BIOLOGICAL MANGANESE OXIDATION BY *PSEUDOMONAS PUTIDA* IN TRICKLING FILTERS

Dissertation

by

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ABSTRACT

Manganese (Mn) is considered a nuisance chemical in drinking water. Manganese causes problems with staining, foul odor, undesirable tastes, and can be corrosive to pipelines. The United States Environmental Protection Agency (US EPA) recommends a secondary maximum contaminant level for Mn below a concentration of 0.05 mg/L. Currently manganese contaminated water is typically treated using expensive and potentially harmful oxidizing agents. Biological treatment techniques have been researched as a viable alternative for removing undesired chemicals from drinking water. In this study, bench scale trickling filters were constructed to compare the Mn removal efficiency between biochemical and abiotic processes. Glass beads between three and five millimeters in diameter were used as the solid media in the trickling filters with and without inoculation of a Mn oxidizing bacterium, *Pseudomonas putida*. Manganese oxidation and removal was found to be significantly greater in trickling filters with *Pseudomonas putida* biofilms after startup times of only 48 hours. Mn oxidation in *Pseudomonas putida* inoculated trickling filters was up to 75% greater than non-inoculated filters. One dimensional advection dispersive models were formulated to describe the transport of Mn in trickling filter porous media. Using the data collected in the experiments, the model predicted that that an average of 10 mg/L of influent Mn (II) concentration can be decreased by 78.56% with a filter depth of only 10 cm. The rapid startup time and the high Mn removal capacity of trickling filters inoculated with *Pseudomonas putida* can potentially become a mainstream treatment system in conjunction with sand filters.

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NOMENCLATURE

M – Mass

L – Length

T - Time

r_{Mn} – Manganese oxidation rate [M/L^3T]

S – Substrate concentration [M/L^3]

S_o – Initial substrate concentration [M/L^3]

X – Biomass concentration [M/L^3]

X_o – Initial biomass concentration [M/L^3]

μ_{max} – Maximum specific growth rate [1/T]

Y – Yield coefficient [$M_{biomass} / M_{substrate}$]

K_s – Half saturation constant [M/L^3]

t – Time [T]

λ – First order decay constant [1/T]

γ – Zero order growth constant [M/L^3T]

x – Distance [L]

D – Diffusion Coefficient [L^2/T]

D_o – Diffusion coefficient of solute in free water body [L^2/T]

D_{lh} – Hydrodynamic dispersion coefficient [L^2/T]

α - Dispersion degree [L]

v – Average porous flow velocity [L/T]

v_D – Darcy velocity [L/T]

θ – Volumetric water content

V – Volume [L^3]

R – Retardation factor

K – Empirical distribution constant [L^3/M]

Ø - Porosity of solid media

ρ – Density [M/L^3]

CHAPTER I

INTRODUCTION

I.1 Mn in the environment

Manganese is a nuisance chemical in drinking water. Concentrations of Mn as low as 0.1 mg/L have shown to cause problems with staining, undesirable taste, bad odor, and corrosion of distribution pipes. The United States Environmental Protection Agency (EPA) and The European Commission recommend a Secondary Maximum Contaminant Level for Mn below a concentration of 0.05 mg/L (Environmental Protection Agency 55; European Commission L330/50-L330/51). Chronic exposure to high doses of manganese (Mn) may cause harmful neurological effects; but there is not enough data to make a quantitative assessment of Mn toxicity (Environmental Protection Agency 55). Manganese is the fifth most abundant transition metal and second most abundant heavy metal (Tebo et al. 287-328). Manganese has been detected in roughly 70% of ground water and 97% of surface water assayed in the United States (Environmental Protection Agency 55). Manganese concentrations in freshwater typically range from 0.001 to 0.2 mg/L (Environmental Protection Agency 55). Currently treatment facilities that specifically target manganese typically use chemical treatment processes.

Manganese is typically found in nature in its +2, +3, and +4 oxidation state. However Mn(III) is unstable and will quickly dissociate to Mn(II) or precipitate as Mn(IV) unless it is chelated to another molecule. Mn(II) is readily soluble in water

below neutral pH, making it difficult to remove while Mn(IV) forms insoluble, black or brown precipitate above 8 pH. Because Mn(IV) forms a precipitate it can be easily removed with common filtration or sedimentation treatment processes. Natural chemical oxidation from Mn(II) to Mn(IV) is a slow process at standard pH and redox conditions and can take six to seven years for oxidation to begin (Tebo et al. 287-328; Diem and Stumm 1571-1573; Junta and M. 4985-4999; Tekerlekopoulou, I., and D. 292-301; Wilson 1311-1317). The autocatalytic oxidation rate of Mn is three to six times slower relative to biological oxidation rates (Tekerlekopoulou, I., and D. 292-301; Tebo S883-S905; Brouwers et al. 1573-1582). This indicates that aeration and precipitation alone cannot sufficiently remove manganese.

I.2 Removal of Mn from source water

The following are the eight conventional treatment processes used for removing manganese and iron from drinking water (Mouchet 158-167):

- Aeration followed by sand filtration or dual media
- Chemical oxidation followed by filtration
- Filtration with a medium that acts as an ion or electron exchanger (greensand, sand coated with manganese dioxide, and zeolites of volcanic origin)
- Conventional treatment with lime softening
- Using sodium silicate, phosphates, and/or polyphosphates as sequestering agents
- In situ treatments where oxygenated water is pumped into the aquifer using feed wells to create a treatment area around the main well.
- Biological filtration

I.2.1 Aeration

Although manganese can be oxidized by aeration, it is a slow process. Oxidation reduction potential (E_h) and pH conditions required for Mn oxidation is not typically found in natural waters. Conventional diffused aeration systems and hypolimnetic aeration systems are used to oxidize Mn(II) in reservoirs so that precipitated Mn(IV) can be later removed with filtration. Conventional diffused aeration systems pump air into the bottom of water bodies. This causes bubbles to form providing mixing and oxygenation to the water (Casale, M., and F. 187). Hypolimentic systems aerate water at different temperature gradients allowing for aeration in layers with low dissolved oxygen without disturbing the natural stratification of the reservoir (Casale, M., and F. 187).

I.2.2 Chemical Mn oxidation

Oxidizing chemicals are typically used as disinfectants. Conventional water treatment techniques use oxidizing agents such as oxygen, chlorine, ozone, chlorine dioxide, and potassium permanganate in combination with filters or coagulation and sedimentation to remove Mn. Chlorine is used as the primary disinfectant in 90% of treatment facilities in the United States (Kohl and S. 184). Theoretical reaction stoichiometry and corresponding stoichiometric ratios for Mn(II) with different oxidizing agents are as follows (Casale, M., and F. 187):

- O_2 (aq) 0.29 mg O_2 : 1 mg Mn
    - $Mn^{2+} + 1/2O_2 + H_2O \rightarrow MnO_2\ (s) + 2H^+$
- HOCl 1.30 mg HOCl : 1 mg Mn

- $Mn^{2+} + HOCl + H_2O \rightarrow MnO_2\ (s) + Cl^- + 3H^+$

- O_3 (aq) 0.88 mg O_3 : 1 mg Mn
  - $Mn^{2+} + O_3 + H_2O \rightarrow MnO_2\ (s) + O_2 + 2H^+$
- ClO_2 2.45 mg ClO_2 : 1 mg Mn
  - $Mn^{2+} + 2ClO_2 + 2H_2O \rightarrow MnO_2\ (s) + 2ClO_2 + 4H^+$
- $KMnO_4$ 1.92 mg $KMnO_4$: 1 mg Mn
  - $3Mn^{2+} + 2KMnO_4 + 2H_2O \rightarrow 5MnO_2\ (s) + 2K^+ + 4H^+$

Free chlorine does not efficiently oxidize Mn. Manganese oxidation rates using chlorine are slow in water bodies with neutral and acidic pH, causing chlorine dosages to be much higher than stoichiometric requirements. Studies have shown that three hours of contact time at neutral pH with chlorine dosages four times greater than stoichiometric requirements reduces 1 mg/L of Mn to 0.7 mg/L while only one hour of contact time is required at pH 9 to reduce 1 mg/L of Mn to less than 0.05 mg/L (Casale, M., and F. 187). Studies have also shown Mn oxidation rates decrease as temperature decreases (Casale, M., and F. 187).

Ozone (O_3) directly and efficiently oxidizes Mn at relatively low doses. However ozonation is not typically used due to its large capital costs and excess doses can potentially oxidize manganese to permanganate, which results in pink effluent water (Casale, M., and F. 187). High concentrations of organic material, humic material, and iron have shown to inhibit Mn oxidation (Casale, M., and F. 187).

Chlorine dioxide (ClO_2) has shown to have similar Mn oxidation mechanisms and rates as potassium permanganate. Mn(II) oxidation by chlorine dioxide has also shown to improve with high pH, temperature, and humic carbon. Research has shown that chlorine dioxide is an effective oxidant for removing low initial Mn(II) concentrations. Chlorine dioxide used with sedimentation has shown to remove up to 95% of Mn in water treatment plants.(Kohl and S. 184).

These oxidizing agents can be expensive and may form harmful byproducts such as bromate, trihalomethanes, and haloacetic acids. The EPA has strict drinking water regulations for these contaminants and each byproduct is potentially carcinogenic and mutagenic (Richardson et al. 178-242).

I.2.3 Filtration with an electron exchanging medium

Mn(II) can be adsorbed to the surface of filtration media such as zeolite, greensand, or pyrolusite (MnO_2). Greensand is a granular filter medium processed from glauconite sand with an effective size of 0.30-0.35 mm (Casale, M., and F. 187). Greensand needs to be conditioned by oxidizing agents such as chlorine or potassium permanganate ($KMnO_4$). Conditioning increases adsorption by converting Mn^{2+} on the surface of greensand into MnO_2 (s). Greensand facilitates both adsorptive and oxidative processes. Manganese removal rates have shown to be 4.5 g of Mn(II) per 0.028 m^3 of filter media (Casale, M., and F. 187). Media removal capacity increases with increasing solution pH.

Granular pyrolusite can be used to remove manganese through adsorption. Similar to greensand filters pyrolusite filters can use chlorine to regenerate the filter media. The main advantage of pyrolusite filters are their relatively high filtration rates, typically limited from 2.0 to 2.74 $L/m^2 \cdot sec$, but can be as high as between 6.8 and 10.2 $L/m^2 \cdot sec$ (Casale, M., and F. 187). The main disadvantage of pyrolusite filters is the relative high specific gravity (4.0) of the media. The high specific gravity requires a high backwash flow to fluidize filter beds and provide necessary cleaning (Casale, M., and F. 187).

Zeolite softening removes Mn(II) from water through cationic exchange. Backwashing zeolite media is typically done with brine solution prepared from sodium chloride (NaCl) to remove manganese and other cations (Casale, M., and F. 187).

Granular activated carbon (GAC) filters have shown to remove up to 2.55 mg of Mn per gram of GAC (Jusoh et al. 347-353). Another treatment process that was able to achieve above 95% Mn(II) removal included; polyelectrolytes at 8.5 pH, a 0.5 stoichiometric dose of potassium permanganate ($KMnO_4$), and 35 µm membrane filtration (Roccaro et al. 205-214). The disadvantages of this process were long holding times and high cost of membrane filtration; potassium permanganate is an expensive oxidant and may act as a skin irritant.

I.2.4 Lime softening

Lime (CaO) and soda ash (Na_2CO_3) are typically used as softening agents in water treatment systems. They are also useful in removing Mn because they can

increase pH to the point where Mn becomes insoluble (pH > 9.5) (Casale, M., and F. 187). Studies have shown Mn removal between 98 and 100% can be achieved at pH between 9.4 and 9.8. Due to the relatively high cost of using lime for Mn removal, this technique is not typically used unless water softening is also required (Casale, M., and F. 187).

I.2.5 Sequestering agents

Sequestering agents do not remove Mn from drinking water but rather control the negative aesthetic affects by preventing oxidation. Use of sequestering agents is usually limited to very low levels and for sequestering to be effective, manganese must be in the bicarbonate form (Casale, M., and F. 187). Typical sequestering agents are sodium silicate, trisodium phosphate, hexametaphosphate, zinc orthophosphate, and polyphosphates. Sequestering agents have shown to lose dispersing properties in water that is heated or boiled. Polyphosphates use should be limited because it has shown to increase bacterial regrowth and have adverse effects on phosphate levels (Casale, M., and F. 187).

I.2.6 *In situ* treatment

In situ aeration techniques are used for ground water sources. Injection of aerated water near pumping sites in groundwater sources has shown to reduce Mn(II) at several drinking water treatment facilities (Casale, M., and F. 187). However, some studies suggest that aeration of certain reservoirs does not reduce Mn(II) and microorganisms are the source of most Mn oxidation (Casale, M., and F. 187).

I.2.7 Biological Mn oxidation

Biological oxidation of manganese has the potential to be a cost effective and safe means of removing manganese from wastewater and drinking water when coupled with common filtration or clarification methods. Although biological treatment processes have not been widely utilized in drinking water treatment processes, they have proven to be excellent for wastewater treatment. Mn(II) oxidizing bacteria have shown to increase oxidation rates between three and five orders of magnitude. Biological oxidation of manganese is a viable alternative for Mn removal due to increased oxidation rates (Diem and Stumm 1571-1573; Diem and Stumm 1571-1573; Tekerlekopoulou, I., and D. 292-301). Manganese oxidation can be carried out by a wide variety of bacteria. Bacterial genera that can oxidize manganese are *Leptothrix, Crenotrix, Bacillus, SG-1, Hyphomicrobium, Metallogenium, Siderocapsa, Siderocystis,* and *Pseudomonas* (Mouchet 158-167; Gouzinis et al. 2442-2450). In this research project *Pseudomonas putida* was used to oxidize Mn(II) in bench scale trickling filters. *Pseudomonas putida* is a rod-shaped gram negative bacterium capable of oxidizing Mn(II). The strains capable of oxidizing Mn(II) are MnB1 and GB-1 (Tebo et al. 287-328; Brouwers et al. 1762-1768). Environmental conditions best suited for growth are 30^{o}C and pH 6.8 (Yang and Humphrey 1211-1235; Hill and R. 1599-1615).

Biologically produced manganese dioxide precipitates have shown to have more porous structures with greater surface areas than manganese produced using physical and chemical processes. (Casale, M., and F. 187)(Ulrich). The most common structure of biogenic Mn oxides formed by *Pseudomonas putida* is hexagonal phyllomanganate.

Biogenic Mn structures have high specific surface areas that ranges between 98 and 224 m^2/g, two to four times greater than synthetic Mn oxides. This means heavy metals can be strongly adsorbed to the biogenic Mn oxides (Villalobos et al. 2649-2662). This may promote additional autocatalytic Mn oxidation and water treatment.

I.3 Research objectives

The objective of the research was to inoculate porous media with *Pseudomonas putida* to significantly increase Mn oxidation rates and lower filter start up times. Mathematical models were developed to describe the biological manganese oxidation and growth of *Pseudomonas putida* in the trickling filters.

In the following chapter methods used to inoculate the trickling filters with *Pseudomonas putida* and measure Mn(II) oxidation will be described first. Results from the representative filter studies and modeling the Mn(II) removal and *Pseudomonas putida* growth will be presented then.

CHAPTER II

BIOCHEMICAL MANGANESE REMOVAL IN TRICKLING FILTERS USING *PSEUDOMONAS PUTIDA*

II. 1 Introduction

Removal of manganese (Mn) from water can be an expensive and time consuming process. Oxidizing Mn(II) to Mn(IV), the precipitate form of Mn, is generally accomplished using common chemical oxidants such as chlorine. It can take up to three hours to oxidize 1 mg/L to 0.7 mg/L of Mn(II) at neutral pH with a chlorine dosage four times greater than stoichiometric requirements (Casale, M., and F. 187). Biological oxidation of Mn may be a viable alternative to conventional treatment methods. Studies have shown that down flow homogenous biological filters are best suited for Mn removal (Yang et al. 1447-1454). Costs can be optimized by changing nutrient inputs, such as oxygen and carbon sources, or the type of microorganisms, such as *Pseudomonas*, *Leptothrix*, *Bacillus*, *Crenotrix*, *Hyphomicrobium*, *Metallogenium*, *Siderocapsa*, and *Sidercystis*. Active biofilms in attached growth biological filters such as trickling filters can take several months to establish although some inoculation procedures have reduced start up time to only two weeks (Stembal et al. 509-518).

Trickling filters have been closely examined for use as biological filters, because the large pore spaces provide aerobic conditions throughout the filter. Trickling filters have large ventilation ports near the bottom of the filters; the ventilation ports usually provide sufficient air supply from natural wind forces (Environmental Protection Agency

7). This means biological manganese filters will not require large energy inputs to maintain aerobic conditions, which will promote Mn oxidation.

Trickling filters are "aerobic treatment systems that utilize microorganisms attached to a medium to remove organic matter from wastewater" (Environmental Protection Agency 7). Typical trickling filter process flow diagrams can be seen in Figure 1 (Tchobanoglous, F., and D. 887-981). Trickling filter medium is comprised of rocks or plastic packing material that is either cross-flow or vertical-flow in design. These designs are considered non-submerged fixed-film biological reactors, and are typically either tertiary or secondary treatment processes. Advantages of trickling filters compared to other biological treatment processes, such as activated sludge, are as follows (Tchobanoglous, F., and D. 887-981):

- Less energy required
- Simple operation with no issues of mixed liquor inventory control and sludge wasting
- No problems of bulking sludge in secondary clarifiers
- Better sludge thickening properties
- Less equipment maintenance
- Better recovery from shock

Disadvantages of trickling filters are (Tchobanoglous, F., and D. 887-981):

- Lower effluent quality in terms of BOD and TSS
- Greater temperature sensitivity
- Odor
- Uncontrolled solids sloughing events

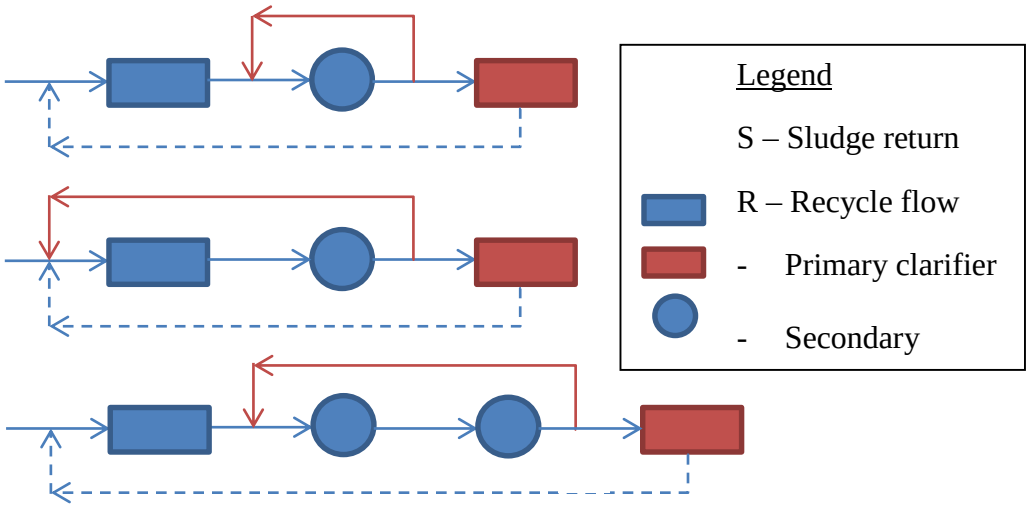

Figure 1. Common trickling filter process flow diagrams (Tchobanoglous, F., and D. 887-981)

Filter medium depth ranges from 0.9 to 2.5 m for rock media and 3 to 12 m for plastic media. Biofilm, typically 0.1 to 0.2 mm thick, forms over several months as wastewater continuously flows over the trickling filter medium (Environmental Protection Agency 7; Tchobanoglous, F., and D. 887-981). Wastewater treatment occurs mainly due to microbial degradation of target substrates as it flows over the biofilm. Biofilm thickness is greatest at the top of trickling filters and is maintained around 0.2 mm due to hydraulic shearing of water flow.

To ensure low cost and efficient water treatment, filter depth should be optimized. A pilot scale study showed that a filter depth of approximately 0.8 m is needed to decrease 1.06 mg/L of feed Mn to below 0.05 mg/L in the effluent (Stembal et

al. 327-335). As filtration rates increased from 12 m/hr to 24 m/hr less Mn was removed from the water effluent.

Biological filtration have shown to have Mn removal rates between 60% and 95% using mixed cultures (Gouzinis et al. 2442-2450; Stembal et al. 327-335; Pacini, A., and G. 4463-4475). Those studies typically had long startup times ranging between several weeks and months to treat water with Mn concentration typically between 0.5 mg/L and 1 mg/L. According to the biological filtration studies mentioned above a Mn removal rate of 95% is needed to meet the EPA's Secondary Maximum Contaminant Level (0.05 mg/L) for most Mn impaired water bodies. *Pseudomonas putida* was inoculated to filter media to facilitate Mn oxidation under neutral pH. Filter set up, Mn oxidation studies under laboratory conditions, and modeling Mn oxidation and microbial growth are described in this chapter.

II.2 Materials and methods

Initially the filter was designed as a rapid sand filter using a 0.60 m (2 ft) long PVC pipe 10.16 cm (4 in) in diameter. The 0.60 m long PVC pipe was too cumbersome and difficult to handle for a bench scale project and was replaced using a repurposed methane filter. The repurposed methane filter had difficulty draining properly and was also determined to be too large. It was then decided that large sand filters may not provide enough aeration for Mn to be oxidized. The filter was soon redesigned to be a trickling filter that used pea pebbles, greater than 0.5 cm in diameter, as a solid media and a 0.30 m (1 ft) long PVC pipe 5.08 cm (2 in) inches in diameter. A mesh screen was stretched

across the bottom of the PVC pipe to keep the solid media in place. There was anecdotal evidence from the preliminary experiments that indicated that consistent source of light aided the manganese oxidizing bacterium, *Pseudomonas putida*, to oxidize Mn(II). So, the PVC trickling filters were replaced with glass columns of 4 cm diameter which also helped to allow observation of manganese oxidation without disturbing the solid media. During preliminary studies, an increase in Mn oxidation was observed in filters inoculated with *Pseudomonas putida* even though the biofilms were not well established. . This led to the addition of clear glass beads as the solid media *in lieu* of pea pebbles. Pea pebbles were kept at the bottom of the column to prevent the glass beads from leaving. Three more preliminary trials showed increased Mn oxidation and biofilm formation in comparison to previous trials. The dark brown biofilms and oxidized manganese oxides were forming on the surface of the solid media within 48 hours.

The final design consisted of two bench scale trickling filters. One of the filters was inoculated with *Pseudomonas putida* the other filter was used as a control and was not inoculated. The trickling filters consisted of 500 mL glass dispensing burets, 400 mm high and with 4 cm internal diameters. The support media consisted of a top layer with 130 mL (10 cm depth) of solid glass beads, 3 to 5 mm in diameter, and a bottom layer with 50 mL (four centimeter depth) of pea pebbles with a mean diameter of 7.5 mm. The porosity (θ) of the glass bead layer was 0.62 and the porosity of the pebble layer was 0.45. Liquid media was pumped through the system using two Masterflex L/S peristaltic pumps. The liquid media was stored in a 500 mL shake flask at the outlet of the filter. The liquid media in the shake flask was then recirculated through the trickling filter. pH

measurements were carried out using a Beckman Coulter pHi 570 pH meter. pH measurements were carried out daily to ensure the liquid media pH stayed near neutral pH. A Thermo Scientific Genesys 10S UV – Vis spectrophotometer was used to monitor bacterial growth and Mn(II) concentration.

II.2.1 *Pseudomonas putida* growth and trickling filter inoculation process.

Pseudomonas putida cultures were preserved in glycerol stock and stored in a freezer at -20˚C. The maintenance cultures were later thawed in a 4ºC incubator several hours before an enrichment culture was created. An enrichment culture was prepared by adding 0.1 mL of glycerol stock inoculated with *Pseudomonas putida* to100 mL of Tebo liquid medium (refer to Table 1 and 2) in multiple 250 mL shake flasks. A $MnCl_2$ concentration of 8×10^{-4} M was added to each shake flask. The enrichment cultures that showed signs of Mn(II) oxidation between 24 and 120 hours were used to inoculate the trickling filters. The trickling filter was inoculated with *P. putida* by adding 1 mL of the enrichment culture directly to the solid media of a filter and one milliliter of the enrichment culture to the shake flask containing the liquid medium being continuously pumped through the trickling filter. This was done every hour for up to four hours and then absorbance measurements were taken.

Table 1. Composition of Tebo liquid growth medium (Villalobos et al. 2649-2662) for inoculating trickling filters with *Pseudomonas putida*. All compounds were mixed into one liter of deionized water then autoclaved at 120°C for 20 minutes.

Chemical	Concentration
Yeast extract	0.5 g/L
Casamino acids	0.5 g/L
Glucose	1.0 g/L
$CalCl_2$	$2x10^{-3}$ M
$MgSO_4$	$3.3x10^{-2}$ M
$FeCl_3$	$3.7x10^{-4}$ M
Trace element solution	1.0 mL/L

Table 2. Composition of trace element solution in Tebo liquid growth medium. All components were mixed into one liter of deionized water then autoclaved at 120°C for 20 minutes (Villalobos et al. 2649-2662).

Chemical	Concentration
$CuSO_4 \cdot 5H_2O$	10 mg/L
$ZnSO_4 \cdot 7H_2O$	44 mg/L
$CoCl_2 \cdot 6H_2O$	20 mg/L
$Na_2MoO_4 \cdot 2H_2O$	13 mg/L

Tebo liquid growth medium (Table 1) was pumped into both the trickling filters and recycled at a rate of 100 mL/min for 48 hours in a continuous down flow operation (figure 2). A 48 hour recycle time was chosen because it was seen from preliminary trials that 48 hours was the minimum amount of time taken for *Pseudomonas putida* to attach to the support media and grow enough to form a visible biofilm. Both filters were open to the air. One filter was inoculated with *Pseudomonas putida*, while the other was not

inoculated with any bacteria. Every hour the absorbance for three 1 mL samples of the liquid growth media in each trickling filter was measured with a spectrophotometer set at 600 nm wavelength along a 1 cm light path. This was done to monitor the growth and attachment of bacteria in both trickling filters.

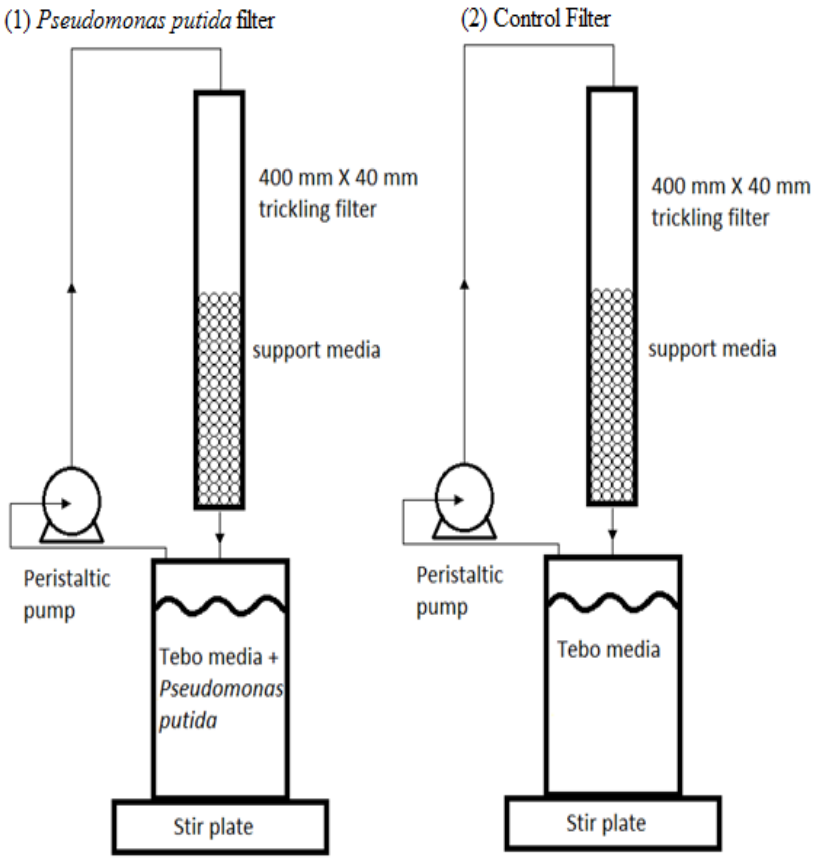

Figure 2. Setup for inoculating trickling filter with *Pseudomonas putida* and a control trickling filter with airborne bacteria. The filter on the left (1) was inoculated with Pseudomonas putida and fed Tebo liquid growth medium for 48 hours while open to the air. The filter on the right (2) was a control and was not inoculated with any specific bacteria but was open to the air and fed Tebo liquid growth medium for 48 hours.

Tebo liquid growth medium for *Pseudomonas putida* was used to stimulate bacterial growth in the trickling filters (Villalobos et al. 2649-2662). Normally Tebo growth medium is prepared by adding 8×10^{-4} M $MnCl_2$ to the solution; however this was not added during the inoculation process, but was added later after the *Pseudomonas putida* grew in the trickling filter for 48 hours.

II.2.2 Determination of Manganese in trickling filter effluent.

Deionized water with feed manganese concentration of 8×10^{-4} M was pumped into the trickling filters at a rate of 0.25 mL/min. Simultaneously, 250 mL of Tebo liquid growth media was fed to the filter at rate of 100 mL/min and the filter effluent was collected in the feed tank and recycled (Figure 3). Every hour the concentration of Mn in the effluent should increase by 4.8×10^{-8} M, if no Mn is removed. Three 10 mL samples from the Tebo media were taken every two hours from each trickling filter to measure the Mn(II) concentration. The Mn(II) concentration was measured using the persulfate method (American Water Works Association and American Public Health Association). The experiment was repeated three times. After the final trial was completed, the support media was analyzed using scanning electron microscope (SEM) imaging. The SEM preparation and imaging was carried out by Dr. Vaddiraju's laboratory. The SEM imaging was done to characterize the Mn in the trickling filters.

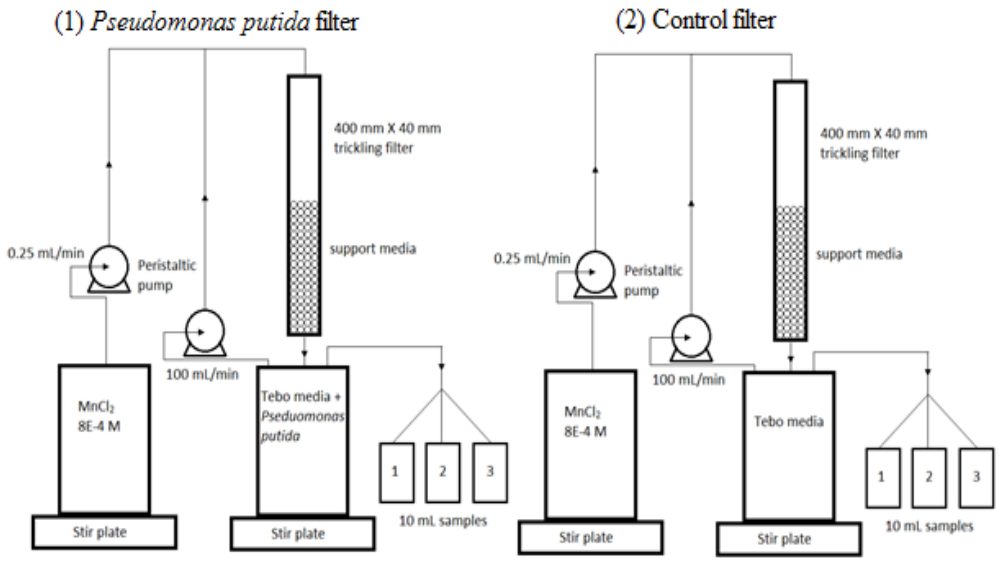

Figure 3. Flow diagram for Mn removal and sample collection in inoculated and control bench scale trickling filters. The figure above shows how samples were taken to measure Mn from the effluent of the filter inoculated with *Pseudomonas putida* (1) and the control filter (2).

The persulfate method for measuring Mn(II) concentration was done as follows. First a sample was diluted or concentrated to 90 mL. Next, one drop of H_2O_2 and 5 mL of special reagent was added to the solution followed by the addition of 1 g of $(NH_4)_2S_2O_8$. Then the solution was boiled for 1 min. This solution was diluted to 100 mL after cooling down for 1 min and analyzed using a spectrophotometer set at 525 nm along a one centimeter light path. The special reagent was prepared by dissolving 75g $HgSO_4$ in 400 mL of HNO_3 concentrate and 200 mL DI water. Next 200 mL of 85% H_3PO_4 and 35 mg $AgNO_3$ was added to the solution. The solution was diluted to one liter with DI water then cooled.

A standard curve for the persulfate method was prepared using known concentrations of permanganate between 0 and 1500 µg/100 mL (Figure 4). The standard curve was created by analyzing known concentration of Mn using a Thermo Scientific Genesys 10S UV – Vis spectrophotometer. A linear relationship was observed between absorbance and Mn concentration (Figure 4) and Mn concentration was determined by using the following equation ($r^2 = 0.9913$):

$$Absorbance = 0.0004 * Mn\ concentration$$

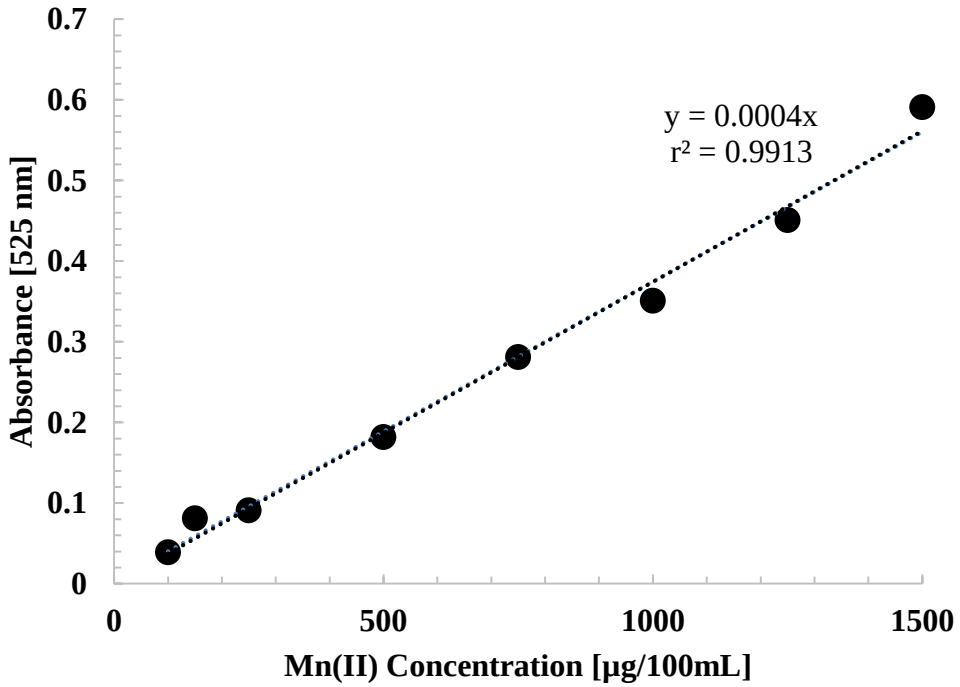

Figure 4. Determining Mn concentration by persulfate method. The standard curve was developed for measuring Mn(II) in 100 mL of sample using the persulfate method

II.2.3 Modeling manganese removal using advective dispersive equations.

The one dimensional advective-dispersive equation (ADE) describes chemical transport under changing fluid flow conditions (Genuchten and W. 151). This equation can be used to simulate Mn transport in the filter and determine appropriate filter depth. While filter depth is likely the largest factor that affects Mn concentration other important factors such as, filtration rate, substrate consumption rate, and the type of solid media can also affect the effluent quality. The following equations describe the advective dispersive transport:

$$D\frac{\partial^2 S}{\partial x^2} - v\frac{\partial S}{\partial x} - R\frac{\partial S}{\partial t} = \lambda S - \gamma$$

Where S is the aqueous phase substrate concentration (µg/L), D is the diffusion coefficient (cm^2/min), x is the depth (cm), t is time (min), R is the retardation coefficient, γ is a zero order Mn production constant (µg/mL·min), and λ is a first order Mn consumption constant (1/min):

The retardation coefficient can be determined using the following equation:

$$R = 1 + \rho K/\theta$$

$$\theta = V_{water}/V_{total}$$

Where ρ is the water density, K is the empirical distribution constant, and θ is the volumetric water content.

Where the diffusion coefficient (D) is approximately equal to the hydrodynamic diffusion coefficient (D_{lh}) and $D_o a e^{b\theta}$ is negligible.

$$D = D_o a e^{b\theta} + D_{lh}$$

$$D_{lh} = \alpha v$$

The hydrodynamic diffusion coefficient is equal to the Darcy velocity (v_D) multiplied by the dispersion degree (α), and divided by the volumetric water content (θ).

$$v = v_D/\theta$$

An analytical solution for the one dimensional advective dispersive model can be found by assuming boundary conditions. The boundary conditions were determined to be flux-type and semi-infinite for the trickling filter due to steady state conditions and the continuous flow of Mn contaminated water through the filter, where S_o is the initial substrate concentration.

$$-D\frac{dS}{dx} + vS\,|_{x=0} = vS_o$$

$$\frac{dS}{dX}(\infty, t) = 0$$

Zero order production rate (γ) of Mn(II) is assumed to be negligible in this experiment. The concentration of Mn(II) at distance (x) in the trickling filter can be found using the above boundary conditions as follows:

$$S(x) = \frac{\gamma}{\lambda} + \frac{\left(S_o - \frac{\gamma}{\lambda}\right) 2v}{u + v} \exp\left(\frac{(v-u)x}{2D}\right)$$

$$u = v\sqrt{1 + \frac{4\lambda D}{v^2}}$$

II.3 Results and discussion

II.3.1 Attachment and growth of Pseudomonas putida and airborne bacteria.

Pseudomonas putida was grown for 48 hours attached to the solid media in the trickling filter after the first inoculation with the enrichment culture. The time it took for the enrichment culture to show signs of Mn oxidation varied by several days. This may be the reason for wide range of growth rates shown later. The absorbance values measured in the reservoir of the Tebo liquid growth media began to decline between five and fifteen hours (Figure 5). It is assumed that between five and fifteen hours is when *Pseudomonas putida* began to attach to the solid media. After 15 hours the glass beads would start to turn a pale yellowish color. The trickling filters became saturated with *Pseudomonas putida* around 48 hours where stationary phase reached and maximum Mn oxidation happened

In Trial 1 *Psuedomonas putida* reached maximum growth rate after seven hours. The specific growth rate of *P. putida* was 0.0065 min^{-1} (doubling time =106 min). However, the manganese oxidation was the lowest compared to other tirals showing that the oxidation was not dependent on *P. putida* growth rate. Innoculation time, nutrient input, environmental factors, final cell concentration, and the state of the enrichment

culture are likely to contribute more to Mn(II) oxidation. The control trickling filter had a similar growth rate to the trickling filter innoculated with *Pseudomonas putida*. The control trickling filter showed negligible Mn oxidation and biofilm formation (Figure 6).

The second trial took longer to start up (Figure 5), but resulted in the highest Mn(II) oxidation (*as seen in* Figure 7). The specific growth rate was found to be 0.0049 min^{-1} (doubling time = 140 min). The control trickling filter had a much slower start up and lower Mn oxidation than the trickling filter inoculated with *Pseudomonas putida* (Figure 5).

The final trial had the fastest start up time and the highest percent reduction of Mn(II) (*as seen in* Figure 5). The control trickling filter had a much slower startup time than the trickling filter inoculated with *Pseudomonas putida* (Figure 5). Because there is no data during the initial growth phase of third trial, that the specific growth rate was estimated to be 0.0057 min^{-1} (doubling time = 122 min), the average of the previous trials. Negligible amount biofilm was observed in the control filter as well (Figure 8).

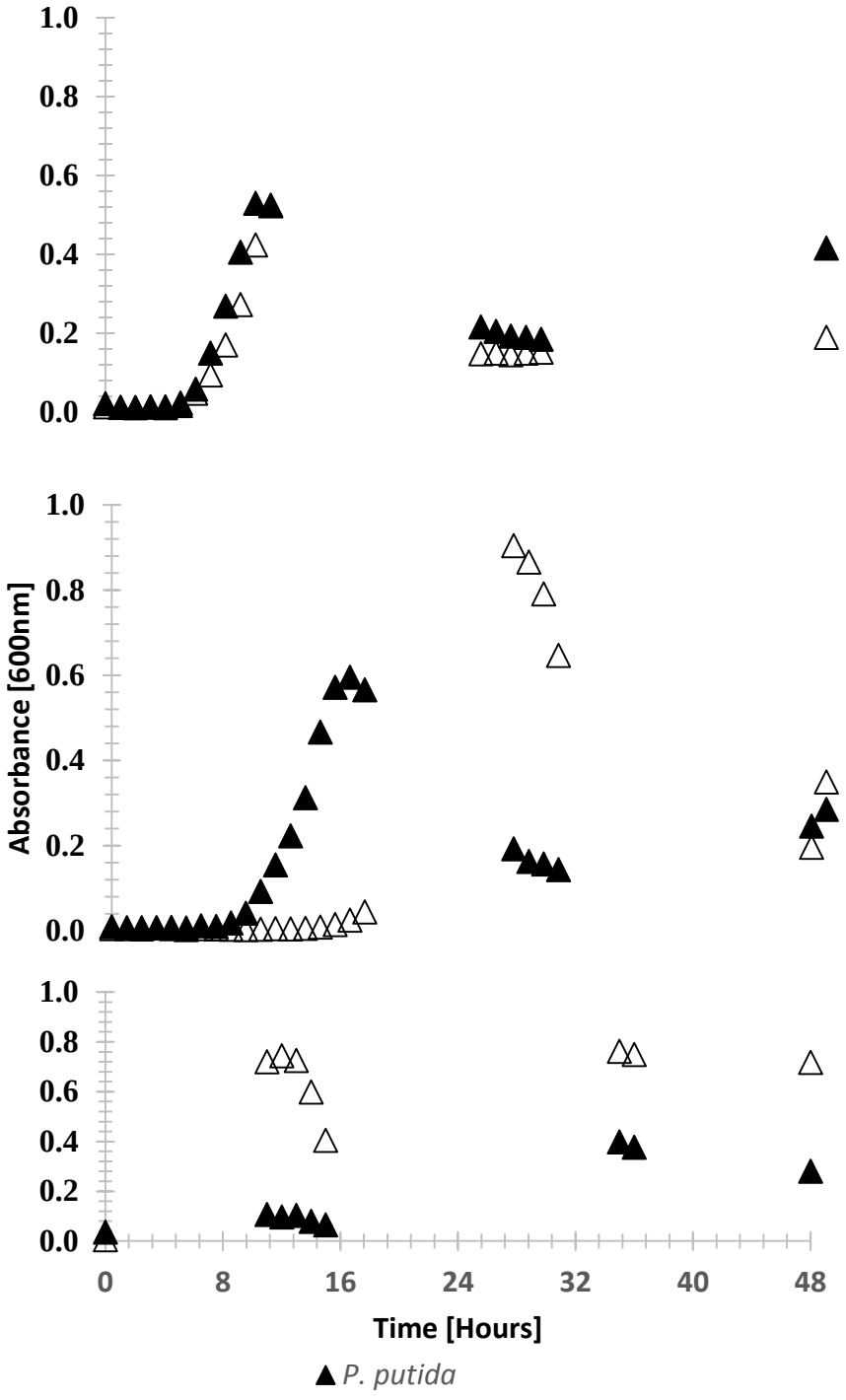

Figure 5. Growth and attachment in continuous flow trickling filters inoculated with *P. putida* and airborne bacteria (control). Growth and attachment was accomplished using Tebo liquid growth medium. The figure above shows data from trials one (top), two (middle), and three (bottom).

The growth rate and concentration of ambient bacteria in the control filter varied greatly in each trial (Figure 6). The growth and absorbance of the control filters appears to mimic the growth of the respective *P. putida* filters, despite the fact that there was typically more lag time for the control filters to develop biofilm. The lag time for *Pseudomonas putida* in each experiment varied between zero and eight hours (Figure 5). Each inoculated filter appeared to follow the same pattern of initial growth and attachment but varied greatly as the inoculation time increased.

II.3.2 Manganese oxidation and removal from trickling filters.

Once the *Pseudomonas putida* had grown for 48 hours, manganese contaminated water with average concentration of 58.69 μg/mL, was pumped through each trickling filter at a rate of 0.25 mL/min. At the same time Tebo liquid growth media was still being recirculated through the trickling filter at a rate of 100 mL/min. Once Mn concentration in the effluent of the filter inoculated with *Pseudomonas putida* reached steady state feeding was stopped and samples were taken from each filter for SEM imaging. Although the control filter showed signs of Mn oxidation and adsorption to the solid media, oxidation in the trickling filter inoculated with *Pseudomonas putida* was obviously much greater due to the large amount of black and brown Mn oxide precipitates that formed on the solid media of the trickling filters (Figures 6, 7, 8, and 9).

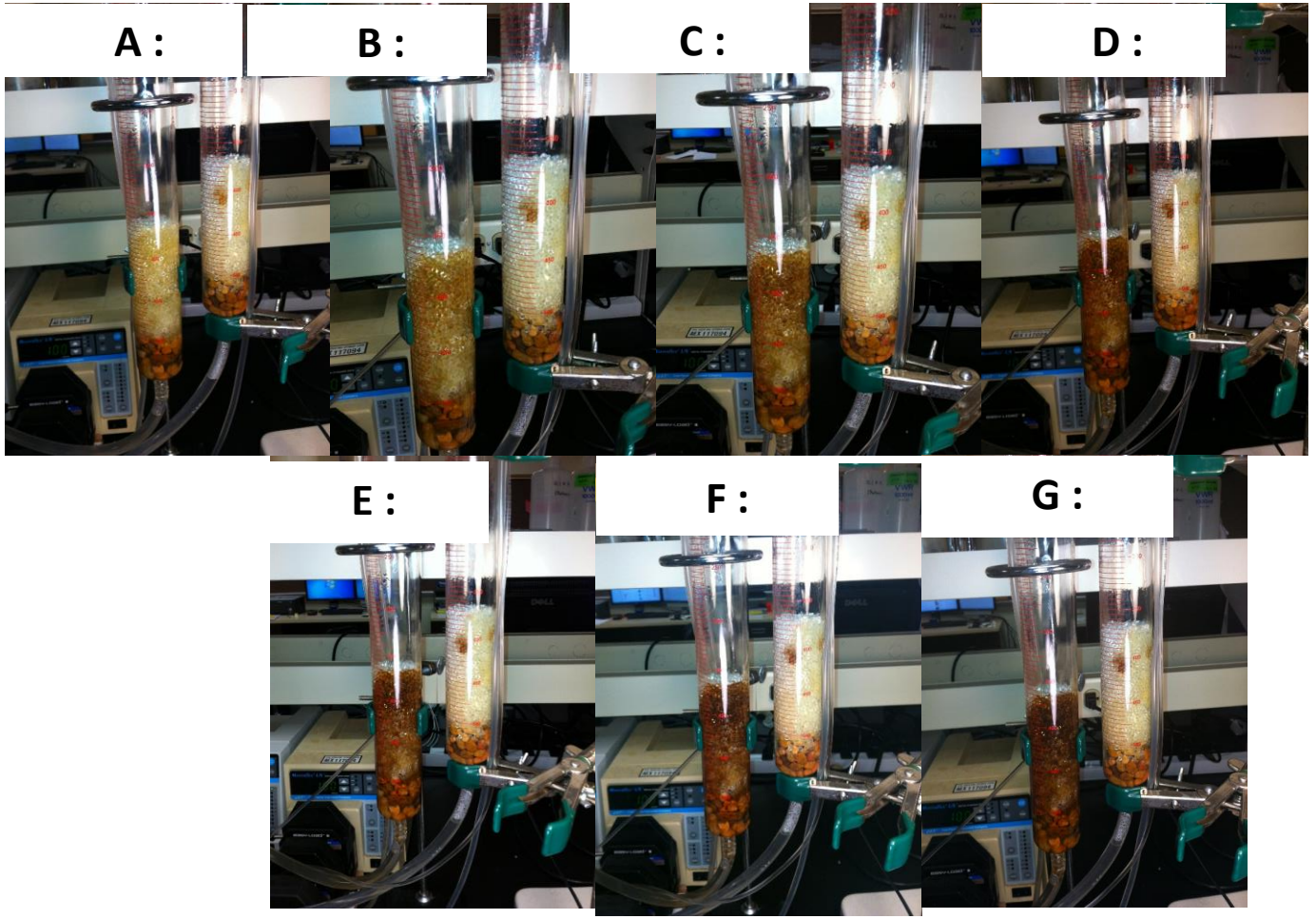

Figure 6. Mn oxidation progression trial 1. The progression of Mn oxidation and adsorption to the solid media in two hour intervals during the first trial. The inoculated filter (left) shows signs of Mn oxidation with the brown/black precipitate attached to the solid media, while the control filter shows minor signs of Mn oxidation (right).

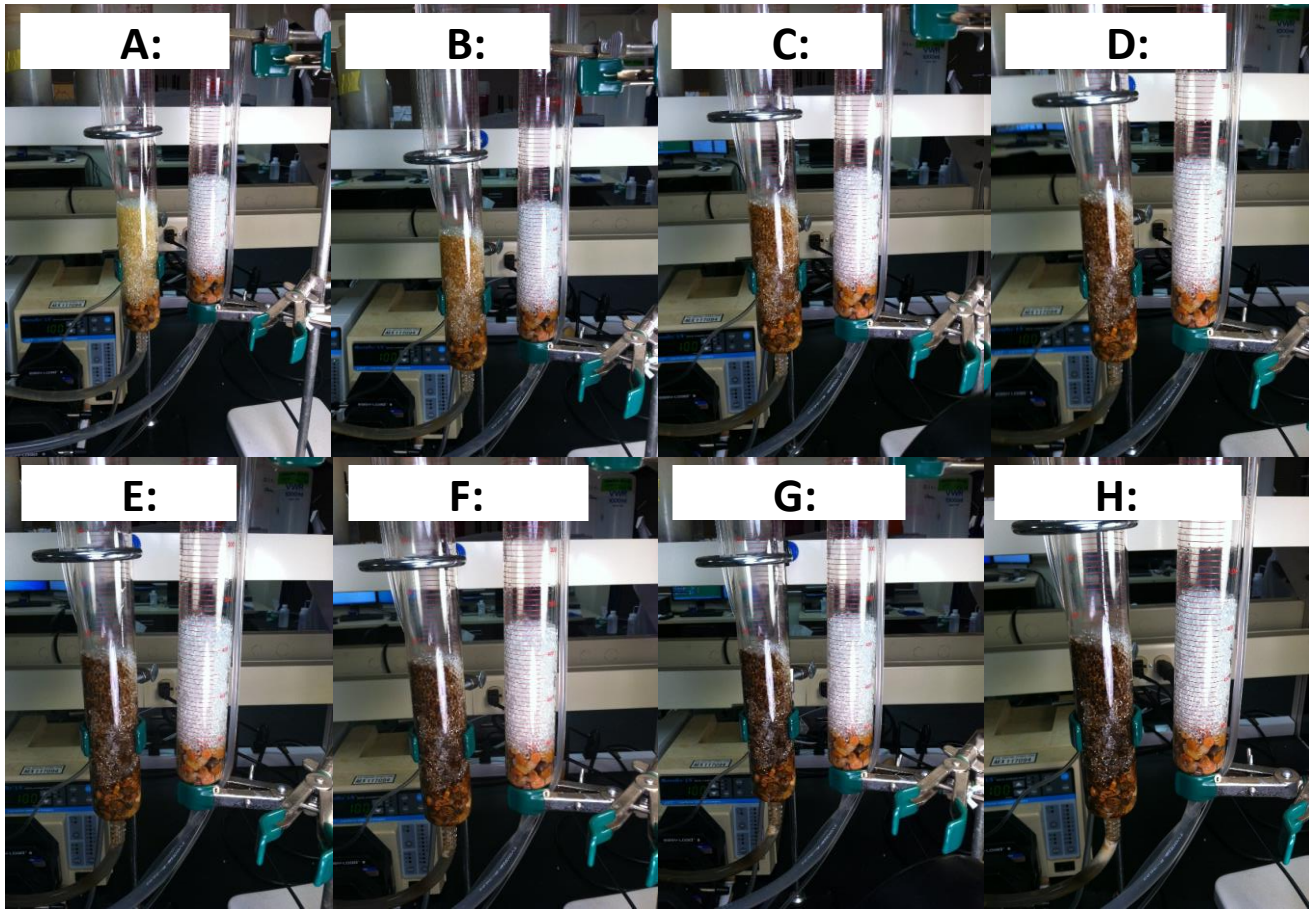

Figure 7. Mn oxidation progression trial 2. The progression of Mn oxidation and attachment to the solid media in two hour intervals during the second trial. The inoculated filter (left) shows signs of Mn oxidation with the brown/black precipitate attached to the solid media, while the control filter shows no signs of Mn oxidation (right).

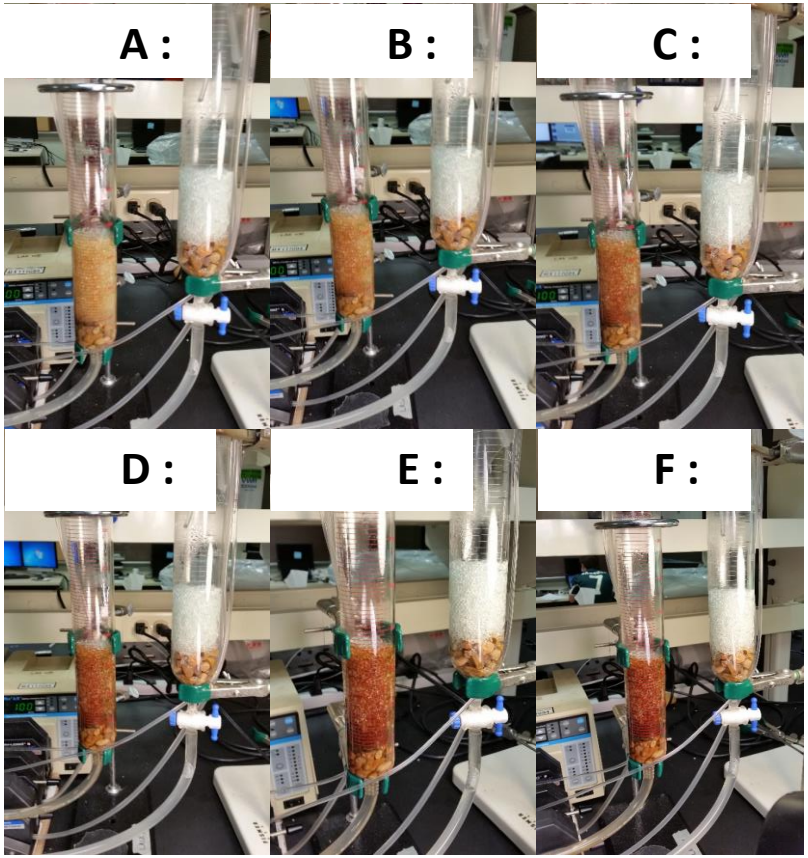

Figure 8. Mn oxidation progression trial 3. The progression of Mn oxidation and adsorption to the solid media in two hour intervals during the third trial. The inoculated filter (left) shows signs of Mn oxidation with the brown/black precipitate attached to the solid media, while the control filter shows no signs of Mn oxidation (right). Before Mn(II) contaminated water was introduced into the system *Pseudomonas putida* can be seen attached to the solid media as a yellowish color (A). The final figure (F) shows Mn attached to the filter after the system reached steady state, and Mn was being removed from the water effluent at the maximum possible rate.

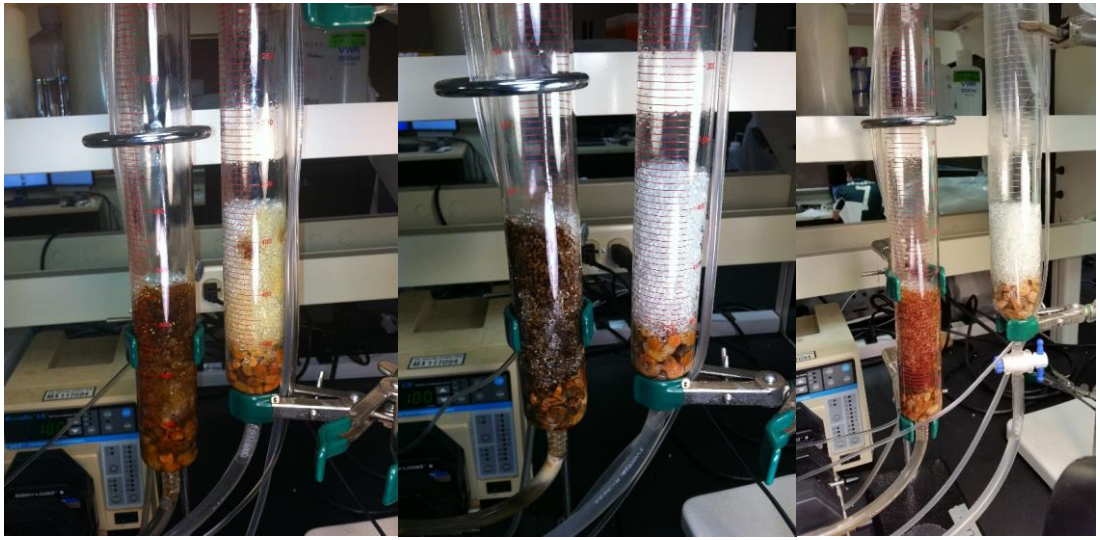

Figure 9. Steady state trickling filter comparison of each experimental trial. The figure above shows a comparison between the first, second, and third (left to right) experiments after the inoculated trickling filters had reached steady state

During the first experimental trial the Mn(II) concentration in the trickling filter inoculated with *Pseudomonas putida* was significantly lower than the control filter (Figure 10). Although some of the Mn began to oxidize and adsorb to the control filter after 10 hours (Figure 6). It appears that the *Pseudomonas putida* did not reach its maximum growth rate until nine hours during this phase of the experiment (Figure 5). This is likely the reason for much higher Mn(II) concentration in the effluent when compared to the other two trials (Figure 10).

The highest decrease in initial Mn(II) concentration was observed during second trial (Figure 10). The control filter showed no signs of biofilm formation (Figure 8) and had almost no Mn removal (Figure 10).

The third trial had the highest Mn percent reduction in the filter inoculated with *Pseudomonas putida (Table 3)* but the total amount of Mn oxidized was less than the second trial (Figure 10 and Table 3).

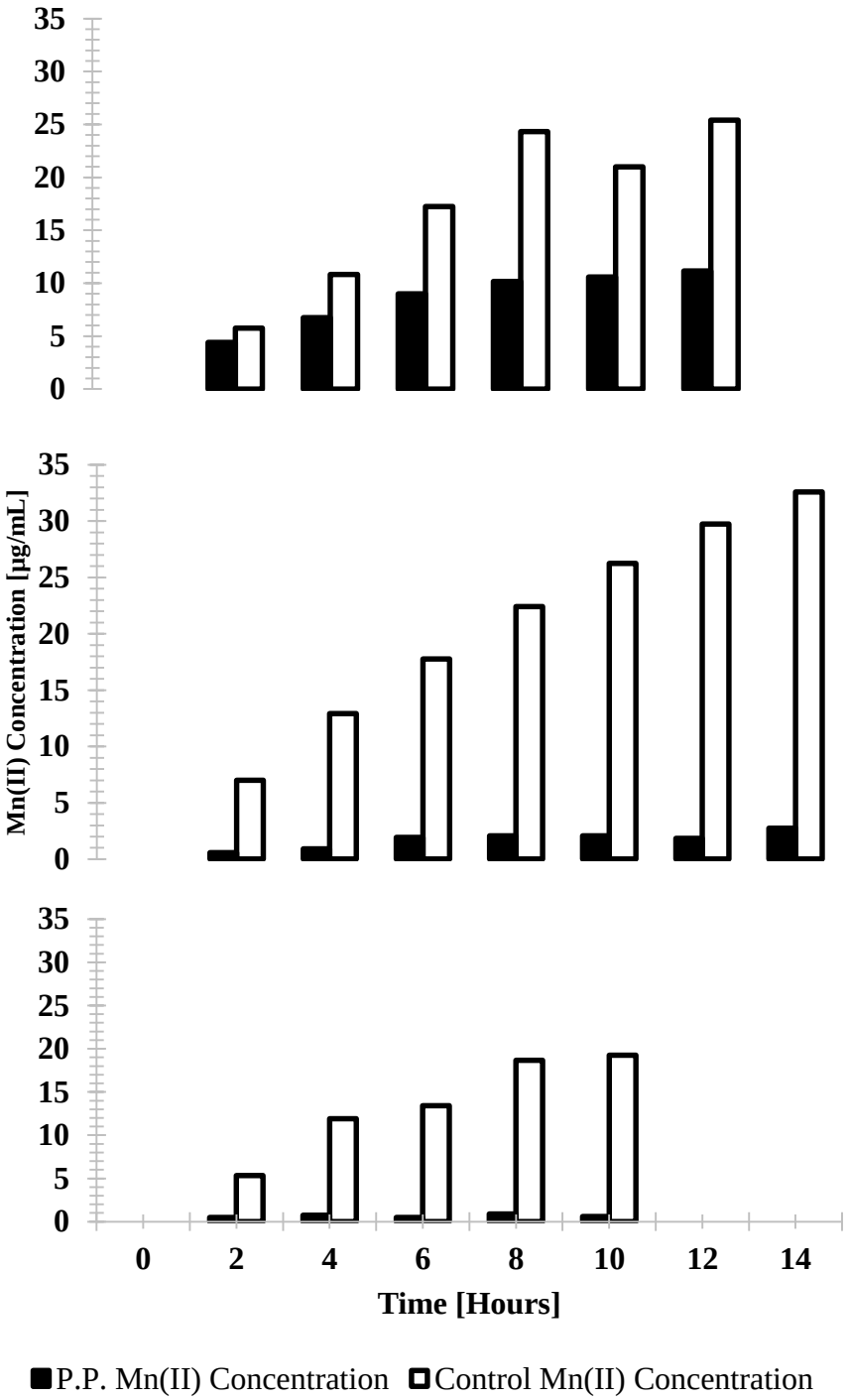

Figure 10. Mn(II) concentration in the effluent of *Pseudomonas putida* inoculated trickling filters vs. control trickling filters. Mn(II) concentration in the effluent of the inoculated and control trickling filters for the first (top), second (middle), and third (bottom) trials.

Table 3. Percent removal of manganese in filters inoculated with *P. putida* and no inoculation.

Trial #	Mn (II) removal percentage (%)	
	Filter inoculated with *P. putida*	Filter with no inoculation
1	38	14
2	82	13
3	91	16

Control filters had negligible Mn(II) oxidation as compared to filters inoculated with *P. putida* in all trials (Table 3). Manganese removal in control filters can be attributed mainly to abiotic and some biotic losses. Previous studies have shown from 90% to 95% removal of Mn(II) with mixed bacterial cultures using much lower Mn concentration than used in this research (Stembal et al. 327-335; Pacini, A., and G. 4463-4475). In one of the laboratory studies to achieve 90% removal it took up to eight weeks. The removal percentages in *P. putida* filters were three to six orders of magnitude more than control filters (Table 3). This is consistent with other research results indicating biological filtration could oxidize Mn between three and six orders of magnitude greater than autocatalytic oxidation (Tekerlekopoulou, I., and D. 292-301; Tebo S883-S905; Brouwers et al. 1573-1582). Mn oxidation by *Pseudomonas putida* has proven to be consistently greater than autocatalytic oxidation but the results vary dramatically. It should be noted that autocatalytic oxidation is shown to oxidize Mn consistently. This can be overcome by proper treatment design of biological filtration in conjunction with other chemical processes to account for the variability in Mn removal.

II.3.3 Statistics.

A one-tailed *t*-test with unequal variance was used to determine whether or not the concentration of Mn(II) in the effluent of the trickling filter inoculated with *Pseudomonas putida* was significantly lower than the control filter. It was found that the concentration of Mn(II) in each trial was significantly lower in the *Pseudomonas* filter at $\alpha = 0.05$ (Table 4). Although trial two had the most significant difference between the Mn(II) concentration in the effluent, trial three had the greatest percent reduction of Mn(II) overall. The null hypothesis was that the concentration of Mn(II) in the effluent of the trickling filter inoculated with *Pseudomonas putida* is significantly greater than or equal to the control filter. The alternate hypothesis was the concentration of the Mn(II) in the effluent of the trickling filter inoculated with *Pseudomonas putida* is significantly less than the control filter. Statistics on each trial results rejected the null hypothesis and supported the alternate hypothesis.

Table 4. t-test to determine if there is a significant difference between inoculated and control trickling filters effluent Mn(II) concentrations. p-values comparing Mn(II) concentration in the effluent of a filter inoculated with *Pseudomonas putida* and a control filter.

Trial #	p-value	df	Significantly lower
1	0.048	6	Yes
2	0.002	7	Yes
3	0.008	5	Yes

A two tailed *t*-test was used to determine if the Mn(II) concentration in the effluent of the inoculated filters is significantly different between each run (Table 5). It was found that each trial was significantly different than the other trials at $\alpha = 0.05$. This

may be caused by different environments within the inoculated filters, different *P. putida* cell concentrations, or *Pseudomonas putida* cultures that had adapted to the presence of Mn differently in each trial. Each comparison rejected the null hypothesis, the Mn(II) concentration was significantly identical when comparing trials one, two, and three. The alternate hypothesis claimed each trial was significantly different when compared to each other.

Table 5 - t-test to determine if the effluent Mn(II) concentrations in each experimental trial in the inoculated filters are significantly different. The p-values below show that the Mn(II) concentration found in the effluent of the inoculated filters is significantly different when comparing each experimental trial.

Trial comparison	**p-value**	**df**	**Significantly different**
1 and 2	0.008	6	Yes
1 and 3	0.004	7	Yes
2 and 3	0.020	5	Yes

II.3.4 Characterizing biogenic manganese oxides in trickling filters using scanning electron microscope images.

Scanning electron microscope (SEM) images of Mn oxides can indicate the source of Mn oxidation. The bright crystalline structure of biogenic Mn oxides adsorbed to the surface of the glass beads in the *Pseudomonas putida* filter was much more pronounced in the SEM images when compared to the surface of the glass beads in the

control filter (Figure 11). This indicates higher amount of Mn oxidized and adsorbed in the inoculated filters as compared to control filters. The biogenic oxidized Mn appears to be far more porous and jagged when compared to other SEM images of Mn(IV) that were oxidized chemically (Hu et al. 308-313). The increased porosity of biogenic Mn oxides has a much larger specific surface area than that of chemically oxidized Mn, making it a stronger oxidant. The greater oxidation potential of the biogenic Mn oxides that accumulate around the cell membrane of *Pseudomonas putida* (not distinguishable in the SEM pictures) may protect the cell from other harmful chemicals.

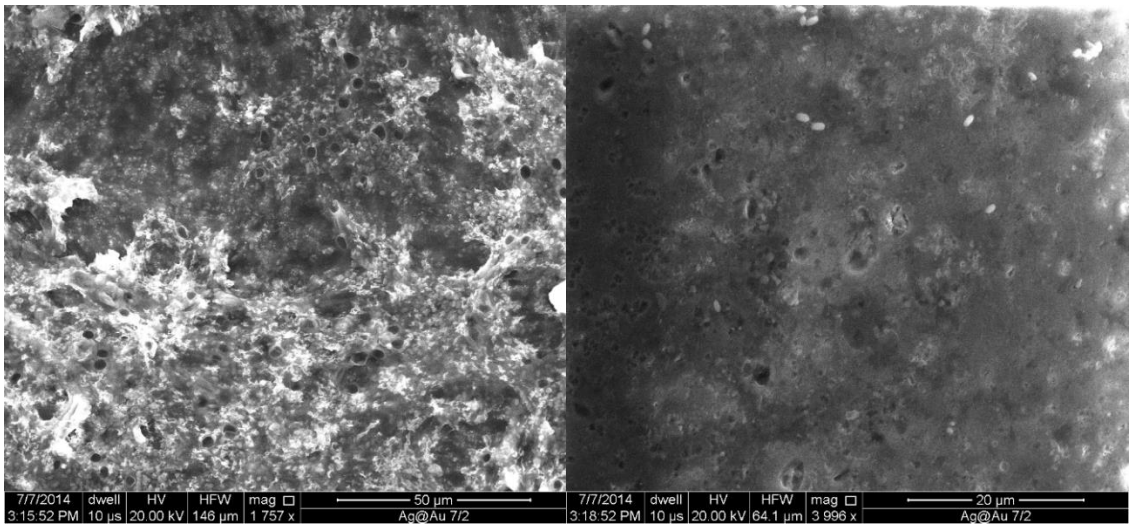

Figure 11. SEM image comparison of Mn particles on the solid media of inoculated and control trickling filters. SEM pictures showing a high concentration of crystalline Mn oxides adsorbed to glass beads of the *Pseudomonas putida* filter (left) compared to relatively little Mn oxidation on the solid media of the control filter (right).

II.3.5 Advective dispersive and first order kinetic models for Mn(II) effluent concentration in trickling filters inoculated with *Pseudomonas putida*.

CXTFIT was used to create a one dimensional advective-dispersive model (ADE) for each trial. CXTFIT is a computer program for estimating solute concentrations and modeling solute transport (Tang et al. 1200). The predicted concentration of Mn in the effluent of the trickling filter was found using CXTFIT and assuming intermediary decay coefficients and retardation factors equal to one. The final decay coefficients and retardation factors were found by minimizing the average squared difference between the observed and predicted data. An overall model was created by using the average values of the decay coefficient and the retardation factor obtained by three trials. The average decay coefficient for Mn(II) was determined to be 3.29 min^{-1} and the average retardation factor was determined to be 282.86. The retardation factor is comparable to other transport research results dealing with transport of oxidizing agents through well-developed biofilms (Hu et al. 936-941; Davison, Pitts, and Stewart 2920-2927). The half-life for biological manganese oxidation estimated using the decay coefficient is much lower than previous research (Katsoyiannis and A. 1922-1932). Advection dispersion models for estimating Mn concentration were created using the following assumptions:

- Porosity ($\emptyset$) = volumetric water content
- Dispersivity (α) = 0.5
- $D_o a e^{b\emptyset}$ negligible

The distance (x) to which Mn contaminated water needed to flow through in the trickling filter was estimated to be 10 cm under the laboratory conditions. The advection velocity (v) and diffusion coefficient (D) was determined to be 20.98 cm/min and 10.49 cm^2/min respectively, using the equations shown in the Materials and Methods section. The advection dispersion model fitted using data from the *Pseudomonas putida* inoculated filter collected in first trial resulted in a decay coefficient of 1.01 min^{-1} and a retardation factor of 1 (Figure 12 and Table 6). The advection dispersion model fitted using data from the non-inoculated filter collected in first trial resulted in a decay coefficient of 0.36 min^{-1} and a retardation factor of 1 (Figure 13 and Table 6). An average of 38% of Mn was removed during this trial while the AD model predicted an average removal of 38% of Mn. This was the lowest percent removal of Mn from the effluent water (Table 3). Trial one also had the smallest decrease for observed and predicted Mn in the effluent. The amount of observed and predicted Mn that was oxidized was 5.23 µg/mL and 5.22 µg/mL respectively. The high concentration of Mn in the effluent is due to the low removal rate in the first 6 hours of measurement. Increasing the inoculation period would likely improve the removal rates.

The model for the second trial had a decay coefficient of 3.40 min^{-1} and a retardation factor of 597.07 (Figure 12 and Table 6). The advection dispersion model fitted using data from the non-inoculated filter collected in second trial resulted in a decay coefficient of 0.31 min^{-1} and a retardation factor of 1 (Figure 13 and Table 6). The average observed and predicted percent removal of Mn was 81.78% and 82.81% respectively. Trial two had the largest decrease for observed and predicted Mn

concentration in the effluent. Observed and predicted Mn concentration was decreased by 7.59 µg/mL and 7.67 µg/mL respectively.

The decay coefficient was 5.46 min^{-1} and a retardation factor of 250.52 for trial three (Figure 12 and Table 6). The advection dispersion model fitted using data from the non-inoculated filter collected in third trial resulted in a decay coefficient of 0.39 min^{-1} and a retardation factor of 1 (Figure 13 and Table 6). The observed and predicted percent Mn removal were 90.58% and 90.65% respectively (Figure12). The observed and predicted Mn concentrations were both decreased by 6.17 µg/mL.

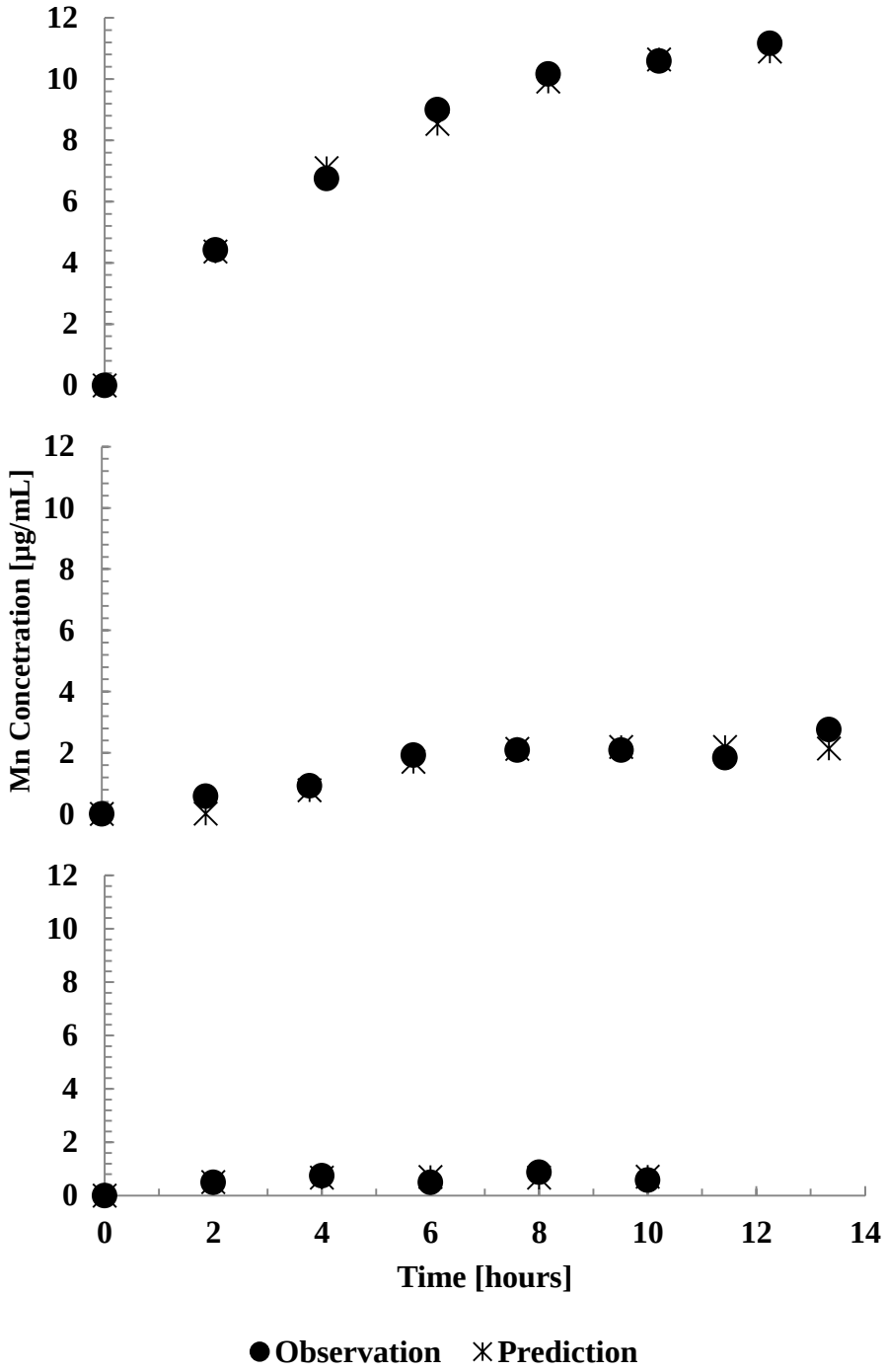

Figure 12. Predicted vs. observed Mn(II) concentrations in Pseudomonas putida inoculated trickling filters using an advection dispersion model. Comparison between the predicted model and the observed data for Mn(II) concentration in the effluent of the Pseudomonas putida trickling filter for the first (top), second (middle), and third (bottom) experiments, with r^2 values.

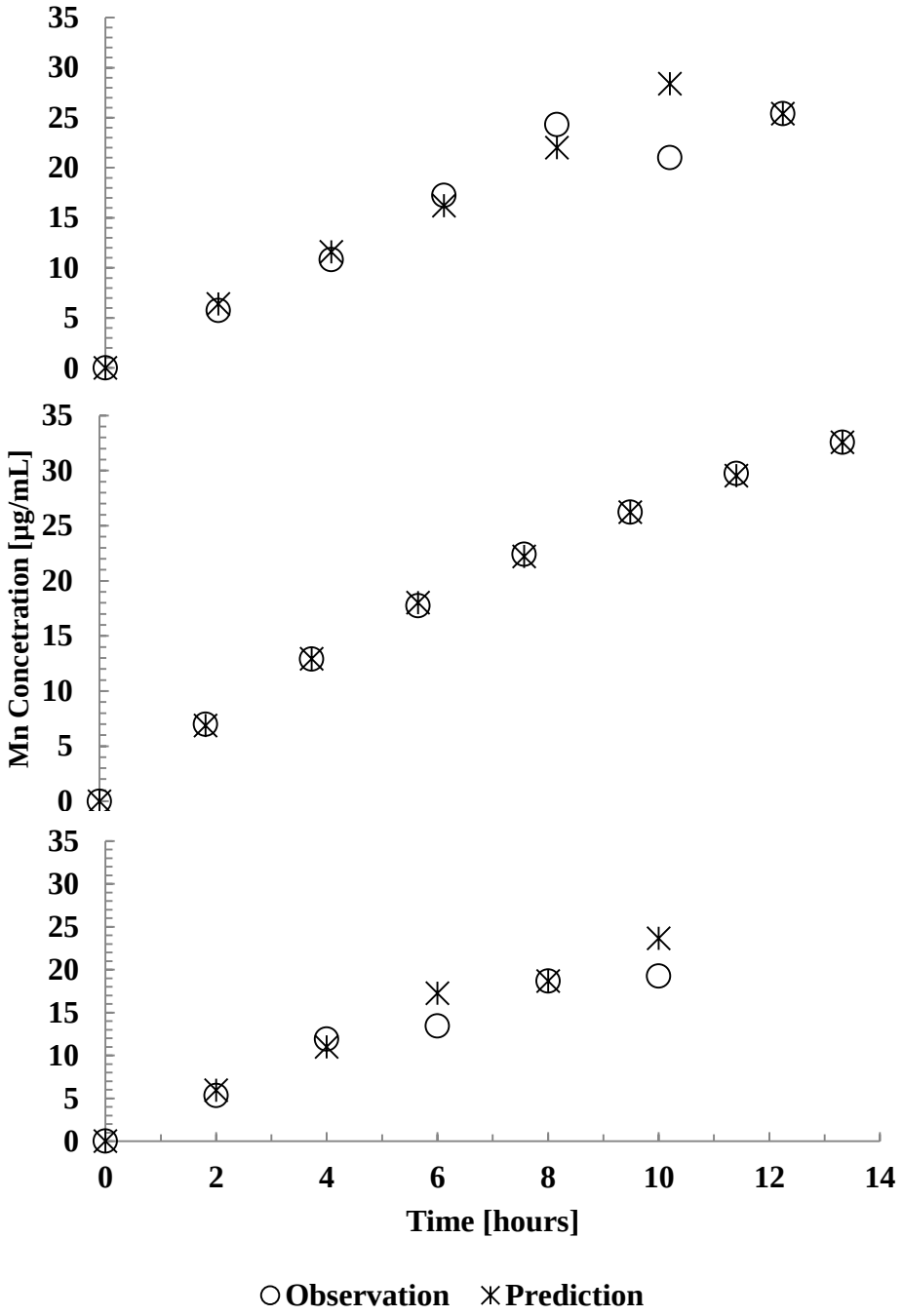

Figure 13. Predicted vs. observed Mn(II) concentrations in control trickling filters using an advection dispersion model. Comparison between the predicted model and the observed data for Mn(II) concentration in the effluent of the control trickling filter for the first (top), second (middle), and third (bottom) experiments, with r^2 values.

Table 6. Decay coefficients and retardation factors for inoculated and non-inoculated trickling filters.

Trial	Inoculated decay coefficients	Non-inoculated decay coefficients	Inoculated retardation factors	Non-inoculated retardation factors
1	1.01	0.36	1.00	1.00
2	3.40	0.31	597.07	1.00
3	5.46	0.39	250.52	1.00
average	3.29	0.35	282.86	1.00

The decay coefficients and the retardation factors listed above were averaged to create a model to predict Mn removal from *Pseudomonas putida* inoculated filters and non-inoculated filters using the ADE (Figure 14 and Table 6). In the modeled *Pseudomonas putida* inoculated filters the average values for the decay coefficient and retardation factor were 3.30 min^{-1} and 282.86, respectively assuming an initial influent concentration 7.16 µg/mL. In the modeled non-inoculated filters the average values for the decay coefficient and retardation factor were 0.35 min^{-1} and 1. It was noted that an increase of 100's or 1000's was needed for the retardation factor to make a small but noticeable difference in the Mn Concentration. The retardation factors found using CXTFIT have a noticeable effect on the predicted Mn concentration during the first 6 hours of operation, but had a negligible effect on the concentration of Mn in the effluent of the trickling filters afterwards. The predicted Mn concentration is mostly influenced by the decay coefficient. A retardation factor of 1 can be assumed when using the ADE,

which also implies Mn oxidation in both inoculated and non-inoculated trickling filters follow first order kinetics.

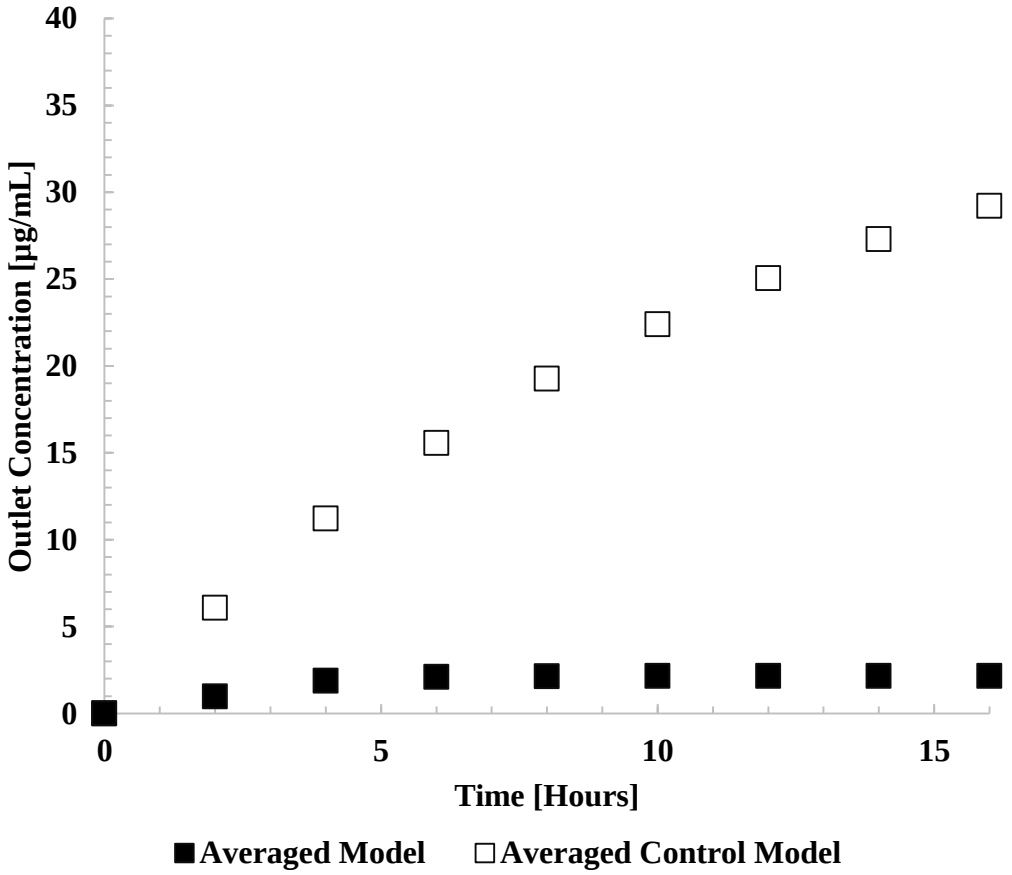

Figure 14. Advection dispersion model to predict Mn(II) concentrations in inoculated and non-inoculated trickling filters effluents. ADE models for inoculated and non-inoculated trickling filters using the averaged decay coefficients and retardation factors.

Because Mn was introduced to the filter as step input, a flux type boundary condition can be assumed for the ADE. Likewise the majority of the liquid media was recycled through the trickling filter therefore a semi-infinite boundary condition can be

assumed for the trickling filter. These boundary conditions allow for the following analytical solution:

$$S(x) = \frac{\gamma}{\lambda} + \frac{\left(S_o - \frac{\gamma}{\lambda}\right) 2v}{u + v} \exp\left(\frac{(v-u)x}{2D}\right)$$

$$u = v\sqrt{(1 + \frac{4\lambda D}{v^2})}$$

Using the above analytical solution with the average decay coefficient (3.30 min^{-1}) the Mn concentration at varying filter depths can be determined (Figure 16). The trickling filters had support media depths of approximately 10 cm of glass beads and 4 cm of pea pebbles. There was no visible manganese precipitated and coated on pea pebbles (Figure 6, 7, 8, and 9); so the support media depth of the trickling filter was assumed to be10 cm. According to the ADE model, a trickling filter with 10 cm of solid media with a continuous Mn(II) input of approximately 10 µg/mL at 0.25 mL/min will have a bulk effluent Mn(II) concentration of 2.36 mg/L (Figure 15). The AD model predicts an effluent Mn concentration in a 10 cm trickling filter that is similar to the effluent Mn concentration from trial two, but the actual removal rates in trials two and three (Table 3) indicated the ADE may underestimate the Mn concentration.

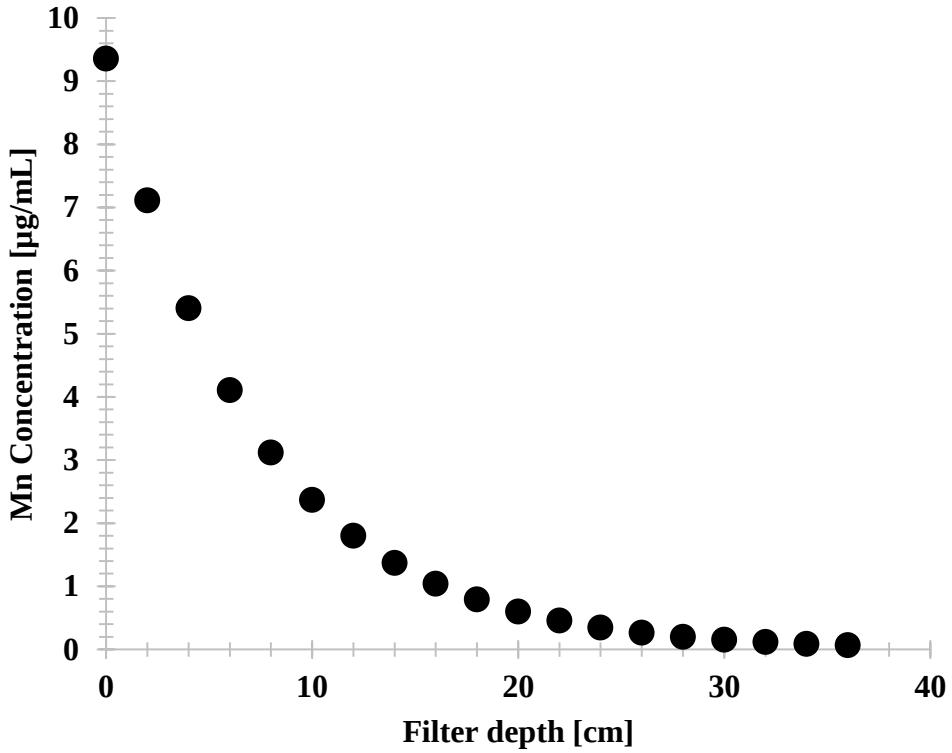

Figure 15. Bench Scale Trickling Filter ADE: semi-infinite, continuous flow. Theoretical Mn concentration at different depths for a bench scale tricking filter with influent Mn concentration of 10 µg/mL and advection velocity of 20.98 cm/ min.

Assuming the decay coefficient in the trickling filter matches that of the average prediction model (3.30 min^{-1}) the ADE model indicates that a filter depth of 38 cm is needed to reduce influent concentration of 10 µg/mL to 0.05 µg/mL. If the influent concentration resembled typical environmental conditions where the Mn concentration was a maximum of 2 µg/mL the support media depth would only need to be 26.5 cm. For full scale operation a support media of 0.21 m will be needed to reduce 1 mg/L of Mn to 0.05 mg/L (Figure 16). Pilot scale biological filters with mixed cultures using

similar flow rates and influent Mn concentration required filter depths of approximately 0.8 m to achieve the same level of removal (Stembal et al. 327-335). This indicates that trickling filters inoculated with *Pseudomonas putida* may be much more efficient than mixed culture biological filters.

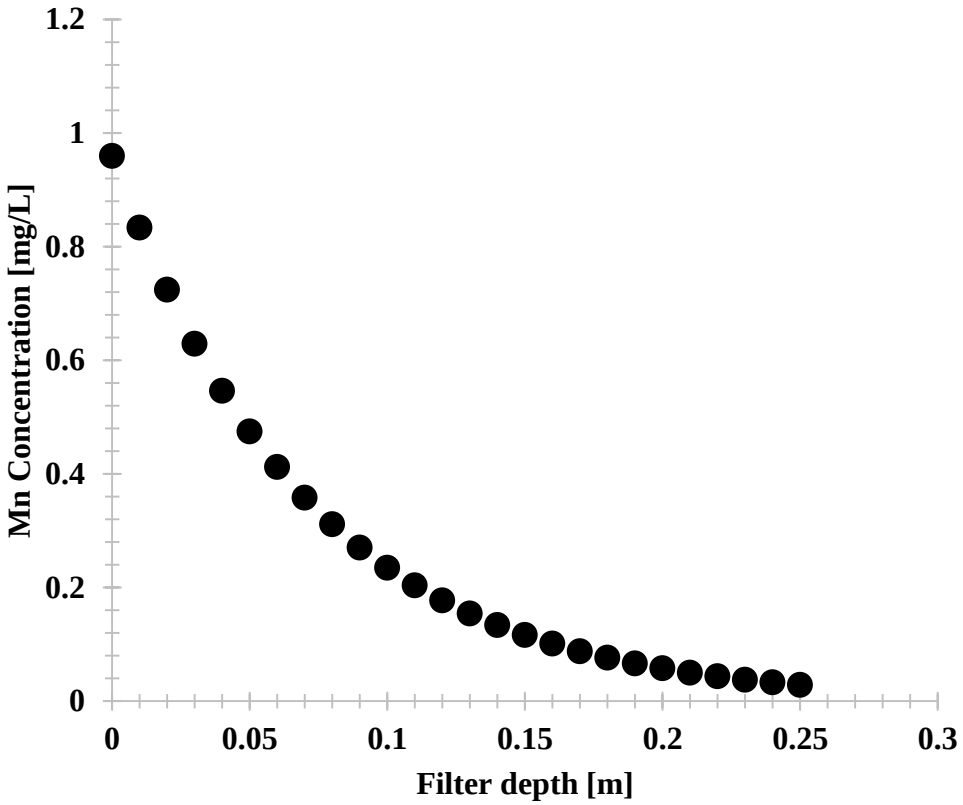

Figure 16. Full Scale Trickling filter ADE: semi-infinite, continuous flow. Theoretical Mn concentration at different depths for a full scale tricking filter with influent Mn concentration of one milligram per liter and advection velocity of 12.58 m/hr.

Mn oxidation was found to follow first order decay kinetics so the half-life could be found using the following equation:

$$Half\ Life = \frac{\ln(2)}{\lambda}$$

The half-life for biochemical Mn oxidation in trickling filters inoculated with *Pseudomonas putida* was determined to be 0.225 min. The Mn oxidation rate was found to be 17.7 times faster in *Pseudomonas putida* inoculated trickling filters than a similar study that utilized *Leptothrix ochracea* and *Gallionella ferruginea* in a two stage up flow fixed-bed filter to remove Mn from groundwater sources (Katsoyiannis and A. 1922-1932). This shows that *Pseudomonas putida* inoculated filters have potential for increased Mn(II) oxidation rates in comparison to biological filters that utilize other Mn oxidizing bacteria and mixed cultures.

II.4 Conclusions

The startup time for trickling filters inoculated with *Pseudomonas putida* was only 48 hours, which is much less than typical biological filters currently in use. Bench scale trickling filters inoculated with *Pseudomonas putida* have been shown to remove up to 90% of Mn(II) from the influent water resulting in six times greater removal rates than well aerated trickling filters inoculated with only ambient bacteria. The biogenic Mn oxides produced during the process appears to be very porous and have jagged shapes according to the SEM images. A fate and transport model of the Mn(II) in the *Pseudomonas putida* inoculated filters was formulated using a one dimensional advective dispersive equation. The decay coefficient for the ADE model was

determined to be 3.30 min^{-1}. The retardation factor was determined to be negligible (1). The bench scale experimental results using *P. putida* inoculated trickling filters showed much higher Mn(II) removal rates than mixed culture biological filters.

CHAPTER III

SUMMARY AND RECOMMENDATIONS

Trickling filters inoculated with *Pseudomonas putida* had significantly increased Mn(II) oxidation rates when compared to well aerated trickling filters exposed to ambient conditions under laboratory conditions. The time it took to achieve Mn removal of up to 90% using *Pseudomonas putida* was only 48 hours. A one-dimensional advective dispersive equation was used to simulate Mn removal in the inoculated filters. The ADE model predicted an average Mn removal rate of 72% in a bench scale trickling filter with a depth of 10 cm. The model also predicted full scale trickling filters with a solid media depth of 280 cm and an advection velocity of 12.58 m/hr can reduce Mn at a concentration of 1 mg/L to below 0.05 mg/L.

Although 48 hours appeared to be a sufficient startup time, the actual inoculation period should be delegated to the operators' best judgment due to the large variability in growth and attachment seen in the inoculation stage of the experiment. Forty-eight hours is a short startup time compared to other inoculation procedures. Additional research should be conducted to see if a more accurate model can be created to simulate and predict fate and transport of Mn(II) in porous media. Possible alternative models such as the non-equilibrium two-region mobile/immobile model, or the stochastic-convective transport model may be better suited for analysis in some cases. Further studies are required to assess the feasibility of inoculating other types of biological filters with *Pseudomonas putida* such as rapid sand filters for removing Mn from drinking

water. Further studies involving different filter configurations, flow rates, initial Mn(II) concentrations, and nutrient inputs should be conducted before considering biological oxidation using *P. putida* for full scale operation. Obtaining consistent Mn(II) oxidation rates in different filter set up while using *P. putida* is a prerequisite for large scale implementation of the process. The effects of long term operation are unknown, but the process may still be used during filter startup to treat Mn contaminated water for short periods of time while uniform biofilm layers are established.

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