



## PILOT-SCALE BIOFILTRATION OF IRON- AND MANGANESE-CONTAMINATED GROUNDWATER AT LOW IN-SITU TEMPERATURES AT A WATER TREATMENT PLANT IN SASKATCHEWAN

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**Abstract:** Iron (Fe) and manganese (Mn) are common elements of concern in groundwater in the Canadian Prairies. Biological filtration that stimulates indigenous Fe- and Mn-oxidizing microorganisms that are naturally present in groundwater is often considered a cost-effective water treatment option. One of the challenging aspects of biological treatment is that low temperatures significantly hinder microbial metabolic activity. This study focuses on enhancing cold-adapted, indigenous microbial populations for Fe and Mn oxidation at the in situ low temperatures (8 °C) of a pilot-scale biofilter at the Langham water treatment plant in Saskatoon. The pilot-scale biofiltration system consists of two aerated biofilters connected in series, designed to remove Fe in Filter 1 and Mn in Filter 2. The growth of biofilms was promoted either on conventional plastic filter media or on anthracite. Rapid oxidization of iron occurred through both filters in one month (99% removal,  $p < 0.05$ ). After several months, Mn removal was successfully achieved in Filter 2 when it contained anthracite (97% removal,  $p < 0.05$ ). Scanning Electron Microscopy/Energy Dispersive X-ray Spectroscopy (SEM/EDS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analyses confirmed the removal of Fe and Mn to meet water quality criteria. Adsorption of Mn on anthracite, which was confirmed by an additional batch experiment, likely promoted the biological removal, bacterial immobilization, and/or physicochemical removal of Mn in Filter 2. Culture-dependent microbial assessments coupled with the leucoberbelin blue method indicated the presence of Mn-oxidizing bacteria in the biofiltration system.

### 1 INTRODUCTION

Manganese is a commonly occurring element found in soil and water and is the second most abundant transition metal on earth after iron. In the environment, manganese usually exists in its reduced state (Mn (II)) as oxides, carbonates, and silicates. In Canada, groundwater is used for many industrial and agricultural purposes and 30% of the population relies on it for domestic use. Iron and manganese are two important contaminants in Canadian groundwater (Health Canada 1979). If reduced manganese (Mn (II)) in aquifers is not removed at a treatment facility, it can be oxidized in the distribution system or in consumers' homes by residual disinfectants, bacteria, or household oxidants (such as bleach). This Mn can precipitate and cause black discoloration of water, and can cause scaling in pipes and fixtures. Manganese scaling in distribution systems could occur at concentrations as low as 0.02 mg/L (Sly et al. 1989, Bean 1974, Griffin 1960). Considering this, Sly et al. (1989) argued that the Environmental Protection Agency (EPA) drinking water guideline level (standard) for manganese should be lowered from 0.05 mg/L to 0.01 mg/L (Kohl and Medlar 2006). The EPA has set a non-enforceable Secondary Maximum Contaminant Level (SMCL) of 0.05 mg/L of Mn in drinking water to address the aesthetic issues. However, a target of 0.01-0.02 mg/L is considered more appropriate to minimize the potential risk for water discoloration and scaling (Sly et al. 1989) and to minimize the neurotoxic effects associated with consumption of manganese in drinking water (Kondakis et al. 1989).

The Mn concentration in groundwater and surface water generally ranges from 0.02 mg/L to 4 mg/L in Saskatchewan and it is influenced by seasonal fluctuations in geochemical conditions. The major sources of manganese contamination in aquifers are industrial wastes, mine tailings, and natural or anthropogenic



geochemical changes. Manganese-contaminated groundwater sources usually have high soluble Mn (II) concentrations year round; therefore, soluble Mn must first be oxidized for it to be precipitated and removed through standard filtration. Manganese is not easily oxidized by oxygen at neutral pHs; therefore, additional treatment is required to oxidize manganese in the aqueous phase.

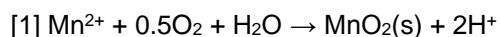
## 2 BACKGROUND

Manganese is not readily oxidized by oxygen, making it difficult to separate by physical means at neutral pH conditions in water treatment plants, and a specialized removal process is necessary to remove it from the aqueous phase. Biofiltration is considered a promising alternative approach to removing manganese from groundwater that can eliminate adverse effects associated with physical and chemical treatments. Biofiltration involves the biotic oxidation of manganese by microbial communities, which can enhance manganese oxidation in aqueous environments. The oxidized manganese solids are separated from the water using sand and/or gravel in the biofiltration unit. The table below reviews several common biofiltration systems by listing the mechanism associated with manganese oxidation in groundwater and the geochemical conditions required, including pH and oxidation reduction potential (ORP).

Table 1: Different methods for the treatment of manganese in groundwater (modified from Snyder, 2013).

Manganese treatment	pH	ORP	Mn removal (%)	Scale	References
Aeration, biofiltration and sand filtration	7.5-8.0	361-423	85-95	Pilot scale	Pacini et al. 2005
Biofiltration, polystyrene bead biofilter and sand filtration	7.2	340	90	Bench scale	Katsoyiannis and Zouboulis 2004
Aeration, biofiltration and sand filtration	>7.5	300-400	100	Pilot scale	Mouchet 1992
Biofiltration and sand filtration	7.2	N/A	60	Pilot scale	Qin et al. 2009
Biofiltration	6.5-7.5	295-368	100	Full scale	Burger et al. 2008

Mn-oxidizing bacteria are ubiquitous in nature and they take advantage of Mn (II) oxidation for such benefits as protection against the surrounding environment and having a stored electron acceptor for their respiration cycle (Edwards et al. 2003). Manganese oxidation is used in cellular functions such as energy derivation and possibly for chemo-litho-autotrophic growth (Boogerd and de Vrind 1987, de Vrind et al. 1986, Tebo et al. 1997). In optimal conditions, manganese-oxidizing bacteria oxidize reduced Mn (II) as:



Manganese-oxidizing bacteria in biofiltration units have been characterized to understand phylogenetic relationships and their ability to oxidize manganese, and these studies have concluded that the presence of manganese-oxidizing bacteria in biofilters can increase manganese removal from 25% (based solely on



chemical oxidation) to almost 100%, with combined physical, chemical and biological oxidation (Gouzinis et al. 1998).

### 3 RATIONALE AND OBJECTIVES

Currently, 42.8% of Saskatchewan's total population in urban, municipal and rural areas use groundwater for domestic purposes (Statistics Canada, 1996). Along with that, Saskatchewan is a national leader in growth associated with natural resource development and industrial activities. As a result, strong demands have risen from domestic and industrial sectors for clean water supplies. The groundwater supply at the Langham water treatment plant, Saskatoon, at times contains unacceptably high levels of iron (5 mg/L), manganese (1 mg/L) and humic substances. As a consequence, water at this site does not meet the drinking water standards without treatment. Currently, chlorination-based water treatment is employed at the site to meet the drinking requirements. To meet future requirements and to minimize the cost and adverse effects associated with chemical oxidation, a pilot scale biological filtration unit has recently been designed, constructed, and operated at the water treatment plant to accelerate the removal rate of iron and manganese. The biofiltration unit will be integrated into the existing water treatment plant once it is scaled up.

Removing iron by physical/biological treatment with aeration requires a pH greater than 7.2 and a redox potential of 0.2 V in the filtration unit, which is easy to achieve. However, manganese is very stable in natural conditions (pH: 7-8, and redox potential: -0.1 to +0.2 V). Oxidizing Mn requires a redox potential of 0.3 V or more unless chemical oxidants are provided. Since chemical oxidation is laborious and costly, biological oxidation, which is inexpensive, plays a crucial role in manganese oxidation in this pilot scale filtration unit. This unit is designed to enhance microbial oxidation. It contains filter media that provide more surface area for the biofilms to form on, and these biofilms can in turn oxidize the influent manganese/iron within the filtration unit. Sand and gravel are used as the supporting materials to prevent the flow of iron/manganese oxides through the effluent water. A challenge in designing this system is the low temperature of the groundwater source. The average annual groundwater temperatures typically range from 7 to 10 °C. It is known that cold temperatures hinder the metabolic activity of microbial populations and it can become problematic for enhancing microbial oxidation in the biofiltration unit.

In this study, we aim to design an effective biofiltration unit that can achieve the removal of manganese from the groundwater to meet drinking water standards at low temperature profiles. Another aim of this study is to identify the key rate-limiting factors for manganese oxidation in the biofiltration unit.

### 4 METHODOLOGY

#### 4.1 Biofiltration unit design and operation

A new biofiltration system was specifically designed for the removal of iron and manganese from the local groundwater supply. Based on laboratory experiments, a downward flow configuration of a two-stage pilot-scale biofilter was built at the Langham water treatment plant. This pilot has the capacity to treat water flow rates ranging from 3.78 to 11 L/min supplied directly from a groundwater source. The first filter is designed to remove iron biologically, whereas manganese is removed in the second filter by a biological process. In order to see biological activity and biofilm formation throughout the filter, the filters were made using a transparent polyvinyl chloride (PVC) column with a height of 1.5 m, a diameter of 0.3 m and an effective working volume of 50 liters. Two types of filter media were used: plastic media and granular anthracite. These were tested in the filtration unit to examine their comparative biological activity and Mn oxidation capabilities.

#### 4.2 Sampling

The water samples from untreated groundwater, effluent from Filter 1, effluent from Filter 2, and backwash (water and solids inside the filter are flushed back) from Filters 1 and 2 are collected in sterile 50 ml vials in several sets for the laboratory experiments and stored at -20 °C for future experiments. Immediate microbial assessment studies were conducted as soon as fresh samples were collected from the filtration unit. 2% (w/v) nitric acid is added to the fresh samples for the ICP-MS analyses.



### **4.3 Analytical methods**

#### **4.3.1 Groundwater chemistry**

The water chemistry (pH, alkalinity, hardness, major cations and anions, and metals) of samples of untreated water, effluent from Filter 1, effluent from Filter 2, backwash water from Filter 1 and backwash water from Filter 2 was characterized using ICP-MS (Saskatchewan Research Council). Changes in the iron and manganese concentrations were statistically compared using one-way ANOVA tests. We also performed preliminary geochemical speciation analyses to identify the manganese species present in the aqueous and solid phases using a geochemical modeling tool, PHREEQC (USGS).

#### **4.3.2 Scanning Electron Microscopy and Energy Dispersive Spectrometry (SEM/EDS) analyses**

SEM imaging was used to observe and characterize the biofilms formed on the plastic media surfaces in Filters 1 and 2 in the biofiltration treatment unit. The samples (coated substances from the plastic media) were prepared by air drying, were sliced using aseptic blades, fixed to aluminum stubs using carbon tape, and then carbon-coated for 5 minutes. The elemental composition of the samples was determined using an EDS-based X-ray detector on the SEM.

#### **4.3.3 Culture-dependent microbial assessment**

Assessing the presence of manganese-oxidizing oligotrophic bacterial populations is an important part of this study as they are the major constituents in biofiltration units expected to biologically oxidize Mn. Growth media was optimized for the growth of oligotrophic manganese-oxidizing bacteria from the groundwater system as described in Beukes and Schmidt (2012), Nealson (2006) and Tebo (2007).

Manganese-oxidizing bacterial consortia were enriched from Filters 1 and 2 by inoculating 1 mL of backwash water from each of the filters into 100 mL of mineral salt, sodium succinate, vitamin and phosphate (MSVP) media with 100  $\mu$ M of Mn (II) salt solution, and incubated at 18 °C while shaking at 100 rpm. These consortia were continuously enriched by adding 20 mL of fresh media every 7 days for one month until brown precipitates formed on the sides of the flasks. Manganese-oxidizing bacterial populations were isolated by inoculating the consortia developed from the filter backwash onto optimized agar plates, along with 100  $\mu$ L of sterile 100  $\mu$ M Mn (II) salt solution spread on the surface of the agar gel. The plates were incubated at 17 °C for a month until colony forming units were visible. Manganese oxidation by manganese-oxidizing bacteria was indicated by the appearance of a dark blue colour after adding 1 mL of 0.04% (w/v) leucoberberlin blue assay in 45 mM acetic acid to the bacterial culture/isolates and incubating for 15 minutes in the dark (Dick et al. 2008, Tebo 2007).

#### **4.3.4**

#### **4.3.5 Manganese adsorption studies for anthracite**

Anthracite is widely used in filtration systems for its hardness, durability and particle size. Anthracite has also shown the ability to adsorb cations in filtration units, including manganese, and can provide surface areas for microbial growth (Wang 2009). To understand the adsorption of manganese on anthracite, time dependent adsorption tests were performed. Three types of anthracite samples were prepared: (1) Abiotic samples, for which granular anthracite was sterilized by autoclaving, (2) Abiotic/sterile control samples, which were treated with UV irradiation for 30 minutes in a biosafety cabin, and (3) Fresh (untreated) anthracite samples, which were used to examine the behaviour of non-sterilized samples. A Mn (II) solution was added to the anthracite-water system, resulting in an initial Mn (II) concentration of 0.5 mg/L. The adsorption experiments were conducted on a shaker at 100 rpm at 22 °C. Mn concentrations were measured at 0, 0.25, 0.5, 1, 2, 4, and 8 hours, using a manganese colorimeter (Hach Method 8149; Range: 0.006 - 0.700 mg/l).



## 5 RESULTS AND DISCUSSION

### 5.1 Groundwater chemistry

The drinking water standards for iron and manganese are 0.3 mg/L and 0.01 mg/L, respectively (Kohl and Medlar 2006). Our ICP-MS analyses of the groundwater at the Langham site have shown high concentrations of iron (2.82 mg/L) and manganese (0.88 mg/L), which are both above drinking water standards. Table 2 explains the groundwater chemistry of the contaminated groundwater at the Langham water treatment plant.

The geochemical modeling (PHREEQC, USGS) of manganese speciation in the groundwater suggested that  $Mn^{2+}$  (aq) is the most abundant form of Mn, and Mn-oxides (solid) are limited in the raw groundwater samples. The groundwater pH was 7.9 and the redox potential was in the range of -0.2 to +0.1 V. The PHREEQC model indicated that a redox potential greater than +0.2 V is required in this groundwater to promote manganese oxidation and separation from the aqueous phase. Since it is hard to achieve a higher redox potential under natural conditions, enhancing the microbial activity of indigenous, cold-adapted, manganese-oxidizing bacteria that are naturally present in the contaminated groundwater could potentially activate and accelerate the oxidation of manganese at low temperatures.

Table 2: Chemical properties and composition of untreated groundwater samples

Untreated ground water	
pH	7.87
Electric conductivity ( $\mu$ S/cm)	1573
Total alkalinity (mg/L)	421
Total hardness (mg/L)	905
Nitrate (mg/L)	0.04
Calcium (mg/L)	220
Magnesium (mg/L)	87
Potassium (mg/L)	7.0
Sodium (mg/L)	30
Sulfate (mg/L)	527
Bicarbonate (mg/L)	513.3
Iron (mg/L)	2.82
Manganese (mg/L)	0.88
Sum of ions (mg/L)	1397

When plastic media were used as the filter media, the filtration unit achieved a 99% removal of iron and 60% removal of manganese after 6 months of operation. When using anthracite as the filter media, the effluent from the treatment system met drinking water standards; the filtration unit achieved a 99% removal of both iron and manganese from the groundwater source. Using anthracite as a filter media was more efficient for removing the iron and manganese from the groundwater compared to the plastic media. The manganese adsorption properties of anthracite provide a possible explanation for the improved manganese removal from the groundwater (Figure 4). The change in the iron and manganese concentrations and the statistical significance of the removal are depicted in Figure 1 and Table 3.



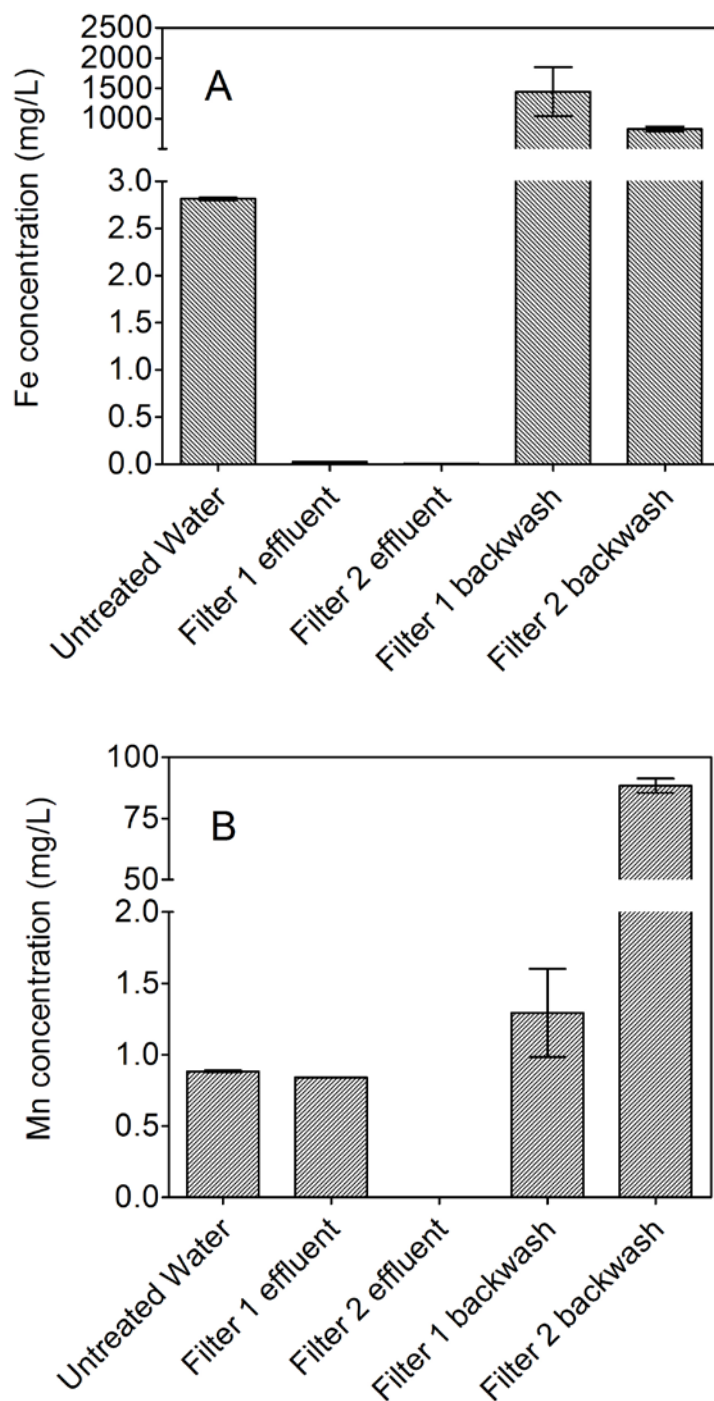


Figure 1: Iron and manganese concentrations of different water samples from the filtration unit: **A.** The change in the iron concentration in the effluents of the filtration unit, **B.** The change in the manganese concentration in the effluents of the filtration unit.



Table 3: Statistical significance (one way ANOVA) and comparison of removal of iron and manganese from the biofiltration unit

Dunnett's multiple comparison test (Manganese)	Iron		Manganese	
	Mean difference (mg/L)	Significance ( $p < 0.05$ )	Mean difference (mg/L)	Significance ( $p < 0.05$ )
Untreated water vs. Filter 1 effluent	2.79	Yes	0.0433	Yes
Untreated water vs. Filter 2 effluent	2.81	Yes	0.882	Yes

## 5.2 SEM/EDS analyses

Figure 2 shows the elemental composition of plastic media (control) and that carbon is the main element present. Figure 2B also shows the elemental composition of coated plastic media from Filter 2 and that it contains significant quantities of elements like carbon, oxygen, phosphorus, iron and manganese. From Figure 2A and 2B, it can be observed that the coating on the plastic filter media is dominated by iron compounds. Based on the ORP and pH conditions in the system, we expect that the manganese and iron are present primarily in the form of oxides. In the biofilm on the plastic media in Filter 2, manganese is present in smaller quantities compared to iron. It is indicated that aqueous manganese (II) was removed from the groundwater in the filter media and immobilized on the filter media.

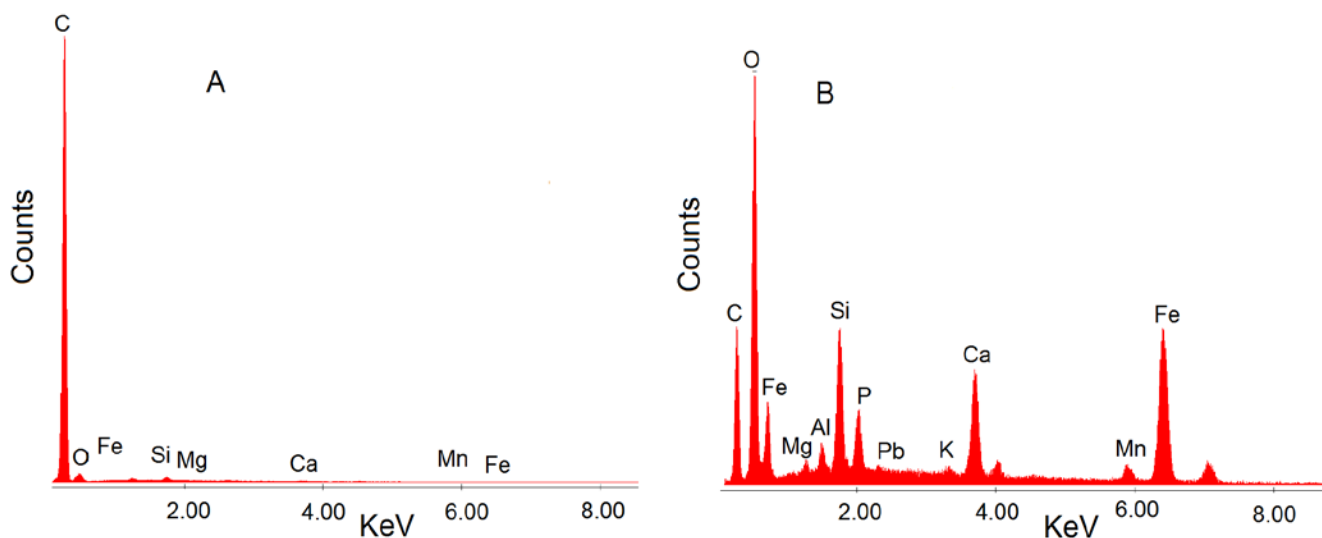


Figure 2: Elemental composition of **A.** Fresh plastic filter media indicating the presence of carbon as major element and **B.** Coated plastic filter media from Filter 2 indicating the presence of iron and manganese along with other elements through X-ray detector (Energy Dispersive Spectrometer).

### 5.3 Culture-dependent microbial assessment

The presence of manganese-oxidizing bacterial populations in the filtration unit was confirmed by enriching bacterial consortia in optimized culture media. The microbial populations produced brown precipitates, which accumulated on the sides of the flasks as shown in Figure 3A. The brown precipitates were then qualitatively confirmed to contain Mn using the leucoberbelin blue assay, which produces a color change from brown to dark blue color in the presence of Mn (III) or Mn (IV). Brown-coloured bacterial isolates were obtained on the optimized agar plates using enriched consortia as the inoculum, as shown in Figure 3B and 3C. These colonies were confirmed as manganese-oxidizers through the color change to blue upon addition of the leucoberbelin blue reagent. The culture-dependent analyses confirmed the presence of manganese-oxidizing bacterial populations in the biofiltration unit.

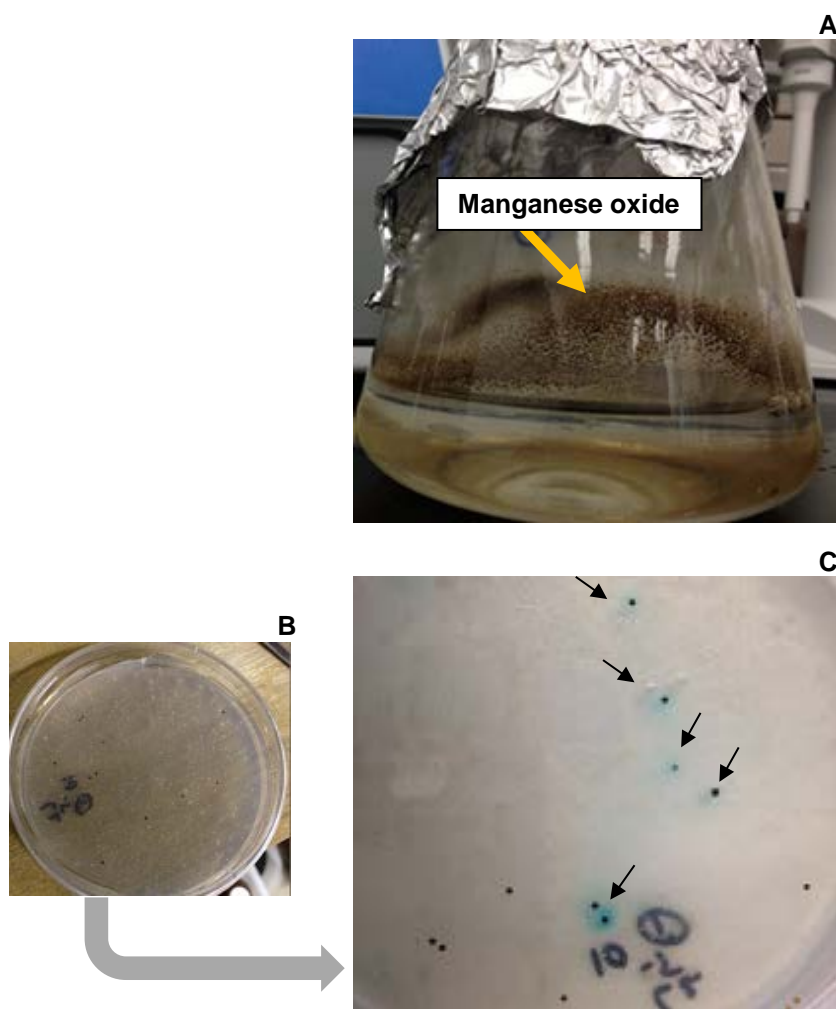


Figure 3: **A.** The culture-dependent microbial assessment of manganese-oxidizing bacteria in optimized nutrient media; the yellow arrow points to the brown layer formed on the walls of the flask, which was qualitatively confirmed to be composed of manganese oxides using the leucoberbelin blue test. **B.** The





brown colonies represent manganese-oxidizing bacteria with manganese oxides, **C**. The leucoberbelin blue test showing the color change of brown colonies upon assay addition.

#### 5.4 Manganese adsorption

The anthracite-manganese adsorption studies showed that there is a gradual increase in the adsorption of Mn (II) on anthracite between 0 and 8 hours, with a maximum adsorption of 0.2 mg/L in the solid phase in the fresh (untreated) anthracite set (Figure 4). The same trend is observed in UV-treated and autoclaved anthracite, as shown in Figure 4. The untreated anthracite had a greater adsorption rate compared to UV-treated and autoclaved anthracite. The adsorption experiments provided insight into the potential advantages of using anthracite as an adsorptive material for aqueous Mn (II), which would likely accelerate the removal of soluble manganese in the biofiltration unit.

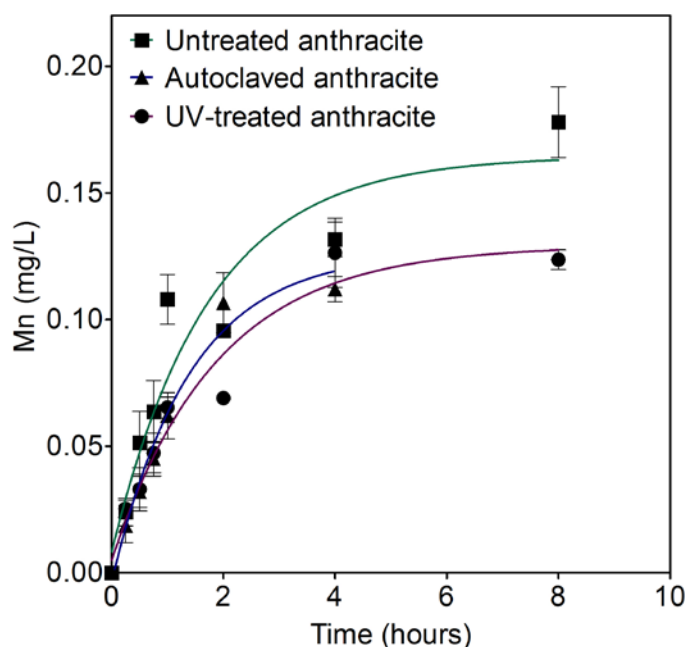


Figure 4: Mn (II) concentrations in solid phase (adsorbed to anthracite) over time for UV-treated, autoclaved and untreated anthracite at 22 °C.

## 6 CONCLUSION

The biofiltration unit design for the removal of iron and manganese from contaminated groundwater at the Langham water treatment plant was successful. We observed that the type of filter media and the growth of the manganese-oxidizing bacteria are rate-limiting factors and key design considerations that influence the rate and extent of manganese removal from groundwater at low in-situ temperatures in the pilot-scale biofiltration unit. The knowledge about geochemical and microbial growth conditions obtained from the pilot-scale feasibility study will be used to conduct further, more detailed kinetic and microbial community studies related to manganese oxidation at the low site temperatures.



## 7 ACKNOWLEDGEMENT

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