. After protease treatments, *Clostridium*, a member of the Deltaproteobacteria, was dominant and increased in relative abundance with increasing protease dose (26%, 31%, and 58% for the 1%, 1.5%, and 3% doses, respectively). As reported previously by Fowler et al. (2016), an increase in the relative abundance of the *Clostridium* results in increased downstream conversion of smaller molecules to methanogenic intermediates. For the lysozyme 1.5% treatment, two different genera appeared: *Clostridium* (23%) and *Anaerovorax* (37%). However, in the lysozyme 1.5% treatment, *Marinilabiaceae* was the dominant family with a relative abundance of 79%. The dominant genera observed in most of the lime treatment conditions and BES were *Desulfosporosinus* (29%–4%), *Desulfotomaculum* (9%–3%), and some genera of

the family of *Anaerolinaceae* (14%–6%). Previous reports by Sutton et al. (2013) linked the presence of the family of *Anaerolineaceae* with the anaerobic degradation of oil-related compounds such as *n*-alkanes.

The dominant archaeal genera in the control microcosms were *Methanosaeta* (3%–9%), *Methanolinea* (3%–8%), *Methanoculleus* (3%–7%), and *Candidatus Methanoregula* (0.2%–4%) (Figure 5.10b). A slight decrease in most of the methanogen-affiliated genera was observed in the control condition after 23 weeks of incubation. For the protease treatment (1% and 2%), *Candidatus Methanoregula*, a hydrogenotrophic methanogen, was dominant over the other genera that were suppressed after treatment compared with control. Most of the treatments reduced the relative abundance of *Methanosaeta*, an acetoclastic methanogen, after 23 weeks incubation; the highest reduction occurred with protease 1%, from 8% to 0.55% relative abundance. *Methanosaeta* is mainly found in wetlands and is considered one of the most active methanogens responsible for producing large amounts of CH_4 (Fowler et al. 2016). *Methanolinea* (a known hydrogenotrophic methanogen) decreased more in most treatment conditions than in the control; with protease 1%, *Methanolinea* decreased from 3% to 0.4% and with BES 50 µM it decreased to 0.3%. The lime doses of 1600 ppm and 3500 ppm resulted in the lowest reduction in archaea genera compared with other treatment conditions.

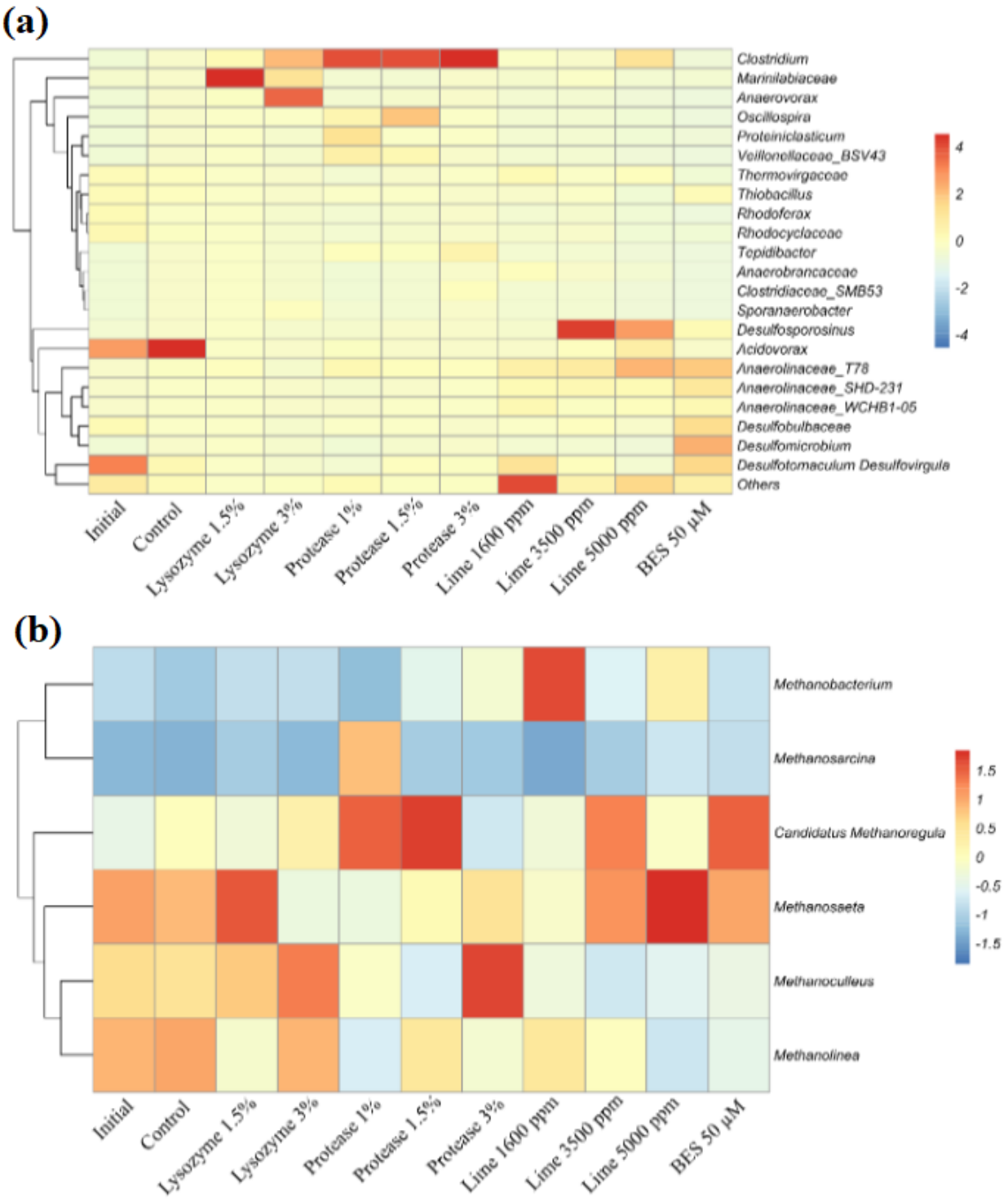

Figure 5.10. (a) Changes in the relative abundance at the genus level for bacteria, (b) for the archaea for the control at the beginning of the experiment (Initial), the control at the end of the experiment (Control), and week 23 for all other treatment conditions.

5.3.5 Principal Component Analysis (PCA)

PCA was performed to analyze the relationship between the treatment conditions and microbial community abundances at the phylum and genus levels (Figure 5.11). The treatment conditions differently impacted the abundances of the microbial communities. At the phylum level, the control and lime 1600 ppm treatments clustered together in the same quadrant (top right) (Figure 5.11a). *Euryarchaeota* had a strong relationship with the control and lime 1600 ppm treatments (Figure 5.11b). The relative abundance of *Euryarchaeota* agreed with previous studies that focused on biomethane production (Magdalena et al. 2019). Another study reported that *Euryarchaeota* comprises hydrogentrophic and acetotrophic methanogens (Fowler et al. 2016). Other treatment conditions, such as lysozyme 1.5% and 3% and protease 1.5% and 3%, were closely associated and had a strong relationship to the abundances of Bacteroidetes and Firmicutes. The lime 3500 and 5000 ppm and BES 50 μM treatments were located in the same quadrant (right bottom) and were closely associated with Chloroflexi, Actinobacteria, and Proteobacteria.

The relationship between the treatment conditions and archaeal genera was also evaluated using PCA (Figure 5.11c and b). Similar to the phylum level, the control and lime 1600 ppm treatments were located in the same quadrant (right bottom), closely correlated with hydrogenotrophic methanogens (*Methanobacterium*, *Methanoculleus*, and *Methanolinea*) (Zakaria and Dhar 2019). High CH_4 production in the control condition may be attributed to the abundance of the hydrogenotrophic methanogens *Methanobacterium*, *Methanoculleus*, and

Methanolinea. Therefore, hydrogenotrophic methanogens provide the predominant pathway for CH_4 production in the control microcosm, with lower activity of acetoclastic methanogens. Similarly, the lime 3500 ppm and protease 1.5% treatments were clustered together in the right-top quadrant with the hydrogenotrophic methanogen *Candidatus Methanoregula* (Yamamoto et al. 2014). The protease 1% and BES 50 μM treatments were located on the top-left quadrant and were associated with *Candidatus Methanoregula*. *Methanosarcina* was located in a separate quadrant and showed no association with any other genera. These results illustrated that both hydrogenotrophic (i.e., *Methanoregula*) and acetoclastic (i.e., *Methanosarcina*) methanogens governed the pathway for CH_4 production after protease 1% treatment. The lysozyme 1.5%, lysozyme 3%, protease 3%, and lime 5000 ppm treatments were very weakly associated with archaeal abundance (Figure 5.10c). These findings agree with the CH_4 results where protease 3%, lysozyme 3%, and lime 1500 ppm treatments resulted in inhibition of CH_4 production.

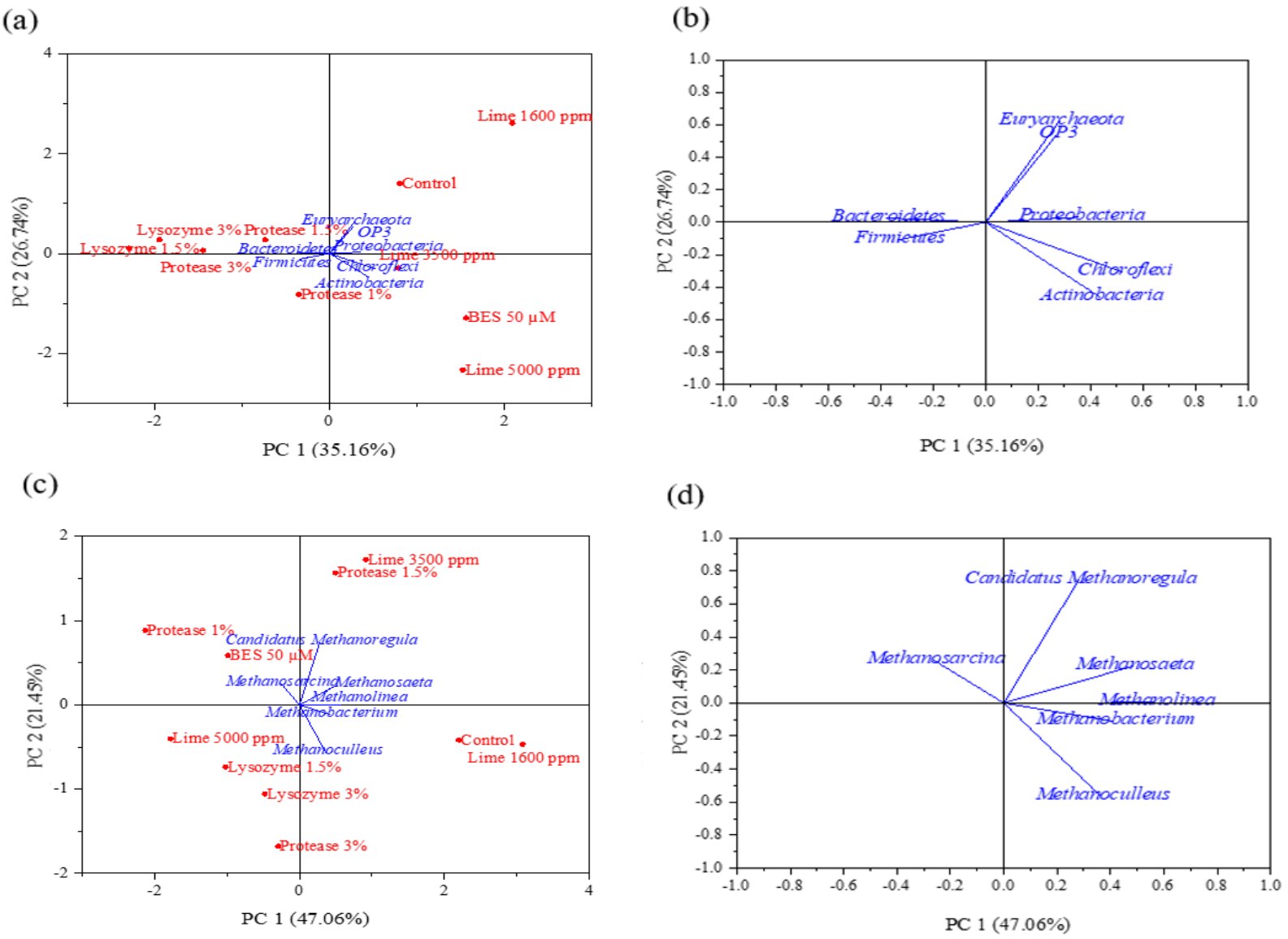

Figure 5.11. PCA loading plot and biplot between different treatment conditions and abundances of the microbial communities at the (a, b) phylum levels, and (c, d) archaeal genera level.

5.3.6 Significance of Results

Despite the effectiveness of enzymes (e.g., protease) in inhibiting methanogenic activity, due to their high costs as compared to lime the feasibility of enzymatic treatment is questionable (Cammarota & Freire, 2006; Ramkumar et al., 2016; Silvetti et al., 2017). However, there has been considerable research on the low-cost production of various enzymes for different industrial applications (including wastewater treatment), which may make enzymatic treatment economically sound in the near future (Cammarota & Freire, 2006; Ramkumar et al., 2016; Silvetti et al., 2017). While this study focused on understanding the fundamental roles of enzymes and lime treatment in mitigating methane emission from oil sands tailings management, a detailed economic and life-cycle assessment in future research would be critical for selecting the most viable method.

5.4 Conclusions

In summary, 3% protease was the most effective for methanogenesis inhibition. Microcosm acidification (pH of 4.9 after week 23) due to VFAs generation in protease-treated tailings may have contributed to the methanogenic inhibition. Alternatively, the formation of toxic compounds, including high nitrogen content after protease treatment, could adversely affect the microorganisms. Among different lime doses, 5000 ppm resulted in the lowest CH_4 production, while enzyme treatments resulted in relatively less CH_4 production. All lime doses resulted in the lowest hydrocarbon degradation rates compared with the control and other treatment conditions. However, at the end of 23 weeks of

incubation, 5000 ppm lime provided the lowest toxicity (11.3 TU) to *V. fischeri* than enzyme treatments at higher doses (124.8 TU for protease 3% and 81.3 TU for lysozyme 3%). PCA analysis revealed that lysozyme 3%, protease 3%, and lime 5000 ppm have a weak association with archaeal abundance; therefore, these treatments inhibited CH_4 production. Further investigation is still warranted on the effect of enzyme doses and identifying the methanogenesis inhibition mechanisms.

5.5 References

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6 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions and significance of results

The key findings and significance of results from each experimental chapter are summarized below:

Chapter 3. Impact of lime treatment on tailings dewatering and cap water quality under an oil sands end pit lake scenario. As the first study to examine the effectiveness of lime treatment under an EPL scenario, generally, the performance metrics and indicative parameters revealed the role of lime addition towards the reclamations benefits within EPLs. Higher water recovery % from the FFT layer was achieved after using a high lime dose of 3500 ppm (i.e., pH > 11.5) compared with low lime doses and control. Lower alkalinity for the cap water was maintained at high lime doses of 3500 ppm, which achieved low alkalinity environment for an aquatic ecosystem in EPLs. Furthermore, cation exchange and clay settling occurred at high lime doses (pH > 11.5). In terms of microbial cell numbers in the underlying FFT, when pH > 10 (i.e., high lime doses 1600, 3500, and 4000 ppm), the microbial cell numbers in the underlying FFT impacted negatively. At high lime doses, minimal reduction of organics (PHC) compared with moderate reduction at a low lime dose (650 ppm). Lime treatment demonstrated high toxicity effects on *V. fischeri* in the FFT layer. The atmospheric CO_2 reduced the pH of the cap water over time due to the regeneration of the carbonate buffer. Hence it reduced the toxicity of the cap water. The study introduced the proof of concept for the lime treatment in EPL in order to proceed with larger-scale applications of lime treatment. The current findings assisted in the optimization of the lime treatment

with an understanding of the cation exchange occurrence during high lime dosage treatment. The study highlighted the associated risks for the effect of the movement of COPCs into the cap water and toward the aquatic life within an EPL scenario.

Chapter 4. Enzyme-assisted dewatering of oil sands tailings: Significance of water chemistry and biological activity. This was the first study to examine the effectiveness of enzymatic treatment in the oil sands sector to accelerate the dewatering of FFT. The dissolution of carbonate minerals during the release of methane gas increased ionic strength and decreased the diffuse double layer (DDL) of the FFT. A significant increase in water recovery from FFT using lysozyme was shown due to the increase in both ionic strength and zeta potential and the decrease in DDL thickness. The link between using enzymes (i.e., cellulase and lysozyme) and the ebullition of methane gas resulting from the methanogenic activity were illustrated, which created pathways for the trapped water release. Lysozyme was the most effective in charge neutralization of clay particles, which could be attributed to the positive charge carried by lysozyme under pH conditions of tailings (pH< 7.5). Lysozyme treatment reduced the NAFC concentrations in pore water. However, the released pore water from the lysozyme treatment had the highest toxicity compared with the other enzyme treatments. The study presented for the first time the concept of the enzymatic treatment in oil sands applications. The conclusions from the work endorsed the enzymatic treatment as a promising method for the dewatering of the oil sands during lysozyme treatment. The study highlighted the associated risks for the increase of toxicity in pore water released from the enzymatic treatment due to the release of organic fractions.

Chapter 5. Significance of enzyme and lime treatment in the mitigation of methane emissions from oil sands tailings. This study examined the mitigation of methane emissions from oil sands tailings using lime and enzymatic treatment. Increasing the enzyme dose (i.e., lysozyme and protease) to 3% suppressed the CH_4 production and reduced the degradation of hexane compared with lime treatment. Protease treatment caused a reduction in the pH below 5.0, which promotes the formation of VFA and reduce the CH_4 production. Another potential mechanism could be the elevated nitrogen levels (toxic compounds) after protease treatment, affecting the microbial community. Lime treatment demonstrated a low toxicity effect on *V. fischeri* compared with other enzymatic treatment. All lime doses illustrated the lowest degradation of hydrocarbons and highest reduction in 16S rRNA and archaeal gene copies compared with control and other treatment conditions. The PCA analysis identified a feeble association with the archaeal abundance for lysozyme 3%, protease 3%, and lime 5000 ppm. Therefore, an inhibition effect for the CH_4 production was embraced by the lysozyme 3%, protease 3%, and lime 5000 ppm. For the first time the concept of the mitigation of methane emissions from oil sands tailings using the enzymatic and lime treatment was presented. The study emphasized the associated benefits for the enzymatic treatment on the inhibition of CH_4 production. The outcomes from this research work recommended applying lysozyme 3%, protease 3%, and lime 5000 ppm for the inhibition of CH_4 production.

6.2 Recommendations

Based on the results, some recommendations for future work are summarized as follows:

1. **Impact of lime treatment on tailings dewatering and cap water quality under an oil sands end pit lake scenario:**

- Future work is warranted to optimize the lime treatment and the occurrence of cation exchange in the EPL scenario.
- Monitoring EPL systems and the levels of contamination in cap water layer associated with the movement of COPCs will help in understanding the fate and transport of such contaminants and will support establishing an inclusive standard that reflect on the influenced environment characteristics, intended water usage, and reclaim substitutes.
- Oil Sands Industrial companies and researchers are advised to report the mine closure plan results, commissioned EPLs and EPL piloting's studies (Syncrude BML) of their monitoring programs for EPLs to verify the EPL cap layer water chemistry and the movement of COPCs mechanism to well derive a controlled condition understanding for EPL approaches.

2. **Enzyme-assisted dewatering of oil sands tailings: Significance of water chemistry and biological activity:**

- Further investigation is still warranted to investigate the release of organic fractions from FFT and their associated potential risks.
- Future studies that consider different oil sands tailings with different characteristics and higher/lower solid content, organics load such as NAFCs and PHCs would evaluate the capability of the enzyme dosage and demonstrate and quantify the individual contribution of microbial community species with

regards to the enzyme treatment and biodegradation process to the overall dewatering.

3. **Significance of enzyme and lime treatment in the mitigation of methane emissions from oil sands tailings:**

- Future studies that include different tailings sources and age with higher loads of PHCs would evaluate and quantify the capability of enzyme treatment in association with the archaeal abundance, and the individual contribution of the various species to the inhibition of GHGs and to the overall treatment.
- In comparison to single enzyme, a combination of two or multi enzyme assisted process may offer a distinctive collaborative advantage to inhibit the GHGs which should be further examined.
- It could be beneficial to further investigate ways to further enrich the enzymes' role and the enzymes performance in an EPL system/scenario and evaluate the impact of enzyme treatment on improving the inhibition mechanism in EPL.

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APPENDIX A. STANDARD CURVES

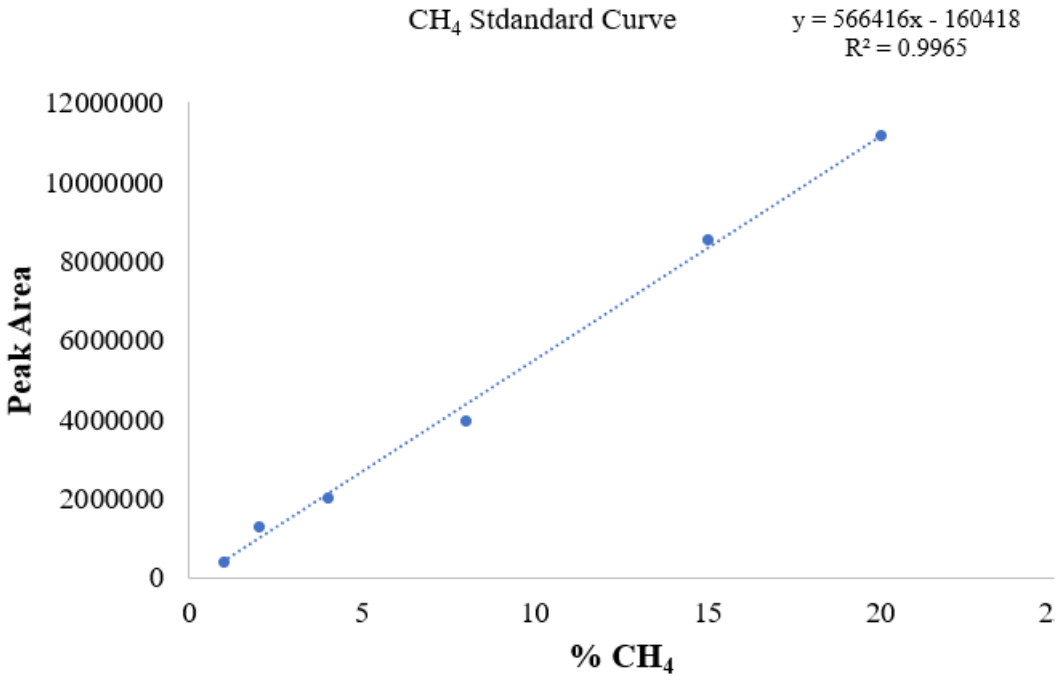

Figure A1. Methane determination calibration curve using standards.

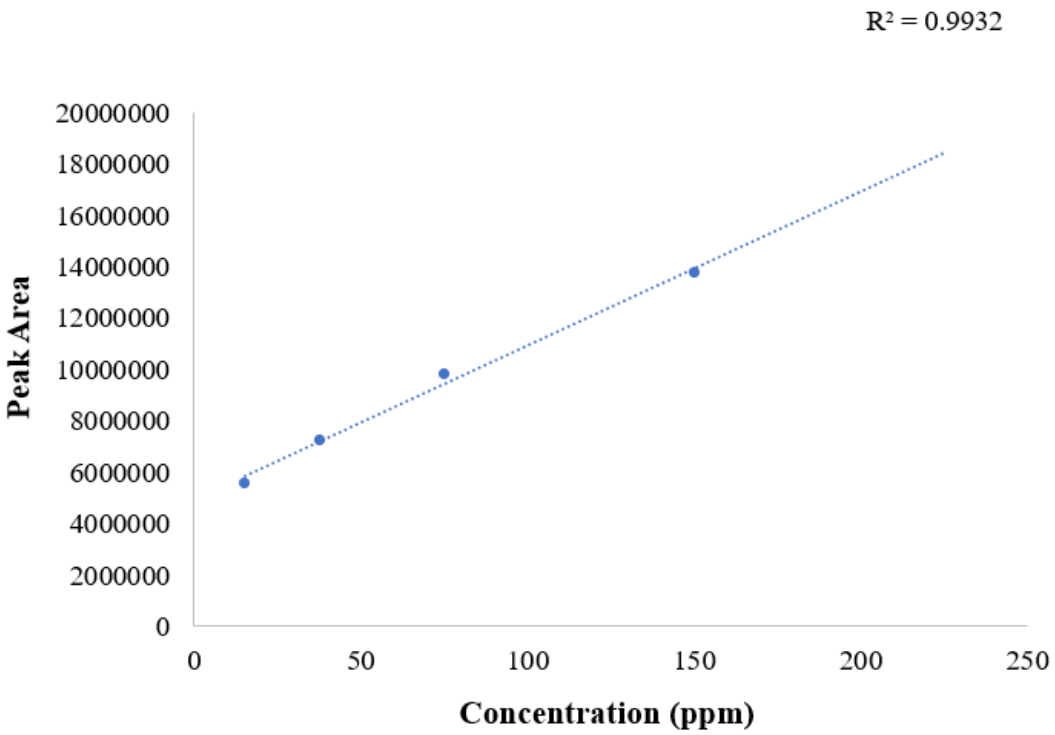

Figure A2. Toluene determination calibration curve using standards.

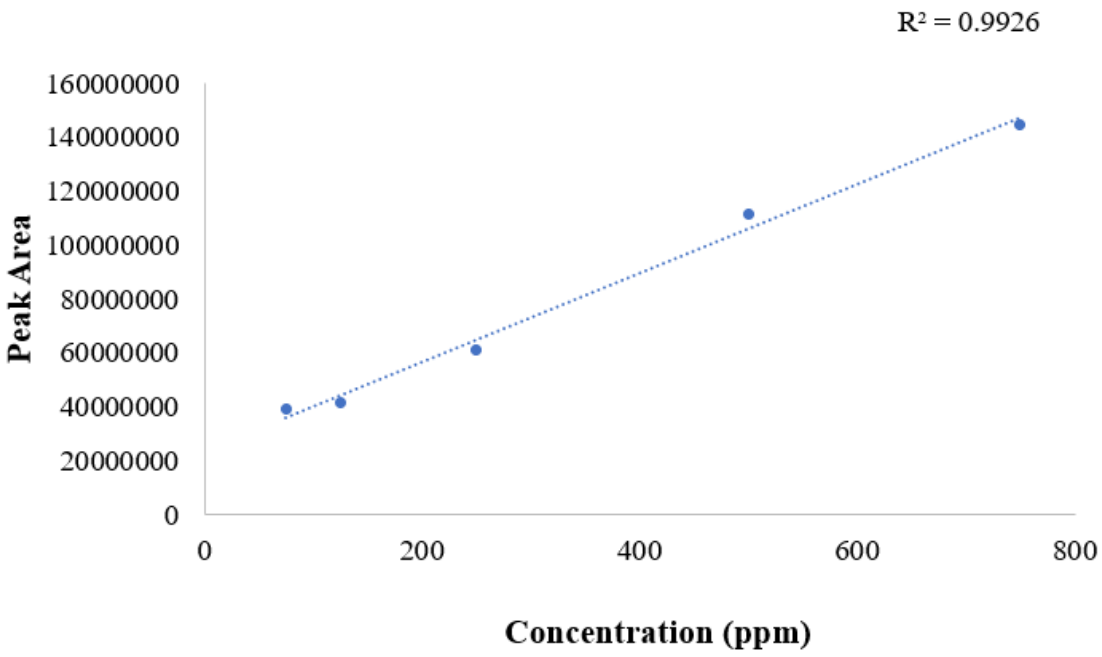

Figure A3. Hexane determination calibration curve using standards.

APPENDIX B. SUPPLEMENTARY INFORMATION

Table B1. Methane production/ concentration in (mmol) throughout the experiment for all treatment conditions, control/untreated FFT and negative control 2-Bromoethanesulfonate (BES).

		METHANE (MMOL)												
WEEK		0	1	3	5	7	9	11	13	15	17	19	21	23
PROTEASE 1%	P1	0.8	1.1	1.1	1.1	1.2	1.1	1.1	1.0	1.0	1.1	1.1	1.1	1.4
	P2	0.8	1.5	1.5	1.5	1.4	1.5	1.3	1.3	1.3	1.5	1.2	0.0	1.5
	P3	0.9	1.2	1.4	1.4	1.3	1.2	1.2	1.2	1.1	1.4	1.0	0.0	1.0
PROTEASE 1.5%	P4	1.2	1.9	2.1	2.1	2.0	2.0	1.8	1.7	1.8	2.0	1.8	1.7	1.5
	P5	1.2	1.7	2.0	2.1	2.0	2.0	1.8	1.6	1.6	2.0	1.6	0.0	1.3
	P6	1.2	1.3	1.4	1.6	1.5	1.6	1.4	1.4	1.3	1.4	1.2	0.0	1.3
PROTEASE 3%	P7	1.9	2.2	1.8	2.1	2.0	1.8	1.5	1.4	1.4	1.6	1.3	1.5	0.9
	P8	2.2	1.6	1.8	2.0	1.8	1.8	1.6	0.0	1.5	1.6	1.5	0.0	1.0

		METHANE (MMOL)												
WEEK		0	1	3	5	7	9	11	13	15	17	19	21	23
	P9	2.5	2.4	2.2	2.5	1.9	2.2	1.4	0.1	1.3	1.9	1.7	0.0	2.0
LYSOZYME 1.5%	LZ1	1.6	2.1	2.1	2.1	2.3	2.6	2.5	3.0	2.4	3.6	2.7	2.9	2.2
	LZ2	1.6	1.8	1.8	2.2	2.1	2.1	2.1	2.6	2.5	4.1	3.6	0.0	3.3
	LZ3	1.5	1.7	1.9	2.0	1.7	2.4	1.9	2.7	2.1	3.8	4.0	0.0	2.0
LYSOZYME 3%	LZ4	1.5	1.5	1.5	1.4	1.3	1.4	1.4	1.3	1.4	1.4	0.1	1.2	0.9
	LZ5	1.5	1.6	1.3	1.0	1.3	1.5	1.5	1.4	1.3	1.5	1.3	0.0	1.1
	LZ6	1.6	1.1	1.8	1.0	1.4	1.6	1.5	1.4	1.4	1.7	1.4	1.4	1.2
LIME 1600 PPM	LM1	1.2	1.3	1.6	1.5	1.3	1.4	1.3	1.3	1.1	1.4	1.1	1.1	1.2
	LM2	1.2	1.2	1.3	1.3	1.1	1.3	1.1	11.1	1.2	1.4	1.1	1.1	1.0

		METHANE (MMOL)												
WEEK		0	1	3	5	7	9	11	13	15	17	19	21	23
	LM3	1.2	0.9	0.8	0.8	0.8	0.9	0.8	0.9	0.8	0.3	0.2	0.3	0.8
LIME 3500 PPM	LM4	1.5	1.4	1.6	1.6	1.5	1.5	1.3	1.4	1.2	1.5	1.4	1.3	1.3
	LM5	1.3	1.3	1.2	1.2	1.1	1.1	0.9	1.0	0.1	1.0	0.8	0.8	0.7
	LM6	1.1	1.1	1.3	1.0	1.0	0.9	0.8	1.0	0.1	1.1	1.2	0.8	0.7
LIME 5000 PPM	LM7	1.7	1.6	2.2	1.9	1.9	1.7	1.5	1.5	0.1	1.8	0.1	0.0	0.1
	LM8	2.0	2.0	2.1	2.2	2.1	1.7	1.5	1.8	0.1	1.9	1.6	0.0	1.4
	LM9	1.8	1.8	1.5	1.8	1.8	1.5	1.7	1.5	0.1	1.8	1.5	1.3	1.3
BES 50 UMOL/L	BES 1	0.6	0.7	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.1	0.0	0.7
	BES 2	0.5	0.6	1.1	1.0	1.0	0.9	0.9	0.9	0.9	1.1	0.9	0.9	0.8

METHANE (MMOL)

WEEK		0	1	3	5	7	9	11	13	15	17	19	21	23
	BES 3	0.7	0.6	0.8	0.8	0.8	0.7	0.7	0.7	0.6	0.8	0.6	0.0	0.6
CONTROL	Control 1	1.4	2.0	1.9	1.9	2.4	2.5	2.6	2.6	2.7	2.7	2.8	2.7	2.1
	Control 2	1.5	1.4	2.0	2.1	2.1	2.6	2.6	2.7	2.7	2.6	2.7	2.7	1.8
	Control 3	1.5	2.1	1.8	2.0	2.4	2.5	2.7	2.7	2.7	2.8	2.7	2.6	1.8

Figure B1. Pressure filter (OFITE; Bench-Mount Filter Press with Hose and Regulator; #140-31) used at Chapter 3 to increase the solids content of raw FFT to 50% (wt./wt.) at 100 psi.

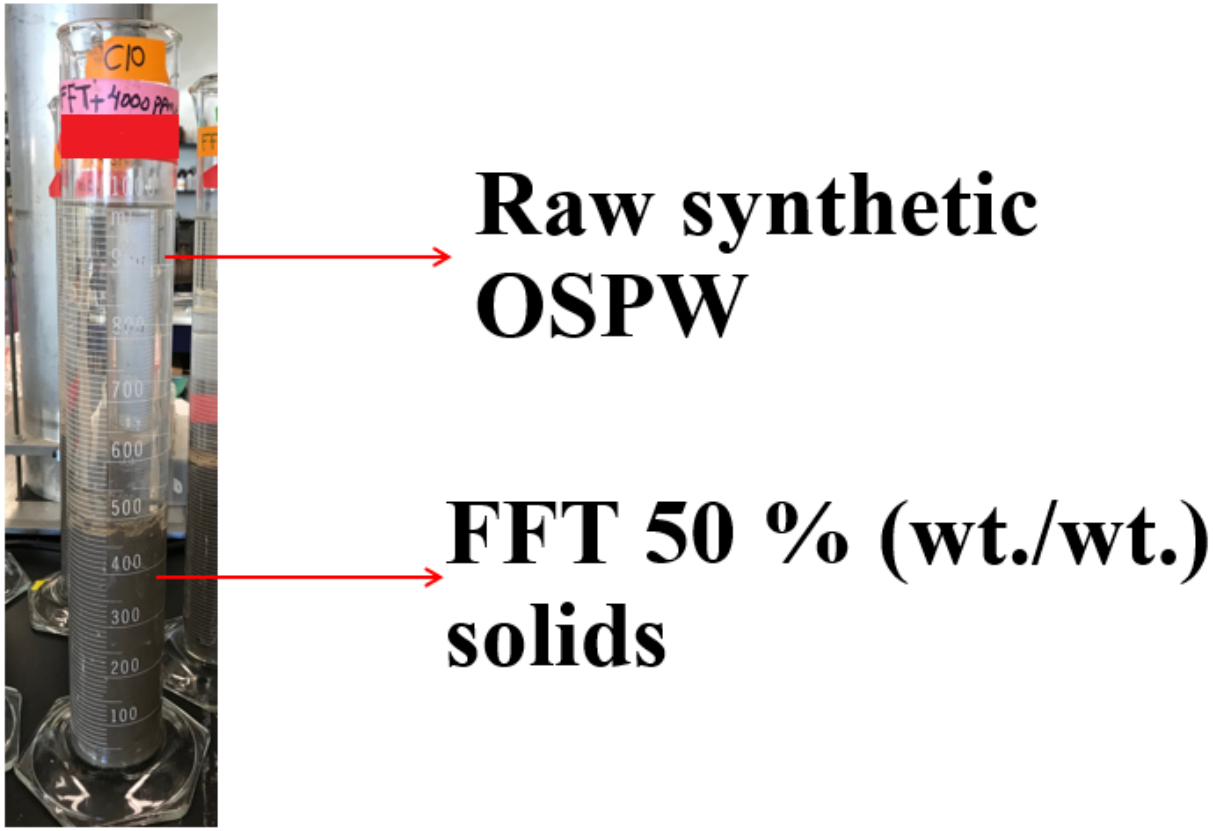

Figure B2. The experimental set-up for Chapter 3 consists of 500 ml lime treated or untreated FFT (50% solids) covered with 500 ml Raw synthetic OSPW. All columns were open to the atmosphere.

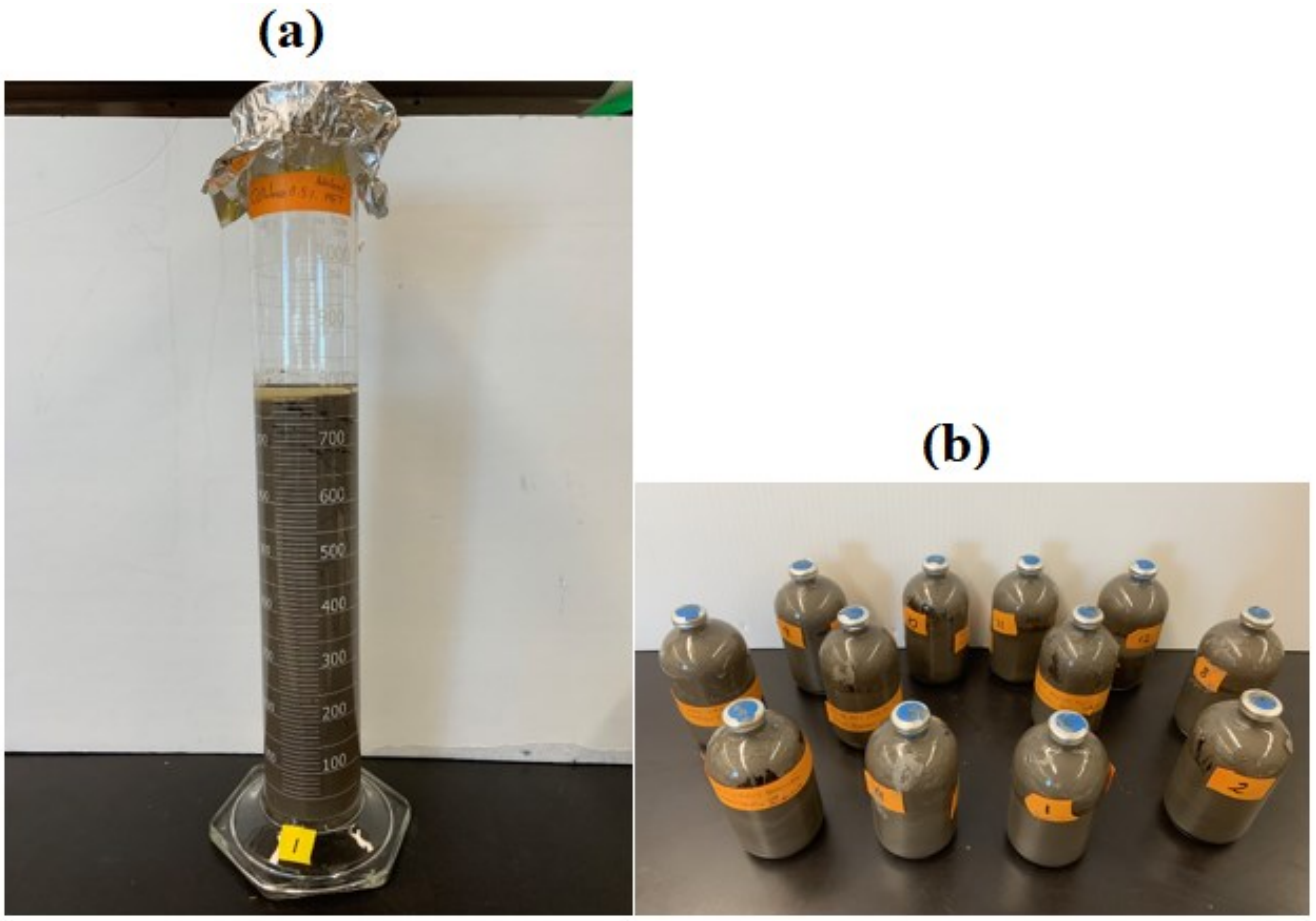

Figure B3. The experimental set-up for Chapter 4, (a) 1L column consists of 800 ml treated or untreated FFT and sealed with parafilm and aluminum foil. (b) 158 mL microcosms consist of 75 mL of FFT and 25 mL of anaerobic media.

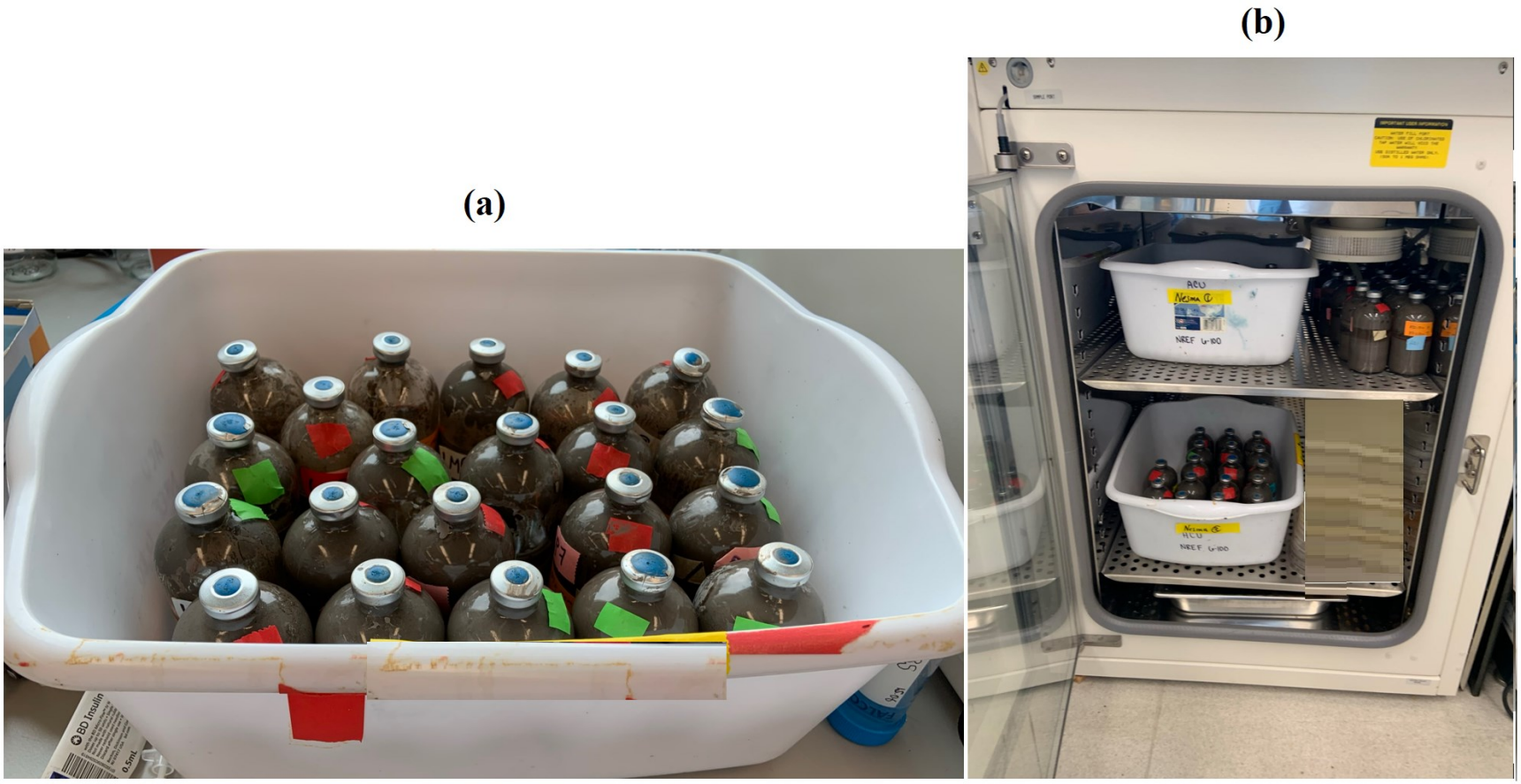

Figure B4. The experimental set-up for Chapter 5, (a) 158 mL microcosms consists of 65 mL FFT and 35 mL of anaerobic media control, treated with enzyme, lime or BES, (b) All the microcosms incubated at 30 ºC incubator in dark conditions.