HYPERSALINE POTASH MINE TAILINGS AND BRINE: MICROBIAL COMMUNITIES AND METAL BIOSORPTION APPLICATIONS

A Thesis Submitted to the College of

Graduate and Postdoctoral Studies

in Partial Fulfillment of the Requirements

for the Degree of Master of Science

in the Department of Civil, Geological, and Environmental Engineering

University of Saskatchewan

Saskatoon, Canada

By

Nicola Janz Harris

PERMISSION TO USE

In presenting this thesis/dissertation in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis/dissertation in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis/dissertation work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis/dissertation or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis/dissertation.

DISCLAIMER

Reference in this thesis/dissertation to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its endorsement, recommendation, or favoring by the University of Saskatchewan. The views and opinions of the author expressed herein do not state or reflect those of the University of Saskatchewan, and shall not be used for advertising or product endorsement purposes.

Requests for permission to copy or to make other uses of materials in this thesis/dissertation in whole or part should be addressed to:

Head of the Department of Civil, Geological, and Environmental Engineering
University of Saskatchewan
57 Campus Drive
Saskatoon, Saskatchewan
S7N 5A9 Canada

ABSTRACT

Brine and tailings produced by potash mining operations create hypersaline environments where only highly salt-tolerant organisms are capable of living – generally microbes. Microbial communities within analogous hypersaline environments such as salterns, evaporite deposits, and salt lakes have been characterized in the peer-reviewed literature and individual organisms have been used for various applications in biotechnology such as in cosmetics or pharmaceuticals. Bacterial biomass has also been broadly investigated as a metal biosorbent. However, microbial communities in potash mine tailings and brine and their potential applications in environmental technology has not been extensively studied. These unique microbial communities and biomaterials may offer new ways to manage industrial wastes or remediate contaminated sites under highly saline conditions.

In this thesis, the microbial communities within brine, coarse tailings, and fine tailings from a Saskatchewan potash mine were examined. Culture-independent high-throughput amplicon sequencing of the 16S rRNA gene (V4 region) and culture-dependent plating techniques were employed to examine community compositions and salinity tolerance. The brine and tailings materials were all pH neutral, sodium-dominated, and highly saline (370 g/l for brine and > 835 g/kg for tailings). High-throughput sequencing results (206164 total reads) identified a mixed community of archaea and bacteria within the brine pond sample, and bacterially dominated communities in the coarse and fine tailings. *Proteobacteria* were the most predominant phylum for all samples, making up 41-89% of subsampled sequences, and included high read counts in both classes *Gammaproteobacteria* and *Betaproteobacteria*. Twenty-two unique isolates that were relatives of genera observed in the high-throughput sequencing results were identified from spread plates. Isolates included known halophilic and halotolerant Archaea (*Haloferax* and *Halorubrum* species) and Bacteria (including *Halomonas*, *Marinobacter*, and *Dietzia* species). Salt tolerance of 0-25% (w/v) NaCl was demonstrated by 13 of the isolates, while all isolates were capable of growth in the presence of at least 15% (w/v) NaCl.

The halotolerant bacterial isolate *Croceicoccus* sp. FTI14, selected for its fast growth in 3% (w/v) NaCl amended media, was evaluated as a potential biosorbent for the removal of dissolved Cu(II) and Cr(VI) from saline groundwater (0.55 M ionic strength). Biosorption performance of the oven-dried and finely ground material was evaluated using batch biosorption experiments at varied ionic strengths, coupled with synchrotron-based scanning X-ray transmission microscopy (STXM) and Fourier Transform Infrared (FTIR) spectroscopy. Cu(II) uptake by dried FTI14 was 1.7-7.8 times higher than Cr(VI) uptake and metal uptake decreased when ionic strength of the solution was increased. At pH 4-5 and with 40 mg/l initial metal concentrations, FTI14 removed 40.3 ± 0.7% of the dissolved Cu(II) from deionized water and 19.3 ± 0.1% from saline groundwater solutions. Biosorption isotherms for Cu(II) fit both Langmuir (R^2 values of at least 0.80) and Freundlich models (R^2 values of at least 0.86), while the Cr(VI) isotherm fit the Freundlich model only (R^2 value = 0.97). STXM images showed that the adsorbent was a mixture of whole cells and indistinct biomass as well as demonstrated a spatial association between metal and biomass. FTIR spectra data suggested a change in amide functional groups, potentially the proteins on the biomass surface, after metal exposure. Findings suggest that the removal of metals from salt-impacted water is possible using biosorbents derived from salt-tolerant bacteria.

This is the first study to utilize high throughput sequencing to investigate the membership and diversity of microbial communities in potash tailings and brine. It contributes to the broader understanding of halophilic and halotolerant microbes in natural and engineered environments, as well as investigates a potential environmental engineering application of biomaterials derived from them.

Keywords: halophile, halotolerance, hypersaline environment, microbial community, potash tailings, potash brine, biosorption, metals, salinity, contaminated groundwater, remediation

ACKNOWLEDGEMENTS

First and foremost, I thank my supervisors Dr. Wonjae Chang and Dr. Joyce McBeth for giving me the opportunity to work on this project, their always-open doors, and their guidance through graduate studies.

I am very grateful for my committee members, Dr. Terry Fonstad and Dr. Catherine Niu, and my external reviewer, Dr. Darren Korber, whose expertise and input were irreplaceable. Thank you to Jay Dynes for his proficiency and guidance in the use of the STXM beamline at the Canadian Light Source.

Thank you to Al Shpyth, Richard Weishaupt, Kathlene Jacobson, Jeff Meadows, Mark Getzlaf, and Wayne Brownlee for their technical expertise and to Cory Laferriere for his guidance during my internship at Agrium VPO.

I would also like to thank the Natural Sciences and Engineering Research Council of Canada (NSERC), the International Minerals Innovation Institute (IMII), Mitacs Accelerate Cluster, The Mosaic Company, Potash Corporation, and Agrium Inc. for providing the principal funding for this study. Additional thanks to the College of Engineering Graduate Research Fellowship at the University of Saskatchewan for further financial support.

Thanks to my lab mates, colleagues, and friends for their encouragement and help. Also, to Jarvis and the Canadian Light Source for letting me use the life sciences lab and to Helen for all her help in the Environmental Engineering lab. And finally, thank you to Matt for supporting me every step of the way.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS & SYMBOLS

Å	Angstrom
AAS	Atomic absorption spectrophotometer
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
B	Langmuir constant
CFU	Colony forming units
c_i	Ion concentration
C_f	Final concentration (mg/l)
C_o	Initial concentration (mg/l)
CLS	Canadian Light Source
CMCF-BM	Canadian Macromolecular Crystallography Facility bending magnet beamline
COD	Crystallography Open Database
DI	Deionized water
DNA	Deoxyribonucleic acid
E	Empfindung
eV	Electron volt
FTIR	Fourier transform infrared
GW	Groundwater
GW+Na	Saline groundwater (0.55 M ionic strength)
I	Ionic strength
k	Freundlich constant
ICP-OES	Inductively coupled plasma optical emission spectroscopy
ICP-MS	Inductively coupled plasma mass spectrometry
M	Mass (g)
meq	Milliequivalents

n	Affinity constant
NB	Nutrient broth
OD	Optical density
OTU	Operational taxonomic units
PCR	Polymerase chain reaction
q	Solute uptake (mg/g)
q_{max}	Maximum uptake (mg/g)
R-2A	Reasoner's 2A
RDP	Ribosomal Database Project
rRNA	Ribosomal ribonucleic acid
SRC	Saskatchewan Research Council
STXM	Scanning transmission x-ray microscope
TEM	Transmission electron microscopy
TMA	Tailings management area
V_f	Final volume (l)
V_o	Initial volume (l)
z_i	valency

1 GENERAL INTRODUCTION

The responsible management of mining by-products is essential to achieve sustainability in the Canadian mining industry. Physicochemical properties of industrial effluents and solid tailings are often well-characterized and regulated to prevent introduction into natural, recreational, or drinking water systems. However, the microbiology of these materials is often overlooked. The microbes in tailings or mine water can sometimes be detrimental if released to the environment, but more often they present an opportunity for controlling the transport and fate of inorganic and organic contaminants. To seize this opportunity, the microbial species present in these materials first needs to be identified, and then control and/or use of the community or specific species can be considered.

Canada was the largest potash producer and exporter in the world in 2015, with ten Saskatchewan mines producing the potassium-containing salts used as fertilizer (Jasinski, 2014; Marshall, 2015). The tailings produced by potash mining and processing are composed mostly of NaCl, KCl, and $MgCl_2$ (Tallin et al., 1990), and the retention ponds for brine in tailings management areas can have a sum of ions on the order of 350 g/l due to run-off from tailings piles (Maathuis & Van der Kamp, 2002). When at saturation concentrations, salts are lethal for all but a few biological species – microorganisms in particular – and organisms that are able to live in extreme environments such as potash tailings and brine are considered extremophiles (Gupta et al., 2014).

Other environments that contain similarly high salt concentrations include salterns, evaporite deposits, and salt lakes, and studies of these environments have revealed surprisingly diverse communities of microorganisms (DasSarma & DasSarma, 2012). A characterization of potash brine and tailings that includes the microbiology will contribute to the understanding of microbial communities in highly saline environments as well as provide the potash mining industry with a better picture of their mine tailings management areas. However, little attention has been paid to the microbial communities in Saskatchewan's potash brine and tailings and the associated biotechnology-based research for potential applications of these extremophiles.

Actual and suggested engineering applications of biomaterials derived from extremophiles include water and waste treatment, energy production, biodegradable plastics production, and use as pharmaceutical intermediates (Margesin & Schinner, 2001). Microbes found in potash tailings or brine could potentially be used as metal adsorbents in water treatment, such as the removal of metals from industrial-contaminated groundwater. Biological material contains unique surface molecules, such as peptidoglycan or enzymes, that are able to attract and react with metal ions in solution, enabling microbes to sorb various micronutrients from their environment and contributing to metal fate and transport (Ams et al., 2013). This mechanism is effective in metabolically-inactive or heat-killed organisms which can be used as biosorbents for the treatment of metal-contaminated waters, an application that has been demonstrated with many types of biological material including algae, bacteria, fungi (Volesky & Holan, 1995), and food processing waste such as crab shells (Niu et al., 2007). Some of these materials have shown excellent sorption or ion exchange capacity, comparable with commercial synthetic cation exchange resins (Ahluwalia & Goyal, 2007) and often perform better than traditional sorbents (Gabr et al., 2008).

It has been suggested that the adsorption of metals from saline systems by organisms adapted to living in hypersaline environments is greater than adsorption from saline systems by non-halophiles (Ams et al., 2013). A biosorbent developed from potash tailings or brine could offer an alternative remediation technology for the removal of metals from saline water and could be potentially used in groundwater pump and treat operations, or in industrial or other wastewater treatment plants.

1.1 Research Objectives

The microbial community in potash brine and tailings is largely unknown and the main purpose of this study is to identify the bacteria and archaea present in these communities. A secondary objective of the study is to evaluate the development of isolated halotolerant microbes as biosorbents for metal remediation in salt-impacted water, an understudied area of the biosorption literature. The use of cutting-edge analytical tools, including high-throughput amplicon sequencing and scanning transmission x-ray microscope (STXM) imaging, will enable in-depth analysis of microbial communities and the potential biosorbent.

The specific objectives of this study include the following:

1. Identify microbial community.

a. Compare the microbial communities in potash mine brine, coarse tailings, and fine tailings
b. Isolate and characterize heterotrophic, aerobic microbes from these materials
c. Explore how the microbiology relates to chemical compositions of potash brine and tailings

2. Assess feasibility of halotolerant microbes as a biosorbent.
    a. Prepare a halotolerant or halophilic isolate as adsorbent
    b. Evaluate effectiveness of biosorbent for metal removal under saline and non-saline conditions using batch adsorption experiments coupled with STXM and Fourier Transform Infrared (FTIR) spectra

1.2 Research Significance

This study examines the microbial community within potash tailings and brine, an environment that has only been previously described using isolation techniques. The use of high-throughput amplicon sequencing allows for a snapshot of the entire community, including those microbes that are difficult to isolate. Information obtained from community analysis lays the groundwork for further metabolic characterization, biogeochemical studies, and development of biotechnological applications. This benefits the scientific community by contributing to the growing understanding of extreme halophile distribution in natural and engineered environments.

The study goes on to investigate the potential use of this waste microbial community in a biotechnological application: biosorption of metals. It contributes to biosorption research by looking at adsorption of metals in natural groundwater and salt-impacted groundwater, as well as by using a halotolerant organism in a salt-impacted system – something that has not been focussed on in peer-reviewed literature. It benefits the potash industry by suggesting a means to repurpose a process by-product for alternative uses. As well, biosorption in salt-impacted water benefits many industries that have concerns for the co-occurrence of salt and metal contamination of waterways.

1.3 Scope

Brine, fine tailings, and coarse tailings were chosen for study as key representatives of the by-products produced by the potash milling process. A sample of each was provided by an active

potash mine site in Saskatchewan, Canada. Physicochemical studies throughout this thesis focused on salinity, measured as total salinity or ionic strength. Microbial community studies focused on the 16S rRNA gene (V4 region) and the study of isolated microbes was limited to readily culturable, heterotrophic, and aerobic species from brine and tailings.

Adsorption studies focused on the batch biosorption of copper and hexavalent chromium, examples of metals that are commonly found in industrial wastes and represent both cationic and anionic metal species. Ionic strength was adjusted using sodium chloride and the effect of different ions was not studied.

1.4 Thesis Organization

This document is written as a manuscript-style thesis according to the guidelines established by the College of Graduate Studies and Research and the Department of Civil, Geological, and Environmental Engineering at the University of Saskatchewan. It includes a general introduction (Chapter 1), literature review (Chapter 2), two manuscripts (Chapters 3 and 4), and overall conclusions and recommendations for future research (Chapter 5). The first manuscript is fundamental research, describing the microbial community within potash tailings and brine. It has been adapted and reformatted from a manuscript that will be submitted for publication. The second manuscript is applied research, where an isolate from potash tailings was developed as a metal biosorbent for salt-impacted groundwater remediation, and will be submitted for publication as part of a larger project.

2 LITERATURE REVIEW

2.1 Potash Mining

2.1.1 Potash mine and tailings management area

Potash mining in a conventional mine occurs approximately 1000-1600 m underground, where the temperature remains a constant +27°C. The milling process was described in detail by Perucca (2003) and is summarized here (Figure 2-1). Large pieces of ore are removed from the ore bed using boring machines and are stored underground until needed for processing. Once brought to the surface, the milling process begins by mechanically crushing the ore into smaller pieces that are then scrubbed in agitation tanks with a brine solution to remove clay insolubles. The potash is separated from the sodium chloride by flotation using organic reagents, then skimmed from the top of the flotation solution and centrifuged. The refined potash product is then fire-dried and sized before sale. The remaining salty tailings, insolubles, and brine water are disposed of in piles on the mine site in the tailings management area (TMA, Figure 2-2). Liquids in the TMA drain into brine ponds adjacent to these piles (UNEP, 2001). The term brine is broadly used to describe water with high concentrations of salt and the brine ponds in the potash industry can reach concentrations of approximately 350 g/l (Maathuis & Van der Kamp, 2002).

The high salinity – a measure of the total ionic composition of water – of potash mine surface and subsurface environments makes them extreme habitats where only highly-adapted microbes such as halophilic bacteria can survive. The potash extraction and refinement process is not sterile and microbes can be introduced to the system at each step of potash processing, however sources of halophilic organisms will contribute to the metabolically-active portion of the microbial community. These may include the ore bed, organic chemicals used in potash flotation (Robbins & Ingledew, 1976), atmospheric deposition including windblown dust, avian carriers, and soils in contact with the tailings piles and brine ponds and ditches. In addition to the inhospitable salinity levels, there are few sources of carbon, nitrogen, and the other nutrients needed to support

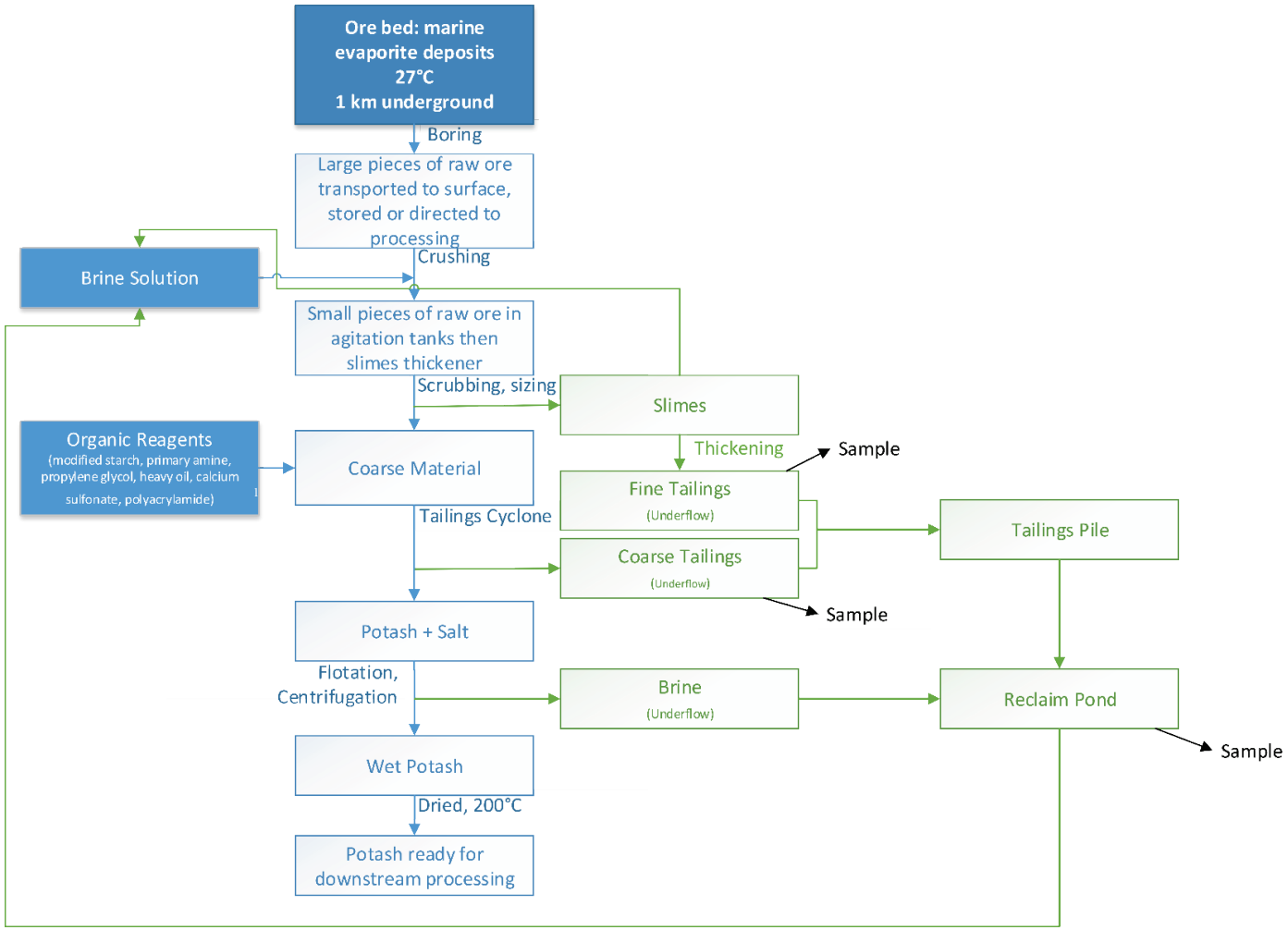


Figure 2-1 Schematic drawing of potash extraction and refining process for the Mosaic Colonsay conventional potash mine. The sampling points of fine and coarse tailings, as well as reclaim pond water, were used for physicochemical and microbial community characterizations in this study. [1] (Perucca, 2003)

Figure 2-2 Tailings piles at the PotashCorp (PCS) Cory mine site in Cory, Saskatchewan, on the order of 30 m high.

microbial life in potash brine and tailings. Potential organic carbon and nitrogen sources include the evaporite bed and chemical reagents used in the refinement processes.

2.1.2 Analogous saline environments

Hypersaline environments are defined as having a dissolved salt concentration higher than the salinity of seawater (35 g/l, DasSarma & DasSarma, 2012). Due to the high salt concentrations, these environments have several unique chemical characteristics. First, measurement of pH in high ionic strength solutions is complicated due to a lack of appropriate buffers and electrodes (Ams et al., 2013). In fresh water, the pH of a solution is often used to indicate the alkalinity (the measure of compounds in solution that can neutralize an acid) of a sample. However, when salinity is higher than 50 g/l, it no longer indicates the alkalinity as at this point the pH will decrease with increasing salinity and in no correlation to the alkalinity (Javor, 1989). Alkalinity must be measured as total alkalinity or by acid titration which will overcome the effects of inaccurate pH readings or ion complexing resulting from the high concentration of ions. The salt concentration also affects the specific heat capacity and concentration of dissolved gases, both of which decrease with increasing

salt concentration. This means that a saline environment will be on average warmer and contain less dissolved oxygen than a freshwater counterpart.

Studies of hypersaline environments have shown examples of thriving species from all domains of life. Eukaryotic microorganisms are not very common at high salinities, but the green-alga *Dunaliella* can still be found in diverse hypersaline environments including the harsh conditions of the Dead Sea (Oren, 2002) and the black yeast *Hortaea werneckii* can grow over a full range of NaCl concentrations from 0 g/l up to 321 g/l (Lenassi et al., 2007). Archaea tend to dominate the microbial community in solar salterns at salinities above 230 g/l but Bacteria have quite a few representatives across hypersaline environments as well (DasSarma & DasSarma, 2012). These communities can vary greatly geographically even when environments with similar ionic composition are compared (Vreeland, 2012). An example of a common halophilic bacterium in these environments is *Salinibacter ruber*, whose highly-conserved gene sequences have been recovered from various salterns and natural salt lakes world-wide (Vreeland, 2012).

2.2 Salt-Adapted Microorganisms

2.2.1 Halophilic and halotolerant bacteria and archaea

Halotolerant or halophilic organisms are able to live in environments with high salinity. They are extremophiles – organisms that are found in extreme or harsh environments – and are specially-adapted to live in environments with high salt concentrations. The distinction between halotolerant and halophilic organisms is that the former are able to grow in a broad range of salinities, while the latter require high salinity for growth (Golikowa, 1930; Rubentschik, 1929). Non-halophiles grow best at salinities less than 20 g/l, and halophiles are further characterized as being slight, moderate, and extreme halophiles, growing in salinities of 20-50 g/l, 50-200 g/l, and greater than 200 g/l, respectively (Larsen, 1962).

High salt concentrations create a particularly harsh environment for microbes, producing high osmotic pressure in the cells; they are considered to be toxic to most forms of life (Gunde-Cimerman et al., 2009). Biological membranes are water permeable and so halophilic organisms need to maintain a cytoplasm that is at least isosmotic to the surrounding environment and low in toxic sodium ions (Oren, 2002). Two main strategies have been studied that allow microbes to maintain their cellular osmotic balance: the accumulation of K^+ or Cl^- ions, or the accumulation of organic solutes (Oren, 2002). The osmotic pressure affects the availability of usable water for these

organisms and the adaptations they employ to overcome it are of great interest for biotechnological studies.

Halophiles are metabolically diverse, with representative species of most metabolic processes (Oren, 2002). It is suggested that the processes that have not been observed in halophiles have energetic constraints with regards to the cost of maintaining an isosmotic cell matrix (Oren, 1999). Oxygenic photosynthesis, anoxygenic photosynthesis, aerobic respiration, and fermentation have all been observed in cultures with NaCl at saturation levels (approximately 300-350 g/l, Oren, 2002) and as such may be possible metabolisms present in potash waste materials.

2.2.2 Applications for halophilic bacteria and archaea

The unique properties of halophilic organisms have started to be utilized in different applications of biotechnology (Table 2-1). Halophilic organisms have properties especially appealing to industries that need to deal with high salt concentrations, including the oil and gas sector, salterns, and the food industry. For example, the presence of *Halobacteriaceae* sp. is encouraged in saltern crystallizer ponds because they produce a carotenoid pigment that absorbs light and increases pond evaporation rates (Oren, 2002). Halophiles offer unique cellular features including unique proteins such as the polyhydroxyalkanoates produced by *Haloferax mediterranei* that can be used to make a new type of biodegradable plastic (Oren, 2002). Their ability to thrive in high salinity conditions has also made halophiles valuable in bioremediation processes for brine spills such as those encountered in the petroleum industry (DasSarma & DasSarma, 2012).

2.3 Biosorption

Biosorption is a physicochemical reaction relevant to biotechnology, especially for the remediation of dissolved contaminants from contaminated water. It employs physical or chemical reactions to bind a molecule from a gas or liquid phase onto the surface of solid biological material. The process is competitive in terms of cost and efficiency with more conventional adsorbents including activated carbon and ion exchange resins (Table 2-2) (Aryal & Liakopoulou-Kyriakides, 2015). Biomass is able to adsorb many metals, dyes, fluoride, pharmaceuticals, and organic contaminants (Michalak et al., 2013). For this thesis, the biosorption of metals by bacterial biomass was examined.

Table 2-1 Biotechnological applications for halophilic and halotolerant microbial species.

Product	Process	Species
β-carotene [a]	Food supplements: antioxidant and food coloring agent	*Dunaliella* sp.
Bacteriorhodopsin [a]	Computer memory and processing, photoelectric converters, etc.	*Halobacter salinarum*
Biosurfactants [b]	Microbially-enhanced oil recovery	*Bacillus licheniformis*
Carotenoid pigment [c]	Light absorption, increase in saltern evaporation	*Dunaliella* sp., *Halobacteriaceae* sp.
Cell biomass [a]	Additive in cosmetic anti-wrinkle cream	*Dunaliella* sp.
Chemical oxygen demand (COD) removal [b]	Biological waste treatment	*Halobacter halobium*
Ectoine, hydroxyectoine, and compatible solutes [a, b]	Biomolecule and cell stabilizers, salt antagonists, stress-protective agents, moisturizer in cosmetics	*Halomonas elongata, Desulfovibrio gigas, Clostridium pasteurianum, Marinococcus* M52
Exopolysaccharides [b]	Microbially-enhanced oil recovery	*Halobacterium salinarum, Haloferax volcanii, Halobacterium distributum, Halomonas sp.*
Heat shock proteins, gene transfer of halotolerance to plants [b]	Soil salinity issues	*Aphanothece halophytica*
Hydrocarbon, polycyclic aromatic hydrocarbon, halogenated organic compound breakdown [b]	Biological waste treatment	*Streptomyces albaxialis, Halomonas sp., Methylomicrobium sp., Rhodococcus rhodochrous, Brevibacterium sp.*
Hydrogen production [b]	Energy	Various photosynthetic bacteria
Hydrolase [b]	Enzymes used in cell study and biomolecule production	*Pseudomonas sp., Haloferax alicantei, Alteromonas sp., Alterococcus agarolyticus, Halobacterium salinarum, Bacillus sp.*
Ice-nucleation activity (INA) [b]	Artificial snow and ice production, ice cream production	*Pseudomonas syringae*

Product	Process	Species
Isomerase [b]	Enzymes used in cell study and biomolecule production	*Methanopyrus kandleri*
Liposomes [b]	Target-specific medicines or cosmetics	*Halobacterium eutirubrum*
Long-chain polyunsaturated fatty acids [b]	Food supplements	*Shewanella* sp., *Olwellia* sp.
Organophosphorus acid anhydrases [b]	Chemical warfare detoxification	*Alteromonas JD6.5*
Poly (γ-D-glutamic acid) [b]	Thickener, humectant, sustained-release, or drug carrier for food or pharmaceuticals	*Natrialba* sp.
Polyhydroxyalkanoates (PHA) [a]	Biodegradable plastics	*Haloferax mediterranei*
Salt sequestration via N-fixation [b]	Soil salinity issues	*Anabaena torulosa*
Soy sauce, fish sauce [b]	Fermentation	*Lactobacillus plantarum, Halobacterium salinarum, Halococcus* sp., *Bacillus* sp., *Tetragenococcus halophile*

[a] (Oren, 2002); [b] (Margesin & Schinner, 2001); [c] (Javor, 1989)

Biosorption can be more cost effective, environmentally friendly, and efficient than traditional metal-adsorbing materials as well as other methods for metal-removal including chemical precipitation and electrochemical techniques (Vijayaraghavan & Yun, 2008). For example, the algae *Sargassum natans* was found to have a binding capacity of 420 mg of gold per gram of biomass; in comparison, the binding capacity of a commercial ion exchange resin was approximately 350 mg per gram (Volesky & Kuyucak, 1988). While activated carbon in the same study had a binding capacity of close to 500 mg, it is much more expensive than the algae and cannot be regenerated for reuse.

2.3.1 Mechanism of metal biosorption

Due to the complex chemical nature of biomass, the mechanisms involved in binding metals are not always well-defined. Studies have suggested a variety of mechanisms including ion exchange, complexation, chelation, redox reactions, physical adsorption, and microprecipitation (Volesky, 2001). These reactions are driven by concentration gradients and high affinity binding sites on the biomass.

Depending on the mechanism of the specific system, the reverse reaction – desorption – is often possible after a material has removed a solute (Atkinson et al., 1998; Fomina & Gadd, 2014). The ability to desorb the contaminant from the biosorbent purposefully is advantageous; the user could concentrate the metal and either return it to the process or sell it for profit, and potentially reuse the biosorbent. The ideal recovery process would be highly efficient and cause minimal damage to the biosorbent. Strong acids, bases, and complexing agents have been employed to elute the metals after the sorption process (Wang & Chen, 2009).

Bioaccumulation

Biosorption is a metabolically-passive method to concentrate a substrate and should not be confused with the process of bioaccumulation where substrates are actively taken up by live biomass (Aryal & Liakopoulou-Kyriakides, 2015). Live biological species can be capable of sequestering a wide variety of heavy metals, but toxicity thresholds limit the maximum uptake. In comparison, inactive biomass material is limited by the availability of binding sites on the surface without a toxic concentration threshold. Further, there is more cost involved in bioaccumulation operations as nutrients and specific growth conditions need to be applied to the system

Table 2-2 Comparison of inorganic, organic, and biological adsorbent sources.

Type	Example	Uses	Advantages	Disadvantages
Inorganic				
Siliceous materials [a]	Silica beads, glass, dolomite	Desiccant, separation of hydrocarbons, cations	Abundant, low cost, high surface area, mechanical strength	pH <8 only, can have irreversible sorption processes
Activated Alumina (Al_2O_3) [b]	n/a	Catalyst (or catalyst support), desiccant, fluoride	High surface area, mechanical strength, can act as acid or base	Manufactured, high temperature pre-treatment
Aluminosilicates [a, c]	Clays, Zeolites	Cation sorption, ion exchange, heavy metals, phenols	Can be pH-independent, low cost, abundant, high sorption capacity, selective, mechanical strength	Low permeability
Organic				
Activated Carbon [a]	Charcoal, Biochar	Air particulates, water particulates, dyes, metals	Effective, large capacity, large surface area, chemical structure easily modified, well studied	Expensive, non-selective, regeneration difficult
Biological materials				
Wood Products [a, c, d]	Sawdust, bark	Anion sorption (metals and dyes), heavy metals, organic compounds	Abundant, low cost, basic binding sites, hydrophobic sites, ion exchange	pH-dependent, discoloration of water
Algae or Seaweed [c]	n/a	Heavy metals, dyes	Abundant, low cost, ion exchange, high sorption capacity	Used in other industries (competition), swelling and disintegration in column
Chitin and Chitosan [a, c]	Crab shells	Anion sorption (metals and dyes)	Abundant, low cost, contains high levels of –NH and –OH functional groups, chemical stability, highly-selective	High processing required, nonporous, pH dependent, processing creates toxic waste
Bacterial Cells [e]	n/a	Heavy metals, cation or anion sorption, dyes	Highly selective, works in low concentrations, available as industrial waste	Low mechanical strength, slow sorption process, pH-dependent, ionic strength-dependent

[a] (Crini, 2006); [b] (Naiya et al., 2009); [c] (Bailey et al., 1999); [d] (Li et al., 2010); [e] (Vijayaraghavan & Yun, 2008)

(Vijayaraghavan & Yun, 2008). Srinath et al. (2002) compared biosorption and bioaccumulation of hexavalent chromium (Cr(VI)) in *Bacillus* strains and found that they could adsorb a maximum of 39.9 mg Cr/g (dry weight) versus the maximum of 34.5 mg/g that live strains could accumulate. While these two values are similar, the bioaccumulation process required an additional step to dilute the Cr(VI) solution to an initial concentration of 50 mg Cr(VI)/l. In a similar study, *Pseudomonas aeruginosa* living cells were able to sequester 79 mg/g of lead and 70 mg/g of nickel, but their non-living counterparts were able to adsorb 123 mg/g and 113.6 mg/g respectively (Gabr et al., 2008).

2.3.2 Parameters affecting biosorption

To provide a proper analysis of a material's biosorption capabilities, adsorption characterization experiments need to operate under optimized conditions. Parameters that influence biosorption reactions include solution pH, temperature, other solutes, mixing rate, biosorbent particle size, and the nature of the reactive groups on the biosorbent.

The most significant of these parameters is the solution pH, which affects both the sorbent and the metal species in solution. The overall surface charge of a biosorbent will often dictate what metal species it is able to bind – a negatively-charged surface will attract positively-charged metal species and a positively-charged surface will attract negatively-charged metal species. Solution pH controls the protonation of binding sites and changes which sites are available for adsorption reactions. Generally, when the pH is low and binding sites are protonated the adsorbent will attract anions and when the pH is raised (Park et al., 2010), the binding sites will be deprotonated and attract cations.

The pH also affects the speciation of a metal in the solution. A basic pH may cause some metals to precipitate (Britton, 1943), which can contribute to the overall metal removal in practice but needs to be controlled in equilibrium biosorption studies. Also, hydrolysis reactions or hydration of metals in various pH ranges can affect the size of the metal ion in solution; the charge on these hydroxides are pH-dependent (Volesky & Holan, 1995).

Acidification of the solution commonly occurs during metal biosorption reactions, thus the pH often needs to be monitored and controlled over the course of the equilibrium reaction (Volesky & Holan, 1995). Again, while this is essential for experimental studies, this is not practical in industrial-scale applications and that must be taken into consideration.

The temperature at which the experiment takes place has been found to have much less of an impact on the outcome than the pH (Aksu et al., 1992). How much temperature will affect the processes will depend on the exact binding mechanism, as physical adsorption reactions tend to be exothermic and need lower temperatures for sorption while chemical adsorption reactions tend to be endothermic and need a temperature that meets the heat of enthalpy for the reaction (Volesky, 2003). Further, it has been found that temperature has little impact on biosorption processes between +20 and +35°C (Veglio & Beolchini, 1997).

Ionic strength has generally been found to negatively impact adsorption capacity. Inhibition may be due to chloride competition at active sites, changes that affect the electrical double layer, or complexes between the metal ions and the salt ions (Dönmez & Aksu, 2002). High concentrations of other ions in solution may also introduce binding competition, especially for electrostatic-dependent processes. In one study, however, it was found that using halophilic bacteria as an adsorbent lead to an increase in adsorption at an ionic strength of 4 M compared to adsorption at 2 M (Ams et al., 2013). This finding provides important implications for the present thesis (Chapter 4), which explores the feasibility of metal adsorption under saline conditions by a halotolerant bacterium derived from potash tailings.

The particle size of an adsorbent affects the kinetics of the adsorption reaction and the flow dynamics in an industrial-scale process. To enhance the reaction kinetics, a small-sized particle will maximize the surface area available for the reaction. In batch reactions, this is one of the advantages of using bacteria as an adsorbent, as the smallest particle that can be attained will be the size of one cell. However, to achieve a particle that will both have sufficient surface area and maintain proper pressure in a packed column or other industrial processes, it is best to maintain biosorbent particles between 0.5 and 1.5 mm in diameter (Volesky, 2003). There are immobilizing methods that can be used to increase bacterial sorbent particle size.

Finally, reactive groups on the surface of the biosorbent can be affected by various pre-treatments including acid-activation, growth conditions before the biomass was harvested, and the lifecycle point at which a cell was harvested (Gupta et al., 2000).

2.3.3 Biosorption isotherm and models

Assessment of a solid-liquid sorption system is initially based on small-scale batch sorption tests carried out to equilibrium. These batch equilibrium experiments mainly aim to determine the

affinity and sorption capacity of an adsorbent to each adsorbate at optimal operation parameters, as well as to elucidate the mechanisms and kinetics of the reaction. This type of experiment needs to be conducted first before upscaling the process or applying it to a continuous operation mode.

A biosorption isotherm is the plot of solute uptake at equilibrium against the equilibrium solution concentration and can be used to compare adsorbents at the same system parameters. Generally, as the final solution concentration increases, the solute uptake will also increase until all the binding sites are filled. As the simplest isotherm, the relationship forms a single curve. This isotherm offers a clear picture of the maximum solute adsorbed (q_{max}), as well as the affinity between sorbate and adsorbent at low concentrations as shown by the initial slope of the curve.

Modelling can become complicated as the classic model assumptions may not always apply to systems with numerous types of binding sites, sorbate solution chemistry, and the precipitation of accumulating metal phases and mechanisms (Fomina & Gadd, 2014; Volesky & Holan, 1995). Common models include the Langmuir model (Langmuir, 1918) and the Freundlich model (Freundlich, 1906), neither of which describe mechanisms involved, but instead reflect the experimental isotherm curves.

2.3.4 Application for industrial wastewater

Industrial effluents contain a mixture of various organic chemicals and metals and this greatly affects adsorption performance compared to a system with only one or two solutes. The performance of an adsorbent needs to be evaluated in these conditions in addition to the optimized systems.

Ionic strength is regulated in laboratory controlled systems, but the competing ions present in industrial effluents will lower adsorption performance (Cotoras et al., 1992). The increased ionic strength in solution introduces competition for binding sites, changes in metal activity, and affect the properties of the electric double layer for system kinetics (Vijayaraghavan & Yun, 2008). It especially affects adsorption mechanisms that use electrostatic attraction.

Studies of multi-solute systems in the literature demonstrate the suppression of individual metal binding and a specific order of preference in the mixed metals. This binding preference can be manipulated by pre-treatment as demonstrated with the fungi *Aspergillus fumigatus*, where the order of decreasing adsorption for iron-coated biomass was Fe > As > Zn > Mn > Pb instead of

the natural biomass order Fe > Zn > Mn > As > Pb (Jalili Seh-Bardan et al., 2013). Aksu et al. (2002) observed that the effect of these multiple solutes increases with increasing concentrations.

There are several commercialized biosorption products available on the market, some of which are listed in Table 2-3. These are generally made of immobilized microbial material including algae, bacteria, and fungi. Products, including AlgaSORB^{TM}, AMT-BIOCLAIM^{TM}, and Bio-fix, offer high removal rates in dilute systems and the ability to recycle the adsorbent material many times. AMT-BIOCLAIM is capable of metal recovery from waste systems containing cyanide and therefore can work in metal finishing operations (Atkinson et al., 1998), while Bio-fix has demonstrated effective metal recovery in waters of acid mine drainage (Garnham et al., 1997).

Biological sorption materials have limitations that need to be addressed before broad use on an industrial scale is feasible (e.g. Michalak et al., 2013; Park et al., 2010). Firstly, raw biomass offers some unique mechanical strength and swelling issues. This is often dealt with by using various immobilization techniques, including embedding in a silica gel or polysulfone matrix. The extra processing steps for these approaches add time and cost to the process and this needs to be minimized to keep the economic edge that biomass has on chemical ion exchange resins. Secondly, unprocessed material often leaches organic materials or dyes into the wastewater, creating a secondary contamination of the water. Some options being explored include pre-treatment with acid- or alkali-wash.

Table 2-3 Examples of commercialized biosorption products.

Product	Material	Specifications	Reference
AlgaSORB™	*Chlorella vulgaris*	Metal ions Dilute waters (1-100 mg/l) Immobilized in silica or polyacrylamide gel More than 100 biosorption-desorption cycles	Garnham et al. (1997)
AMT-BIOCLAIM™	*Bacillus subtilis* treated in caustic solution	Metal ions Up to 2.90 mmol Pb/g 99% removal from dilute system Regenerable Works in cyanide solutions	Brierley et al. (1991) Kuyucak (1990) Veglio & Beolchini (1997)

Product	Material	Specifications	Reference
Bio-fix	Mixture (Sphagnum peat moss, algae, yeast, bacteria, and/or aquatic flora)	Toxic heavy metals Immobilised in polysulfone Zn adsorption 4-fold higher than ion exchange resins More than 120 biosorption-desorption cycles Used in acid mine drainage	Garnham et al. (1997) Volesky (1990)

2.3.5 Halophilic biosorbents

Species that can live in high salt concentrations need to tightly control ion permeability and these bacterial cells can have distinctive cell envelope characteristics and increased negative charge in phospholipids (Kushner & Kamekura, 1988; Schneegurt, 2012; Vreeland, 1987) that may contribute to improved metal adsorption capabilities. Adaptations can include a cell wall with increased hydrophobicity, an increase in the concentration of ion pumps (Oren, 2002), or the presence of S-layer proteins. In one study, the negatively-charged S-layer was found to increase copper and iron adsorption but decrease manganese and zinc adsorption (Schut et al., 2011). The use of halophilic organisms for metal adsorption has not been studied in-depth in the published literature, and most of the published research on this topic has taken place in the last 15 years (Table 2-4).

Table 2-4 Metal adsorption by halophilic microorganisms in the literature.

Halophile	Adsorbate	Metal Uptake[1]	Reference
Dunaliella	Cr(VI)	102.5 mg/g	Dönmez & Aksu (2002)
Halomonas	V	91.8%	Ghazvini & Mashkani (2009)
	Pb, Cd	90, 50%	Amoozegar et al. (2012)
	Pb, Cd	24.15, 23.88 mg/g	Rajesh & Rajesh (2015)

Halophile	Adsorbate	Metal Uptake[1]	Reference
Nostoc punctiformes exopolysaccharide	Cr, Na	144.68 mg/g	Sharma et al. (2009)
Purple nonsulfur bacteria	Pb, Cu, Cd, Zn, Na	39, 20, 7, 5, 31%	Panwichian et al. (2010)
Halobacteria	Zn, As, Cd	68.6, 36, 39.8 %	Williams et al. (2013)
	Ni, Al, Hg	50.8, 110.25, 7.4 ppm	Williams et al. (2012)
	Mn	108.44 mg/g	Naik & Furtado (2014)
Chromohalobacter sp.	Np	89%	Ams et al. (2013)
Pseudomonas aeruginosa	Co-EDTA complex	80.4%	Paraneeiswaran et al. (2014)

[1] Metal uptake as reported, not necessarily at maximum

2.3.6 Bacterial biosorbent preparation

The methods for the preparation of bacterial adsorbents vary widely within the literature. The most common techniques involve suspending live cells in distilled water or autoclaving or oven-drying cell suspensions (e.g. Bai et al., 2014; Naik & Furtado, 2014).

Live, inactive cells are often used because of the simplicity of the procedure. Nutrient media is removed and washed from the cells, and when they are re-suspended in distilled water the cells are assumed to be metabolically inactive. This technique is unable to represent biosorption as the sole uptake mechanism and toxicity levels in solution will still affect the adsorbent integrity. In peer-reviewed literature, Bai et al. reported higher levels of lead uptake in living cells than in autoclaved *Bacillus subtilis* (Bai et al., 2014).

Autoclaves are used in the laboratory to sterilize equipment using high temperature and pressure and will effectively kill bacteria cells, including endospores. This process will often lyse cells, leaving cell debris suspended in the solution that can bind aqueous metals (Aryal & Liakopoulou-Kyriakides, 2015). However, autoclaving may destroy or damage some of the metal binding sites and cell structure, reducing binding capacity and the ability to reuse the biosorbent (Bai et al., 2014; Schut et al., 2011).

The final option, oven-dried biomass, has had good adsorption results in the literature. Drying cells using a mid-range temperature (+55 to +65°C) should inactivate the cells while leaving the components on the cell surface relatively untouched and a high surface-to-volume ratio. Better uptake of a wide range of metals has been demonstrated in oven-dried cells compared to living cells of chromium-resistant isolates (Srinath et al., 2002), *Myxococcus xanthus*, and *Saccharomyces cerevisiae* (Avery & Tobin, 1992; Omar et al., 1997). Drying the material also enables long-term stability and storage of biological adsorbents at low costs (Srinath et al., 2002).

2.3.7 Metal adsorbates

Metals are both a valuable resource and a challenge in industry. Because they are found in many ores and soils and have a broad range of chemical uses, the effluent from both mining and other industrial processes typically contain toxic metals at concentrations exceeding regulatory guidelines. Spills from these waste waters and chronic low-level discharges in industrial effluents are common sources of elevated levels of metals in the environment (Aryal & Liakopoulou-Kyriakides, 2015; Wang & Chen, 2009). In Saskatchewan alone, there are 287 federally-controlled contaminated sites with metal concentrations above background levels and that may pose a risk to human health or the environment, as reported in the Federal Contaminated Site Inventory (Environment Canada, 2016), and these numbers do not include privately-managed sites. Metals are often essential to biological processes in low concentrations, acting as catalysts in the active sites of enzymes, but at higher concentrations they pose a toxicological risk to the environment and human health.

When it comes to metal adsorption, metals of interest can be sorted into categories (Volesky, 2003). Firstly, the adsorption of toxic or radioactive metals (i.e. nickel, chromium, copper, uranium) would allow an industrial effluent to be treated sufficiently to meet discharge standards. Precious metals, including gold and silver, can be concentrated and sold as a commodity. Finally, there are metals like manganese, which is not toxic at low levels but requires removal to meet aesthetic guidelines. Copper and chromium are both toxic at high concentrations and are common contaminants in industrial wastewater in Saskatchewan (e.g. Shaw et al., 2011; Tenenbaum, 2009).

Copper

Copper is considered a deleterious substance as its presence degrades the quality of a water system for both fish and humans (Environment Canada, 2012b). Because of this, the Government of Canada has set its drinking water guidance to 1 mg/l and the maximum authorized average monthly concentration under the Canadian Fisheries Act is 0.30 mg/l (Environment Canada, 2012a). It is primarily present in industrial wastewater as $CuCO_3$ and commonly found in the effluents of paper, petroleum, copper/brass plating, copper-ammonium, mining, and dye industries (Aksu et al., 1992; Yilmazer & Saracoglu, 2009). Conventional removal of copper from these waters include chemical or electrochemical processes, ion exchange, and evaporative recovery (Aksu et al., 1992). However, copper has a high affinity to both soil and organic ligands and adsorption is a plausible option for copper removal (McLean & Bledsoe, 1996). Reported values of copper adsorption can be found in Table 2-5, including the highest reported adsorption value of 381 mg/g on a protein from *Bacillus firmus* (Salehizadeh & Shojaosadati, 2003) and the second highest value of 270 mg/g reported in 1984 (Norberg & Persson, 1984).

Table 2-5 Highest reported values of metal uptake in copper biosorption.

Biosorbent	Metal Uptake[1] (mg/g)	Reference
Bacillus firmus protein	381	Salehizadeh & Shojaosadati (2003)
Zoogloea ramigera	270	Norberg & Persson (1984)
Aspergillus terreus	224	Gulati et al. (1999)
Penicillium simplicissimum	112.3	Li et al. (2008)
Thiobacillus ferrooxidans	198.5	Ruiz-Manriquez et al. (1998)
Spirulina sp.	196	Chojnacka et al. (2005)
Aspergillus terreus	160-180	Gulati et al. (2002)
Arthrobacter sp.	148	Veglio et al. (1997)
Penicillium chrysogenum	108.3	Deng & Ting (2005)
Pseudomonas putida	96.9	Uslu & Tanyol (2006)

[1] Metal uptake as reported, not necessarily at maximum

Chromium

Chromium is not mined in Canada; however, it is present in many anthropogenic wastes. It is a component found in bitumen, the extracted component in oil sands (Tenenbaum, 2009), but it is also commonly found in liquid discharges from pulp mills (EPA, 2010), metal smelters and refineries, metal finishing plants, and petroleum refineries (Environment Canada, 1994). It is estimated that at least 27 tonnes of chromium are released from industrial sources in Canada each year (Environment Canada, 1994).

Chromium occurs in the environment in two valences, Cr(III) and Cr(VI). Trivalent chromium is naturally-occurring, cationic, and a micronutrient requirement for humans. In its hexavalent form, however, chromium occurs as anions and is toxic to biological systems. In solution, the chemical form of Cr(VI) depends on the solution pH, where $HCrO_4^-$ is predominant at pH < 6.5, CrO_4^{2-} (chromate) predominates at pH 6.5, and $Cr_2O_7^{2-}$ (dichromate) is most common when present in concentrations higher than 10 mM and pH 2-6 (McLean & Bledsoe, 1996). Hexavalent chromium is highly mobile, but can be easily chemically-reduced to Cr(III) which is readily adsorbed by soils (McLean & Bledsoe, 1996). In Canada, hexavalent chromium is classified as "Carcinogenic to Humans" and no level of exposure is considered safe (Act, 1993). The maximum contaminant level allowed by the US EPA for total chromium (chromium-6 and chromium-3) concentration in drinking water is 0.1 mg/l (EPA, 2010). Literature reported values for chromium adsorption can be found in Table 2-6. The highest reported value of Cr(III) adsorption is 714.3 mg/g (Calfa & Torem, 2008), while the highest reported Cr(VI) adsorption value is much lower at 294.0 mg/g (Aksu et al., 2002).

Table 2-6 Highest reported values of metal uptake in chromium biosorption .

Valence	Biosorbent	Metal Uptake[1] (mg/g)	Reference
III	*Rhodococcus opacus*	714.3	Calfa & Torem (2008)
VI	Activated sludge	294.0	Aksu et al. (2002)
VI	*Aeromonas caviae*	284.4	Loukidou et al. (2004)
Not specified	Orange peel (outer skin)	275	Masri et al. (1974)
Not specified	Senna leaves	250	Masri et al. (1974)
VI	*Pachymeniopis* sp.	225	Lee et al. (2000)

Valence	Biosorbent	Metal Uptake[1] (mg/g)	Reference
VI	*Spirulina* sp.	185	Chojnacka et al. (2005)
VI	Rice hulls	164.3	Roy et al. (1993)
VI	*Chlorella minutissima*	162.2	Roy et al. (1993)
VI	*Staphylococcus xylosus*	143	Ziagova et al. (2007)
Not specified	Orange peel (inner white skin)	125	Masri et al. (1974)

[1] Metal uptake as reported, not necessarily at maximum

3 ARCHAEAL AND BACTERIAL COMMUNITIES IN HYPERSALINE POTASH MINE TAILINGS AND BRINE[1]

[1] This chapter is written in joint authorship with Wonjae Chang (supervisor), Joyce M McBeth (co-supervisor), and Jonathan M Vyskocil. The study is part of the IMII research program led by Dr. Wonjae Chang (Principal Investigator). Nicola Harris designed and conducted culturing experiments and all lab work. Jonathan Vyskocil prepared and executed the sequencing data pipeline in mothur. Phylogenetic data analysis, figure preparation, and interpretation was done by Nicola Harris, Jonathan Vyskocil, and Joyce McBeth. All other data analysis, interpretation, and manuscript preparation was conducted by Nicola Harris under the supervision of Wonjae Chang and Joyce McBeth. The tables, figures, and references cited herein have been reformatted to fit the thesis style.

3.1 Abstract

Conventional potash mining practices produce large quantities of hypersaline tailings and brine. Other environments such as salt lakes or oilfield brine can have similar sodium-dominated salt concentrations as these materials, but potash by-products also contain limited carbon sources. The present study builds our understanding of previously-understudied microbial community compositions in potash mine wastes. Specifically, we have characterized three types of hypersaline potash by-products – brine, coarse tailings, and fine tailings – from a potash mine in Saskatchewan, Canada. Both high-throughput amplicon sequencing of the 16S rRNA gene (V4 region) and culture-dependent plating techniques were employed to examine community composition and salinity tolerance. High-throughput sequencing identified a mixed community of archaea and bacteria within the brine pond sample and bacterially-dominated communities in the coarse and fine tailings. Twenty-two unique isolates that were relatives of genera observed in the high-throughput sequencing results were identified from spread plates. Isolates included known halophilic and halotolerant Archaea (*Haloferax* and *Halorubrum* species) and Bacteria (including *Halomonas*, *Marinobacter*, and *Dietzia* species) and the majority of these isolates demonstrated a wide salinity tolerance (NaCl concentrations from 0-25% (w/v)). Some of the isolated species may have utility to potash companies to enhance the evaporation of brine ponds while others may be utilized for the biodegradation of hydrocarbons in highly saline conditions. This is the first study to utilize high-throughput sequencing to investigate membership and diversity of microbial communities within potash tailings and brine. It also contributes to the broader understanding of the distribution of halophilic and halotolerant microbes in natural and engineered environments.

3.2 Introduction

Canada was the largest producer and exporter of potash in the world in 2015, playing a large role in global fertilizer production (Marshall, 2015). Potash ore is sourced from an ore bed deposited by the evaporation of an ancient marine basin and is composed mainly of sylvite (KCl) and halite (NaCl), with some insoluble clays and minerals (Holter, 1969). Extraction and refinement of the raw ore produces two tonnes of NaCl salts for every tonne of refined KCl salts (Tallin et al., 1990), which generates large amounts of potash brine and tailings. Brine contains total salt concentrations on the order of 350 g/l (Maathuis & Van der Kamp, 2002) and small

amounts of process water from the mill including organic reagents (e.g. oils, amines, and/or flocculants) (Perucca, 2003; Vonhof, 1975). Tailings are composed mostly of NaCl salts, with lesser amounts of KCl, $MgCl_2$, and insoluble clays and minerals (Tallin et al., 1990; Vonhof, 1975). In Saskatchewan potash mines, brine is stored in retention ponds, recirculated into the mill, or disposed of through deep-well injection and the tailings are deposited in piles within the tailings management area (TMA) (Reid & Getzlaf, 2004; Tallin et al., 1990; UNEP, 2001).

Environments with similarly high sodium salinity have recently become the focus of several microbial characterization studies, expanding the pool of knowledge on microbial communities and processes in extreme conditions (Narasingarao et al., 2012; Oren, 2015; Schubert et al., 2010). Environments where the salt concentrations are near saturation can house diverse microbial communities despite the harsh environmental conditions, but community make-up can vary worldwide depending on the ionic composition (for example, K:Na weight ratios), and nutrient levels (Park, 2012; Vreeland, 2012). Broadly, archaea tend to dominate the microbial community in environments having salinities above 230 g/l, such as the crystallizer ponds in solar salterns, but bacteria have also been observed at these high salinities (Antón et al., 2000; DasSarma & DasSarma, 2012). Microbial community studies of environments with similar chemical characteristics to potash mine wastes (near-saturation salt levels, low nutrient and carbon loadings, and neutral pH), are expected to report microbial communities similar to those in potash brine and tailings. Such environments include man-made solar salterns and naturally occurring evaporite deposits and salt lakes,

Solar salterns are anthropogenic environments originating from seawater and can be chemically-similar to potash brine, with sodium-dominated ion compositions. Crystallizer ponds, the last and most concentrated evaporative ponds in solar salterns, contain total salt concentrations of up to 370 g/l (Ghai et al., 2011) although they can range from oligotrophic to eutrophic nutrient states (Dillon et al., 2013). Microbial studies have found microbes from all three domains of life that may also be found in potash tailings or brine communities, including organisms from the Eukarya: the alga *Dunaliella* and brine shrimp *Artemia* (Gunde-Cimerman et al., 2009; Litchfield & Gillevet, 2002). Archaeal diversity is generally higher than bacterial diversity (Ghai et al., 2011) and genera can include *Halorubrum*, and *Haloquadratum* (Dillon et al., 2013; Ghai et al., 2011; Gunde-Cimerman et al., 2009). Crystallizer ponds can also contain benthic mats comprised of cyanobacteria and anoxygenic phototrophs, layered according to gradients in light, oxygen, and

sulfide (Caumette, 1993), and communities often include the Proteobacteria genera *Oceanicola*, *Salinibacter*, *Vibrio, Flavobacterium,* and *Pseudomonas* (Antón et al., 2002; Fernández et al., 2014; Ghai et al., 2011).

Members of microbial communities found within evaporite deposits, including the ore bed that potash is mined from, may also be present in potash brine and tailings. As saline lakes or marine environments evaporate, solid layers of salts (halite or sylvite, for example) are deposited and can contain fluid inclusions with microbial life. These brine inclusions can have various ionic and organic material compositions, depending on the composition of the original body of water (Timofeeff et al., 2001). Culture studies have achieved isolations of mostly halophilic, Archaeal species from class *Halobacteria* (Haloarchaea) (Grant et al., 1998; Schubert et al., 2010) as well as one halotolerant bacterium that was closely related to a *Bacillus* species (Vreeland et al., 2000). Molecular studies have also found DNA sequences that are related to Haloarchaea genera including *Haloarcula, Halobacterium, Halorubrum, Haloferax,* and *Halogeometricum* (Gramain et al., 2011; Park et al., 2009; Radax et al., 2001), with some evidence for the presence of bacterial DNA (Fish et al., 2002).

Although salt lakes have much broader ranges of salinity and nutrient levels than potash brine and tailings and thus are not a perfect analogue, they have been well-characterized with regards to their chemistry and microbiology and provide useful information on microbes that are adapted to survive at high salinities. Great Salt Lake in Utah has similar sodium-dominated ion ratios to potash brine and tailings and the northern arm of the lake has salinity reaching 330 g/l (Javor, 1989). The northern portion of Great Salt Lake, however, also has high biodiversity of both Bacteria and Archaea due to the presence of a petroleum seep with nitrogen and sulfur containing asphaltic oil that provides abundant carbon and nutrients for microbes (Tazi et al., 2014). Many of the operational taxonomic units (OTU) found there are unique from other hypersaline environments, including haloarchaeal representatives and hydrocarbon-degrading *Proteobacteria* (Tazi et al., 2014).

Culturing studies have been conducted using potash mine materials as a source for microbial isolates. Various haloarchaeal species have been isolated, including *Arhodomonas* sp. (Genbank accession no. HQ833040), *Haloarchaeum* sp. (JN227878-JN227881), and *Haloferax* sp. (JX669135, JN787949-JN787950) from a flotation enrichment step and mineral samples from a potassium mining company in Russia (Saralov et al., 2012a; Saralov et al., 2012b; Saralov et al.,

2013) and *Halobacterium* sp., *Halococcus* sp., and *Haloarcula* sp. from a brine pool and mineral samples within a British potash mine (Norton et al., 1993). As well, halobacterial isolates (*Pseudomonas* sp.) were found in the substrate covering of a potash tailings pile in Germany (Koch et al., 2012). The microbial communities associated with potash tailings and brine described in the current study has not been previously described in peer-reviewed literature or assessed using high-throughput sequencing techniques.

The present research aims to characterize the Bacterial and Archaeal communities that live within potash brine and tailings to provide insight into their compositions and diversity, contribute to the growing understanding of the distribution of extreme halophiles in natural and engineered environments, and suggest some implications of the microbes for the potash industry and in biotechnological applications. To accomplish this, the study's objectives were to (i) compare the microbial community in potash mine brine, coarse tailings, and fine tailings, (ii) isolate and characterize heterotrophic, aerobic microbes from these materials, and (iii) explore how the microbiology relates to chemical compositions of potash brine and tailings.

3.3 Materials and Method

3.3.1 Sampling and chemical analyses

Samples were collected using bleach-sterilized containers in June 2015 and July 2016 from a potash mine in Saskatchewan (SK), Canada, including brine from a retention pond and coarse and fine tailings from the mill. Subsamples of the June 2015 samples were sent to the Environmental Analytical Laboratory at the Saskatchewan Research Council (SRC; Saskatoon, SK, Canada) and tested for major cations (Ca^{2+}, K^{+}, Mg^{2+}, Na^{+}, SO_4^{2-}) using inductively coupled plasma optical emission spectrometry (ICP-OES), chloride with the ferricyanide method, HCO_3^{-} by titration, nitrate using colorimetric methods, organic carbon content using persulfate oxidation, and the presence of metals by inductively coupled plasma mass spectrometry (ICP-MS), all using standard methods (American Public Health Association et al., 2012; USEPA, 1983). Nutrient levels of the July 2016 samples were measured at the SRC, including ammonia (total Kjeldahl nitrogen), nitrate (hydrazine reduction), organic carbon (persulfate oxidation), and phosphorus (ICP-MS) using standard methods. The pH of brine and porewater from tailings samples (July 2016) was measured using a Hach HQ40d portable pH meter (Loveland, CO, USA). Salinity was calculated using the concentrations of major ions in each sample (Ca^{2+}, K^{+}, Mg^{2+}, Na^{+}, SO_4^{2-}, Cl^{-},

HCO_3^-, and NO_3^-). Results are reported as the mean of triplicate analyses ± standard error, if applicable (Table 3-1).

3.3.2 DNA extraction

All DNA extractions were conducted using standard aseptic handling techniques. Brine was prepared for total DNA extraction by filtering 5 L samples through sterile, disposable bottle top filters (0.20 µm Thermo Scientific™ Nalgene™ Rapid-Flow™) in triplicate. Tailings samples were weighed into approximately 10 g aliquots in triplicate and all sample types were stored at –80°C until extractions were performed. DNA was extracted using the MO BIO PowerWater® Kit (brine) or the MO BIO PowerMax® Soil DNA Isolation Kit (tailings) following the supplier's recommended protocol with minor modification (brine samples were initially vortexed for 10 min instead of 5 min). Tailings extractions were vacuum concentrated with infrared at + 50°C (CentriVap® micro IR Vacuum Centrifugal Concentrator, Labconco). All extractions were quantified using a Qubit® 2.0 Fluorometer and Qubit™ dsDNA HS Assay kit (Life Technologies) and the DNA quality was assessed using an Epoch Microplate Spectrometer with a Take3 plate (Biotek) at 260/230 and 260/280 nm.

3.3.3 High-throughput amplicon sequencing analysis

DNA extractions were sent to RTL Genomics (Lubbock, Texas, USA) for Illumina MiSeq high-throughput amplicon sequencing of the V4 region of the 16s rRNA gene using the universal (bacterial/archaeal) primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Caporaso et al., 2011).

Sequencing data was analyzed using the mothur software package (Schloss et al., 2009) and MiSeq data standard operating procedure (Kozich et al., 2013). Briefly, sequences were trimmed and aligned to the Silva reference database, chimeras were removed, and sequences were screened for a maximum length of 300 base pairs. Reads were classified using the Silva rRNA Database v119 and an 80% bootstrap value cut-off for taxonomic assignments. The sequence reads were then assigned to operational taxonomic units (OTUs) based on 97% sequence identity and the OTUs were classified. Sampling coverage was estimated using Good's Coverage. Data were subsampled to 22020 sequences per sample for calculating the Yue and Clayton measure of similarity, analysis of molecular variance (AMOVA), community richness (Chao1 and ACE), and

alpha-diversity (inverse Simpson). The ACE richness estimates consider an abundant OTU to have ten or more individuals.

3.3.4 Microbial isolation and identification

Media was prepared using either nutrient broth (NB; Sigma Aldrich, N7519) or Reasoner's 2A agar (R-2A; Sigma Aldrich, 17209), one of four levels of sodium chloride amendment (0, 3, 15, and 25% (w/v) NaCl), and 20 g/l agar (total) then autoclaved at 121°C for 15 minutes. Both nutrient sources are nonspecific and target heterotrophic microorganisms, however NB is used broadly as a rich nutrient source while R-2A is nutrient-poor and often used for groundwater studies. Standard aseptic handling techniques were used for media inoculation and culturing. Undiluted brine or tailings were spread onto agar plates with three biological replicates within 12 h of sampling. Final plate counts were performed two weeks after the last isolates appeared and the concentration of colony forming units (CFUs) calculated. Single colonies showing unique colony or growth characteristics were picked and streaked onto the same plate-type they were isolated on three times to procure pure microbial cultures.

DNA was extracted from pure cultures using an UltraClean® Microbial DNA Isolation Kit (MoBio). Partial 16S rRNA genes were amplified by polymerase chain reaction (PCR) on a Veriti™ Thermal Cycler (Applied Biosystems). The PCR mix contained Econotaq® Plus 2X Master Mix (Lucigen) and the following primer combinations: (i) 27F-1492R for bacteria or (ii) 519F-1492R for archaea (5'-AGAGTTTGATCMTGGCTCAG-3' and 5'-GGWTACCTTGTTACGACTT-3', respectively; 5'-CAGCMGCCGCGGTAATWC-3'). The presence and size of the amplicons was confirmed using gel electrophoresis (Lonza FlashGel™ System) on 1.2% agarose and with a run time of 8 min. The PCR products and sequencing primers (27F, 519F, and 1492R) were sent to the McGill University and Génome Québec Innovation Centre for Sanger sequencing (Montreal, Canada).

The 16S rRNA gene sequences obtained for forward and reverse strands were trimmed, assembled, and manually-curated using Sequencher software (Gene Codes Corporation). Consensus sequences were then compared to existing genome libraries using the Ribosomal Database Project (RDP) version 11.4 from Michigan State University to determine species similarity and assign taxonomic affiliation. Phylogenetic trees were constructed using isolates, near-relative type-strains, and halotolerant microbes that are often found in highly saline

environments with MEGA7: Molecular Evolutionary Genetics Analysis software version 7.0. The maximum likelihood method was used with the Tamura-Nei substitution model, *Methanospirillium hungatei* (Archaea) or *Aquifex aeolicus* (Bacteria) as outgroups, and 1000 bootstrap replicates (Kumar et al., 2015; Tamura et al., 2011).

3.3.5 Isolate characterization

Twenty-two isolate groups having similar gene sequences (≤ 1 base pair different, ambiguous base pairs considered acceptable) were identified and representatives were chosen for phenotypic characterization. Salt (0, 3, 15, and 25% NaCl (w/v)) and temperature (4, 22, and 37°C) tolerances as well as growth at different nutrient levels (NB and R-2A) were tested using streak plates. With the exception of the tested variable, the growth conditions for each test were identical to those used in each strain's isolation (e.g. FTI17 was isolated at 37°C using R-2A agar with 3% (w/v) NaCl amendment, thus all salinity tests for this isolate were conducted at 37°C on R-2A agar). A negative result was assigned after two weeks without observed growth. Gram staining (VWR) and light microscopy (Nikon Eclipse LV100) was utilized to check consistency of cell shape and type for each representative with the nearest identified type-strain relative.

3.3.6 Nucleotide sequence accession numbers

Sanger sequencing results were submitted to the GenBank nucleotide archive (http://www.ncbi.nlm.nih.gov/genbank/) under accession numbers KX344919-KX344960. Sequences obtained from high-throughput amplicon sequencing data were submitted to the European Nucleotide Archive (http://www.ebi.ac.uk/ena) under project number PRJEB15442 and accession numbers ERS1355422-ERS1355425.

3.4 Results

3.4.1 Chemistry of potash brine and tailings

The brine and pore water from coarse and fine tailings were all neutral in pH (6.99-7.49) and all samples were highly saline (370 g/l for brine and > 835 g/kg for tailings; Table 3-1). Sodium was the dominant cation, with milliequivalent concentrations greater than the next most predominant cation, potassium, by a factor of 2.1 (fine tailings) to 20.1 (coarse tailings). In comparison to the salt concentrations, toxic metal concentrations were much lower and generally

found on the scale of µg/l or µg/g (Supplementary Materials Table 3-4). Total organic carbon levels in brine and coarse tailings were 1 mg/l and ≤ 10 mg/kg, respectively, both below the 16.8 mg/l cut-off suggested by Schut et al. (1997) and thus many of the microbes living within them could be oligotrophic. The fine tailings contained a higher concentration of organic carbon (32 mg/kg) as well as a higher proportion of insoluble material than coarse tailings (27.5% as compared to 1.12%), including clays and metals (Supplementary Materials Figure 3-5 and Table 3-4).

Table 3-1 Physicochemical characteristics of the potash brine and tailings and number of isolates for each sample type. Mean values are presented ± standard error.

Parameter	Brine (mg/l, except as noted)	Coarse Tailings (mg/kg, except as noted)	Fine Tailings (mg/kg, except as noted)
Physical and major ions			
pH	7.49 ± 0.01	6.99 [a]	7.11 ± 0.01
Total Alkalinity	60[a]	NT	NT
Total Salinity	370 g/l [a]	1120 ± 20 g/kg	835 ± 5 g/kg
Bulk Density	NT	0.957 g/cm^3 [a]	0.839 g/cm^3 [a]
Water insoluble portion	NT	1.12%[a]	27.6 ± 0.6%
Ca^{2+}	2000[a]	2570 ± 90	32500 ± 3000
Cl^-	219000 ± 1000	700000 ± 20000	460000 ± 3200
K^+	58200 ± 300	33200 ± 3900	132000 ± 8000
Mg^{2+}	1800[a]	1330 ± 30	24100 ± 2100
Na^+	89500 ± 200	379000 ± 6500	160000 ± 2000
SO_4^{2-}	1800[a]	3900 ± 200	28700 ± 1600
Carbon and nutrients			
Organic Carbon	1[a]	≤ 10	32 ± 2
Inorganic Carbon	14[a]	<10	116 ± 4
Total N	1.2 ± 0.1	≤ 10	143 ± 12
Total P	<1	< 10	113 ± 7
Isolation Experiments			
Observed CFU [b]	++	+	+++
No. distinct isolates	6	5	19

Parameter	Brine (mg/l, except as noted)	Coarse Tailings (mg/kg, except as noted)	Fine Tailings (mg/kg, except as noted)

NT – not tested;

[a] Standard error is 0

[b] (+++) high, (++) medium, (+) low observed CFU compared to other plated samples

3.4.2 High-throughput amplicon sequencing analysis

Illumina MiSeq high-throughput amplicon sequencing was successful in two of the three brine extractions and one coarse tailings extraction. Amplification failures in all sample types were likely due to low DNA concentrations in extractions (≤ 0.01 ng/µl). To achieve a successful amplification, the fine tailings extractions were pooled and concentrated further at the sequencing facility; due to the different treatment and high degree of processing, this sample data should be interpreted with caution, and comparisons with the other sequencing data presented here are only general.

A total of 206164 high-quality reads were obtained from all successfully sequenced samples, and samples ranged from 22037 (brine) to 89567 (coarse tailings) reads each. The most prevalent phylum in all samples was the *Proteobacteria* (40.6-89.3% of subsampled sequences), with high read counts for both classes *Gammaproteobacteria* and *Betaproteobacteria* (Figure 3-1). The highest number of sequences occurring within both brine and coarse tailings samples belonged to *Halomonas* sp., while substantial *Marinobacter* sp. sequences were present in the brine samples (up to 4.6% of sequence reads). The fine tailings data also had a high percentage of *Proteobacteria* sequences (40.5%), but differed from brine and coarse tailings because they showed high proportions of Cyanobacteria (16.5%, including *Coleofasciculus* sp. and *Lyngya* sp.), and many sequences related to chemolithotrophs including *Deferribacter* sp., *Sulfurospirillum* sp., and *Sedimenticola* sp. The Archaeal phylum *Euryarchaeota* was a considerable portion (up to 23.2%) of brine samples and sequences included the haloarchaea *Halorubrum* sp., *Haloarcula* sp., and *Haloferax* sp. Although differences in community composition can be observed, the overall difference in community make-up between sample-types was not found to be statistically significant (AMOVA, p-value 0.328).

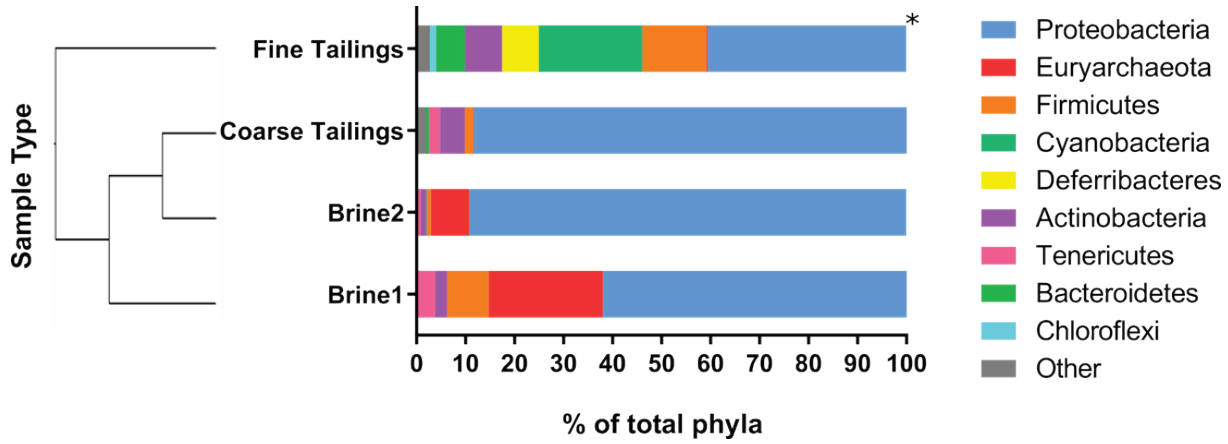


Figure 3-1 Phylogenetic distribution of most abundant phyla in the potash brine and tailings samples. Sequences reads for each sample were subsampled to 22020 reads. The dendrogram is based on the Yue and Clayton measure of dissimilarity. (*) indicates sample that was pooled and highly processed before sequencing due to previous failed amplification.

3.4.3 Microbial richness

At 97% similarity cut-off, a total of 1069 OTUs were observed in the four sets of sequencing results. The brine and coarse tailings samples were very similar in the number of observed OTUs and in estimators of OTU richness (Table 3-2). The fine tailings sample had a higher number of observed OTUs (619) and subsequently had the highest richness estimators. Chao1 is the most commonly used richness estimator and is a robust test that predicts the minimum number of species present in a sample (Shen et al., 2003), while Ace uses a more complicated model based on the number of individuals shown for each OTU (single, rare, and abundant species) and tends to estimate greater number of species than Chao1. With good sampling coverage Chao1 and Ace estimators converge; this is the case in these results.

The Inverse Simpson diversity index, ranging from 1 (no diversity) to infinity, was calculated to compare these communities using OTU abundance and evenness. Brine ranged from 1.45 to 3.24, while coarse tailings were on the low end of that range (1.57). Fine tailings, on the other hand, had a comparably higher diversity index (52.89).

Table 3-2 Sample richness described as total number of observed OTUs, Good's coverage, species richness estimators, and diversity indices calculated for the microbial communities from the potash brine and tailings samples using a sub-sampled population (22020 reads).

Sample	Total OTUs	Good's Coverage	Estimated Richness		Estimated Diversity
			Chao1	Ace	Inverse Simpson
Brine 1	157	0.996	375.6	382.8	3.24
Brine 2	128	0.998	255.5	289.0	1.45
Coarse	148	0.997	286.3	439.3	1.57
Fine	536	0.992	1160.6	1713.0	52.89

3.4.4 Isolation culture-based analysis

Microbial growth was observed on spread plates made from all three types of potash waste material for the full range of salinity tested (0-25% (w/v) NaCl). Fine tailings had the highest plate counts overall while coarse tailings had the lowest (Table 3-1, and Supplementary Materials Table 3-5). The highest colony counts were observed on plates with 3-15% (w/v) NaCl amendments for both brine and fine tailings and brine samples only had 3 colonies appear on the 0% (w/v) NaCl plates. Coarse tailings produced the highest colony counts on plates with 0-3% (w/v) NaCl amendments. Colonies were slower to appear on the NB media than on R-2A agar, suggesting an adjustment period was required for growth. A total of 50 colonies with unique colony or growth characteristics were chosen for genetic-based identification and further testing.

Analysis of the partial 16S rRNA gene sequences of 42 pure cultures revealed the presence of 22 unique isolates (Table 3-3). Of these, 2 were isolated from brine, 1 from coarse tailings, 14 from fine tailings, and 5 isolates were observed in more than one sample-type. Similarity values provided by Ribosomal Database Project (RDP) indicate that the majority of unique isolates showed > 99% sequence similarity to previously cultured type strains reported in the 16S rRNA gene database and four different isolates showing > 97% sequence similarity.

The four archaeal isolates belong to the phylum *Euryarchaeota*, class *Halobacteria* (Haloarchaea), like the sequences observed in brine high-throughput sequencing data (Figure 3-2), and belonged to either genus *Halorubrum* or genus *Haloferax*. The eighteen bacterial isolates

belonged to four distinct phyla: one isolate to *Bacteroidetes*, three to *Actinobacteria*, three to the *Firmicutes*, and eleven to *Proteobacteria* (eight within class *Gammaproteobacteria* and three within class *Alphaproteobacteria;* Figure 3-3). Genus-level relatives (at least 97% sequence similarity) of all isolated species, including *Vibrio*, *Salinibacter*, and *Flavobacterium* species, also appeared in the high-throughput amplicon sequencing data.

Table 3-3 Distinct potash isolates as determined by 99% similarity of the 16S rRNA gene, the best match in the RDP database, the sample type each was isolated from, and characteristics.

Distinct isolates	RDP match (similarity score, %)	Sample Type	Isolation Media[1]	Gram Stain	Cell Shape	Salt tolerance (% (w/v) NaCl)
FTI21	*Halomonas gudaonensis* (99.5)	brine, coarse tailings, fine tailings	R-2A (15%)	-	short rods	0-25
FTI23	*Halomonas shengliensis* (99.8)	brine, coarse tailings, fine tailings	R-2A (15%)	-	short rods	3-25
BI05	*Salicola salis* (98.2)	brine, coarse tailings	R-2A (25%)	-	rods	0-25
FTI24	*Halorubrum saccharovorum* (99.9)	brine, fine tailings	R-2A (25%)	nt	nt	3-25
FTI19	*Halomonas andesensis* (99.1)	coarse tailings, fine tailings	R-2A (3%)	-	short rods	0-25
BI06	*Alcanivorax venustensis (*99.5)	brine	R-2A	-	short rods	0-25
BI09	*Halorubrum californiense* (99.2)	brine	NB (25%)	nt	nt	15-25
CTI06	*Staphylococcus epidermidis* (100)	coarse tailings	R-2A	+	cocci	0-25
FTI05	*Bacillus pumilus* (99.9)	fine tailings	NB	+	rods	0-25
FTI12	*Bacillus thuringensis* (100)	fine tailings	R-2A	+	rods	0-25

Distinct isolates	RDP match (similarity score, %)	Sample Type	Isolation Media[1]	Gram Stain	Cell Shape	Salt tolerance (% (w/v) NaCl)
FTI14	*Croceicoccus naphthovorans* (97.6)	fine tailings	R-2A	-	short rods	0-15
FTI08	*Dietzia maris* (99.8)	fine tailings	NB	+	cocci	0-25
FTI07	*Gordonia alkanivorans* (99.8)	fine tailings	NB	+	cocci	0-15
FTI25	*Haloferax prahovense* (99.8)	fine tailings	R-2A (25%)	nt	nt	0-25
FTI16	*Halomonas meridiana* (99.8)	fine tailings	R-2A	-	rods	0-15
FTI26	*Halorubrum lipolyticum* (99.5)	fine tailings	NB (25%)	nt	nt	15-25
FTI17	*Marinobacter adhaerens* (99.8)	fine tailings	R-2A (3%)	-	rods	0-25
FTI11	*Microbacterium phyllosphaerae* (99.8)	fine tailings	NB	+	rods	0-25
FTI06	*Pseudomonas xanthomarina* (99.5)	fine tailings	NB	-	rods	0-25
FTI09	*Skermanella aerolata* (99.2)	fine tailings	NB	-	rods	0-15
FTI13	*Sphingomonas jaspsi* (98.3)	fine tailings	R-2A	-	rods	0-15
FTI18	*Zunongwangia profunda* (99.7)	fine tailings	R-2A (3%)	-	rods	0-25

[1] NaCl amendment denoted in parentheses as w/v, if applicable

Isolates demonstrated wide ranges of salt tolerance (Table 3-3). Using descriptions by DasSarma and DasSarma (2012), moderate salt tolerance (0-15% (w/v) NaCl) was demonstrated by 5 bacterial isolates, all from fine tailings, and high salt tolerance (0-25% (w/v) NaCl) was

demonstrated by 13 of the isolates from various sample types, including an archaeal isolate. Two of the archaeal isolates were extremely halophilic (15-25% (w/v) NaCl), and one bacterial and one archaeal isolate were moderately halophilic (3-15% (w/v) NaCl). All brine isolates were capable of growth at 25% (w/v) NaCl amendments, but tailings samples contained some species having lower tolerance. All unique isolates demonstrated growth on both R-2A and NB plates. They were also capable of growth at 4°C, 22°C, and 37°C indicating that they are tolerant of mesophilic and psychrophilic conditions.

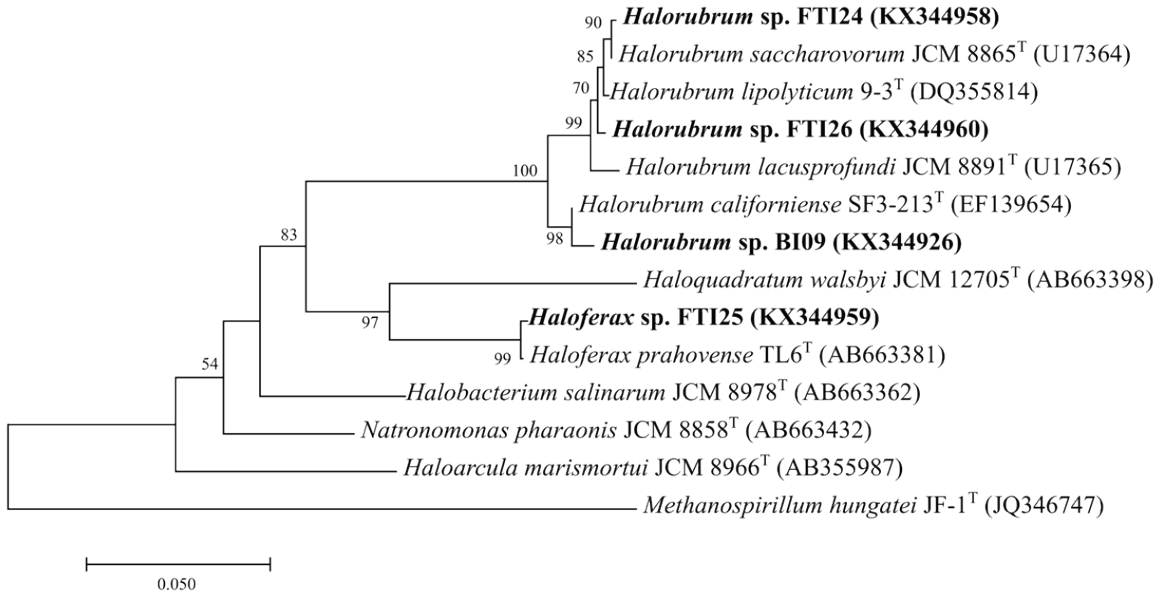


Figure 3-2 Maximum likelihood phylogenetic tree of archaeal isolates. Bootstrap values are shown for branches with ≥ 50% bootstrap support.

3.5 Discussion

Brine and coarse tailings samples had similar chemistry with regards to carbon, nutrients, and metals. In comparing high-throughput amplicon sequencing and culturing experiments, these sample-types also yielded similar OTU counts, taxonomic composition with a predominant *Gammaproteobacteria* portion, and number of isolates (Figure 3-4). The tailings pile, whose run-off feeds into the brine pond, contains 10 times as much coarse tailings as fine tailings (Potashcorp, 2010), thus these similarities are likely due to the relationship between the materials in the TMA.

Observed genera from these samples are consistent with genera that are often seen in hypersaline environments, suggesting that there are viable communities existing within tailings

and brine. The haloarchaea that are common in salterns, evaporites, and salt lakes were observed in both high-throughput amplicon sequencing and isolates from brine, but absent from coarse

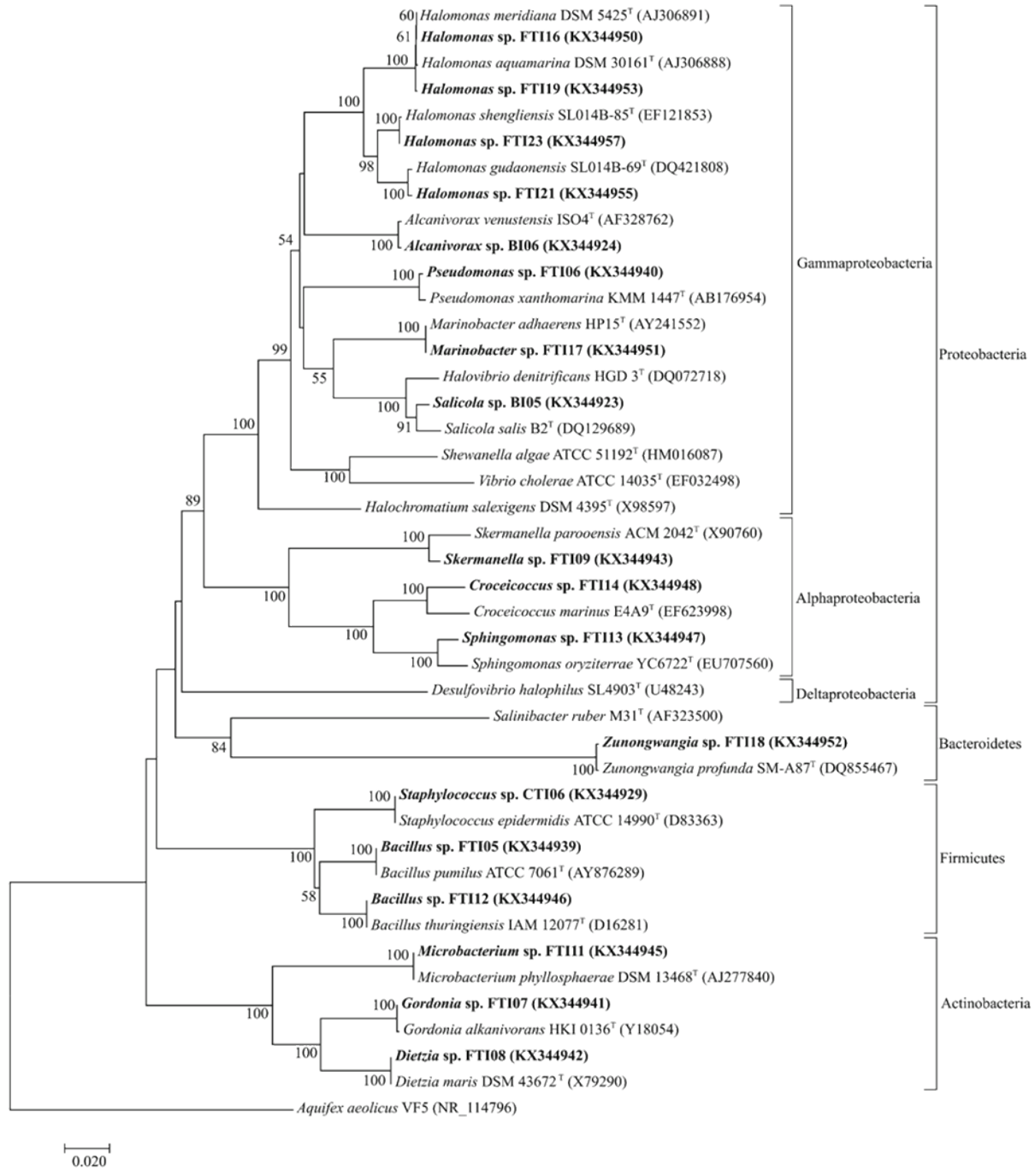


Figure 3-3 Maximum likelihood phylogenetic tree of bacterial isolates. Bootstrap values are shown for branches with ≥ 50% bootstrap support. Phyla and class breakdown of Proteobacteria are indicated on the right.

tailings results. Specifically, the genera *Halorubrum*, isolated from brine samples, has also been found in saltern crystallizer ponds (Dillon et al., 2013) and in a halite evaporite (Gramain et al., 2011). The most prevalent bacterial phyla in these samples – *Proteobacteria, Firmicutes,* and *Actinobacteria* – are also commonly observed in salterns and salt lakes (Ghai et al., 2011; Hollister et al., 2010). Bacterial isolates were mostly *Gammaproteobacteria* and genera that are commonly observed in marine environments and salt lakes (*Alcanivorax*, *Halomonas*, and *Staphylococcus* (a *Firmicute*)). *Salicola* sp. BI05, a brine and coarse tailing isolate, was closely related to strains that have been isolated from salterns and salt flats (Kharroub et al., 2006; Maturrano et al., 2006).

Conversely, the fine tailings sample, having insoluble mineral phases and a higher carbon content, was chemically different from brine and coarse tailings samples which may have led to the difference in observed community compositions and diversities. The higher inorganic carbon content of these materials (116 mg/kg) may explain the presence of autotrophic OTUs in the high-throughput amplicon sequencing results. Autotrophs were not targeted with the tested isolation conditions, but examples were observed in molecular data from the fine tailings sample. Chemolithotrophs included *Nitrosomonas* and *Halothiobacillus*, while phototrophs included various halotolerant *Cyanobacteria*. Autotrophs were not observed in the other two samples.

Fine tailings yielded 14 isolated species (11 OTUs) that were not seen in the other sample types (Figure 3-4). Many of these were relatives of species reported to be hydrocarbon degraders like those found in the Northern Arm of Great Salt Lake, and their presence could be related to the higher observed organic carbon content (32 mg/kg) in this sample. Close-relative species that have reported hydrocarbon degrading capability include *Dietzia maris* (Bødtker et al., 2009), *Pseudomonas xanthomarina* (Isaac et al., 2013; Sopeña et al., 2014), *Sphingomonas jaspsi* (Ferrera-Rodríguez et al., 2013; Zhou et al., 2012), and *Bacillus thuringiensis* (Al-Saleh et al., 2009).

It was expected that the high salinity of the brine and tailings would select for extremely halophilic microbes in these communities, but this was not consistently observed on isolation plates or in isolate growth tests. Salt tolerance observed in isolates was generally broader than that previously tested and/or reported in their closest relatives (e.g. Behrendt et al., 2001; Fernández-Martínez et al., 2003; Satomi et al., 2006). The archaeal isolates FTI24, FTI26, and FTI25 demonstrated tolerance of lower salinity than that reported in near-relatives, including *Haloferax* sp. FTI25 which grew on a 0% (w/v) NaCl plate in the present study (Cui et al., 2006; Enache et

al., 2007; Nuttall & Dyall-Smith, 1993). Two of the bacterial isolates, *Halomonas* sp. FTI23 and *Gordonia* sp. FTI07, grew on plates both higher and lower in salinity than previous reports (Kummer et al., 1999; Wang et al., 2007a), and eleven other isolates demonstrated higher salinity tolerance than their type-strain counterparts. The ability of most of the isolates to grow on the near-saturation 25% (w/v) NaCl plates suggest that they are capable of growing within tailings and brine. The observed halotolerant characteristics instead of true halophilic characteristics could be due to the presence of microniches of different salinities within these materials, or reflect the different sources of microbes in this environment. Microbes could have been introduced to these materials from many different sources with a wide range of salinity and organic load, including the ore body, organic chemicals used in the milling process, wind deposition, and soils in contact with the TMA, and some species such as *Bacillus* sp. may be tolerating the salinity as spores instead of growing.

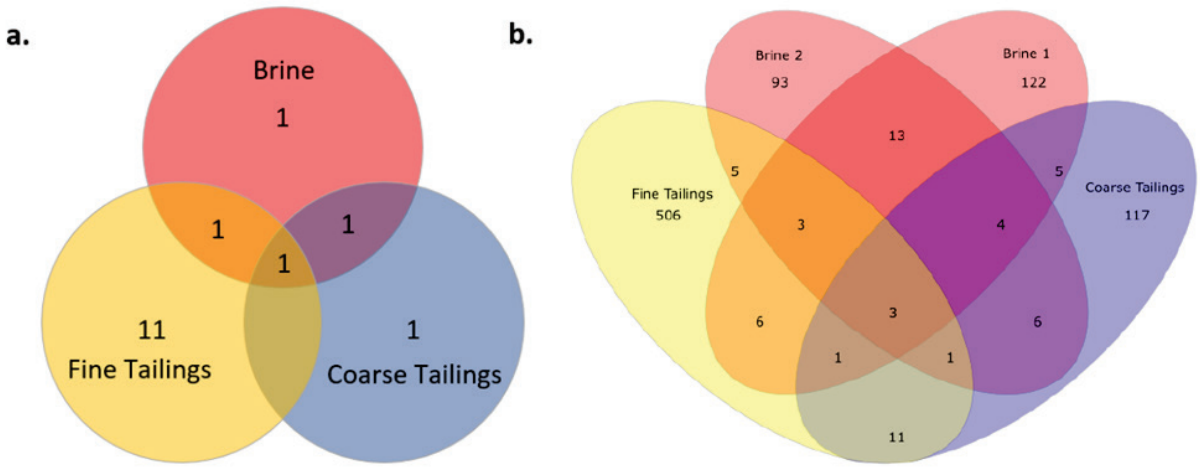


Figure 3-4 Venn diagrams showing shared OTUs (97% sequence similarity) in sample types for (a) isolated microbes and (b) high-throughput sequencing.

Regarding temperature tolerance, many close relatives also reported growth at the tested range of 4-37°C (e.g. Kaeppel et al., 2012; Romano et al., 2006; Xu et al., 2009), or have not been previously tested. Many halophilic archaea and bacteria demonstrate temperature tolerance, including psychrophilic species observed in Antarctic saline lakes (Bowers et al., 2009; DasSarma & DasSarma, 2012), thus the presence of species that can grow at 4°C is not surprising. Growth

was observed at lower temperatures in *Halomonas* sp. FTI21, *Halomonas* sp. FTI23, *Halorubrum* sp. BI09, *Gordonia* sp. FTI07, and *Haloferax* sp. FTI25 than their closest relatives (Enache et al., 2007; Kummer et al., 1999; Pesenti et al., 2008; Wang et al., 2007a; Wang et al., 2007b). DNA comparison showed high similarity scores in the 16S rRNA gene between isolates and their closest type-strain relatives (mostly > 99%), and thus, the broader temperature tolerances observed in this study may be due to environmental exposure in brine isolates, introduced genomic DNA through lateral gene transfer as plasmids, or a function of the type of media used in tolerance tests.

Low organic carbon concentrations (< 32 mg/kg) and the ability to perform spread plate colony counts without sample dilution suggest that there are low levels of biomass in these materials. The metagenomic data, however, indicate microbes with a broad range of metabolic functions. The fine tailings high-throughput sequencing yielded sequences related to sulfate-reducing bacteria that can have implications in greenhouse gas emissions and iron corrosion including *Desulfothermus* sp. and *Desulfocapsa* sp., as well as the sulfur-reducing bacteria *Sulfurospirillum* sp. (Finster et al., 1997; Finster et al., 2013; Nunoura et al., 2007). Methanogenic archaea may be present in the tailings, indicated by relatives of *Methanobrevibactor* detected in the coarse tailings and of *Methanocaldococcus* detected in the fine tailings, and could emit methane a greenhouse gas (Cheng et al., 2009). Nitrogen fixing *Rhizobia*, important organisms in nitrogen cycling processes, were detected in both brine and fine tailings (Güereña et al., 2015). Other processes may occur that affect metal-cycling, including chromium reduction (*Arthrobacter* relatives in fine tailings and *Clostridium* relatives in both tailings samples (Camargo et al., 2004; Inglett et al., 2011)), iron redox reactions (*Acidiferrobacter* and *Deferribacter* relatives in fine tailings), and arsenic transformations (*Pseudomonas* sp. were detected in all high-throughput sequencing results and was isolated from fine tailings (Koechler et al., 2015). The extent of these processes within the tailings pile needs to be confirmed with further studies.

An interesting application for the highly pigmented microbes observed in the system would be to encourage growth and use the increase in pigments and carotenoids for stimulating brine pond evaporation, a strategy that is used in saltern evaporation ponds (Javor, 1989). Increasing evaporation would decrease the need for deep-well injection of excess brine, which has recently been linked to increased seismic activity (Verdon et al., 2016). This technology has been mentioned but not thoroughly studied within the peer-reviewed literature (Davis, 2000; Rocha et al., 2012).

Potash brine and tailings may also be a good source for biotechnologically-relevant microbes adapted to extreme environments. Microorganisms from similar environments have been used in antibiotic production, hydrocarbon degradation, surfactant production, biological waste treatment, and heavy metal biosorption (Margesin & Schinner, 2001; Oren, 2002). The versatile halotolerance demonstrated by isolates, especially those that may possess hydrocarbon-degrading capabilities, would be useful in remediation or waste treatment in highly saline conditions such as those seen in the oil industry.

3.6 Conclusion

This study presents the first characterization of microbial communities within hypersaline potash brine and tailings environments. High-throughput sequencing results showed three communities dominated by *Proteobacteria*, and a mixed Archaea and Bacteria community in the brine sample. Isolation experiments yielded both Haloarchaea and Bacteria species. Isolates demonstrated a broad salt tolerance – many of them capable of growth in 0-25% (w/v) NaCl amendments – extending the current understanding of salt tolerance in these species. Some of the isolated species may have utility to potash companies to enhance the evaporation of brine ponds, and others may be capable of degrading organic pollutants in highly saline conditions. The high salt tolerance among isolates and similar genera with previously characterized hypersaline communities suggest that a viable microbial community exists in these materials in spite of low biomass.

3.7 Acknowledgements

We acknowledge the technical advice and assistance provided by Kathlene Jacobson, Al Shpyth, Jeff Meadows and Richard Weishaupt through the International Minerals Innovation Institute (IMII). We thank the Canadian Light Source (Saskatoon, Canada) for use of the Life Sciences Lab and computing resources, and Jarvis Stobbs for his technical support with lab equipment. We gratefully acknowledge Tim Vogel, Chris Maierhoffer, and Justin Hayunga for sample acquisition, Samira Sumaila for assistance with XRD analysis, and Viorica Bondici and Kathlene Jacobson for constructive discussions.

3.8 Supplementary Materials

3.8.1 X-ray diffraction (XRD)

For mineralogical analysis, samples were dried in a + 65°C oven for one week then ground to a fine powder using mortar and pestle. The fine powder was packed into 0.034" polyimide tubing, sealed with Loctite® 454™ Prism® Instant Adhesive, and mounted onto a sample holder. These samples were taken to the Canadian Macromolecular Crystallography Facility bending magnet beamline (CMCF-BM) at the Canadian Light Source, mounted on a sample holder, and analyzed using powder x-ray diffraction at a wavelength of 0.6888 Å and detector distance of 250.011 mm. Using GSAS-II software (Toby & Von Dreele, 2013), the resulting x-ray diffraction patterns were calibrated using lanthanum hexaboride (LaB_6) as a standard, then integrated to linearize the spectra using a blank polyimide tube to minimize background if needed. Mineral phases were identified using Match! 2 software to match diffraction patterns of the samples to reference diffraction patterns using the Crystallography Open Database (COD) (Downs & Hall-Wallace, 2003; Gražulis et al., 2009; Gražulis et al., 2012).

Spectra were consistent with the chemistry data obtained from the SRC (Table 3-1) and indicated that Halite (NaCl) is the major fraction of all three sample types (43.56-66.69% of peak area), while Sylvite (KCl) is the second highest fraction (13.74-34.15%) (e.g. Figure 3-5). These two mineral phases alone were able to account for more than 69% of peak intensity for all three sample types. Fine tailings had an additional three phases to cover a total of 94.90% of the diffraction peak profile: dolomite, anhydrite, and quartz, all phases that are expected with the Saskatchewan ore bed.

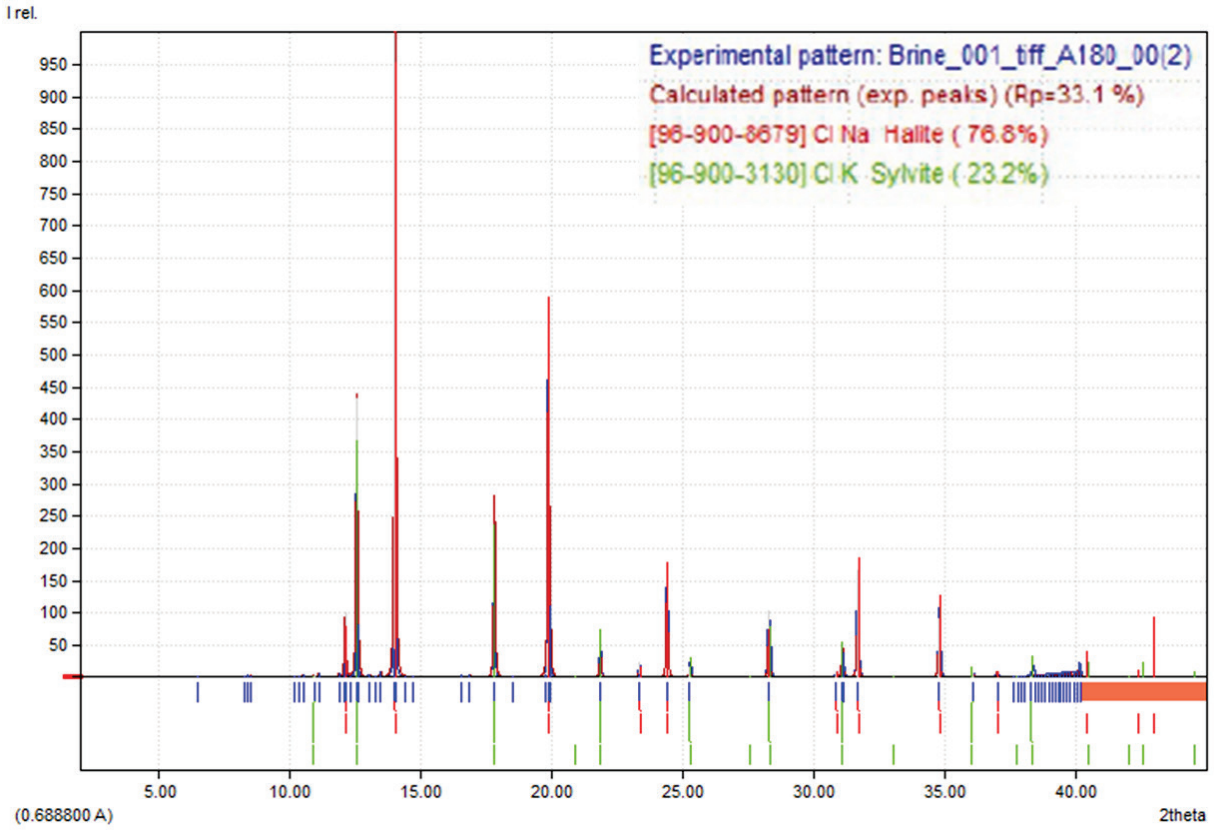


Figure 3-5 Example of XRD spectra showing evaporated brine mineralogy.

3.8.2 ICP-MS analysis

Table 3-4 Metal composition of potash brine and tailings. Mean values are presented as ± standard error.

Parameter [a]	Brine (mg/l, except as noted)	Coarse Tailings (mg/kg, except as noted)	Fine Tailings (mg/kg, except as noted)
Aluminum	0.76 ± 0.01	640 ± 20	12200 ± 700
Arsenic	0.01 [b]	0.23 ± 0.03	1.2 ± 0.1
Barium	0.82 ± 0.01	2.73 ± 0.13	41 ± 3
Beryllium	ND	ND	0.4 [b]
Boron	ND	ND	24 ± 1
Chromium	0.17 [b]	0.70 [b]	17 ± 2
Cobalt	ND	ND	2.6 ± 0.3
Copper	0.75 [b]	ND	2.3 ± 0.5
Iron	0.80 ± 0.01	340 ± 10	6000 ± 300
Lead	ND	0.1 [b]	0.5 [b]
Manganese	3.0 [b]	7.5 ± 0.2	83 ± 7
Molybdenum	0.04 [b]	ND	0.1 [b]
Nickel	0.12 [b]	0.6 [b]	11.5 ± 1.0

Parameter [a]	Brine (mg/l, except as noted)	Coarse Tailings (mg/kg, except as noted)	Fine Tailings (mg/kg, except as noted)
Selenium	0.13 ± 0.01	ND	0.2 [b]
Silver	≤ 0.009	ND	ND
Strontium	36 [b]	14 [b]	98 ± 8
Tin	ND	ND	0.3 [b]
Titanium	ND	19 ± 1	90 ± 7
Uranium	ND	ND	0.3 [b]
Vanadium	0.01 [b]	0.8 [b]	18 ± 1
Zinc	0.25 ± 0.01	ND	11 ± 1

ND – not detected

[a] Tested and not detected in any samples: Antimony, Cadmium, and Thallium

[b] Standard error is 0

3.8.3 Colony forming units

Table 3-5 Potash isolation plate counts.

Media (w/v NaCl)	Observed Colony Counts Per Plate			Mean Observed Colonies	Mean Colony Counts (/g sample)
Brine					
R-2A	0	0	3	1	10
R-2A (3%)	192	214	nc	203	2030
R-2A (15%)	nc	nc	270	270	2700
R-2A (25%)	nc	nc	180	180	1800
NB	0	0	0	0	0
NB (25%)	47	150	0	65.7	657
Coarse Tailings					
R-2A	11	51	162	74.67	194
R-2A (3%)	141	TNTC	TNTC	TNTC	TNTC
R-2A (15%)	TNTC	50	TNTC	TNTC	TNTC
R-2A (25%)	20	8	2	10	26
NB	147	40	48	78.3	135
NB (25%)	55	19	4	26	58
Fine Tailings					
R-2A	4	7	4	5	50
R-2A (3%)	TNTC	TNTC	TNTC	TNTC	TNTC
R-2A (15%)	TNTC	TNTC	TNTC	TNTC	TNTC
R-2A (25%)	354	TNTC	TNTC	TNTC	TNTC
NB	17	4	8	9.7	97
NB (25%)	173	206	139	172.7	1727

nc - not countable; TNTC - too numerous to count

4 A FEASIBILITY STUDY TO DEVELOP BACTERIAL BIOSORBENTS FROM POTASH MINE TAILINGS FOR THE REMOVAL OF CU(II) AND CR(VI) FROM SALINE GROUNDWATER[2]

[2] This chapter was written in joint authorship with Wonjae Chang, James J Dynes, and Joyce M McBeth. The study is part of the IMII research program led by Dr. Wonjae Chang (Principal Investigator). Nicola Harris designed and conducted experiments. STXM images were collected by Nicola Harris and James Dynes, and STXM data analysis and figure preparation was done by James Dynes. FTIR figure preparation was done by Blain Paul. All other data analysis, interpretation, and manuscript preparation was conducted by Nicola Harris under the supervision of Wonjae Chang and Joyce McBeth. The tables, figures, and references cited herein have been reformatted to fit the thesis.

4.1 Abstract

Removal of metals from contaminated water can be accomplished using biosorbents derived from waste biomass. Biosorption studies however, have indicated that the presence of salts can greatly decrease metal adsorption; this may be due to increased ionic strength, or competition for binding sites. This study investigated the use of halotolerant *Croceicoccus* sp. FTI14, isolated from hypersaline potash mine tailings, as a biosorbent for the removal of dissolved Cu(II) and Cr(VI) from saline groundwater (0.55 M ionic strength). Biosorption performance of the oven-dried and finely-ground material was evaluated using batch biosorption experiments at varied ionic strengths, coupled with scanning X-ray transmission microscopy (STXM) and Fourier Transform Infrared (FTIR) technology. With 40 mg/l initial metal concentrations, FTI14 was capable of 40.3 ± 0.7% (16.3 ± 0.5 mg/g) and 19.3 ± 0.1% (7.8 ± 0.1 mg/g) Cu(II) removal from deionized water and saline groundwater, respectively. The observed Cu(II) uptake (meq/g) was higher than Cr(VI) by a factor of 6.3-28.7 and uptake decreased as ionic strength increased. Adsorbent structure and association between metal and biomass was visualized using STXM and FTIR spectra, and the results were consistent with a change in amide functional groups on the biomass after metal exposure. These findings suggest that removal of metals from salt-impacted water is possible using biosorbents derived from salt-tolerant bacteria.

4.2 Introduction

Metal contamination is a serious concern due to its persistence in the environment, its tendency to accumulate through the food chain, and the toxic effects it has on many organisms (Aryal & Liakopoulou-Kyriakides, 2015). In 2016, there were more than 3000 active metal-contaminated sites managed by the Canadian government, according the Canadian Federal Contaminated Site Inventory (Environment Canada, 2016). Metals can be released into the environment through spills or chronic low level discharges in wastewater or industrial effluents (Volesky & Holan, 1995), often in association with inorganic salts (Chen et al., 2010; Panno et al., 2006).

Biosorption is a growing field in biotechnology used for environmental remediation and wastewater treatment (Vijayaraghavan & Yun, 2008). Many types of biological material including algae, seaweed, bacteria, fungi, plant material, and agriculture and food process wastes such as

crab shells have demonstrated metal adsorption capability (Crini, 2006; Michalak et al., 2013; Niu et al., 2007). These materials can remove metals that form cations in solution such as Cu(II) and those that form anions in solution such as Cr(VI) (Table 4-1). Biological materials have excellent sorption capacity, comparable with commercial synthetic cation exchange resins (Ahluwalia & Goyal, 2007) and often perform better than traditional sorbents, especially in dilute systems (Gabr et al., 2008). Biosorption is effective when contaminant concentrations are below 100 mg/l, where chemical and electrochemical processes are ineffective (Wang & Chen, 2009). Further, because biosorbent materials are generally derived from natural or waste material sources, biosorption is often considered to be more cost-effective than technologies such as ion exchange and reverse osmosis (Ahluwalia & Goyal, 2007; Nourbakhsh et al., 1994; Vijayaraghavan & Yun, 2008).

Table 4-1 Highest reported values of metal uptake of Cu(II) and Cr(VI) in biosorption literature.

Metal (valence)	Biosorbent	Metal Uptake[1] (mg/g)	Reference
Cu(II)	*Bacillus firmus* protein	381	Salehizadeh & Shojaosadati (2003)
Cr(VI)	Activated sludge	294.0	Aksu et al. (2002)
Cr(VI)	*Aeromonas caviae*	284.4	Loukidou et al. (2004)
Cu(II)	*Zoogloea ramigera*	270	Norberg & Persson (1984)
Cr(VI)	*Pachymeniopis* sp.	225	Lee et al. (2000)
Cu(II)	*Aspergillus terreus*	224	Gulati et al. (1999)
Cu(II)	*Penicillium simplicissimum*	112.3	Li et al. (2008)
Cu(II)	*Thiobacillus ferrooxidans*	198.5	Ruiz-Manriquez et al. (1998)
Cu(II)	*Spirulina* sp.	196	Chojnacka et al. (2005)
Cr(VI)	*Spirulina* sp.	185	Chojnacka et al. (2005)
Cr(VI)	Rice hulls	164.3	Roy et al. (1993)
Cr(VI)	*Chlorella minutissima*	162.2	Roy et al. (1993)
Cu(II)	*Aspergillus terreus*	160-180	Gulati et al. (2002)
Cu(II)	*Arthrobacter* sp.	148	Veglio et al. (1997)
Cr(VI)	*Staphylococcus xylosus*	143	Ziagova et al. (2007)
Cu(II)	*Penicillium chrysogenum*	108.3	Deng & Ting (2005)

Metal (valence)	Biosorbent	Metal Uptake[1] (mg/g)	Reference
Cu(II)	*Pseudomonas putida*	96.9	Uslu & Tanyol (2006)

[1] Metal uptake as reported, not necessarily at maximum

Biological material contains charged surface molecules that are able to attract and react with charged metals, dyes, or fluorides as well as hydrophobic sites that can bind organic molecules (Çabuk et al., 2006; Green-Ruiz, 2006; Michalak et al., 2013; Nacèra & Aicha, 2006; Öztürk, 2007; Uslu & Tanyol, 2006). In metal biosorption, these materials are used independent of metabolic mechanisms to physically or chemically bind and remove metals from a gas or liquid phase and can be used to treat dilute wastewater or environmental spills (Aryal & Liakopoulou-Kyriakides, 2015).

Bacterial biomass can have particularly high metal uptake capacities and cell surface carries an overall negative charge at neutral pH levels (Öztürk et al., 2004). *Lactobacillus acidophilus* was able to remove 800 mg of arsenic per gram of dried biomass (Singh & Sarma, 2010), almost doubling its own weight in removed metal. Similarly, an uptake capacity of 714 mg of Cr(III) was reported per gram of *Rhodococcus opacus* (Calfa & Torem, 2008). Due to their small size of 0.01-0.091 μm^3 (Fauteux et al., 2015; Huete-Stauffer et al., 2016), bacteria have a high surface area that favors adsorption kinetics, and often present hydrophilic and gel-like surfaces that encourage adsorption (Volesky, 2003). The bacterial cell wall presents several functional groups identified in binding dissolved metals, including the amino, amido, sulfydryl, and carboxyl groups in various proteins, and hydroxyls in polysaccharides (Volesky & Holan, 1995; Wang & Chen, 2009). However, the availability of a functional group for adsorption can be affected by steric or conformational barriers.

While many biosorption studies report findings from single-solute systems, natural- and wastewaters contain a mixture of chemicals and minerals that greatly impact adsorption performance (Cotoras et al., 1992). Inhibition may be caused by competition at binding sites, changes that affect the electrical double layer or metal activity, or complexes forming between metal and salt ions such as chloride (Shukla et al., 2002; Vijayaraghavan & Yun, 2008). Adsorption mechanisms that use electrostatic attraction are especially affected (Volesky, 2003). For example, Dönmez and Aksu (2002) found that *Dunaliela* algae could remove 37.7 mg/g of Cr(VI) in solution

with 0% (w/v) NaCl, but this was decreased to 13.4 mg/g in solution with 20% (w/v) NaCl. An advantage, however, of using biological adsorption materials is that the diversity and specificity of available binding sites can allow for selective adsorption (i.e. the selective adsorption of Cu(II) over Na), a property that can make synthetic adsorbents costly.

In general, biosorption has been proposed as a promising economic and environmentally friendly alternative to conventional metal removal methods (Gadd, 2009), but the presence of salts in the system can greatly impact metal removal performance (Wang et al., 2016; Zhu et al., 2015). Ams et al. (2013) suggested that the adsorption of metals from hypersaline systems by halophilic organisms – those that are adapted to living in hypersaline environments – is increased compared to adsorption by non-halophiles. Their study found that using halophilic bacteria as an adsorbent led to an increase in adsorption at an ionic strength of 4 M compared to adsorption at 2 M. Species that can live in high salt concentrations need to tightly control ion permeability, resulting in bacterial cells that can have distinctive cell envelope characteristics and increased negative charge in phospholipids (Kushner & Kamekura, 1988; Schneegurt, 2012; Vreeland, 1987) that may contribute to improved metal adsorption capabilities. Adaptations can include proteins with highly negatively-charged residues, a cell wall with increased concentration of ion pumps, or the accumulation of compatible amino acids or sugars within the cell (DasSarma & DasSarma, 2012; Zhuang et al., 2010). The effects of these adaptations on metal uptake are not yet clearly defined.

The available literature for metal adsorption by halophilic and halotolerant bacteria is somewhat limited, with most of the published studies on this topic having taken place in the last ten years. Metal removal ranges from 5% Zn uptake by purple non-sulfur bacteria (Amoozegar et al., 2012) to 92% removal of Hg by *Bacillus* sp. (Green-Ruiz, 2006). Further, few studies have been conducted using halophilic or halotolerant bacterial adsorbents in the presence of salts (for example: Amoozegar et al., 2012; Ams et al., 2013; Ghazvini & Mashkani, 2009), other metal ions (Panwichian et al., 2010), industrial waste water, or natural water.

Waste biomass derived from hypersaline environments such as potash mine tailings have not been extensively considered as potential metal biosorbents under saline conditions. The specific objectives for the current study were: (1) isolate a halotolerant bacterium from hypersaline mine tailings and prepare the biomass as a biosorbent, (2) conduct batch biosorption experiments to measure the adsorption of copper (Cu(II)) and chromium (Cr(VI)) as examples of cationic and anionic contaminants, respectively, and compare performance in deionized water, groundwater,

and saline groundwater, and (3) evaluate effectiveness of the biosorbent using atomic absorption spectrophotometry (AAS), scanning X-ray transmission microscopy (STXM), and Fourier Transform Infrared (FTIR) analysis. This study will demonstrate the feasibility of using a halotolerant biosorbent from potash mine tailings to remediate metals from saline groundwater at concentrations relevant to contaminated sites.

4.3 Materials and Method

4.3.1 Isolation, strain selection and culturing

Isolates were obtained from potash mine tailings using nutrient broth (NB; Sigma Aldrich, N7519) or Reasoner's 2A agar (R-2A; Sigma Aldrich, 17209) spread plates with NaCl amendments between 0 and 25% (w/v). Standard aseptic handling techniques were used for all culturing and DNA extractions. After pure cultures were obtained, DNA was extracted using an UltraClean® Microbial DNA Isolation Kit (MoBio) and the 16S rRNA gene was amplified by polymerase chain reaction (PCR) on a Veriti™ Thermal Cycler (Applied Biosystems) using the 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R primers (5'-CAGCMGCCGCGGTAATWC-3'). Sanger sequencing of the 16s rRNA gene performed at the McGill University and Génome Québec Innovation Centre (Montreal, Canada) was used to identify close relatives.

Using Sequencher software (Gene Codes Corporation), the 16S rRNA gene sequences were trimmed, assembled, and manually curated, then the consensus sequence was compared using the Ribosomal Database Project (RDP) from Michigan State University. A maximum likelihood phylogenetic tree was constructed using MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 with the consensus sequence and near-relative type-strains. The tree was determined using the Tamura-Nei substitution model, *Aquifex aeolicus* as an outgroup, and 1000 bootstrap replicates (Kumar et al., 2015; Tamura et al., 2011).

Isolates were tested for salt- (0, 3, 15, and 25% NaCl (w/v)) and temperature tolerance (4, 22, and 37°C). Microscopy was performed (Nikon Eclipse LV100) using Gram staining (VWR) to determine cell shape and type. Growth curves of fast-growing halotolerant isolates were determined by optical density measured at 600 nm using an Epoch Microplate Spectrometer, after growth in Reasoner's 2A broth (R-2A, Himedia M1687) with 30 mg/l NaCl amendment and transfer to a 96-well plate with flat-bottomed wells (Supplementary Materials Figure 4-15).

Croceicoccus sp. FTI14 was selected for biosorption studies due to its fast growth. Cultures were grown in R-2A broth in a 37°C shaking water bath (80 rpm) for 2-4 days until solution was turbid and bright yellow in colour.

Potash tailings samples were analyzed for: major cations (Ca^{2+}, K^{+}, Mg^{2+}, Na^{+}, and SO_4^{2-}) using inductively coupled plasma optical emission spectrometry (ICP-OES) method; chloride with the ferricyanide method; HCO_3^{-} by titration; nitrate with colorimetric methods; and metals using inductively coupled plasma mass spectrometry (ICP-MS). These analyses were performed at the Environmental Analytical Laboratory at the Saskatchewan Research Council (SRC; Saskatoon, Canada) using standard methods (American Public Health Association et al., 2012; USEPA, 1983).

4.3.2 Biosorbent preparation

Dried biomass adsorbent materials were prepared following an adapted procedure from Srinath et al. (2002) (Figure 4-1). After the growth period, cultures were poured into 50 ml conical tubes and centrifuged at 4000×g for 10 minutes to separate biomass from the growth media. Supernatant was then poured off and the biomass was rinsed with sterilized Milli-Q water to remove any residual media. Biomass was oven-dried overnight at 65 ± 5°C (Aksu et al., 2002), resulting in hard pellets. These were then ground to a fine powder using an electric hand-held grinder and weighed. The prepared adsorbent was stored in a desiccator at room temperature until used in further experiments.

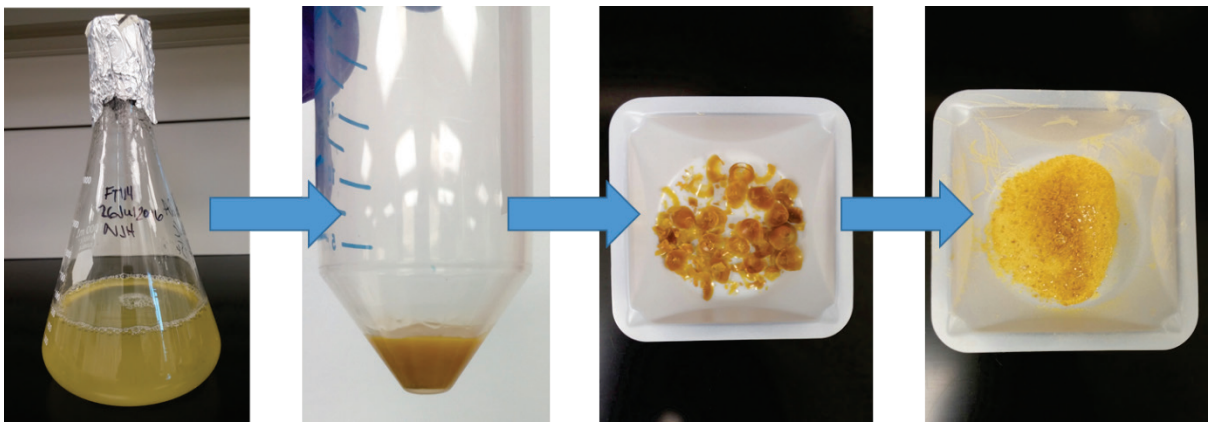


Figure 4-1 Dried FTI14 preparation procedure: culture growth, centrifugation, oven-drying, and grinding to fine powder.

4.3.3 Solution preparation

Groundwater was sampled from a rural well in Saskatchewan, Canada. It was tested for major ions by the Environmental Analytical Laboratory at the SRC using the same methods described in Section 4.3.1. Ionic strength was calculated using major ions (Ca^{2+}, K^{+}, Mg^{2+}, Na^{+}, SO_4^{2-}, Cl^{-}, HCO_3^{-}, and NO_3^{-}) according to the formula:

$$I = \frac{1}{2}\sum c_i \, z_i^2 \tag{4.1}$$

where c_i is the molar concentration of an ion species and z_i is its valency.

All glassware was washed with 0.1 M nitric acid then rinsed three times with Milli-Q water, and bottles for chemical storage were then autoclaved. Stock metal solution (40 mg/l) was prepared by mixing 3.133 ml of $CuSO_4$ 0.1 M solution (Fluka 35185) or 12 mL K_2CrO_4 1/30 M solution (Fluka 35157) in 500 ml of deionized water (DI), groundwater (GW), or groundwater with 30 g/l NaCl amendment (GW+Na). Initial concentrations were chosen to be more representative of contamination levels than those commonly used in biosorption experiments (Chergui et al., 2007; Naik & Furtado, 2014; Öztürk, 2007), but high enough for detection in atomic absorption spectrophotometry (AAS), STXM, and FTIR analysis. A Hach HQ40d portable pH meter (Loveland, CO, USA) was used to measure solution pH, which was adjusted using HCl and NaOH. Solutions were filter-sterilized into glass bottles using sterile disposable bottle top filters (0.20 µm Thermo Scientific™ Nalgene™ Rapid-Flow™).

4.3.4 Metal biosorption experiments

Batch adsorption experiments were performed at pH levels based on the literature: 4.0-5.0 for dissolved Cu(II) and 2.0-3.0 for dissolved Cr(VI) (Zouboulis et al., 2004). The optimum pH for Cu(II) biosorption is often reported to be pH 5.0-6.0 in the literature (Naja & Volesky, 2008; Subbaiah et al., 2011). However, negative control tests were conducted (without the addition of biomass) at approximately pH 5.5 and resulted in 75 and 90% removal of Cu(II) from GW and GW+Na, respectively (data not shown). Tests were repeated with pH 4.5 and no abiotic removal of Cu(II) was observed. A target pH of 4.0-5.0 was chosen for further tests. The adsorption experiments were conducted in triplicate in 50 ml conical centrifuge tubes using 10 ml of metal

ion solution and 1 g/l biosorbent concentrations. All treatments were conducted at 22 ± 1°C and agitated on a shaker at 200 rpm. Tests were conducted first using 40 mg/l Cu(II) and Cr(VI) ion solutions in DI, GW, and GW+Na, then with concentrations adjusted to 8, 16, 24, and 32 mg/l with DI for both metals and GW and GW+Na for Cu(II). Negative control experiments were conducted in triplicate without the addition of biomass.

More than 85% of Cu(II) uptake occurred within the first 30 min and the system reached a maximum uptake after 120 minutes of contact time (Supplementary Materials Section 0 and Figure 4-16). Subsequent tests were all conducted using a 120 min (2h) shaking period. After shaking, tubes were centrifuged at 4000 rpm for 10 min (Eppendorf 5804 R). Supernatant was analyzed for pH and residual metal ion concentrations in triplicate, then averaged. Metal concentrations were measured using a flame atomic absorption spectrophotometer (AAS) (Thermo Scientific iCE 3000) at 324.8 and 357.9 nm for Cu and Cr, respectively. Results are reported as the average of the three replicate test values ± standard error. Solute uptake was calculated using the following equation (Vijayaraghavan & Yun, 2008):

$$Q = \frac{V_o C_o - V_f C_f}{M} \tag{4.2}$$

where Q is the solute uptake (mg/g), C_o and C_f are the initial and final solute concentrations (mg/l), respectively; V_o and V_f are the initial and final solution volume in liters, respectively; and M is the mass of dried FTI14 (g).

T-tests were used to compare initial and final mean values in biosorption experiments and one-way ANOVA with Bonferroni was utilized for the comparison of mean uptake in DI, GW, and GW+Na adsorption systems, both using GraphPad Prism 6.0 (GraphPad Software Inc.). Linear regression was performed for affinity calculations from isotherms to determine R^2 values using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp.).

4.3.5 Biosorption modeling and statistical analysis

Isotherms were compared to the Langmuir and Freundlich adsorption models based on the nonlinear squares method (Volesky & Holan, 1995). The Langmuir isotherm model uses the following equation:

$$q = (q_{max} K_L C_f)/(1 + K_L C_f) \quad (4.3)$$

where q is solute uptake (mg/g), q_{max} is the maximum uptake (mg/g), C_f is the final solute concentration (mg/l), and K_L is the Langmuir constant (L/mg), related to affinity. The Freundlich isotherm model uses the form:

$$q = K_F C_f^{1/n} \quad (4.4)$$

where q is solute uptake (mg/g), C_f is the final solute concentration (mg/l), n is the Freundlich exponent (dimensionless) and K_F [(mg/g)(L/mg)$^{1/n}$] is the Freundlich constant.

The fit of the models was assessed with Akaike's Information Criterion (AIC):

$$\text{AIC} = N \ln \frac{\text{SSE}}{N} + 2N_\text{p} + \frac{2N_p(N_p+1)}{N-N_p-1} \quad (4.5)$$

where N is the number of data points and N_p is the number of parameters in the model. The AIC values were compared using the evidence ratio:

$$\text{Evidence ratio} = e^{-0.5\Delta} \quad (4.6)$$

where Δ is the absolute value of the difference in the AIC values of the two models.

Statistical analyses were run using GraphPad Prism 6.0 (GraphPad Software Inc.). Graphs show mean values with error bars indicating standard error.

4.3.6 Synchrotron-based STXM analysis

Residual adsorbent from 40 mg/l Cu(II) biosorption experiments in all water types and Cr(VI) biosorption experiments in DI was characterized using synchrotron-based STXM analysis to identify the location of metals within the biomass. Adsorbent samples were rinsed with sterile Milli-Q water then 1-5 µl of adsorbent suspension in water were deposited onto a Si_3N_4 window (1x1 mm, thickness 75 nm on 200 µm thick Si chip, 5x5 mm, Norcada Inc., Edmonton, Canada) following a procedure similar to Obst et al. (2009). Each sample was slowly spread across window

area by gravity – allowing the adsorbent to stick to the window surface – and air-dried. They were pre-screened using optical microscopy to choose representative areas of each sample type.

Imaging and spectromicroscopy was performed on the Soft X-ray Spectromicroscopy (SM) beamline at the Canadian Light Source (CLS) in Saskatoon, Canada, using the STXM end station (Kaznatcheev et al., 2007). The beamline was operated in the energy range of 130-2500 eV, with a spatial resolution of 30 nm, and resolving power of 3000 E/ΔE. Image difference maps were derived by subtracting the off-resonance image (280 eV for C 1s or 925 eV for Cu) from the on-resonance image (288.2 eV for peptide bond of proteins or 931 eV for Cu(II) and 934.3 eV for Cu(I)) to confirm the presence of biomass or Cu, respectively. Composite maps were derived from C 1s (280-320 eV), Cu 2p (922-940 eV), and the Cr 2p edges (566-616 eV) stacks by spectral fitting with selected reference spectra for spatial correlation analysis (Dynes et al., 2006). Reference compounds included human serum albumin (protein), xanthan gum (polysaccharide), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (lipid), $CuSO_4$, Cu_2O, and K_2CrO_4.

4.3.7 Fourier transform infrared (FTIR) spectroscopy

IR spectra of dried FTI14 before and after exposure to 32 mg/l Cu(II) or Cr(VI) in DI were recorded at room temperature on a Renishaw Invia Reflex Raman Microscope (Renishaw, Gloucestershire, UK) fitted with a IlluminatIR II FTIR microscope accessory (Smith's Detection, Danbury, CT). 256 scans were accumulated for each spectrum in the region of 4400-400 cm^{-1} and compared to the spectrum produced by dried FTI14 before metal exposure. All samples were rinsed with DI and then air-dried overnight. They were then prepared as KBr pellets using 1 mg of sample carefully ground with 99 mg of KBr.

4.3.8 Metal leaching

After biosorption reactions were performed, spent biomass from 40 mg/l systems was tested for leaching properties. Metal and biomass solutions were separated by centrifugation, then the supernatant poured off. Biomass was then suspended in DI (approximate pH of 5.8) in duplicates without shaking for 24 hours at room temperature (22 ± 1°C). Mixtures were centrifuged at 4000 rpm for 10 min and the supernatant analyzed for dissolved metal concentrations.

4.4 Results and Discussion

4.4.1 Isolate and tailings characteristics

Isolate FTI14 formed small, round colonies that were bright yellow on R-2A plates with 0% NaCl (w/v) amendment (Figure 4-2). Growth tests determined that FTI14 was capable of growth on both NB and R-2A plates and at 4-37°C. Growth was observed without salt amendment as well as at 3 and 15% NaCl (w/v) amendments, indicating that it was moderately halotolerant (DasSarma & DasSarma, 2012). Light microscopy revealed that cells were Gram negative, short rods that were arranged in chains (data not shown).

Comparison of the sequenced 16s rRNA gene with type strains in the Ribosomal Database Project (RDP) indicated a match with *Croceicoccus naphthovorans* E4A9 (97.6% similarity score). An *Alphaproteobacteria*, the *Croceicoccus* genus was recently described within the *Erythrobacteracea* family and is noted for its yellow colour. Other members of this genus were also isolated from saline environments – *C. marinus* from deep sea sediment (3.4% salinity) and *C. naphthovorans* from a marine biofilm (Huang et al., 2015; Xu et al., 2009). *C. naphthovorans* was also identified as a potential polycyclic aromatic hydrocarbon (PAH) degrader, but no members of this genus have been studied in-depth for biotechnology applications, including biosorption studies. Other close relatives (> 95% similarity scores) of FTI14 include

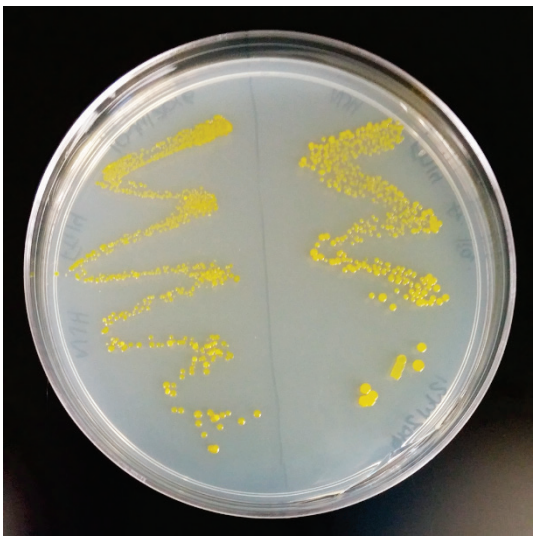


Figure 4-2 Isolate FTI14 plated on R-2A without NaCl amendment.

Novosphingobium sp. and *Sphingopyxis* sp., both within the *Sphingomonadacea* family (Figure 4-3).

The tailings used to isolate FTI14 contained a high level of salts (total salinity of 835 g/kg), which is likely to be the limiting factor affecting microbial life in those materials. In comparison the toxic metal and metalloid levels were low and inconsequential with regards to tolerance levels in microbial organisms (< 0.5 mmol/l, as used by Nieto et al. (1989)). For example, the Cu detected in the tailings was 2.3 mg/kg (0.03 mmol/l) and the total Cr detected was 17 mg/g (0.27 mmol/l).

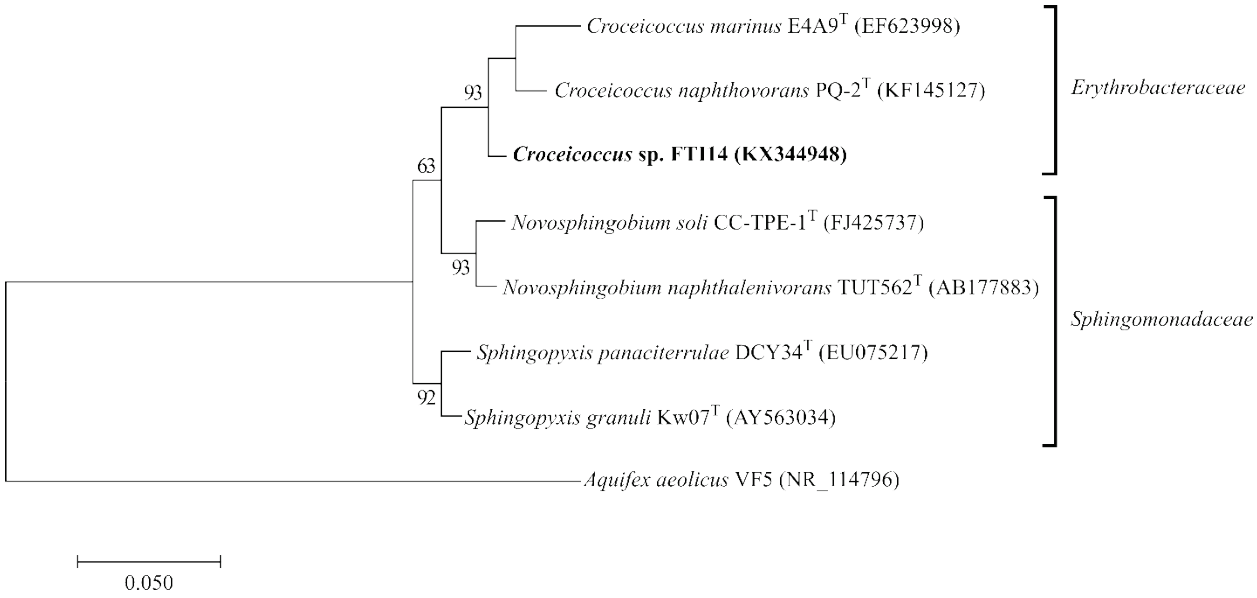


Figure 4-3 Maximum likelihood phylogenetic tree of *Croceicoccus* sp. FTI14 and closest type-strain relatives. Bootstrap values are shown for branches with ≥ 50% bootstrap support and *Aquifex aeolicus* was used as an outgroup. Taxonomic families are indicated on the right.

4.4.2 Cu(II) and Cr(VI) biosorption

Negative controls demonstrated changes in Cu(II) and Cr(VI) concentration for the tested conditions to be ≤ 3.04 mg/l and in pH of ≤ 0.02 (Figure 4-4 and Figure 4-5). Because both concentration and pH changes were minimal, the experimental set-up was considered to have no effect and no adjustments were made to the adsorption results. No statistical significance was observed between initial and final values (p values ≥ 0.01).

Biosorption solution pH affects both the dried biomass and the metal species in solution. Generally speaking, the lower pH (2-3) for Cr(VI) reactions will protonate binding sites on dried FTI14 and allow it to attract the $HCrO_4^-$ ions that are predominant at pH < 6.5 (McLean & Bledsoe, 1996; Park et al., 2010). The slightly higher pH for Cu(II) reactions (4-5) allows some of the potential binding sites to be deprotonated and attract the cationic Cu^{2+} ions. More basic pH levels can cause some metals to precipitate, which could contribute to the overall metal removal process but would have obscured the biosorption reactions studied here (Britton, 1943).

With a 40 mg/l initial metal concentration and in DI, Cu(II) uptake by dried FTI14 was found to be 16.3 ± 0.5 mg/g, which was 40.3 ± 0.7% of the Cu(II) in the system (Figure 6). The adsorbent had a lower uptake for Cr(VI) with 9.6 ± 0.2 mg/g (22.9 ± 0.7% removal). When comparing the adsorption of Cd(II) and Cr(VI), Ziagova et al. (2007) found similar uptake differences between the cationic and anionic metals. The lower anion uptake under these conditions may be due to steric hindrance caused by the large $HCrO_4^-$ molecule that is the dominant form of Cr(VI) at a pH of 2 (McLean & Bledsoe, 1996), or the lack of positively-charged binding sites associated with cell membranes (Loukidou et al., 2004).

Metal uptake decreased as the ionic strength increased in solution (Figure 4-6). Although both Cu(II) and Cr(VI) GW systems were within the drinking water objective set by the Water Security Agency in Saskatchewan for summation of ions (1500 mg/l), GW had an increased ionic strength of 0.03 M. In GW, adsorption was observed to be 9.9 ± 0.1 mg/g for Cu(II) (24.0 ± 0.5% removal) and 2.7 ± 0.5 mg/g for Cr(VI) (5.8 ± 1.0%), both lower than in DI. GW+Na had an ionic strength of 0.55 M and observed metal uptake was further decreased to 7.8 ± 0.1 mg/g Cu(II) (19.3 ± 0.1%) and 1.0 ± 0.3 mg/g Cr(VI) (2.1 ± 0.6%). Changes in concentration were all statistically significant at the 95% certainty cut off, while Cu adsorption systems and the Cr in DI system were significant at the 99% certainty cut off (Cu(II) p-values all < 0.0001, Cr(VI) in GW p-value = 0.0047 and Cr(VI) in GW+Na p-value = 0.0392).

Cr(VI) adsorption was drastically decreased in GW and negligible in GW+Na. While Cu(II) adsorption in GW+Na varied from DI by 21%, it only varied from GW by less than 5%, suggesting that Cu(II) adsorption was not substantially affected by the additional NaCl. In a study of vanadate uptake by *Halomonas* sp., decreases similar to the latter were observed (8.4%)using 50 mg/l initial vanadate concentrations when 2% (w/v) NaCl was introduced to DI (Ghazvini & Mashkani, 2009). A bigger difference was seen with Cr(VI) uptake by *Dunaliella* sp. using 56.6

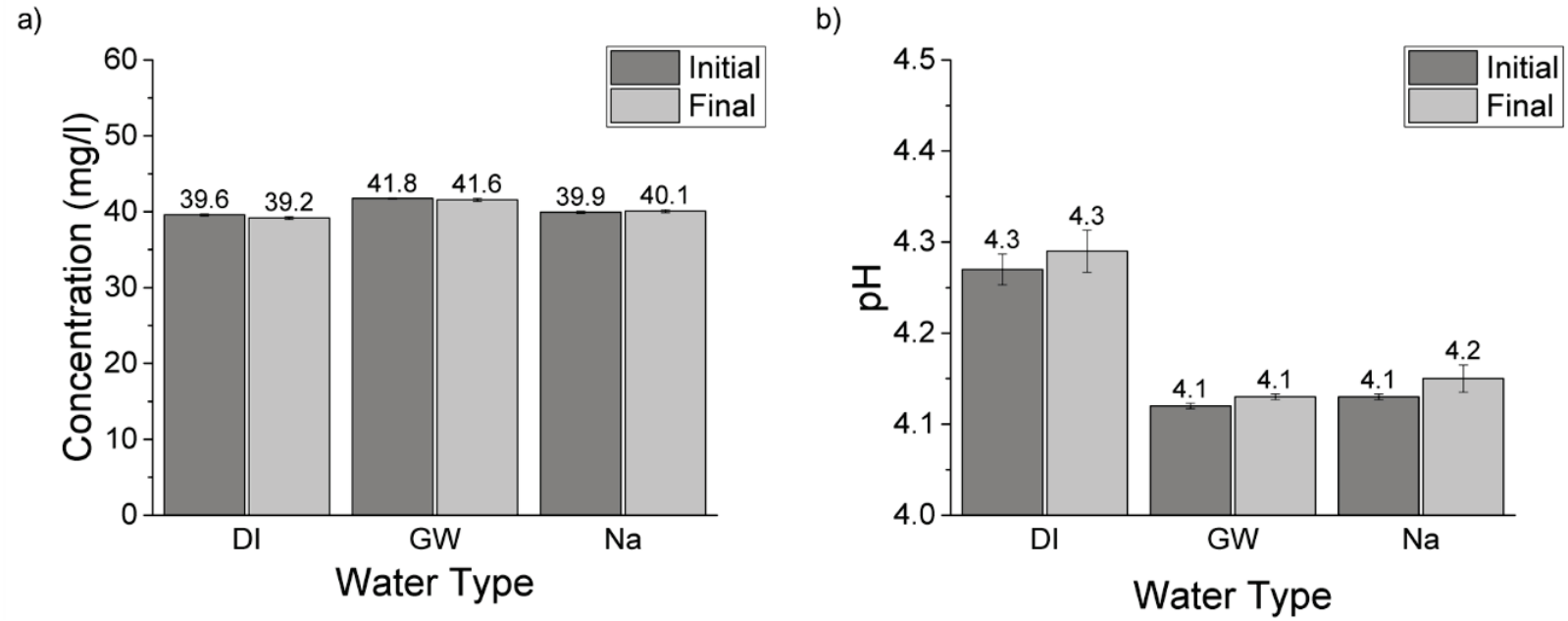


Figure 4-4 Negative controls for Cu(II) biosorption showing (a) initial and final concentrations and (b) pH for deionized water (DI), groundwater (GW), and saline groundwater (Na).

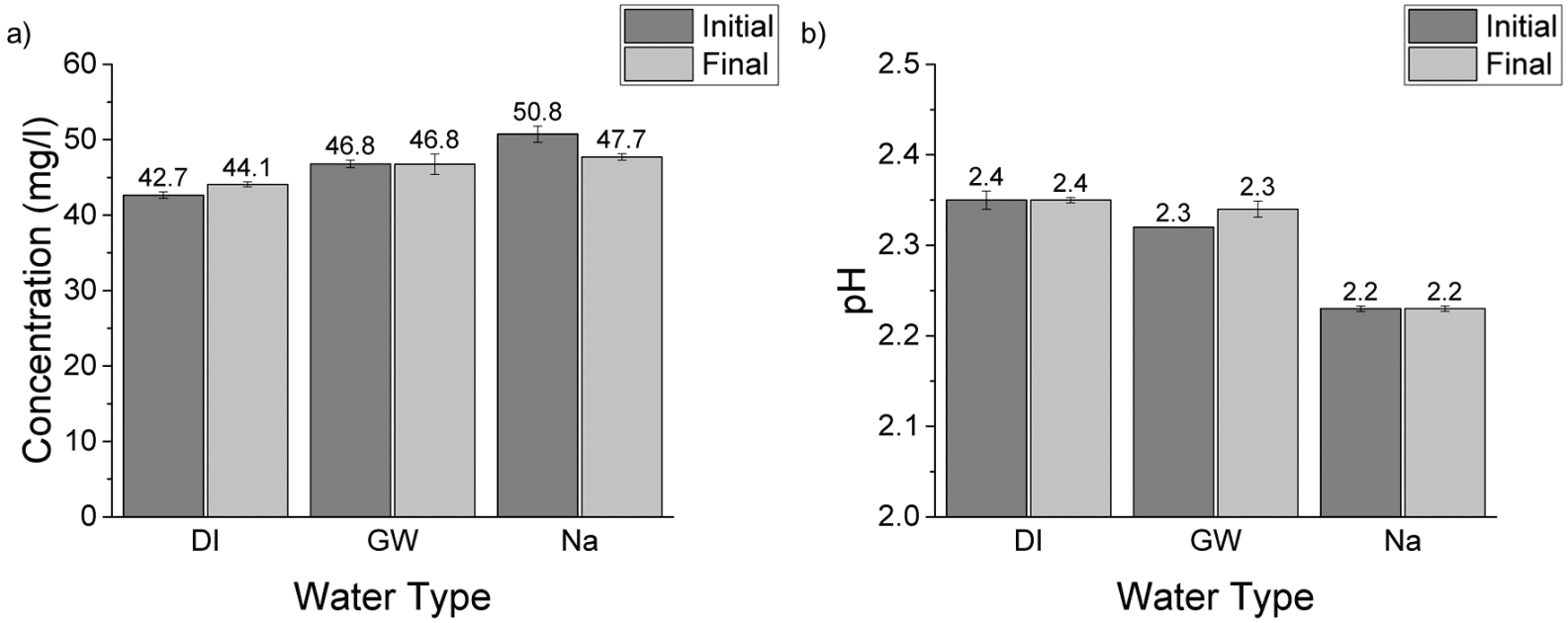


Figure 4-5 Negative controls for Cr(VI) biosorption showing (a) initial and final concentrations and (b) pH for deionized water (DI), groundwater (GW), and saline groundwater (Na).

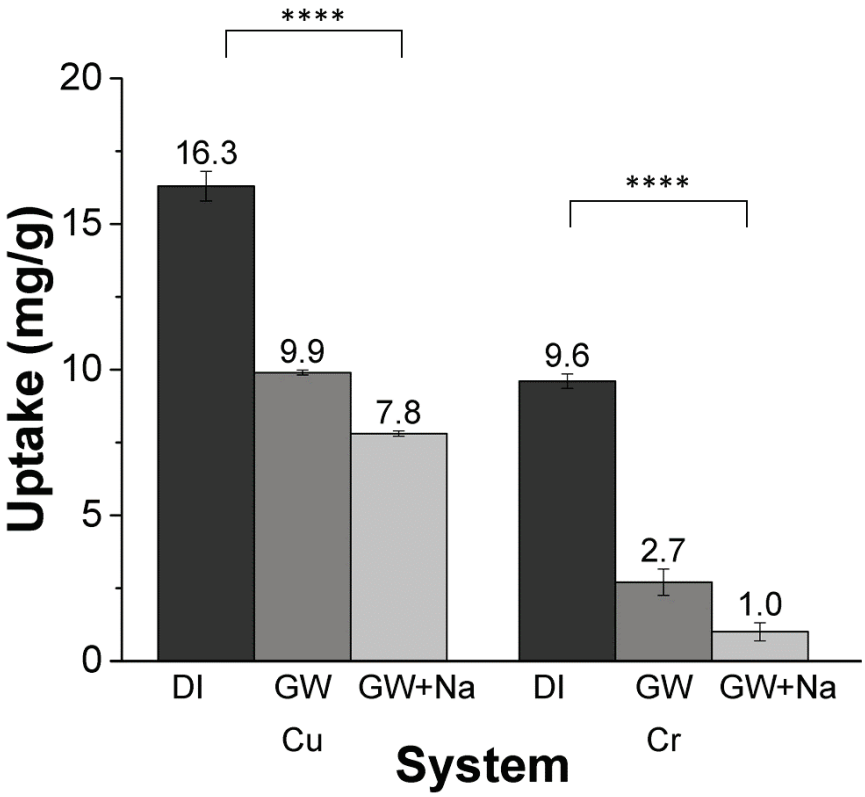


Figure 4-6 Effect of water type (DI, GW, and GW+Na) on metal uptake (mg of metal per dry g of adsorbent) in 40 mg/l systems; (****) significant at p < 0.0001 values.

mg/l initial Cr(VI) concentrations, when 5% (w/v) NaCl was added to DI removal varied by 26% (Dönmez & Aksu, 2002). While neither study investigated the cause of the decrease in metal adsorption, both suggested that it could be caused by either competition for binding sites or interference by the increased ionic strength on the activity of both binding sites and metals.

Although the final concentrations observed in these experiments do not meet the current standards set by Health Canada Guidance (1.0 mg/l for Cu and 0.05 mg/l for total Cr), the initial concentrations of 40 mg/l are higher than those generally observed in contaminated groundwater sites. Both Cu and Cr are trace metals, and are usually present in both clean and contaminated groundwater on the scale of ppb (µg/l). However, Cu has been observed at 2.78 mg/l in a New Jersey study (Page, 1981) and Cr at 0.08 mg/l in a monitoring well near a closed mine-site in Hungary (Czop et al., 2011).

4.4.3 Effect of biosorption on effluent pH

The pH level rose in all systems after the 120 min contact time (Figure 4-7). With an initial pH of 4.2-4.4, the Cu(II) tests saw a rise in pH of approximately 0.5, while the Cr(VI) tests increased from 2.2-2.4 by approximately 0.1. Statistical analyses suggest some degrees of statistical significance (p values <0.001), but the shift was not enough to move any of the batch systems outside of the targeted pH range (4.0-5.0 for Cu(II) and 2.0-3.0 for Cr(VI)) and therefore pH was not adjusted during the reaction.

Acidification is commonly expected during metal biosorption reactions due to ion exchange with the H^+ ions on the adsorbent surface (e.g. Thevannan et al., 2010). The absence of this effect suggests that metal binding mechanisms in the current study may not include ion-exchange reactions, particularly with H^+ ions. Loukidou et al. (2004) observed a similar shift in pH (2.5 to 2.6) during Cr(VI) biosorption experiments and identified this as a potential cause of the effect.

4.4.4 Adsorption isotherms

Generally, as the final metal ion concentration increased, the solute uptake also increased (Figure 4-8). The tested concentrations represent a low-level metal contamination in groundwater (1-100 mg/l (Gupta et al., 2000)) and may only make up the initial slope of a traditional isotherm curve because it has not yet levelled off. Further testing at higher concentrations would show the full isotherm shape and determine if a maximum uptake can be observed, as is predicted with monolayer adsorption models.

At low concentrations, the relationship between metal uptake and the final concentration is expected to be linear and the affinity of an adsorbent can be calculated as the average linear slope of each system. The affinity between the metal and FTI14 at these concentrations was the highest for Cu(II) in DI (0.740, $R^2 = 0.897$) and decreased as the ionic strength increased (0.340, $R^2 = 0.951$ for GW and 0.266, $R^2 = 0.977$ for GW+Na). Cr(VI) affinity in DI (0.266, $R^2 = 0.987$) was similar to the CuGW+Na system. Under these conditions, the order of affinity the adsorbent had in each system matched the observed uptake amounts (CuDI > CuGW > CuGW+Na = CrDI).

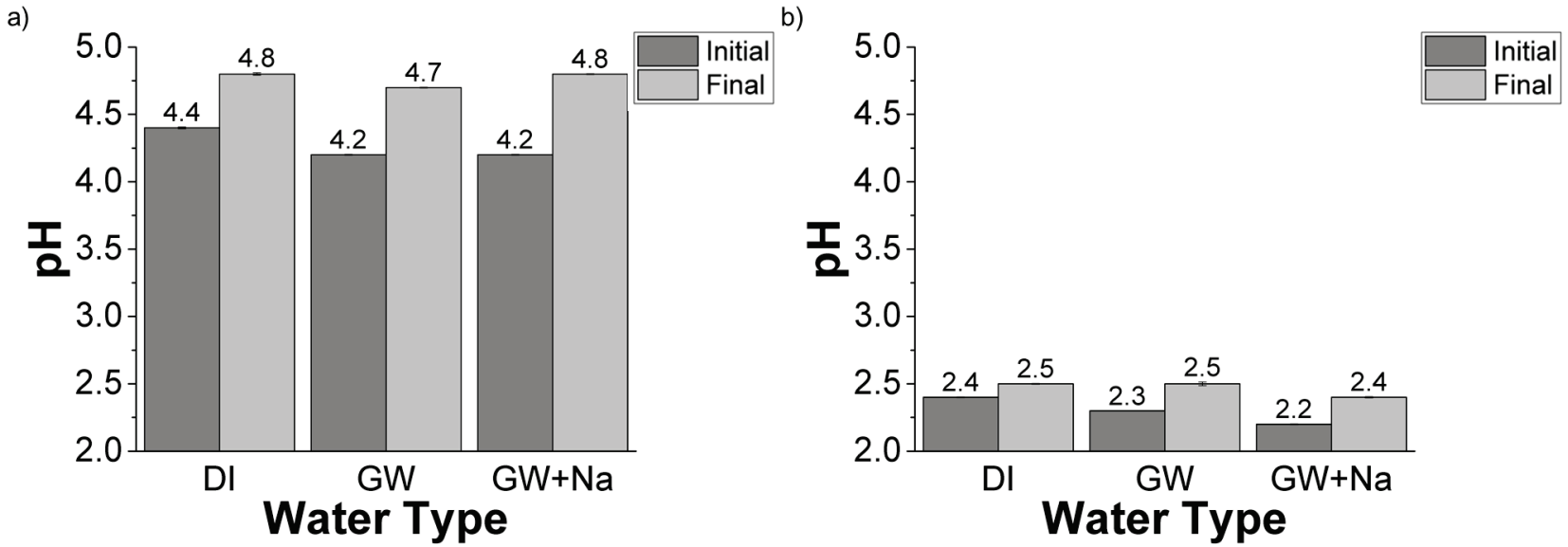


Figure 4-7 Effect of biosorption batch reactions on effluent pH for (a) Cu(II) and (b) Cr(VI).

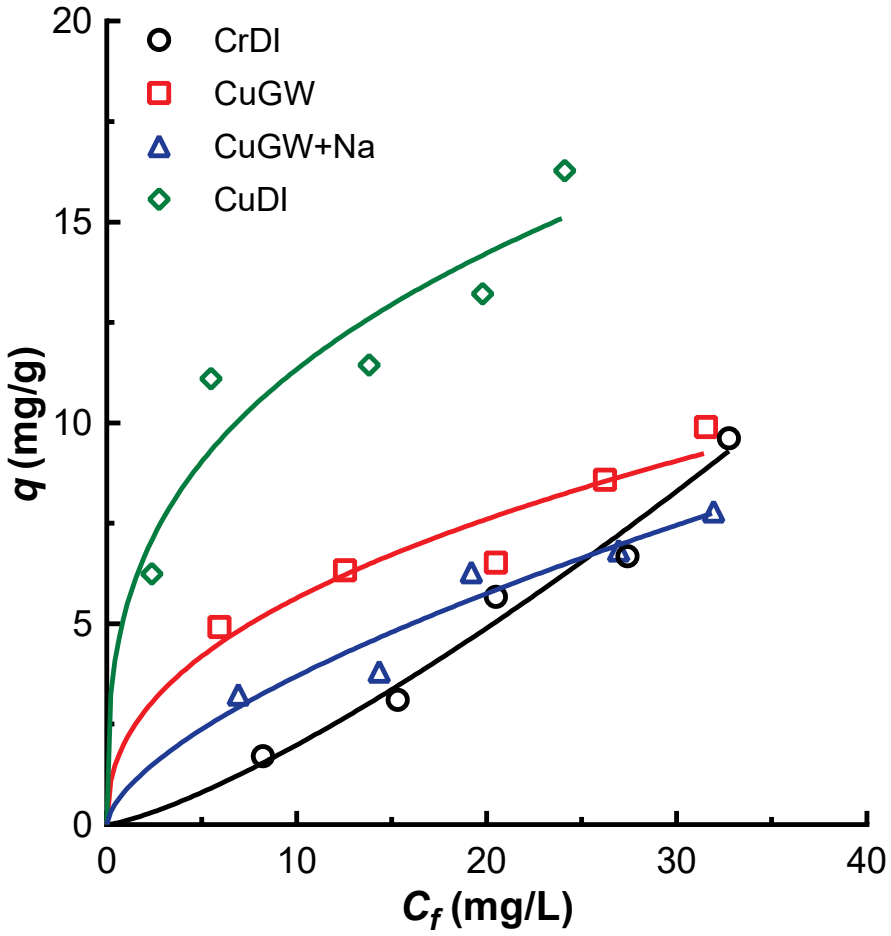


Figure 4-8 Biosorption isotherms for final metal concentration (C_f) against Cu(II) uptake from deionized water (DI), groundwater (GW), and saline groundwater (GW+Na), and Cr(VI) uptake from DI. Lines indicate the fit of the Freundlich adsorption model.

4.4.5 Adsorption models

Two common biosorption models, the Langmuir model (Langmuir, 1918) and the Freundlich model (Freundlich, 1906), were used to fit the experimental data and determine uptake parameters and statistical fit (Figure 4-8 and Table 4-2). All Cu(II) systems mathematically fit both models but the Freundlich model provided a better fit as indicated by evidence ratios of 1.29-3.33 (R^2 values $\geq$ 0.86). The evidence ratio for Cu(II) in GW implies that the Freundlich model is 3.33 times more likely to be valid than the Langmuir model. Due to a high dependency observed between parameters produced by the Cr(VI) system, the Langmuir model cannot be used with that

data. The Freundlich model, however, also provides a good fit for Cr(VII) uptake in DI (R^2 value of 0.97). The constants from the Cu(II) Freundlich model, K_F and n, decrease with increasing ionic strength, indicating a decreasing affinity between dried FTI14 and Cu(II) under these conditions. The Cu(II) systems also demonstrate $n > 1$, showing favourable adsorption reactions (Aksu et al., 2002). The smallest Freundlich constants are seen with Cr(VI), indicating that dried FTI14 has less affinity to Cr(VI) than Cu(II).

Using the Langmuir model, maximum uptake of Cu(II) was calculated to be 16.7 mg/g for DI, 12.1 mg/g for GW, and 15.7 mg/g for GW+Na. These values are similar to the uptake observed at the highest-tested initial concentration but because the isotherm has not necessarily levelled-off, the Langmuir model may not describe the system appropriately. Adsorption values reported in the literature have been much higher than this, with Cu(II) uptake up to 381 mg/g on a purified protein from *Bacillus firmus* (Salehizadeh & Shojaosadati, 2003) and 270 mg/g onto the bacterium *Zoogloea ramigera* (Norberg & Persson, 1984). Although maximum uptake of Cr(VI) cannot be calculated, the literature reports higher values than the observed uptake with FTI14, including 294.0 mg/g using activated sludge (Aksu et al., 2002) and 284.4 mg/g using *Aeromonas caviae* (Loukidou et al., 2004). Further testing at higher concentrations may still demonstrate higher uptakes than those observed in the current study.

The Langmuir model was developed for chemical, monolayer adsorption and represents homogeneous binding sites, while the Freundlich model accounts for heterogeneous adsorption surfaces and assumes that the energy of activation differs with each type of binding site (Aksu et al., 2002). The latter both describes the surface of dried FTI14 biomass more accurately and represented the observed uptake more closely. Although the underlying adsorption mechanisms and assumptions of the two models are different, both models have been found to represent many bacterial-metal adsorption reactions in the literature (Çabuk et al., 2006; Cotoras et al., 1992; Hasan et al., 2012; Öztürk, 2007).

4.4.6 STXM analysis

STXM images recorded at the C 1s edge (280-320 eV) revealed that prepared FTI14 biomass was present as both whole cells and indistinct biomass. The form of the biosorbent is important when considering the dynamics of biosorption reactions, where individual cells will provide more surface area than biomass with damaged cell walls that has clustered together. Long

Table 4-2 Langmuir and Freundlich adsorption parameters for Cu(II) and Cr(VI) isotherms with 8 to 40 mg/l initial metal concentrations.

		Langmuir model				Freundlich model				Comparison	
Metal	**Water Type**	**q_{max} (mg/g dry weight)**	**K_L (l/mg)**	**R^2**	**Sy.x**	**n**	**K_F (mg/g)**	**R^2**	**Sy.x**	**Δ AIC**	**Evidence Ratio**
Cu(II)	DI	16.7 ± 2.1	0.27 ± 0.12	0.84	1.67	3.05 ± 0.81	5.3 ± 1.3	0.86	1.58	0.51	1.29
	GW	12.1 ± 2.6	0.09 ± 0.05	0.80	1.03	2.32 ± 0.56	2.1 ± 0.6	0.87	0.81	2.41	3.33
	GW+Na	15.7 ± 6.0	0.03 ± 0.02	0.91	0.70	1.57 ± 0.32	0.8 ± 0.3	0.92	0.66	0.65	1.39
Cr(VI)	DI	--	--	--	--	0.77 ± 0.10	0.1 ± 0.1	0.97	0.61	--	--

rods were observed in the dried FTI14 control and in biomass after Cu(II) exposure in both GW tests, short rods after exposure to Cu(II) in DI, and indistinct clumps of biomass were observed in all systems (Figure 4-9). No patterns in shape were observed with the presence or absence of Na or the increased salts concentrations, as would be expected if differences in form were due to physical disruption during the biosorption reaction. An interesting observation, however, is that the dried FTI14 used for Cu(II) in GW+Na system appears to be lacking polysaccharides, possibly due to the saline solution stripping away the outer layer (Figure 4-10). Microscopy comparing dried bacterial biosorbents before and after metal exposure is not very common in the literature,

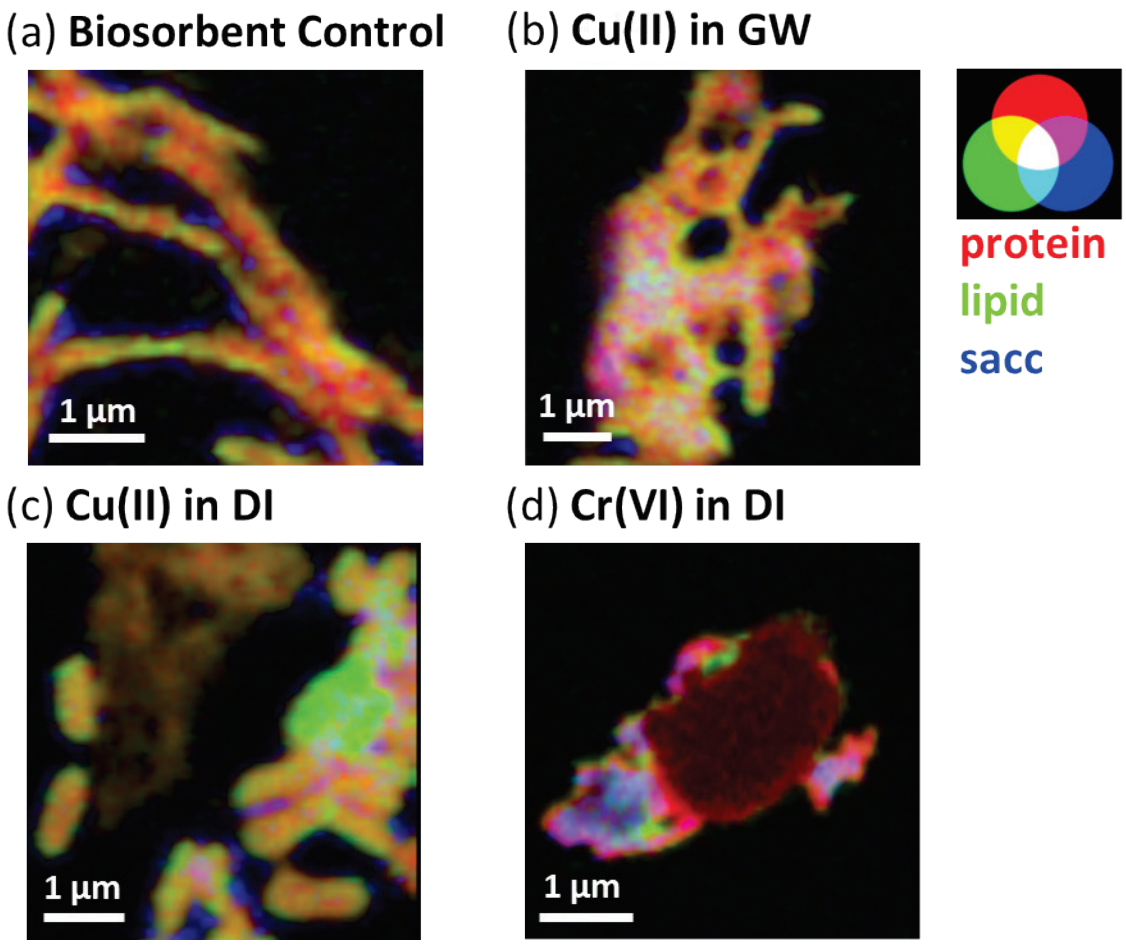


Figure 4-9 STXM generated colour overlay maps of organics associated with dried FTI14. Maps show long rods in (a) dried FTI14 control, and (b) biomass after Cu(II) exposure in GW, short rods in (c) biomass after Cu(II) exposure in Di, and indistinct biomass (d) biomass after exposure to CR(VI) in DI.

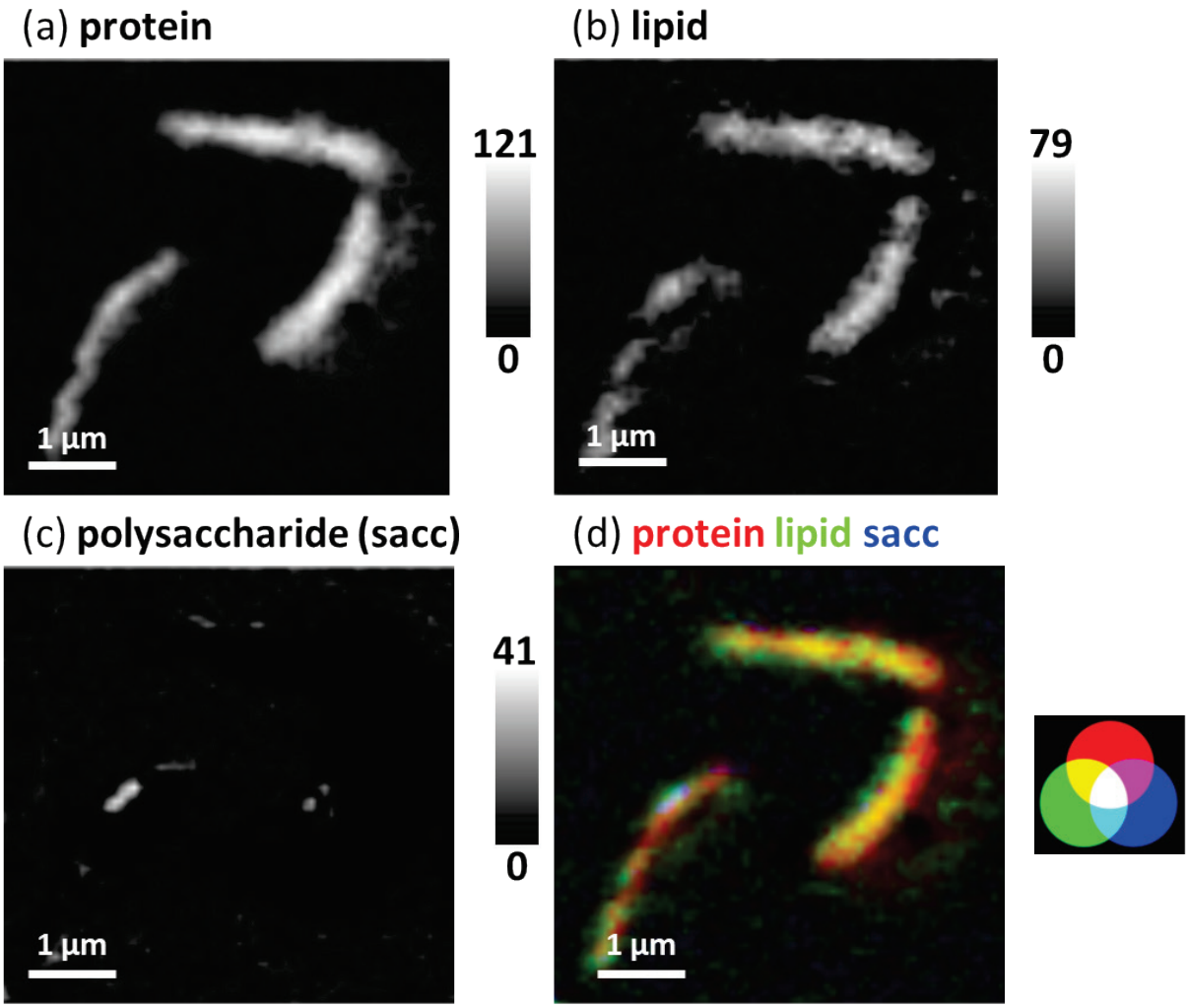


Figure 4-10 STXM generated component maps for Cu biosorption from GW+Na. Maps show (a) proteins, (b) lipids, (c) small amounts of polysaccharides, and (d) a colour overlay of the three.

however whole cells have also been observed in autoclaved biosorbents after metal exposure using transmission electron microscopy (TEM) (François et al., 2012).

Images were also recorded to examine the relationship between the metals and dried FTI14 biomass. Spatial correlation between organic material and metals was observed in both the CuDI and CrDI samples, suggesting that there was an association between these materials resulting from a biosorption reaction. Cu was detected in the CuDI samples, although the signal was weak (< 0.01 optical density (OD)). Both Cu(II) and Cu(I) oxidation states were observed spatially associated with biomass as evident by two peaks in the Cu 2p spectrum, one associated with Cu(II) (931 eV) and one with Cu(I) (934.3 eV) (Figure 4-11) (Grioni et al., 1992). The presence of Cu(I) may be

due to radiation damage from imaging rather than occurring during adsorption as it was not observed in the CuGW or CuGW+Na samples (images not shown) (Yang et al., 2011). The Cr detected in the CrDI sample had a spectrum similar to that of the Cr standard (Figure 4-12). Visualization of the relationship between the metals and the biocomponent using synchrotron technology is not traditionally utilized by biosorption researchers and although the signal obtained in this study is weak, the technology may prove useful in biosorption studies studying higher concentrations of substrate.

4.4.7 FTIR analysis

Analysis using FTIR further confirmed the association between dried FTI14 and the metals. The spectrum obtained from the dried FTI14 before metal exposure, used as a negative control, showed distinct peaks at 2960, 1659, 1530, 1451, 1384, and 1250 cm^{-1} (Figure 4-13). The strong peak at 1529.7 cm^{-1} assigned to N-H groups shifted slightly down to 1512.7 cm^{-1} after Cu(II) exposure and 1518.7 cm^{-1} after Cr(VI) exposure (Table 4-3). The focus of this study was not on the mechanism of adsorption, but this suggests that the amide ($-NH^2$) functional groups often associated with proteins in bacteria were involved in the uptake of both Cu(II) and Cr(VI). The change was more pronounced in the system with Cu(II) ions which could be due to the higher uptake concentration or a stronger interaction. This observation is consistent with Subbaiah et al. (2011) in Cu(II) adsorption onto fungi as well as Gupta and Rastogi (2008) in Cr(VI) adsorption onto cyanobacteria. Both studies also observed peak shifts associated with hydroxide, carbonyl, or carboxyl groups, however these were not observed in the present study. The tested range of concentrations did not indicate that the biomass had reached saturation with regards to binding sites, and therefore the metals may bind amide groups as a first preference. Further experiments using higher metal concentrations would confirm if peaks from other functional groups also shift during binding site saturation.

4.4.8 Metal leaching

The ability to retain adsorbed metals is an important characteristic in the application of a biosorbent for use in water remediation and the low leached-metal values observed here are

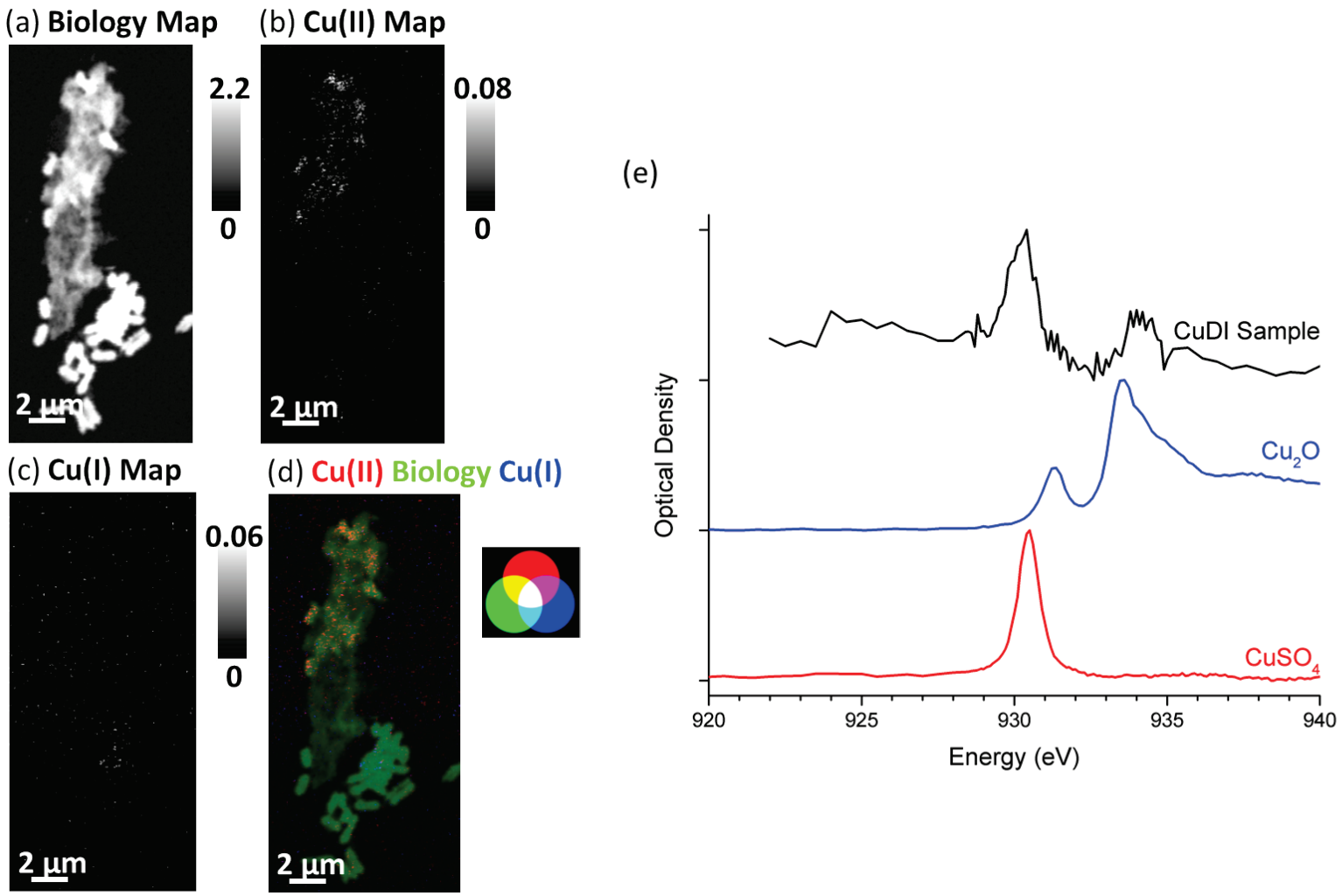


Figure 4-11 STXM generated component maps for Cu biosorption from DI. Maps show (a) biological material, (b) Cu(II), (c) Cu(I), and (d) a colour overlay of the three. Cu spectra are shown (e) including $CuSO_4$ and Cu_2O controls.

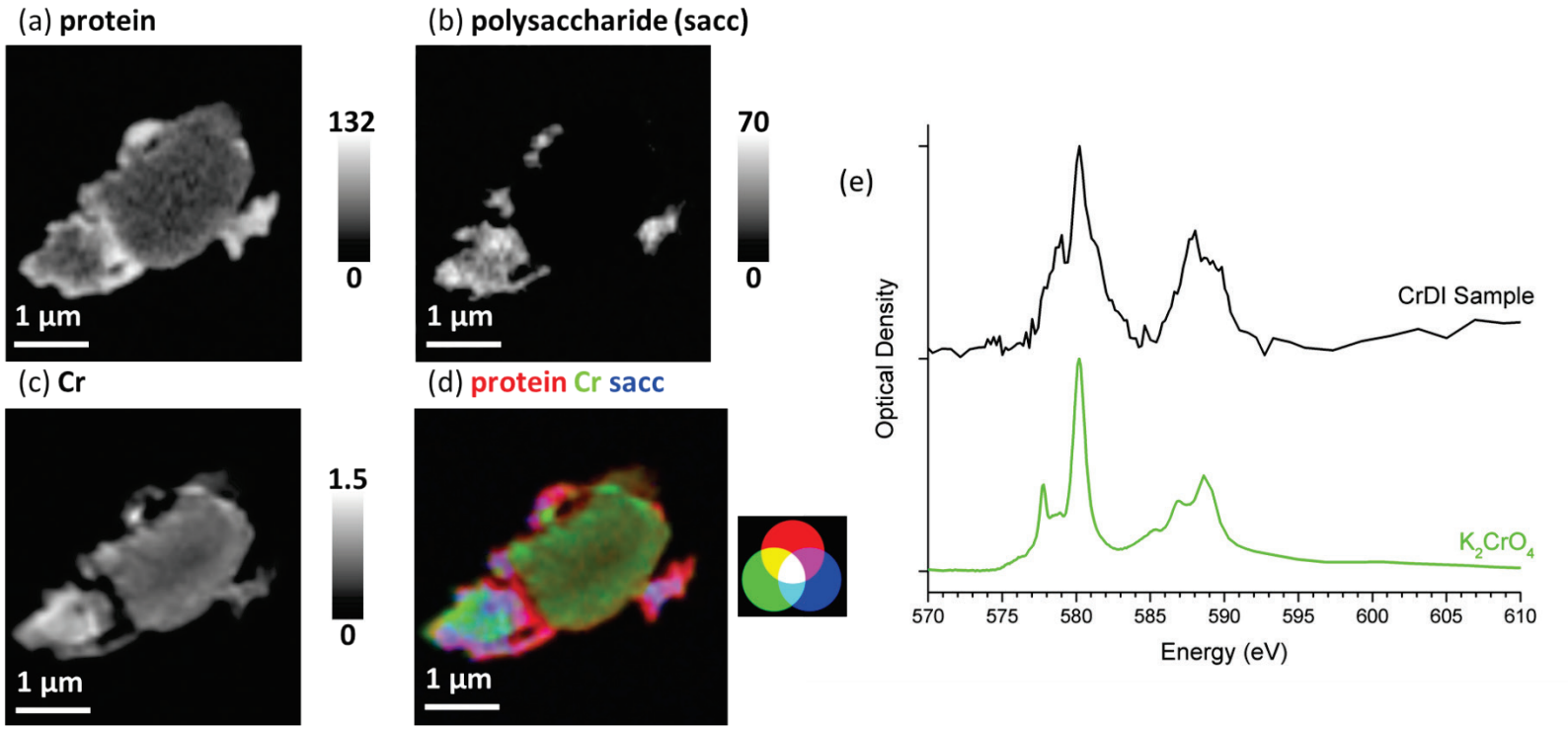


Figure 4-12 STXM generated component maps for Cr biosorption from DI. Maps show (a) proteins, (b) polysaccharides), (c) Cr, and (d) a colour overlay of the three. Cr spectra are shown (e) including K_2CrO control.

Table 4-3 FTIR absorption bands and suggested corresponding functional groups before and after metal biosorption in deionized water.

	Wavenumber (cm^{-1})		
Suggested functional group	**Unloaded biomass (control)**	**Cu-loaded biomass (Cu)**	**Cr-loaded biomass (Cr)**
Carbonyl (C─H)	2959.5	2959.5	2959.5
Carboxyl (C═O)	1658.9	1658.9	1658.9
Amide (N─H)	1529.7	1512.7	1518.7
Carboxyl (C─O)	1451.2	1451.2	1451.2
Carbonyl (C─H)	1383.7	1383.7	1383.7
Sulfate (S─O)	1249.5	1249.5	1249.5

(Akar et al., 2009; Bueno et al., 2008)

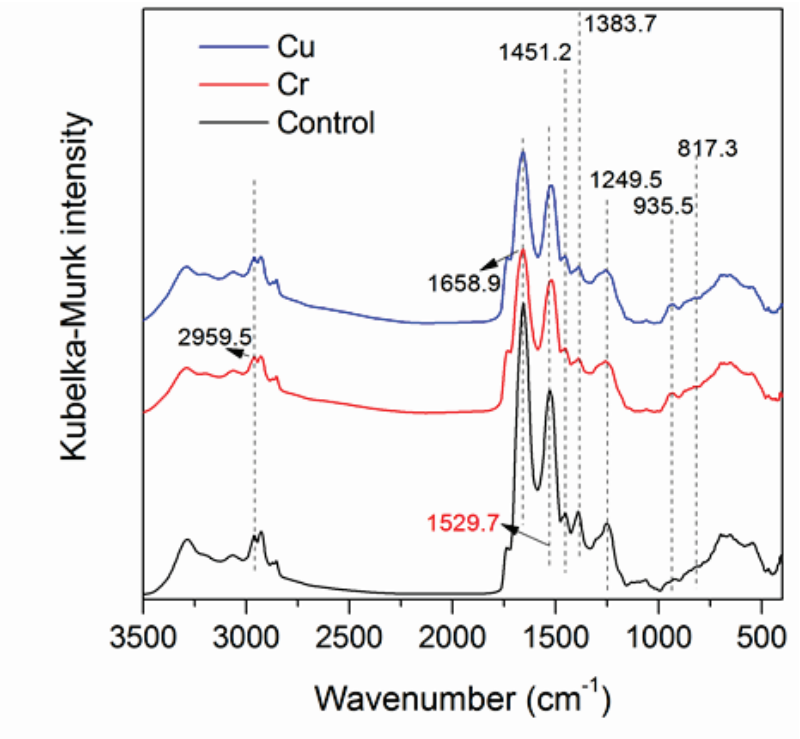


Figure 4-13 FTIR spectra of biomass before biosorption (Control) and after exposure to Cu(II) and Cr(VI), indicating amide interaction with the metal ions.

promising in that regard. Dried FTI14 was found to leach 1.4 ± 0.2 mg/g (10-14%) of the adsorbed Cu(II) and 2.0 ± 0.7 mg/g (40-75%) of the adsorbed Cr(VI) (Figure 4-14). The only statistically-relevant amount of leached Cu(II) was less than 1 mg/g (p value = 0.0063), while the statistically-relevant leached Cr(VI) was higher at approximately 4 mg/g (p value = 0.0018). These results suggest that dried FTI14 can retain adsorbed Cu(II) in clean (metal- and salt-free) solutions and further suggests that the mechanisms involved are not simply concentration-dependent ion exchange reactions. Implications during water remediation applications may include the requirement of a chemical, such as hydrochloric acid, to desorb the metals before reusing spent dried FTI14 in subsequent reactions.

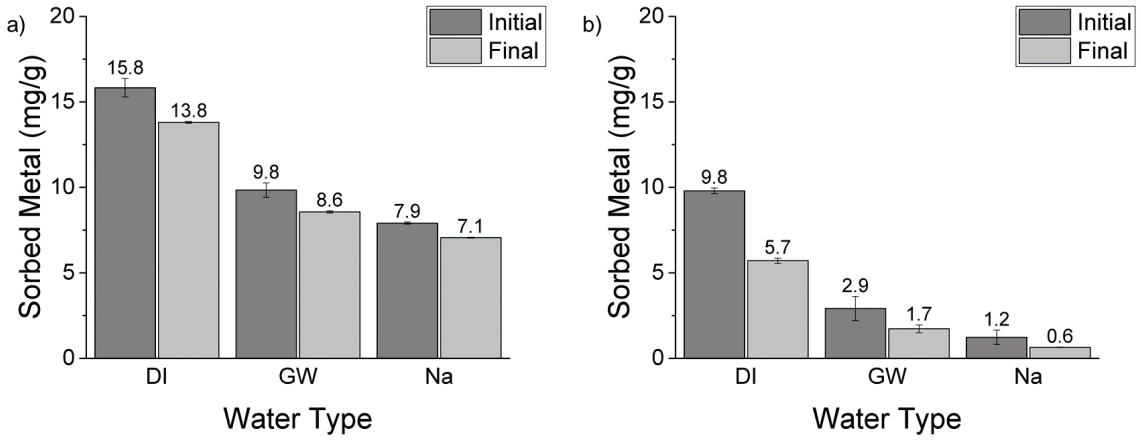


Figure 4-14 Leaching tests showing initial and final adsorption for (a) Cu(II) and (b) Cr(VI).

4.4.9 Implications, potential applicability and recommendations

Effective Cu(II) adsorption was demonstrated in DI, GW and GW+Na at low pH (pH 4.2-4.4) using a halotolerant biosorbent. These experiments were conducted at field-relevant contaminant levels in natural groundwater amended with NaCl, and successful adsorption in these conditions suggests that halotolerant bacteria could be considered as low-cost remedial agents in existing technologies such as pump and treat remediation of contaminated groundwater or for a polishing step in industrial wastewater treatment. The technology can be applied as fixed- or moving-bed column reactors (Michalak et al., 2013) or bag filtration-based biosorption systems where the adsorbent is kept in a mesh bag to allow easy separation (Banfalvi, 2006; Naja et al.,

2006). In addition to metal-contaminated water, biosorption in this study was demonstrated at low pH values and the remediation of metals from acid mine drainage (AMD) is a potential application for this biotechnology (Kim et al., 2014).

Before a scaled-up application of this technology is possible, many issues need to be addressed. Firstly, a practical method for selecting appropriate bacterial strains for adsorption needs to be developed. Next, a more economical growth media would need to be used to keep overall costs of the process low. Finally, tests would need to be conducted to evaluate immobilization strategies (for example embedding in silicon beads or biofilm) to improve strength in a column system or separation from the liquid phase. Preliminary studies for the immobilization of dried FTI14 biomass using zeolite have been conducted to evaluate zeolite adsorption potential under the conditions tested in this study (Supplementary Materials, Section 4.7.3).

4.5 Conclusion

The feasibility for development of a biosorbent from a halotolerant isolate derived from potash mine tailings and its use in the removal of Cu(II) and Cr(VI) in deionized water, groundwater, and saline groundwater was investigated. Dried FTI14 demonstrated a higher sorption capacity for Cu(II) ions than Cr(VI) in single-solute systems, and the presence of salt ions in the system suppressed adsorption of metal ions. Adsorption reactions for Cu(II) fit both Langmuir and Freundlich adsorption models while Cr(VI) fit the Freundlich model. STXM results showed that after metal exposure dried FTI14 was composed of both intact and broken-down cells and tentatively demonstrated an association between biomass and metals. FTIR spectra suggest that the ions were associated with proteins on the surface of dried FTI14. These observations demonstrate the development of halotolerant microbial biosorbents and that they can remove metals from saline groundwater.

4.6 Acknowledgements

We acknowledge the technical advice and assistance provided by Kathlene Jacobson, Al Shpyth, Richard Weishaupt, and Jeff Meadows through the International Minerals Innovation Institute (IMII). Thank you to Blain Paul for help with FTIR data analysis and spectra preparation. We thank the Canadian Light Source (Saskatoon, Canada) for use of the Life Sciences Lab and Jason Maley at the Saskatchewan Structural Sciences Centre (SSSC) for help obtaining FTIR

scans. We gratefully acknowledge Helen Yin for lab and equipment support, Ashley Siemens for groundwater sample collection, and Nick Gibb for constructive discussions. The STXM analyses described in this study was performed at the Canadian Light Source, which is supported by the Natural Sciences and Engineering Research Council of Canada, the National Research Council Canada, the Canadian Institutes of Health Research, the Province of Saskatchewan, Western Economic Diversification Canada, and the University of Saskatchewan.

4.7 Supplementary Materials

4.7.1 Growth curve

Growth curves of select isolates indicated that FTI14 reached the end of the exponential phase after approximately 8 hours of growth and achieved the highest absorbance (0.118) and subsequently the highest density of the tested isolates (Figure 4-15). The end of exponential growth phase was identified at 11, 12, and 24 hours for FTI06, FTI23, and FTI21, respectively, and was not observed within the tested timeframe for FTI08. FTI14 was selected to be developed as a biosorbent due to its fast growth, which allowed more adsorbent to be grown.

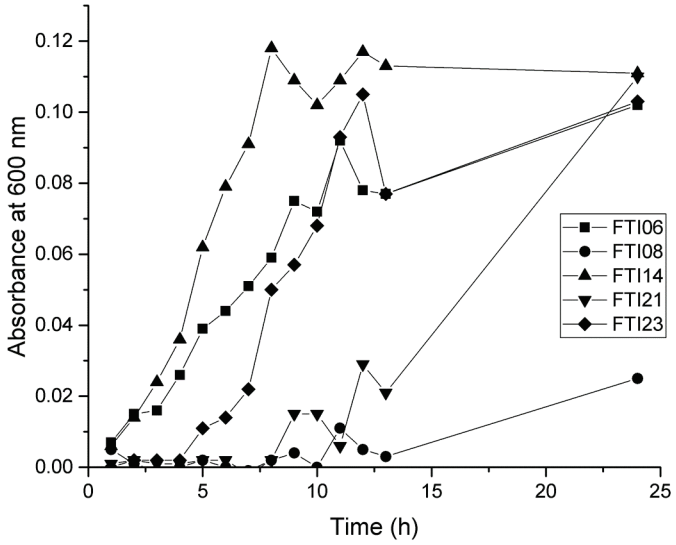


Figure 4-15 Growth curves of select isolates in R-2A with 30 mg/l NaCl amendment, shown as absorbance at 600 nm over time.

4.7.2 Contact time

Required contact time was tested using 40 mg/l Cu(II) ion solution in DI and 1 g/l prepared dried biomass with three knock-out replicates taken at 30, 60, 120, 180, 240, and 1440 min. at pH 4.5. Maximum uptake was reached after 120 minutes of contact time.

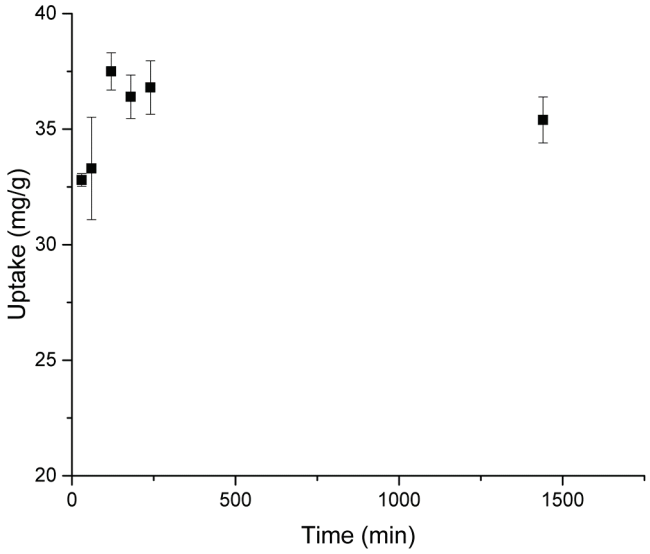


Figure 4-16 Cu(II) biosorption by FTI14 as a function of contact time.

4.7.3 Zeolite adsorption

Zeolite is a type of clay that has demonstrated effective cationic metal adsorption using ion exchange (Wang & Peng, 2010). It was tested for metal uptake and affect on the effluent pH using the same methods and conditions as the biosorption experiments outlined in Section 4.3.4. These tests were conducted using untreated Bear River Zeolite (BRZ™, USA).

After 120 min with 1 g/l of zeolite, Cu(II) concentrations were reduced from 40 mg/l in DI (p value < 0.0001), but not substantially affected in GW or GW+Na (Figure 4-17). The zeolite had an uptake of 5.725 ± 0.3 mg Cu(II)/g from DI (14.5 ± 0.5% removal), a lower value than that seen with FTI14. In GW, uptake was greatly impacted and was only 0.651 ± 0.1 mg Cu(II)/g (1.58 ± 0.3%). Cu(II) uptake by zeolite in the literature has been reported higher than the values seen here, therefore the limited adsorption may be due to the (unaltered) particle size, type of zeolite used, or a function of pH. Panayotova (2001) obtained almost 74% removal of Cu(II) from 50 mg/l initial concentration using pH 5.

The final pH for Cu(II) biosorption rose in all water-types, including GW+Na. This suggests that pH was being affected by a reaction separate from the Cu(II) adsorption. The change in pH was larger than that observed in biosorption experiments.

Both the Cr(VI) concentrations and pH were only negligibly affected by the zeolite (Figure 4-18), which was expected due to the presence of negatively charged binding sites in raw zeolite. It should be noted that chemically-modified forms of zeolite have reported successful Cr(VI) adsorption; at a final Cr(VI) concentration of 32 mg/l, an uptake of approximately 7 mg/g was reported (Ghiaci et al., 2004) which is only slightly lower than the 9.6 mg/g achieved in biosorption tests for the current study.

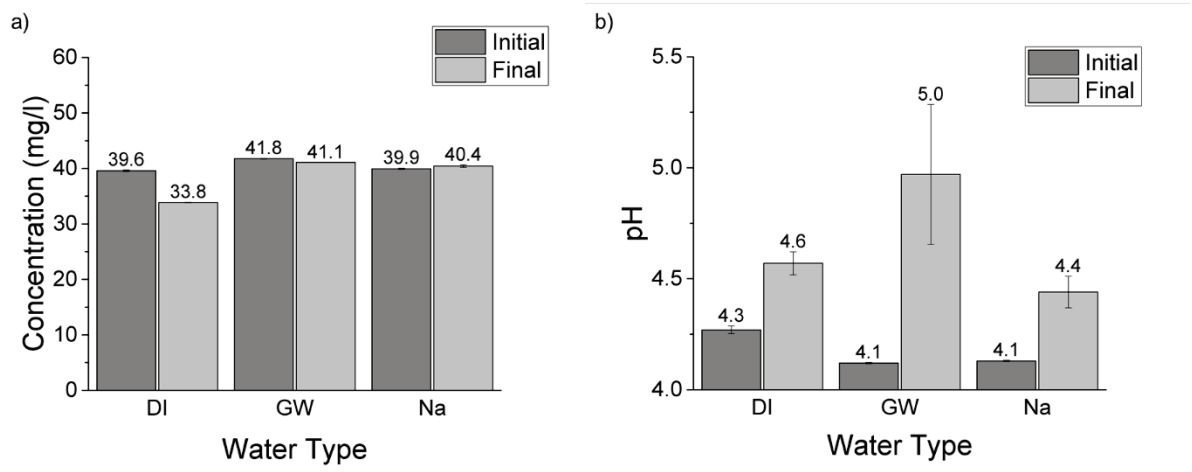


Figure 4-17 Zeolite tests for Cu(II) showing (a) initial and final concentrations and (b) pH.

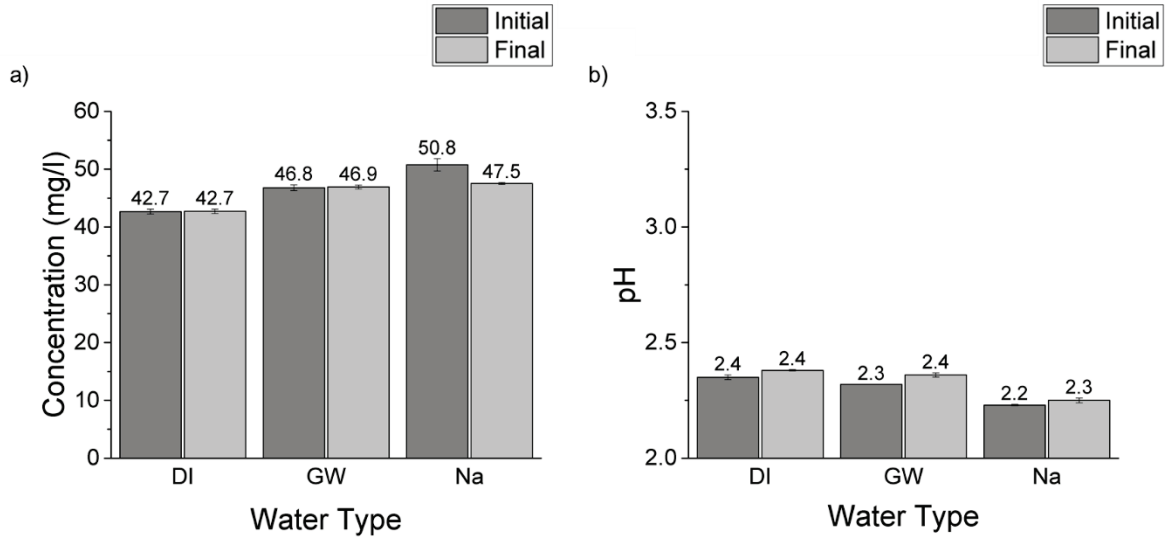


Figure 4-18 Zeolite tests for Cr(VI) showing (a) initial and final concentrations and (b) pH.

5 CONCLUSIONS

5.1 Key Findings

The microbial communities found within potash tailings and brine shared many genera with other hypersaline environments such as solar salterns, evaporites, and salt lakes. The most prevalent phylum in all samples was the *Proteobacteria* (40.6-89.3% of subsampled sequences), and *Actinobacteria* and *Firmicutes* were also prevalent. Haloarchaeal genera that are common in salterns and evaporites were observed in both high-throughput amplicon sequence reads and isolates from brine and fine tailings, but absent from coarse tailings results, indicating there are mixed Bacterial and Archaeal communities in those two materials.

Spread plates of fine tailings had the highest colony counts and most number of distinct isolates, while coarse tailings had the least. All isolates were relatives of species that were observed in high-throughput sequencing results and included known halophilic and halotolerant Archaea (*Haloferax* and *Halorubrum* species) and Bacteria (including *Halomonas*, *Marinobacter*, and *Dietzia* species). It was expected that the high salinity of the brine and tailings would select for extremely halophilic microbes in these communities, but isolates demonstrated a broad range of salt tolerance (0-25% (w/v) NaCl amendments) and both halotolerant and halophilic strains were observed. All isolates grew on both media-types and at the full range of temperatures tested. The observed growth characteristics extend the current understanding of both salt- and temperature-tolerances.

The Archaeal isolates were brightly-coloured and related to species used in salterns to enhance brine evaporation (Davis, 2000; Rocha et al., 2012). This is a use for indigenous microbes that the potash industry may be able to duplicate in their own brine ponds. Some fine tailings isolates were related to hydrocarbon degraders, including *Dietzia maris* (Bødtker et al., 2009), *Pseudomonas xanthomarina* (Isaac et al., 2013; Sopeña et al., 2014), *Sphingomonas jaspsi* (Ferrera-Rodríguez et al., 2013; Zhou et al., 2012), and *Bacillus thuringiensis* (Al-Saleh et al., 2009), and can potentially be used for bioremediation of hydrocarbon-contaminated soils and groundwater in saline conditions.

One of the isolates, a bacterium related to *Croceicoccus* sp. with 0-15% (w/v) NaCl tolerance and fast growth was chosen to be prepared and tested as a biosorbent. *Croceicoccus* sp. FTI14 was grown in liquid media, then concentrated and dried in an oven, and ground to a fine powder. In batch biosorption experiments, dried FTI14 demonstrated a higher affinity for Cu(II) ions than Cr(VI). The presence of salt ions in the groundwater system suppressed adsorption of metal ions, decreasing each time ionic strength was increased. FTIR spectra indicated a shift in an amide group peak, suggesting that the ions were associated with proteins on the surface of dried FTI14. STXM images showed that, after preparation and metal exposure, dried FTI14 was present as both intact cells (long and short rods) and indistinct biomass, indicating that some but not all the cells lysed during the process. The overlay maps of biological material and metals visualized the association between the biomass and metals.

5.2 Future Research Directions

5.2.1 Community composition

While previous studies have found select isolates from potash mines and effluents, this is the first study of the microbial community as a whole. Future work could expand this research by: performing additional replicate analyses to allow for more statistical analyses, characterizing the community within the tailings piles with borehole sampling, and looking at time-relevant sampling to include seasonal fluctuations.

5.2.2 Stimulating evaporation of brine ponds

Many of the archaeal isolates found in this study were closely related to archaea found in saltern evaporation ponds (Dillon et al., 2013; Fernández et al., 2014). The saltern industry takes advantage of these brightly coloured organisms by using their pigments to enhance the evaporation of seawater (Davis, 2000; Javor, 1989). The brightly-coloured archaeal and bacterial isolates identified in this thesis could potentially be used for enhancing evaporation in brine ponds for potash mining operations and other industries. To enhance brine pond evaporation would mean a decrease in deep-well injections of excess brine, a practice that has been associated with increased seismic activity (Verdon et al., 2016), and the reintroduction of (clean) process water into the environment.

5.2.3 Scale-up and optimization of biosorption process

The current study looked at biosorption by one strain isolated from potash tailings, chosen for its fast growth characteristics. There is currently no methodology for selecting effective biosorbents and while fast growth is an important factor in making a biosorbent, it does not indicate biosorption capability of a strain or consortium. A process for pre-selecting biomass for biosorption before conducting individual batch experiments is needed to improve on the time required for these studies.

Improvements to lower the cost of media and shorten growth time will also be required before this technology can be applied in water treatment as large quantities of biomass are required for full-scale application. Further, optimized system performance (i.e. initial pH, reaction temperature, mixing rate, and particle size (if applicable)) needs to be compared to *in situ* performance to understand the full cost and benefits of a biosorption system. Finally, scale-up will require improvements to the biomass separation step, such as pilot-scale continuous column or bag-filtration batch treatments, and studies regarding the regeneration of the biosorbent and cycle lifetime.

6 REFERENCES

Act, C.E.P. 1993. Priority substances list assessment report. *Environment Canada and Health Canada, Ottawa, Ontario*, 1-56.

Ahluwalia, S.S., Goyal, D. 2007. Microbial and plant derived biomass for removal of heavy metals from wastewater. *Bioresource Technology*, **98**(12), 2243-2257.

Akar, S.T., Gorgulu, A., Anilan, B., Kaynak, Z., Akar, T. 2009. Investigation of the biosorption characteristics of lead(II) ions onto *Symphoricarpus albus*: batch and dynamic flow studies. *Journal of Hazardous Materials*, **165**(1), 126-133.

Aksu, Z., Açıkel, Ü., Kabasakal, E., Tezer, S. 2002. Equilibrium modelling of individual and simultaneous biosorption of chromium(VI) and nickel(II) onto dried activated sludge. *Water Research*, **36**(12), 3063-3073.

Aksu, Z., Sag, Y., Kutsal, T. 1992. The biosorption of copper(II) by *C. vulgaris* and *Z. ramigera*. *Environmental Technology*, **13**(6), 579-586.

Al-Saleh, E., Drobiova, H., Obuekwe, C. 2009. Predominant culturable crude oil-degrading bacteria in the coast of Kuwait. *International Biodeterioration & Biodegradation*, **63**(4), 400-406.

American Public Health Association, American Water Works Association, Water Environment Federation. 2012. Standard methods for the examination of water and wastewater.

Amoozegar, M.A., Ghazanfari, N., Didari, M. 2012. Lead and cadmium bioremoval by *Halomonas* sp., an exopolysaccharide-producing halophilic bacterium. *Progress in Biological Sciences*, **2**(1), 1-11.

Ams, D.A., Swanson, J.S., Szymanowski, J.E., Fein, J.B., Richmann, M., Reed, D.T. 2013. The effect of high ionic strength on neptunium(V) adsorption to a halophilic bacterium. *Geochimica et Cosmochimica Acta*, **110**, 45-57.

Antón, J., Oren, A., Benlloch, S., Rodríguez-Valera, F., Amann, R., Rosselló-Mora, R. 2002. *Salinibacter ruber* gen. nov., sp. nov., a novel, extremely halophilic member of the Bacteria from saltern crystallizer ponds. *International Journal of Systematic and Evolutionary Microbiology*, **52**(2), 485-491.

Antón, J., Rosselló-Mora, R., Rodríguez-Valera, F., Amann, R. 2000. Extremely halophilic bacteria in crystallizer ponds from solar salterns. *Applied and Environmental Microbiology*, **66**(7), 3052-3057.

Aryal, M., Liakopoulou-Kyriakides, M. 2015. Bioremoval of heavy metals by bacterial biomass. *Environmental Monitoring and Assessment*, **187**(1), 1-26.

Atkinson, B., Bux, F., Kasan, H. 1998. Considerations for application of biosorption technology to remediate metal-contaminated industrial effluents. *Water SA*, **24**(2), 129-135.

Avery, S.V., Tobin, J.M. 1992. Mechanisms of strontium uptake by laboratory and brewing strains of *Saccharomyces cerevisiae*. *Applied and Environmental Microbiology*, **58**(12), 3883-3889.

Bai, J., Yang, X., Du, R., Chen, Y., Wang, S., Qiu, R. 2014. Biosorption mechanisms involved in immobilization of soil Pb by *Bacillus subtilis* DBM in a multi-metal-contaminated soil. *Journal of Environmental Sciences*, **26**(10), 2056-2064.

Bailey, S.E., Olin, T.J., Bricka, R.M., Adrian, D.D. 1999. A review of potentially low-cost sorbents for heavy metals. *Water Research*, **33**(11), 2469-2479.

Banfalvi, G. 2006. Removal of insoluble heavy metal sulfides from water. *Chemosphere*, **63**(7), 1231-1234.

Behrendt, U., Ulrich, A., Schumann, P. 2001. Description of *Microbacterium foliorum* sp. nov. and *Microbacterium phyllosphaerae* sp. nov., isolated from the phyllosphere of grasses and the surface litter after mulching the sward, and reclassification of *Aureobacterium resistens* (Funke et al. 1998) as *Microbacterium resistens* comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, **51**(4), 1267-1276.

Bødtker, G., Hvidsten, I.V., Barth, T., Torsvik, T. 2009. Hydrocarbon degradation by *Dietzia* sp. A14101 isolated from an oil reservoir model column. *Antonie Van Leeuwenhoek*, **96**(4), 459-469.

Bowers, K.J., Mesbah, N.M., Wiegel, J. 2009. Biodiversity of poly-extremophilic bacteria: Does combining the extremes of high salt, alkaline pH and elevated temperature approach a physico-chemical boundary for life? *Saline Systems*, **5**(1), 1.

Brierley, J.A., Brierley, C.L., Decker, R.F., Goyak, G.M. 1991. Contacting aqueous solution containing metal cation with causticized, immobilized *Bacillus* biomass, separation, Google Patents.

Britton, H.T.S. 1943. The application of electrometric methods to the study of some ionic reactions. *Ann. Rep. Prog. Chem.*(40), 43-59.

Bueno, B., Torem, M., Molina, F., De Mesquita, L. 2008. Biosorption of lead (II), chromium(III) and copper(II) by *R. opacus*: Equilibrium and kinetic studies. *Minerals Engineering*, **21**(1), 65-75.

Çabuk, A., Akar, T., Tunali, S., Tabak, Ö. 2006. Biosorption characteristics of *Bacillus* sp. ATS-2 immobilized in silica gel for removal of Pb(II). *Journal of Hazardous Materials*, **136**(2), 317-323.

Calfa, B.A., Torem, M.L. 2008. On the fundamentals of Cr(III) removal from liquid streams by a bacterial strain. *Minerals Engineering*, **21**(1), 48-54.

Camargo, F., Bento, F., Okeke, B., Frankenberger, W. 2004. Hexavalent chromium reduction by an actinomycete, *Arthrobacter crystallopoietes* ES 32. *Biological Trace Element Research*, **97**(2), 183-194.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences*, **108**(Supplement 1), 4516-4522.

Caumette, P. 1993. Ecology and physiology of phototrophic bacteria and sulfate-reducing bacteria in marine salterns. *Experientia*, **49**(6-7), 473-481.

Chen, Y., Pan, B., Li, H., Zhang, W., Lv, L., Wu, J. 2010. Selective removal of Cu(II) ions by using cation-exchange resin-supported polyethyleneimine (PEI) nanoclusters. *Environmental Science & Technology*, **44**(9), 3508-3513.

Cheng, Y., Mao, S., Liu, J., Zhu, W. 2009. Molecular diversity analysis of rumen methanogenic Archaea from goat in eastern China by DGGE methods using different primer pairs. *Letters in Applied Microbiology*, **48**(5), 585-592.

Chergui, A., Bakhti, M., Chahboub, A., Haddoum, S., Selatnia, A., Junter, G. 2007. Simultaneous biosorption of Cu^{2+}, Zn^{2+} and Cr^{6+} from aqueous solution by *Streptomyces rimosus* biomass. *Desalination*, **206**(1-3), 179-184.

Chojnacka, K., Chojnacki, A., Gorecka, H. 2005. Biosorption of Cr^{3+}, Cd^{2+} and Cu^{2+} ions by blue–green algae *Spirulina* sp.: kinetics, equilibrium and the mechanism of the process. *Chemosphere*, **59**(1), 75-84.

Cotoras, D., Viedma, P., Cifuentes, L., Mestre, A. 1992. Sorption of metal ions by whole cells of *Bacillus* and *Micrococcus*. *Environmental Technology*, **13**(6), 551-559.

Crini, G. 2006. Non-conventional low-cost adsorbents for dye removal: a review. *Bioresource Technology*, **97**(9), 1061-1085.

Cui, H.-L., Tohty, D., Zhou, P.-J., Liu, S.-J. 2006. *Halorubrum lipolyticum* sp. nov. and *Halorubrum aidingense* sp. nov., isolated from two salt lakes in Xin-Jiang, China. *International Journal of Systematic and Evolutionary Microbiology*, **56**(7), 1631-1634.

Czop, M., Motyka, J., Sracek, O., Szuwarzyński, M. 2011. Geochemistry of the hyperalkaline Gorka Pit Lake (pH> 13) in the Chrzanow region, southern Poland. *Water, Air, & Soil Pollution*, **214**(1-4), 423-434.

DasSarma, S., DasSarma, P. 2012. Halophiles. *eLS*.

Davis, J.S. 2000. Structure, function, and management of the biological system for seasonal solar saltworks. *Global NEST Journal*, **2**(3), 217-226.

Deng, S., Ting, Y.P. 2005. Fungal biomass with grafted poly (acrylic acid) for enhancement of Cu(II) and Cd(II) biosorption. *Langmuir*, **21**(13), 5940-5948.

Dillon, J.G., Carlin, M., Gutierrez, A., Nguyen, V., McLain, N. 2013. Patterns of microbial diversity along a salinity gradient in the Guerrero Negro solar saltern, Baja CA Sur, Mexico. *Frontiers in Microbiology*, **4**.

Dönmez, G., Aksu, Z. 2002. Removal of chromium(VI) from saline wastewaters by *Dunaliella* species. *Process Biochemistry*, **38**(5), 751-762.

Downs, R.T., Hall-Wallace, M. 2003. The American Mineralogist crystal structure database. *American Mineralogist*, **88**(1), 247-250.

Dynes, J.J., Lawrence, J.R., Korber, D.R., Swerhone, G.D., Leppard, G.G., Hitchcock, A.P. 2006. Quantitative mapping of chlorhexidine in natural river biofilms. *Science of the Total Environment*, **369**(1), 369-383.

Enache, M., Itoh, T., Kamekura, M., Teodosiu, G., Dumitru, L. 2007. *Haloferax prahovense* sp. nov., an extremely halophilic archaeon isolated from a Romanian salt lake. *International Journal of Systematic and Evolutionary Microbiology*, **57**(2), 393-397.

Environment Canada. 1994. Chromium and its compounds (Priority substances list assessment report). in: *Environment Canada and Health Canada, Ottawa, Ontario*, pp. 1-56.

Environment Canada. 2016. Federal Contaminated Site Inventory.

Environment Canada. 2012a. Metal Mining Effluent Regulations, Minister of Justice, pp. 1-55.

Environment Canada. 2012b. Metal mining guidance document for aquatic environmental effects monitoring. Gatinaue, QC.

EPA, U.S. 2010. National Primary Drinking Water Regulations. in: *Title 40*, (Ed.) C.o.F. Regulations, pp. 367-639.

Fauteux, L., Cottrell, M.T., Kirchman, D.L., Borrego, C.M., Garcia-Chaves, M.C., del Giorgio, P.A. 2015. Patterns in abundance, cell size and pigment content of aerobic anoxygenic phototrophic bacteria along environmental gradients in northern lakes. *PLoS One*, **10**(4), e0124035.

Fernández-Martínez, J., Pujalte, M.J., García-Martínez, J., Mata, M., Garay, E., Rodríguez-Valera, F. 2003. Description of *Alcanivorax venustensis* sp. nov. and reclassification of *Fundibacter jadensis* DSM 12178T (Bruns and Berthe-Corti 1999) as *Alcanivorax jadensis* comb. nov., members of the emended genus *Alcanivorax*. *International Journal of Systematic and Evolutionary Microbiology*, **53**(1), 331-338.

Fernández, A.B., Vera-Gargallo, B., Sánchez-Porro, C., Ghai, R., Papke, R.T., Rodriguez-Valera, F., Ventosa, A. 2014. Comparison of prokaryotic community structure from Mediterranean and Atlantic saltern concentrator ponds by a metagenomic approach. in: *The Proceedings from Halophiles 2013, T he International Congress on Halophilic Microorganisms*, Frontiers Media SA, pp. 116.

Ferrera-Rodríguez, O., Greer, C.W., Juck, D., Consaul, L., Martínez-Romero, E., Whyte, L. 2013. Hydrocarbon-degrading potential of microbial communities from Arctic plants. *Journal of Applied Microbiology*, **114**(1), 71-83.

Finster, K., Liesack, W., Tindall, B. 1997. *Sulfurospirillum arcachonense* sp. nov., a new microaerophilic sulfur-reducing bacterium. *International Journal of Systematic and Evolutionary Microbiology*, **47**(4), 1212-1217.

Finster, K.W., Kjeldsen, K.U., Kube, M., Reinhardt, R., Mussmann, M., Amann, R., Schreiber, L. 2013. Complete genome sequence of *Desulfocapsa sulfexigens*, a marine deltaproteobacterium specialized in disproportionating inorganic sulfur compounds. *Standards in Genomic Sciences*, **8**(1), 58.

Fish, S.A., Shepherd, T.J., McGenity, T.J., Grant, W.D. 2002. Recovery of 16S ribosomal RNA gene fragments from ancient halite. *Nature*, **417**(6887), 432-436.

Fomina, M., Gadd, G.M. 2014. Biosorption: current perspectives on concept, definition and application. *Bioresource Technology*, **160**, 3-14.

François, F., Lombard, C., Guigner, J.-M., Soreau, P., Brian-Jaisson, F., Martino, G., Vandervennet, M., Garcia, D., Molinier, A.-L., Pignol, D. 2012. Isolation and characterization of environmental bacteria capable of extracellular biosorption of mercury. *Applied and Environmental Microbiology*, **78**(4), 1097-1106.

Freundlich, H. 1906. Over the adsorption in solution. *Journal of Physical Chemistry*, **57**(385), e470.

Gabr, R., Hassan, S., Shoreit, A. 2008. Biosorption of lead and nickel by living and non-living cells of *Pseudomonas aeruginosa* ASU 6a. *International Biodeterioration & Biodegradation*, **62**(2), 195-203.

Gadd, G.M. 2009. Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. *Journal of Chemical Technology and Biotechnology*, **84**(1), 13-28.

Garnham, G., Wase, J., Forster, C. 1997. The use of algae as metal biosorbents. in: *Biosorbents for Metal Ions*, pp. 11-37.

Ghai, R., Pašić, L., Fernández, A.B., Martin-Cuadrado, A.-B., Mizuno, C.M., McMahon, K.D., Papke, R.T., Stepanauskas, R., Rodriguez-Brito, B., Rohwer, F., Sánchez-Porro, C., Ventosa, A., Rodríguez-Valera, F. 2011. New abundant microbial groups in aquatic hypersaline environments. *Scientific Reports*, **1**, 135.

Ghazvini, P.T.M., Mashkani, S.G. 2009. Effect of salinity on vanadate biosorption by *Halomonas* sp. GT-83: Preliminary investigation on biosorption by micro-PIXE technique. *Bioresource Technology*, **100**(8), 2361-2368.

Ghiaci, M., Kia, R., Abbaspur, A., Seyedeyn-Azad, F. 2004. Adsorption of chromate by surfactant-modified zeolites and MCM-41 molecular sieve. *Separation and Purification Technology*, **40**(3), 285-295.

Golikowa, S. 1930. Eine Gruppe von obligat halophilen Bakterien, gezüchtet in Substraten mit hohem NaCl-Gehalt. *Zentr Bakteriol Parasitenk, Abt II*, **80**, 35-41.

Gramain, A., Díaz, G.C., Demergasso, C., Lowenstein, T.K., McGenity, T.J. 2011. Archaeal diversity along a subterranean salt core from the Salar Grande (Chile). *Environmental Microbiology*, **13**(8), 2105-2121.

Grant, W.D., Gemmell, R.T., McGenity, T.J. 1998. Halobacteria: the evidence for longevity. *Extremophiles*, **2**(3), 279-287.

Gražulis, S., Chateigner, D., Downs, R.T., Yokochi, A., Quirós, M., Lutterotti, L., Manakova, E., Butkus, J., Moeck, P., Le Bail, A. 2009. Crystallography Open Database–an open-access collection of crystal structures. *Journal of Applied Crystallography*, **42**(4), 726-729.

Gražulis, S., Daškevič, A., Merkys, A., Chateigner, D., Lutterotti, L., Quirós, M., Serebryanaya, N.R., Moeck, P., Downs, R.T., Le Bail, A. 2012. Crystallography Open Database (COD): an open-access collection of crystal structures and platform for world-wide collaboration. *Nucleic Acids Research*, **40**(D1), D420-D427.

Green-Ruiz, C. 2006. Mercury(II) removal from aqueous solutions by nonviable *Bacillus* sp. from a tropical estuary. *Bioresource Technology*, **97**(15), 1907-1911.

Grioni, M., Van Acker, J., Czyžyk, M., Fuggle, J. 1992. Unoccupied electronic structure and core-hole effects in the x-ray-absorption spectra of Cu_2O. *Physical Review B*, **45**(7), 3309.

Güereña, D.T., Lehmann, J., Thies, J.E., Enders, A., Karanja, N., Neufeldt, H. 2015. Partitioning the contributions of biochar properties to enhanced biological nitrogen fixation in common bean (*Phaseolus vulgaris*). *Biology and Fertility of Soils*, **51**(4), 479-491.

Gulati, R., Saxena, R., Gupta, R. 2002. Fermentation waste of *Aspergillus terreus*: a potential copper biosorbent. *World Journal of Microbiology and Biotechnology*, **18**(5), 397-401.

Gulati, R., Saxena, R., Gupta, R. 1999. Fermentation waste of *Aspergillus terreus*: a promising copper bio-indicator. *Current Science*, **77**(10), 1359-1366.

Gunde-Cimerman, N., Ramos, J., Plemenitaš, A. 2009. Halotolerant and halophilic fungi. *Mycological Research*, **113**(11), 1231-1241.

Gupta, G., Srivastava, S., Khare, S., Prakash, V. 2014. Extremophiles: an overview of microorganism from extreme environment. *International Journal of Agriculture, Environment and Biotechnology*, **7**(2), 371.

Gupta, R., Ahuja, P., Khan, S., Saxena, R., Mohapatra, H. 2000. Microbial biosorbents: meeting challenges of heavy metal pollution in aqueous solutions. *Current Science*, **78**(8), 967-973.

Gupta, V., Rastogi, A. 2008. Sorption and desorption studies of chromium(VI) from nonviable cyanobacterium *Nostoc muscorum* biomass. *Journal of Hazardous Materials*, **154**(1), 347-354.

Hasan, H.A., Abdullah, S.R.S., Kofli, N.T., Kamarudin, S.K. 2012. Isotherm equilibria of Mn^{2+} biosorption in drinking water treatment by locally isolated *Bacillus* species and sewage activated sludge. *Journal of Environmental Management*, **111**, 34-43.

Hollister, E.B., Engledow, A.S., Hammett, A.J.M., Provin, T.L., Wilkinson, H.H., Gentry, T.J. 2010. Shifts in microbial community structure along an ecological gradient of hypersaline soils and sediments. *The ISME Journal*, **4**(6), 829-838.

Holter, M.E. 1969. The Middle Devonian Prairie Evaporite of Saskatchewan.

Huang, Y., Zeng, Y., Feng, H., Wu, Y., Xu, X. 2015. *Croceicoccus naphthovorans* sp. nov., a polycyclic aromatic hydrocarbons-degrading and acylhomoserine-lactone-producing bacterium isolated from marine biofilm, and emended description of the genus *Croceicoccus*. *International Journal of Systematic and Evolutionary Microbiology*, **65**(5), 1531-1536.

Huete-Stauffer, T.M., Arandia-Gorostidi, N., Alonso-Sáez, L., Morán, X.A.G. 2016. Experimental Warming Decreases the Average Size and Nucleic Acid Content of Marine Bacterial Communities. *Frontiers in Microbiology*, **7**(730).

Inglett, K.S., Bae, H.-S., Aldrich, H., Hatfield, K., Ogram, A. 2011. *Clostridium chromiireducens* sp. nov., isolated from Cr(VI)-contaminated soil. *International Journal of Systematic and Evolutionary Microbiology*, **61**(11), 2626-2631.

Isaac, P., Sánchez, L.A., Bourguignon, N., Cabral, M.E., Ferrero, M.A. 2013. Indigenous PAH-degrading bacteria from oil-polluted sediments in Caleta Cordova, Patagonia Argentina. *International Biodeterioration & Biodegradation*, **82**, 207-214.

Jalili Seh-Bardan, B., Othman, R., Abd Wahid, S., Sadegh-Zadeh, F., Husin, A. 2013. Biosorption of heavy metals in leachate derived from gold mine tailings using *Aspergillus fumigatus*. *CLEAN–Soil, Air, Water*, **41**(4), 356-364.

Jasinski, S.M. 2014. Minerals Yearbook: Potash, Advance Release]: US Geological Survey.

Javor, B. 1989. *Hypersaline environments: microbiology and biogeochemistry*. Springer Science & Business Media.

Kaeppel, E.C., Gärdes, A., Seebah, S., Grossart, H.-P., Ullrich, M.S. 2012. *Marinobacter adhaerens* sp. nov., isolated from marine aggregates formed with the diatom *Thalassiosira weissflogii*. *International Journal of Systematic and Evolutionary Microbiology*, **62**(1), 124-128.

Kaznatcheev, K., Karunakaran, C., Lanke, U., Urquhart, S., Obst, M., Hitchcock, A. 2007. Soft X-ray spectromicroscopy beamline at the CLS: commissioning results. *Nuclear Instruments and Methods in Physics Research Section A: Accelerators, Spectrometers, Detectors and Associated Equipment*, **582**(1), 96-99.

Kharroub, K., Aguilera, M., Quesada, T., Morillo, J.A., Ramos-Cormenzana, A., Boulharouf, A., Monteoliva-Sanchez, M. 2006. *Salicola salis* sp. nov., an extremely halophilic bacterium isolated from Ezzemoul sabkha in Algeria. *International Journal of Systematic and Evolutionary Microbiology*, **56**(11), 2647-2652.

Kim, I., Lee, M., Wang, S. 2014. Heavy metal removal in groundwater originating from acid mine drainage using dead *Bacillus drentensis* sp. immobilized in polysulfone polymer. *Journal of Environmental Management*, **146**, 568-574.

Koch, S., Majewski, E., Schmeisky, H., Schmidt, F.R. 2012. Critical evaluation of phosphate solubilizing pseudomonads isolated from a partially recultivated potash tailings pile. *Current Microbiology*, **65**(2), 202-206.

Koechler, S., Arsène-Ploetze, F., Brochier-Armanet, C., Goulhen-Chollet, F., Heinrich-Salmeron, A., Jost, B., Lièvremont, D., Philipps, M., Plewniak, F., Bertin, P.N. 2015. Constitutive arsenite oxidase expression detected in arsenic-hypertolerant *Pseudomonas xanthomarina* S11. *Research in Microbiology*, **166**(3), 205-214.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K., Schloss, P.D. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and Environmental Microbiology*, **79**(17), 5112-5120.

Kumar, S., Stecher, G., Tamura, K. 2015. MEGA7: Molecular Evoluitonary Genetics Analysis version 7.0. Molecular Biology and Evolution.

Kummer, C., Schumann, P., Stackebrandt, E. 1999. *Gordonia alkanivorans* sp. nov., isolated from tar-contaminated soil. *International Journal of Systematic and Evolutionary Microbiology*, **49**(4), 1513-1522.

Kushner, D.J., Kamekura, M. 1988. Physiology of halophilic eubacteria. *Halophilic Bacteria*, **1**, 109-140.

Kuyucak, N. 1990. Feasibility of biosorbents application. in: *Biosorption of Heavy Metals*, Vol. 4, CRC Press Inc Boca Raton, Florida, pp. 372-377.

Langmuir, I. 1918. The adsorption of gases on plane surfaces of glass, mica and platinum. *Journal of the American Chemical Society*, **40**(9), 1361-1403.

Larsen, H. 1962. Halophilism. *The Bacteria*, **4**, 297-342.

Lee, D.-C., Park, C.-J., Yang, J.-E., Jeong, Y.-H., Rhee, H.-I. 2000. Screening of hexavalent chromium biosorbent from marine algae. *Applied Microbiology and Biotechnology*, **54**(3), 445-448.

Lenassi, M., Vaupotic, T., Gunde-Cimerman, N., Plemenitas, A. 2007. The MAP kinase HwHog1 from the halophilic black yeast *Hortaea werneckii*: coping with stresses in solar salterns. *Saline Systems*, **3**(3).

Li, X., Liao, D., Xu, X., Yang, Q., Zeng, G., Zheng, W., Guo, L. 2008. Kinetic studies for the biosorption of lead and copper ions by *Penicillium simplicissimum* immobilized within loofa sponge. *Journal of Hazardous Materials*, **159**(2), 610-615.

Li, Y., Chen, B., Zhu, L. 2010. Enhanced sorption of polycyclic aromatic hydrocarbons from aqueous solution by modified pine bark. *Bioresource Technology*, **101**(19), 7307-7313.

Litchfield, C., Gillevet, P. 2002. Microbial diversity and complexity in hypersaline environments: a preliminary assessment. *Journal of Industrial Microbiology and Biotechnology*, **28**(1), 48-55.

Loukidou, M.X., Zouboulis, A.I., Karapantsios, T.D., Matis, K.A. 2004. Equilibrium and kinetic modeling of chromium(VI) biosorption by *Aeromonas caviae*. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **242**(1), 93-104.

Maathuis, H., Van der Kamp, G. 2002. Analytical methods to predict vertical brine migration and comparison with actual observations. *Ground and Water: Theory to Practice*, 1087-1091.

Margesin, R., Schinner, F. 2001. Potential of halotolerant and halophilic microorganisms for biotechnology. *Extremophiles*, **5**(2), 73-83.

Marshall, B. 2015. Facts and Figures of the Canadian Mining Industry, (Ed.) W.C.E. Associates, The Mining Association of Canada.

Masri, M., Reuter, F.W., Friedman, M. 1974. Binding of metal cations by natural substances. *Journal of Applied Polymer Science*, **18**(3), 675-681.

Maturrano, L., Valens-Vadell, M., Rosselló-Mora, R., Antón, J. 2006. *Salicola marasensis* gen. nov., sp. nov., an extremely halophilic bacterium isolated from the Maras solar salterns in Peru. *International Journal of Systematic and Evolutionary Microbiology*, **56**(7), 1685-1691.

McLean, J.E., Bledsoe, B.E. 1996. Behavior of Metals in Soils. in: *US EPA Environmental Assessment Sourcebook*, pp. 19.

Michalak, I., Chojnacka, K., Witek-Krowiak, A. 2013. State of the Art for the Biosorption Process—a Review. *Applied Biochemistry and Biotechnology*, **170**(6), 1389-1416.

Nacèra, Y., Aicha, B. 2006. Equilibrium and kinetic modelling of methylene blue biosorption by pretreated dead *Streptomyces rimosus*: effect of temperature. *Chemical Engineering Journal*, **119**(2), 121-125.

Naik, S., Furtado, I. 2014. Equilibrium and Kinetics of Adsorption of Mn^{2+} by Haloarchaeon *Halobacterium* sp. GUSF (MTCC3265). *Geomicrobiology Journal*, **31**(8), 708-715.

Naiya, T.K., Bhattacharya, A.K., Das, S.K. 2009. Adsorption of Cd(II) and Pb(II) from aqueous solutions on activated alumina. *Journal of Colloid and Interface science*, **333**(1), 14-26.

Naja, G., Mustin, C., Volesky, B., Berthelin, J. 2006. Stabilization of the initial electrochemical potential for a metal-based potentiometric titration study of a biosorption process. *Chemosphere*, **62**(1), 163-170.

Naja, G., Volesky, B. 2008. Optimization of a biosorption column performance. *Environmental Science & Technology*, **42**(15), 5622-5629.

Narasingarao, P., Podell, S., Ugalde, J.A., Brochier-Armanet, C., Emerson, J.B., Brocks, J.J., Heidelberg, K.B., Banfield, J.F., Allen, E.E. 2012. De novo metagenomic assembly reveals abundant novel major lineage of Archaea in hypersaline microbial communities. *The ISME Journal*, **6**(1), 81-93.

Nieto, J., Fernandez-Castillo, R., Marquez, M., Ventosa, A., Quesada, E., Ruiz-Berraquero, F. 1989. Survey of metal tolerance in moderately halophilic Eubacteria. *Applied and Environmental Microbiology*, **55**(9), 2385-2390.

Niu, C.H., Volesky, B., Cleiman, D. 2007. Biosorption of arsenic(V) with acid-washed crab shells. *Water Research*, **41**(11), 2473-2478.

Norberg, A.B., Persson, H. 1984. Accumulation of heavy-metal ions by *Zoogloea ramigera*. *Biotechnology and Bioengineering*, **26**(3), 239-246.

Norton, C.F., McGenity, T.J., Grant, W.D. 1993. Archaeal halophiles (halobacteria) from two British salt mines. *Microbiology*, **139**(5), 1077-1081.

Nourbakhsh, M., Sag, Y., Özer, D., Aksu, Z., Kutsal, T., Caglar, A. 1994. A comparative study of various biosorbents for removal of chromium (VI) ions from industrial waste waters. *Process Biochemistry*, **29**(1), 1-5.

Nunoura, T., Oida, H., Miyazaki, M., Suzuki, Y., Takai, K., Horikoshi, K. 2007. *Desulfothermus okinawensis* sp. nov., a thermophilic and heterotrophic sulfate-reducing bacterium isolated from a deep-sea hydrothermal field. *International Journal of Systematic and Evolutionary Microbiology*, **57**(10), 2360-2364.

Nuttall, S.D., Dyall-Smith, M.L. 1993. Ch2, a novel halophilic archaeon from an Australian solar saltern. *International Journal of Systematic and Evolutionary Microbiology*, **43**(4), 729-734.

Obst, M., Dynes, J., Lawrence, J., Swerhone, G., Benzerara, K., Karunakaran, C., Kaznatcheev, K., Tyliszczak, T., Hitchcock, A. 2009. Precipitation of amorphous $CaCO_3$ (aragonite-like) by cyanobacteria: a STXM study of the influence of EPS on the nucleation process. *Geochimica et Cosmochimica Acta*, **73**(14), 4180-4198.

Omar, N.B., Merroun, M.L., Peñalver, J.M.A., Muñoz, M.T.G. 1997. Comparative heavy metal biosorption study of brewery yeast and *Myxococcus xanthus* biomass. *Chemosphere*, **35**(10), 2277-2283.

Oren, A. 1999. Bioenergetic aspects of halophilism. *Microbiology and Molecular Biology Reviews*, **63**(2), 334-348.

Oren, A. 2015. Halophilic microbial communities and their environments. *Current Opinion in Biotechnology*, **33**, 119-124.

Oren, A. 2002. *Halophilic Microorganisms and their Environments*. Springer.

Öztürk, A. 2007. Removal of nickel from aqueous solution by the bacterium *Bacillus thuringiensis*. *Journal of Hazardous Materials*, **147**(1), 518-523.

Öztürk, A., Artan, T., Ayar, A. 2004. Biosorption of nickel(II) and copper(II) ions from aqueous solution by *Streptomyces coelicolor* A3(2). *Colloids and Surfaces B: Biointerfaces*, **34**(2), 105-111.

Page, G.W. 1981. Comparison of groundwater and surface water for patterns and levels of contamination by toxic substances. *Environmental Science & Technology*, **15**(12), 1475-1481.

Panayotova, M. 2001. Kinetics and thermodynamics of copper ions removal from wastewater by use of zeolite. *Waste Management*, **21**(7), 671-676.

Panno, S., Hackley, K.C., Hwang, H., Greenberg, S., Krapac, I., Landsberger, S., O'kelly, D. 2006. Characterization and identification of Na-Cl sources in ground water. *Ground Water*, **44**(2), 176-187.

Panwichian, S., Kantachote, D., Wittayaweerasak, B., Mallavarapu, M. 2010. Isolation of purple nonsulfur bacteria for the removal of heavy metals and sodium from contaminated shrimp ponds. *Electronic Journal of Biotechnology*, **13**(4), 3-4.

Paraneeiswaran, A., Shukla, S.K., Rao, T.S., Prashanth, K. 2014. Removal of toxic Co-EDTA complex by a halophilic solar-salt-pan isolate *Pseudomonas aeruginosa* SPB-1. *Chemosphere*, **95**, 503-510.

Park, D., Yun, Y.-S., Park, J.M. 2010. The past, present, and future trends of biosorption. *Biotechnology and Bioprocess Engineering*, **15**(1), 86-102.

Park, J., Vreeland, R., Cho, B., Lowenstein, T., Timofeeff, M., Rosenzweig, W. 2009. Haloarchaeal diversity in 23, 121 and 419 MYA salts. *Geobiology*, **7**(5), 515-523.

Park, J.S. 2012. Effects of different ion compositions on growth of obligately halophilic protozoan *Halocafeteria seosinensis*. *Extremophiles*, **16**(1), 161-164.

Perucca, C.F. 2003. Potash processing in Saskatchewan-A review of process technologies. *CIM bulletin*, **96**(1070), 61-65.

Pesenti, P.T., Sikaroodi, M., Gillevet, P.M., Sanchez-Porro, C., Ventosa, A., Litchfield, C.D. 2008. *Halorubrum californiense* sp. nov., an extreme archaeal halophile isolated from a crystallizer pond at a solar salt plant in California, USA. *International Journal of Systematic and Evolutionary Microbiology*, **58**(12), 2710-2715.

Potashcorp. 2010. 2010 Online Sustainability Report – Environmental.

Radax, C., Gruber, C., Stan-Lotter, H. 2001. Novel haloarchaeal 16S rRNA gene sequences from Alpine Permo-Triassic rock salt. *Extremophiles*, **5**(4), 221-228.

Rajesh, V., Rajesh, N. 2015. An indigenous *Halomonas* BVR1 strain immobilized in crosslinked chitosan for adsorption of lead and cadmium. *International Journal of Biological Macromolecules*, **79**, 300-308.

Reid, K.W., Getzlaf, M.N. 2004. Decommissioning planning for Saskatchewan's potash mines. British Columbia Technical and Research Committee on Reclamation.

Robbins, J., Ingledew, W. 1976. Potash guar gum-a source of halophilic bacteria. *Canadian Journal of Microbiology*, **22**(11), 1658-1661.

Rocha, R.D.M., Costa, D.F., Lucena-Filho, M.A., Bezerra, R.M., Medeiros, D.H., Azevedo-Silva, A.M., Araújo, C.N., Xavier-Filho, L. 2012. Brazilian solar saltworks -- ancient uses and future possibilities. *Aquatic Biosystems*, **8**(8).

Romano, I., Lama, L., Nicolaus, B., Poli, A., Gambacorta, A., Giordano, A. 2006. *Halomonas alkaliphila* sp. nov., a novel halotolerant alkaliphilic bacterium isolated from a salt pool in Campania (Italy). *The Journal of General and Applied Microbiology*, **52**(6), 339-348.

Roy, D., Greenlaw, P.N., Shane, B.S. 1993. Adsorption of heavy metals by green algae and ground rice hulls. *Journal of Environmental Science & Health Part A*, **28**(1), 37-50.

Rubentschik, L. 1929. Zur nitrifikation bei hohen salzkonzentrationen. *Zentralblatt Bakteriologie II Abteilung*, **77**, 1-18.

Ruiz-Manriquez, A., Magana, P., Lopez, V., Guzman, R. 1998. Biosorption of Cu by *Thiobacillus ferrooxidans*. *Bioprocess Engineering*, **18**(2), 113-118.

Salehizadeh, H., Shojaosadati, S. 2003. Removal of metal ions from aqueous solution by polysaccharide produced from *Bacillus firmus*. *Water Research*, **37**(17), 4231-4235.

Saralov, A., Baslerov, R., Reutskikh, E., Kuznetsov, B. 2012a. *Halarchaeum solikamskense* sp. nov., a thermotolerant neutrophilic haloarchaeon from the foamy products of flotation enrichment of potassium minerals. *Microbiology*, **81**(5), 589-595.

Saralov, A., Kuznetsov, B., Reutskikh, E., Baslerov, R., Panteleeva, A., Suzina, N. 2012b. *Arhodomonas recens* sp. nov., a halophilic alkane-utilizing hydrogen-oxidizing bacterium from the brines of flotation enrichment of potassium minerals. *Microbiology*, **81**(5), 582-588.

Saralov, A.I., Baslerov, R.V., Kuznetsov, B.B. 2013. *Haloferax chudinovii* sp. nov., a halophilic archaeon from Permian potassium salt deposits. *Extremophiles*, **17**(3), 499-504.

Satomi, M., La Duc, M.T., Venkateswaran, K. 2006. *Bacillus safensis* sp. nov., isolated from spacecraft and assembly-facility surfaces. *International Journal of Systematic and Evolutionary Microbiology*, **56**(8), 1735-1740.

Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J. 2009. Introducing mothur: open-source, platform-

independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, **75**(23), 7537-7541.

Schneegurt, M.A. 2012. Media and Conditions for the Growth of Halophilic and Halotolerant Bacteria and Archaea. in: *Advances in Understanding the Biology of Halophilic Microorganisms*, Springer, pp. 35-58.

Schubert, B.A., Lowenstein, T.K., Timofeeff, M.N., Parker, M.A. 2010. Halophilic archaea cultured from ancient halite, Death Valley, California. *Environmental Microbiology*, **12**(2), 440-454.

Schut, F., Prins, R.A., Gottschal, J.C. 1997. Oligotrophy and pelagic marine bacteria: facts and fiction. *Aquatic Microbial Ecology*, **12**(2), 177-202.

Schut, S., Zauner, S., Hampel, G., König, H., Claus, H. 2011. Biosorption of copper by wine-relevant lactobacilli. *International Journal of Food Microbiology*, **145**(1), 126-131.

Sharma, M., Rani, N., Kamra, A., Kaushik, A., Bala, K. 2009. Growth, exopolymer production and metal bioremoval by Nostoc punctiforme in Na and Cr(VI) spiked medium. *Journal of Environmental Research And Development Vol*, **4**(2).

Shaw, S.A., Hendry, M.J., Essilfie-Dughan, J., Kotzer, T., Wallschläger, D. 2011. Distribution, characterization, and geochemical controls of elements of concern in uranium mine tailings, Key Lake, Saskatchewan, Canada. *Applied Geochemistry*, **26**(12), 2044-2056.

Shen, T.-J., Chao, A., Lin, C.-F. 2003. Predicting the number of new species in further taxonomic sampling. *Ecology*, **84**(3), 798-804.

Shukla, A., Zhang, Y.-H., Dubey, P., Margrave, J., Shukla, S.S. 2002. The role of sawdust in the removal of unwanted materials from water. *Journal of Hazardous Materials*, **95**(1), 137-152.

Singh, A.L., Sarma, P.N. 2010. Removal of Arsenic(III) from Waste Water Using *Lactobacillus acidophilus*. *Bioremediation Journal*, **14**(2), 92-97.

Sopeña, F., Laiz, L., Morillo, E., Sanchez-Trujillo, M.A., Villaverde, J., Jurado, V., Saiz-Jimenez, C. 2014. Phenanthrene biodegradation by *Pseudomonas xanthomarina* isolated from an aged contaminated soil. *CLEAN–Soil, Air, Water*, **42**(6), 785-790.

Srinath, T., Verma, T., Ramteke, P., Garg, S. 2002. Chromium(VI) biosorption and bioaccumulation by chromate resistant bacteria. *Chemosphere*, **48**(4), 427-435.

Subbaiah, M.V., Vijaya, Y., Reddy, A.S., Yuvaraja, G., Krishnaiah, A. 2011. Equilibrium, kinetic and thermodynamic studies on the biosorption of Cu(II) onto *Trametes versicolor* biomass. *Desalination*, **276**(1), 310-316.

Tallin, J., Pufahl, D., Barbour, S. 1990. Waste management schemes of potash mines in Saskatchewan. *Canadian Journal of Civil Engineering*, **17**(4), 528-542.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**(10), 2731-2739.

Tazi, L., Breakwell, D.P., Harker, A.R., Crandall, K.A. 2014. Life in extreme environments: microbial diversity in Great Salt Lake, Utah. *Extremophiles*, **18**(3), 525-535.

Tenenbaum, D.J. 2009. Oil sands development: a health risk worth taking. *Environmental Health Perspectives*, **117**(4), A150-A156.

Thevannan, A., Mungroo, R., Niu, C.H. 2010. Biosorption of nickel with barley straw. *Bioresource Technology*, **101**(6), 1776-1780.

Timofeeff, M., Lowenstein, T., Brennan, S., Demicco, R., Zimmermann, H., Horita, J., Von Borstel, L. 2001. Evaluating seawater chemistry from fluid inclusions in halite: examples from modern marine and nonmarine environments. *Geochimica et Cosmochimica Acta*, **65**(14), 2293-2300.

Toby, B.H., Von Dreele, R.B. 2013. GSAS-II: the genesis of a modern open-source all purpose crystallography software package. *Journal of Applied Crystallography*, **46**(2), 544-549.

UNEP. 2001. Environmental Aspects of Phosphate and Potash Mining, United Nations Environment Programme (UNEP) and International Fertilizer Industry Association (IFA) Paris, France.

USEPA. 1983. Methods for Analysis of Water and Wastes. in: *EPA Methods 353.1*, U.S. Environmental Protection Agency. Washington, DC.

Uslu, G., Tanyol, M. 2006. Equilibrium and thermodynamic parameters of single and binary mixture biosorption of lead(II) and copper(II) ions onto *Pseudomonas putida*: effect of temperature. *Journal of Hazardous Materials*, **135**(1), 87-93.

Veglio, F., Beolchini, F. 1997. Removal of metals by biosorption: a review. *Hydrometallurgy*, **44**(3), 301-316.

Veglio, F., Beolchini, F., Gasbarro, A. 1997. Biosorption of toxic metals: an equilibrium study using free cells of *Arthrobacter* sp. *Process Biochemistry*, **32**(2), 99-105.

Verdon, J.P., Kendall, J.-M., Horleston, A.C., Stork, A.L. 2016. Subsurface fluid injection and induced seismicity in southeast Saskatchewan. *International Journal of Greenhouse Gas Control*.

Vijayaraghavan, K., Yun, Y.-S. 2008. Bacterial biosorbents and biosorption. *Biotechnology Advances*, **26**(3), 266-291.

Volesky, B. 1990. *Biosorption of Heavy Metals*. Taylor & Francis.

Volesky, B. 2001. Detoxification of metal-bearing effluents: biosorption for the next century. *Hydrometallurgy*, **59**(2), 203-216.

Volesky, B. 2003. *Sorption and biosorption*. BV Sorbex.

Volesky, B., Holan, Z.R. 1995. Biosorption of Heavy Metals. *Biotechnology Progress*, **11**(3), 235-250.

Volesky, B., Kuyucak, N. 1988. Biosorbent for gold. U.S. Patent No. 4,769,223.

Vonhof, J. 1975. Waste disposal problems near potash mines in Saskatchewan, Canada. *International Association for Hydrological Sciences*, **103**, 191-215.

Vreeland, R.H. 2012. *Advances in Understanding the Biology of Halophilic Microorganisms*. Springer.

Vreeland, R.H. 1987. Mechanisms of halotolerance in microorganisms. *Critical Reviews in Microbiology*, **14**(4), 311-356.

Vreeland, R.H., Rosenzweig, W.D., Powers, D.W. 2000. Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal. *Nature*, **407**(6806), 897-900.

Wang, B., Song, H., Wang, C., Shuang, C., Li, Q., Li, A. 2016. Evaluation of nitrate removal properties of magnetic anion-exchange resins in water. *Journal of Chemical Technology and Biotechnology*(91).

Wang, J., Chen, C. 2009. Biosorbents for heavy metals removal and their future. *Biotechnology Advances*, **27**(2), 195-226.

Wang, S., Peng, Y. 2010. Natural zeolites as effective adsorbents in water and wastewater treatment. *Chemical Engineering Journal*, **156**(1), 11-24.

Wang, Y.-N., Cai, H., Chi, C.-Q., Lu, A.-H., Lin, X.-G., Jiang, Z.-F., Wu, X.-L. 2007a. *Halomonas shengliensis* sp. nov., a moderately halophilic, denitrifying, crude-oil-utilizing bacterium. *International Journal of Systematic and Evolutionary Microbiology*, **57**(6), 1222-1226.

Wang, Y.-N., Cai, H., Yu, S.-L., Wang, Z.-Y., Liu, J., Wu, X.-L. 2007b. *Halomonas gudaonensis* sp. nov., isolated from a saline soil contaminated by crude oil. *International Journal of Systematic and Evolutionary Microbiology*, **57**(5), 911-915.

Williams, G.P., Gnanadesigan, M., Ravikumar, S. 2013. Biosorption and bio-kinetic properties of solar saltern Halobacterial strains for managing Zn^{2+}, As^{2+} and Cd^{2+} metals. *Geomicrobiology Journal*, **30**(6), 497-500.

Williams, G.P., Gnanadesigan, M., Ravikumar, S. 2012. Biosorption and bio-kinetic studies of halobacterial strains against Ni^{2+}, Al^{3+} and Hg^{2+} metal ions. *Bioresource Technology*, **107**, 526-529.

Xu, X.-W., Wu, Y.-H., Wang, C.-S., Wang, X.-G., Oren, A., Wu, M. 2009. *Croceicoccus marinus* gen. nov., sp. nov., a yellow-pigmented bacterium from deep-sea sediment, and emended description of the family *Erythrobacteraceae*. *International Journal of Systematic and Evolutionary Microbiology*, **59**(9), 2247-2253.

Yang, J., Regier, T., Dynes, J.J., Wang, J., Shi, J., Peak, D., Zhao, Y., Hu, T., Chen, Y., Tse, J.S. 2011. Soft X-ray induced photoreduction of organic Cu(II) compounds probed by X-ray absorption near-edge (XANES) spectroscopy. *Analytical Chemistry*, **83**(20), 7856-7862.

Yilmazer, P., Saracoglu, N. 2009. Bioaccumulation and biosorption of copper(II) and chromium(III) from aqueous solutions by *Pichia stipitis* yeast. *Journal of Chemical Technology and Biotechnology*, **84**(4), 604-610.

Zhou, L., Li, H., Zhang, Y., Wang, Y., Han, S., Xu, H. 2012. Abundance and diversity of *Sphingomonas* in Shenfu petroleum-wastewater irrigation zone, China. *Environmental Science and Pollution Research*, **19**(1), 282-294.

Zhu, C., Liu, F., Xu, C., Gao, J., Chen, D., Li, A. 2015. Enhanced removal of Cu(II) and Ni(II) from saline solution by novel dual-primary-amine chelating resin based on anion-synergism. *Journal of Hazardous Materials*, **287**, 234-242.

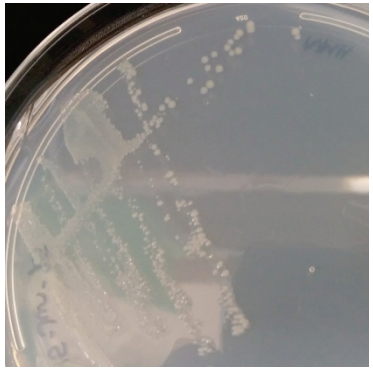
Zhuang, X., Han, Z., Bai, Z., Zhuang, G., Shim, H. 2010. Progress in decontamination by halophilic microorganisms in saline wastewater and soil. *Environmental Pollution*, **158**(5), 1119-1126.

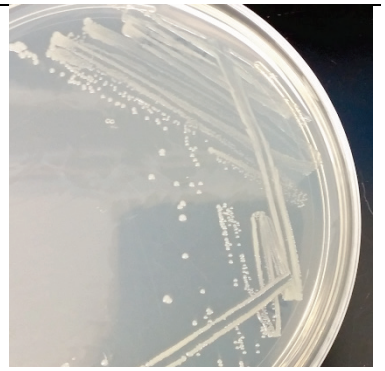
Ziagova, M., Dimitriadis, G., Aslanidou, D., Papaioannou, X., Tzannetaki, E.L., Liakopoulou-Kyriakides, M. 2007. Comparative study of Cd(II) and Cr(VI) biosorption on *Staphylococcus xylosus* and *Pseudomonas* sp. in single and binary mixtures. *Bioresource Technology*, **98**(15), 2859-2865.

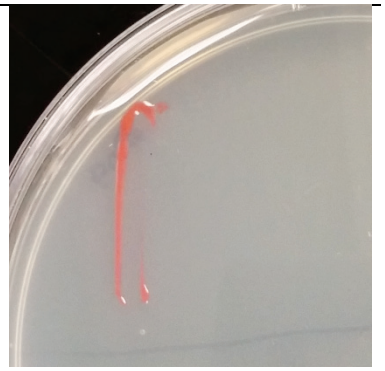
Zouboulis, A., Loukidou, M., Matis, K. 2004. Biosorption of toxic metals from aqueous solutions by bacteria strains isolated from metal-polluted soils. *Process Biochemistry*, **39**(8), 909-916.

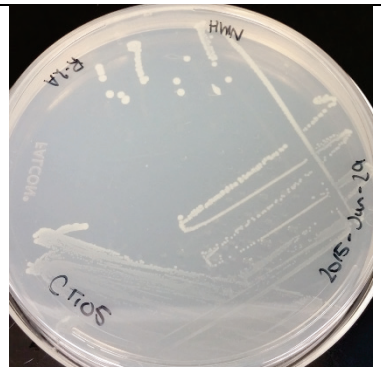
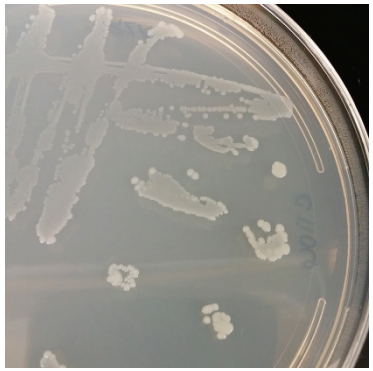
7 APPENDIX A – Isolate Inventory

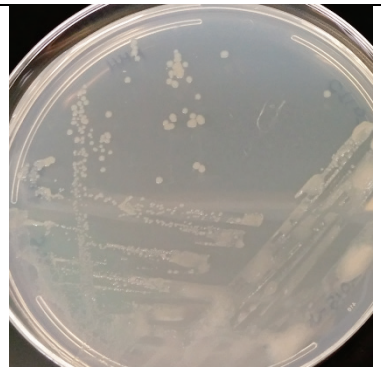
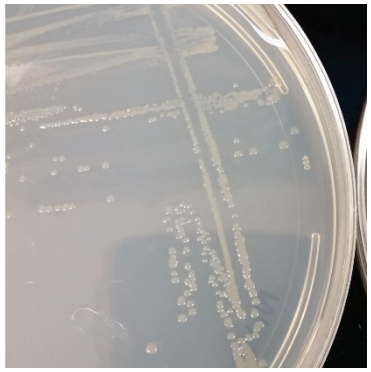
Table A-1 Isolate Inventory

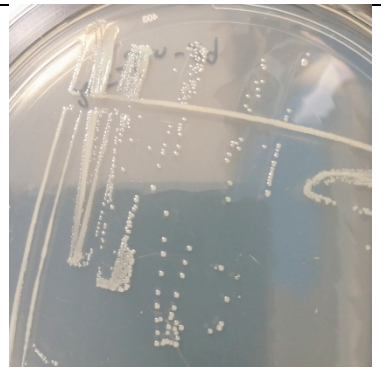
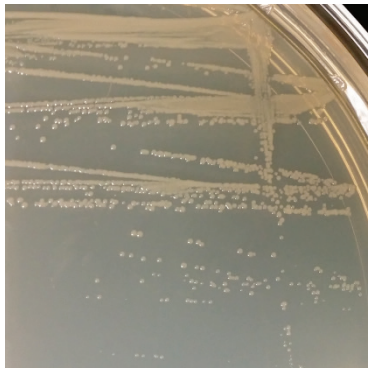
Isolate ID	Sample Source	Original Spread Plate (NaCl amendment (w/v))	Isolation Plate Growth Time (days)	Preliminary RDP Type-Strain Match	Colony Characteristics				Photo
					Colour	Size	Shape	Other	
BI01[a]	Brine	R-2A (3%)	--	--	white	small	circular	glistening	--
BI02	Brine	R-2A (3%)	1	*Halomonas gudaonensis*	white	small	circular	glistening	
BI03	Brine	R-2A (15%)	6	*Halomonas shengliensis*	light pink	small - medium	circular	--	--

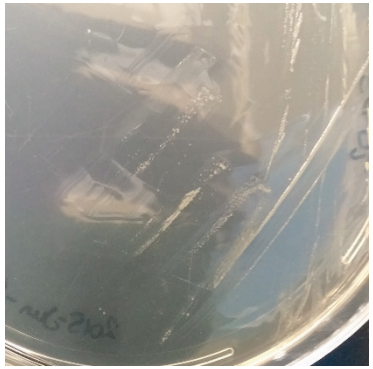
Isolate ID	Sample Source	Original Spread Plate (NaCl amendment (w/v))	Isolation Plate Growth Time (days)	Preliminary RDP Type-Strain Match	Colony Characteristics				Photo
					Colour	Size	Shape	Other	
BI04	Brine	R-2A (15%)	6	*Halomonas gudaonensis*	white	small	circular	--	
BI05	Brine	R-2A (25%)	4	*Salicola salis*	off-white	tiny	circular	--	--
BI06	Brine	R-2A	6	*Alcanivorax venustensis*	white	small	circular	--	--
BI07	Brine	R-2A (25%)	4	--	red	tiny	circular	--	--
BI08	Brine	R-2A (25%)	4	*Halorubrum saccharovorum*	pink	tiny	circular	--	--

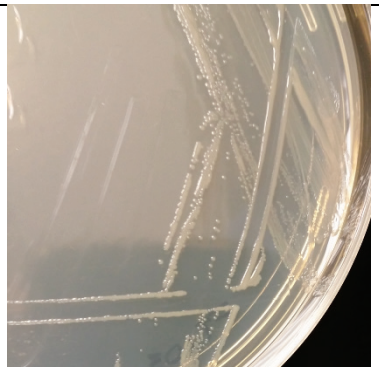
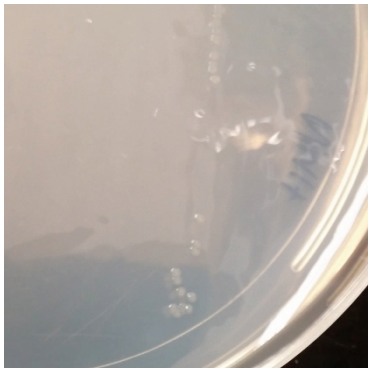
Isolate ID	Sample Source	Original Spread Plate (NaCl amendment (w/v))	Isolation Plate Growth Time (days)	Preliminary RDP Type-Strain Match	Colony Characteristics				Photo
					Colour	Size	Shape	Other	
BI09	Brine	NB (25%)	4	*Halorubrum californiense*	red	tiny	circular	--	
CTI01[a]	Coarse Tailings	NB	6	--	light orange	med	circular	--	--
CTI02	Coarse Tailings	NB	1	*Halomonas andesensis*	light orange	large	irregular	--	--
CTI03[a]	Coarse Tailings	NB	6	--	white	med	circular	--	--
CTI04[a]	Coarse Tailings	NB	7	--	off-white	small	circular	bullseye	--

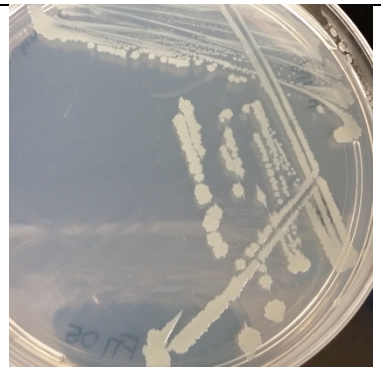
Isolate ID	Sample Source	Original Spread Plate (NaCl amendment (w/v))	Isolation Plate Growth Time (days)	Preliminary RDP Type-Strain Match	Colony Characteristics				Photo
					Colour	Size	Shape	Other	
CTI05	Coarse Tailings	R-2A	1	*Staphylococcus epidermidis*	pink	small	circular	umbonate	
CTI06	Coarse Tailings	R-2A	1	*Staphylococcus epidermidis*	white	med	circular	--	

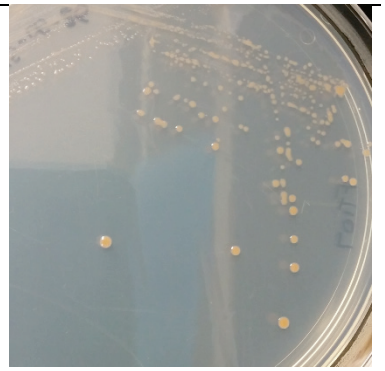
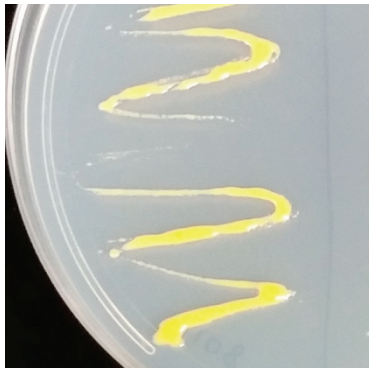
Isolate ID	Sample Source	Original Spread Plate (NaCl amendment (w/v))	Isolation Plate Growth Time (days)	Preliminary RDP Type-Strain Match	Colony Characteristics				Photo
					Colour	Size	Shape	Other	
CTI07	Coarse Tailings	R-2A (3%)	1	*Halomonas gudaonensis*	off-white	small	circular	--	
CTI08	Coarse Tailings	R-2A (3%)	2	*Halomonas gudaonensis*	off-white	med	circular	bullseye	

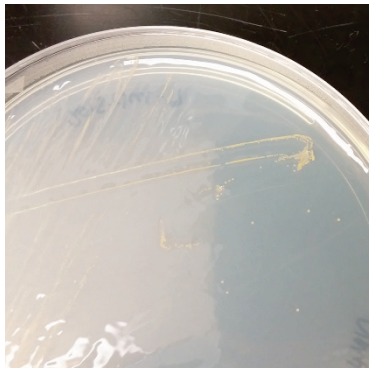
Isolate ID	Sample Source	Original Spread Plate (NaCl amendment (w/v))	Isolation Plate Growth Time (days)	Preliminary RDP Type-Strain Match	Colony Characteristics				Photo
					Colour	Size	Shape	Other	
CTI09	Coarse Tailings	R-2A (15%)	2	*Halomonas gudaonensis*	white	small	circular	--	
CTI10	Coarse Tailings	R-2A (15%)	2	*Halomonas shengliensis*	peach	small	circular	--	
CTI11	Coarse Tailings	R-2A (25%)	4	*Salicola salis*	off-white	small	circular	bullseye	--
CTI12	Coarse Tailings	R-2A (25%)	4	*Salicola salis*	white	tiny	circular	--	--

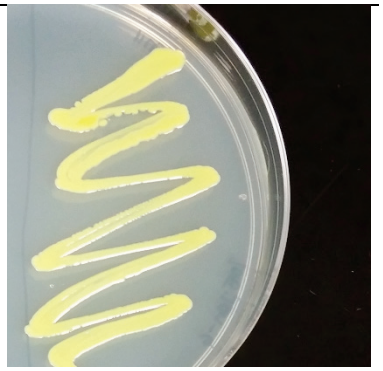
Isolate ID	Sample Source	Original Spread Plate (NaCl amendment (w/v))	Isolation Plate Growth Time (days)	Preliminary RDP Type-Strain Match	Colony Characteristics				Photo
					Colour	Size	Shape	Other	
CTI13[a]	Coarse Tailings	R-2A (25%)	4	--	off-white	small	irregular	--	--
CTI14[a]	Coarse Tailings	R-2A (25%)	4	--	red	tiny	circular	--	--
CTI15[a]	Coarse Tailings	NB (25%)	4	--	red	tiny	circular	--	--
FTI01	Fine Tailings	R-2A (3%)	3	*Halomonas gudaonensis*	yellow	small	circular	--	--
FTI02	Fine Tailings	R-2A (3%)	3	*Halomonas shengliensis*	light pink	small	circular	--	

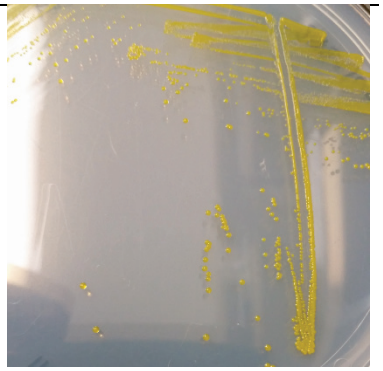
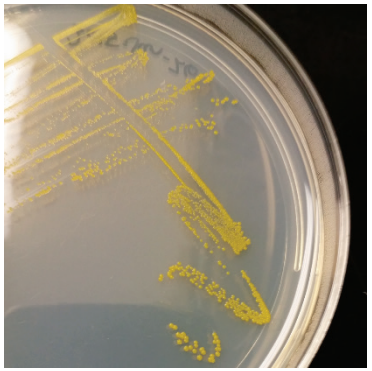
Isolate ID	Sample Source	Original Spread Plate (NaCl amendment (w/v))	Isolation Plate Growth Time (days)	Preliminary RDP Type-Strain Match	Colony Characteristics				Photo
					Colour	Size	Shape	Other	
FTI03	Fine Tailings	R-2A (3%)	2	*Halomonas gudaonensis*	off-white	small	circular	bullseye	
FTI04	Fine Tailings	R-2A (3%)	2	*Marinobacter adhaerens*	white	small	circular	--	

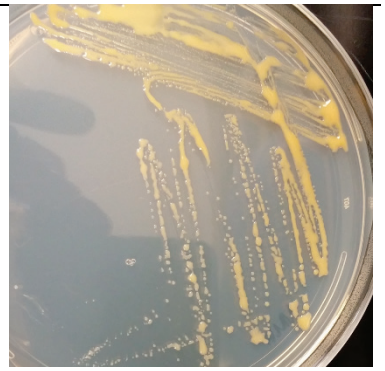
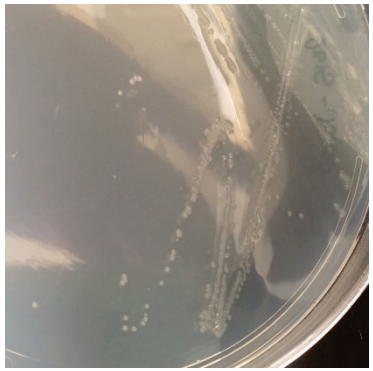
Isolate ID	Sample Source	Original Spread Plate (NaCl amendment (w/v))	Isolation Plate Growth Time (days)	Preliminary RDP Type-Strain Match	Colony Characteristics				Photo
					Colour	Size	Shape	Other	
FTI05	Fine Tailings	NB	1	*Bacillus pumilus*	white	large	irregular	--	
FTI06	Fine Tailings	NB	1	*Pseudomonas xanthomarina*	yellow	med	circular	glistening, umbonate	

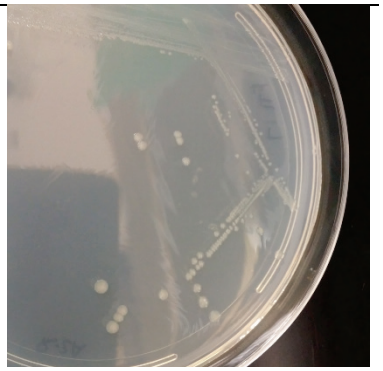
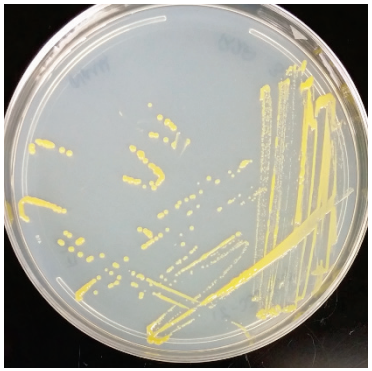
Isolate ID	Sample Source	Original Spread Plate (NaCl amendment (w/v))	Isolation Plate Growth Time (days)	Preliminary RDP Type-Strain Match	Colony Characteristics				Photo
					Colour	Size	Shape	Other	
FTI07	Fine Tailings	NB	1	*Gordonia alkanivorans*	orange	small	circular	--	
FTI08	Fine Tailings	NB	2	*Dietzia maris*	off-white	med	circular	flat	

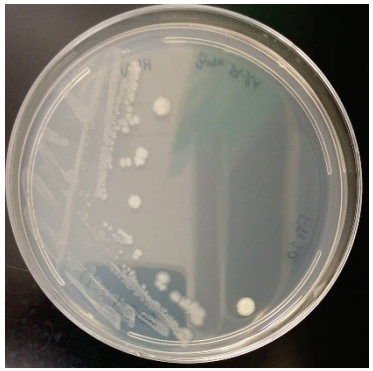
Isolate ID	Sample Source	Original Spread Plate (NaCl amendment (w/v))	Isolation Plate Growth Time (days)	Preliminary RDP Type-Strain Match	Colony Characteristics				Photo
					Colour	Size	Shape	Other	
FTI09	Fine Tailings	NB	2	*Skermanella aerolata*	light pink	med	circular	mucoid	
FTI10	Fine Tailings	NB	2	*Dietzia maris*	dark yellow	tiny	circular	--	

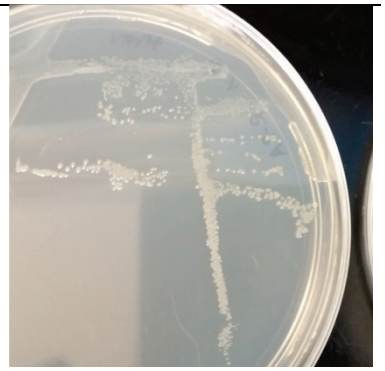
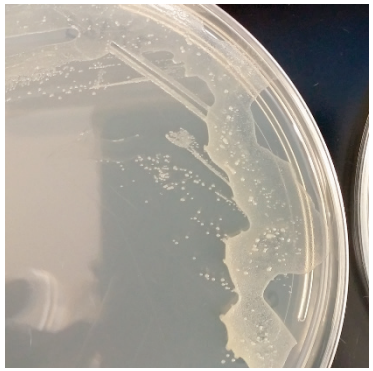
Isolate ID	Sample Source	Original Spread Plate (NaCl amendment (w/v))	Isolation Plate Growth Time (days)	Preliminary RDP Type-Strain Match	Colony Characteristics				Photo
					Colour	Size	Shape	Other	
FTI11	Fine Tailings	NB	1	*Microbacterium phyllosphaerae*	yellow	med	circular	glistening	
FTI12	Fine Tailings	R-2A	1	*Bacillus thuringiensis*	white	large	irregular	--	

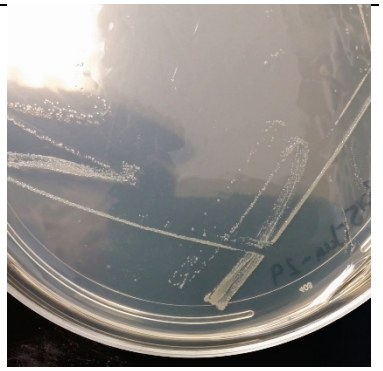
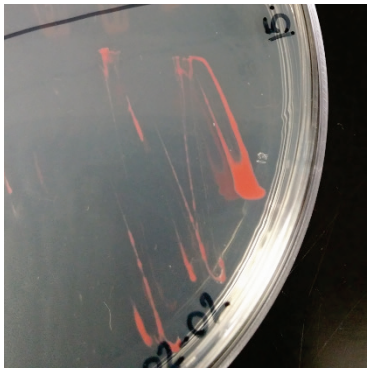
Isolate ID	Sample Source	Original Spread Plate (NaCl amendment (w/v))	Isolation Plate Growth Time (days)	Preliminary RDP Type-Strain Match	Colony Characteristics				Photo
					Colour	Size	Shape	Other	
FTI13	Fine Tailings	R-2A	1	*Sphingomonas jaspsi*	orange	small	circular	--	
FTI14	Fine Tailings	R-2A	1	*Croceicoccus marinus*	yellow	small	circular	--	

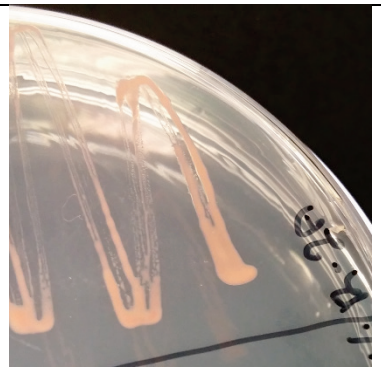
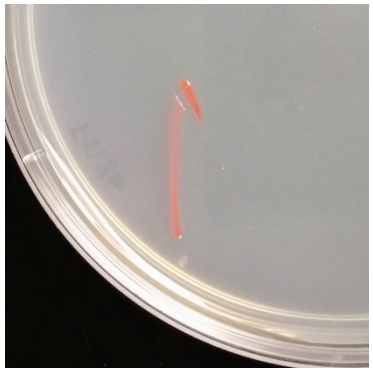
Isolate ID	Sample Source	Original Spread Plate (NaCl amendment (w/v))	Isolation Plate Growth Time (days)	Preliminary RDP Type-Strain Match	Colony Characteristics				Photo
					Colour	Size	Shape	Other	
FTI15	Fine Tailings	R-2A	1	*Dietzia maris*	light orange	small	circular	--	
FTI16	Fine Tailings	R-2A	2	*Halomonas meridiana*	clear	small	circular	--	

Isolate ID	Sample Source	Original Spread Plate (NaCl amendment (w/v))	Isolation Plate Growth Time (days)	Preliminary RDP Type-Strain Match	Colony Characteristics				Photo
					Colour	Size	Shape	Other	
FTI17	Fine Tailings	R-2A (3%)	1	*Marinobacter adhaerens*	white	med	circular	bullseye	
FTI18	Fine Tailings	R-2A (3%)	1	*Zunongwangia profunda*	yellow	small	circular	--	

Isolate ID	Sample Source	Original Spread Plate (NaCl amendment (w/v))	Isolation Plate Growth Time (days)	Preliminary RDP Type-Strain Match	Colony Characteristics				Photo
					Colour	Size	Shape	Other	
FTI19	Fine Tailings	R-2A (3%)	1	*Halomonas andesensis*	light pink	small	circular	bullseye	
FTI20	Fine Tailings	R-2A (3%)	1	*Halomonas andesensis*	white	large	irregular	lobate	

Isolate ID	Sample Source	Original Spread Plate (NaCl amendment (w/v))	Isolation Plate Growth Time (days)	Preliminary RDP Type-Strain Match	Colony Characteristics				Photo
					Colour	Size	Shape	Other	
FTI21	Fine Tailings	R-2A (15%)	2	*Halomonas gudaonensis*	white	tiny	circular	--	
FTI22	Fine Tailings	R-2A (15%)	5	*Halomonas shengliensis*	pink	tiny	circular	--	

Isolate ID	Sample Source	Original Spread Plate (NaCl amendment (w/v))	Isolation Plate Growth Time (days)	Preliminary RDP Type-Strain Match	Colony Characteristics				Photo
					Colour	Size	Shape	Other	
FTI23	Fine Tailings	R-2A (15%)	2	*Halomonas shengliensis*	light orange	tiny	circular	--	
FTI24	Fine Tailings	R-2A (25%)	4	*Halorubrum saccharovorum*	red	tiny	circular	--	

Isolate ID	Sample Source	Original Spread Plate (NaCl amendment (w/v))	Isolation Plate Growth Time (days)	Preliminary RDP Type-Strain Match	Colony Characteristics				Photo
					Colour	Size	Shape	Other	
FTI25	Fine Tailings	R-2A (25%)	4	*Haloferax prahovense*	pink	small	circular	glistening	
FTI26	Fine Tailings	NB (25%)	4	*Halorubrum lipolyticum*	red	tiny	circular	--	

[a] isolate was unable to be isolated under tested growth conditions

8 APPENDIX B – STXM Results

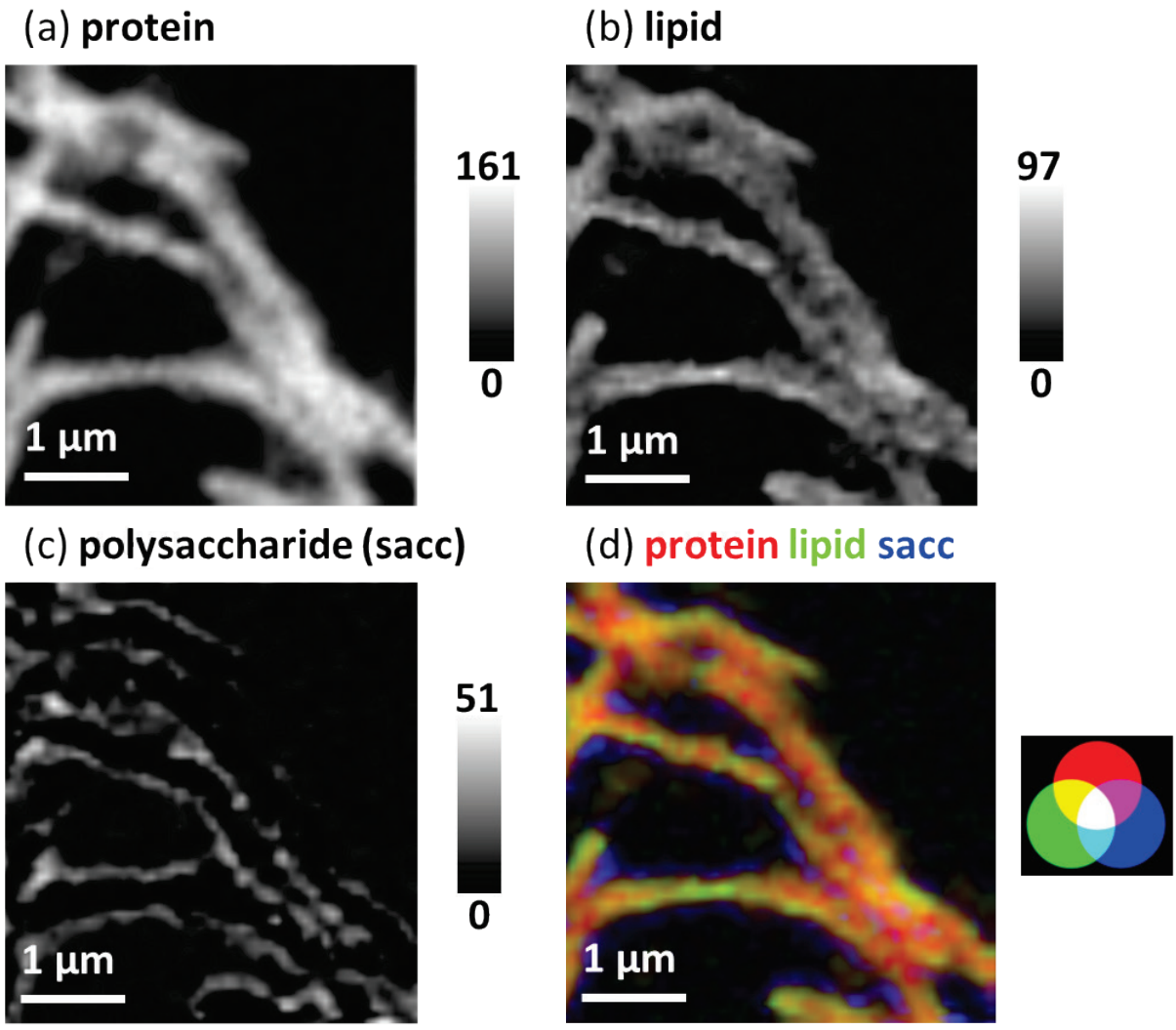


Figure B-1 Component maps of organics associated with the prepared biosorbent before metal exposure. Maps show (a) proteins, (b) lipids, (c) polysaccharides, and (d) a colour overlay of the three.

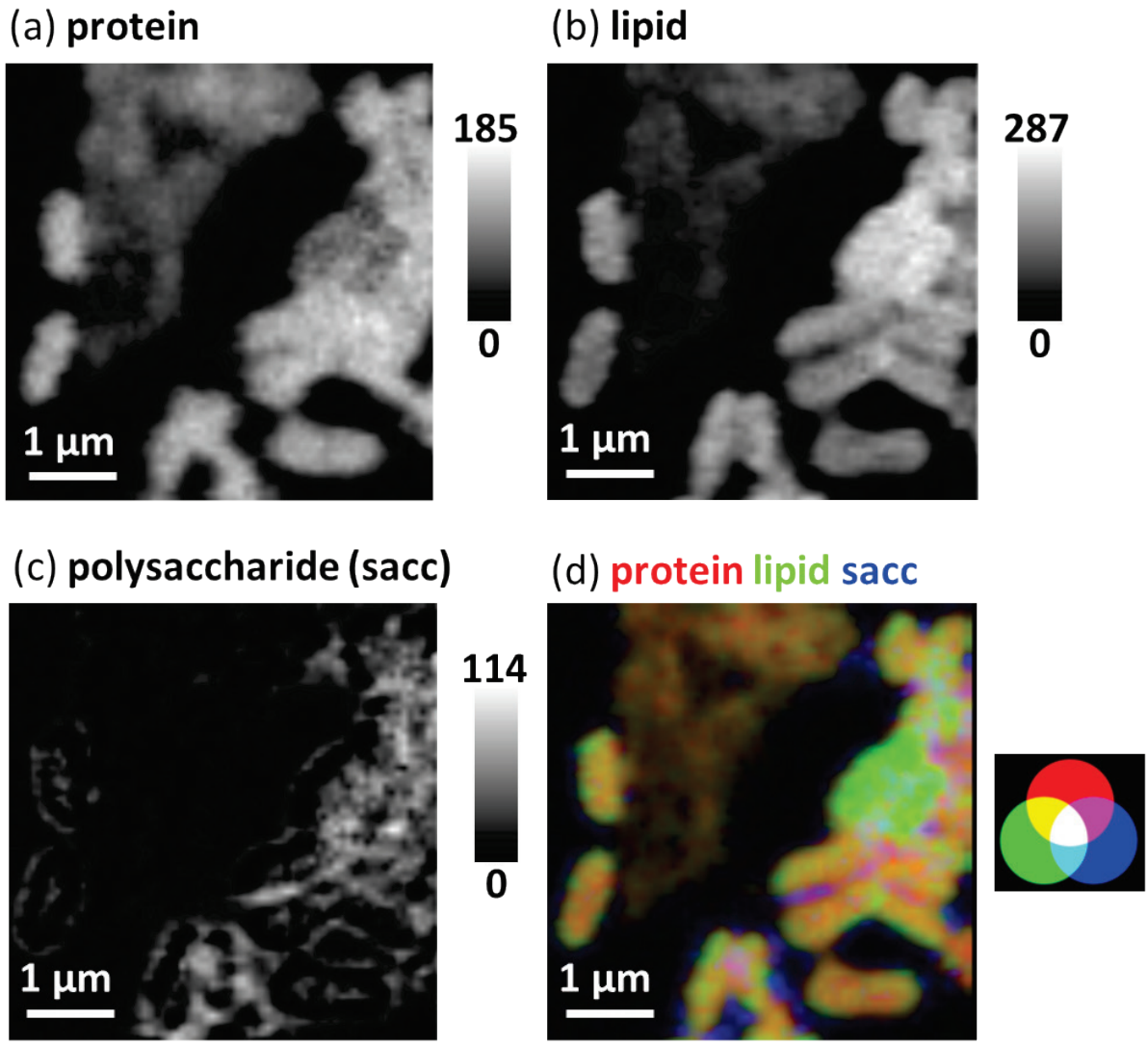


Figure B-2 Component maps of organics associated with the prepared biosorbent after biosorption of Cu from DI. Maps show (a) proteins, (b) lipids, (c) polysaccharides, and (d) a colour overlay of the three.

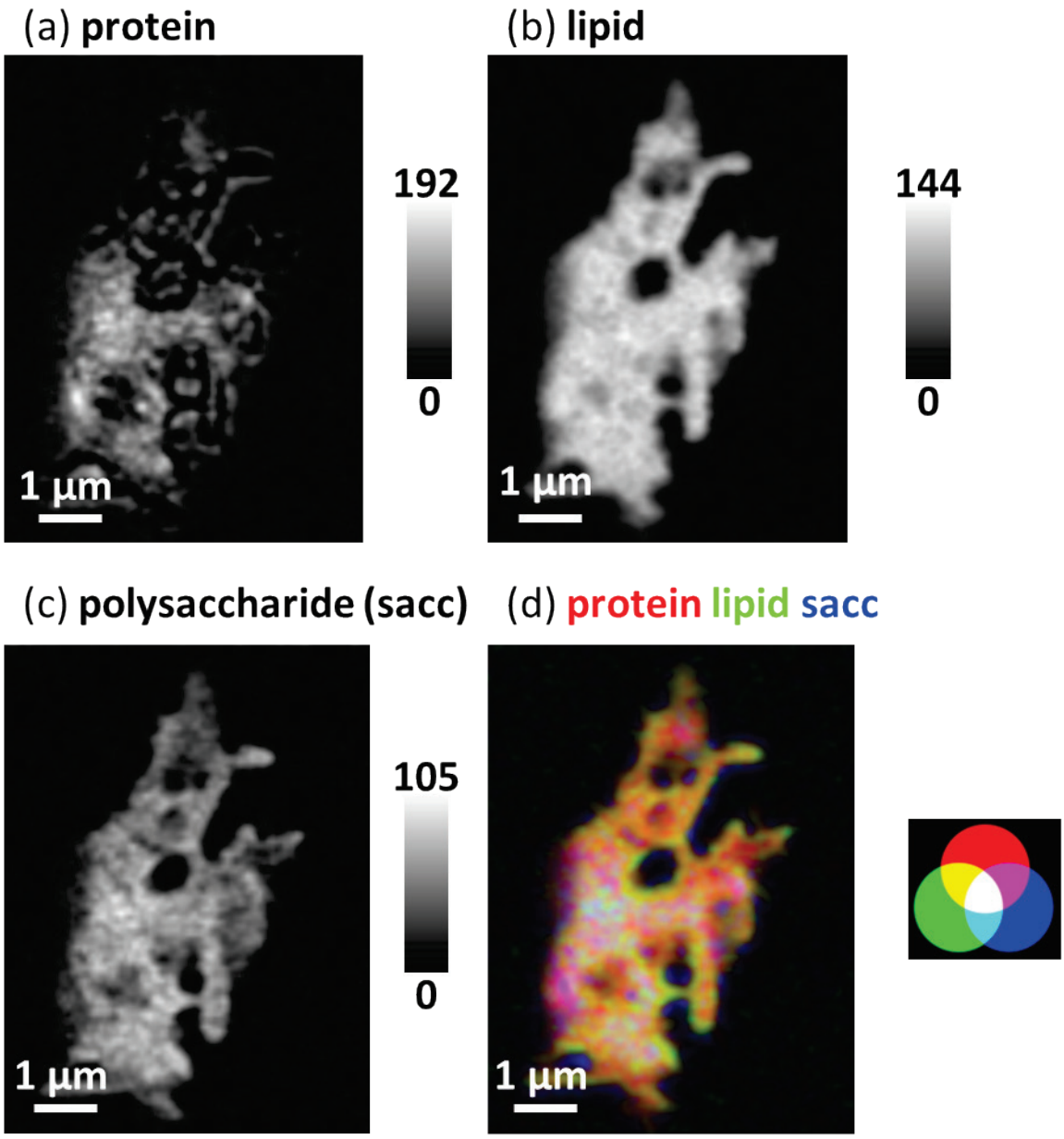


Figure B-3 Component maps of organics associated with the prepared biosorbent after biosorption of Cu from GW. Maps show (a) proteins, (b) lipids, (c) polysaccharides, and (d) a colour overlay of the three.

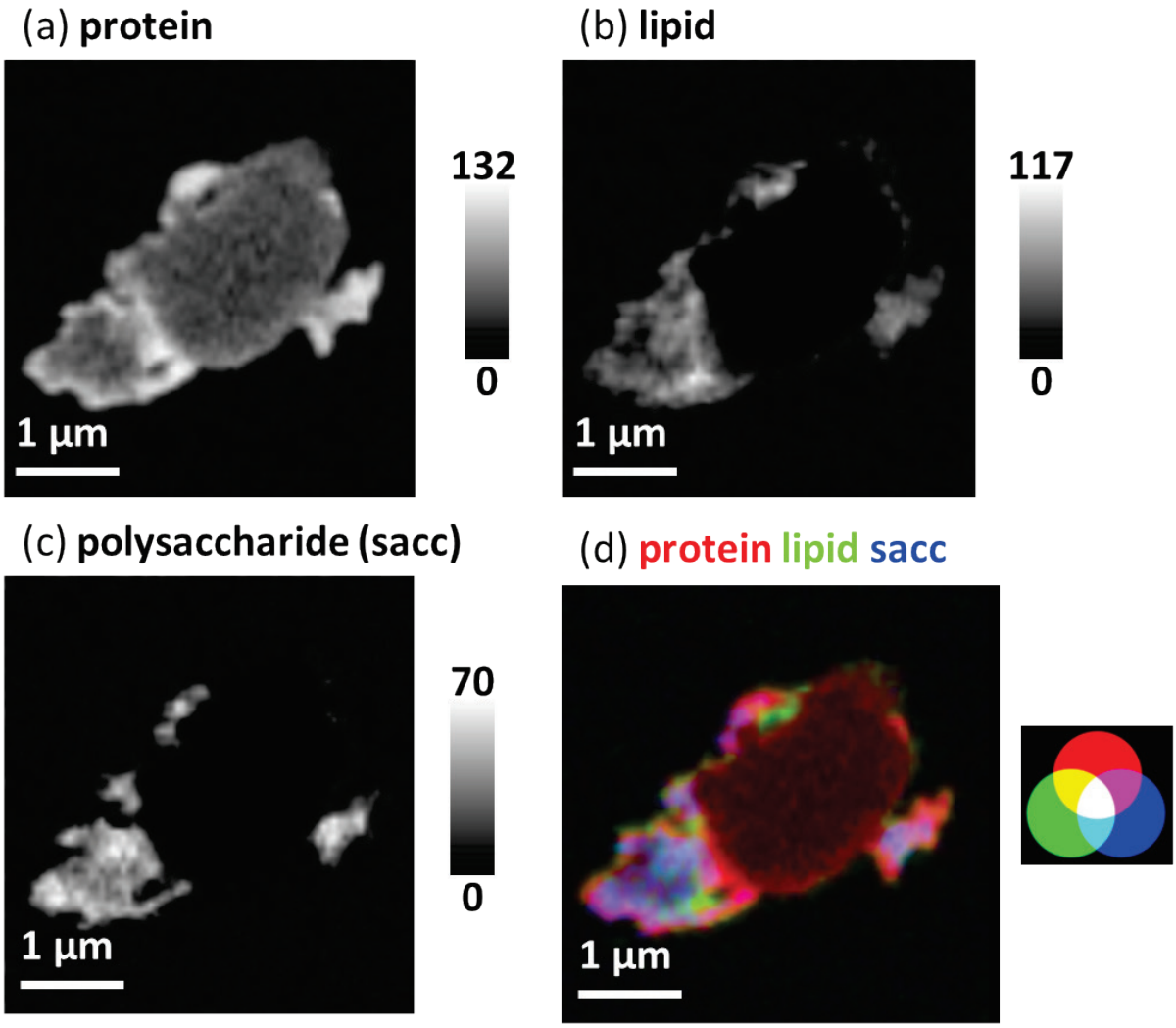


Figure B-4 Component maps of organics associated with the prepared biosorbent after biosorption of Cr from DI. Maps show (a) proteins, (b) lipids, (c) polysaccharides, and (d) a colour overlay of the three.