INVESTIGATION OF BIOLOGICAL FILTRATION FOR REMOVAL OF
MANGANESE FROM DRINKING WATER

by

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Dedication

This is dedicated to my loving wife, Marcia and my late grandfather, Howard, who both know the value of hard work and education.

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Abstract

Reduced iron and manganese in drinking water can cause aesthetic concerns and manganese has potential neurotoxic effects. Biofiltration is a proven but poorly understood method for removal of manganese from drinking water. In this research media selection for biofiltration is investigated in bench-top and pilot-scale experiments. Treatments using sand as filter media had higher rates of manganese removal, and pilot-scale sand filters supported 36,666 and 25,000 colony forming units per gram (CFU/g) of media while anthracite and recycled glass supported 11,111 and 6,349 CFU/g of media, respectively. Biological filtration proved to be an effective treatment option for removal of iron and manganese for the municipality where it was piloted. Sand filter removal rates ranged from 88.0% to 91.9% for iron and 84.3% to 87% for manganese. Canadian Drinking Water Quality Guidelines (CDWQG) were met for 44 of 44 samples for iron and for 35 of 44 samples for manganese. Deoxyribonucleic acid (DNA) sequencing indicated that manganese oxidising bacteria (MOB) accounted for 4.0% of total bacteria and that 10 phyla made up 95.5% of the bacteria in all filter samples.

List of abbreviations and symbols used

°C	degrees Celsius
AO	aesthetic objective
CFU	colony forming units
CFU/g	colony forming units per gram
CDWQG	Canadian drinking water quality guidelines
DNA	deoxyribonucleic acid
EBCT	empty bed contact time
Fe	iron
GAC	granular activated carbon
IQ	intelligence quotient
MAC	maximum allowable concentration
mg/L	milligram per litre
min	minute
ml	millilitre
Mn	manganese
$MnSO_4$	manganese sulfate
MOB	manganese oxidising bacteria
mV	millivolts
$NaHCO_3$	sodium bicarbonate
NaOH	sodium hydroxide
ORP	oxidation-reduction potential
OTU	operational taxonomic unit
PBS	phosphate buffered saline
PSI	pound per square inch
PRV	pressure reducing valve
RPM	revolutions per minute
µg/L	microgram per litre

Acknowledgements

Chapter 1 Introduction

1.1   Project rationale

Manganese (Mn) is naturally found in reduced form in groundwater drawn from aquifers containing manganese bearing minerals as well as in anaerobic surface water, such as a the hypolimnion of a stratified lake (Mouchet, 1992). Of drinking water supplies: 20% of groundwater systems and 4.4% of surface water systems have raw water concentrations above 0.05 mg/L (Kohl & Medlar, 2001). Traditionally manganese was a concern for drinking water due to laundry staining and causing taste and odour issues. Black water and laundry staining can occur at concentrations as low as 0.02 mg/L (MWH, 2005). Even treated water that meets guidelines may also present concerns for utilities as low-level breakthrough can lead to an accumulation of Mn precipitates in the distribution system. Small decreases in distribution system ORP may cause dissolution (re-suspension) of the particulate Mn, resulting in colored water episodes (Kohl & Medlar, 2006).

The current standard for manganese in drinking water in Canada is an aesthetic objective (AO) of 0.05 mg/L. However, Health Canada has proposed a guideline of a maximum allowable concentration (MAC) of 0.1 mg/L based on neurological effects on rodents and an aesthetic objective of 0.02 mg/L based on consumer complaints of discoloured water. More recent research has indicated that high levels of manganese in drinking water has neurotoxic effects (Bouchard et al., 2011). Children exposed to high levels of manganese in drinking water reported lower IQs (Wassermen et al., 2006) and poorer motor skills (Dion et al., 2016).

In order to remove manganese from drinking water it must be oxidized from the Mn(II) valence state, which is soluble in water, to the Mn (IV) valence state, which forms insoluble manganese oxide precipitates. Conventional treatment methods for removal of iron and manganese from drinking water are oxidation through the use of greensand filtration or addition of a strong oxidant such as chlorine or potassium permanganate (Mouchet, 1992). When chlorine is used as the oxidant doses as high as 5 mg/L are often

required which can have a negative impact on DBP formation control (MWH, 2005). In water treatment processes the use of chemicals should be limited in order to reduce costs and limit the impact of chemical residuals and by-product formation in the treated water (Gallard & von Gunten, 2002). Biological filtration has been gaining interest in recent years since it does not require chemical addition (Tobiason et al., 2016; Cai et al., 2015), and according to Mouchet (1992) the other primary advantages of biofiltration are high filtration rate, high retention capacity, flexibility of operation, and reduced capital and operating costs.

Growstone is a hydroponic medium made from recycled glass bottles and has not been previously used as media for biofiltration. It is produced by crushing glass into a powder, melting it in a kiln, allowing it to cool and harden then crushing it to the desired size. Growstone is highly porous which could potentially provide a greater surface area to support biological growth if used as biofilter media.

By using recycled glass (Growstone) as an alternative media source it could further reduce the cost and environmental impact of using biological treatment for manganese removal from drinking water.

1.2   Research Objectives

There are two main objectives of this research

(a) to investigate the role that biofilter media plays in the removal of manganese as a substrate for biological growth by comparing 'Growstone', a type of recycled glass, to conventional biofilter media under laboratory environment, and

(b) to evaluate biological filtration with Growstone for the removal of iron and manganese at the pilot-scale level.

This was done by comparing different media through bench-scale experiments using simulated groundwater as well as pilot-scale experiments using groundwater naturally high in iron and manganese.

1.3 Thesis Organization

Each chapter in this thesis describes an experiment with a specific objective. Chapter 3 describes bench-top experiments designed to compare the effectiveness of different media materials by inoculating biofilters with a known manganese oxidising bacterium (*Leptothrix discophora SP-6*), or a native biofilm grown from water high in manganese. Chapter 4 describes a pilot-scale study designed to asses media performance in a real-world scenario as well as determine effectiveness of biofiltration at that site. Chapter 5 compiles the conclusions derived from each experiment and outlines the overall picture.

Chapter 2 Literature Review

2.1   Chemistry of manganese in water

Manganese (Mn) is the earth's second most abundant transition metal (Tebo et al., 2004).
Mn oxides occur in soils, sediments and water as coatings on soil and sediment particles
and as discrete particles (Tebo et al., 1997). Mn(II) is favored at low pH and the absence
of oxygen (low ORP) (Tebo et al., 2004). It is released through the weathering of igneous
and metamorphic rock and then oxidised to the Mn(III), Mn(IV) states. There are over 30
known types of Mn(III), Mn(IV), or mixed Mn(III,IV) oxides (Post, 1999). Bacteria (and
some fungi) are known to naturally catalyze the oxidation of Mn(II) to Mn(IV). Owing to
the high activation energy, the oxidation of Mn(II) to Mn(III) and Mn(IV) is largely
catalyzed by microorganisms. This biological oxidation is generally fast compared to
abiotic oxidation, suggesting that biological oxidation dominates in the environment
(Tebo et al., 2004). Naturally occurring manganese oxides are believed to be mainly
biogenic or derived from the alteration of biogenic oxides. Generally speaking,
manganese is favored in the Mn(II) state in the absence of oxygen and at a low pH. In the
presence of oxygen and a high pH Mn(III) and Mn(IV) are favored and form insoluble
precipitates (Tebo et al., 2004)
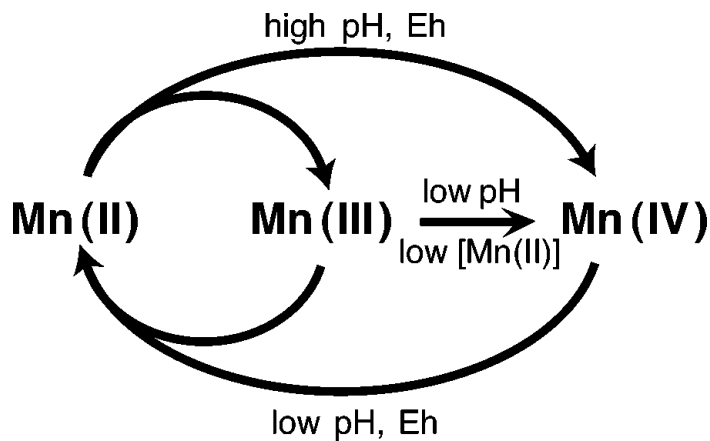
Figure 1- The Mn cycle of oxidation states found in nature. Reproduced from (Tebo et al., 2004)

The oxidation of manganese is an autocatalytic reaction. The oxides that are formed
adsorb Mn(II) and catalyze its oxidation to Mn(IV) (Stumm & Morgan, 1996; Davies &

Morgan, 1989). Next to oxygen, manganese oxides are some of the strongest naturally occurring oxidising agents in the environment (Tebo et al., 2005). This autocatalytic effect aids treatment by increasing the rate of reaction once manganese oxides are formed on the media. A large amount of Mn^{2+} is actually removed by adsorption on to the surface of the manganese oxides that have formed.

At pH values of most natural waters aqueous Mn(II) is the predominant form of Mn, as shown in Figure 1. Mn(II) can be removed from water by oxidation but aeration alone is not effective unless the pH is greater than 9 (MWH, 2005).

2.2   Chemistry of iron in water

Iron (Fe) is the 4th most abundant element in the earth's crust (MWH, 2005). Iron is relatively soluble in a reducing environment, such as low oxygen groundwater or surface water. It can dissolve into the water from Fe-bearing soils or rock formations or into anoxic hypolimnion from reduced lake sediments and is found in the reduced, or ferrous (Fe(II)) form (MWH, 2005). Treatment of iron in drinking water typically involves oxidizing ferrous iron to the ferric (Fe(III)) form, which will form a precipitate and can be removed via sedimentation or filtration. The current standard for iron in drinking water in Canada is an AO of 0.3 mg/L, based on taste and staining of laundry and plumbing fixtures.

High levels of manganese alone can be a problem in an aquifer but it is not uncommon for an aquifer to have high levels of both iron and manganese at the same time (Bottomly, 1984). When iron and manganese are found together it can present a problem for efficient manganese removal. Since iron has a lower oxidative potential it will be oxidised before any Mn removal takes place. Generally, Fe will be spontaneously chemically oxidized by oxygen at pH values greater than 5 (Emerson & Moyer, 1997) and will tend to produce ferric hydroxide under a positive redox potential (Eh) (Figure 2). But Iron can form a strong complex with natural organic matter (NOM), so oxygen, chlorine, chlorine dioxide, and potassium permanganate are often unable to oxidise iron in many waters (Knocke et al., 1991).
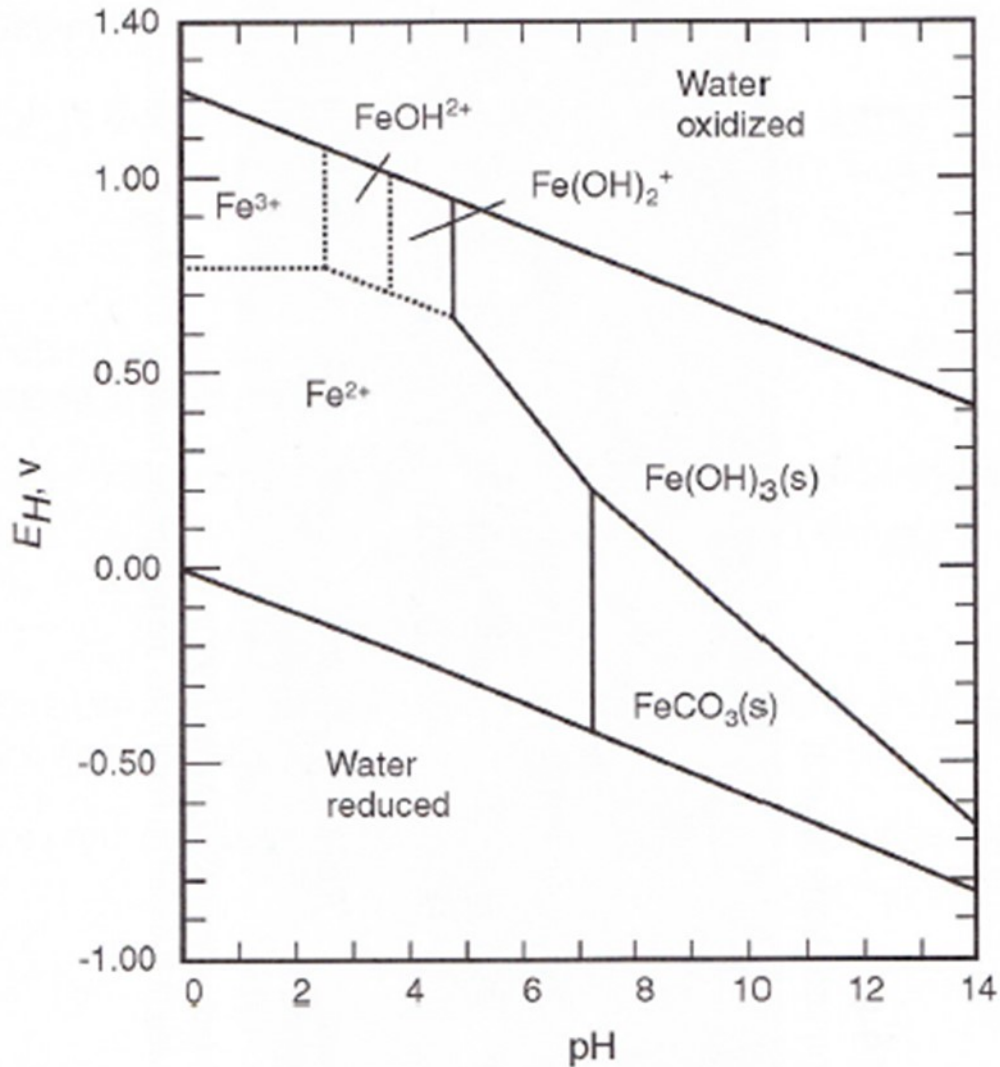
Figure 2- Forms of iron in water as a function of redox potential versus pH (MWH, 2005)

2.3 Biological oxidation of manganese

Biological manganese oxidation is widespread among a large number of phylogenetically diverse bacteria. A list of known manganese oxidising bacteria is given in Table 1. Most oxidizers are gram positive or belong to the phylum Proteobacteria (Tebo et al., 1997). Mn(II) oxidation appears to be extracellular (Tebo et al., 1997). Although the mechanisms by which bacteria oxidize manganese and the biological function it serves are poorly understood, there are 3 known mechanisms by which microorganisms can remove manganese: direct intracellular oxidation as part of the metabolic pathway (MOB

use Mn as an electron donor and oxygen as an electron acceptor), extracellular adsorption where Mn(II) is adsorbed onto negatively charged extracellular polymer substances, and the catalysis of Mn(II) oxidation by biopolymers generated by microorganisms (Mouchet, 1992; Stumm & Morgan, 1996; Tebo et al., 2004; Tobiason et al., 2016).

While Fe(II) oxidation to Fe(III) will proceeded abiotically with the addition of air (MWH, 2005), manganese oxidising bacteria play an important role in the oxidation of Mn(II) to Mn(IV) (Nitzsche et al., 2015). Although oxidation from Mn(II) to Mn(IV) is thermodynamically favorable there is no direct evidence that bacteria use the energy produced from it (Tebo et al., 2005). Some other theories as to what function it does serve are for the protection of the bacterium from toxic heavy metals, ultraviolet light or predators, storage of an electron acceptor for use in harsh conditions, toxicity control and the breakdown of organic matter into usable substrates and the scavenging of micronutrient trace metals (Tebo et al., 2004; Han et al., 2013).

Table 1- List of known manganese oxidising bacteria

Species	Citation
Leptothrix spp	Mouchet, 1992
Leptothrix discophora SP6	Tebo ,2004
Leptothrix discophora SS1	Boogerd, F. C.De Vrind, J. P M, 1986
Pedomicrobium spp	Kohl and Dixon, 2012
Bacillus spp	Kohl and Dixon, 2012
Pseudomonas putida MnB1	Tebo ,2004
HA-01	Tebo ,2004
HA-10	Tebo ,2004
HA-11HA-17	Tebo ,2004
SI85-9A1	Tebo ,2004
SI85-2B	Tebo ,2004
SI92-1	Tebo ,2004
Crenothrix spp	Mouchet, 1992
Hyphomicrobium spp	Mouchet, 1992
Siderocapsa spp	Mouchet, 1992
Siderocystis spp	Mouchet, 1992
Metallogenium spp	Mouchet, 1992
Pseudomonas manganoxidans	Mouchet, 1992
Arthrobacter globiformis,	Dubinina and Zhdanof, 1975
Acinetobacter sp	Beukes et al, 2012
Sphaerotilus, spp	Cai et al, 2015
Clonothrix, spp	Cai et al, 2015
Sphingomonas, spp	Cai et al, 2015
Flavobacterium, spp	Cai et al, 2015
Janthinobacterium, spp	Cai et al, 2015

2.3.1 Media requirements

Biofilter media acts as both a particle screening mechanism and the substrate for biological growth (Lauderdale et al., 2011), so it stands to reason that factors driving selection are cost, grain size distribution, ability to support biological growth and media hardness, since the media must not break down while in use. Granular activated carbon (GAC) has been shown to support between three to eight times more biological growth than anthracite, which is likely due to GAC having a greater surface area available for microbial attachment (Wang et al., 1995; LeChevallier et al., 1992). Granger et al. (2014) and Kohl & Dixon (2012) found that GAC had better removal than anthracite but

Hoyland et al. (2014) found that an anthracite/gravel combination can remove 98% of Mn at pH 6.3, which indicates that both GAC and anthracite are effective as biofilter media for manganese removal.

GAC may provide more robustness over anthracite under these challenging conditions due to high levels of biological activity (Emelko et al., 2006; Wang et al., 1995). Media hardness must also be considered when selecting media, as attrition is a larger concern for GAC than for anthracite (Lauderdale et al., 2011).

2.3.2   Empty bed contact time and kinetic requirements

Empty bed contact time (EBCT) is an important parameter for biofiltration and is determined from the hydraulic loading rate and the filter bed volume. It is essentially the amount of time it takes for water to pass through the media bed of the biofilter. Most biofilters are designed for an EBCT of 10-20 minutes because research has shown that 90% removal of biodegradable organic material and Mn can be removed within this EBCT range (Provost et al., 1995; Bourgine et al., 1994). In a review of over 100 biological treatment plants Bruins et al. (2014) found that only plants that had an EBCT greater than 11.5 minutes achieved complete removal. The same review also determined that other water quality factors such as pH and iron loading could still cause ineffective removal rates even if EBCT was optimal.

Kinetic analysis can be used to calculate the rate at which the oxidation of manganese proceeds. Kinetics are important because it can provide the required time it takes to remove a contaminate efficiently, which is necessary in sizing treatment plants (Katsoyiannis & Zouboulis, 2004). Once the kinetic removal rate is calculated the EBCT necessary for adequate treatment can be calculated which will determine the needed filter size.

Kinetic removal rates can be calculated from the following equation (Cerrato et al., 2010):

$$\frac{d[Mn]_t}{dt} = k[Mn]_t$$

Where: k is the rate constant in min^{-1}.

Once integrated, the following equation can be derived:

$$ln\frac{[Mn]_t}{[Mn]_0} = -kt$$

Where: $[Mn]_t$ is the final manganese concentration

$[Mn]_0$ is the initial manganese concentration

k is the rate constant

t is the empty bed contact time

2.3.3 pH and oxidation reduction potential

Oxidation-Reduction potential (ORP) reflects the amount of oxidants in the water (Tekerlekopoulou et al., 2013). Most raw waters where removal of manganese is a concern are anaerobic and typically have a low ORP. Aeration is needed to raise the ORP and maintain an aerobic environment for MOB survival. According to (Mouchet, 1992) the approximate lower limit of the field of activity where MOB are capable of oxidising Mn(II) to Mn(IV) is an ORP of +300-400 mv and a pH of 7.4-7.5. More recent work has determined biological oxidation of manganese can actually occur at pH values as low as 6.3 (Granger et al., 2014; Hoyland et al., 2014; Burger et al., 2008b). This pH/ORP range is much broader than the range at which physical/chemical oxidation will take place, which can be seen in Figure 3. At higher pH it can be difficult to distinguish between biological and physical/chemical oxidation of manganese (Tobiason et al., 2016)
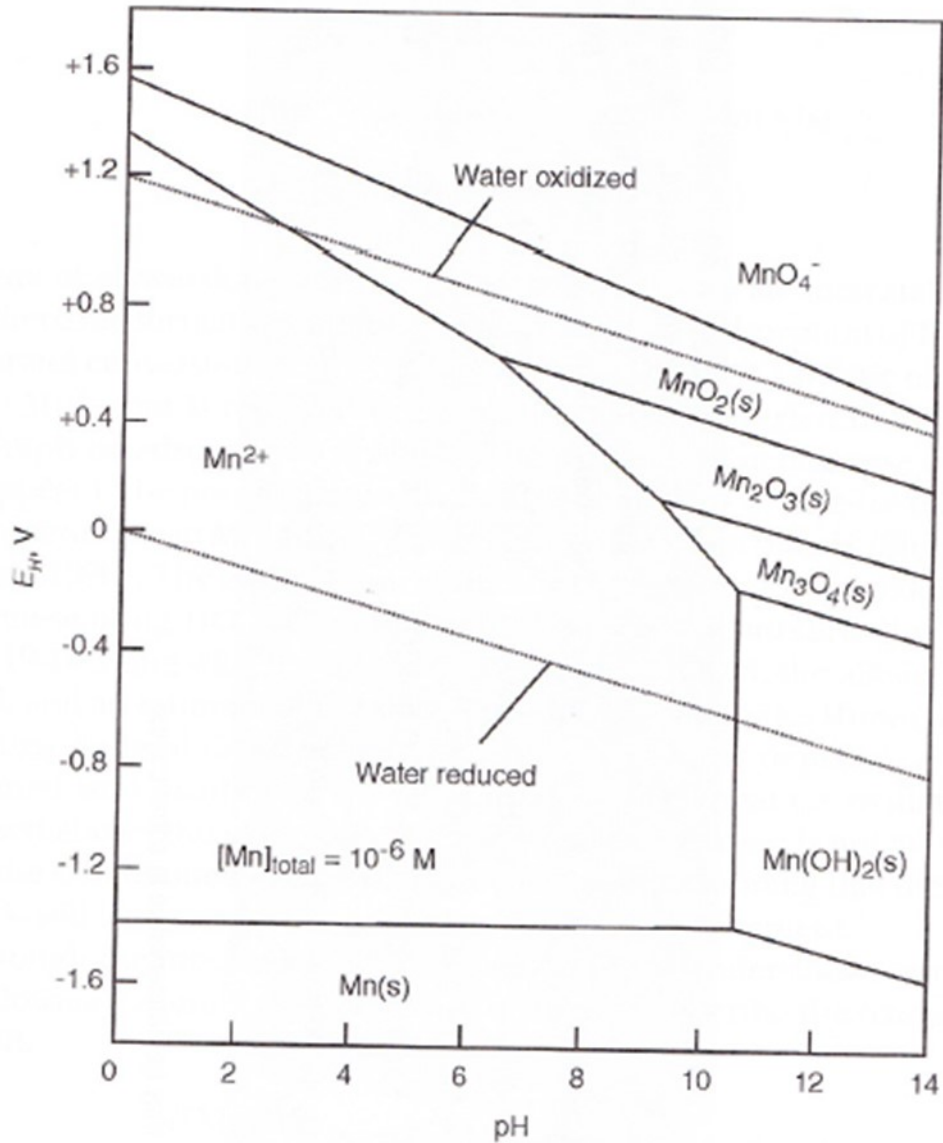
Figure 3-- Forms of manganese in water as a function of redox potential vs pH at 25°C (MWH, 2005)

2.3.4 Dissolved oxygen concentration

Manganese removal is negatively affected by low dissolved oxygen (DO) concentration (Stumm & Morgan, 1996). In general DO concentration strongly influences the performance of biological processes as it is necessary for microorganism growth (Tekerlekopoulou et al., 2013). A DO concentration greater than 3 mg/L is needed to maintain aerobic conditions throughout the filter bed (Tekerlekopoulou et al., 2013). Apart from a minimum value for DO that needed to be met Bruins et al. (2014) found that

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there was no other distinct relationship between DO concentration and manganese removal.

2.3.5   Previous research

Biofiltration for iron and manganese removal is a relatively new field (Tekerlekopoulou et al., 2013), and although it is widespread the assogiated research is still fairly limited. Due to the site-specific nature of most research, a wide range of operating conditions and raw water quality parmeters has been encountered. Previous research can be seen in Table 2.

Table 2-Previous iron and manganese biofiltration research

Treatment process	Plant Location	Raw water concentration		Operational Conditions				Media	Removal rates (%)		Citation
		Fe (µg/L)	Mn (µg/L)	pH	ORP (mV)	DO (mg/L)	EBCT (min)		Fe	Mn	
Bench scale biofiltration with *L. Discophora sp6*	Halifax, Nova Scotia		300	6.5, 7.5	435-485			sand		90%	Burger et al., 2008b
Bench scale biofilters			100-500	6.3-7.3		7.5-8.0	10	anthracite		98%	Hoyland et al., 2014
Bench scale direct biofiltration	Halifax, Nova Scotia	189-2589	148-1069	6, 9	236-329	4.2-10.3	15	anthracite/GAC	38-53	70-91	Granger et al., 2014
Pilot scale biofiltration with pre-aeration	Harbin, China	7000	2300	7.2		5	15	sand	99	34.5-59.3	Qin et al., 2008
Pilot scale aeration with roughing upflow filtration	Avellaneda, Argentina	190-440	180-370	7.0-7.3	325-391	4.6-7.4		sand	95	88	Pacini et al., 2005
Pilot scale aeration with roughing upflow filtration	Las Garzas, Argentina	90-370	1080-1830	7.1-7.4	389-505	1.1-1.9		sand	70	95	Pacini et al., 2005
Pilot scale 2-stage upflow filtration	Berlin, Germany	100	400	7.2	340	3.8	15	polystyrene beads	90	90	Katsoyiannis and Zouboulis, 2004
Full scale biofiltration with aeration	Sorgues, France		0.7-1.0	7.2-7.5	300-400			sand		100	Mouchet, 1992
Full scale biofilter	Shenyang, China	500	570-3000	6.8		0.56		sand	80-100	80-100	Li et al., 2005
Full scale biofilter:	Dorchester, N.B.		930	7.25	295			sand		>99%	Burger et al., 2008a
Full scale biofilter:	Memramcook, New Brunswick		860	6.46	343			sand		>99%	Burger et al., 2008a
Full scale biofilter:	Shediac, New Brunswick		860	7.5	338			sand		>99%	Burger et al., 2008a
Full scale biofilter:	Woodstock, New Brunswick		1390	7.39	368			sand		>99%	Burger et al., 2008a

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Chapter 3 Bench-scale manganese removal through biofiltration

3.1   Introduction

Bio-filtration has been used in Europe from the 1980's (Mouchet, 1992) and in North America from 1999 (Burger et al., 2008a). Biofiltration has proven to be an effective method for Mn removal but little is known about the bacteria involved or the mechanism used.

This study used a bench-scale setup to assess the effectiveness of different types of media in supporting the growth of MOB. Some filters were inoculated with a native biofilm grown from a source water high in manganese and some were inoculated with *Leptothrix discophora SP-6*. All columns were compared to a control column that received no inoculant to assess the importance of the source of bacteria. A native biofilm was grown and used for this experiment in order to provide a scenario more closely matching that of a full-scale plant. Most full-scale plants make use of the MOB present in the raw water and just provide the right conditions for growth on the filter media. Seeding may occur naturally in this way or the filter media or backwash water of another plant that is already operational may be used to shorten the acclimation period of the filter (Kohl & Dixon, 2012; Mouchet, 1992; Burger et al., 2008a).   *L. discophora SP-6* was selected for this experiment because it is a known manganese oxidising bacterium and has been studied extensively (Emerson & Ghiorse, 1993). Bacteria in the genus *Leptothrix* are gram-negative, sheathed bacteria that can oxidise iron and manganese directly (Tebo et al., 2004). *Leptothrix* bacteria also rely on this sheath to protect themselves and attach to surfaces in groundwater aquifers which are their natural habitat (Emerson & Ghiorse, 1993). In *L. discophora*, Mn(II) oxidation is catalyzed by a protein that is localized in the sheath of environmental strains (Tebo et al., 2004). *L. discophora-SP-6* oxidize iron and manganese and deposit oxidized minerals on exopolymers associated with their sheath (Tebo et al., 1997).

The objective of this chapter was to compare the ability of different types of filter media to support biological growth and their effectiveness for biological manganese removal at the bench-scale level.

3.2   Methods

3.2.1   Metals analysis

All metals samples were analysed on an inductively coupled plasma mass spectrometer (Thermo Fisher XSeries 2 ICPMS). Samples were collected in polyethylene test tubes and preserved with two drops of nitric acid. Dilutions were performed with deionized water.

3.2.2   Bacterial Cultures

All *Leptothrix discophora SP-6* cultures were grown in K media from cultures preserved at -80°C. 0.2 mL of preserved culture was added to 200 mL of media and the flasks loosely capped. The cultures were then placed on a shaker table at 75 RPM and incubated at 22°C and allowed to grow for 28 days.

All bacteria cultures were spread plated in triplicate according to section 9215 C in Standard methods for the Examination of Water and Wastewater (22ND edition) from serial dilutions and grown on R2A agar with 17 mg/L manganese added to allow for identification of manganese oxidising bacteria. Plates were incubated at 22°C for 5-7 days in the dark. Plates with dilution factors producing between 30 and 300 colonies were counted. If more than one set of plates produced between 30 and 300 colonies the plate with the lower dilution was counted. If manganese oxidizing bacteria plate counts were needed the same plates were used and incubated in the dark for an additional 21 days at 22°C at which time the black and brown colonies were counted.

3.2.3   Biofilm removal

In order to remove biofilm from filter media a well-mixed sample was collected from the filter. One gram of media was taken from that sample and stomached for 2 minutes in a

sterile stomaching bag with 10 mL of autoclaved phosphate buffered saline (PBS) and used tor serial dilutions and plate counting.

3.2.4 Experimental set-up and operation for columns seeded with *Leptothrix discophora SP-6*

A bench-scale biofilter set-up was modified from Burger et al. (2008b) (Figure 4). Treated drinking water from Halifax, Nova Scotia was used and modified to simulate groundwater. It was first passed through an activated carbon filter to remove chlorine. It was then pumped through 8 chromatography columns filled with filter media using Cole Parmer peristaltic pumps. $MnSO_4$ was added to produce high levels of manganese commonly found in drinking water (0.4-0.8 mg/L) and an NaOH and $NaHCO_3$ solution was added to control pH. The chromatography column influent was mixed at a 1:1 ratio. Aerated column effluent was recycled to the column influent and mixed at a 1:1 ratio with simulated groundwater in order to raise the ORP and ensure that the columns remained aerobic. Samples were taken at the outlet of the columns. Two chromatography columns were filled with each media type used, one was seeded with *Leptothrix discophora SP-6*, a known manganese oxidizing bacterium, and one was left unseeded as a control (see Table 3). Samples were collected 2-3 times a week for metals analysis and pH and ORP were measured once a week. Flow rates for all pumps were taken once a week and pump speed was adjusted in order to maintain proper flow rates.

Figure 4- Bench-scale apparatus

Table 3- Experimental design for columns seeded with *Leptothrix discophora SP-6*

Column ID	Seed source	Media type
1	*L. discophora SP-6*	Anthracite
2	none	Anthracite
3	*L. discophora SP-6*	Sand
4	none	Sand
5	*L. discophora SP-6*	Glass
6	none	Glass
7	*L. discophora SP-6*	Growstone
8	none	Growstone

Columns were initially seeded with *L. discophora SP-6* on May 7th, 2014. No removal was seen from any seeded columns during the first two months. Columns were seeded again July 8th, 2014 and a nutrient cocktail consisting of sodium acetate (1 mg/L as C), sodium nitrate (0.117 mg/L as N), and dipotassium phosphate (0.026 as P) was added to aid biofilm establishment on the media. This was done to obtain a C:N:P ratio of

100:10:1 The nutrients were removed on Dec 4th, 2014 and the experiment was continued until Dec 27th, 2014. All of the columns were disinfected on Jan 26th, 2015 by pumping water with 10 mg/L sodium hypochlorite, then flushed with chlorine free water until the column effluent had no measureable chlorine residual. and seeded for a third time on June 4th, 2015. The apparatus design was modified by switching the flow of recycled effluent to a separate source of filtered, aerated water (Figure 5). This was done to ensure a manganese-free source of aerated water during periods of low manganese removal. During this phase flow rates were taken for the simulated groundwater supply and the aerated water supply after each sample in order for a dilution factor to be calculated and applied to the effluent manganese concentration.
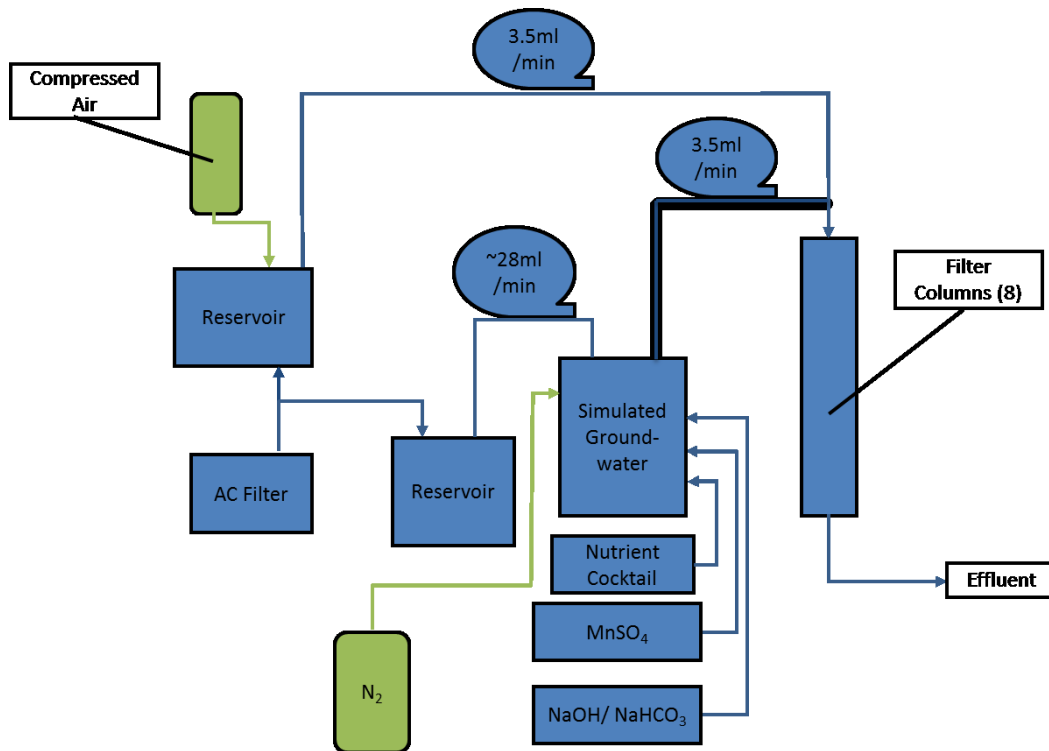
Figure 5- Modified bench-scale apparatus

3.2.5 Seeding procedure for columns seeded with *Leptothrix discophora SP-6*

L. discophora SP-6 cultures were grown as described in section **3.2.1**. The cultures were then then poured into sterile centrifuge tubes and centrifuged at 2000 RPM for 10 minutes. The supernatant was then replaced with PBS and vortexed at 1700 RPM for 1

minute. The cultures were centrifuged and re-suspended in PBS two more times in order to thoroughly wash the cells before seeding. The suspended cultures were added to autoclaved PBS, which was pumped through the four seeded columns at a rate of about 1 mL per min over 24 hours. The bacteria solution contained approximately 9.65×10^5 CFU/mL of *L. discophora SP-6* and each seeded column received about 1.39×10^9 cells of *L. discophora SP-6*. Pure autoclaved PBS was pumped through the control columns at the same flow rate. The PBS was then held in the columns for 24 hours to allow the bacteria more opportunity to attach. The feed was then switched to the simulated groundwater supply. This seeding procedure was used to ensure that each column received the same amount of bacteria.

3.2.6 Experimental set up and operation for columns seeded with a native biofilm

Treated drinking water from Halifax, Nova Scotia was used and modified to simulate groundwater. It was first passed through an activated carbon filter to remove chlorine. It was then pumped through 12 chromatography columns filled with filter media using Cole-Parmer peristaltic pump. $MnSO_4$ was added to produce high levels of manganese commonly found in drinking water (0.4-0.8 mg/L) and an NaOH and $NaHCO_3$ solution was added to control pH. The chromatography column influent was mixed at a 1:1 ratio. Aerated column effluent was recycled to the column influent and mixed at a 1:1 ratio with simulated groundwater in order to raise the ORP and ensure that the columns remained aerobic. Samples were taken at the outlet of the columns. Three chromatography columns were filled with each media type used, two were seeded with a native biofilm grown with water from Bennery Lake and one was left unseeded as a control (Table 4). Samples were collected 2-3 times a week for metals analysis and pH and ORP were measured once a week. Flow rates for all pumps were taken once a week and pump speed was adjusted in order to maintain proper flow rates.
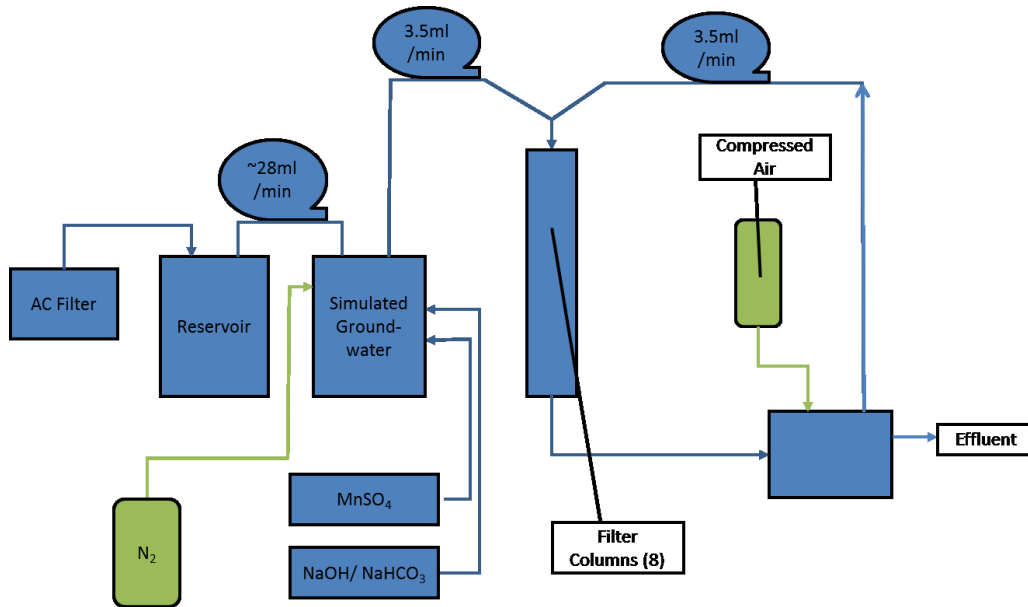
Table 4- Experimental design for columns seeded with a native biofilm

Column ID	Seed Source	Media
9	Native Biofilm	Glass
10	Native Biofilm	Glass
11	none	Glass
12	Native Biofilm	Anthracite
13	Native Biofilm	Anthracite
14	none	Anthracite
15	Native Biofilm	Sand
16	Native Biofilm	Sand
17	none	Sand
18	Native Biofilm	Growstone
19	Native Biofilm	Growstone
20	none	Growstone

3.2.7 Seeding procedure for columns seeded with a native biofilm

Raw water from Bennery lake was pumped through 8 Chromatography columns (Kimble Chase Flex Column, 2.5 cm ID by 20cm) filled with filter media at a rate of 1 mL/min in order to encourage native bacteria to grow on the media. Two chromatography columns were filled with each type of media used: sand, anthracite, glass beads and Growstone. After 2 weeks the columns were collected and set up in a bench-scale biofilter set-up that was modified from Burger et al. (2008b) and described above (Figure 4).

About 1 g of media was removed from each column and stomached as described in section 3.2.2 in order to estimate the amount of bacteria each column started with at the beginning of the experiment. The results can be seen in Table 5. Although each column began with a different amount of bacteria, the amount of bacteria each column did have was a direct result of the medias ability to support biological growth during the seeding phase.

Table 5- Seeding heterotrophic plate counts for biofilm setup Feb 24th, 2015

Column	Weight stomached (g)	Dilution factor	avg. HPC	CFU/g of media
Glass	1	10,000	21	2.E+05
Glass	1	10,000	17	2.E+05
Anthracite	1	10,000	4	4.E+04
Anthracite	1	10,000	49	5.E+05
Sand	1	10,000	13	1.E+05
Sand	1	10,000	30	3.E+05
Growstone	1	10,000	76	8.E+05
Growstone	1	10,000	35	3.E+05

3.2.8   16S rDNA sequencing for community analysis

Media samples were collected from each filter by thoroughly mixing the top 5 cm of filter media and placing in an autoclaved sample bottle and capped. DNA was extracted using the Mo Bio Power Biofilm DNA Isolation kit. No stomaching was necessary as the DNA isolation kit did not require the biofilm to be separated from the media. The extracted samples were sent to the Integrated Microbiome Resource at Dalhousie University for 16s rDNA gene sequencing

In order to determine if *L. discophora SP-6* remained in the biofilm the accession number corresponding to the 16s ribosomal DNA sequence of *L. discophora* strain *SP-6* (L33974) was obtained from Siering and Ghiorse, 1996. The accession number was then used to obtain the operational taxonomic unit (OTU) number from the greengenes website, which could be compared to the 16s rDNA results.

3.3   Results

3.3.1   Columns seeded with *Leptothrix discophora SP-6*

Columns were first seeded on May 7th, 2014 and ran until July 7th, 2014 (Figure 6). No removal was seen during this stage for the sand, glass or Growstone columns. An analysis of variance with a multiple comparison test showed that there was no significant removal from these columns (p-value: 0.107). Biological growth on the media was very minimal and black manganese deposits were not seen in the columns. Since the treated water used

to produce the synthetic groundwater in the experiments is nutrient deficient it is likely that the lack of nutrients was limiting the growth of the biofilm.
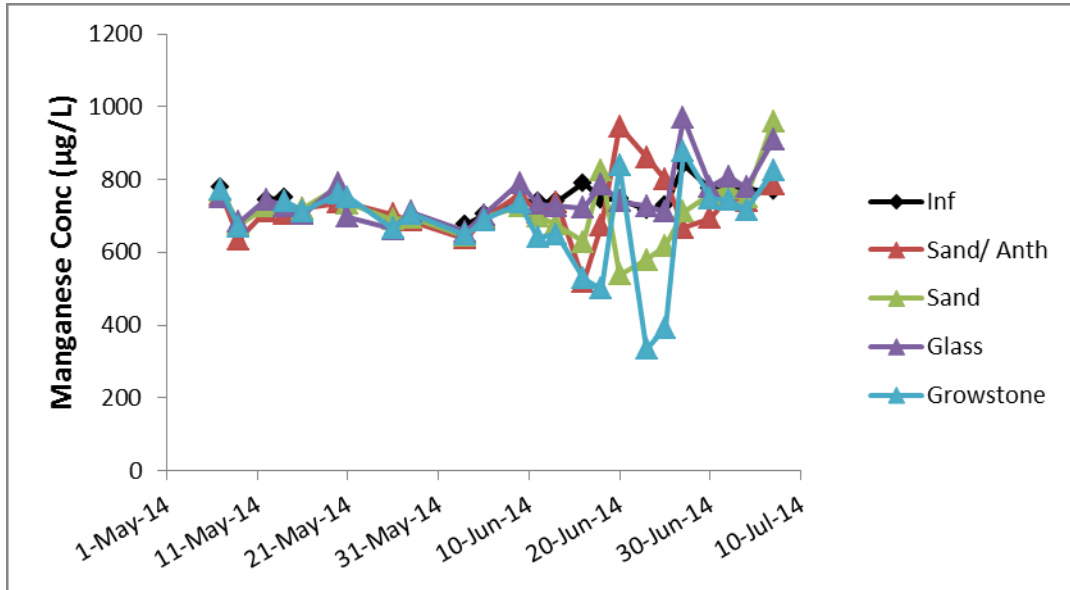
Figure 6-Manganese removal for columns seeded with *Leptothrix discophora SP-6*, May 7th -July 7th, 2014

Columns were seeded again on July 8th, 2014. A nutrient cocktail was added to all columns to increase bacterial growth and to aid biofilm establishment of *L. discophora SP-6* for seeded columns. The nutrient cocktail ran until December 4th, 2014 (Figure 7). During this time a robust biofilm could be seen growing on all columns. The sand and anthracite column had near steady state removal for the period of August 15th to September 24th. An analysis of variance with multiple comparisons indicated that the sand and anthracite column had significant removal (Figure 8) and had significantly more removal than the glass (p-value: 0.0010) and Growstone (p-value: 0.0001) columns as well as all control columns, but was not significantly higher than the sand column (p-value: 0.1159) . The sand column also had removal from October 3rd to November 18th (Figure 9), but an analysis of variance indicated that it was not significant (p-value 0.99).

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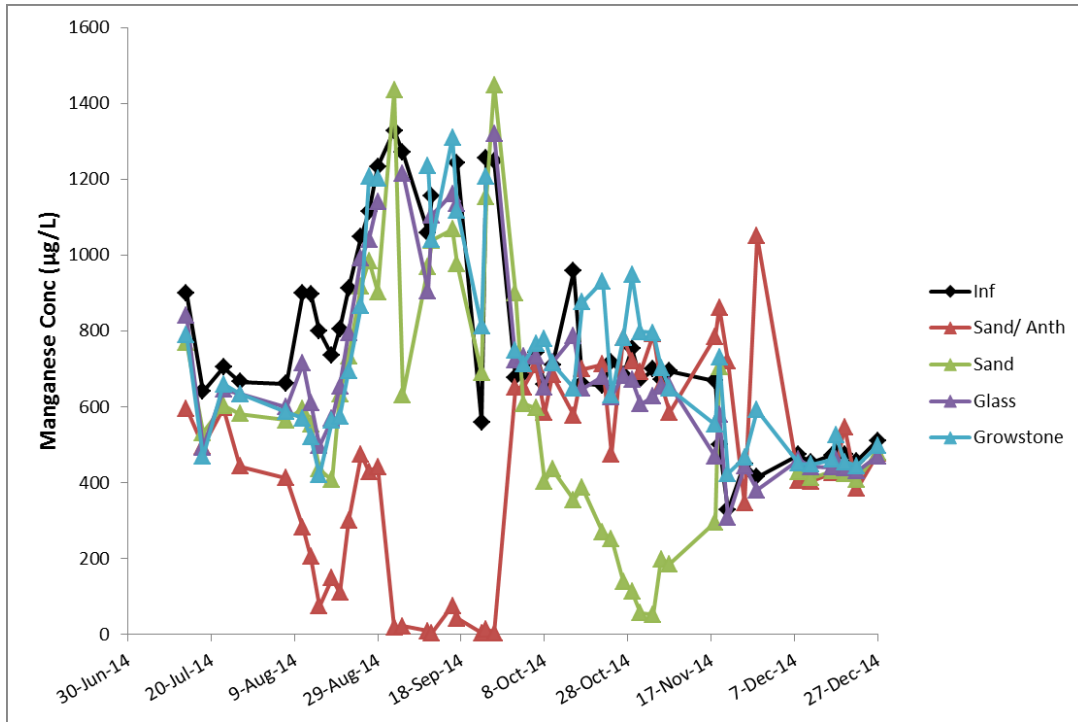
Figure 7- Manganese removal for columns seeded with *Leptothrix discophora SP-6*, July 14th –December 27th, 2014
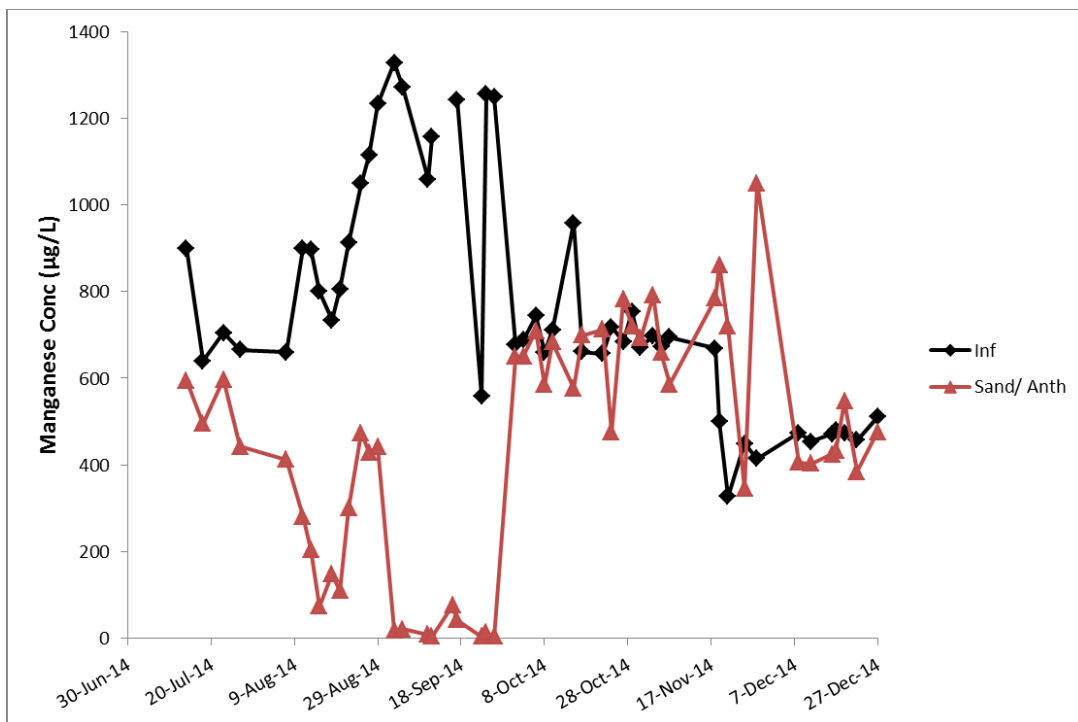
Figure 8-Manganese removal for sand/ anthracite column seeded with *Leptothrix discophora SP-6*, July 14th – December 27th, 2014
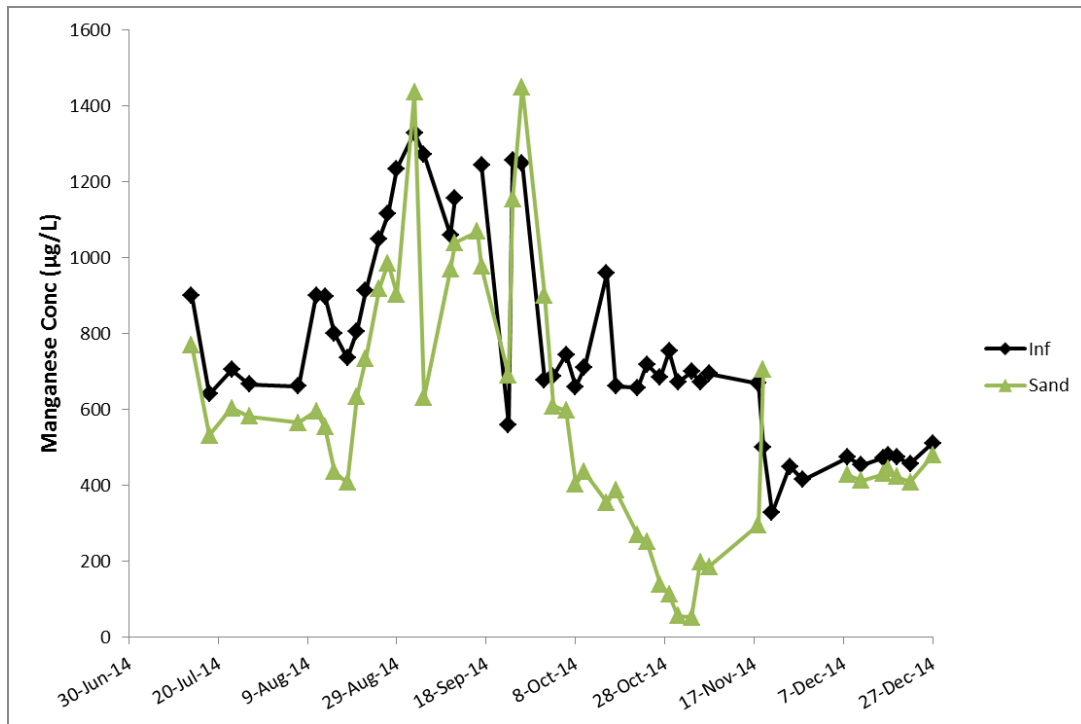
Figure 9-Manganese removal for sand column seeded with *Leptothrix discophora SP-6*, July 14th –December 27th, 2014

During November manganese removal began to decrease while biofilm growth increased to the point where filter fouling and clogging became a continuous problem. Backwashing frequencies were increased from once per week to twice per week in order to reduce the filter fouling but this had little effect. On December 4th, 2014 the nutrient cocktail was removed because it was no longer needed to aid biofilm establishment. Manganese removal still decreased after the nutrient cocktail was removed and the apparatus was shut down on December 27th, 2014. Unfortunately bacterial analysis on the biofilm could not be performed until the end of the experiment so the presence or absence of *L. discophora SP-6* or other manganese oxidising bacteria could not be confirmed during the experiment.

The pH ranged from 6.94 to 7.8 and the ORP was between 451 and 513 throughout the experiment. This is well within the range where MOB can oxidise manganese. It is possible that *L. discophora SP-6* did become established on the two columns where

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removal occurred but was out competed by heterotrophic bacteria by the time bacterial analysis was performed on the media.

Due to the small size of the filter bench-top set up it was impractical to measure DO during the experiment so it is not known if the DO was greater than the 3 mg/L recommended by Tekerlekopoulou et al. (2013) to maintain aerobic conditions throughout the filter bed.

After disinfecting the columns to inactivate any heterotrophic bacteria the apparatus was modified to the design seen in Figure 5 and the columns were seeded again on June 4th, 2015 and ran until August 20th, 2015 (Figure 10), at which time the experiment was ended. An analysis of variance with multiple comparisons was performed on the influent and effluent manganese concentrations for the period of June 4th to August 20th of all columns seeded with *L. discophora SP-6* and determined that there was no significant amount of manganese removal for any treatment (p value: 0.146).
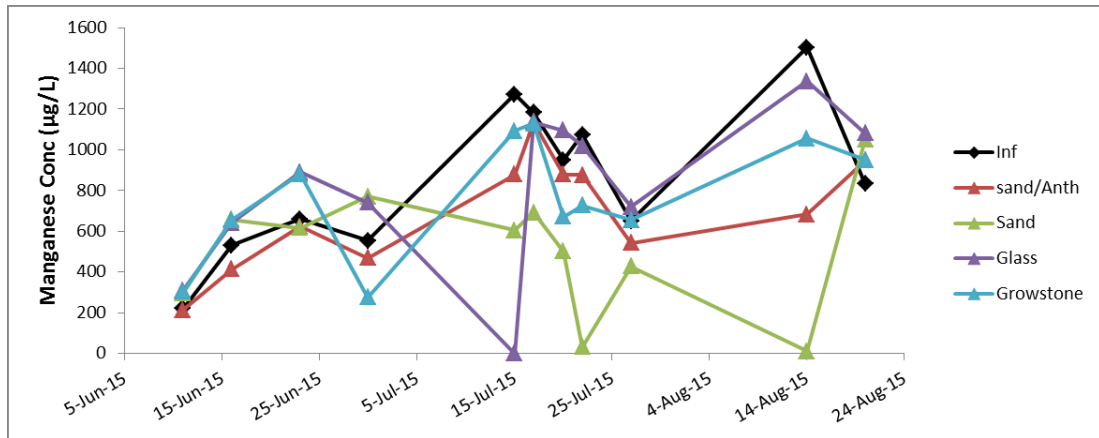
Figure 10-Manganese removal for columns seeded with *Leptothrix discophora SP-6*, June 11th- August 20th, 2015

3.3.2 Columns seeded with a native biofilm

Columns were first seeded on July 8th, 2014 and ran until Nov 7th, 2014 (Figure 11). An analysis of variance was performed on the influent and effluent manganese concentrations of all columns seeded with a native biofilm. There was no significant

difference between influent and effluent manganese concentrations for any treatment (p value: 0.5426).
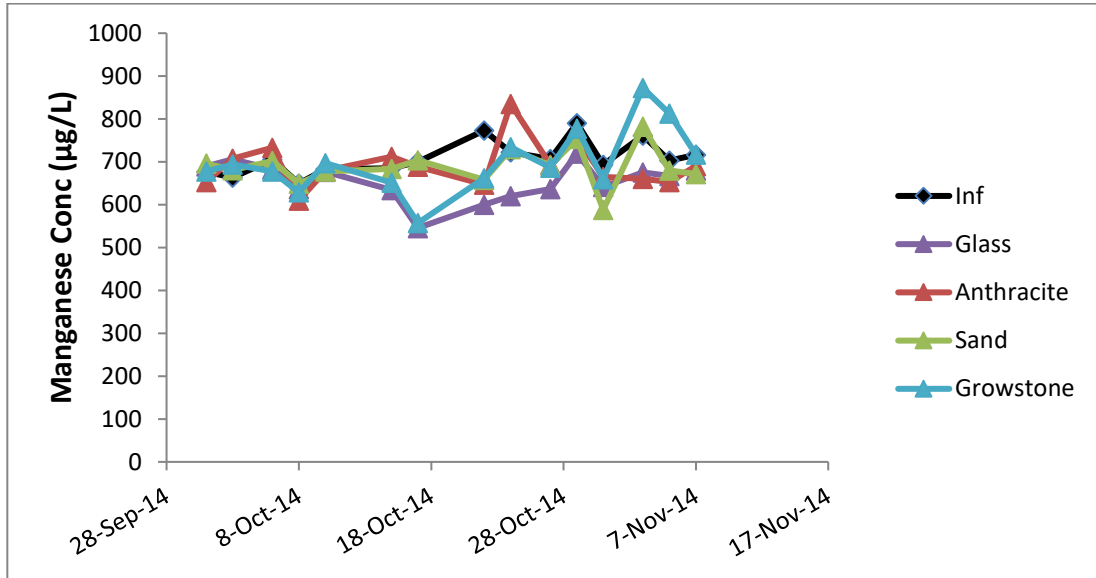
Figure 11- Manganese removal for columns seeded with a native biofilm for the period of October 1st, 2014 to November 7th, 2014
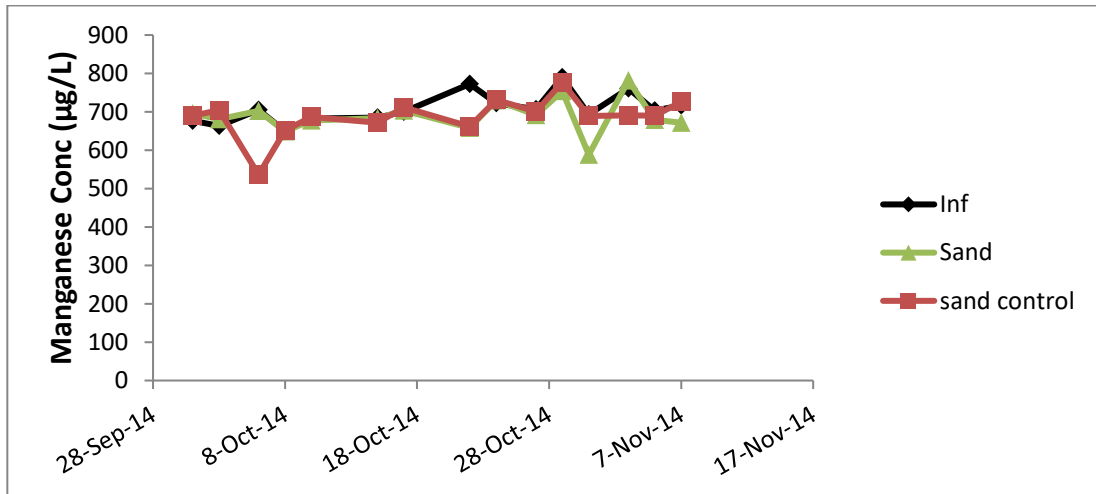
Figure 12- Manganese removal for columns using sand as media for the period of October 1st, 2014 to November 7th, 2014. The treated columns were seeded with a native biofilm while the control remained unseeded

Columns were seeded again on Feb 24th, 2015 and ran until May 15th, 2015. An analysis of variance was performed on the influent and effluent manganese concentrations of all columns seeded with a native biofilm and determined that there was no significant

amount of manganese removal for each treatment (p value: 0.746). There was also no significant difference between the control (unseeded columns) and the seeded columns (p value: 0.236).

3.3.3 Heterotrophic plate count results

Heterotrophic plate counts of biofilm from media samples were done after the final stage of the experiment (Table 6). An analysis of variance showed that there was no significant difference between column heterotrophic plate counts (p-value: 0.328). MOB plate counts were completed on the same plates but no manganese oxidising colonies were found on any of the 8 treatments.

Table 6- Bench-top heterotrophic plate counts

Column	Weight stomached (g)	Dilution factor	avg. HPC	CFU/g of media
Anthracite	1.1	10,000	1,104	1.E+07
Anthracite control	1.2	10,000	895	7.E+06
Sand	1	10,000	1,061	1.E+07
Sand control	1.1	10,000	968	9.E+06
Glass	1	10,000	1,253	1.E+07
Glass control	1.1	10,000	1,020	9.E+06
Growstone	1.2	10,000	1,312	1.E+07
Growstone control	1.1	10,000	408	4.E+06

3.3.4 16S rDNA sequencing for community analysis

The operational taxonomic unit (OTU) number for *L. discophora SP-6* was obtained and compared to the 16s rDNA sequencing results. *L. discophora SP-6* was not present in any of the biofilm samples for this experiment.

A rarefaction curve comparing observed OTUs for the different media types found that there was no significant difference between media types (Figure 13). A rarefaction curve comparing OUTs for the columns seeded with *L. discophora SP-6* found that there was no significant difference between the seeded columns or the control columns (Figure 14).
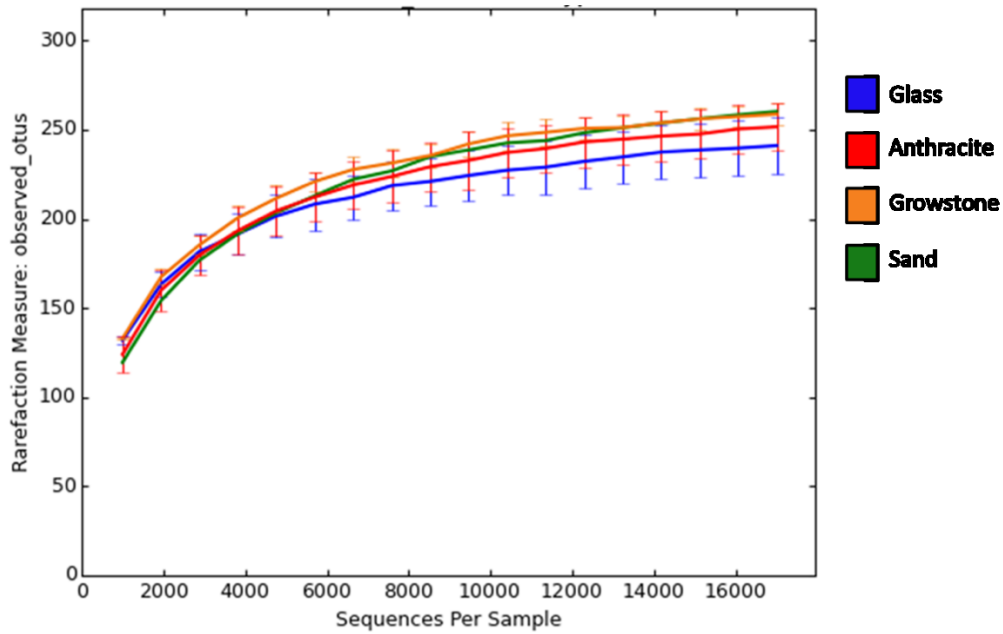
Figure 13- Rarefaction curve of media type for bench-scale media biofilm samples
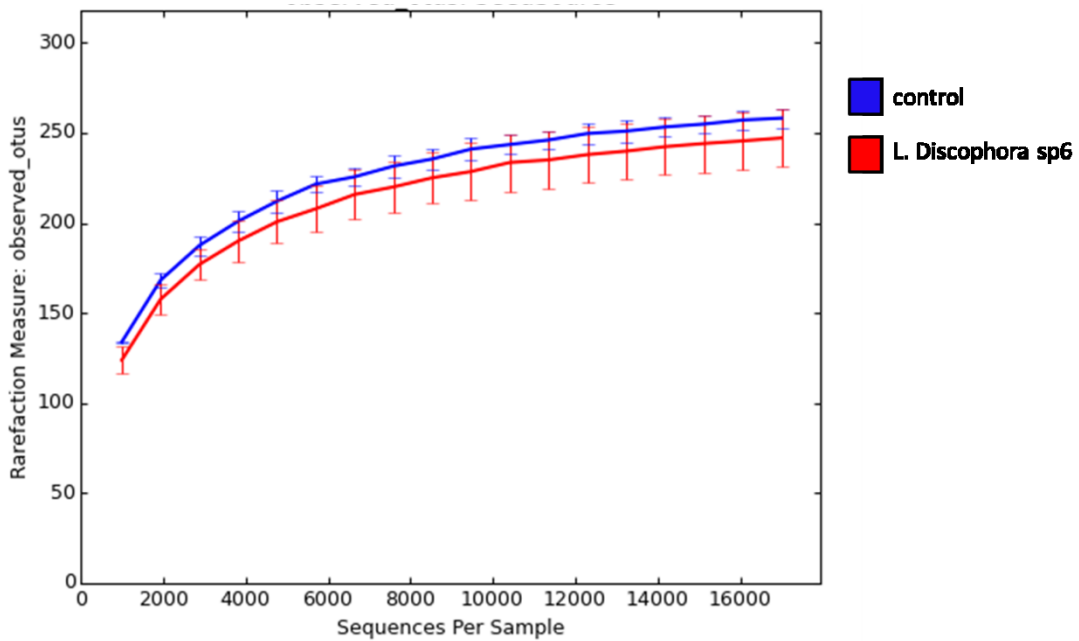
Figure 14- Rarefaction curve of seeded versus control columns for bench-scale media biofilm samples

16S rDNA sequencing indicated that the phylum Proteobacter made up the vast majority of bacteria in all columns and ranged between 52% and 76% of the total diversity in each column (Figure 15). A total of 22 different species made up 66.9% of total diversity in

all columns. The most common species was from the family Syntrophobacteraceae and made up 8.6% of the total population. All species within the family Syntrophobacteraceae are anaerobic. This is an indication that the dissolved oxygen concentrations may have been too low in the filter columns for manganese oxidising bacteria which are aerobic.
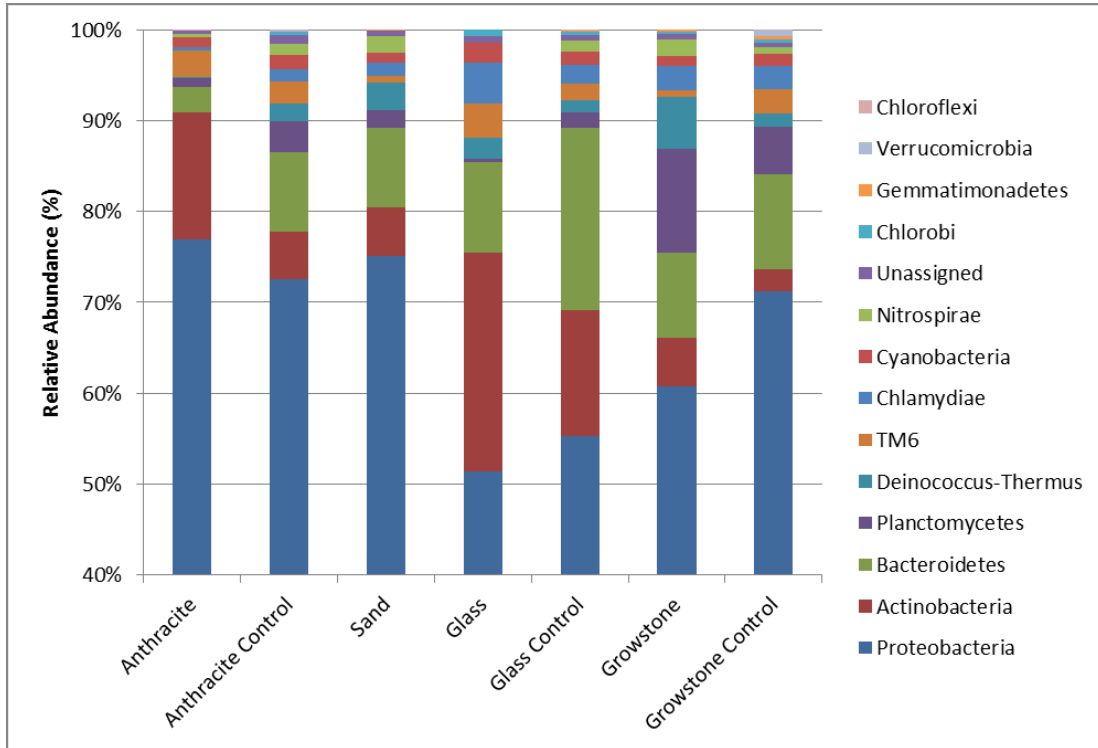
Figure 15- Relative abundance of bacterial phyla for benchtop filter media samples

3.4 Discussion

3.4.1 Columns seeded with *Leptothrix discophora SP-6*

Far less removal was seen than in other bench-top biofilter experiments. In Burger et al. (2008b), bench-scale biofiltration columns had removal from greater than 300 μg/L to below 100 μg/L at pH values of 6.5 and 7.5. During the third attempt at seeding the columns there was no significant removal. Hope & Bott (2004) demonstrated that seeding a recirculating biofilter with *L. discophora SP-6* could shorten maturation times and remove manganese levels as high as 4.7 mg/L.

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It is apparent that *L. discophora SP-6* was not able to become established on any media types during this stage of the experiment since MOB plate counts completed after the third seeding indicated that there were no MOB present. 16s rDNA sequencing confirmed this since *L. discophora SP-6* was not present in DNA samples taken from any filter. This was likely the case during the first seeding as well but it can not be verified since no bacterial analysis was conducted at that stage. It is possible that *L. discophora SP-6* was able to become established during nutrient addition but this could not be confirmed since samples for sequencing could only be taken at the end of the experiment.

Inspection of cultures plated during the seeding procedure under 100x magnification revealed that the *L. discophora SP-6* cultures had indeed retained their sheath-forming ability (Figure 16). This is important since bacteria from the genus *Leptothrix* rely on their sheath to attach to surfaces in their natural habitat (Emerson and Ghiorse, 1992; Emerson and Ghiorse, 1993), and they have been known to lose their sheath-forming ability during culturing. The sheath is thought to be important to manganese oxidation as well since oxidation is catalyzed by a protein that is localized in the sheath of environmental strains (Tebo et al., 2004), and it is also where the oxides that are formed are deposited (Tebo et al., 2004). Since the sheath-forming ability of Leptothrix and therefore its' ability to attach to media was retained it is thought that it was outcompeted by other bacteria or algae.
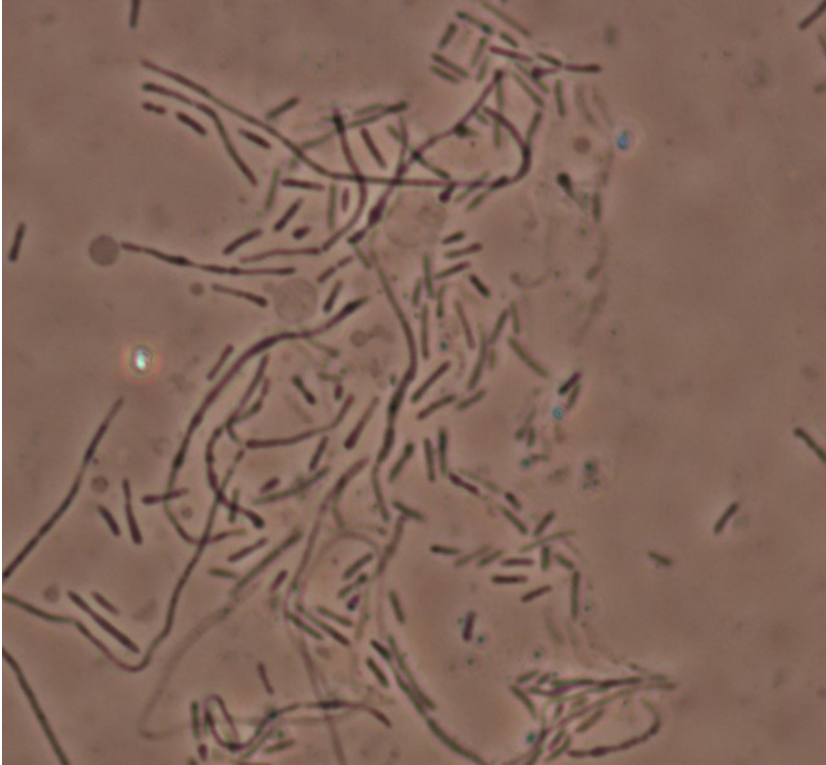
Figure 16- *Leptothrix discophora SP-6* **100x magnification**

On July 8th, 2014, the columns were seeded with *L. discophora SP-6* again and a nutrient cocktail was added. After a two week acclimation period the combination sand and anthracite column began to show significant removal for about two months after which time the removal returned to zero. At this point faster growing heterotrophic bacteria may have begun to out compete the manganese oxidising bacteria due to the nutrient cocktail addition. Bacterial composition of the biofilm was not analyzed at the end of this stage because the results were poor. This information may have given some insight into the mixed results.

The columns were seeded again on June 4th, 2015 after pumping chlorinated water through all columns to disinfect them and flushing them with chlorine free water. The apparatus design was modified by switching the flow of recycled effluent to a separate source of filtered, aerated water. Nutrient addition may have aided manganese oxidizing

bacteria becoming established in the columns since it was partially successful after the July 8th seeding.

Overall the only columns which had any removal were those which had sand as media (one had only sand and the other was a sand and anthracite combination). The best results were seen when nutrient addition was used to aid biofilm development but using the nutrient too long led to biofilter fouling and conditions which likely favored the growth of heterotrophic bacteria rather than manganese oxidising bacteria. It has been well documented that MOB comprise a small amount of total bacteria in manganese biofilters (Hoyland et al., 2014). Granger et al. (2014) found that MOB represented less than 1% of all bacteria during plate counts and may have been out competed by heterotrophs due to slow growth rate. Burger et al. (2008a) also found low numbers of MOB (zero to 10^4 CFU/g of media).

3.4.2   Columns seeded with a native biofilm

No significant removal was seen during this experiment. Each seeded column began with an established biofilm containing greater than 1×10^5 CFU/g of media. The amount of initial bacteria had no effect on column performance since there was no difference between seeded and control columns. It is likely that the biofilm of the seeded columns did not grow or even died off but it cannot be verified since bacterial analysis was not conducted at the end of the experiment.

Nutrient addition may have aided this experiment. Granger et al. (2014) found that bench-scale biofilters treated with 200 mg/L phosphorous had 91% removal of manganese at a pH of 6.

Some previous experiments which had consistent removal of manganese had a longer inoculation procedures (McKee et al., 2016) or used an established biofilm from a full-scale filter (Burger et al., 2008b). Either of these procedures could have created a more robust biofilm in the filter columns which would have aided manganese removal.

ORP was not thought to have been a limiting factor for removal. The minimum ORP for the columns seeded with *L. discophora SP-6* was 406 mV and the minimum ORP for the columns seeded with a native biofilm was 374 mV. This is well above the minimum suggested by Mouchet (1992) of 300 mV.

3.5 Conclusions

Manganese removal was only observed sporadically during these experiments. The combination of sand and anthracite seeded with *Leptothrix discophora SP-6* was the only treatment to show significant removal of manganese. It is unclear as to which media performed the best since removal rates were so low for most of the experiments and neither media supported more biological growth or a higher amount of manganese oxidising bacteria. It could not be proven that Growstone is effective as a media for biological filtration. No columns containing Growstone had effective treatment of manganese during these experiments.

L. discophora-SP-6 was not observed in the biofilm samples of any columns for this experiment, which indicates that conditions were not met for growth since each column received 1.39×10^9 cells of *L. discophora SP-6*. Since biofilm samples could not be taken during the experiment the absence of *L. discophora SP-6* could only be confirmed at the end of the experiment and not during periods where manganese removal was observed.

Chapter 4 Pilot-scale iron and manganese removal through biofiltration

4.1 Introduction

Iron and manganese are often found in reduced form in groundwater drawn from aquifers containing iron and manganese bearing minerals (Mouchet, 1992), and the removal of these minerals presents a large problem for municipalities encountering high levels of iron and manganese in a water supply. Traditionally manganese was a concern for drinking water due to laundry staining and causing taste and odour issues. Black water and laundry staining can occur at concentrations as low as 0.02 mg/L (MWH, 2005). More recent research has indicated that it has neurotoxic effects (Bouchard et al., 2011). Iron and manganese can also form precipitates in municipal distribution systems after being oxidized by residual chlorine, which build up in distribution mains and storage tanks.

The objectives of this chapter was to compare the ability of different types of filter media to support biological growth and investigate their effectiveness for biological iron and manganese removal at the pilot-scale level.

4.2 Methods

4.2.1 Metals analysis

All metals samples were analysed on an inductively coupled plasma mass spectrometer (Thermo Fisher XSeries 2 ICPMS). Samples were collected in polyethylene test tubes and preserved with two drops of nitric acid. Dilutions were performed with deionized water.

4.2.2 Culture of bacteria

All bacteria cultures were spread plated in triplicate according to section 9215 C in Standard methods for the Examination of Water and Wastewater (22ND edition) from serial dilutions and grown on R2A agar with 17 mg/L manganese added to allow for identification of manganese oxidising bacteria. Plates were incubated at 22°C for 5-7

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days in the dark. Plates with dilution factors producing between 30 and 300 colonies were counted. If more than one set of plates produced between 30 and 300 colonies the plate with the lower dilution was counted. If manganese oxidizing bacteria plate counts were needed the same plates were used and incubated in the dark for an additional 21 days at 22°C at which time the black and brown colonies were counted.

4.2.3   Description of pilot setup and location

A pilot system was installed at a well house that supplies a municipal distribution system for a town in Nova Scotia with a population of approximately 3,000 people. The pilot ran from August 2014 to July 2015. The well house is fed by three wells that all have high but varying levels of iron and manganese. This location was chosen because these three wells have the highest levels of iron and manganese and turbidity in the system and provide a worst-case scenario for treatment.

The pilot system consisted of a 5 μm polypropylene cartridge filter to remove turbidity followed by two stages of biofilters (Figure 17). The first stage biofilter consisted of a large pressure tank for iron removal and the second stage consisted of three smaller pressure tanks set up in parallel to allow for media comparison. The first stage biofilter was filled with 106.5 L of sand and each of the second stage biofilters were filled with 35.5L of sand, anthracite or Growstone. Raw water flow first passed through the cartridge filter followed by the first stage biofilter. After the first stage biofilter water was then pumped through a venturi which aerated the water in order to increase the ORP and maintain an aerobic environment in the system. A portion of the aerated water was then recycled back to the inlet of the first stage biofilter while the rest was diverted to the second stage biofilters. Flow through the venturi could be adjusted via a pressure reducing valve (PRV) on an opposing line if an increase or decrease in aeration was required. The ratio of raw water to recycled water entering the first stage biofilter was maintained at 1:1.
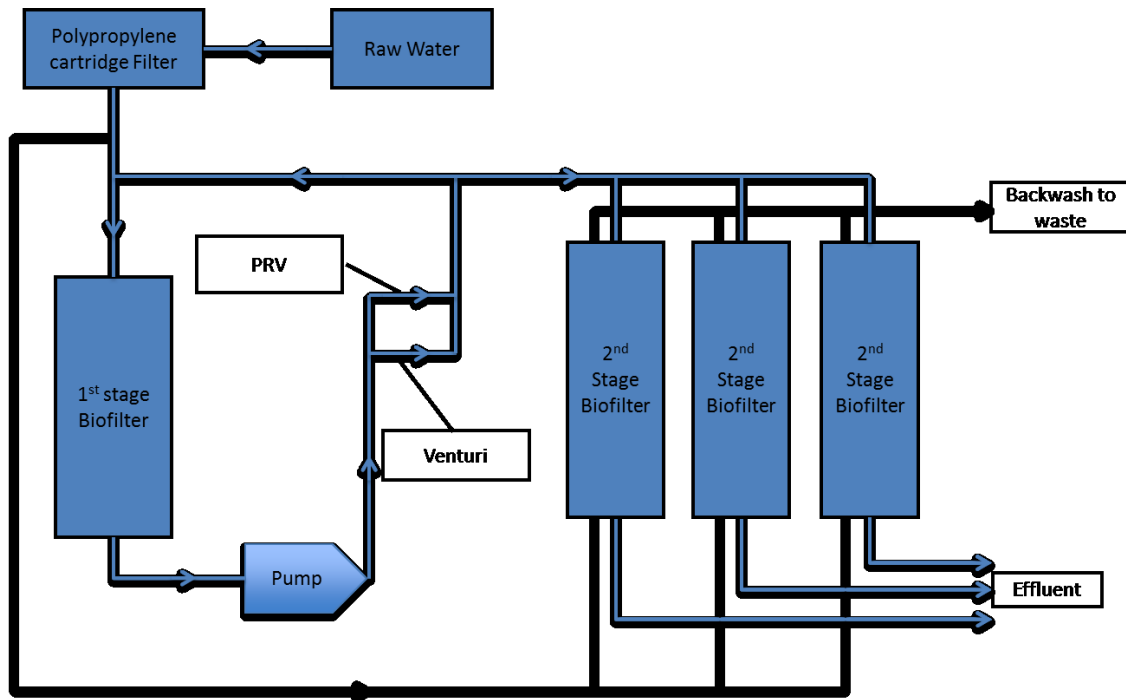
Figure 17- Pilot system Process Diagram

4.2.4 Pilot operation

A flow rate of 5.68 L/min plus a recycle rate of 5.68 L/min was used for the first stage biofilter, which gave an empty bed contact time (EBCT) of 9.4 minutes. A flow rate of 1.89 L/min was used for the second stage biofilters in order to maintain an empty bed contact time of 18.8 minutes in all three tanks. All biofilters were backwashed when the pressure differential rose above 10 psi, which was approximately every one to two weeks. Backwash flow rates were approximately 4.92 L/min. This was high enough for filter bed fluidization but low enough to prevent filter media from being washed out during the backwash.

Samples were collected before the cartridge filter, after the cartridge filter, after the first stage biofilter and after each of the second stage biofilters. Samples were prepared onsite for metals analysis using inductively coupled plasma mass spectrometry at the Centre for Water Resource Studies at Dalhousie University. Oxidation reduction potential (ORP) and pH were measured onsite using a Hanna Instruments HI991002 handheld pH/ORP meter, which was calibrated daily for pH using pH 4.00 and pH 7.00 buffers. The Hanna

Instruments HI991002 handheld pH/ORP meter is factory calibrated and can not be calibrated by the user but was verified weekly using Hach ORP standard.
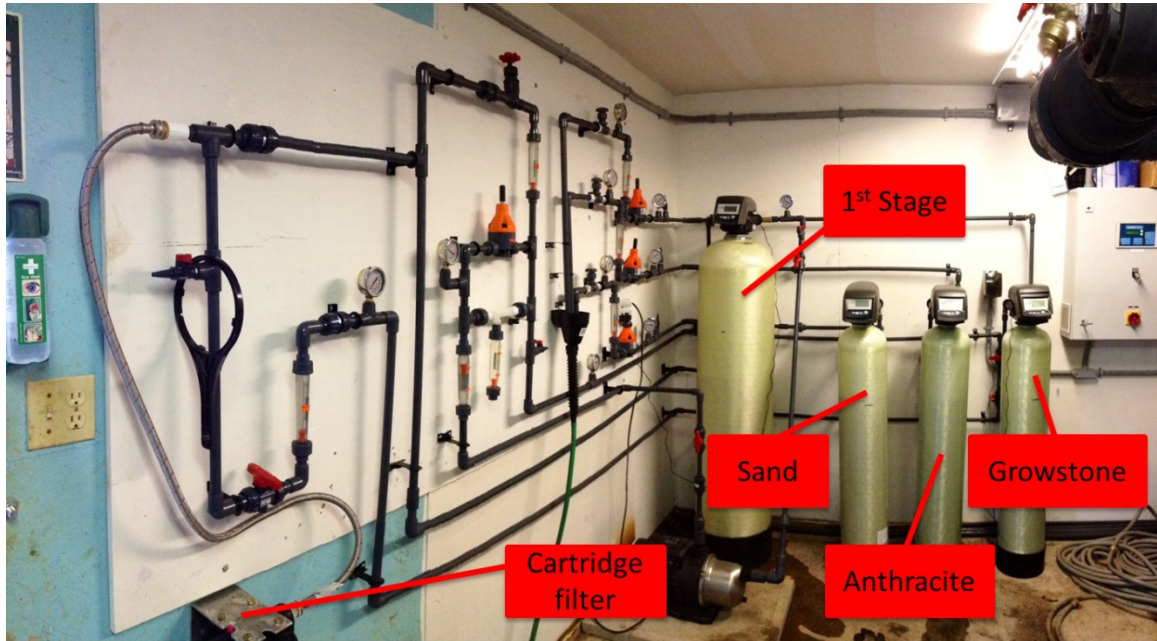
Figure 18- Picture of Pilot Setup

The operation of the pilot system is divided into 3 phases:

- Phase 1: Initial start-up, August 2nd, 2014- October 16th, 2014
- Phase 2: Low raw water iron and manganese levels, October 16th, 2014- January 25th, 2015
- Phase 3: Repaired anthracite filter bed, March 23rd, 2015- July 27th, 2015

During Phase 1 the pilot system was operated as described in section 4.2.1. During phase 2 Well #10 was shut down due to lower water demand. This was done in order to improve water quality since Well #10 produces high levels of iron and turbidity. Phase 2 was characterized by a large reduction in the levels of iron and manganese in the supply water to the pilot system. Turbidity was not measured at this time but a reduction in cartridge filter fouling was observed.

During Phases 1 and 2 particulate anthracite was noticed in the effluent of the anthracite filter, which was thought to be causing some high iron and manganese values. After Phase 2 was complete the pilot system was shut down in order for work to be done to the anthracite filter in order to eliminate this . On March 23rd, 2015, the downspout was replaced in the anthracite filter, the media was removed and washed in a sterile bucket and the underdrain depth was increased from 6.5 cm to 13 cm.

4.2.5 16S rDNA sequencing for community analysis

Media samples were collected from each filter by thoroughly mixing the top 15 cm of filter media and placing in an autoclaved sample bottle and capped. DNA was extracted using the Mo Bio Power Biofilm DNA Isolation kit. The extracted samples were sent to the Integrated Microbiome Resource at Dalhousie University for 16s rDNA gene sequencing.

4.3 Results

4.3.1 Phase 1: Initial start-up, August 2nd, 2014- October 16th 2014

August 2nd -11th was an acclimation period for the three manganese filters during which little removal took place. During this time high levels of iron removal were seen in the first stage but iron oxidation rates are much faster than manganese (Katsoyiannis & Zouboulis, 2004) and the process of manganese oxidation is somewhat more complex (Mouchet, 1992).

Overall the first stage biofilter had about a 34.8% removal rate for iron (Figure 19). A total of 12 samples were taken after the acclimation period. The sand filter had the highest iron and manganese removals at 91.9% and 84.3% respectively (Figure 20). The sand filter met guidelines 100% of the time for iron and 83% of the time for manganese. The anthracite filter had an average of 9% removal for iron and 80.4% for manganese and met guidelines 83% of the time for iron and 41.6% of the time for manganese. The Growstone filter had average removal rates of 45.5% and 49.2% for iron and manganese respectively and met guidelines 91.6% of the time for iron and 33% of the time for manganese. Both stages were combined when calculating percent removal and the quality

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of the water entering the second stage filters was assumed to be the same for all three filters.

Figure 19-Pilot iron removal rates August 2nd, 2014 to October 15th, 2014
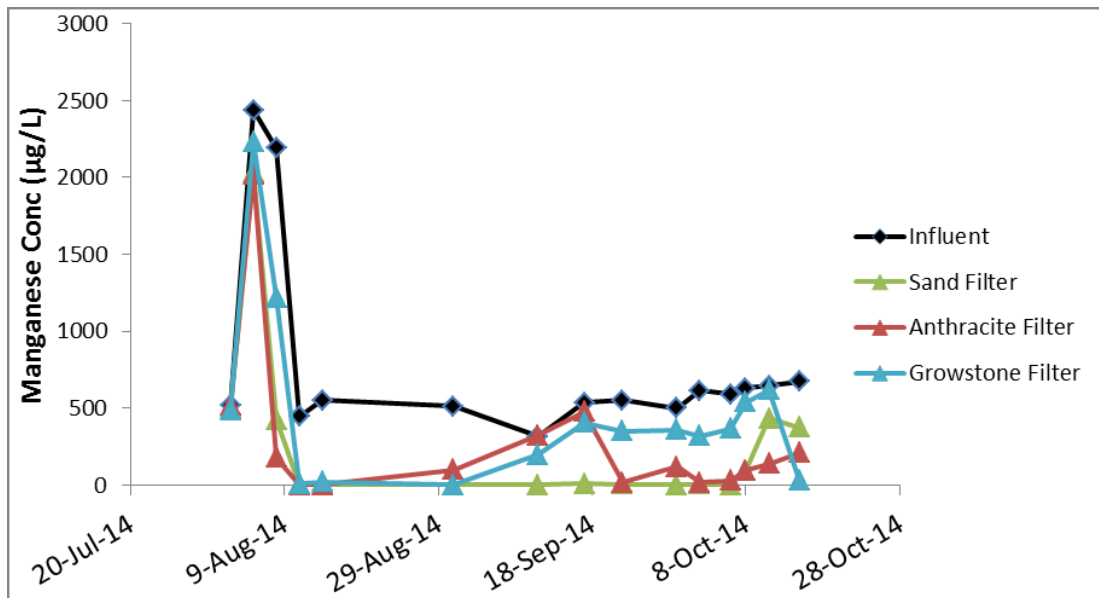
Figure 20-Pilot manganese removal rates August 2nd, 2014 to October 15th, 2014

During this stage the anthracite results were confounded by particulate anthracite in the effluent of the filter and it is difficult to draw accurate conclusions from the anthracite results.

4.3.2 Phase 2: Low raw water iron and manganese levels October 16th- January 25th

Raw water met both iron and manganese guidelines 100% of the time (14 out of 14 samples). Final iron and manganese concentrations were often higher than raw water concentrations (Figures 21 and 22). It is likely that low pH (less than 6.5) caused reducing conditions in the filters, which allowed manganese oxides formed on the media to revert back to soluble form and pass through the filter (Tebo et al., 2005; Raveendran et al., 2001; Abu Hasan et al., 2012)

Figure 21- Pilot iron removal rates October 19th, 2014 to January 25th, 2015
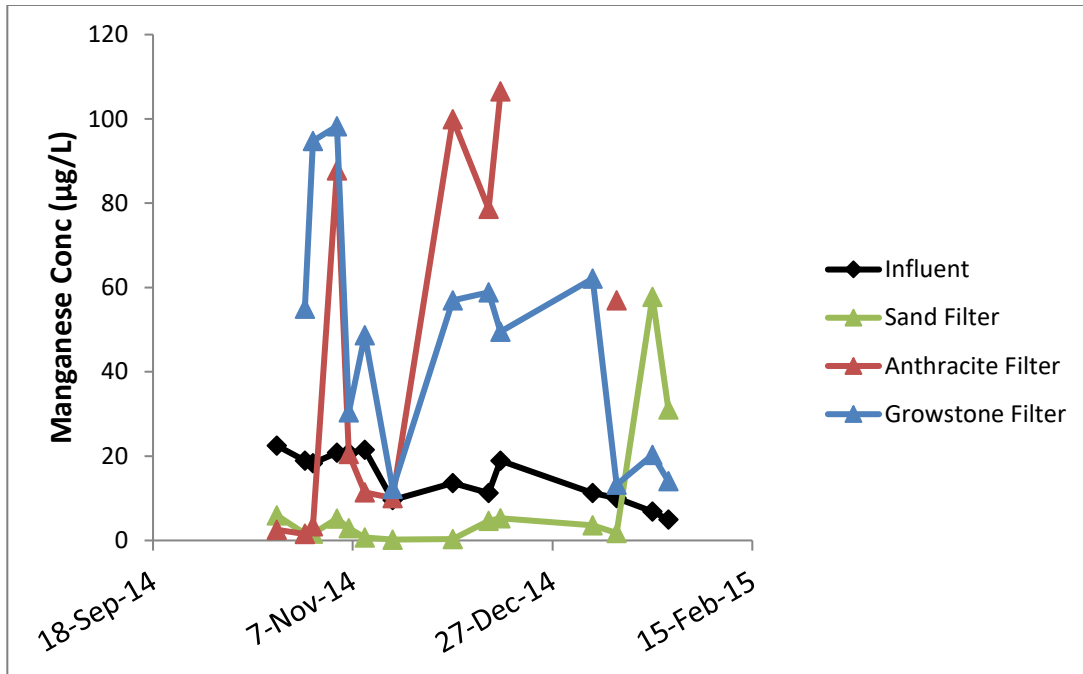
Figure 22- Pilot manganese removal rates October 19th, 2014 to January 25th, 2015

4.3.3   Phase 3: Repaired anthracite filter bed, March 23rd, 2015- July 27th, 2015

The period of April 3rd to May 7th was characterized by very high levels of iron and manganese removal but after May 7th raw water iron and manganese levels dropped considerably and effluent iron and manganese levels were often higher than influent levels (Figures 23 and 24). A total of 18 samples were taken and the sand filter had the best performance and met manganese guidelines 66% of the time with 87% removal, and met iron guidelines 100% of the time with 88% removal. The anthracite filter and Growstone filter iron levels were often higher than influent levels. The anthracite filter and Growstone filter had the same manganese levels as the 1st stage filter prior to May 7th, which indicates no removal taking place in the anthracite and Growstone filters. After May 7th these two filters were often higher than the first stage filter. Only the sand filter had lower iron levels than the influent but a t-test showed it was not significantly lower (p-value: 0.0576). An ANOVA with multiple comparisons test with Bonferroni correction showed that the sand filter had significantly lower manganese levels than both the anthracite and Growstone filters (p-values 0.0101 and 0.0102 respectively) and that there was no significant difference between the anthracite and Growstone manganese

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removal (p-value 0.999). Both stages were combined when calculating percent removal and the quality of the water entering the second stage filters was assumed to be the same for all three filters.
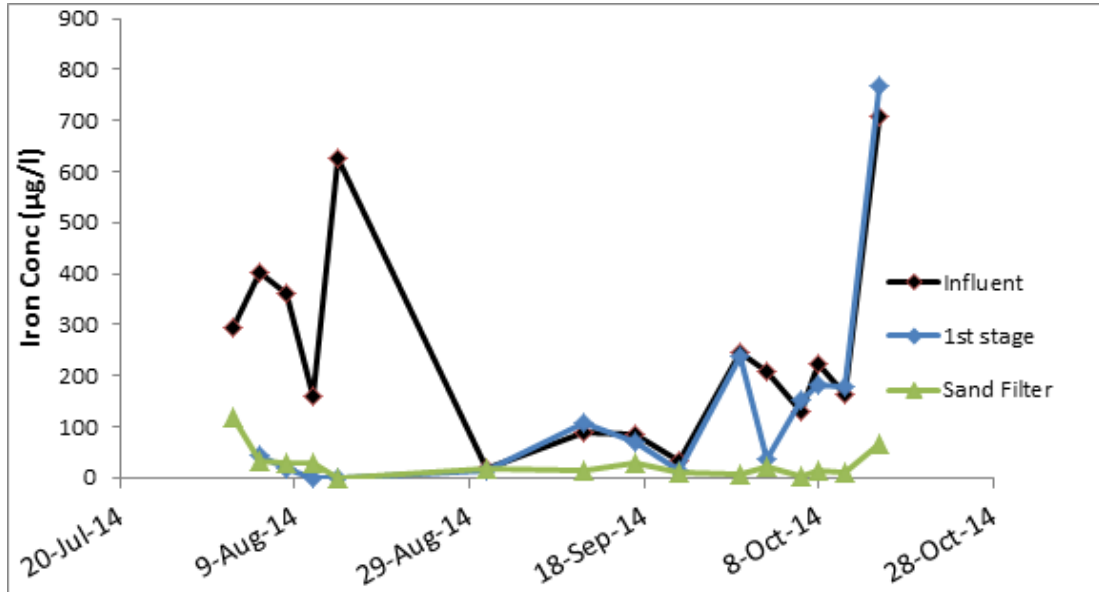

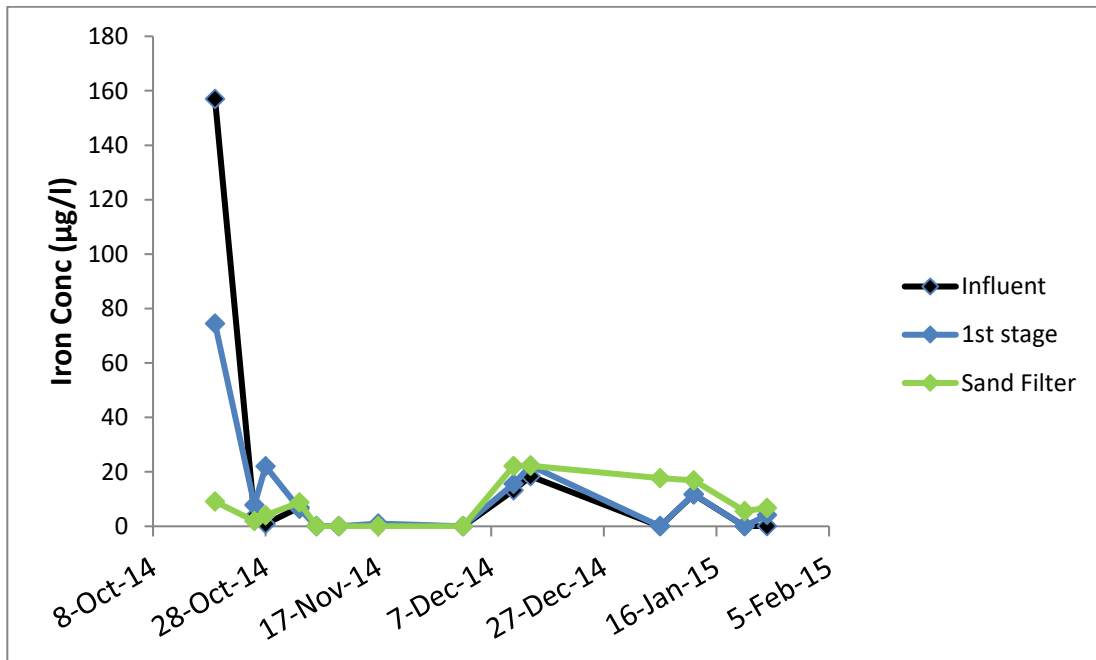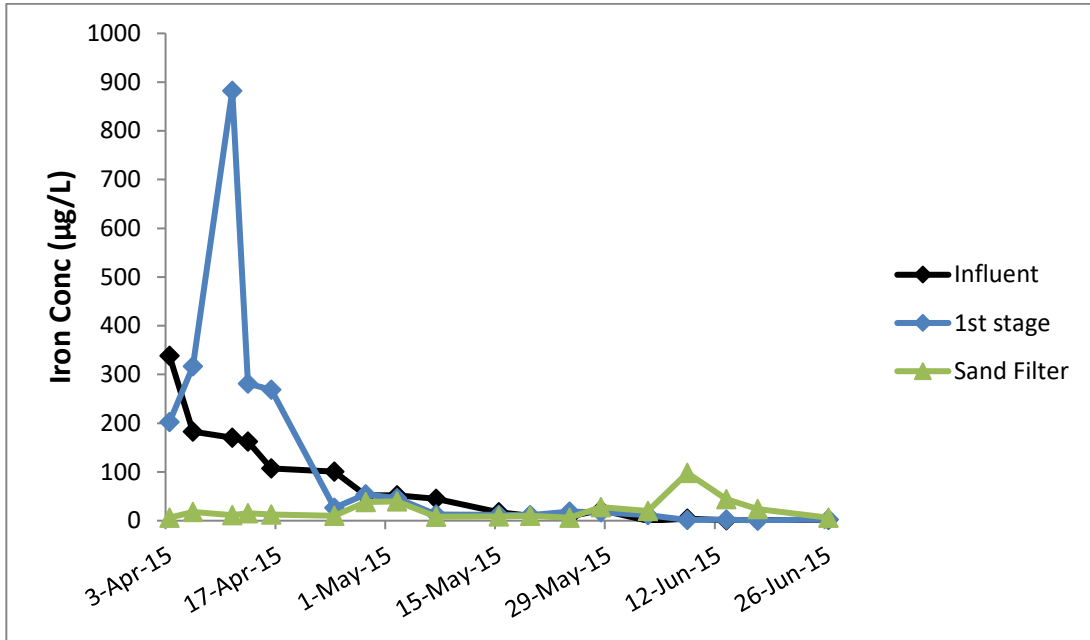**Figure 23- Pilot Iron removal rates April 3rd, 2015 to June 30th, 2015**
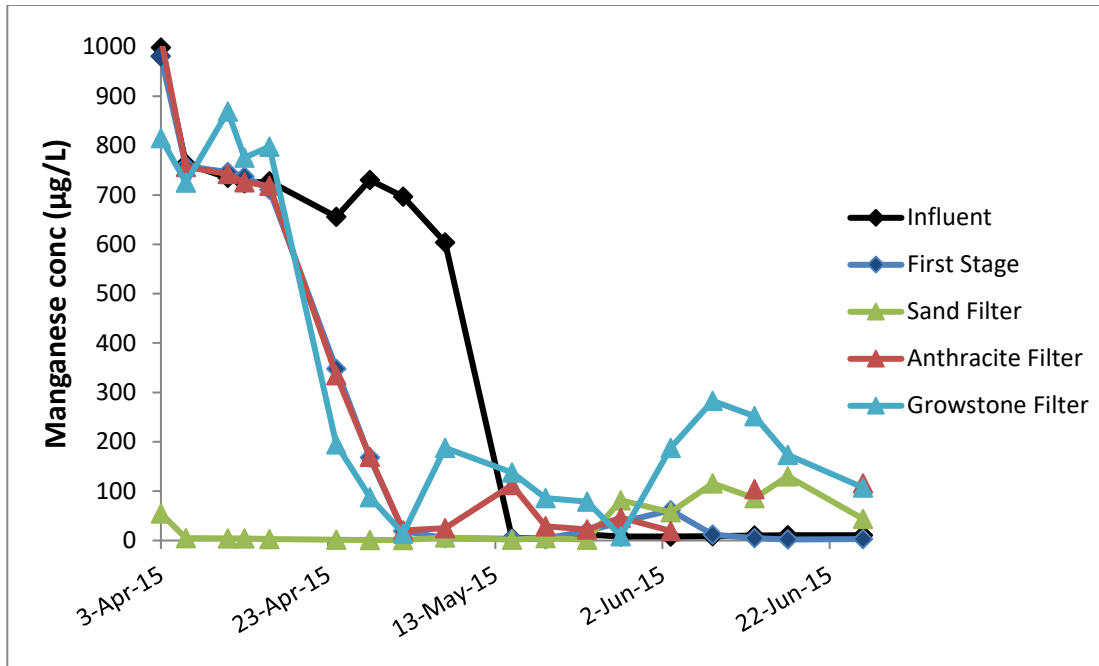
Figure 24- Pilot Manganese removal rates April 3rd, 2015 to June 30th, 2015

4.3.4   pH and oxidation reduction potential

The ORP ranged between 112 and 368 mV and averaged 210 mV. The pH ranged between 6.3 and 7.1 and averaged 6.39 (Figure 25).
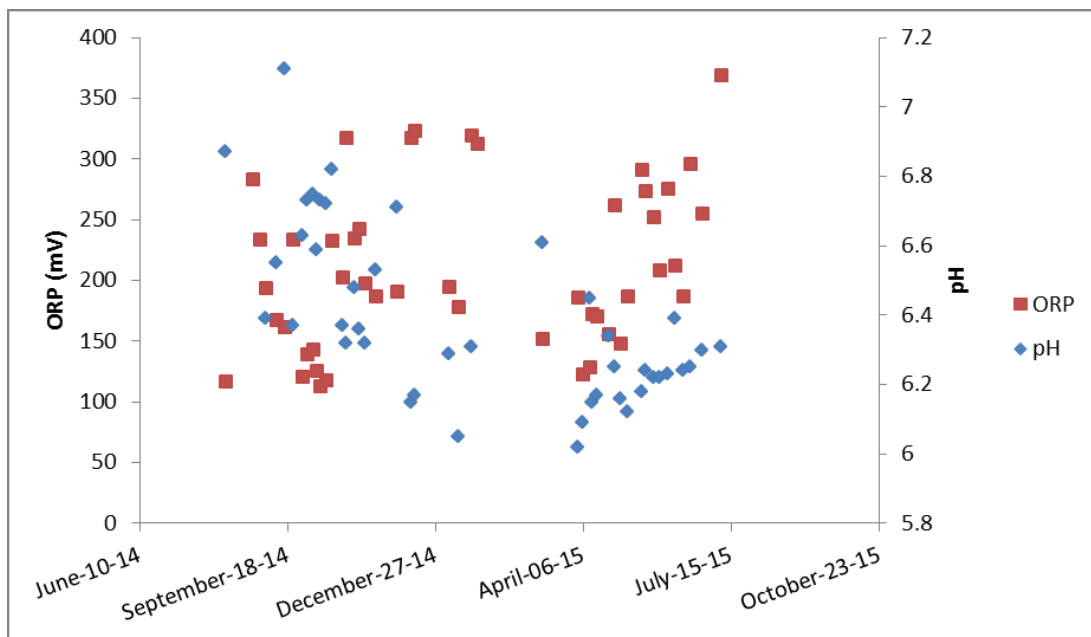
Figure 25- Pilot pH and oxidation reduction potential (ORP) range

4.3.5 Biological results

Spread plating indicated that sand from the first stage biofilter supported the most growth of both heterotrophic bacteria and manganese oxidising bacteria. The sand filter supported the most growth of the second stage biofilters for both HPC and MOB (Table 7). An analysis of variance with a Bonferroni multiple comparisons test showed that the plate counts for the first stage biofilter were significantly higher than the anthracite and Growstone filters for both MOB and HPC and that the plate counts for the sand filter were significantly higher than the Growstone filter but not the anthracite filter. The first stage and sand biofilters also showed thicker biofilm than the anthracite and Growstone filters on SEM images of filter media taken after the experiment (Figure 26)

Table 7- Pilot hetertrophic and manganese oxidising bacteria plate counts

Column	Weight stomached (g)	Dilution factor	avg. HPC	avg. MOB plate count	CFU/g of media	MOB/g of media
1st Stage (sand)	2	10,000	7.33	4.67	36667	23333
sand	2	10,000	5.00	4.00	25000	20000
anthracite	2.1	10,000	2.33	1.33	11111	6349
Growstone	2.1	10,000	1.33	0.33	6349	1587
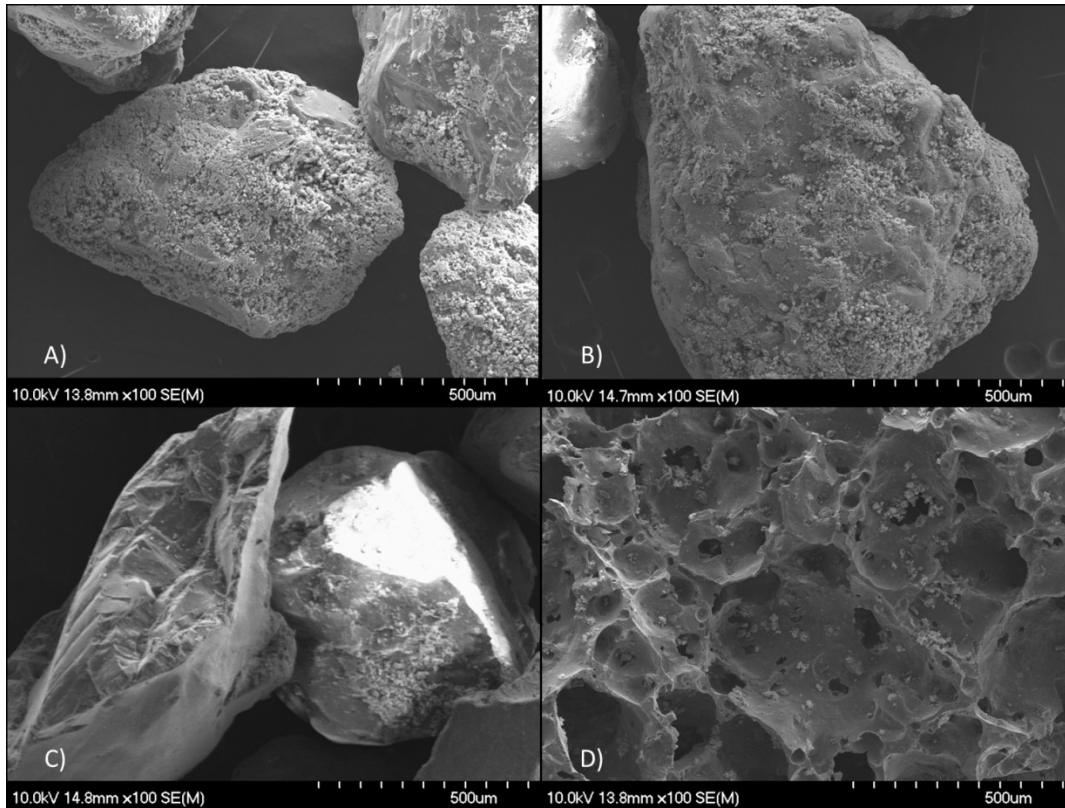
Figure 26- SEM Images of Pilot Biofilter media from: A) first stage; B) sand; C) anthracite and D) Growstone biofilters

4.3.6   16S rDNA sequencing for community analysis

16s rDNA sequencing indicated that the species composition (species diversity and relative abundance of each species) of the first stage filter and the sand filter were more similar than the three second stage filters. This is shown in Figure 27, a beta diversity plot of biofilter media samples. The samples taken from the sand filters were also more closely related to each other than the samples taken from the anthracite or Growstone filters were to each other, which showed a more uniform population. Rarefaction curves for pilot media biofilm indicated that neither media had significantly higher number of OTU per sample (Figure 28), and therefore neither media had greater biodiversity.
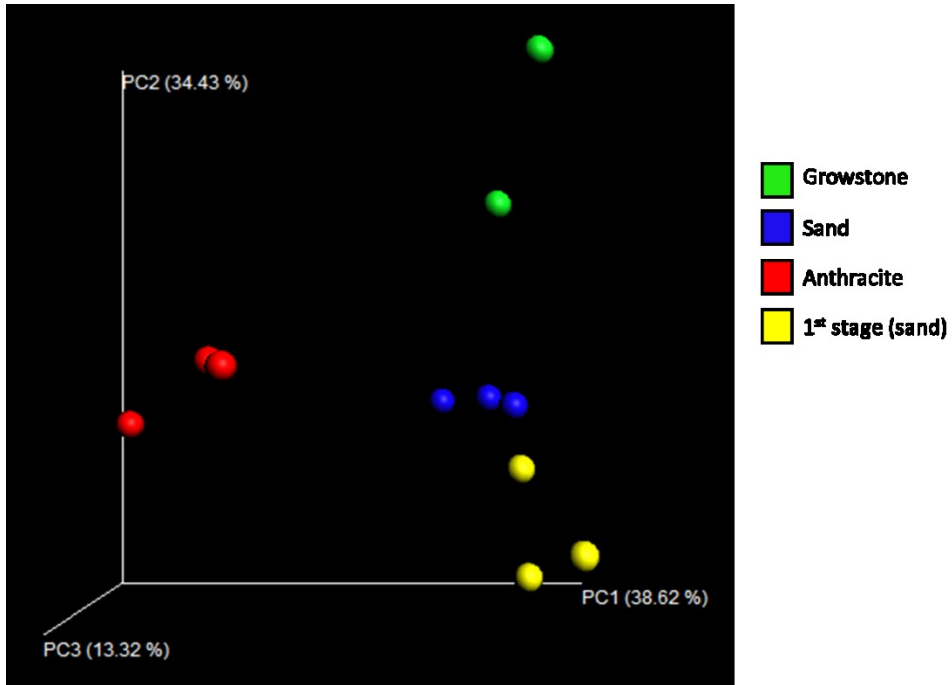
Figure 27- 16S rDNA sequencing Beta diversity plot for Pilot media biofilm
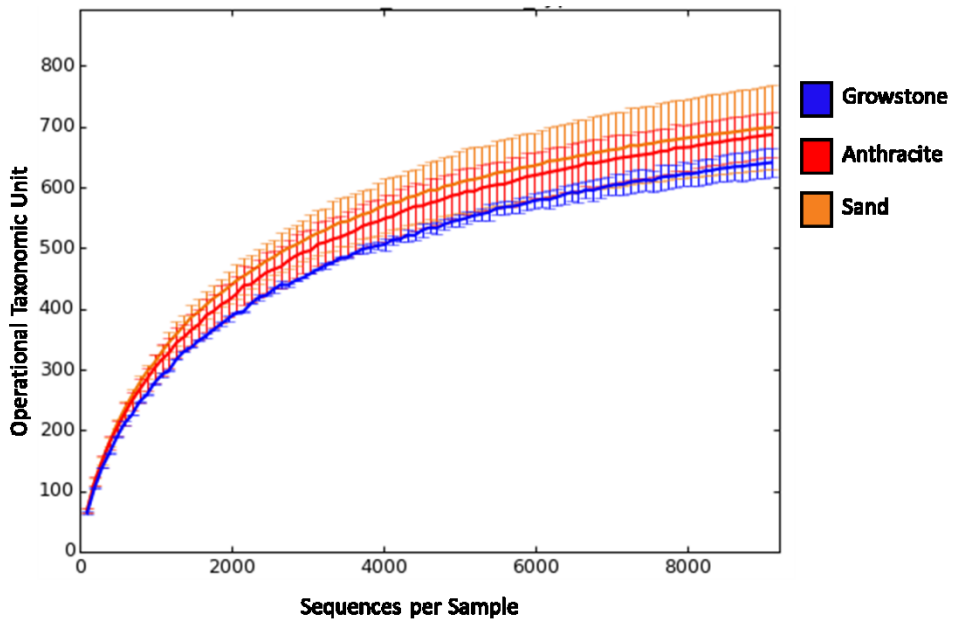
Figure 28- 16S rDNA sequencing Rarefaction curve for Pilot media biofilm

Phylum Proteobacter made up the vast majority at between 60% and 72% of bacteria in each filter. The 10 phyla shown in Figure 29 made up 95.5% of the bacteria in all filter samples together. They were: Proteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria, Chlamydiae, Nitrospirae, Gemmatimonadetes, Verrucomicrobia, Chloroflexi and WS3. There were another 19 phyla which comprised the remaining 4.5% of species diversity. The composition of phylum Proteobacteria was further broken down in Figure 30.
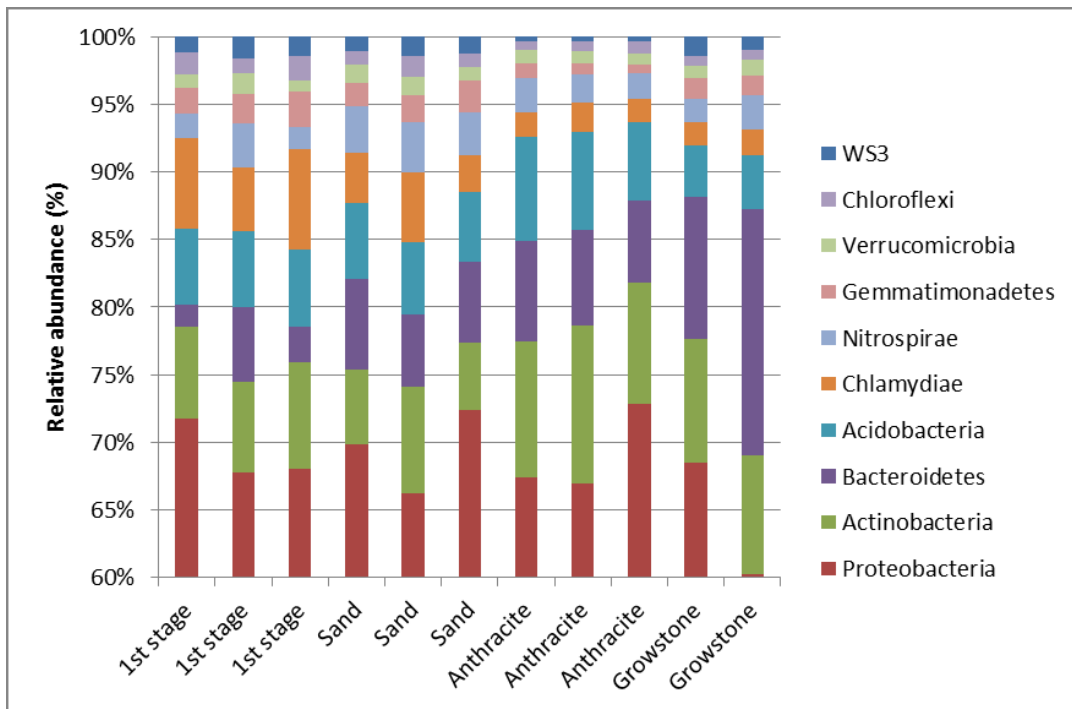
Figure 29- Relative abundance of bacterial phyla for pilot filter media samples
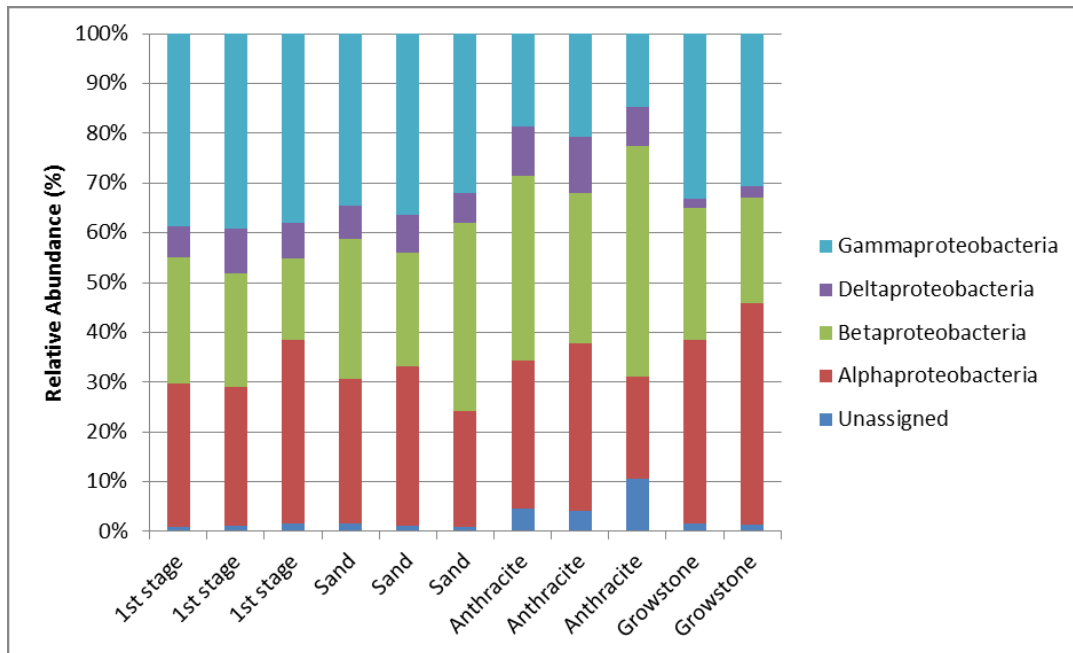
Figure 30- Relative abundance of phylum Proteobacter for pilot filter media samples

The genera *Bacillus, Pedomicrobium, Hyphomicrobium and Crenothrix*
All contain MOB and were found in all filter samples (Figure 31) with the exception of *Bacillus,* which was found only in the Growstone samples. No other species listed in Table 1 were found in any filter samples. MOB comprised about 4.0% of total bacteria in all samples. The sand filter samples had the highest relative abundance of MOB averaging 7.1%, followed by the Growstone filter (3.6%), and the 1st stage sand filter (2.4%). The anthracite filter samples had the lowest relative abundance of MOB with an average of 1.7%. On March 23rd, 2015 the anthracite filter was taken out of service and repaired. During this process the media was temporarily removed. Since MOB are slow growing this may have contributed to the low proportion of MOB in the filter.
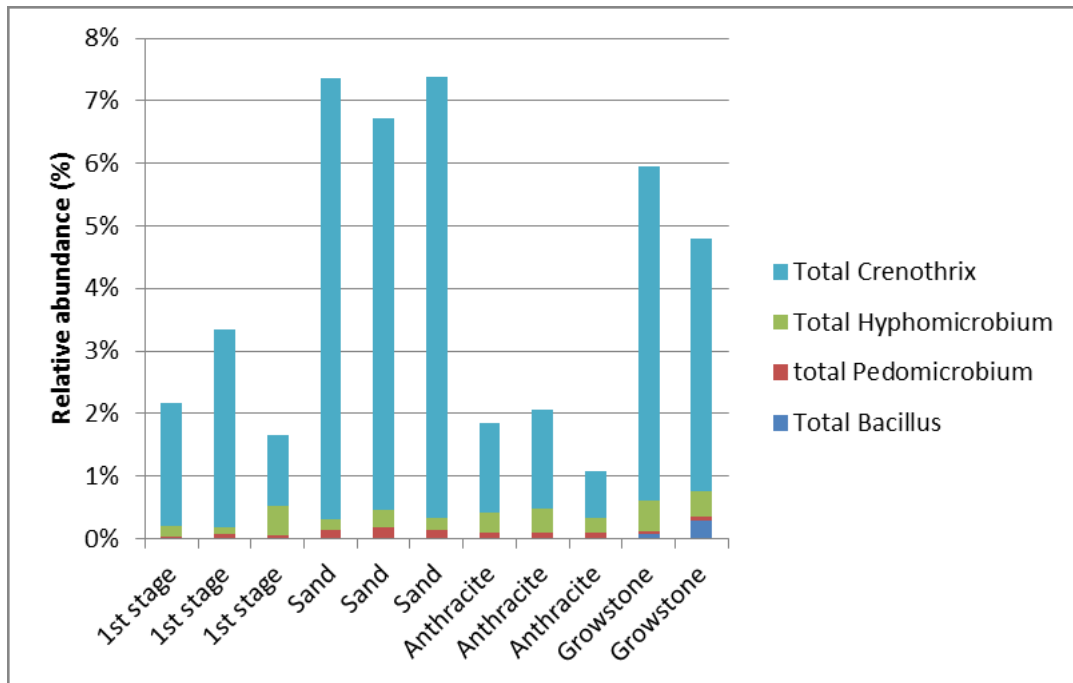
Figure 31- Relative abundance of manganese oxidising bacteria for pilot filter media samples

4.4 Discussion

Throughout the three phases the sand filter had higher removal rates and met guidelines more often for iron and manganese than the anthracite or Growstone filter. During the first phase the removal rate for iron was only 34.8% in the first stage which indicates that most of the iron removal was taking place during the second stage. Spread plates indicated that the sand filter also supported higher levels of growth of both heterotrophic bacteria and manganese oxidizing bacteria. The spread plates were further supported by SEM images, which showed greater amounts of biofilm on the sand filter and first stage filter.

The sand filter often had removal rates of greater than 90% for manganese but removal was not consistent throughout the entire experiment. Past research has shown that consistent manganese removal greater than 90% is possible in full-scale treatment plants (Burger et al., 2008a; Bruins et al., 2014).

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When raw water manganese levels were low removal rates were also low, having a Pearson correlation coefficient of 0.787 (p-value: 0.0001). High effluent levels of iron and manganese only occurred when raw water levels were very low (below CDWQ guidelines). The conditions may be favoring heterotrophic bacteria and allowing them to dominate the biofilm on the filter media. Unfortunately it was not possible to analyze the filter media until the end of the experiment so it was not possible to prove or disprove this theory through any biofilm population analysis.

The ORP range during this experiment was lower than the 300-400 mV recommended by Mouchet (1992), but more recent research has shown that biologic manganese oxidation can occur at values as low as 240 mV (Granger et al., 2014) but not as low as seen in this experiment. The ORP/pH range in this study is compared to previous biofiltration research in Figure 32. Because high rates of removal took place when the ORP was low a direct correlation between ORP and manganese removal could not be drawn. According to Raveendran et al. (2001), Mn(IV) that is deposited on the filter media can be reduced back to Mn(II) when dissolved oxygen levels are greater than 4 mg/L. DO levels ranged between 8-12 mg/L during phase 3 of the experiment so it is possible that the high levels of effluent manganese were caused by reduction of deposited manganese oxides. In a study of 4 full-scale manganese biofiltration plants, which all consistently obtained close to 100% removal, all had an ORP greater than 295 mVp and 3 had a pH greater than 7 (Burger et al., 2008a).
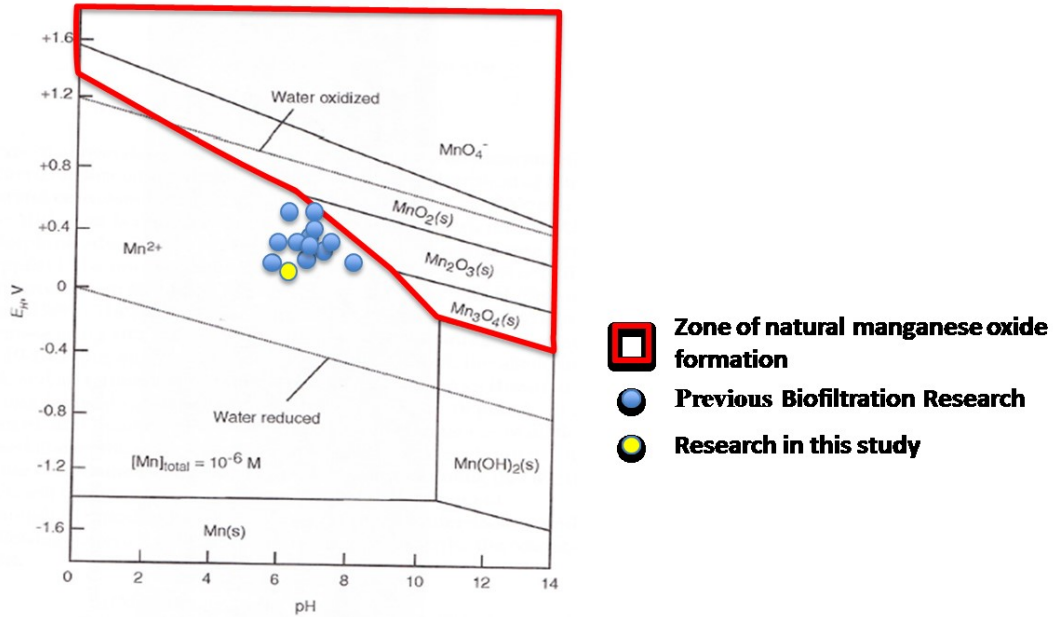
Figure 32- Forms of manganese in water as a function of redox potential vs pH at 25°C, (adapted from MWH, 2005)

Temperature and pH are also known to play a large role in the oxidation of iron and manganese during biofiltration. In a review of ammonium, iron and manganese removal by biofiltration units Tekerlekopoulou et al. (2013) states that raw water temperatures in the range of 10-30°C are suitable for the growth of iron and manganese oxidising bacteria. Hoyland et al. (2014) found that manganese removal rates were as low as 50% when water temperatures dropped below 10°C. Although temperatures were often below 10°C and even as low as 6.4°C, it was found that there was no significant correlation between temperature and manganese removal with a Pearson correlation coefficient of 0.102 (p-value: 0.5907).

The pH ranged between 6.3 and 7.1. Bruins et al. (2014) found that a pH of 7.1 was the lower limit of the range where removal could be achieved, but other research has shown that removal can take place at a pH as low as 6.5 (Burger et al., 2008a; Burger et al., 2008b; Granger et al., 2014). There was no significant correlation between sand filter removal and pH (Pearson correlation coefficient of 0.151, p- value 0.3859), but pH adjustment could be trialed to see if removal rates could be improved. High levels of

manganese in the effluent could be from reduction of deposited MnOx to Mn(II) (Tebo et al., 2005). Hasan et al. (2012) also found this to be the case in their research, (Raveendran et al., 2001). Poor removal could be caused by a combination of low temperatures and barely meeting the minimum threshold for ORP.

4.4.1   Kinetic removal rates

Kinetics are important because it can provide information on the EBCT needed for biological Mn oxidation (Cai et al., 2015). Kinetic removal rates were calculated for the second stage sand filter because results from the anthracite and Growstone filters were not consistent enough to calculate accurate values. Calculated k values ranged from 0.29 min^{-1} to 0.09 min^{-1} and the half-life for manganese removal in the second stage sand filter was between 2.14 min to 7.59 min for removal rates greater than 80%. This is consistent with values found in the literature. Katsoyiannis & Zouboulis (2004), found that biologically mediated Mn removal had a half-life of 3.98 min and a k value of 0.174 min^{-1} when treating raw water manganese levels from 0.4 mg/L down to 0.05 mg/L. Katsoyiannis had slightly more favorable conditions for biological oxidation (ORP of 330-340mv, pH 7.2, DO 3.8) and slightly lower and more consistent raw water manganese concentrations. Cai et al. (2015) used lab scale biofilters for simultaneous removal of iron manganese and ammonia and found that k values ranged from 0.147 min^{-1} to 0.153 min^{-1} and half-life values ranged from 4.69 min to 4.56 min.

This experiment demonstrated the effectiveness of biofiltration in simultaneously reducing iron levels from above 200µg/L to below 50 µg/L and manganese levels from above 700 µg/L to below 50 µg/L in a two stage process. Simultaneous removal of both iron and manganese often took place during the first stage of the treatment process, which has been observed in other experiments (Abu Hasan et al., 2012; Tekerlekopoulou & Vayenas, 2008). This simplifies the treatment process and could reduce costs for a full-scale biofiltration system (Li et al., 2005). Sand had higher iron and manganese removal rates and met guidelines more often than anthracite and Growstone. Sand also supported the highest levels of both heterotrophic bacteria and manganese oxidising bacteria.

DNA sequencing showed that the first stage filter and the second stage sand filter had more closely related populations than the three second stage filters, which indicates that the type of media can be more important to bacterial growth than treatment stage. The anthracite filter samples had the lowest amount of MOB relative to the other filters but did not run continuously over the duration of the experiment as the other filters did which may have negatively impacted the growth of MOB. MOB comprised about 4% of total bacteria. This does not agree with heterotrophic and MOB plate counts in this research but is consistent with values obtained by Hoyland et al. (2014) and is slightly higher than previous research (Granger et al., 2014; Burger et al., 2008a) which found that MOB comprised less than 1% of HPC.

Crenothrix was the most abundant MOB in all filters. Some species of Crenothrix are also capable of oxidising methane (Stoecker et al., 2006) therefore, Crenothrix may be present at higher abundances than other MOB in pilot biofilters due to the presence of methane in the groundwater. Raw water was not sampled for methane during this study but it is a common concern in groundwater in this region of Nova Scotia. Without the presence of Crenothrix MOB in all biofilters would comprise less than 1% of HPC which would more closely agree with previous research (Granger et al., 2014; Burger et al., 2008a). Crenothrix was more common in the second stage sand and Growstone filters than in the first stage filter. Since the majority of manganese oxidation is taking place in the second stage it is likely that Crenothrix is having some impact on manganese oxidation.

In a review of over 100 groundwater treatment plants Bruins et al. (2014) found that a minimum empty bed contact time of 11.5 minutes was needed for high levels of manganese removal. Greater removal efficiency could have been achieved in the first stage by increasing the empty bed contact time above this minimum threshold. Unfortunately this research was published after the pilot was already in operation.

4.5 Conclusions

Biological filtration proved to be an effective treatment option for removal of iron and manganese for the municipality where it was piloted with removal rates above 90% for iron and above 80% for manganese for all periods where raw water concentrations were above respective guidelines. Pilot results also indicated that sand was more effective as media for biological filtration. Sand supported more biological growth and also supported a higher proportion of MOB (7.1%) than anthracite (1.6%) or Growstone (3.6%). Sand had higher removal efficiencies for iron and manganese than anthracite or Growstone. Sand filters supported 3.67×10^4 in the first stage filter and 2.50×10^4 CFU/g of media in the second stage filter while anthracite and recycled glass supported 1.11×10^4 and 6.35×10^3 CFU/g of media respectively. The sand filter had the highest removal rates which ranged from 88.0% to 91.9% for iron and 84.3% to 87% for manganese. CDWQG were met for 44 of 44 samples for iron and for 35 of 44 samples for manganese using sand biofiltration.

Chapter 5 Conclusions and Recommendations

5.1   Bench-scale manganese removal through biofiltration

Manganese removal was only observed sporadically during these experiments. The combination of sand and anthracite seeded with *Leptothrix discophora SP-6* was the only treatment to show significant removal of manganese. It is unclear as to which media performed the best since removal rates were so low for most of the experiments and neither media supported more biological growth or a higher amount of manganese oxidising bacteria. It could not be proven that Growstone is effective as a media for biological filtration. No columns containing Growstone had effective treatment of manganese during these experiments.

5.2   16S rDNA sequencing for community analysis

DNA sequencing showed that the pilot was able to support a much higher diversity of bacteria than the bench-top columns seeded with *L. discophora SP-6*. *L. discophora SP-6* was also not observed in the biofilm samples from any of the columns seeded with it, which indicates that conditions were not met for growth since each column received 1.39×10^9 cells of *L. discophora SP-6*.

DNA sequencing indicated that MOB accounted approximately 4.0% of total bacteria and that 10 phyla made up 95.5% of the bacteria in all filter samples. Sand supported the highest proportion of MOB.

5.3   Pilot-scale iron and manganese removal through biofiltration

Biological filtration proved to be an effective treatment option for removal of iron and manganese for the municipality where it was piloted. Canadian Drinking Water Quality Guidelines were met for 100% of samples for iron and 79.5% of samples for manganese. Removal rates were above 90% for iron and above 80% for manganese for all periods where raw water concentrations were above respective guidelines. Pilot results also indicated that sand was more effective as media for biological filtration. Sand supported

more biological growth and had higher removal efficiencies for iron and manganese than anthracite or Growstone.

5.4   Recommendations

16s rDNA sequencing of raw water being treated in the pilot-scale experiments could be used to compare the composition of the bacterial community of the filter media biofilm to that of the raw water being treated. This would make it possible to track how the composition of bacteria is changing during treatment and allow for further insight into how a healthy biofilm functions. Sequencing of filter media biofilm at different stages of the experiment would also demonstrate how or if the community is changing over time.

Piloting experiments using dual media filters with a lower portion of sand while different media is used for the top portion of the filter only. The lower portion of the filter would allow for more consistent filtration and stability while the top, bioactive portion of the filter could be changed. This would allow the researcher to more effectively compare media solely on the ability to support biological growth. This would be particularly effective for Growstone which had a tendency to compact at grain sizes more comparable to sand and anthracite but supported large populations of bacteria.

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