BIOGEOCHEMICAL CYCLING OF MANGANESE IN DRINKING WATER SYSTEMS

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ABSTRACT

This work represents an interdisciplinary effort to investigate microbiological and chemical manganese (Mn) cycling in drinking water systems using concepts and tools from civil and environmental engineering, microbiology, chemistry, surface science, geology, and applied physics.

Microorganisms were isolated from four geographically diverse drinking water systems using selective Mn-oxidizing and -reducing culture media. 16S rRNA gene sequencing revealed that most are bacteria of the *Bacillus* spp. (i.e., *Bacillus pumilus* and *Bacillus cereus*). These bacteria are capable of performing Mn-oxidation and -reduction under controlled laboratory conditions. Pseudo-first order rate constants obtained for microbiological Mn-oxidation and -reduction (aerobic and anaerobic) of these isolates ranged from 0.02 - 0.66 days^{-1}. It is likely that spores formed by *Bacillus* spp. protect them from chlorine and other disinfectants applied in drinking water systems, explaining their ubiquitous presence.

A new method was developed using X-ray photoelectron spectroscopy (XPS) to identify Mn(II), Mn(III), and Mn(IV) on the surfaces of pure oxide standards and filtration media samples from drinking water treatment plants. A necessary step for the comprehensive analysis of Mn-cycling in drinking water systems is to characterize the chemical properties of filtration media surfaces. Analyses of filtration media samples show that, while Mn(IV) was predominant in most samples, a mixture of Mn(III) and Mn(IV) was also identified in some of the filtration media samples studied. The use of both the XPS Mn 3s multiplet splitting and the position and

shape of the Mn 3p photo-line provide added confidence for the determination of the oxidation state of Mn in complex heterogeneous environmental samples.

XPS was applied to investigate Mn(II) removal by $MnO_x(s)$-coated media under experimental conditions that closely resemble situations encountered in drinking water treatment plants in the absence and presence of chlorine. Macroscopic and spectroscopic results suggest that Mn(II) removal in the absence of chlorine was mainly due to adsorption, while in the presence of chlorine was due to oxidation. Mn(IV) was predominant in all the XPS analyses while Mn(II) was detected only in samples operated without chlorine. Future research should apply XPS under different experimental conditions to understand the specific mechanisms affecting Mn(II) removal by $MnO_x(s)$-coated media.

DEDICATION

The author dedicates this work to Dr. G.V. Loganathan (an exemplary human being, teacher, mentor, researcher, and outstanding engineer; proficient in physics and mathematics), Daniel Patrick O'Neil (great songwriter and Beatles fan, had lots of fun playing together…), Matthew Gregory Gwaltney and Brian R. Bluhm (great friends, can't think of Brian without Matt together in the Patton graduate student room **-** ping pong…), Jeremy Michael Herbstritt (very funny and energetic friend, not "the best" soccer player in the world…), and Juan Ramón Ortiz-Ortiz (very talented friend and a great percussionist although we never had the chance to play together). Their talent, human quality, contributions and achievements will forever be a source of inspiration.

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AUTHOR'S PREFACE & ATTRIBUTION

This dissertation is a detailed report of an interdisciplinary investigation performed since the Fall Semester 2005 to better understand biological and chemical cycling of manganese applied to drinking water systems. This document is composed of four chapters which stand as individual articles that are either in review or will be submitted to peer-reviewed articles. Chapter I will be submitted as a review article to the journal *Critical Reviews in Environmental Science and Technology*. Chapter II has been accepted to *Water Research*. Chapter III is in review in *Environmental Science and Technology*. Chapter IV will also be submitted to *Environmental Science and Technology*.

The chapters were organized as a function of the following logical research approach: a) review background about microbiological and chemical factors affecting Mn-cycling in both natural and engineered systems to link relevant concepts and identify gaps in the literature; b) evaluate the presence of Mn-oxidizing and - reducing microorganisms in drinking water systems that could affect Mn deposition and release; c) develop a method to determine Mn-oxidation states in anthracite media oxide-coated samples using X-ray photoelectron spectroscopy (XPS) to obtain a chemical characterization of the solid-microbe-solution interface; and d) apply XPS to investigate Mn(II) removal by Mn-oxide coated in order to understand specific mechanisms affecting Mn treatment in drinking water.

The work described was a highly collaborative effort; direct input was received from the following persons at Virginia Tech: Dr. Andrea M. Dietrich (Civil and Environmental Engineering, provided advice related to all the chapters of this dissertation), Dr. William R.

Knocke (Civil and Environmental Engineering, provided advice related to all the chapters of this dissertation), Dr. Joseph O. Falkinham III (Biological Sciences, provided advice related to the microbiological aspects covered in Chapters 1 and 2), Dr. Michael F. Hochella Jr. (Geosciences, provided advice related to the XPS and surface science analyses covered in Chapters 1, 3 and 4), Dr. Amy Pruden (Civil and Environmental Engineering, provided advice related to the molecular microbiology analyses presented in Chapter 2), Chad W. McKinney (Civil and Environmental Engineering, performed the molecular microbiology analyses presented in Chapter 2), Thomas F. Cromer (Chemistry, performed XPS analyses presented in Chapters 3 and 4), and Andrew Jones (assisted in the experimental work presented in Chapter 4) . The names of the co-authors have been provided accordingly in each chapter of the dissertation.

CHAPTER 1: LITERATURE REVIEW - Chemical and Microbiological Cycling of Manganese Applied to Drinking Water Systems

José M. Cerrato, Andrea M. Dietrich, William R. Knocke, Joseph O. Falkinham III, Michael F. Hochella Jr.

ABSTRACT

The focus of this literature review is biogeochemical cycling of Mn in natural systems and applications in engineered drinking water systems that affect Mn treatment, release, and deposition. The continuous investigation of interactions between microbiological and chemical factors is considered key to understanding biogeochemical Mn cycling in natural systems. Microbiological factors affecting Mn-release and deposition in engineered drinking water systems are often overlooked since it is assumed that disinfection processes "suppress" biological activity and hence only chemical factors are relevant. This review summarizes microbiological and chemical Mn oxidation and reduction in natural systems, chemical and biological processes for Mn removal in drinking water systems, and Mn-release and deposition in drinking water systems. Research challenges and tools for the investigation of microbiological and chemical cycling in both natural and engineered systems are also described.

1.1. Introduction

1.1.1. Broader Impacts and Objective

Manganese (Mn) is a transition metal that plays a key role in relevant environmental biogeochemical processes. Its fate and transport is affected by interactions with natural

(inorganic and organic) chemicals and a wide range of microorganisms. Chemical and microbiological oxidation and reduction reactions have a major effect on the formation and dissolution of Mn mineral phases that affect the release, sequestration, bioavailability and toxicity of heavy metal contaminants. The occurrence of Mn in engineered drinking water systems can cause undesired effects in water quality. Mn is also an essential trace element for human beings; however, excessive intake can cause adverse health effects.

Mn is ubiquitous in groundwater and surface water sources of drinking water. Negative consequences associated with the presence of Mn in drinking water systems include the formation of biofilms and secondary disinfectant consumption, contributing to microbiological activity in the distribution system and growth of opportunistic pathogens (Camper and Jones, 2002, Cerrato et al., 2006, Digiano and Zhang, 2005, Lechevallier et al., 1987, Norton et al., 2003, Sly et al., 1990). The occurence of Mn in drinking water distribution systems also causes water discoloration, staining on plumbing fixtures and consumer complaints (Dietrich, 2006, Hargette and Knocke, 2001). Soluble Mn(II) concentrations as low as 0.02 mg/L can cause drinking water to turn yellow or black due to oxidation to particulate $MnO_{x(s)}$ (Carlson and Knocke, 1999, Sly et al., 1990). Many water utilities throughout the United States have attributed particulate matter problems in their distribution system to the presence of Mn (Booth and Brazos, 2004). Water distribution pipe infrastructure from drinking water systems containing excessive Mn can be negatively affected by the formation of a "surface coating". This Mn surface coating is well incorporated into galvanized iron pipe causing the formation of corrosion tubercles, but can be readily dislodged from PVC pipe by flowing water (Cerrato, 2005, Cerrato et al., 2006).

Engineered drinking water systems present a special case as water treatment and distribution processes link the natural environment of surface and ground waters with the anthropogenic environment of household faucets and sinks. Although chemical and microbiological interactions similar to those in natural systems occur in engineered water treatment plants and water distributions systems when Mn is present, the microbiological factors affecting the cycling of manganese in drinking water are often ignored or overlooked. Limited information exists documenting the contribution of chemical versus microbiological processes affecting manganese release and deposition in disinfected drinking water systems.

Other reviews have either focused exclusively on the biogeochemistry of Mn in natural systems (Borch et al., 2010, Brown et al., 1999, Ehrlich, 1987, Nealson and Popa, 2005, Nealson and Saffarini, 1994, Tebo et al., 2005) or on Mn removal and deposition in water treatment plants and distribution systems (American Water Works Association, 1999, Mouchet, 1992, Vaishya and Gupta, 2003). This review presents relevant literature related to biogeochemical cycling of Mn in both natural and engineered systems, and provides a brief discussion about research challenges and analytical tools relevant to the study of biogeochemical interactions.

1.1.2. Regulations and Health Effects of Manganese

Due to negative aesthetic effects caused by Mn in drinking water, the U.S. Environment Protection Agency (USEPA) has established a non-enforceable secondary maximum contaminant limit (SMCL) of 0.05 mg L^{-1} Mn (American Water Works Association, 1999); the World Health Organization has set a guide value of 0.4 mg L^{-1} (World Health Organization, 1998). Customers who experience water discoloration problems lose confidence in the service

that their water utilities provide (Whelton et al., 2007). The loss in confidence can be a costly and difficult problem for water utilities to resolve since it contributes to public scrutiny of the water supply industry (Hargette and Knocke, 2001).

Although concerns with Mn in drinking water are predominantly related to aesthetic problems, studies indicate a possible relation between Mn chronic exposure in humans and neurotoxic effects (Kondakis et al., 1989, Michalke et al., 2009, 2007). Inhalation and ingestion are the major paths of Mn intake into the human body (Michalke et al., 2007). The most significant anthropogenic sources of Mn-exposure are Mn-mining ores, associated with severe behavioral and muscle changes (Donaldson, 1987, Michalke et al., 2007). Low level Mn-exposure via inhalation can also be neurotoxic (Elsner and Spangler, 2005, Kondakis et al., 1989). While drinking water can be both ingested and inhaled during showering or other water uses, adverse effects due to ingestion of excess Mn in drinking water are seldom reported (Michalke et al., 2007).

1.2. <u>Biogeochemistry of Manganese in Natural Drinking Water Sources</u>

1.2.1. Manganese in the Environment

Soluble Mn can be found in natural water supply sources including groundwater and the hypolimnetic regions of surface water reservoirs. Mn is involved in various environmental ion exchange reactions since it reacts at the pe-pH potential boundaries characteristic of natural systems. Manganese (Mn) is an abundant transition metal and is found in alternating valence states that can be readily transformed under physical-chemical conditions commonly found in nature (Nealson and Saffarini, 1994, Stumm and Giovanoli, 1976). Mn can exist in the oxidation

states 0, +2, +3, +4, +6, and +7; the values of +2, +3, and +4 are the most important in natural systems (Davies and Morgan, 1989, Ehrlich and Newman, 2009). The reduced form, Mn(II), is soluble while oxidized forms, Mn(III) and Mn(IV), are usually present as oxyhydroxides (Nelson et al., 2002).

Both Mn and iron (Fe) are present in natural waters and undergo similar distribution patterns. The solid phase Fe(III) oxides are found below Mn(IV) oxides at the oxic zone of the water column in natural environments because iron is more readily oxidized than manganese (Nealson and Popa, 2005). Mn(II) is found above Fe(II) because the electron potential of Mn(IV) is higher than that of Fe(III) (Nealson and Saffarini, 1994). Oxidized forms of manganese are more readily reduced than those of iron because Fe(II) can reduce most Mn(IV) phases (Burdige et al., 1992, Nealson and Saffarini, 1994). Table 1-1 provides a comparison of different electron acceptors which permits one to distinguish those that are more energetically favored than others (Nealson and Saffarini, 1994).

The oxidation of Mn is significant in the control of carbon redox cycling in the environment (Nealson and Saffarini, 1994). Mn-oxidizing and -reducing microorganisms play a major role in the geochemical cycle of manganese in aquatic sediments (both in freshwater and marine environments), submerged soils, and groundwater (Ehrlich and Newman, 2009, Lovley, 1991). Mn accumulation in the sediments of natural surface water reservoirs and in soils occurs in the form of oxides, carbonates, and silicates (Ehrlich and Newman, 2009, Nealson and Saffarini, 1994). Mn(II) is the stable form in reducing environments (Davies and Morgan, 1989). Mn(II) persists in reducing environments because Mn(III) and Mn(IV) oxides are reduced by natural organics (Ehrlich and Newman, 2009, Morgan, 2005, Nealson and Saffarini, 1994, Stone and

Morgan, 1984b) and because of the slow oxidation kinetics of Mn(II) with oxygen (Davies and Morgan, 1989). The formation and stability of Mn(III) oxide solids during the chemical and microbiological oxidation of Mn(II) have been reported (Junta and Hochella, 1994, Murray et al., 1985, Webb et al., 2005a). A summary of relevant factors affecting Mn-oxidation and reduction in natural waters is presented in Table 1-2.

1.2.2. Factors affecting Abiotic Mn-oxidation and –reduction

1.2.2.1. Abiotic Mn oxidation

Oxygen is one of the key factors affecting abiotic Mn-oxidation. Mn(II) oxidation by oxygen is a key processes in that affects cycling of Mn(II), Mn(III), and Mn(IV) in natural waters. Kinetics of Mn(II) oxidation with oxygen in natural waters have been reported in numerous studies (Davies and Morgan, 1989, Kessick and Morgan, 1975, Morgan, 1967b, Morgan, 2005). Rates of manganese oxidation by oxygen at the pH range (6-9) of natural waters are typically slow (Morgan, 1967b); the magnitude of half-life constants is typically in the order of several days. The abiotic oxidation of Mn(II) can be described by the following general equation (Morgan, 2005, Stumm and Morgan, 1996):

$$-\frac{d[Mn(II)]}{dt} = k_1[Mn(II)] + k_2[Mn(II)][MnO_{x(s)}] + k_3[Mn(II)][Substrate]$$
$$\ldots\ldots\ldots\ldots\ldots(1)$$

This equation represents the homogeneous oxidation of Mn accompanied by a heterogeneous catalytic action in the presence of newly oxidized Mn surfaces and of externally added mineral substrates (Davies and Morgan, 1989, Junta-Rosso et al., 1997). The term "homogenous" refers

to abiotic Mn(II) oxidation in solution by oxygen only, while the term "heterogenous" refers to abiotic Mn(II) in the presence of surface substrates.

Pseudo-first order kinetics have been used to describe the homogenous oxidation of Mn simplifying Equation (1) (Davies and Morgan, 1989):

$$-\frac{d[Mn(II)]}{dt} = k_1[Mn(II)]$$ ……………………………………………………………….……(2)

Equation (1) provides a more applicable model than Equation (2) in the context of natural systems as a variety of mineral surface substrates are commonly encountered. The following expressions describe the elementary steps for the oxidation of Mn(II) species with oxygen and their second order rate constants ($M^{-1}s^{-1}$) at zero ionic strength (Morgan, 2005, Rosso and Morgan, 2002):

$Mn^{+2} + O_2 \rightarrow Mn^{+3} + O_2$ log k = -17.82…..…………………………..………(3)

$Mn(OH)^+ + O_2 \rightarrow Mn(OH)^{+2} + O_2^-$ log k = -9.2……..……………………………………(4)

$Mn(OH)_2 + O_2 \rightarrow Mn(OH)_2^+ + O_2^-$ log k = -3.1…………………………………………(5)
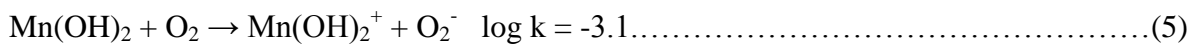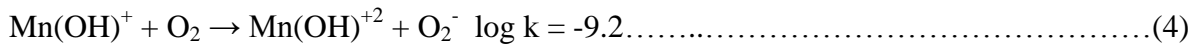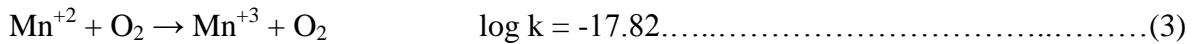
Metal oxide surfaces have a catalytic effect on Mn(II) oxidation in the presence of oxygen (Davies and Morgan, 1989, Junta and Hochella, 1994, Morgan, 2005). Manganese oxides encountered in rocks and soils have a strong reactivity with respect to sorption and redox chemistry (Hochella, 2002, Murray, 1974). Microscopic and spectroscopic evidence has shown that "steps" (surface shape indentations extending in the horizontal and vertical direction) in the surface of metal oxides are the most reactive sites for initiating adsorption-oxidation reactions for the heterogeneous oxidation of Mn(II) (Junta-Rosso et al., 1997, Junta and Hochella, 1994).

Spectroscopic studies have shown the formation and stability of Mn(III) oxide solids as a result of Mn-oxidation catalyzed by metal oxide surfaces (Junta and Hochella, 1994, Murray et al., 1985). Mn oxides are scavengers of heavy metal cations in the environment (Ehrlich and Newman, 2009). Metals such as Cu, Cd, Co, Pb, and As can be removed by complexation with manganese oxides (Ehrlich and Newman, 2009, Hochella, 2002, Nealson and Saffarini, 1994). Oxidation of MnO_x with naturally occurring organics may be an important sink for heavy metals in natural waters (Bertino and Zepp, 1991).

1.2.2.1. Abiotic Mn reduction

Reduction and dissolution of Mn are catalyzed by natural organic matter (NOM) and other organic and inorganic compounds (Canfield et al., 1993, Jun and Martin, 2003, Stone, 1987b, Stone and Morgan, 1984a, Stone and Morgan, 1984b). Reduced transition metals [particularly Fe(II) in several complexes], sulfides, and polyphenols are reductants commonly found in natural waters (Jun and Martin, 2003, Stone and Morgan, 1984b, Stumm and Morgan, 1996, Villinski et al., 2001). Non-enzymatic reduction of Mn(IV), Mn(III), and Fe(III) can cause the release of trace metals and phosphate into water supplies (Bertino and Zepp, 1991, Lovley et al., 1991). Adsorption of NOM, phosphate and bicarbonate to Mn(IV) and Mn(III) mineral surfaces may protect minerals from microbial Mn-reduction (Borch et al., 2007, Lovley, 1991). Extracellular organic and inorganic compounds generated by living organisms can affect the dissolution and speciation of manganese in the environment (Stone, 1997).

1.2.3. Microbial Mn(II)-oxidation

Biomineralization of Mn affects several key processes relevant to natural water environments as Mn-oxidizing microorganisms are ubiquitous. A wide variety of bacteria capable of performing Mn-oxidation have been identified in the Firmicutes [i.e. Bacillus and Halobacillus spp.], Proteobacteria [i.e. *Pseudomonas, Nitrosomonas, Pedomicrobium,* and *Erythrobacter* spp.], and Actinobacteria [i.e. Arthrobacter and Streptomyces spp.] (Tebo et al., 2005).

The following microorganisms have been well-studied and used as model Mn(II) oxidizing bacteria: gram positive spore-forming *Bacillus* sp. strain SG1, the γ-proteobacteria *Pseudomonas putida* MnB1 and GB-1, and the β-proteobacterium sheath-forming *Leptothrix discophora* strain SS-1 (Boogerd and Devrind, 1987, Devrind et al., 1986b, Francis et al., 2001, Tebo et al., 2005). Strains of *Bacillus* and *Pseudomonas* are capable of Mn‑oxidation (Devrind et al., 1986b, Dick et al., 2008, Okazaki et al., 1997, Tebo et al., 2005) and Mn‑reduction (Devrind et al., 1986a, Ghiorse and Ehrlich, 1976, Lovley, 1991, Nealson and Saffarini, 1994). Some species of *Leptothrix* are facultative aerobes that can oxidize both ferrous and manganous salts (Madigan et al., 2000). Heterotrophic species of iron-oxidizing bacteria of the genera *Sphaerotilis*, *Leptothrix*, *Chlonothrix*, and *Siderobacter* precipitate manganese and iron on their sheaths (Boogerd and Devrind, 1987, Ehrlich and Newman, 2009, Tebo et al., 2005). Certain species of *Methalogenium* are capable of oxidizing manganous oxide, manganous sulfate, or manganous carbonate to $MnO_{x(s)}$ for obtaining part of their energy. Other species are heterotrophic and contribute to the deposition of manganese and iron oxides in lake sediments together with other iron and manganese oxidizers (Jaquet et al., 1982, Kuznetsov, 1970, Oborn, 1964, Perfil'ev and

Gabe, 1969, Wetzel, 1995). Mn-oxidation can also be performed by fungi, i.e. *Trametes versicolor* and *Stropharia rugosoannulata* (Ehrlich and Newman, 2009, Tebo et al., 2005).

The formation of freshwater and marine manganese micronodules has been associated with bacterial activity (Ehrlich, 1998). Despite the well known catalytic role of Mn oxide surfaces (Junta and Hochella, 1994, Tebo, 1991), Mn(II) oxidation is many times faster in the presence of Mn oxidizing bacteria than under abiotic conditions in natural systems (Morgan, 2000, Nealson and Saffarini, 1994). Microbial oxidation of Mn(II) takes place at rates of up to 5 orders of magnitude greater than those for abiotic oxidation (Bargar et al., 2000).

Kinetics of microbial Mn-oxidation in natural waters have been represented by the Michaellis-Menten equation (Lehninger, 1975, Okazaki et al., 1997, Tebo and Emerson, 1986):

$$V = \frac{V_{max}[Mn(II)]}{(K_M + [Mn(II)])}$$ ……………………………………..…………………………………(6)

Where V is the reaction rate, V_{max} is the maximum reaction rate, and K_M is the Michaelis constant (substrate concentration at which the rate of conversion is half of V_{max}). V_{max} values range form to 5×10^{-4} to 70 nM hr^{-1} while K_M from 10^{-3} to 5 μM (Morgan, 2000, Okazaki et al., 1997, Tebo and Emerson, 1986).

The mechanisms for microbial oxidation of Mn are still unknown. Physiological studies suggest that not all microorganisms use the same Mn-oxidizing mechanism. Limited phylogenetic and molecular analyses have been performed for the majority of Mn-oxidizing bacteria (Ehrlich and Newman, 2009). Microbial Mn-oxidation can be enzymatic and nonenzymatic (Ehrlich and Newman, 2009). Molecular studies have shown that certain cultures belonging to the *Pseudomonas*, *Leptothrix*, and *Bacillus* genera require a multicopper oxidase for

the oxidation of Mn(II); these cultures deposit oxidized Mn on their cells, sheath, or spore, respectively (Ehrlich and Newman, 2009, Spiro et al., 2009, Tebo et al., 2005). While the oxidation of Mn(II) to Mn(IV) requires a two-electron transfer reaction, there is genetic and biochemical evidence that the activity of this multicopper oxidase is only known to engage one-electron transfers from substrate to O_2 (Spiro et al., 2009, Webb et al., 2005a). Non enzymatic Mn-oxidation can take place when microorgansisms produce one or more metabolic endproducts [i.e. extracellular compounds and hydroxycarboxylic acids] that cause chemical oxidation (Ehrlich and Newman, 2009).

1.2.4. Microbial Mn-reduction

Biologically mediated reduction is accountable for excessively high Mn and Fe in natural sedimentary environments such as aquatic sediments and ground water (Lovley, 1991, 2000). Isolates of the *Pseudomonas*, *Shewanella*, *Geobacter*, *Desulforomonas*, *Desulfovibrio*, *Arthrobacter*, and *Bacillus* species are capable of Mn and Fe reduction (Devrind et al., 1986a, Ehrlich and Newman, 2009, Lovley, 2000, Nealson and Saffarini, 1994). Many Mn(IV) reducing bacteria have found to be strict or facultative anaerobic organisms which are able to use a variety of other electron acceptors. *Desulforomonas acetoxidans* is capable of utilizing iron, manganese, sulfur, and malate as electron acceptors (Nealson and Saffarini, 1994). *Shewanella putrefaciens* can use oxygen, Fe(III), Mn(IV), NO_3^-, NO_2^-, $S_2O_3^-$, SO_3^-, fumarate and others as terminal electrons (Myers and Nealson, 1988a). Humic substances can mediate microbial Mn-reduction (Lovley et al., 1998). Microbiological reduction under both "aerobic" and "anaerobic" conditions has been reported (Ehrlich, 1987, Myers and Nealson, 1988a). Cells of marine *Bacillus* 29 grown in the presence of Mn(II) were able to reduce MnO_2 in the presence of

glucose "aerobically" and "anaerobically" (Ehrlich, 2008, Ghiorse and Ehrlich, 1976). A variety of Mn-reducers can also reduce Fe (Nealson and Saffarini, 1994). Mn and Fe are both electron acceptors that have a redox potential low enough to be non-toxic to microorganisms and high enough to be energetically favorable to the cell when coupled to organic carbon oxidation (Nealson and Saffarini, 1994). Mn(IV) and Fe(III) reducers are often capable of using a variety of electron acceptors, including oxygen and nitrate (Nealson and Popa, 2005, Nealson and Saffarini, 1994). Mn-reduction can also be performed by fungi (Ehrlich and Newman, 2009).

Pseudo-first order kinetics have been used to represent microbial Mn-reduction in natural waters (Dollhopf et al., 2000):

$$\frac{d[Mn_{reduced}]}{dt} = k_{obs}[Mn_{reduced}]$$ ……………………….…………………………………..(7)

Values for k_{obs} range from 0.005 to 0.0165 min^{-1} (Dollhopf et al., 2000).

As for Mn-oxidation, the mechanisms for microbial reduction of Mn are still unknown. Microbial Mn-reduction can be enzymatic and nonenzymatic, as happens for Mn-oxidation (Ehrlich and Newman, 2009). *Shewanella oneidensis* strain MR-1, are facultative aerobes known to reduce Mn(IV) oxides only through an anaerobic respiration process (Myers and Nealson, 1988a). Other microorganisms, such as *Geobacter metallireducens* and *Desulfovibrio desulfuricans,* are strict anaerobes and reduce Mn in an anaerobic respiration process to provide electrons for carbon oxidation (Lovley and Phillips, 1988, Lovley and Phillips, 1994). The c-type cytochromes (linked to mtrA, mtrB, MtrC, omcA and cyma genes) localized in the outer-membrane of *Shewanella oneidensis* are involved in enzymatically mediated Mn(IV) reduction

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for anaerobic respiration (Bretschger et al., 2007, Myers and Myers, 1992, Wigginton et al., 2007b).

Bacillus 29 and Baclillus sp. strain SG1 isolated from marine and freshwater environments can reduce Mn(IV) aerobically and anaerobically (Devrind et al., 1986a, Ehrlich, 1987, Ehrlich and Newman, 2009, Ghiorse and Ehrlich, 1976). The biochemical pathways of the microorganisms that perform Mn(IV) reduction under aerobic and anaerobic conditions are still unknown (Ehrlich and Newman, 2009). Indirect non-enzymatical Mn-reduction can take place through the production of microbial metabolites that are strong enough to cause the dissolution of Mn oxides (Ehrlich and Newman, 2009, Stone, 1987a, 1997).

1.3. <u>Manganese Removal in Drinking Water Systems</u>

1.3.1. General Aspects of Mn Treatment Processes in Drinking Water

Water treatment plants remove soluble Mn when present at elevated levels in natural water sources. Processes typically applied in conventional water treatment are primary disinfection, chemical oxidation, coagulation, sedimentation, filtration, and secondary disinfection. Soluble Mn(II) can be intentionally removed by chemical oxidation, followed by liquid separation and combined adsorption/oxidation onto manganese oxide coated filtration media (Coffey et al., 1993, Knocke et al., 1988, Knocke et al., 1991a). Manganese removal in waters with high organic loading is difficult to achieve since dissolved Mn can form complexes with natural organic matter (Carlson et al., 1997, Potgieter et al., 2005). Mn oxidation state +7 is important in the context of drinking water treatment because potassium permanganate ($KMnO_4$) is commonly applied as a chemical oxidant. Mn oxidation states +2, +3, and +4, are also relevant to engineered drinking water treatment as they are to natural systems.

Biological filtration has emerged as a cost-effective technology that utilizes microbiological oxidation for Mn removal from drinking water (Katsoyiannis and Zouboulis, 2004, Mouchet, 1992). Membrane filtration as a viable method for Mn removal from drinking water has also been investigated (Choo et al., 2005, Suzuki et al., 1998). In-line pre-chlorination in conjunction with ultrafiltration has been used as an alternative treatment method for Fe and Mn removal (Choo et al., 2005). Membrane fouling caused by Mn-oxide particles deposited during backwashing is a major problem associated with the application of ultrafiltration with chlorination for Mn removal (Choo et al., 2005).

1.3.2. Chemical Oxidation

Differing from natural systems in which oxygen is the prevalent oxidant, stronger oxidants are used in engineered water treatment plants to promote rapid abiotic Mn-oxidation. Ozone, potassium permanganate, chlorine dioxide, and chlorine are chemical oxidants commonly used in water treatment (Hao et al., 1991, Knocke et al., 1987, Knocke et al., 1991a, Van Benschoten et al., 1992, von Gunten, 2003). Second order rate kinetics have been used to represent chemical oxidation of Mn(II) (Hao et al., 1991, Jacobsen et al., 1998, Morgan, 2005, Van Benschoten et al., 1992). A summary of the kinetic data obtained from the literature for the oxidation of Mn(II) with ozone, potassium permanganate, chlorine dioxide, chlorine, and oxygen is presented in Table 1-3.

Ozone is an effective and widely used oxidant; it is a very unstable gas in aqueous solutions and, for this reason, it must be generated on-site for its utilization in water treatment (American Water Works Association, 1999, Reckhow et al., 1991). Natural organic matter exerts considerable ozone demand when present in high concentrations in the raw water (Hoigne and

Bader, 1983a, b, Reckhow et al., 1991, von Gunten, 2003). Ozonation requires supplemental catalytic action by filtration/adsorption to granular activated carbon (GAC) to remove manganese when present in complex organic compounds, as often encountered in groundwater (White, 1998). Potassium permanganate and colloidal solids of insoluble manganese dioxide can be formed as a product of soluble manganese oxidation by ozone (Reckhow et al., 1991, von Gunten, 2003).

Potassium permanganate ($KMnO_4$) is another oxidant that has been widely used for Mn-oxidation in drinking water treatment (Knocke et al., 1991b, Van Benschoten et al., 1992); it contains Mn in the $+7$ oxidation state. A three-electron transfer takes place when $KMnO_4$ is applied during water treatment for oxidation of soluble manganese for which Mn(VII) is then reduced to insoluble Mn(IV) (American Water Works Association, 1999). Sufficient time should be allowed for oxidation of soluble manganese by potassium permanganate to take place prior to solid liquid separation processes. Typical detention times that exist between oxidant addition and coagulation, 3 to 9 minutes, are not sufficient for soluble Mn oxidation to take place with a stoichiometric $KMnO_4$ dose (Carlson and Knocke, 1999). Pink water problems have been reported by treatment plant operators as a result of unreacted $KMnO_4$ (American Water Works Association, 1999, Knocke et al., 1990). Chlorine dioxide (ClO_2) is also used as an oxidant for water treatment. Similar to ozone, ClO_2 must be generated on site and is a very strong oxidant, effective for Mn removal in pH and temperature conditions for natural waters (Knocke et al., 1991b, Van Benschoten et al., 1992). Regulated by-products chlorite and chlorate can be formed during ClO_2 generation (American Water Works Association, 1999, Knocke et al., 1990).

Chlorine is the most commonly used oxidant and disinfectant in water treatment. Kinetics for oxidation of Mn(II) by free chlorine are much slower than observed with ferrous iron (Knocke et al., 1990). Although oxidation of soluble manganese by chlorine is a slow process (Knocke et al., 1987, Mathews, 1947, White, 1986), a significant increase in the rate of this reaction has been shown at pH values above 8.5 and 9 (Knocke et al., 1990). A disadvantage of using chlorine is the potential formation of trihalomethanes (THM) and haloacetic acids (HAA); disinfection by-products formed from the chlorination of organic compounds in natural waters (Johnson and Jensen, 1986, Symons et al., 1975). Many water utilities have abandoned chlorination to use alternative oxidants to avoid the formation of these disinfection by-products to comply with the "EPA Stage 1 Disinfectants and Disinfection Byproduct Rule" (Knocke et al., 1987, Tobiason et al., 2008). It is impractical to use dissolved oxygen (D.O.) as an oxidizer for manganese treatment since kinetics for oxidation of soluble manganese by D.O.at pH values typical of natural waters (6-8) are very slow (Davies and Morgan, 1989, Morgan, 1967a, b, Sung and Morgan, 1981). Effective oxidation of soluble Mn and D.O. can occur at pH values greater than 9.5 (Knocke et al., 1990, Morgan, 1967b).

1.3.3. Mn-removal by Adsorption/Oxidation Using Oxide Coated Media

The addition of free chlorine together with the use of oxide-coated filter media removes soluble Mn(II) in a rapid and efficient way (Hargette and Knocke, 2001, Knocke et al., 1988, Knocke et al., 1991a, Tiwari et al., 2007, Tobiason et al., 2008). This can be achieved using synthetically coated manganese greensand and pyrolusite, or taking advantage of an intrinsic phenomenon known as the "natural greensand effect" (NGE) (Griffin, 1960, Hargette and

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Knocke, 2001, Knocke et al., 1988, Knocke et al., 1987). The NGE results from a natural process of manganese deposition and subsequent formation of oxide coatings in filtration media (i.e. sand and anthracite); this process is referred to as "filter aging" (Cleasby, 1975, Griffin, 1960, Knocke et al., 1987). A scanning electron microscope (SEM) back-scattered electron image of the outer coatings of an anthracite media sample is illustrated in Figure 1-1.

The specific mechanisms for Mn-removal using adsorption/oxidation with Mn-coated media are still unclear. Naturally formed Mn-oxide-coated media surfaces coupled with the application of an oxidant catalyze adsorption/oxidation reactions of soluble Mn for its subsequent removal from drinking water (Knocke et al., 1991a). The removal usually occurs spontaneously after the formation of the $MnO_x(s)$ coating on filter media and the addition of free-chlorine just before filtration (Hargette and Knocke, 2001). Mn removal by oxide coated media is better achieved at pH values ~ 7.3 or higher rather than at slightly acidic pH (~6) (Hargette and Knocke, 2001). In the absence of oxidant, Mn(II) is removed by sorption onto $MnO_{x(s)}$ until all surface sorption sites are saturated (Knocke et al., 1991a). Although it has been shown that filter backwashing causes $MnO_{x(s)}$ surface coatings to dislodge from filter media (Hargette and Knocke, 2001, Merkle et al., 1996), sufficient Mn-coatings are retained in filter media after filtration is resumed during regular filter operation for efficient Mn(II) removal (Hargette and Knocke, 2001). Manganese-oxide-coated sand has also been used to remove heavy metals such as Cu(II), Pb(II), and As(II) from water and wastewater (Bissen and Frimmel, 2003, Han et al., 2006, Hu et al., 2004).

1.3.4. Biofiltration: engineered microbiological oxidation processes for drinking water treatment

Biological filtration implements microorganisms known to mediate Mn-oxidation ubiquitous in natural systems (i.e. *Leptothrix*, *Gallionella*, *Crenothrix*, *Hyphomicrobium*, *Siderocapsa*, and *Metallogenium* genera) in an engineered system (Mouchet, 1992). Biological filtration for treatment of Mn and Fe has gained popularity since it does not require the use of chemicals, resulting in lower operation and maintenance costs (Burger et al., 2008b, Mouchet, 1992). As observed in the context of natural systems, many of these microorganisms are capable of oxidizing both Mn and Fe (Mouchet, 1992, Pacini et al., 2005). Biological treatment of Mn performed by *Leptothrix discophora* SP-6 (Hope and Bott, 2004), *Leptothrix ochracea* and *Gallionella ferruginea* (Katsoyiannis and Zouboulis, 2004), and *Gallionella and Leptothrix spp.* (Pacini et al., 2005) has been investigated, yielding satisfactory results. The sheaths of the *Leptothrix* and spirally twisted stalks of the *Gallionella* genera that can be observed making use of an optical microscope (Mouchet, 1992).

As explained in the preceding sections, the physiology and genetics of Mn-oxidizing bacteria in natural systems are poorly understood (Ehrlich and Newman, 2009, Li et al., 2005, Tebo et al., 2005). Thus, there are still many gaps related to the operational characteristics and mechanisms for the efficient application of engineered biological filtration (Burger et al., 2008b, Katsoyiannis and Zouboulis, 2004, Pacini et al., 2005). A good control of the pH and redox (Eh) conditions is required for optimal operation of biological filtration; Mn removal takes place at dissolved oxygen concentrations greater than 5 mg/L and pH values greater than 7.4 to 7.5 (Mouchet,

1992). Efficient Mn removal by *L. discophora* SP-6 has been reported at a pH level as low as 6.5 (Burger et al., 2008b). Uniform sand-coarse medium has been found to produce better results for biofiltration than mixed sand media (Li et al., 2005). Mn removal in biologically active filters can take place due to biotic Mn-oxidation and abiotic adsorption/oxidation achieved by Mn-oxides in the filter media; the contribution of each mechanism is difficult to determine (Pacini et al., 2005). *L. ochracea* in biologically active filters can produce a mixed amorphous Mn(III/IV) oxide that concentrates in the surface bacterial exopolymers (Katsoyiannis and Zouboulis, 2004). Future research should investigate the specific mechanisms affecting microbiological Mn-oxidation for the optimal performance of engineered biological filtration processes applied to treat drinking water.

1.3.5. Mn-reduction in drinking water treatment plants

The factors and mechanisms affecting Mn release in many drinking water utilities and, hence, the solutions to mitigate further financial losses are still unknown. Anaerobic sludge in sedimentation basins can stimulate microbiological activity and cause the release of Mn to drinking water (Hoehn et al., 1987). Mn accumulation and release may result in high costs for water utilities; a water treatment plant spent millions of dollars to replace filter media that accumulated Mn over decades and consequently resulted in chronic Mn release (Gabelich et al., 2006). Discontinuation of chlorine application through filtration basins could potentially cause the release of Mn accumulated on filter media (Gabelich et al., 2006). A trace of Mn was detected in $FeCl_3$; the use of this coagulant in water treatment may lead to the accumulation of Mn in filtration media which is then released into the water (Gabelich et al., 2006).

1.4.   Manganese Deposition in Drinking Water Distribution Systems

When soluble Mn is not adequately removed at the water treatment plant it then enters the distribution system where it is subsequently oxidized to $MnO_{x(s)}$ either by secondary disinfectant residuals or by bacteria (Hasselbarth and Ludemann, 1972, Sly et al., 1990). Customer complaints due to discoloration, taste, and plumbing fixture and staining problems in finished water have been reported when Mn effluent concentrations from treatment plant facilities are as low as 0.02 mg L^{-1} Mn (Carlson and Knocke, 1999, Gregory and Carlson, 2003, Hargette and Knocke, 2001, Sly et al., 1990). Mn-deposits in distribution systems can cause negative effects to pipe infrastructure.

Mn-precipitates deposited in the distribution system can cause reduction in pipe diameter and ultimately lead to water flow obstruction (Costello, 1984, Kothari, 1988). Deposition of Mn within biofilms and the formation of Mn-micronodules in high density polyethylene (HDPE) and polyvinyl chloride (PVC) has been reported (Murdoch and Smith, 1999, Murdoch and Smith, 2000). Mn deposition has been linked to the corrosion in stainless steel and iron pipe surfaces; microbiologically influenced corrosion has been associated with these phenomena (Dickinson and Lewandowski, 1996, Little et al., 1998). Mn-deposits can be easily dislodged from PVC pipes while they are well incorporated in iron pipes potentially affecting surface physical-chemical reactions (Cerrato et al., 2006).

The specific chemical and biological mechanisms that affect Mn release and deposition in drinking water distribution systems are still unclear. Chemical and microbial processes can affect Mn-oxidation and deposition in drinking water distribution systems (Cerrato et al., 2006,

Sly et al., 1990). Chemical factors were associated with Mn-deposition in areas of a distribution system with high disinfectant residuals while microbiological processes were linked to areas that had insufficient disinfectant residuals to control biofilm growth (Sly et al., 1990). *Pseudomonas*, *Hyphomicrobium*, and *Metallogenium* spp, are some of the microorganisms linked with Mn-deposits in drinking water distribution systems (Ehrlich and Newman, 2009, Murdoch and Smith, 2000, Sly et al., 1990, Tyler and Marshall, 1967). There is limited information about the role of microbial reduction and Mn-release in drinking water systems exposed to disinfectants (Chaudhari and Shrivastava, 2003, Davina and D'Souza, 2000, Petrunic et al., 2005). Resistance of *Bacillus* spp. to chlorine and monochloramine has been shown for spores in suspension and spores associated to biofilms in drinking water systems (Gibbs et al., 2004, Morrow et al., 2008, Morrow and Cole, 2009, Rice et al., 2005, Rose et al., 2005, Szabo et al., 2007).

1.5.   Research methods and challenges

What biochemical mechanisms and factors control the activity of microbiological Mn-oxidation and -reduction? How can some aerobic bacteria use oxygen as a terminal electron acceptor and reduce Mn aerobically? These are some of the many questions that still need to be answered related to microbiological Mn-oxidation and -reduction. The development of molecular, microscopic, and spectroscopic tools in the past decade have greatly increased the possibilities of studying, both in the laboratory and *in situ,* important geochemical processes and uncovering new mechanisms (Ehrlich and Newman, 2009, Geesey et al., 2002, O'Day, 1999).

1.5.1. Microscopic and spectroscopic tools

Information obtained from macroscopic solution chemistry methods has been linked to that obtained from solid phase microscopy and spectroscopy surface science techniques in trying to

gain a mechanistic understanding of physical, chemical, and microbiological processes that affect biogeochemical Mn cycling. Excellent reviews have been published providing a detailed description about a wide variety of surface sensitive techniques applied to the study of chemical and microbial transformations in the environment (Brown and Sturchio, 2002, D'Amore et al., 2005, Flores and Toca-Herrera, 2009, Geesey et al., 2002, Hochella, 1988, O'Day, 1999). This review will only focus on some of these surface sensitive techniques which have been summarized in Table 1-4.

1.5.1.1. Solution phase analyses

Total concentrations of Mn can be measured in solution using inductively coupled plasma (ICP) and atomic absorption (AA) (Clesceri et al., 1998). However, a limitation of these two methods is that the determination of "dissolved" concentrations is often challenging due to colloidal and nano-size particles that can pass through filter membranes used to "operationally define" dissolved species (Stumm and Morgan, 1996). Solution oxidation state information can be obtained using the "leucoberbelin method" (Okazaki et al., 1997, Stein et al., 2001). The leucoberbelin method can distinguish between +2 and oxidation states equal or greater than +3; but can't distinguish between oxidation states +3, +4, and +7 (Gabelich et al., 2006, Okazaki et al., 1997). Therefore, it is usually necessary to link the information obtained from this solution chemistry method with other surface sensitive techniques.

1.5.1.2. Solid Phase Micro-topography and Elemental Content Analyses

Surface micro-topography and chemical elemental composition can be studied by scanning electron microscopy/energy dispersive X-ray spectroscopy (SEM/EDS). Several studies have

used SEM/EDS to relate specific surface shape characteristics and quantitative information about Mn content distribution both in natural and engineered water systems (Hu et al., 2004, Junta and Hochella, 1994, Merkle et al., 1996, O'Reilly and Hochella, 2003). A scanning electron micrograph of an oxide coated anthracite filtration media sample is illustrated in Figure 1-1. A limitation of SEM is that it is a vacuum based technique and certain instrumentation can only be operated under dry conditions.

Environmental scanning electron microscopy facilitates the analysis of wet (100% humidity) samples and has been used for the study of relevant biogeochemical processes in natural systems (Callow et al., 2003, Sommers et al., 2002). This tool can also be applied to reveal detailed information about surface irregularities in solid surfaces of engineered drinking water systems. A scanning electron micrograph operated under environmental mode at 100% humidity of an anthracite filtration media sample collected from a water treatment plant is shown in Figure 1-2. Various surface irregularities and details can be appreciated at this resolution, indicative of the micro-topographic complexity of this anthracite filtration media sample.

Transmission electron microscopy (TEM) and atomic force microscopy (AFM) are very powerful surface imaging techniques. TEM a vacuum based microscopy technique based on the transmission of electrons or secondary electrons, offering very high resolution imagining, in the order of a few nm (Geesey et al., 2002, Hochella et al., 2005). TEM EDS is useful for obtaining information related to size distribution, surface area, and chemical analyses of nanoparticles (Wigginton et al., 2007a). TEM SAED (transmission electron microscopy/selected area electron diffraction) can be used to obtain information of mineral phase and internal structure of

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crystalline solids (Wigginton et al., 2007a). AFM is a non vacuum based technique that uses atomic level force to provide high resolution imaging for direct visualization at the angstrom to micrometer scale (O'Day, 1999). AFM is also a powerful tool for obtaining information related to surface area and size distribution of nanoparticles (Flores and Toca-Herrera, 2009, McGuire et al., 2003, O'Day, 1999, Wigginton et al., 2007a). AFM has been used both in natural (Grantham and Dove, 1996, Wigginton et al., 2007c) and engineered systems (Merkle, 1995, Merkle et al., 1996).

1.5.1.3. Solid Phase Oxidation State Analyses

X-ray photoelectron spectroscopy (XPS) is a well established surface analysis technique for the determination of Mn oxidation states. Several investigations have used XPS to identify Mn oxidation states in natural (Junta and Hochella, 1994, Murray et al., 1985, Nesbitt and Banerjee, 1998) and in engineered systems (Han et al., 2006, Katsoyiannis and Zouboulis, 2004, Merkle et al., 1996). XPS uses X-rays as the photon source and the kinetic energies of photo-electrons are detected in order to obtain binding energies for elemental and oxidation state information (Hochella, 1988). The Mn $2p_{3/2}$ peak is used often to obtain oxidation state information because it yields the highest intensity signal of all other XPS Mn orbitals (Han et al., 2006, Katsoyiannis and Zouboulis, 2004, Nesbitt and Banerjee, 1998). However, the Mn 2p peaks are broad and the photo-peak shifts as a function of oxidation state are relatively small so there use is not practical (Junta and Hochella, 1994, Suzer et al., 1999). Determination of the Mn 3s multiplet splitting has been reported as a robust method to determine oxidation state information (Junta and Hochella, 1994, Murray et al., 1985). Applied physicists have studied the use of the Mn 3p

photo-peak to provide oxidation state information (Nelson et al., 2003, Nelson et al., 1999, 2007). Chapter 3 of this dissertation provides a detailed description about the use of the Mn 3p photopeak to determine oxidation state information in Mn-oxide coated anthracite filtration media. A limitation of XPS is that it is a vacuum technique so samples need to be dry for analyses.

X-ray diffraction (XRD) is a surface analysis technique used to analyze crystalline solid phases of Mn. The application of XRD to study natural and engineered environments is often limited by the amorphous character of Mn solid phases commonly encountered in biogeochemical processes (Merkle et al., 1996, Webb et al., 2005a, Webb et al., 2005b). X-ray absorption spectroscopy (XAS) and Raman spectroscopy can be used to analyze "wet" or "fully hydrated" samples. Several studies have used the near edge structure (XANES - provides oxidation state information) and the extended fine structure (EXAFS - provides chemical structure information) of XAS to study relevant biogeochemical processes (Bargar et al., 2008, Brown and Sturchio, 2002, Webb et al., 2005a, Webb et al., 2005b). Raman spectroscopy measures quantum state transitions related to molecular vibrations, relying on inelastic scattering from a laser source in the visible, near infrared, and near ultraviolet range. Raman spectroscopy can also be used to study Mn redox in natural (D'Amore et al., 2005, Geesey et al., 2002, Kempe et al., 2010) and engineered systems (Maliyekkal et al., 2009, Yang et al., 2009).

1.5.2. Isolation of abiotic and microbiological processes: The challenge of obtaining negative microbiological controls in solid samples

Differentiating the individual contribution of chemical and microbiological factors to biogeochemical cycling of Mn in drinking water is difficult (geeseyPacini et al., 2005, Sly et al.,

1990). Obtaining a negative microbiological control is a major challenge to perform studies under controlled experimental conditions to further understand chemical, physical and microbiological interactions that affect Mn release in drinking water systems. A study showed that methods commonly used to achieve microbial controls (i.e. NaN_3, $HgCl_2$, heat sterilization, and sonification) affect particulate Mn samples through dissolution, disaggregation, interference with adsorption, or particle ageing (Shiller and Stephens, 2005). These results were also supported experimentally after exposure of anthracite filter media to autoclaving. A simple experimental setup consisting of 100 mL of chlorine-free tap water and 10g of either anthracite filter media contained in a 250 mL Erlenmeyer flask was used. Triplicate samples of 5 mL of sample were obtained at defined times (Day 0, Day 7, and Day 49) from three independent experimental setups and filtered through a 0.45 µm membrane. Dissolved Mn was measured using a Perkin-Elmer 5100 flame atomic absorption spectrophotometer (Waltham, MA, USA). The results obtained from this study indicate that Mn was released into solution after exposure of anthracite media to autoclaving (Figure 1-3).

Bacillus sp have been found in anthracite media of filtration basins in drinking water treatment plants (see Chapter II). *Bacillus* spp. spores can resist exposure to high pressures; studies performed in the food sciences suggest that the application of different pressurization cycles can be a very efficient way to inactivate spores formed by *Bacillus* spp. (Furukawa et al., 2000b, Furukawa et al., 2004, Robertson et al., 2008). The disadvantage of this method is that samples are exposed to high pressures that could cause the alteration their physical and chemical surface properties. Backwashing in filtration basins of water treatment plants can cause physical and chemical alterations to filtration media, causing surface Mn-oxide coatings to dislodge

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(Hargette and Knocke, 2001, Merkle et al., 1996). Typical operating pressures in filtration basins of drinking water treatment plants range from 50-75 psig (American Water Works Association, 1999), approximately three orders of magnitude lower than those used in pressurization processes applied in the food sciences to inactivate *Bacillus* spp. spores which range from 15,000 - 88,000 psi (Furukawa et al., 2000a, Robertson et al., 2008).

1.6.   Conclusions

Linking fundamental scientific mechanisms investigated in natural systems to the applied context of engineered systems is a necessary step to further understand Mn cycling in drinking water. Special emphasis should be given to microbiological and chemical interactions that are many times overlooked in the context of "disinfected" drinking water systems. The connection between macroscopic experimental data with microscopic and spectroscopic analyses is essential to enhance the understanding of specific microbiological and chemical mechanisms that still remain unknown in both natural and engineered water systems. The following gaps in the literature have been addressed in this dissertation: a) the presence of Mn-oxidizing and -reducing microorganisms in chlorinated drinking water systems is overlooked; b) identification of Mn oxidation states in anthracite filtration media surfaces is a major challenge; c) the specific mechanisms for Mn-removal using adsorption/oxidation with Mn-coated media are still unclear.

1.7.   References

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Table 1-1. Comparison of different electron acceptors (Adapted from Nealson and Saffarini 1994).

Electron Acceptor[a]	$pe^0(pH)^b$	Free Energy (kJ/M glucose)[c]
Oxygen ($O_2 \rightarrow H_2O$)	+13.75	-3190
Nitrate ($HNO_3 \rightarrow N_2$)	+12.65	-3030
Manganese ($MnO_2 \rightarrow Mn^{+2}$)	+8.9	-2920 (pyrolusite, MnO_2)
Nitrate ($HNO_3 \rightarrow N_2 + NH_3$)	+6.15	-2750
Iron ($Fe_2O_3 \rightarrow Fe^{+2}$)	-0.80	-1410 (hematite, Fe_2O_3)
Iron [$FeO(OH) \rightarrow Fe^{+2}$]	-0.80	-1330 [goethite, $FeO(OH)$]
Sulfate ($SO_4^{-2} \rightarrow S^{-2}$)	-3.5	-380
CO_2 (Organic Carbon $\rightarrow CO_2$)	-4.13	-350

[a] Electron acceptors have been illustrated with the reduced species obtained from the equations for generalized oxidation of organic carbon provided by Nealson and Saffarini (Nealson and Saffarini, 1994)
[b] $pe^0(pH)$ values refer to the electron activity of oxidant and reductant at neutral pH, as calculated by Zehnder & Stumm (Zehnder and Stumm, 1988).
[c] Free Energies are taken from calculations performed by Froelich et al (Froelich et al., 1979).

Table 1-2. Summary of some relevant abiotic and biotic factors affecting Mn-oxidation and –reduction in natural waters.

	Mn-oxidation	**Mn-reduction**
Abiotic	▪ **Oxygen** (Davies and Morgan, 1989, Morgan, 1967b, Morgan, 2005) ▪ **Oxygen and metal oxide surfaces** (Davies and Morgan, 1989, Junta and Hochella, 1994, Morgan, 2005) ▪ **Natural organic matter** (Bertino and Zepp, 1991, Stumm and Morgan, 1996)	▪ **Fe(II)** (Stumm and Morgan, 1996, Villinski et al., 2001) ▪ **Natural organic matter and various organic compounds** (Bertino and Zepp, 1991, Jun and Martin, 2003, Stone and Morgan, 1984a, Stone and Morgan, 1984b, Stumm and Morgan, 1996) ▪ **Sulfides** (Stumm and Morgan, 1996, Thamdrup et al., 1993)
Biotic	▪ *Leptothrix spp.*, *Sphaerotilis spp.*, *Chlonothrix spp.*, **and** *Siderobacter spp.* (Boogerd and Devrind, 1987, Ehrlich and Newman, 2009, Tebo et al., 2005) ▪ *Bacillus* **spp.,** *Pseudomonas* **spp., and Fungi** (Devrind et al., 1986b, Ehrlich and Newman, 2009, Okazaki et al., 1997, Tebo et al., 2005)	▪ *Shewanella* **(formerly** *Alteromonas***) spp.** (Ehrlich and Newman, 2009, Myers and Nealson, 1988a) ▪ *Bacillus* **spp.,** *Pseudomonas* **spp., and Fungi** (Ehrlich and Newman, 2009, Ghiorse and Ehrlich, 1976, Lovley, 1991, Nealson and Saffarini, 1994)

Table 1-3. Summary of kinetic data obtained from the literature for Mn(II) oxidation with oxidants commonly used in drinking water treatment.

Oxidant	pH	k (M^{-1}s^{-1}) [a]	t$_{1/2}$ (s) [b]	Reference
Ozone (O$_3$)	7.0	1.50 x 10^3	~23.0	(Jacobsen et al., 1998)
Potassium Permanganate (KMnO$_4$)	7.0	6.15	3.0	(Van Benschoten et al., 1992)
Chlorine Dioxide (ClO$_2$)	6.3	4.75	4.8	(Van Benschoten et al., 1992)
Chlorine	7.0	9.00	5.4 x 10^3	(Reckhow et al., 1991, Soborski, 1990)
Oxygen (O$_2$)	9.3	2.50 x 10^{-2}	~4.6 x 10^5	(Davies and Morgan, 1989, Morgan, 2005)

[a] k = second order rate constant
[b] t$_{1/2}$ = manganese(II) half-life

Table 1-4. Summary of some relevant surface analysis techniques used to study biochemical cycling of Mn in natural and engineered environments.

Surface Technique	Measurement	Depth of Analysis[a]	Advantage	Limitation
SEM	Imaging	1 μm	Good resolution/	Vacuum required
ESEM	Imaging	1 μm	100% humidity	Limited resolution
TEM	Imaging	10-100 nm	High resolution	Vacuum required
EDS	Elemental content	150 nm	Quantitative analyses of surface elemental distribution	High detection limit/limited chemical information
XPS	Elemental content/ Oxidation State	3-5 nm	Well established and effective near surface technique	Vacuum required
XRD	Chemical structure/ Oxidation state	3-756 μm	Existing large library of patterns	Exclusive for crystalline solids
XAS (XANES)	Oxidation state	Tenths of μm	Nondestructive (analyses of wet samples)	Beamline access/limited spectra available
XAS (EXAFS)	Chemical structure	Tenths of μm	Nondestructive (analyses of wet samples)	Beamline access/limited spectra available
Raman	Oxidation state	10 μm	Nondestructive (analyses of wet samples)	

[a] Information obtained from existing literature reviews (D'Amore et al., 2005, Geesey et al., 2002).

Figure 1-1. Scanning electron microscope (SEM) back-scattered electron image of the outer coatings of an anthracite filtration media sample.
Source: Tobiason, J.E., Islam, A.A., Knocke, W.R., Goodwill, J., Hargette, P., Bouchard, R., and Zuranvsky, L., *Characterization and Performance of Filter Media for Manganese Control.* © 2008 Awwa Research Foundation. Reprinted with Permission.
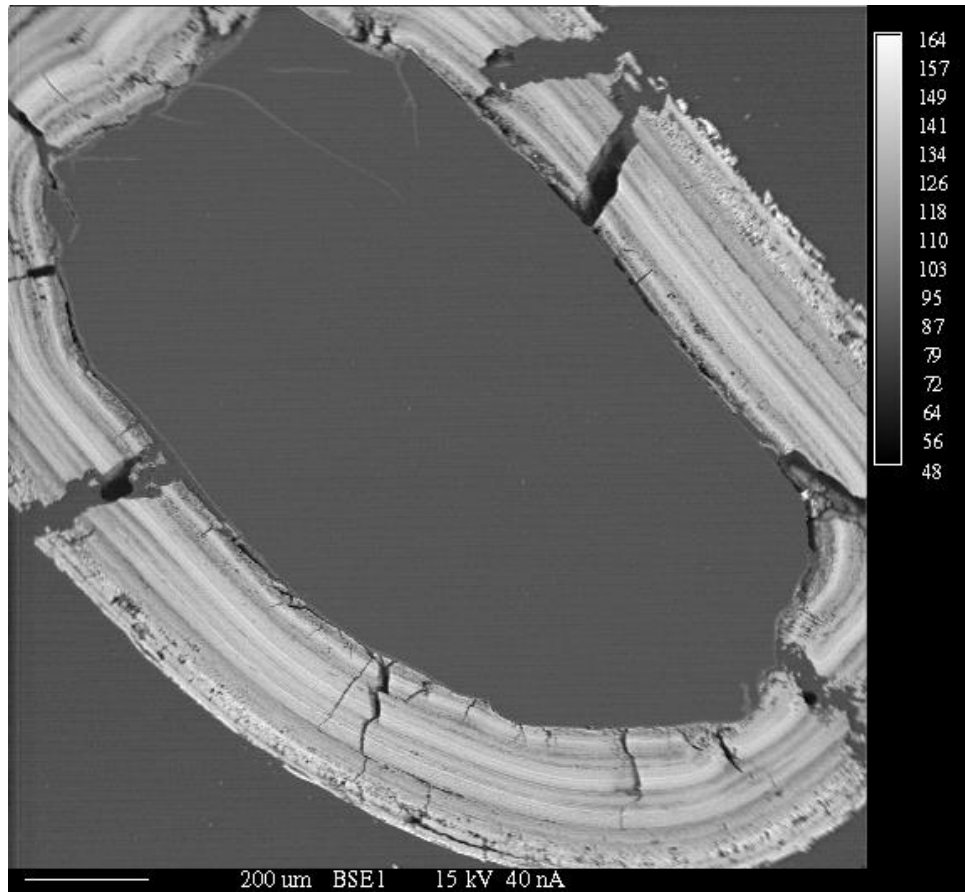
Figure 1-2. ESEM image of a wet anthracite filtration media sample (Cerrato, et al., unpublished data).
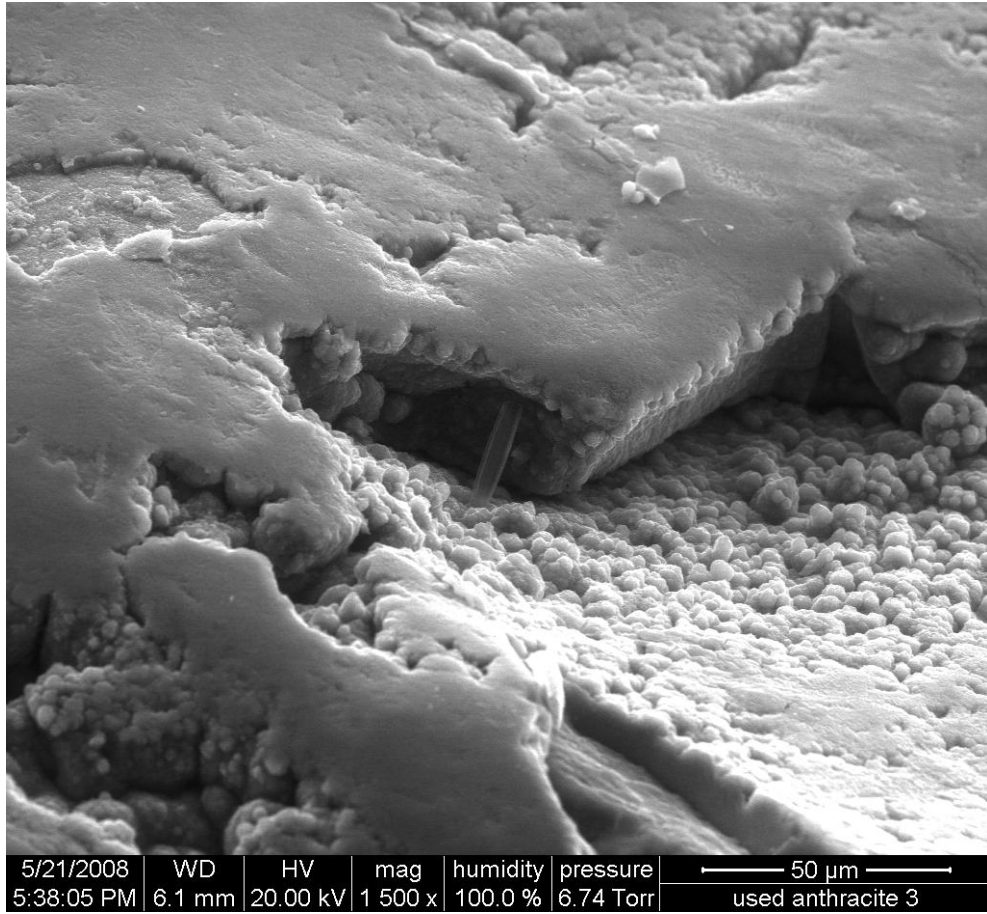
Figure 1-3. Dissolved Mn (mg/L) released to solution as a function of time after exposure of anthracite filter media to autoclaving (standard error bars shown for triplicate samples, n=3, Cerrato et. al., unpublished data).
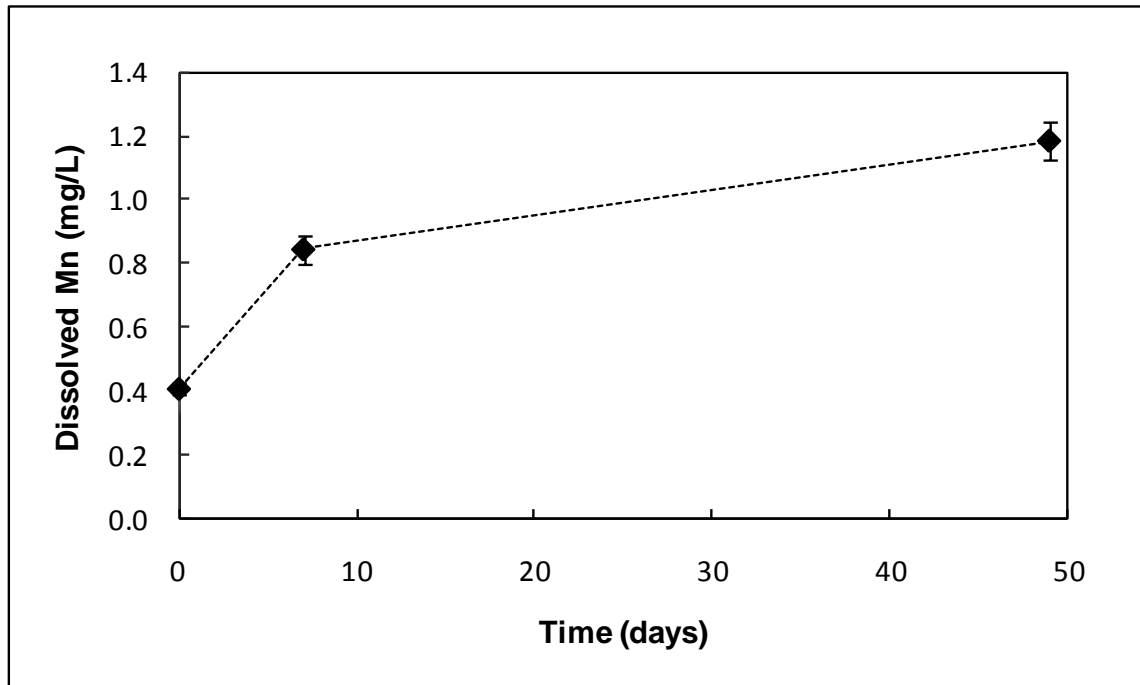
CHAPTER 2: Manganese-Oxidizing and -Reducing Microorganisms Isolated from Biofilms in Chlorinated Drinking Water Systems

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ABSTRACT

The interaction of chemical, physical and biological factors that affect the fate, transport and redox cycling of manganese in engineered drinking water systems is not clearly understood. This research investigated the presence of Mn-oxidizing and -reducing bacteria in conventional water treatment plants exposed to different levels of chlorine. Mn(II)-oxidizing and Mn(IV)-reducing bacteria, principally Bacillus spp., were isolated from biofilm samples recovered from four separate drinking water systems. Rates of Mn-oxidation and -reduction for selected individual isolates were represented by pseudo-first-order kinetics. Pseudo-first-order rate constants were obtained for Mn-oxidation (range: 0.106 - 0.659 days^{-1}), aerobic Mn-reduction (range: 0.036 - 0.152 days^{-1}), and anaerobic Mn-reduction (range: 0.024 - 0.052 days^{-1}). The results indicate that microbial-catalyzed Mn-oxidation and -reduction (aerobic and anaerobic) can take place simultaneously in aqueous environments exposed to considerable oxygen and chlorine levels and thus affect Mn-release and -deposition in drinking water systems. This has important implications for Mn-management strategies, which typically assume Mn-reduction is not possible in the presence of chlorine and oxidizing conditions.

2.1.   Introduction

Manganese (Mn) is an abundant transition metal that is known to undergo biochemical oxidation and reduction cycling in freshwater and marine systems (Nealson and Myers, 1992, Nealson and Saffarini, 1994, Stumm and Giovanoli, 1976, Wetzel, 2001). Soluble manganese, Mn(II), can be found in freshwater sources due to electron exchange reactions that take place in the presence of metal-reducing bacteria under anoxic conditions at the water/sediment interface (Nealson and Saffarini, 1994).   Oxidation of soluble Mn(II) to form insoluble $MnO_{x(s)}$ precipitates in drinking water systems can cause aesthetic problems such as water discoloration and fouling, staining on plumbing fixtures, and consumer complaints (Dietrich, 2006, Sly et al., 1990, Whelton et al., 2007). The United States Environmental Protection Agency has set a secondary maximum contaminant level for Mn of 0.05 mg L^{-1} (United States Environmental Protection Agency, 1979). The World Health Organization provides a guide value of 0.4 mg L^{-1} for Mn (World Health Organization, 1998). Mn accumulation and release can be a costly and difficult problem for drinking water utilities to resolve. A study including several water utilities in the United States reported that 17% attributed particulate matter problems in their distribution system to the presence of Mn (Booth and Brazos, 2004). Even though microbiological-mediated redox reactions of Mn have been widely studied in natural systems (Ehrlich and Newman, 2009, Schink, 2000), their role in Mn deposition and release in disinfected drinking water systems is still unclear.

Many studies have focused on microbial Mn-oxidation in drinking water systems, mainly for its association with biological filtration (Burger et al., 2008, Katsoyiannis and Zouboulis, 2004, Lewandowski et al., 1997, Mouchet, 1992, Tekerlekopoulou et al., 2008, Vandenabeele et al.,

1992). Biological filtration has gained popularity since it is a cost-effective technology that makes use of microbial oxidation for Mn-removal from drinking water, minimizing the use of chemical oxidants that could form undesired by-products (Burger et al., 2008, Katsoyiannis and Zouboulis, 2004, Mouchet, 1992). Most of the existing literature evaluates microbial Mn-oxidation in the absence of a disinfectant to simulate the conditions necessary for biomass accumulation and activity required for optimal operation of biofilters. Although studies have been published on microbial Mn-reduction in drinking water systems (Chaudhari and Shrivastava, 2003, Davina and D'Souza, 2000, Petrunic et al., 2005), these are limited when compared to the existing literature on microbial Mn-oxidation. Likewise, there are limited references focused on the role of microbial reduction and Mn‑release in drinking water systems exposed to high levels of disinfectant (Chaudhari and Shrivastava, 2003, Davina and D'Souza, 2000, Petrunic et al., 2005).

The mechanisms explaining the microbiological factors affecting release and deposition of Mn in drinking water treatment and distribution remain poorly understood. A disinfectant (i.e. chlorine, ozone, and chlorine dioxide) applied to a drinking water system may serve both as a chemical oxidant and microbial inhibitor, simultaneously catalyzing chemical and inhibiting biological Mn(II) oxidation (Sly et al., 1990). A recent research study (Gabelich et al., 2006) demonstrated that Mn-desorption from filtration media in water treatment plants was a source of Mn in drinking water systems after cessation of chlorination in filtration basins. A potential chemical interaction between Mn, Fe(III), and Al(III) coagulant residuals was suggested to explain the Mn-release (Gabelich et al., 2006). The possible effect of microbiologically mediated Mn‑release was rejected based on the assumption that high oxygen and disinfectant

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levels found in drinking water treatment plants would suppress microbiological Mn‑reduction (Gabelich et al., 2006).

This study documents the simultaneous existence of manganese oxidizing and reducing ‑ bacteria in chlorinated drinking water systems which include aqueous environments typically exposed to considerable oxygen and disinfectant levels. The specific objectives of the study were to: 1) isolate Mn‑oxidizing and ‑reducing microorganisms from drinking water systems exposed to different levels of chlorine; 2) identify these microorganisms; and 3) measure Mn‑oxidation (aerobic) and ‑reduction (aerobic and anaerobic) rates for selected microorganisms isolated from chlorinated drinking water systems.

2.2.   Materials and Methods

Sample Sites. Samples were collected from two drinking water systems in Virginia, USA (Water Systems 1 and 2), one system in Honduras (Water System 3), and one system in North Carolina, USA (Water System 4). All these systems used free chlorine as the primary disinfectant for pathogen inactivation during water treatment. The characteristics of the four water systems are listed in Table 2-1. Systems 1, 3, and 4 have previously reported Mn‑associated problems. Particularly, System 3 has had a long‑term and chronic problem with elevated levels of Mn (Cerrato et al., 2006). Water samples with no visible suspended matter were obtained from the sedimentation and filtration basins (rapid filtration) of Systems 1, 2, and 3, and from the distribution system of System 3. Samples of anthracite/sand filter media were collected from Systems 1 ‑ 4. Samples were collected in 500 mL autoclaved polyethylene bottles from the filtration media and also from the top (recently deposited) and bottom (accumulated)

sludge layer of the sedimentation basin. Sedimentation basin sludge and filtration media samples were collected to visually obtain approximately 50% solid (i.e. sludge, anthracite or sand) and 50% water. Wet galvanized iron and PVC pipes of 2" diameter were obtained from the distribution network of System 3. The PVC pipes had been in service for 15 years and the galvanized iron pipes for 30 years. Samples were stored at room temperature and shipped via express courier service, taking approximately 5-7 days between collection and laboratory processing.

Mn content of filter material and pipes surfaces. Representative sections of internal pipe surface walls were scraped using a spatula in order to obtain approximately 1g of material. The total Mn content of pipe surface from System 3 and filter media from Systems 1 - 4, including the adherent biofilm, was measured by adding 0.20 g dry weight of each solid to 100 mL of de-ionized water in 125 mL acid washed polyethylene bottles and then adding 4 mL of 12 M HCl and 2 mL 10% hydroxylamine to the solution (Parks et al., 2004). The contents were heated to 90 °C for 24 hours and allowed to cool. The Mn concentration of the resulting solution was then measured using a Perkin-Elmer 5100 flame atomic absorption spectrophotometer (Waltham, MA, USA). The results are reported as mg Mn (g dry weight of sample)$^{-1}$.

Recovery of biofilms from mixed filters and pipe surfaces. For the purpose of this study, a biofilm was defined as including the biological and particulate material dislodged from an individual sample. Biofilm materials were recovered from anthracite or sand media by suspending 1 g aseptically handled media sample in 5 mL chlorine-free sterile tap water in a sterile 50 mL screw cap centrifuge tube and vortexed for 60 seconds. A representative 2 cm x 2

cm section of the inside pipe surface of both PVC and iron pipes was scraped and suspended in 5 mL sterile tap water to obtain a biofilm suspension. The biofilm scraped from the PVC and iron pipe surfaces was homogenized in a sterile tissue homogenizer and transferred to a sterile 16 x 125 mm screw cap tube.

Detection and Enumeration of Mn‑oxidizing Microorganisms. A dilution series of biofilm suspensions was prepared in 5 mL sterile tap water (10^{-1} to 10^{-5}) and 0.1 mL from each dilution tube was spread in duplicate on Mn‑oxidation (Stein et al., 2001) agar media. The Mn‑oxidation agar media consisted of the following per liter of 10 mM HEPES buffer (pH 7.4): 0.001 g $FeSO_4^{.}7H_2O$, 0.15 g $MnSO_4^{.}H_2O$, 2 g Peptone (Becton Dickinson, Sparks, MD), 0.5 g Yeast Extract (Becton Dickinson, Sparks, MD)), and 15 g agar. The Mn‑oxidation broth media consisted of all the above described minus agar. After inoculation, the Mn‑oxidation agar was incubated at 30 °C and examined every week for the appearance of colonies with dark centers or edges as evidence of deposition of dark (oxidized) Mn. Each putative Mn-oxidizing colony type was picked, streaked for isolation and grown in Mn‑oxidation broth and a portion frozen (-70 °C) for storage after glycerol was added to a final concentration of 20 %. Total colony-forming units (CFU) and CFU for each putative Mn-oxidizing isolate per mL of water or g or cm^2 of biofilm in the original sample were calculated..

Detection and Enumeration of Mn‑reducing Microorganisms. A dilution series of biofilm suspensions was prepared in 5 mL sterile tap water (10^{-1} to 10^{-5}) and 0.1 mL of each dilution tube was spread in duplicate on Mn‑reduction (Myers and Nealson, 1988a) agar media. The Mn‑reduction media consisted of the following per liter of 10 mM HEPES buffer (pH 7.4): 0.2 g of

amorphous $MnO_{2(s)}$, 0.2 g yeast extract, 2 g sodium acetate, and 15 g agar. The Mn -reduction broth media consisted of all the above minus agar. Immediately after plates were poured, the Mn-reduction agar was stored in anaerobic jars (GasPak®, Becton Dickinson, Sparks, MD). The inoculated Mn-reduction agar was incubated anaerobically (GasPak®, Becton Dickinson, Sparks, MD) at 30 °C. Putative Mn-reducing isolates were picked based on the disappearance of MnO_2 granules surrounding the colony, streaked for isolation and grown anaerobically in Mn-reduction broth and a portion frozen (-70 °C) for storage after glycerol was added to a final concentration of 20 %. Total CFU and CFU for each putative Mn-reducing isolate per mL of water or g or cm^2 of biofilm in the original sample was calculated.

Molecular Identification of Mn-oxidizing and -reducing bacteria. DNA was extracted from the isolated pure cultures using the FastDNA Spin Kit (MP Biomedicals LLC, Solon, OH) following the manufacturer's protocol and stored at -20 °C prior to use. Polymerase chain reaction (PCR) was performed to amplify the 16S-rRNA genes from the DNA extracts. Each 25 µL reaction mixture consisted of: 5 µL of 5X *Taq*Master PCR Enhancer, 2.5 µL of 10X *Taq* Buffer with Mg^{2+}, an additional 1.5 µL of 25mM Mg^{2+} solution, 0.2 µM of dNTP mix, 0.2 µM of fD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and rP1 (5'-GGWTACCTTGTTACGACTT-3') primers (Weisburg et al., 1991), 0.25 µL of formamide, and 1.75 U of *Taq* DNA polymerase (5 Prime Inc., Gaithersburg, MD). A C1000 Thermal Cycler (Bio-Rad Laboratories Inc., Hercules, CA) was used to perform the DNA amplification. The temperature profile consisted of one cycle of an initial melting step at 95 °C for 3 min; 35-50 cycles at 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 1.5 min, followed by one final extension step at 72 °C for 7 min. The PCR products

were cleaned using ExoSAP-IT according to the manufacturer's protocol (USB Corporation, Cleveland, OH) and sequenced by the Core Laboratory Facility at the Virginia Bioinformatics Institute (Blacksburg, VA) using primer V3F (5'-CCAGACTCCTACGGGAGGCAG-3'). This provided fragments ranging from 695‑1074 bp of the 16S rRNA gene, including the V3 hypervariable region (Chakravorty et al., 2007), for identification. The Basic Local Alignment Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov) was used to identify sequences within the GenBank database sharing the highest DNA sequence similarity with the isolated pure cultures. Sequences were submitted to the GenBank database and are available under accession numbers HM055943-HM055995.

Measurement of Mn. The concentration of oxidized Mn was measured by the leucoberbelin method (Stein et al., 2001). The leucoberbelin method can distinguish between Mn(II) and oxidized Mn, but cannot differentiate Mn(III) from Mn(IV) (Gabelich et al., 2006). Thus, the term oxidized Mn will be used in this study to refer to either Mn(III) or Mn(IV). In a microcentrifuge tube, 0.2 mL of the early stationary phase culture was mixed with 1 mL of 0.04 % leucoberbelin blue I (Aldrich Chemical Co., St. Louis, MO, USA) in 45 mM acetic acid. The concentration of oxidized Mn was measured by comparing the absorbance (620 nm) to a standard curve constructed using 0.1 % (wt/vol) $KMnO_4$. For measurement of Mn‑reduction, sample volumes of 0.7 mL were withdrawn and the amorphous $MnO_{x(s)}$ and cells were separated from the broth by filtration through 0.1 μm pore size filters. In a polystyrene tube, 0.4 mL of the filtrate was mixed with 3.6 mL of de‑ionized water and Mn concentration was measured at a wavelength of 279.5 nm using a Perkin‑Elmer 5100 flame atomic absorption spectrophotometer (Waltham, MA, USA). An additional control was performed to verify the oxidation state of Mn

in the filtrate: 0.1 mL of filtrate was mixed with 0.9 mL of 0.04 % leucoberbelin blue. All the Mn in these filtrates was observed to be soluble Mn(II).

Measurement of Mn(II)-oxidation by individual isolates. Putative Mn-oxidizing isolates were grown in 10 mL Mn-oxidation broth in 16 x 150 mm screw capped tubes and incubated at 30ºC with aeration air flushing (rotation at 60 rpm). Samples were removed at weekly intervals. A negative (abiotic) control was established by filling screw cap tubes only with sterile Mn-oxidation broth. A complementary killed control was also attempted, but was confounded because the agents or methods used to kill the cells (e.g., azide addition, autoclaving, pasteurization) caused increased Mn-release into solution as reported by others (Edenborn et al., 1985, Ross and Bartlett, 1981, Shiller and Stephens, 2005). The concentration of oxidized Mn was measured as described above. The results are reported in the Supplementary Material as mg Mn(III/IV) per liter formed after 2 weeks. An isolate was considered to "oxidize Mn" if the concentration of Mn oxidized by a particular strain was greater than the mean Mn concentration obtained for the control plus 3 times its standard deviation (n = 4, 0.9 ± 0.2 mg L^{-1} Mn). Results were corrected with respect to the abiotic control.

Measurement of Mn(IV)-reduction by individual isolates. Screw capped, 16 x 125 mm tubes containing 5 mL of Mn-reduction broth were prepared and immediately after autoclaving (15 min at 15 psi) placed in an anaerobic jar. The medium was inoculated with a single colony of each putative Mn-reducing isolate. A culture volume of 1 mL was inoculated in 9 mL of fresh Mn-reduction broth medium and incubated with loose caps at 30° C for 30 days under anaerobic conditions without agitation.   An abiotic control was established by filling screw cap tubes only

with sterile Mn-reduction broth. The concentration of reduced Mn was measured as described in the previous section, "measurement of Mn". The results are reported in the Supplementary Material as mg Mn(II) per liter formed after 5 weeks. An isolate was considered to "reduce Mn" if the concentration of Mn transformed by a particular strain was greater than the mean Mn concentration obtained for the control plus 3 times its standard deviation (n = 4, 2.2 ± 0.7 mg L^{-1} Mn). Both aerobic and anaerobic Mn-reduction were measured for selected individual isolates. Aerobic reduction experiments were carried out by inoculating 1 mL of culture in 9 mL of fresh Mn-reduction broth medium and incubating at 30℃ with aeration (rotation at 60 rpm). Samples were removed at weekly intervals and reduced Mn was measured using the same procedure described in the previous section.

Although much lower than that observed in the cultures, a certain degree of Mn-release was observed in the abiotic control for Mn-reduction incubated under anaerobic conditions. Other studies have reported that various organic compounds can cause abiotic reduction of Mn (III) and Mn (IV) oxides (Kostka et al., 1995, Stone and Morgan, 1984). Thus, the HEPES buffer and other organic compounds contained in the Mn-reduction broth media used for culture experiments performed under anaerobic conditions likely influenced this result. For this reason all the results obtained for Mn-reduction in this study were corrected for the abiotic control.

Data Analysis for Rates of Mn-oxidation and -reduction. A nomenclature system was developed to represent the isolates included in this study. The letters MB (Manganese Bacteria) followed by a number are used to refer to each isolate as organized in the Supplementary Material. For example, MB-1 indicates "Manganese Bacteria, isolate 1". Specific isolates were

selected to measure Mn transformation rates based on their Mn-oxidation and -reduction performance in cultures. The intent was to measure rates for isolates with "high", "medium" and "low" Mn-oxidation and -reduction capabilities.

Pseudo-first-order kinetics were used to represent microbial Mn-transformations (either oxidation or reduction):

$$\frac{d[Mn_{transformed}]}{dt} = k_1[Mn_{transformed}] \qquad (1)$$

After separation of variables and integration of equation (1):

$$\ln[Mn_{transformed}] = k_1 t \qquad (2)$$

Data from the log phase of the Mn-oxidation or Mn-reduction curves (Figures 2-1a, 2-3a, and 2-5a) were used for the pseudo-first-order kinetic analysis; points in the lag phase were excluded. Pseudo-first-order rate analysis was performed for selected isolates only when three or more points were obtained in the log phase. Previous studies have also used pseudo-first-order kinetics to represent rates of microbial Mn(IV)-reduction (Dollhopf et al., 2000), Mn(II) disappearance via chemical oxidation with oxygen in an initially homogenous solution (Davies and Morgan, 1989, Morgan, 2005), and biological manganese oxidation and removal in groundwater treatment (Katsoyiannis and Zouboulis, 2004).

2.3. Results

Mn content of filter material and pipes. The total Mn contents of anthracite media for Systems 1, 2, and 4 were 9.7, 3.7, and 30.7 mg Mn (g dry weight of sample)$^{-1}$, respectively. The total Mn content of sand filtration media for Systems 1, 2, and 3, were 0.4, 0.5, and 0.5 mg Mn (g dry weight of sample)$^{-1}$, respectively. The total Mn contents of surface scrapings of PVC and iron pipes collected from System 3 were 11.7 and 1.1 mg Mn (g dry weight of sample)$^{-1}$, respectively. The values are similar to those reported in another study that led to identification of treatment plant anthracite filter material as the source of Mn in the water effluent from the filtration basin (Gabelich et al., 2006). These data show that there is considerable total Mn in the surfaces of filtration media of the four drinking water systems and the pipes of System 3.

Recovery of Mn-**oxidizing and** -**reducing bacteria.** Mn-oxidizing and Mn-reducing bacteria were recovered from biofilms of all four water systems (Table 2-2). Specifically, Mn-oxidizing and Mn-reducing isolates were recovered from biofilm suspensions from sedimentation basins of all four systems, the filtration basins of Systems 2 and 4, and the distribution pipes of System 3. The average Mn concentration transformed by the microorganisms recovered from all these locations ranged from 2.7 to 4.7 mg L^{-1} Mn oxidized and from 7.9 to 25.7 mg L^{-1} Mn reduced. The concentrations of Mn **-**oxidized and **-**reduced by these isolates are comparable to those reported in other studies performed in natural fresh water and marine systems (Burdige and Nealson, 1985, Francis et al., 2001, Myers and Nealson, 1988a, Myers and Nealson, 1988b, Tebo et al., 2005). The CFU of the isolates in the original biofilms ranged from $5x10^1$ to $5x10^4$ per g of biofilm for Mn-oxidizers and from $5x10^1$ to $1.7x10^5$ per g of biofilm for Mn-reducers (Supplementary Material). This represented between 10^{-5} to 10^{-2} percent of the total CFU (Data

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not shown). Of the 20 Mn-reducing isolates recovered, 4 were capable of also performing Mn-oxidation. Mn-reduction by Mn-oxidizing isolates was not investigated

Identification of Mn-oxidizing and -reducing bacteria. Of the 53 bacteria isolated, 47 were identified as *Bacillus* spp. (15 *Lysinibacillus fusiformis,* 17 *Bacillus pumilus,* 7 *Bacillus cereus,* 5 *Bacillus sphaericus,* 2 *Bacillus simplex,* and 1 *Brevibacillus brevis)*, 4 as *Pseudomonas spp. (1 P. aeruginosa and 3 P. saccharophila)*, and 2 *as Brevundimonas nasdae*. These results are presented in more detail in Tables 2-3 and 2-4. Interestingly, some of the isolates identified as *L. fusiformis, B. pumilus,* and *B. cereus* could both oxidize and reduce Mn (Table 2-3). A similar profile of bacterial species was recovered from all four sites (Table 2-4). The percent sequence similarity for all the isolates analyzed ranged from 98-100% as determined by alignment to sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov).

Rates of Mn- oxidation for selected individual isolates. Figure 2-1a shows the accumulation of Mn-oxidized by selected isolates as a function of time. Isolate MB-1 (obtained from the sedimentation basin of Water System 1) was identified as *B. cereus* while isolates, MB-2 (obtained from the sedimentation basin of Water System 2), MB-3 (obtained from the sedimentation basin of Water System 1) and MB-4 (obtained from the filtration basin of Water System 4) were identified as *B. pumilus*. The pseudo-first-order rate constants obtained for Mn-oxidation by selected isolates range from 0.106 - 0.659 days^{-1} and the results are illustrated in Figure 2-2. The mean R^2 value obtained for the regressions illustrated in Figure 2-2 for the pseudo-first-order rate analysis of Mn-oxidation was 0.96 ± 0.04. Note that the concentration of

oxidized Mn was highest well after cells reached stationary phase and the onset of spore-formation (Figure 2-1b) as observed by another study (Francis et al., 2001). The k_1 rate constants of microbial Mn-oxidation for this study are comparable to those reported previously for pseudo-first-order reactions related to Mn(II) disappearance via chemical oxidation in an initially homogenous solution (Davies and Morgan, 1989, Morgan, 2005) and in sea water (Katsoyiannis and Zouboulis, 2004).

Rates of aerobic Mn-reduction for selected individual isolates. Figure 2-3a shows the accumulation of Mn-reduced aerobically by selected isolates as a function of time. Isolates MB-4 (obtained from the filtration basin of Water System 4) and MB-5 (obtained from the distribution system of Water System 3) were identified as *B. pumilus*, MB-6 (obtained from the sedimentation basin of Water System 1) was identified as *B. cereus*, and MB-7 (obtained from the sedimentation basin of Water System 2) was identified as *L. fusiformis*. The results in Figure 2-3b show that there was modest growth among the aerobic Mn-reducing isolates, relative to the Mn-oxidizing isolates. The pseudo-first-order rate constants obtained for aerobic Mn-reduction ranged from 0.036 - 0.152 days^{-1} and the results are illustrated in Figure 2-4. The mean R^2 value obtained for the regressions illustrated in Figure 2-4 for the pseudo-first-order rate analysis of aerobic Mn-reduction was 0.90 ± 0.08. The k_1 rate constants obtained for aerobic Mn reduction by individual isolates are comparable to those reported in a previous study (Dollhopf et al., 2000).

Rates of Mn-anaerobic reduction for selected individual isolates. Figure 2-5a shows the accumulation of Mn reduced anaerobically by selected isolates as a function of time. Selected

isolates MB-4 (obtained from the filtration basin of Water System 4), MB-5 (obtained from the distribution system of Water System 3), and MB-8 (obtained from the filtration basin of Water System 4) were identified as *B. pumilus* while isolate MB-9 (obtained from the filtration basin of Water System 4) was identified as *B. cereus*. The results in Figure 2-5b show that there was no measureable growth of the anaerobic Mn-reducing isolates, which is not unexpected given the low cell yields typical of anaerobic cultures (Francis et al., 2000, Myers and Nealson, 1988a). The pseudo-first-order rate constants obtained for anaerobic Mn-reduction ranged from 0.024 - 0.052 days^{-1} and the results are illustrated in Figure 2-6. The mean R^2 value obtained for the regressions illustrated in Figure 2-6 for the pseudo-first-order rate analysis of anaerobic Mn-reduction was 0.95 ± 0.06. The k_1 rate constants obtained for anaerobic Mn reduction by individual isolates are comparable to those reported in a previous study (Dollhopf et al., 2000).

2.4. Discussion

Mn-oxidizing and -reducing bacteria were isolated from chlorinated drinking water systems, suggesting that bacteria may influence Mn-release and -deposition in such systems. Both Mn-oxidizing and -reducing microorganisms were recovered from the sedimentation basin of Systems 1, 2, and 3. Anaerobic conditions in the sedimentation basin sludge and excessive microbiological growth can allow Mn release to drinking water (Hoehn et al., 1987). The data indicate that chlorinated drinking water systems harbor biofilm populations capable of Mn-oxidation and -reduction, even for treatment plants that have not reported any Mn-related problems. For instance, Systems 1, 3, and 4 chosen for this study have acknowledged Mn problems (e.g. water discoloration) and employ measures to avoid $MnO_{x(s)}$ deposition in the distribution system. System 1 applies biological filtration for Mn removal so no chlorine

residual is maintained in the filtration basins. System 2 has not reported Mn-associated problems, although higher Mn levels have been measured in its source water on a seasonal basis and chlorine is applied at the plant to prevent Mn(II) entering the distribution system. System 3 has significant Mn problems on a seasonal basis and no solution has yet been applied to control this problem. System 4 maintains a high chlorine residual on top of the manganese oxide coated filters, ranging from 3 - 6 mg L^{-1}, to prevent Mn(II) release to the distribution system since previous studies have shown that MnO_x-coatings on filter media surfaces catalyze Mn-oxidation when chlorine and Mn(II) are present in the water (Hargette and Knocke, 2001, Knocke et al., 1990). Despite the differences in geographic location, water treatment practice, and Mn occurrence, most of the species of Mn-oxidizing and -reducing bacteria recovered were found in two or more systems. This indicates the ubiquity of these microorganisms, and if culture bias is considered, then there are likely even more Mn oxidizers and reducers present that were not detected. Drinking water systems provide enormous surfaces for biofilm formation and thus, the total capacity for microbial-catalyzed Mn-oxidation and reduction could be quite high. The data reported in this study suggest that microbial-catalyzed processes should be considered when dealing with Mn deposition and release problems in chlorinated drinking water systems.

The results of this study have direct applicability to drinking water systems that use chlorine as a disinfectant as spore-forming Mn-oxidizing and -reducing microorganisms of the *Bacillus* spp. can survive in these environments under a wide range of stressful conditions. Mn-oxidation by metabolically dormant spores of *Bacillus* spp. has been reported in other studies (Bargar et al., 2000, Dick et al., 2008, Rosson and Nealson, 1982). Spore-formation by these Mn-oxidizing and Mn-reducing bacilli is likely to confound the problem as spores are disinfectant-resistant. The

four water systems selected are supplied by surface water sources, have conventional treatment plants, and are exposed to different chlorine levels. Mn-oxidizing and -reducing bacilli were isolated from water systems exposed to chlorine levels ranging from 0.3 - 0.6 mg L^{-1} as observed in Systems 2 and 3, to chlorine levels that range from 3 - 6 mg L^{-1} as observed in System 4 (Table 2-1). Studies performed under controlled chlorine-demand-free conditions have shown that *Bacillus* spores survive more than 40 minute – 4 hour exposure of 0.8 - 2.0 mg L^{-1} free available chlorine at pH 7-8 (Rice et al., 2005, Rose et al., 2005). Bacteria in drinking water systems could also be protected from biocidal action due to the resistance of cells in biofilms (Codony et al., 2005, Lechevallier et al., 1987). Thus, a substantially higher concentration of chlorine would be required for bacterial inactivation in a real drinking water system versus that required for inactivation of suspended cells under laboratory chlorine-demand-free conditions. Chlorine concentration profile gradients obtained as a function of biofilm depth using a microelectrode showed a sigmoidal shape where the inflection point was located at the biofilm surface, indicating a decrease in chlorine concentration as a function of depth (de Beer et al., 1994a).

Isolates identified in this study as *L. fusiformis, B. pumilus,* and *B. cereus* were able to oxidize and reduce Mn. Some of these isolates were obtained from the same biofilm sample which indicates the simultaneous existence of Mn-oxidizing and -reducing bacteria. Isolate MB-4 was able to oxidize and reduce (aerobically and anaerobically) Mn. MB-4 was isolated from the anthracite filtration media sample obtained from System 4 and was identified as *B. pumilus*. Pseudo-first-order rate constants were obtained for isolate MB-4 for Mn-oxidation, aerobic Mn-reduction, and anaerobic Mn-reduction as shown in Figures 2-2, 2-4, and 2-6. It has been

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previously reported that certain *Bacillus* and *Pseudomonas* species are capable of performing Mn‑oxidation (Dick et al., 2008, Tebo et al., 2005) and Mn‑reduction (Lovley, 1991, Nealson and Saffarini, 1994). Microbiological "aerobic" Mn-reduction as an environmental process has been reported in aerobic regions of the water column of a lake (Bratina et al., 1998, Ehrlich and Newman, 2009) and in marine environments (Ehrlich and Newman, 2009, Ghiorse and Ehrlich, 1976). Specifically, Mn-reduction performed under aerobic conditions by a marine *Bacillus* has been previously demonstrated (Ghiorse and Ehrlich, 1976). This finding could be important as the role of microbiological reduction under aerobic conditions has not been considered to explain Mn‑release in drinking water systems. Oxygen gradients decrease as a function of depth in a cell cluster of a biofilm (de Beer et al., 1994b). Oxygen consumption by cells in biofilms in drinking water systems will allow for growth and metabolism by anaerobic bacteria.

Mn can be released from filters in water treatment plants when chlorination is discontinued (Gabelich et al., 2006, Hargette and Knocke, 2001). Although the laboratory conditions employed in this study are different than those typically observed in a drinking water system, the time at which microorganisms performed aerobic Mn‑reduction (Figures 2-3a and 2-4) is comparable to the time at which Mn‑release was observed in drinking water systems. In one treatment plant, Mn release resulted in effluent concentrations of $0.07 - 0.11$ mg L^{-1} total Mn approximately 17 days after chlorination was discontinued. In that study, it was stated that coagulant and pH played a key role in determining the release of Mn and that microbial‑catalysis was unlikely to have had a significant role in Mn‑release. Another study of Mn release and deposition in distribution systems found that water quality modeling based on chlorine and oxygen levels and accepted kinetic constants could not predict the Mn concentrations observed,

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suggesting that an additional factor, like bacterial catalysis, could have played a role (Cerrato, 2005, Cerrato et al., 2006). Our data show that bacterial Mn-reduction occurred under aerobic conditions by *Bacillus* spp. after 9 days and the Mn transformed over time continued to increase after 16 days of incubation. These data support the implication of bacterial cycling of manganese in water treatment processes.

2.5. Conclusions

- Mn-oxidizing and -reducing bacteria were isolated from biofilms of four geographically diverse drinking water systems. This study provides supporting evidence of the simultaneous existence of Mn-oxidizing and -reducing bacteria in drinking water systems.

- Most of the isolates (88%) recovered from the drinking water systems were identified as *Bacillus* spp. and several of these bacteria were observed to be capable of both oxidizing and reducing Mn. Specific microorganisms identified in this study were *Lysinibacillus fusiformis, Bacillus pumilus, Bacillus cereus, Bacillus sphaericus, Bacillus simplex*, *Brevibacillus brevis*, *Pseudomonas aeruginosa*, and *Brevundimonas nasdae*.

- The pseudo-first-order rate constants obtained for Mn-oxidation range from 0.106 - 0.659 days^{-1}. The pseudo-first-order rate constants obtained for aerobic Mn-reduction and anaerobic Mn-reduction ranged from 0.036 - 0.152 days^{-1} and 0.024 - 0.052 days^{-1}, respectively.

- The results obtained from this study indicate that bacterial influenced Mn‑oxidation and ‑reduction (aerobic and anaerobic) take place in aqueous environments exposed to considerable oxygen and chlorine levels.

2.6.   Acknowledgements

2.7.   References

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Table 2-1. Description of the water systems studied. Note that Water Systems 1, 3, and 4 have previously reported Mn-associated problems.

Water System	Water Source	Description of Treatment Processes	Sample Obtained
1- Virginia (Coastal Region)	Surface water reservoir	Conventional water treatment processes combined with dissolved air flotation for solid removal and biological filtration. Ozone is used as an oxidant and primary disinfectant, and chloramines for secondary disinfection. Dual-media filter beds of anthracite and sand.	- Sedimentation basin (top and bottom sludge) - Filtration basins (anthracite and sand filtration media). - No chlorine is applied in the filtration basins.
2- Virginia (Appalachian Region)	River	Conventional water treatment processes. Chlorine is used for primary disinfection, chlorine dioxide for oxidation, and chloramines for secondary disinfection. Dual-media filter beds of anthracite and sand.	- Sedimentation basin (top and bottom sludge) - Filtration basins (anthracite and sand filtration media) with approximately 0.5 mg L^{-1} Cl_2 residual in the filter effluent.
3- Honduras	Surface water reservoir	Conventional water treatment processes combined with aeration for chemical oxidation. Chlorine is used for primary and secondary disinfection. Filter beds consist of sand media only.	- Sedimentation basin (top and bottom sludge) - Filtration basins (anthracite and sand filtration media) with approximately 0.3 mg L^{-1} Cl_2 residual in the filter effluent. - Distribution system (iron and PVC pipes) with approximately 0.3-0.6 mg L^{-1} Cl_2 residual.
4- North Carolina	Surface water reservoir	Conventional water treatment processes. Dual-media filter beds of anthracite and sand. Chlorine is applied for disinfection and oxide coated media regeneration.	- Cl_2 residual in the filter effluent ranges from 3- 6 mg L^{-1}.

Table 2-2. Average Mn-oxidation and Mn-reduction by isolates recovered from water systems 1, 2, 3, and 4.

	Mn transformations performed by isolates recovered from:					
	Sedimentation Basin		**Filtration Basin (Anthracite and Sand)**		**Distribution System (Iron and PVC)**	
	Oxidizers[a]	**Reducers**[b]	**Oxidizers**	**Reducers**	**Oxidizers**	**Reducers**
			Water System 1			
No. Isolates	16	4	4	0	N.S.[d]	N.S.
Mn (mg L^{-1})[c]	3.4 ± 1.2	25.4 ± 15.2	4.0	0	N.S.	N.S.
			Water System 2			
No. Isolates	4	6	3	3	N.S.	N.S.
Mn (mg L^{-1})	3.2 ± 1.1	13.1 ± 7.0	3.1 ± 1.2	13.6 ± 6.1	N.S.	N.S.
			Water System 3			
No. Isolates	2	1	1	0	1	3
Mn (mg L^{-1})	2.7 ± 2.1	25.6	4.9	0	4.7	7.9 ± 0.6
			Water System 4			
No. Isolates	N.S.	N.S.	2	3	N.S.	N.S.
Mn (mg L^{-1})	N.S.	N.S.	2.7 ± 0.4	20.1 ± 0.3	N.S.	N.S.

[a] Mn oxidized = Mn(II) oxidized to Mn(IV) after 2 weeks ± standard deviation.
[b] Mn reduced = Mn(IV) reduced to Mn(II) after 5 weeks of anaerobic incubation ± standard deviation.
[c] Results corrected for the abiotic controls of Mn-oxidation and -reduction.
[d] N.S. = Not Sampled

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Table 2-3. Identity of isolates and their Mn-oxidation and -reduction (aerobic or anaerobic conditions) capabilities.

Identity [a]	Number of Isolates	Number of Isolates: Mn Transformation		
		Oxidation	Reduction	
			Aerobic	Anaerobic
Lysinibacillus fusiformis[b]	15	5	1	10
Bacillus pumilus[b]	17	14	2	3
Bacillus cereus[b]	7	4	1	3
Lysinibacillus sphaericus	5	3	N.M. [c]	2
Pseudomonas aeruginosa	1	1	N.M.	N.M.
Pseudomonas saccharophila	3	2	N.M.	1
Brevundimonas nasdae	2	2	N.M.	N.M.
Bacillus simplex	2	2	N.M.	N.M.
Brevibacillus brevis	1	1	N.M.	N.M.

[a] The percent sequence similarity for all isolates analyzed ranged from 98-100% as determined by alignment to sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov)

[b]Some strains were capable of performing more than one Mn-transformation.

[c] N.M. = Not Measured

Table 2-4. Identity of the isolates related to the sampling location.

Identity	Number of Isolates	Sampling Location												
		System 1				System 2				System 3				System 4
		Sed[a]		Filt[b]		Sed		Filt		Sed		Filt	DS[g]	Filt
		T[c]	B[d]	A[e]	S[f]	T	B	A	S	T	B	S		A
Lysinibacillus fusiformis	15	4				3	3	2	1		1		1	
Bacillus pumilus	17	4	2	2	1		3						1	4
Bacillus cereus	7	3	1								1		1	1
Bacillus sphaericus	5	2	1			1		1						
Pseudomonas aeruginosa	1												1	
Pseudomonas saccharophila	3	2						1						
Brevundimonas nasdae	2	1						1						
Bacillus simplex	2			1							1			
Brevibacillus brevis	1											1		

[a] Sed = Sedimentation Basin

[b] Filt = Filtration Basin

[c] T = Top sludge

[d] B = Bottom sludge

[e] A = Anthracite filtration media

[f] S = Sand filtration media

[g] DS = Distribution System

Figure 2-1. a. Time course of Mn(II) -oxidation by selected individual isolates. **b.** Time course of aerobic growth in Mn-oxidation broth for individual selected isolates. The control consisted of uninoculated sterile media. MB-1 was identified as *B. cereus* and MB2, MB-3, and MB-4 were identified as *B. pumilus* (GenBank Accession Numbers HM055943-HM055946).
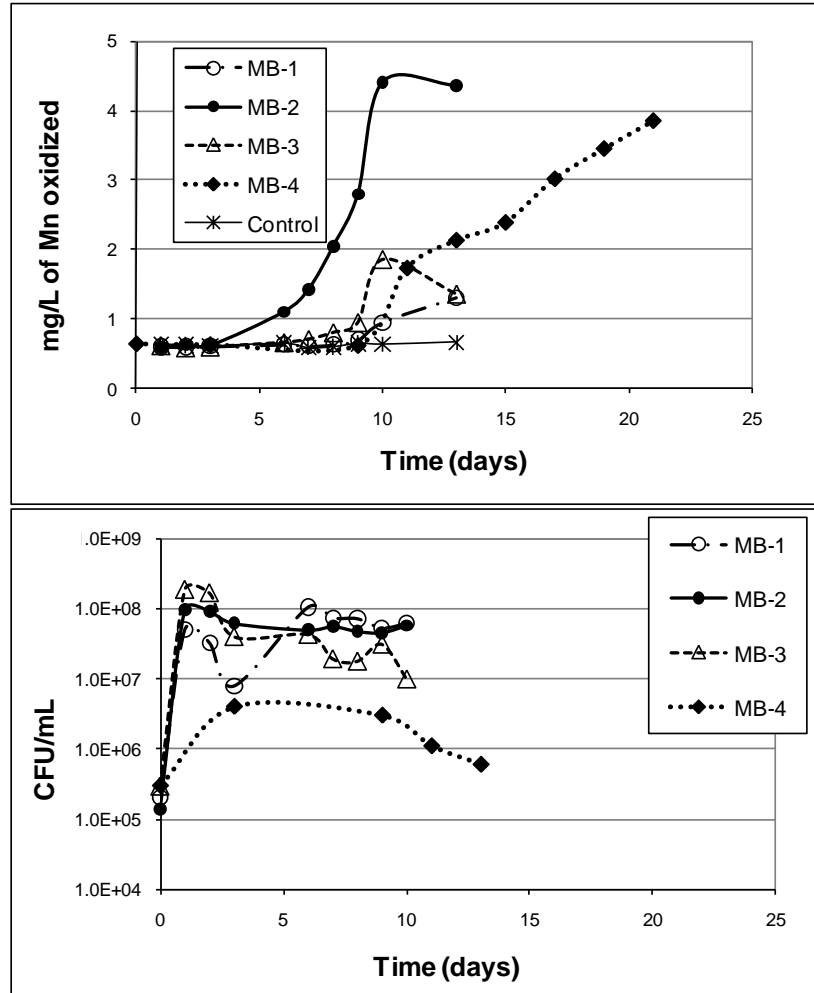
Figure 2-2. Pseudo-first order rate constants for Mn(II) -oxidation by selected individual isolates.
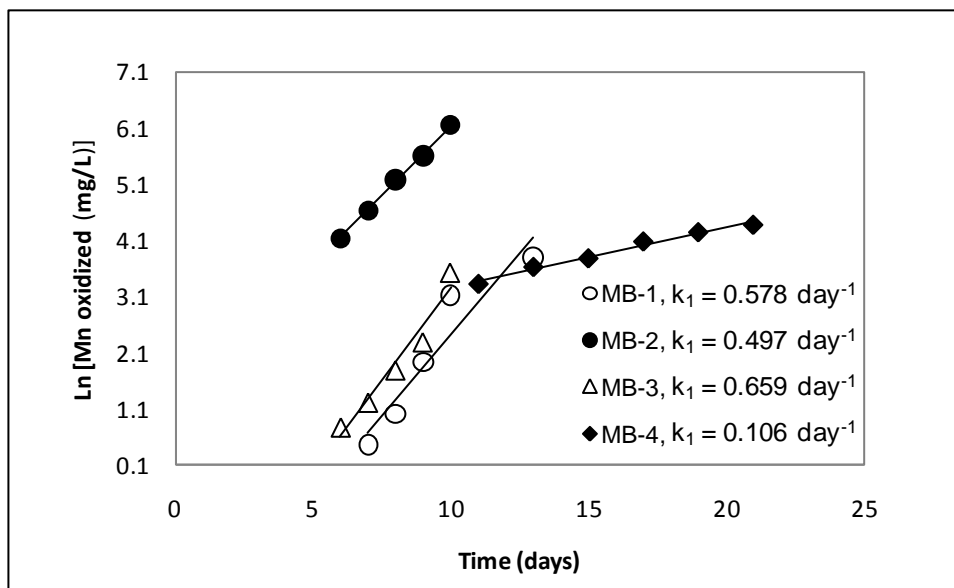
Figure 2-3. a. Time course of "aerobic" Mn(IV) -reduction by selected individual isolates. **b.** Time course of aerobic growth in Mn-reduction broth for individual selected isolates. The control consisted of uninoculated sterile media. MB-4 and MB-5 were identified as *B. pumilus*, MB-6 was identified as *B. cereus,* and MB-7 was identified as *L. fusiformis* (GenBank Accession Numbers HM055946-HM055949).
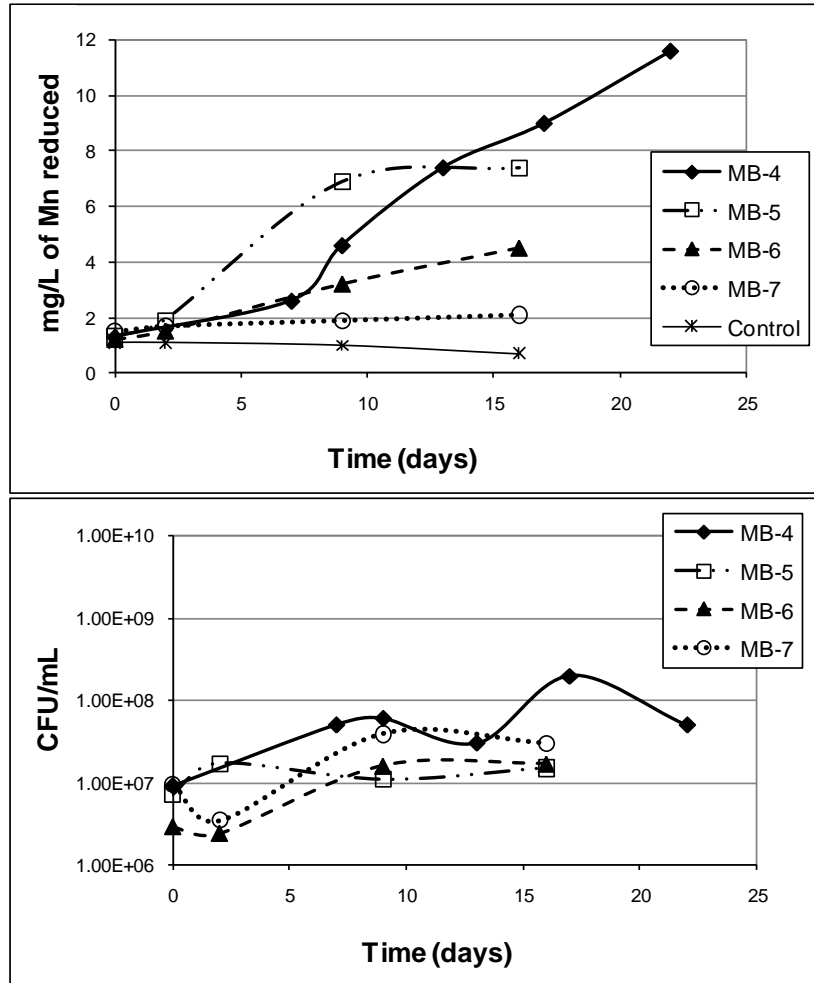
Figure 2-4. Pseudo-first order rate constants for "aerobic" Mn(IV) -reduction by selected individual isolates.

MB-4, $k_1 = 0.117$ day^{-1}
MB-5, $k_1 = 0.152$ day^{-1}
MB-6, $k_1 = 0.147$ day^{-1}
MB-7, $k_1 = 0.036$ day^{-1}

Figure 2-5. a. Time course of "anaerobic" Mn(IV) -reduction by selected individual isolates. **b.** Time course of anaerobic growth in Mn-reduction broth for individual selected isolates. The control consisted of uninoculated sterile media. MB-4, MB-5, and MB-8 were identified as *B. pumilus*, and MB-9 was identified as *B. cereus* (GenBank Accession Numbers HM055946-HM055947, HM055950-HM055951).

Figure 2-6. Pseudo-first order rate constants for "anaerobic" Mn(IV) -reduction by selected individual isolates.

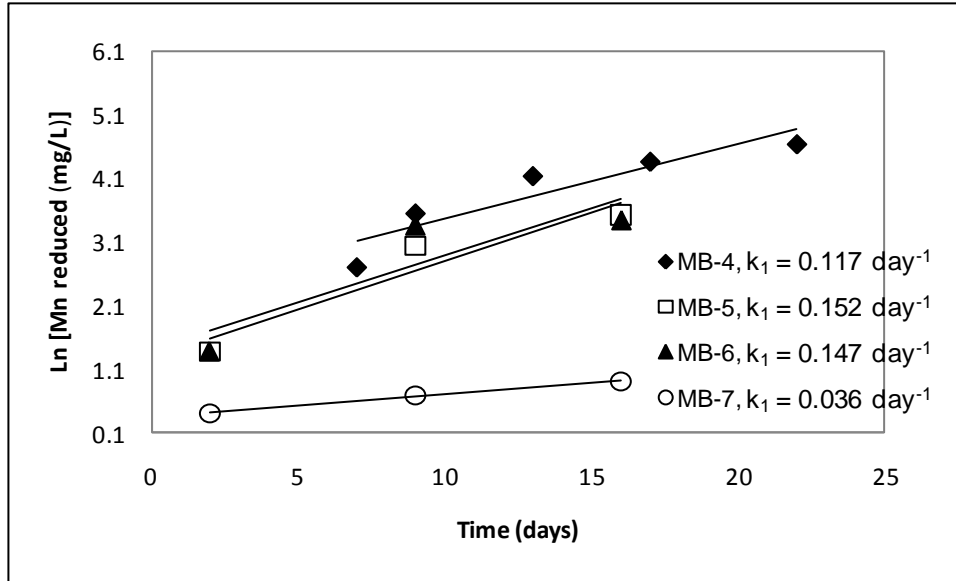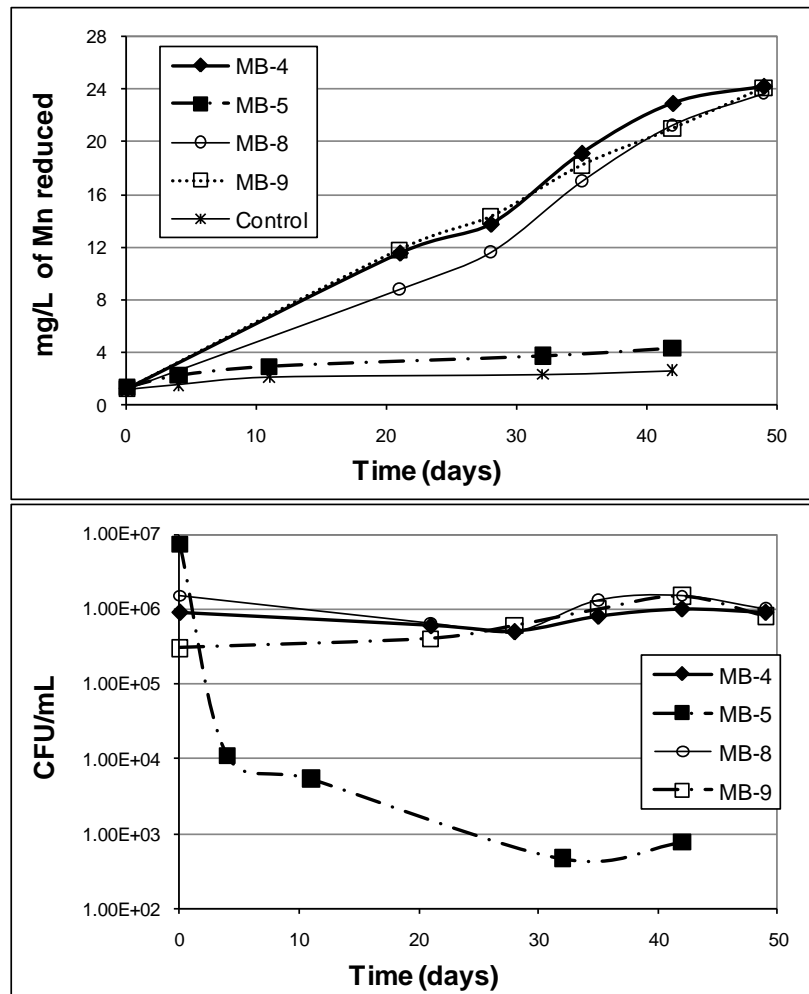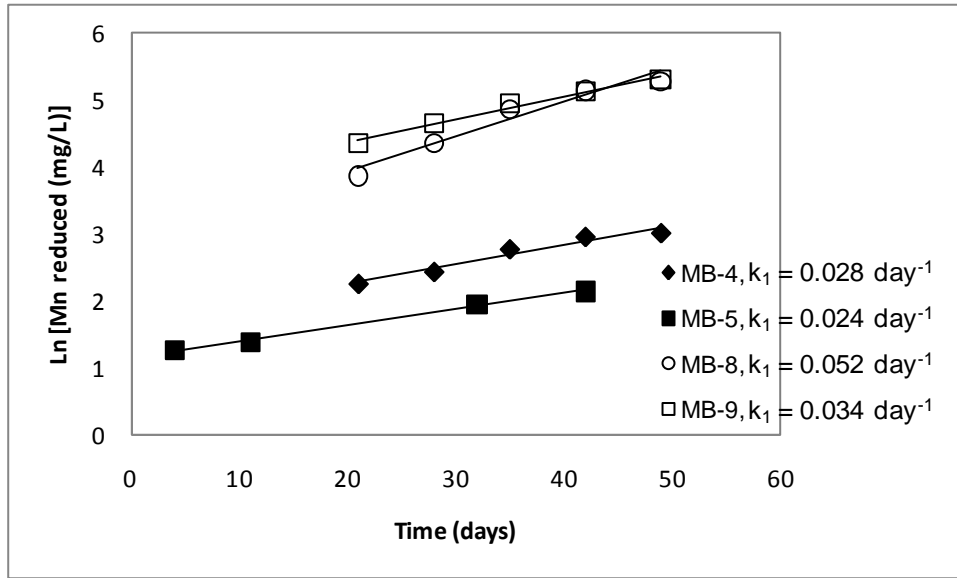# CHAPTER 3: Use of XPS to identify the oxidation state of Mn in solid surfaces of filtration media oxide samples from drinking water treatment plants

José M. Cerrato, Michael F. Hochella Jr., William R. Knocke, Andrea M. Dietrich, and

Thomas F. Cromer

ABSTRACT

X-Ray Photoelectron Spectroscopy (XPS) was used to identify Mn(II), Mn(III), and Mn(IV) in the surfaces of pure oxide standards and filtration media samples from drinking water treatment plants through the determination of the magnitude of the Mn 3s multiplet splitting and the position and shape of the Mn 3p photo-line. The Mn 3p region has been widely studied by applied physicists and surface scientists, but its application to identify the oxidation state of Mn in heterogeneous oxide samples has been limited. This study shows that the use of both the Mn 3s multiplet splitting and the position and shape of the Mn 3p photo-line provides a feasible means of determining the oxidation state of manganese in complex heterogeneous, environmentally important samples. Surface analysis of filtration media samples from several drinking water treatment plants was conducted. While Mn(IV) was predominant in most samples, a mixture of Mn(III) and Mn(IV) was also identified in some of the filtration media samples studied. The predominance of Mn(IV) in the media samples was felt to be related to the maintenance of free chlorine (HOCl) at substantial concentrations (2-5 $mg \cdot L^{-1}$ as Cl_2) across

these filters. XPS could be a useful tool to further understand the specific mechanisms affecting soluble Mn removal using MnO_x-coated filtration media.

KEYWORDS: Manganese, Oxidation State, XPS, Mn 3s, Mn 3p

3.1.   Introduction

Manganese (Mn) is a transition metal that plays a fundamental role in many environmental reactions. Its oxides and oxyhydroxides significantly affect cycling of metals of environmental importance such as arsenic, copper, lead, and zinc (1-5). Mn is also found in drinking water sources (i.e. groundwater and surface water reservoirs) and can cause aesthetic issues such as unpleasant taste, water discoloration, and staining of plumbing fixtures (6-7). For this reason, the United States Environmental Protection Agency (USEPA) has established a secondary maximum contaminant limit of $0.05mg \cdot L^{-1}$ for Mn (8).

The inherent complexity of natural systems implies major challenges to the mechanistic understanding of relevant biogeochemical processes that affect metal cycling in the environment (9). Deduction of microscopic behavior from macroscopic models alone has been shown to lack predictive capability, even in relatively simple systems (10). In addition, solution phase analyses have traditionally been used to try to understand surface-associated biogeochemical interactions, but have yielded limited mechanistic information (11). However, the advancement of spectroscopic and microscopic methods developed in past decades has significantly increased the analytical ability to study biogeochemical processes in the environment at the molecular and atomic levels (10-13). Spectroscopic and microscopic analytical tools coupled with macroscopic analyses and advances in modeling can provide the level of assessment necessary to obtain a

more complete perspective of contaminant reactivity and bioavailability over a range of environmental conditions (14).

X-ray photoelectron spectroscopy (XPS) is the most established surface analysis technique for determining the oxidation state of Mn in near-surface regions of minerals and other materials. The three dominant oxidation states of Mn in the environment are II, III, and IV. Each of these oxidation states has widely different solubilites that can be linked to redox reactions associated with their aqueous/solid state partitioning (2, 11, 15-16). These XPS analyses are often carried out in the 2p region because it has the highest photo-ionization cross section (the most intense signal). However, this is not the most desirable method for determining oxidation states for Mn because the Mn 2p peaks are broad and the photo-peak shifts as a function of oxidation state are relatively small (2, 17).

For manganese, extensive research has been performed in an attempt to better understand both the Mn 3s and Mn 3p regions (18-22) which can also be used for oxidation state information. For example, the Mn(II) initial state $(3s^2 3p^6 3d^5)^6S$ has two possible final states, $(3s^1 3p^6 3d^5)^7S$ and $(3s^1 3p^6 3d^5)^5S$. In the ^7S symmetry state the remaining 3s electron has a spin parallel to the unpaired 3d electron, and in the ^5S state the two spins are anti-parallel (22). This results in two photolines (known as multiplet splitting). The energy difference between these two lines has been reported to be a reliable method to distinguish between oxidation states II, III, and IV in Mn oxides (2, 15, 17, 23). The Mn 3s multiplet splitting becomes smaller as the oxidation state of Mn increases (2, 22).

The Mn 3p line has also been used by applied physicists to determine the oxidation state of Mn (18, 22-24). Photoemissions from the Mn 3p core level can be described by different final states in a manner analogous to Mn 3s (19-20). However, multiplets in the 3p region are inherently more complex than in the 3s region due to the added degree of freedom of the coupling of orbital angular momentum (20), that is, spin orbit splitting. The chemical shift of the Mn $3p_{3/2,1/2}$ spin orbit pair has been associated with the II, III, and IV oxidation states of Mn, where the main Mn 3p line represents the ^7P ionic final state and the higher binding energy shoulders represent several ^5P spin-orbit components (20, 22).

Several studies have determined the oxidation state of Mn in environmental oxide samples using the Mn 2p and Mn 3s regions (2, 15, 25-26). There are limited references focusing on the analysis of the Mn 3p region to identify the oxidation of Mn in environmental oxide samples. The possibility of elucidating the oxidation state of Mn using the 3p photopeak has even been discarded in a previous investigation (23).

The objective of this study is to evaluate the applicability of XPS to identify the oxidation state of Mn in the MnOx(s) coatings often found on filtration media in water treatment plants. Specific focus is given to assessing the use of the Mn 3s multiplet splitting and the Mn 3p photo-line in XPS for differentiating Mn (II), Mn (III) and Mn(IV) in such coatings. Prior research has demonstrated that MnO_x-coated filter media can be very effective in removing soluble Mn during drinking water treatment operations, either via physical-chemical interactions alone (27-29) or when considering a combination of physical-chemical and microbial interactions (30). These studies and others have employed various analysis methods in an attempt to characterize

the forms of Mn present on the media surface. Cited difficulties include the somewhat amorphous nature of the $MnO_x(s)$ solids present as well as the heterogeneity of the metals found in these surfaces. Successful achievement of an XPS-based surface analysis method (as proposed in this study) that would differentiate Mn valence states in such oxide surfaces could help researchers in their efforts to better define the specific mechanisms involved when soluble Mn is removed via this treatment technique.

3.2.   Experimental Section

Mn oxide standards and filtration media samples. The following monovalent oxide powder standards were purchased and used for analyzing Mn oxidation states II, III, and IV using XPS: 99% pure MnO [manganous oxide, Mn(II)] and 99% pure Mn_2O_3 [manganic oxide, Mn(III)], both purchased from Strem Chemicals - Newburyport, MA, USA, and 99.999% pure MnO_2 (manganese dioxide, Mn(IV), purchased from Acros Organics/Thermo Fisher Scientific – Pittsburg, PA, USA). A multivalent Mn oxide standard was made by mixing the Mn_2O_3 (50%) standard and the MnO_2 (50%) standard cited above. A 97% pure $LiMn_2O_4$ (purchased from Sigma Aldrich, St. Louis, MO, USA) with combined III, IV Mn oxidation states was also used.

XPS analyses of pyrolusite ($MnO_2(s)$) filtration media samples were pursued in this study because this mineral can be used to effectively remove both soluble and particulate Mn from drinking water (31). A standard sample (LayneOx™ brand, Layne Christensen Co., Mission Woods, KS) was utilized as a control and will be called "unused" pyrolusite throughout this article (unused in the sense that the material analyzed was fresh). A portion of this unused pyrolusite medium was placed in an experimental filtration column setup that was dosed with

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0.05 - 0.15mg•L^{-1} soluble Mn at a rate of 16 - 20 gpm•ft^{-2} in order to evaluate its Mn sorption capacity and to monitor any changes in its surface chemistry. A sample of this pyrolusite medium was obtained from the experimental filtration column setup after 18 months of operation and will be called "used" pyrolusite for the purposes of this study.

Finally, anthracite coal filtration media from four different conventional water treatment plants, labeled filtration media 1, 2, 3, and 4, were obtained and analyzed with XPS (Table 3-1). Anthracite coal is a commonly used media in granular bed filters at drinking water treatment plants, primarily for the purpose of removing particulate matter (8). Researchers (27-29) have shown that MnO_x(s) coatings will form naturally over time on anthracite coal media if it is exposed to both soluble Mn and HOCl on a fairly continuous basis. These coatings (typically ranging from 2-50 mg extractable Mn per g anthracite media) are quite effective at promoting soluble Mn removal, helping utilities meet treatment criteria regarding Mn content in the water provided to consumers.

Elemental Extractions. A method developed by Knocke *et al*. (27) was used to determine the extractable elemental content on the filtration media samples. Media samples were stored in acid washed polyethylene bottles and air dried within hours of receipt; 4 g of dry media were placed into a 250 mL Erlenmeyer flask with 100 mL of 0.5% Nitric Acid and approximately 600 mg of hydroxylamine sulfate (HAS). The solution was allowed to react for two hours. A solution sample was obtained after this reaction time and filtered through a 0.45µm membrane. The filtrate metal concentrations were analyzed using an inductively coupled plasma mass

spectrophotometer ICP-MS according to Standard Method 3125-B (32). The extractable surface elemental content (mg of element per g media) was calculated using the following equation:

$$EMC = [C * V] / W$$

where:

EMC = extractable metal (mg metal g^{-1}•media)

C = measured soluble metal concentration (mg•L^{-1})

V = volume of solution = 0.1 L

W = mass of media placed in the flask = 4g

The results obtained from the application of this method have a "suggestive" character since it is possible that the metal oxide coatings are not completely digested after the application of hydroxylamine sulfate and nitric acid. Thus, this method is supposed to provide an approximate characterization of the elemental content of filter media surface coatings.

XPS Analysis. A Perkin-Elmer 5400 X-ray photoelectron spectrometer (Physical Electronic Industries, Inc.) consisting of an aluminum X-ray source and two-channel collector was used. The X-ray anode was operated at 12 keV and 250 W and the vacuum was maintained at or below 5×10^{-7} Torr. The pass energy for survey wide scans was 89.45 eV. The pass energy for narrow scans was 17.9 eV. The narrow scan spectra presented for Mn in pure oxide standards represent averages of 100 sweeps with 25 ms for every 0.1 eV step. For filtration media samples, the spectra presented for Mn represent averages of 1000 sweeps with 25 ms for every 0.1 eV step. Note that the average number of sweeps for narrow scan spectra presented for Mn in filtration media samples was approximately 10 times greater than that for pure oxides. This was likely

due to electron energy loss in the detection of the Mn signal when utilizing XPS caused by the major presence of other elements in the near surface region of filtration media samples. Data were analyzed using AugerScan 3.12 (RBD Enterprises Inc.). The oxidation state of Mn was identified by two methods: 1) determination of the Mn 3s multiplet splitting, and 2) careful observations of the position and shape of the Mn 3p photo-line. Narrow scans of the Mn 3s photolines were analyzed using a Shirley background subtraction (16, 33) and least-squares Gaussian-Lorentzian curve-fitting algorithm (2, 15). Samples analyzed in the Mn 3s region were charge referenced to the carbon C1s peak at 285.0 eV. Gold (Au) was vacuum-deposited on all samples analyzed in the Mn 3p region to charge reference the binding energy to the Au $4f_{7/2}$ peak position at 84.0 eV. Narrow scans of the Mn 3p photoline were analyzed using Shirley background subtraction (16, 33) and the data were then normalized so that the peaks for the pure standards and samples could be compared at a common maximum height.

3.3.   Results and discussion

Metal Extractions. Previous studies that used extraction experiments and various surface sensitive analytic techniques demonstrated the presence of various elements in filtration media surface coatings including Al, Fe, Si, and Mn (28-29). The same variety of elements was observed in this study (Table 3-2). The extraction results show that the "unused" and "used" pyrolusite samples contained 38.4 and 45.1 mg Mn per gram of media, respectively. Filtration media 1 contained 30.7 mg Mn per gram of media, showing the highest extractable Mn content among all samples obtained from operating water treatment plants. Filtration media 2 and 3 showed extractable Mn contents of 22.6 and 20.7 mg Mn per gram of media, respectively. Filtration media 4 contained 5.2 mg Mn per gram of media, showing the lowest extractable Mn

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content among all samples obtained from operating water treatment plants. Note that filtration media 1, 2, 3, and 4 had a much higher extractable Al content than the "unused" and "used" pyrolusite samples that may have been due to the application of aluminum sulfate (alum) as the main coagulant used in the water treatment plants from which the samples were collected. The possible co-precipitation of Al with Mn in the surface oxide coatings of filtration media has been hypothesized (29). The extractable Fe in the samples studied ranged from 0.2-1.2 mg Fe per gram of media. It was not surprising that little Fe was detected in the filtration media samples analyzed since it is a common practice to remove iron in the early stages of water treatment by oxidation and solid liquid separation processes (i.e. sedimentation and filtration) (8). Thus, little Fe remains in the water that ultimately passes through the filter media. Extractable content of Cu was detected in all samples except in filtration media 4; filtration media 3 showed the highest extractable Cu content among all samples. The extractable content of 1.2 mg Cu per gram of media in filtration media 3 could be associated with the application of copper sulfate ($CuSO_4$) in the reservoir providing water to this facility as it is routinely applied to control algal growth at various times of the year. Ca, Zn, and Si were the other three elements analyzed from the media samples tested, supporting the variety of elements reported in other studies that characterized the composition of filtration media surface coatings (28-29).

XPS Analysis of Standards. The results obtained from the XPS analyses performed in the Mn 3s region are presented in Table 3-3. Multiplet splittings of 5.7 ± 0.01 eV were obtained for Mn(II), 5.3 ± 0.05 eV for Mn(III), and 4.5 ± 0.02 eV for Mn(IV) standards (n=3, see Table 3-3). These values are consistent with those reported in other studies (2, 15, 17, 22).

The results of the XPS analyses performed in the Mn 3p region for the Mn(II), Mn(III), and Mn(IV) standards are illustrated in Figure 3-1a. Once the background has been subtracted using the Shirley algorithm (16, 33) and the peaks have been size normalized, analyses of the Mn 3p region show the chemical shift of the Mn $3p_{3/2,1/2}$ spin orbit pair. The main 3p line represents the [7]P ionic final state (22). It is interesting to note in this study that the 3p line positions referenced to the Au $4f_{7/2}$ peak position at 84.0 eV gave essentially the same results as the line positions referenced to the carbon C 1s photopeak. However, as shown in previous studies (2, 34), referencing to the Au $4f_{7/2}$ peak position at 84.0 eV is more reliable than arbitrarily assigning a single absolute binding energy to the C1s adventitious carbon photopeak.

The Mn 3s multiplet splitting obtained for the laboratory Mn(III, IV) mixture and the $LiMn_2O_4$ standard [both 50% Mn(III), 50% Mn(IV)] was 5.0 ± 0.1 eV; these values fall between 4.5 eV and 5.3 eV, the mean multiplet splitting values obtained for Mn(IV) and Mn(III), respectively, as would be expected. The results for the XPS analyses of the Mn 3p region are illustrated in Figure 3-1b for the laboratory Mn(III, IV) mixture and in Figure 3-1c for the laboratory Mn(III, IV) mixture versus the Li Mn(III, IV) mixture. Figure 3-1b illustrates that the Mn 3p photo-line for the laboratory Mn(III, IV) mixture is located between the Mn(III) and Mn(IV) photoline positions. Figure 3-1c shows a close match between the Mn3p photopeaks for both the laboratory Mn(III, IV) mixture and the Li Mn(III, IV) standard.

The results obtained for these known standards confirm the applicability of XPS analyses of the Mn 3s and Mn 3p regions to identify Mn oxidation states II, III, and IV. Having established

the validity of these methods using known standards, analyses of filtration media samples from drinking water treatment plants were then pursued.

XPS Analyses of Filtration Media Samples. As expected, analysis of the XPS Mn 3s and Mn 3p regions determined that the oxidation state of Mn in the "unused" pyrolusite sample is IV. The Mn 3s multiplet splitting value obtained for this sample coincides with that of the Mn(IV) standard at 4.5 eV (Table 3-3). The Mn 3p photoline of the "unused" pyrolusite sample closely matches that of the Mn(IV) standard (Figure 3-2a).

Both Mn III and IV oxidation states were identified in the surface of "used" pyrolusite using both the Mn 3s and Mn 3p regions. The Mn 3s multiplet splitting value obtained for the "used" pyrolusite sample coincides with that of the laboratory Mn(III, IV) mixture and the Li Mn(III, IV) standards at 5.0 eV (Table 3-3). The XPS analysis in the Mn 3p region revealed that the photo-peak of the "used" pyrolusite sample was located between that of the Mn(III) and Mn(IV) standards. The Mn 3p photopeaks of "used" pyrolusite and the laboratory Mn(III, IV) mixture standard (Figure 3-2b) show a close match between both peaks. Although, the photo-line of the "used" pyrolusite sample broadens slightly to higher binding energy compared to that of the laboratory Mn(III,IV) mixture. This result indicated that, while a mixture of both Mn(III) and Mn(IV) oxidation states has been identified for "used" pyrolusite through analyses of the Mn 3s and Mn 3p regions, there may have been a slight predominance of Mn(IV) over Mn(III) in this sample.

Mn(IV) was identified in filtration media 1. The Mn 3s multiplet splitting value obtained for filtration media 1 coincides with that of the Mn(IV) standard at 4.5 eV (Table 3-3). Furthermore,

the Mn 3p photo-peaks of filtration media 1 and the Mn(IV) standard illustrated in Figure 3-3a match closely.

A mixture of III, IV oxidation states of Mn was identified in filtration media 2. The Mn 3s multiplet splitting value obtained for filtration media 2 coincided with that of the laboratory Mn(III, IV) mixture and the Li Mn(III, IV) standards at 5.0 eV (Table 3-3). The Mn 3p photo-peak of filtration media 2 was located between that of the Mn(III) and Mn(IV) standards. A close match between the Mn 3p photo-peaks of filtration media 2 and the laboratory Mn(III, IV) standard was observed (Figure 3-3b).

Mn(IV) was identified in filtration media 3 using the Mn 3p photopeak. A close match between filtration media 3 and the Mn(IV) standard was observed in the Mn 3p region (Figure 3-3c). It was not possible to determine the Mn 3s multiplet splitting in filtration media 3. Although the extractable content in filtration media 3 was 20.7 mg Mn per g of media, a weak signal was obtained for the Mn 3s region. It should be noted that the data obtained from the hydroxylamine sulfate extraction does not provide specific information about the amount of Mn contained in the near-surface region detected by XPS. Other studies used microprobe analyses to show that Mn content across the depth of the oxide coating could be highly variable (29). Since the photo-ionization cross section of the 3s region is less than that of the 3p region (22), using the Mn3p photopeak would be more applicable for the identification of Mn oxidation states in this sample.

Mn(IV) was identified in filtration media 4 using the XPS Mn 3p photopeak. Filtration media 4 show the lowest extractable Mn content among all samples, hence, its signal in the Mn 3p region was the noisiest (Figure 3-3d). However, as shown in Figure 3-3d, the Mn 3p photo-

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peak of filtration media 4 closely resembled that of the Mn(IV) standard. The Mn 3s multiplet splitting was not detected in this sample. Filtration media 4 contained 5.2 mg Mn per g of media which was not enough to yield a clear signal for the ^{5}S and ^{7}S peaks in the Mn 3s region (Table 3-3).

Applications related to water treatment. Chlorine is applied for disinfection and regeneration of Mn‑oxide‑coated filter media in the water treatment plants where filtration media 1 and 2 were collected. The chlorine residual maintained in the filter effluent of the water treatment plant where filtration media 1 was collected ranges from 3 to 6 mg•L^{-1}, at least three times greater than that maintained in the filter effluent where filtration media 2 was collected (29). This indicates that filtration media 1 was exposed to more oxidizing conditions compared to those of filtration media 2, which is consistent with the identification of Mn(IV) in filtration media 1 and a mixture of Mn(III) and Mn(IV) in filtration media 2. Chlorine levels ranging from 1 to 2 mg•L^{-1} were applied to the "used" pyrolusite samples for which a mixed Mn(III, IV) oxidation state was identified.

While an investigation that characterized filtration media obtained from drinking water treatment plants reported the identification of Mn(IV) as the predominant species (28), other researchers have identified the presence of an intermediate Mn(III, IV) state (26, 35). The findings of this study confirm that it is possible to encounter both Mn(IV) and Mn(III, IV) oxidation states in filtration media samples. XPS analyses in the Mn 3s and Mn 3p regions can be used with confidence in future investigations to elucidate the mechanisms determining the

predominance of either oxidation state of Mn in filtration media of drinking water treatment plants.

General Application of the XPS Mn 3s and Mn 3p regions. The possibility of using both the XPS Mn 3s and Mn 3p regions increases the ability to verify Mn oxidation state identification in solid surfaces with more certainty, independent of the complexities in chemical composition and structure that characterize unknown environmental oxide samples. The photo-ionization cross section of the 3s region is less than that of the 3p region (22). Therefore, if the content of extractable Mn in the sample is high enough (i.e. greater than 20 mg Mn per gram of media), it is possible to obtain a signal for both the 3s and 3p photo-lines (as observed in the "used" pyrolusite sample and filtration media 1 and 2 for this study), permitting the use of both regions to verify the results. When the sample has insufficient Mn content to produce a detectable signal in the 3s region but high enough to yield a detectable signal in the 3p region (as observed in filtration media 3 and 4 for this study), analyses in the Mn 3p region can still be performed to identify the oxidation state of Mn in the sample. As the results of this study indicate, when using XPS instruments with conventional X-ray sources, it is very difficult or not possible to detect a signal in either the Mn 3s or Mn 3p regions when the extractable Mn content of a filtration media sample is less than 5 mg Mn per gram of media.

XPS analyses in the 3p region can be potentially applied not only to Mn but also to study other transition metals such as Fe, Cr, Sb, Sn, Ti, and V (36).

3.4. Acknowledgements

3.5. References

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Table 3-1. Description of the water treatment plants where the filtration media used for this study were obtained.

Sample	Water Source	Description of Treatment Processes
Filtration Media 1: North Carolina	Surface water reservoir	Conventional water treatment processes; raw water coagulated with ferric chloride. Dual-media filter beds of anthracite and sand. Pre-oxidation with $KMnO_4$ is applied in a seasonal basis. Chlorine is applied for disinfection and oxide coated media regeneration. Cl_2 residual in the filter effluent ranges from 3 - 6 mg•L^{-1}.
Filtration Media 2: Virginia (Coastal Region)	River	Conventional water treatment processes; utilizes coagulation (alum and polymer), superpulsators (flocculation/settling), intermediate ozonation, and dual filtration beds of anthracite and sand media. Pre-oxidation with $KMnO_4$ is applied in a seasonal basis. Chlorine is applied for disinfection and oxide coated media regeneration. Cl_2 residual in the filter effluent ranges from 0.5 - 1 mg•L^{-1}.
Filtration Media 3: Virginia (Coastal Region)	Surface water reservoir	Conventional water treatment processes combined with dissolved air flotation for solid removal and biological filtration. Raw water coagulated with alum and polymer. Ozone is used as an oxidant and primary disinfectant, and chloramines for secondary disinfection. Dual-media filter beds of anthracite and sand. No chlorine is applied in the filtration basins.
Filtration Media 4: Virginia (Appalachian Region)	River	Conventional water treatment processes. Raw water is coagulated with alum. Chlorine is used for primary disinfection, chlorine dioxide for oxidation, and chloramines for secondary disinfection. Dual-media filter beds of anthracite and sand. Cl_2 residual in the filter effluent is approximately 0.5 mg•L^{-1}.

Table 3-2. Average hydroxylamine sulfate extractable elemental content, mg element per g of media, for filtration media samples analyzed (n=3 for all samples).

Sample	mg element per g of media						
	Mn	Al	Fe	Si	Ca	Zn	Cu
"Unused" Pyrolusite	38.4 ± 7.9	1.4 ± 1.0	0.9 ± 0.2	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
"Used" Pyrolusite	45.1 ± 1.36	1.9 ± 0.1	1.6 ± 0.1	0.1 ± 0.0	0.6 ± 0.0	0.1 ± 0.0	0.4 ± 0.0
Filtration Media 1	30.7 ± 1.5	14.4 ± 0.6	1.2 ± 0.1	0.5 ± 0.1	1.1 ± 0.1	0.7 ± 0.0	0.2 ± 0.1
Filtration Media 2	22.6 ± 2.1	13.5 ± 4.7	0.6 ± 0.2	0.3 ± 0.1	1.4 ± 0.2	0.1 ± 0.0	0.1 ± 0.1
Filtration Media 3	20.7 ± 2.3	13.6 ± 0.6	0.4 ± 0.0	0.3 ± 0.0	1.4 ± 0.1	0.1 ± 0.0	1.2 ± 0.4
Filtration Media 4	5.2 ± 0.1	10.8 ± 0.2	0.2 ± 0.0	2.6 ± 0.1	0.6 ± 0.1	0.1 ± 0.0	< 0.1

Table 3-3. Mn 3s multiplet splitting results from this study.

Sample	Curve 1: Mn ^{5}S (eV)	Curve 2: Mn ^{7}S (eV)	Multiplet Splitting (eV)
Standards			
MnO; Mn(II)	89.23	83.54	5.69
MnO; Mn(II)	89.05	83.37	5.68
MnO; Mn(II)	89.23	83.55	5.68
Mn_2O_3; Mn(III)	89.17	83.86	5.31
Mn_2O_3; Mn(III)	88.94	83.72	5.22
Mn_2O_3; Mn(III)	89.13	83.89	5.24
MnO_2; Mn(IV)	88.95	84.42	4.53
MnO_2; Mn(IV)	88.04	83.50	4.54
MnO_2; Mn(IV)	87.86	83.35	4.51
Lab Mn(III, IV) Mixture	88.82	83.73	5.09
Li Mn(III, IV)	88.44	83.44	5.00
Filtration Media			
"Unused" Pyrolusite	88.45	83.95	4.50
"Used" Pyrolusite	88.46	83.46	5.00
Filtration Media 1	88.12	83.58	4.54
Filtration Media 2	88.55	83.55	5.00
Filtration Media 3	N.S.[a]	N.S.	N.S.
Filtration Media 4	N.S.	N.S.	N.S.

[a] N.S. = No Signal

Figure 3-1. Mn 3p (^{7}P) photo-peak spectrum for: **a)** Mn(II), Mn(III), and Mn(IV) standards, **b)** laboratory Mn (III, IV) mixture vs. Mn(III) and Mn(IV) standards, and **c)** laboratory Mn (III, IV) mixture vs. Li Mn(III, IV) oxide.
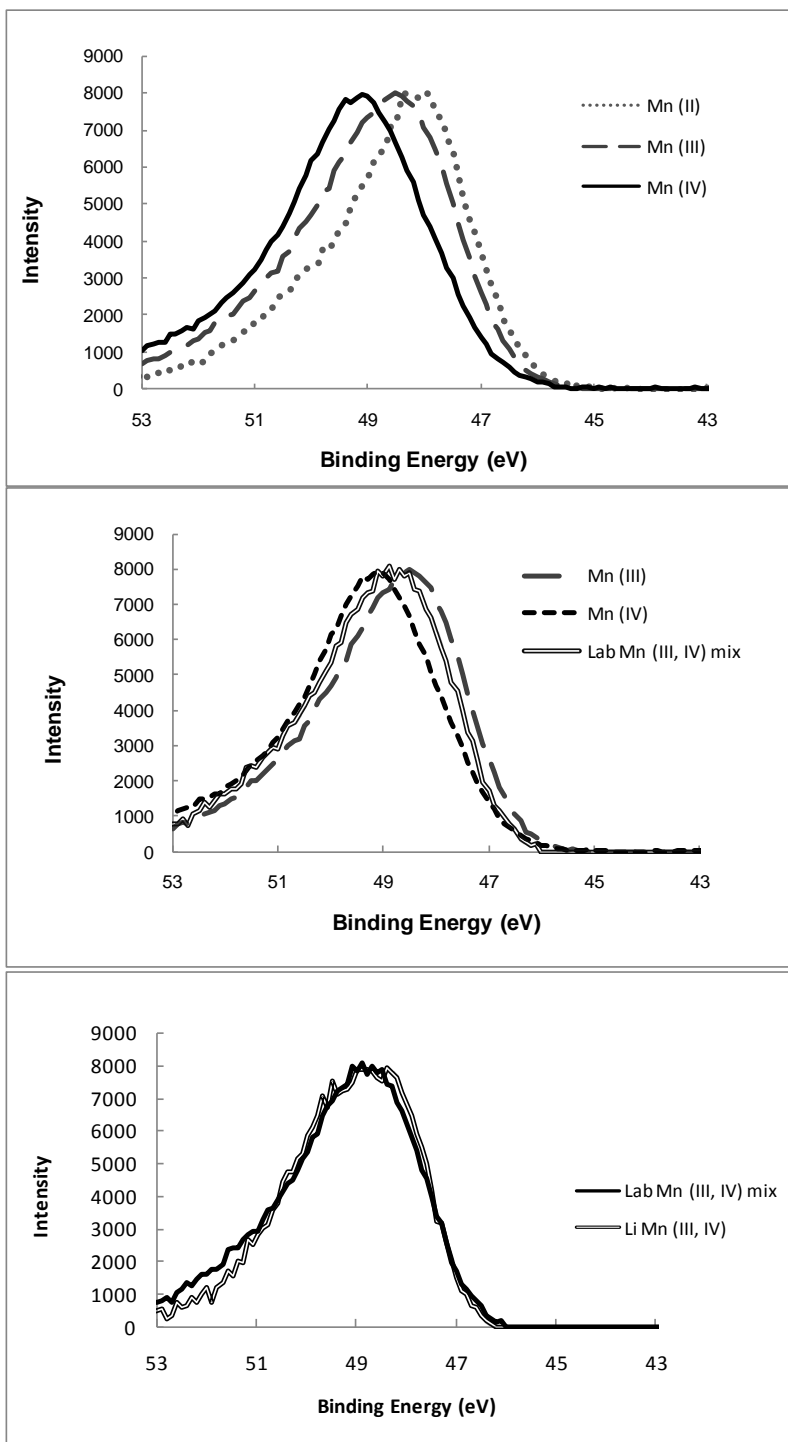
Figure 3-2. Mn 3p (^{7}P) photopeak spectrum for: **a)** "unused" pyrolusite sample vs. Mn(IV) standard; and **b)** "used" pyrolusite sample vs. laboratory Mn(III, IV) mixture.
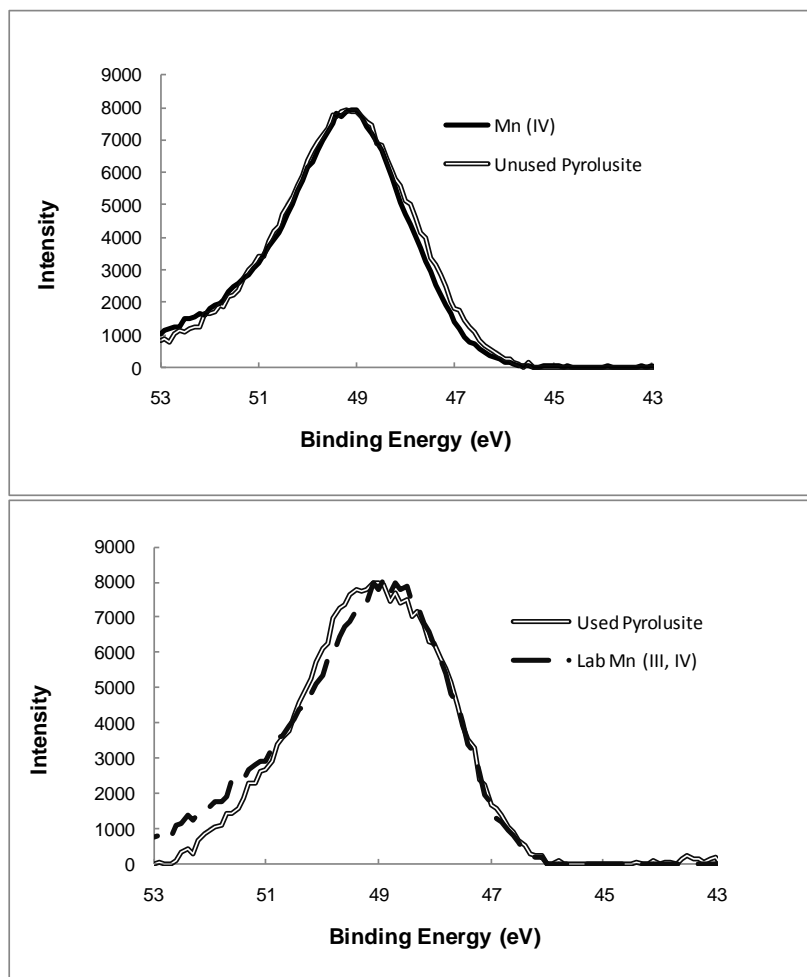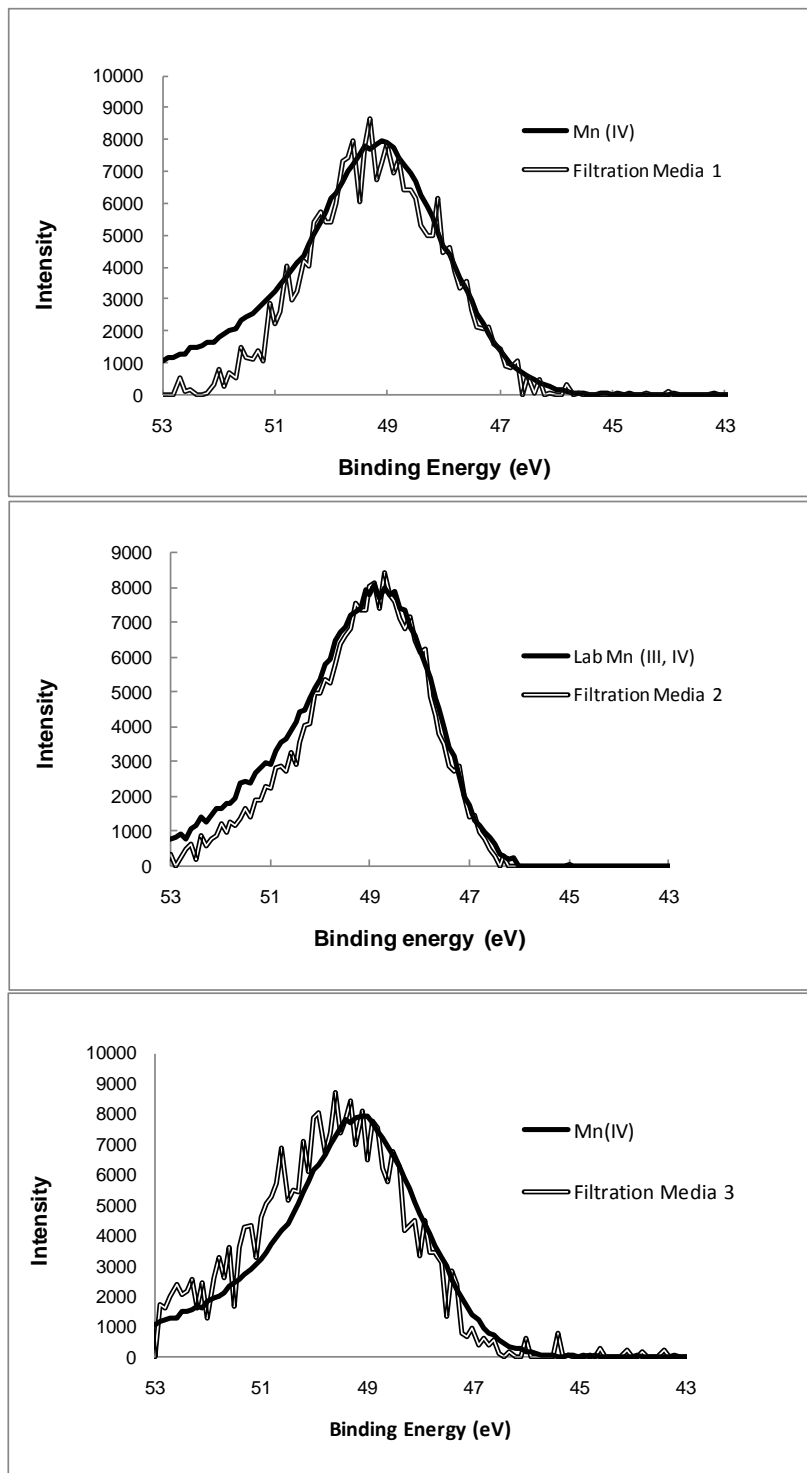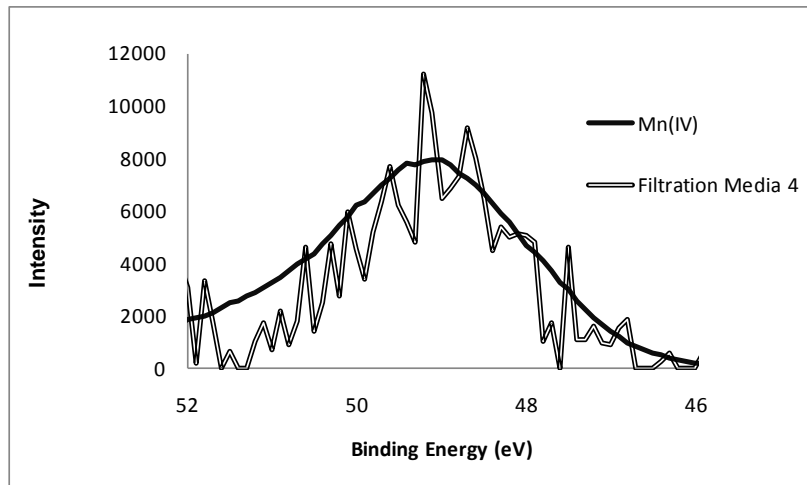
Figure 3-3. Mn 3p (^{7}P) photopeak spectrum for: **a)** filtration media 1 vs. Mn(IV); **b)** filtration media 2 vs. laboratory mixture of Mn(III, IV); **c)** filtration media 3 vs. Mn(IV); and **d)** filtration media 4 vs. Mn(IV).

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CHAPTER 4: Application of XPS to Investigate Soluble Manganese Removal by Oxide-Coated Media

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ABSTRACT:

X-ray photoelectron spectroscopy (XPS) was applied to investigate Mn(II) removal by $MnO_x(s)$-coated media under experimental conditions that closely resemble situations encountered in drinking water treatment plants in the absence and presence of chlorine. Macroscopic and spectroscopic results provide supporting evidence that Mn(II) removal in the absence of chlorine was mainly due to adsorption while in the presence of chlorine was due to oxidation. Mn oxidation states were identified through the determination of the Mn 3s multiplet splitting. Mn(IV) was predominant in all the XPS analyses. Mn(II) was detected in the experimental samples operated in the absence of chlorine. There was no clear spectroscopic evidence about the presence of Mn(III) in the media samples operated in the absence of chlorine. Mn(IV) was identified as the main species present in the samples obtained from columns operated in the presence of chlorine.

4.1. Introduction

Manganese removal through the catalytic action of $MnO_x(s)$-coated media surfaces combined with the application of free chlorine is a very effective water treatment technique (Griffin, 1960, Hargette and Knocke, 2001, Knocke et al., 1988, Knocke et al., 1991). More than twenty years of research have revealed several critical aspects of this water treatment technique: a) efficient

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Mn removal occurred after Mn-oxide coatings naturally developed in filter media through a process referred to as "filter aging" (Griffin, 1960) resulting in an apparent "natural greensand effect" with the presence of various oxidants (Knocke et al., 1988, Knocke et al., 1987); b) effective Mn^{+2} removal can be accomplished on $MnO_x(s)$-coated media in the absence of an oxidant, sorption capacity increases with increasing pH or surface $MnO_{(x)}$ concentration or both (Knocke et al., 1988, Knocke et al., 1991); c) when no oxidant is applied Mn^{+2} removal is due to "sorption" alone (Knocke et al., 1991); d) the application of chlorine together with $MnO_x(s)$-coated media result in effective Mn removal at pH 6 or above resulting in continuous "surface regeneration" over time (Knocke et al., 1988, Knocke et al., 1991); e) filter backwashing may alter the physical and chemical properties of $MnO_x(s)$-coated media (Hargette and Knocke, 2001, Merkle et al., 1996).

Although the research cited above has provided invaluable information about key factors influencing Mn-removal by $MnO_x(s)$-coated media, the specific mechanisms that affect this treatment technique are still unknown. While most of these studies have obtained kinetic information from macroscopic experimental data; there is limited microscopic and spectroscopic evidence that can provide a more specific perspective about possible reaction mechanisms. More recently, researchers have attempted to link macroscopic and microscopic analyses to better understand the role of $MnO_x(s)$-coated media in drinking water treatment (Hu et al., 2004, Liu et al., 2001, Merkle et al., 1996, Sahabi et al., 2009, Tobiason et al., 2008). Most of these studies have applied scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX) to obtain surface micro-topography and elemental composition information. Few investigators have characterized the mineral phase and oxidation state of $MnO_x(s)$-coated media

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surfaces. X-ray diffraction (XRD), a surface analysis technique appropriate for crystalline phases, has also been used to study the mineral phases in $MnO_x(s)$-coated media (Liu et al., 2001, Merkle et al., 1996). However, the applicability of the results obtained by XRD is limited due to the amorphous and heterogeneous nature of $MnO_x(s)$-coated media typically used in drinking water treatment (Liu et al., 2001, Merkle et al., 1996, Tobiason et al., 2008).

X-ray photoelectron spectroscopy (XPS) has also been used to determine Mn-oxidation states in minerals and is a well established surface analysis technique (Hochella, 1988, Junta and Hochella, 1994, Merkle et al., 1996, Murray et al., 1985). A previous study identified a Mn(IV) oxidation state in a synthetically $MnO_x(s)$-coated media using XPS through the determination of the Mn 3s multiplet splitting (Merkle et al., 1996). Other investigators identified a mixed Mn(III) and Mn(IV) oxidation state in the surface of Mn-oxide coated sand (Han et al., 2006) and in the biological products resulting from biofiltration using XPS through the determination of the Mn $2p_{3/2}$ peak (Katsoyiannis and Zouboulis, 2004). However, literature shows that determination of Mn-oxidation states using the XPS Mn $2p_{3/2}$ peak is not a practical approach as the resulting photo-peak shifts from different oxidation states are so small that it is hard to detect their separation (Junta and Hochella, 1994, Suzer et al., 1999).

The objective of this investigation was to apply XPS to better understand the mechanisms that affect Mn-removal by $MnO_x(s)$-coated media in the absence and presence of chlorine. A specific interest was to evaluate the capabilities of XPS to detect the Mn 3s multiplet splitting and differentiate multiple Mn-oxidation states under experimental conditions that closely resemble situations encountered in drinking water treatment plants.

4.2. Materials and Methods

4.2.1. Sample Site. Anthracite media samples were collected in 500 mL autoclaved polyethylene bottles from a drinking water system in North Carolina. This water system consists of a conventional water treatment facility. Its raw water is coagulated with ferric chloride and its filtration basin consists of dual-media filter beds of anthracite and sand. Pre-oxidation with $KMnO_4$ is applied on a seasonal basis. Chlorine is applied for disinfection and $MnO_x(s)$-coated media regeneration; chlorine residual in the filter effluent ranges from 3 - 6 mg/L.

4.2.2. Laboratory Column Experiments. Column tests were performed to evaluate Mn(II) uptake under experimental conditions favorable for sorption (in the absence of chlorine) / oxidation (in the presence of chlorine). Particles of $MnO_x(s)$-coated anthracite filtration media obtained from North Carolina were soaked in a 100 mg/L chlorine solution prior to being placed in the experimental setup. This was done to achieve media regeneration and attack of microorganisms present in the media to reduce the possible effect of microbiologically influenced Mn-redox processes during the time course of the experiments. Glass columns of 0.375 in. internal diameter were packed with regenerated $MnO_x(s)$-coated anthracite media. Due to the high Mn(II) removal capacity of the $MnO_x(s)$ coatings obtained from North Carolina (Knocke et al., 1991, Tobiason et al., 2008), filter column media depths were only 3 in. Influent Mn(II) concentration of 0.3 mg/L were dosed using a manganous chloride stock. Hydraulic loading rates targeted at 5 gpm/ft^2 were applied to the columns using peristaltic pumps to control the flow. The feed water used for these experiments consisted of de-ionized water with 0.5 meq/L as $CaCO_3$ calcium hardness and 1 meq/L as $CaCO_3$ calcium alkalinity as a source for background ions. Stocks of $CaCl_2$ and $NaHCO_3$ were used as the source of calcium and

bicarbonate. Columns were operated at pH 6.3 and pH 7.2 in the absence and presence of chlorine; 1 M NaOH and 1M HCl solutions were used to adjust the feed de-ionized water solutions to these pH values. Experimental chlorine concentrations were targeted at 3 mg/L dosed as sodium hypochlorite at an application point located close enough to the columns so that the target reaction time with Mn(II) before reaching the columns was approximately 5 seconds. This reaction time would have been insufficient to promote oxidation and precipitation of the Mn(II) prior to reaching the $MnO_x(s)$-coated media (Knocke et al., 1987). A "control" column was operated with no influent Mn and no chlorine addition. The experimental treatments and control used to operate these column experiments are illustrated in Figure 1.

Effluent Mn was monitored for 48 hours for the columns operated with chlorine and 72 hours for the columns operated with no chlorine. After this column-loading period, all columns were eluted with a mild acid solution (pH 5.0) to assess the fraction of Mn(II) that was adsorbed (un-oxidized) during column operation. As stated in a previous study (Knocke et al., 1991), it was assumed that operation at pH 5 would promote the release of adsorbed un-oxidized Mn(II) due to competition with H^+ for adsorption sites without promoting reduction of $MnO_x(s)$ to Mn(II). Significant Mn-release was expected from the columns operated under conditions favorable for sorption (no presence of chlorine) while little Mn- release was expected from columns operated under conditions favorable for oxidation (with the presence of chlorine) (Knocke et al., 1991). Effluent Mn concentrations were measured using a Perkin-Elmer 5100 flame atomic absorption spectrophotometer (Waltham, MA, USA) and a Hach DR/2400 spectrophotometer (preprogrammed method for low range concentrations: 0.006 - 0.700 mg/L Mn). Chlorine was measured using a Hach pocket colorimeter II test kit (DPD – total chlorine method).

A check was performed using the leucoberbelin method (Okazaki et al., 1997, Stein et al., 2001) to asses if any oxidized Mn was obtained from the elution. The leucoberbelin method can distinguish between Mn^{+2} and oxidation states Mn^{+3} or higher. Leucoberbelin blue produces a blue color when it reacts with oxidized Mn, but cannot differentiate between Mn(III), Mn(IV), or Mn (VII) (Gabelich et al., 2006, Okazaki et al., 1997). In a microcentrifuge tube, 0.6 mL of the elution sample was mixed with 0.6 mL of 0.04 % leucoberbelin blue I (Aldrich Chemical Co., St. Louis, MO, USA) in 45 mM acetic acid. The concentration of oxidized Mn was measured by comparing the absorbance (620 nm) to a calibration curve constructed using $KMnO_4$ standards.

4.2.3. Analytical Methods.

Elemental Extractions. A method developed by Knocke *et al*. (Knocke et al., 1991) was used to determine the extractable elemental content on the filtration media samples. Media samples were air dried; 4 g of dry media were placed into a 250 mL Erlenmeyer flask with 100 mL of 0.5% Nitric Acid and approximately 600 mg of hydroxylamine sulfate (HAS). The solution was allowed to react for two hours. A solution sample was obtained after this reaction time and filtered through a 0.45μm membrane. The filtrate metal concentrations were analyzed using an inductively coupled plasma mass spectrophotometer ICP-MS according to Standard Method 3125-B (Clesceri et al., 1998).

XPS Analysis. Two particles of MnO_x(s)-coated anthracite filtration media from each of the columns illustrated in Figure 1 were analyzed using XPS**.** A Perkin-Elmer 5400 X-ray photoelectron spectrometer (Physical Electronic Industries, Inc.) consisting of an aluminum X-ray source and two-channel collector was used. The X-ray anode was operated at 12 keV and 250 W and the vacuum was maintained at or below 5 x 10^{-7} Torr. The pass energy for survey

wide scans was 89.45 eV. The pass energy for narrow scans was 17.9 eV. The following standards were purchased and used as a reference to assess Mn oxidation states II, III, and IV: 99% pure MnO [manganous oxide, Mn(II)] and 99% pure Mn_2O_3 [manganic oxide, Mn(III)], both purchased from Strem Chemicals - Newburyport, MA, USA, and 99.999% pure MnO_2 (manganese dioxide, Mn(IV), purchased from Acros Organics/Thermo Fisher Scientific – Pittsburg, PA, USA). The narrow scan spectra presented for Mn in pure oxide standards represent averages of 100 sweeps with 25 ms for every 0.1 eV step. For the anthracite filtration media samples, the spectra presented for Mn represent averages of 1000 sweeps with 25 ms for every 0.1 eV step. Data analyses and curve fitting was performed using AugerScan 3.12 (RBD Enterprises Inc.). The oxidation state of Mn was identified by determination of the Mn 3s multiplet splitting. Narrow scans of the Mn 3s photolines were analyzed using a Shirley background subtraction (Hochella, 1988, Oku et al., 2008) and least-squares Gaussian-Lorentzian curve-fitting algorithm (Junta and Hochella, 1994, Murray et al., 1985). Samples analyzed in the Mn 3s region were charge referenced to the carbon C1s peak at 285.0 eV. The magnitude of the widths and positions used to fit curves in the Mn 3s region were obtained from the analyses performed for pure oxide standards presented in Chapter 3. Multiplet splitting used to fit curves for Mn(II), Mn(III), and Mn(IV) were 5.68, 5.22, and 4.5 eV, respectively; these values are consistent with those reported in the literature (Junta and Hochella, 1994, Murray et al., 1985). The chi square goodness of fit parameter provided by the AugerScan 3.12 (RBD Enterprises Inc.) was also used to assess photo-peak analyses.

4.3.   Results

Mn content of anthracite filter media. The total Mn contents of the anthracite media collected

for this case study was 30.7 mg extractable Mn (g dry weight of sample)$^{-1}$, showing that there is

considerable total Mn content in the surface of this sample. This value is comparable to those

reported in another study (Tobiason et al., 2008).

Mn-removal in the absence and presence of chlorine. The results obtained for Mn-removal in

the absence and presence of chlorine at pH 6.3 and at pH 7.2 are presented in Figure 2 and

Figure 3, respectively. The Mn uptake calculated for the columns operated with no chlorine at

pH 6.3 was 4 mg Mn and the uptake at pH 7.2 was 6.5 mg Mn. These results for the columns

operated in the absence of chlorine indicate that more Mn was removed at pH 7.2 when

compared to pH 6.3, confirming that Mn(II) adsorption capacity in $MnO_x(s)$-coated media

increases in direct proportion to pH increase as suggested in other studies (Knocke et al., 1988,

Knocke et al., 1991). Progressive exhaustion of $MnO_x(s)$-coated media sites available for

sorption of Mn(II) was observed under both pH 6.3 and pH 7.2 over time in the absence of

chlorine. Individual comparisons of both columns operated at pH 6.3 and pH 7.2 clearly indicate

that efficient long-term Mn(II) removal is enhanced by the presence of chlorine due to

continuous "regeneration" of the Mn-oxide coated media surface as suggested in other studies

(Knocke et al., 1988, Knocke et al., 1991, Tobiason et al., 2008). Very mild (<0.01 mg/L Mn) to

levels below the instrumental detection limit (0.006 mg/L) were observed over time for the

"control" (unreacted) column operated with no influent Mn and in the absence of chlorine. Thus,

there was no evidence of soluble Mn released from the $MnO_x(s)$-coated media used in the

columns that could possibly be attributed to chemical and/or microbial factors.

Elution experiments. The results obtained after exposure to a mild acid (pH 5.0) solution of the experimental columns originally operated at pH 6.3 and pH 7.2 in the absence and presence of chlorine are shown in Figure 4 and Figure 5, respectively. Mn release was greater in the columns originally operated in the absence of chlorine, indicating that Mn uptake in these columns was mostly due to "adsorption". No evidence of oxidized Mn was detected with spectrophotometric measurements of the effluent samples using the leucoberbelin blue method; Mn(II) was predominant in the effluent elution samples. Approximately 70% and 76% of Mn(II) adsorbed to the columns operated in the absence of chlorine (shown in Figures 2 and 3) at pH 6.3 and 7.2, respectively, was released during the elution experiments. Low concentrations of Mn were released from the experimental columns originally operated in the presence of chlorine suggesting that most of the Mn(II) uptake was due to "oxidation" reactions. The effluent Mn(II) peaks observed at the initial phase of the elution experiments (illustrated in Figures 4 and 5) and the trends of these results are consistent with the observations reported in a previous study (Knocke et al., 1991).

XPS analyses. The XPS survey scans obtained for the experimental $MnO_x(s)$-coated media samples are illustrated in Figure 6. There is no observable difference between the intensities of the Mn2p peaks of the survey scans shown in Figure 6a for the experimental samples operated in the absence of chlorine. Interestingly, an increase in the intensity of the Mn 2p peaks was detected in the survey scans shown in Figure 6b for the columns operated in the presence of chlorine.

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The results obtained for the curve fitting analyses for the XPS narrow scans of the Mn 3s region are presented in Table 1. The narrow scan photopeak spectrum in the Mn 3s region for the control sample is illustrated in Figure 7. As expected, Mn(IV) was identified in the "control" sample obtained from the column operated with no manganese and no chlorine; no evidence of Mn(II) or Mn(III) was observed in the control.

Mn(IV) was identified as the predominant oxidation state in the XPS analyses performed for media samples obtained from the columns operated at pH 6.3 and pH 7.2 in the absence of chlorine. As confirmed by the elution experiments, XPS analyses also reveal the presence of Mn(II) in the surface of the two particle samples obtained from the column operated at pH 6.3 and pH 7.2 in the absence of chlorine. The results from the curve fitting analyses of these samples are shown in Table 1. The XPS narrow scan in the Mn 3s region for one of the $MnO_x(s)$-coated media particle samples obtained from the column operated at pH 6.3 and pH 7.2 in the absence of chlorine is shown in Figure 8 and Figure 9, respectively. A slight increase in the chi squared goodness of fit parameter was observed in the $MnO_x(s)$-coated media particle samples obtained from the columns operated at pH 6.3 and pH 7.2 in the absence of chlorine when Mn(III) was considered in the analyses. For example, according to the curve fitting results in Table 1, the chi-square goodness of fit parameter for the particle illustrated in Figure 8 was 2.1 when Mn(II) and Mn(IV) were considered in the curve fitting analyses as opposed to 2.2 when Mn(II), Mn(III), and Mn(IV) were considered. Although a slight increase was observed in the chi squared goodness of fit parameter when Mn(III) was considered in the analyses, this increase was not greater than 3% in any of the samples analyzed. Thus, there was no clear evidence about

the detection of Mn(III) by XPS through the determination of the Mn 3s multiplet splitting under the experimental conditions used for his study.

Mn(IV) was identified as the predominant oxidation state in the XPS analyses performed for media samples obtained from the column operated with chlorine at pH 6.3 and pH 7.2. As confirmed by the elution experiments, XPS analyses show no evidence for the presence of Mn(II) in the $MnO_x(s)$-coated media particle samples obtained from the columns operated at pH 6.3 and pH 7.2 in the presence of chlorine. The results from the curve fitting analyses of these samples are shown in Table 1. The XPS narrow scan in the Mn 3s region for $MnO_x(s)$-coated media particle samples obtained from the columns operated at pH 6.3 and pH 7.2 in the presence of chlorine is shown in Figure 10.

4.4. Discussion

The results obtained in this study provide supporting evidence about the findings reported in other studies: a) Mn(II) removal in the absence of chlorine is mainly due to "adsorption", b) Mn(II) removal in the presence of chlorine is mainly due to "oxidation" (Knocke et al., 1988, Knocke et al., 1991, Tobiason et al., 2008).

There was no indication of "autocatalytic oxidation" in the experiments performed without chlorine under conditions that closely resemble situations observed in drinking water treatment plants. Changes in the nature of a manganese oxide surface during the course of an experiment can suggest that "autocatalytic oxidation" has occurred when the surface sites where manganese oxidation takes place are more reactive than the original sites (Davies and Morgan, 1989, Morgan, 1967). There was no clear evidence for the detection of Mn(III) using XPS through the

determination of the Mn 3s multiplet splitting. Nevertheless, macroscopic data obtained from the elution experiments suggest that only 70% and 76% of the Mn uptake at pH 6.3 and pH 7.2, respectively, was released. Approximately 20-30% of the Mn uptake was not released during the elution. These results suggest that, although Mn(III) was not identified by XPS, it is still possible that oxidation could take place in a minor extent in the $MnO_x(s)$ coated media surfaces in the absence of chlorine under the experimental conditions studied.

XPS survey scans of the anthracite media column samples operated in the absence of chlorine, Figure 6a, do not indicate any changes in the intensity of the Mn 2p peaks when compared to the "unreacted" control. An increase in the intensity of the Mn 2p peaks can be observed in Figure 6b for the anthracite media column samples operated in the presence of chlorine, suggesting that a change in the surface conditions has occurred in the experimental samples both at pH 6.3 and pH 7.2. Note that the Mn2p region provides the most intense signal for the Mn photopeaks that appear in an XPS survey scan, explaining why surface changes in Mn concentration are more noticeable in the Mn 2p than in the Mn 3s or Mn 3p regions. These changes could be due to the formation of new $MnO_x(s)$ precipitates resulting from the oxidation of Mn(II) by the catalytic action of chlorine and the $MnO_x(s)$ coated media surfaces. As shown in Figure 10a, a decrease in the Al 2p peak was observed in some narrow scans in the Mn 3s region for $MnO_x(s)$ coated media surfaces obtained from columns operated in the presence of chlorine. This could have been caused by a "masking" of the XPS Al 2p signal due to the formation of $MnO_x(s)$ precipitates in the coated media surfaces after column operation in the presence of chlorine. A better understanding of the physical chemical interactions of Al with Mn

and is necessary to understand how they could potentially affect Mn(II) removal using $MnO_x(s)$ coated media surfaces.

4.5. Conclusions

Macroscopic and spectroscopic data presented in this study suggest that Mn(II) removal in the absence of chlorine is mainly due to "adsorption" and in the presence of chlorine it is due to "oxidation" under experimental conditions that resemble situations encountered in drinking water treatment. XPS was a useful tool for the identification of oxidation states of Mn in $MnO_x(s)$ coated media surfaces. Although Mn(IV) was identified as the predominant oxidation state in the $MnO_x(s)$ coated media surfaces , the presence of Mn(II) was also determined by XPS. There was no clear evidence about the presence of Mn(III) using XPS through the determination of the Mn 3s multiplet splitting under the experimental conditions used in this study.

4.6. Acknowledgements

4.7. References

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Table 4-1. XPS Mn 3s multiplet splitting curve fit information.

Sample	Figure Cited: Curve Fit	Oxidation States Fitted	Chi squared (goodness of fit)	Ratio of Oxidation State Predominance		
				Mn(II)	Mn(III)	Mn(IV)
Control	Figure 7	Mn(IV)	2.3			1.00
pH 6.3, No chlorine, particle 1	Figure 8a	Mn(II), Mn(IV)	2.1	0.41		0.59
pH 6.3, No chlorine, particle 1		Mn(II), Mn(III), Mn(IV)	2.2	0.17	0.23	0.60
pH 6.3, No chlorine, particle 2	Figure 8b	Mn(II), Mn(IV)	2.7	0.26		0.74
pH 6.3, No chlorine, particle 2		Mn(II), Mn(III), Mn(IV)	2.8	0.22	0.32	0.46
pH 7.2, No chlorine, particle 1	Figure 9a	Mn(II), Mn(IV)	4.3	0.29		0.71
pH 7.2, No chlorine, particle 1		Mn(II), Mn(III), Mn(IV)	4.4	0.21	0.30	0.49
pH 7.2, No chlorine, particle 2	Figure 9b	Mn(II), Mn(IV)	4.5	0.41		0.59
pH 7.2, No chlorine, particle 2		Mn(II), Mn(III), Mn(IV)	4.6	0.31	0.24	0.45
pH 6.3, Chlorine, particle 1		Mn(IV)	2.1			1.00
pH 6.3, Chlorine, particle 2	Figure 10a	Mn(IV)	1.6			1.00
pH 7.2, Chlorine, particle 1	Figure 10b	Mn(IV)	3.9			1.00
pH 7.2, Chlorine, particle 2		Mn(IV)	4.1			1.00

Figure 4-1. Controls and treatments used for the operation of the experimental columns.

Mn No Cl$_2$	Mn No Cl$_2$	Mn Cl$_2$	Mn Cl$_2$	No Mn No Cl$_2$
Treatment: Sorption pH=6.3	Treatment: Sorption pH=7.2	Treatment: Surface Oxidation pH=6.3	Treatment: Surface Oxidation pH=7.2	Control No Cl$_2$ No Mn pH=6.3
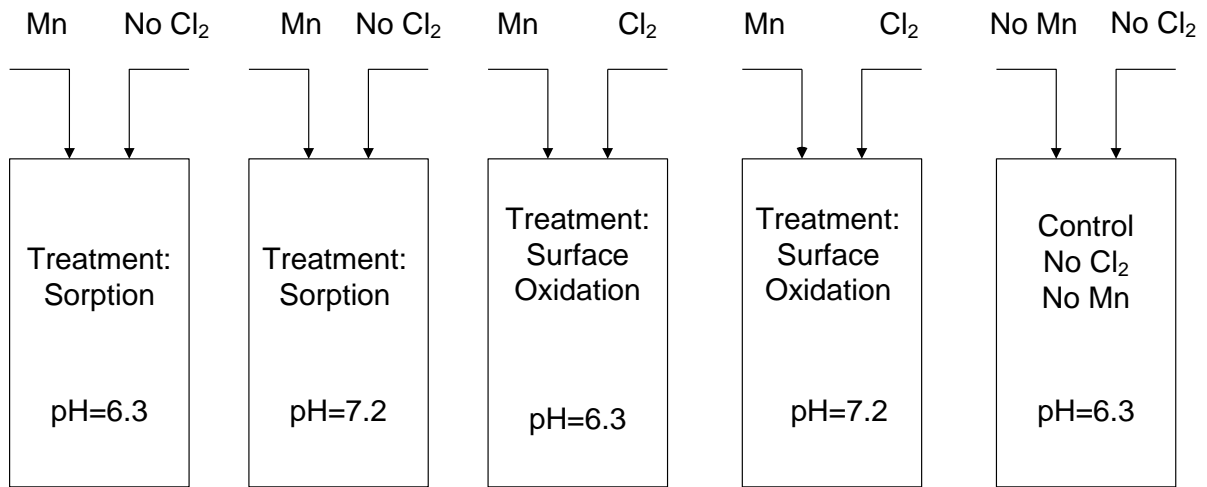
Figure 4-2. Mn(II) uptake of experimental column operated at pH 6.3 with an influent Mn(II) concentration of 0.3 mg/L in the absence and presence of chlorine (target = 3 mg/L HOCl).
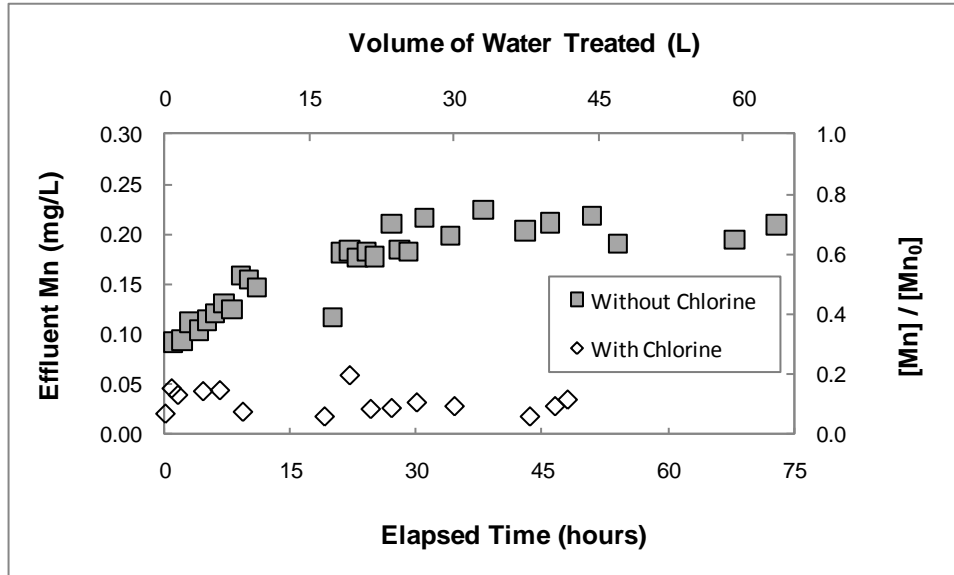
Figure 4-3. Mn(II) uptake of experimental column operated at pH 7.2 with an influent Mn(II) concentration of 0.3 mg/L in the absence and presence of chlorine (target = 3 mg/L HOCl).
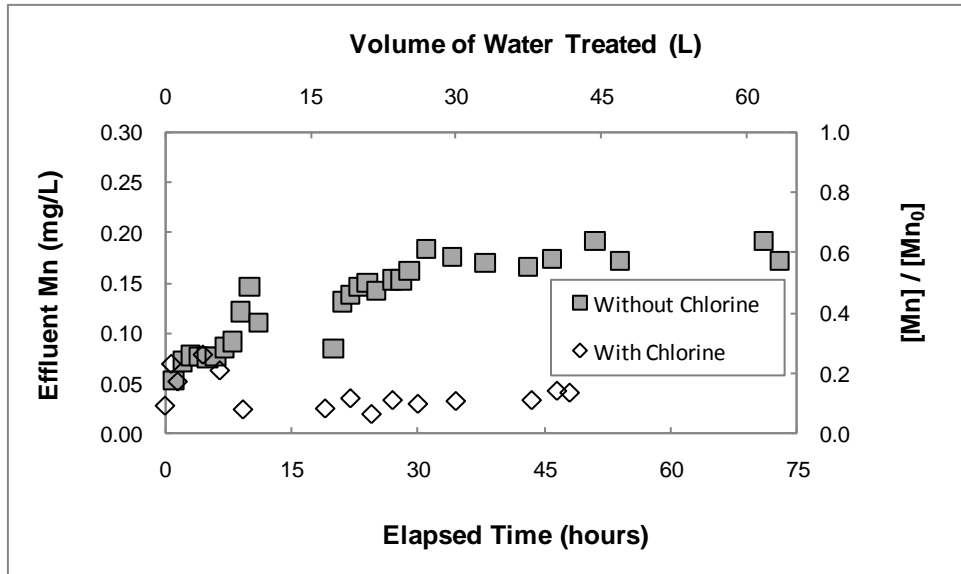
Figure 4-4. Release of Mn(II) from experimental columns originally operated at pH 6.3 with an influent Mn(II) concentration of 0.3 mg/L in the absence and presence of chlorine (target = 3 mg/L HOCl) after elution with a mildly acid solution (pH 5.0).
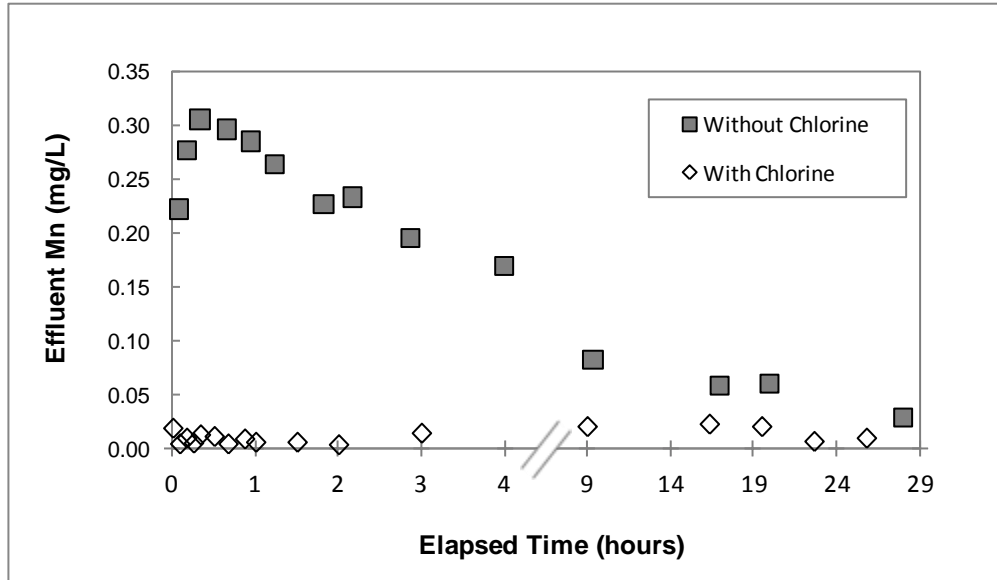
Figure 4-5. Release of Mn(II) from experimental columns originally operated at pH 7.2 with an influent Mn(II) concentration of 0.3 mg/L in the absence and presence of chlorine (target = 3 mg/L HOCl) after elution with a mildly acid solution (pH 5.0).
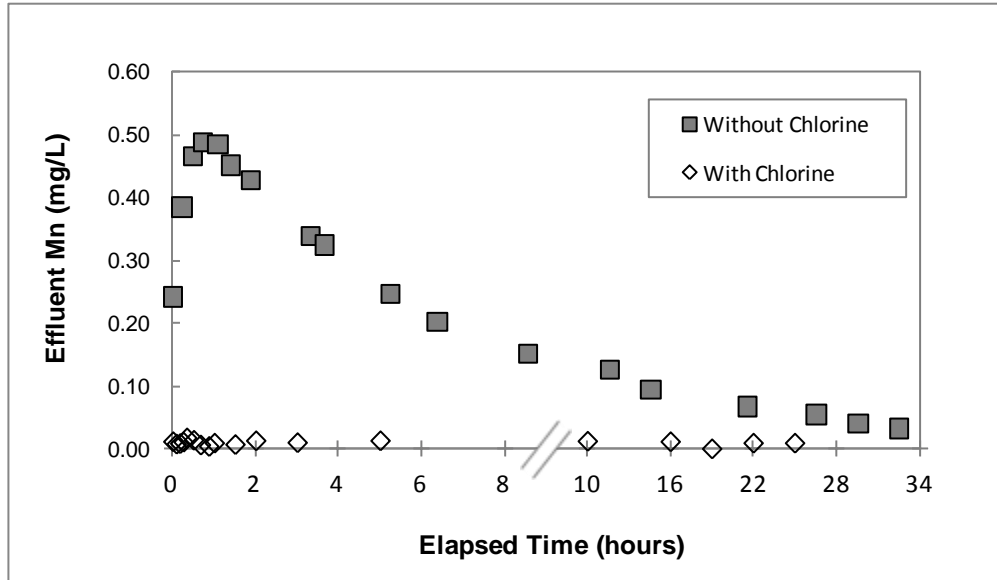
Figure 4-6. XPS survey scans of the control "unreacted" anthracite media and selected experimental column samples operated at pH 6.3 and pH 7.2: **a)** in the absence of chlorine; and **b)** in the presence of chlorine (target = 3 mg/L HOCl).
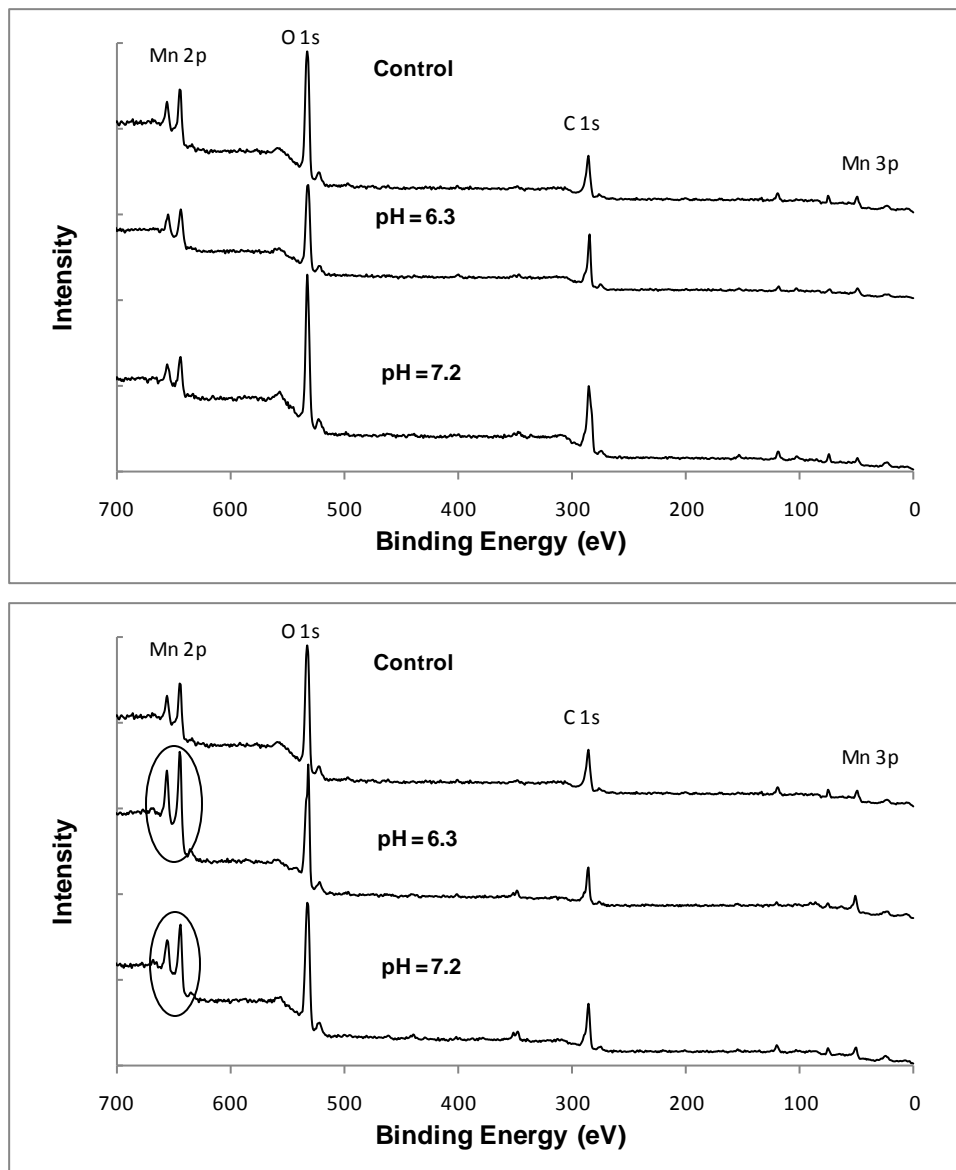
Figure 4-7. XPS narrow scan photopeak spectrum of the Mn 3s region for a particle sample obtained from the control (no chlorine, no manganese) column.
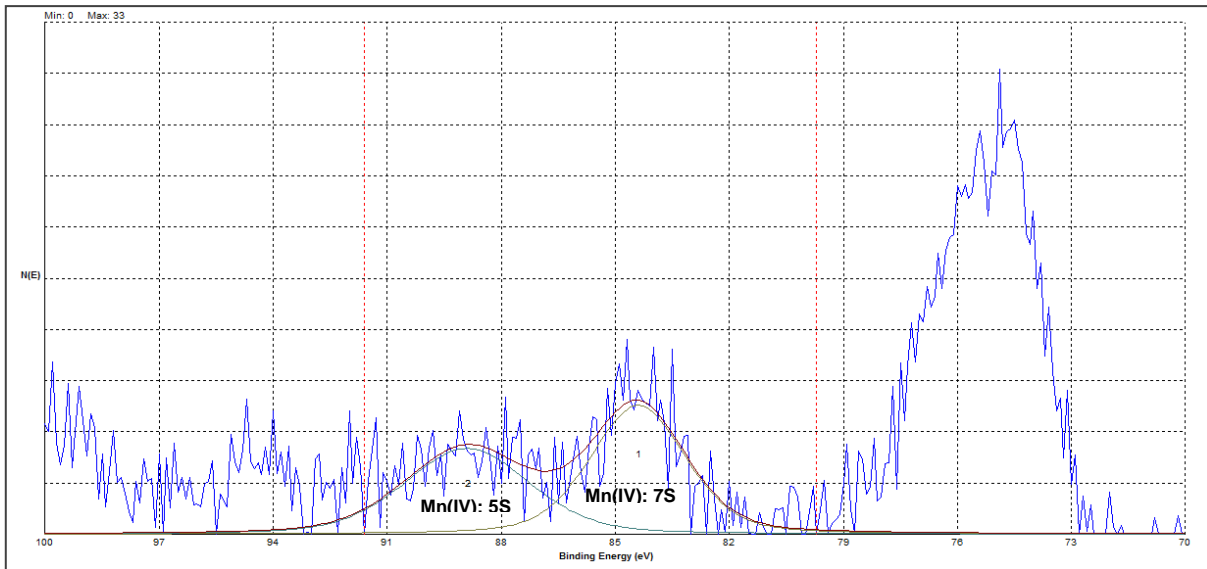
Figure 4-8. XPS narrow scan photopeak spectrum of the Mn 3s region for: **a)** particle 1 obtained from a column operated without chlorine at pH=6.3; and **b)** particle 2 obtained from a column operated without chlorine at pH=6.3.
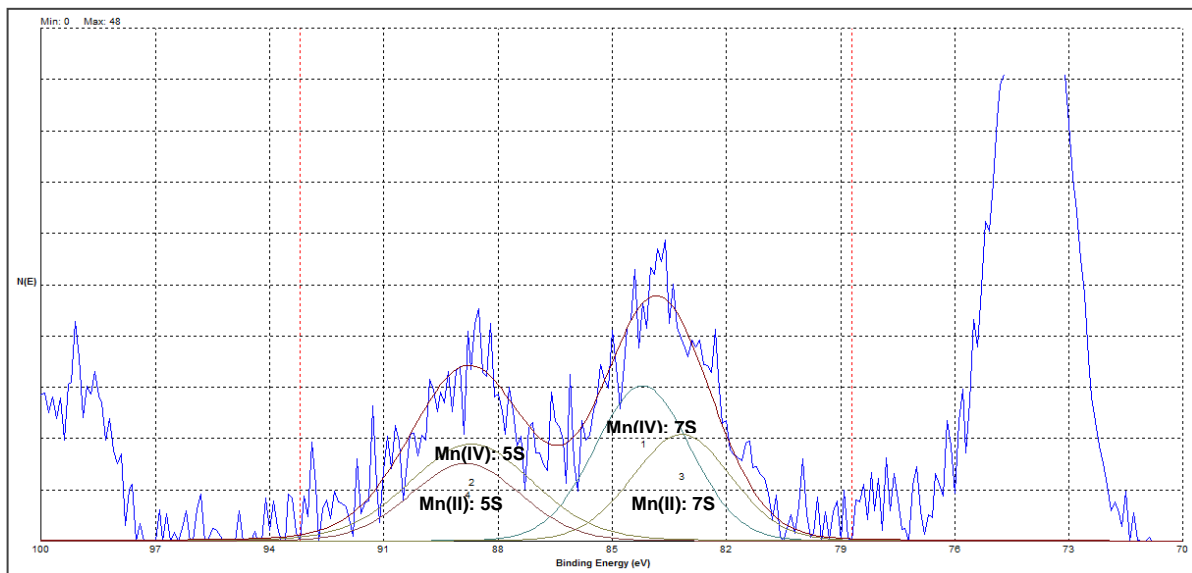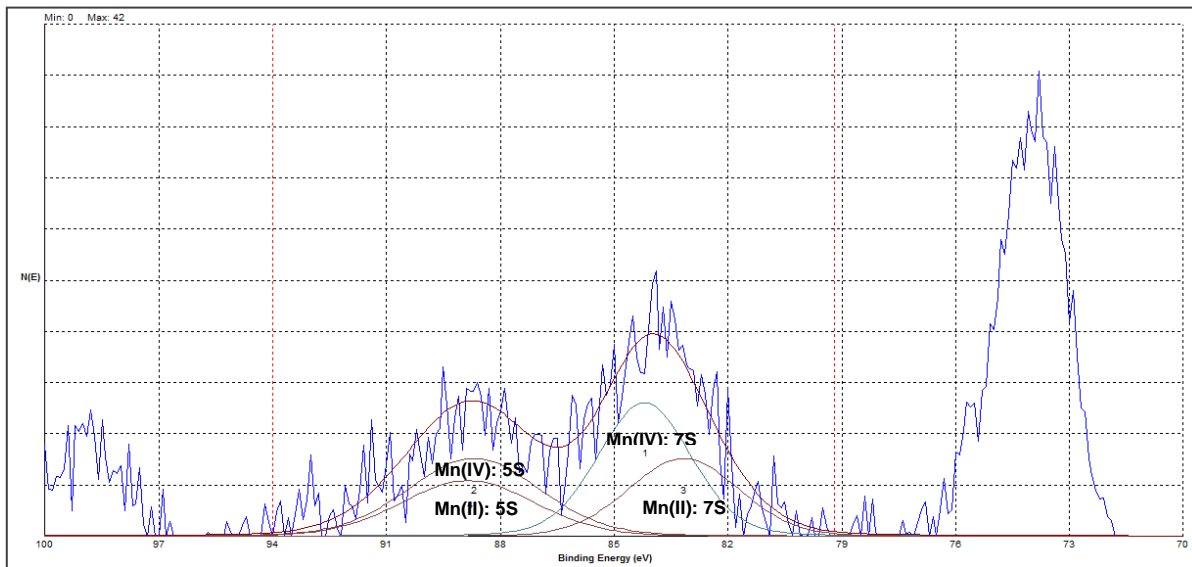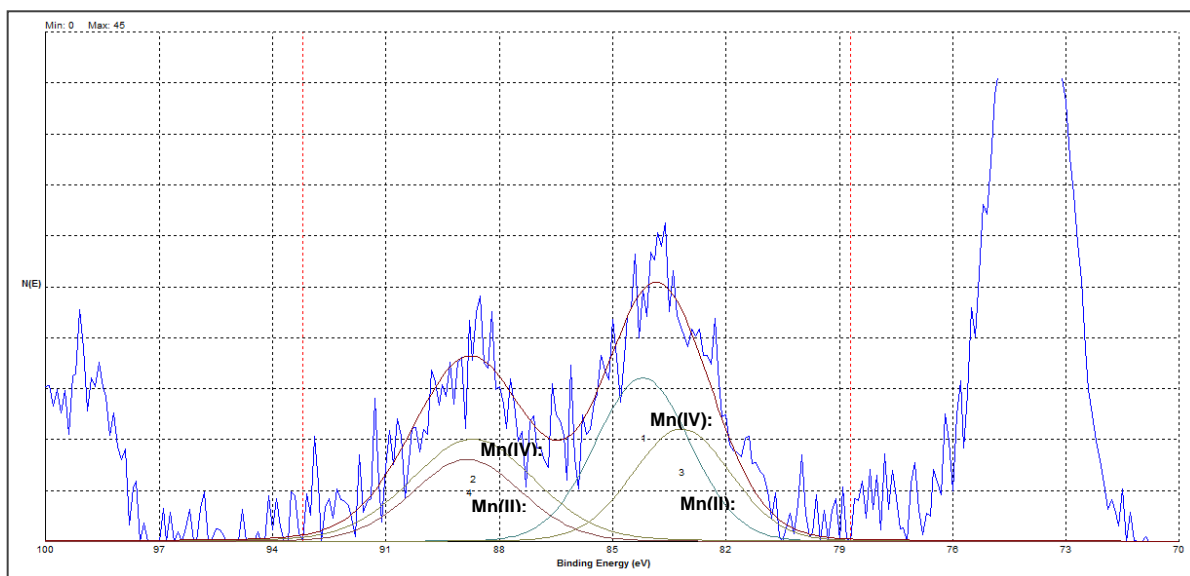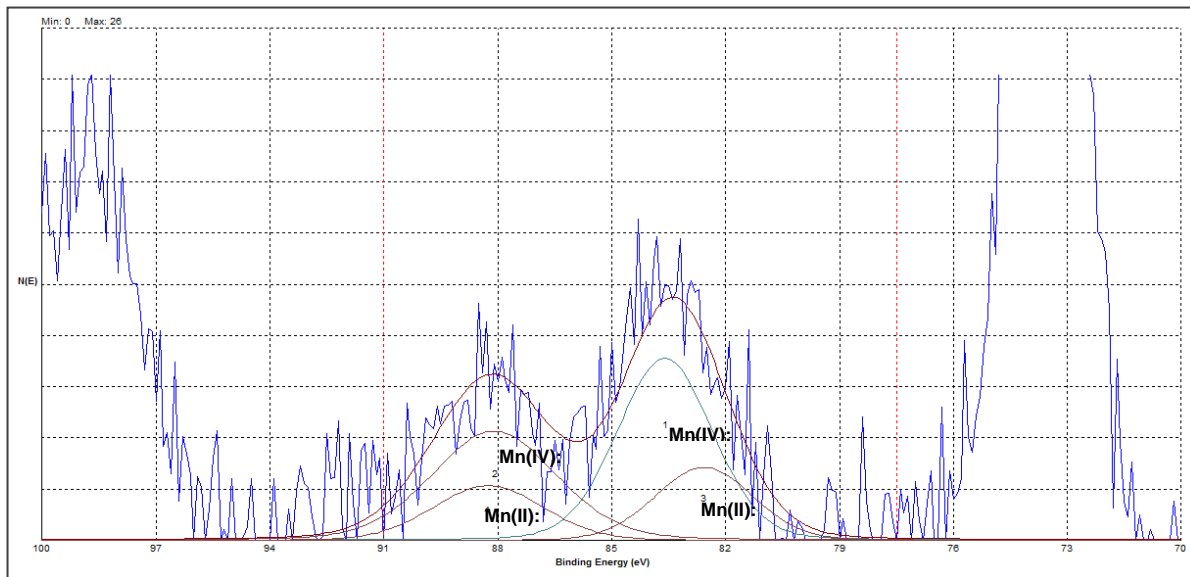
Figure 4-9. XPS narrow scan photopeak spectrum of the Mn 3s region for **a)** particle 1 obtained from a column operated without chlorine at pH=7.2; and **b)** particle 2 obtained from a column operated without chlorine at pH=7.2.
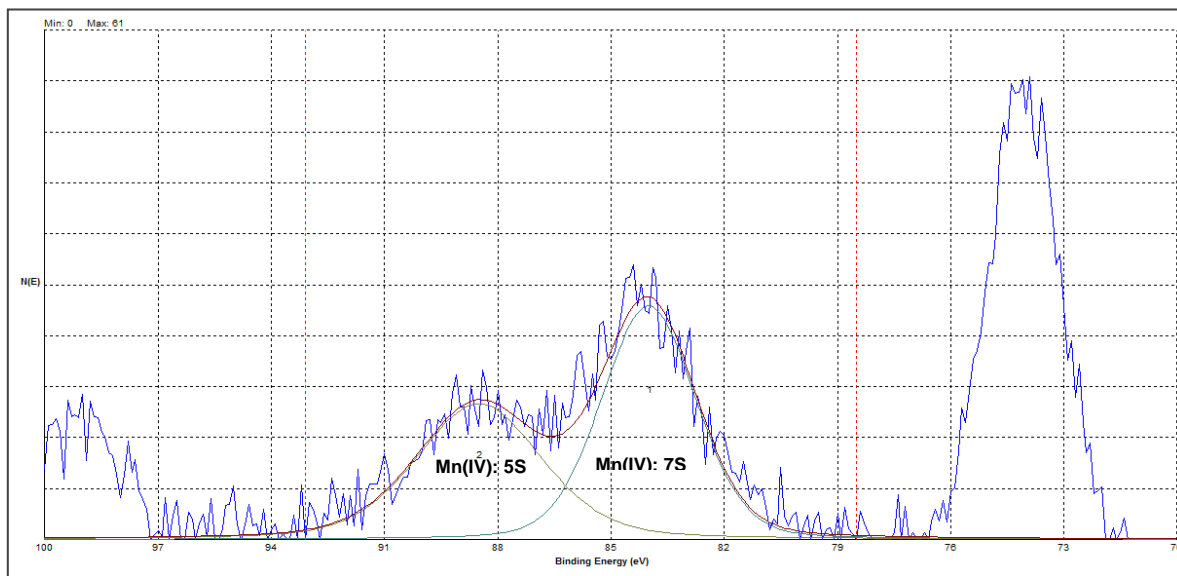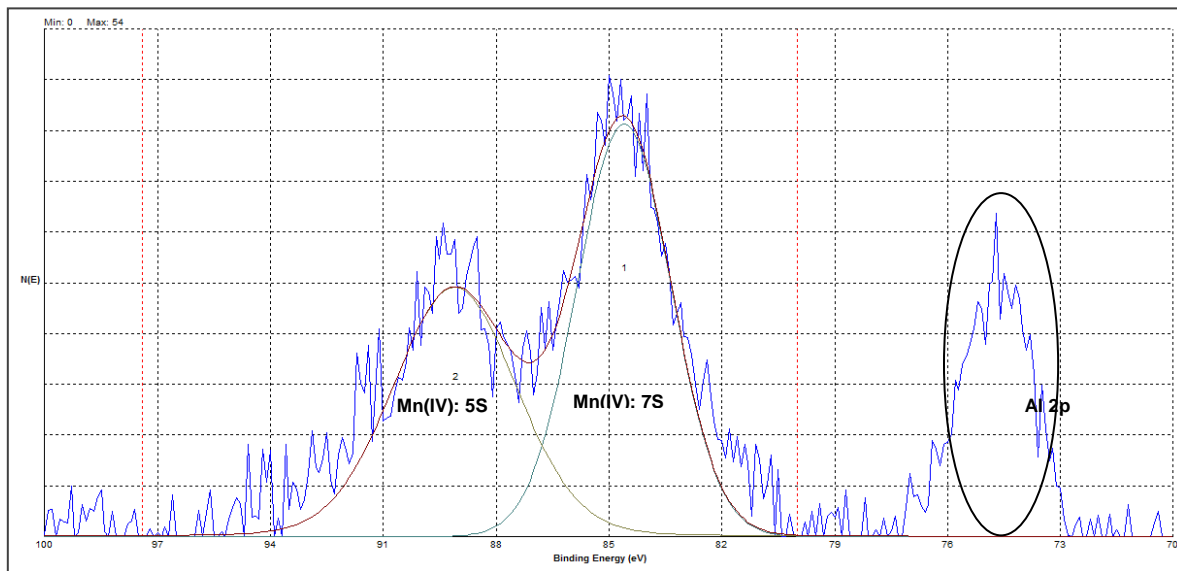
Figure 4-10. XPS narrow scan photopeak spectrum of the Mn 3s region for **a)** particle 2 obtained from a column operated with chlorine at pH=6.3; and **b)** particle 1 obtained from a column operated with chlorine at pH=7.2.

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