Practical Paper

Manganese removal and occurrence of manganese oxidizing bacteria in full-scale biofilters

M. S. Burger, C. A. Krentz, S. S. Mercer and G. A. Gagnon

ABSTRACT

Biological treatment of drinking water to remove manganese (Mn) is becoming more and more common. However, details as to how the technology works remain poorly understood. Some previous studies have focused on bacteria of the genus *Leptothrix*, identifying them as one of the predominant organisms in Mn oxidizing biofilms. This research took media from inside the filters of four Mn biofiltration plants in the province of New Brunswick, Canada. The water was characterized for all four sites and it was found that Mn removal from all plants is virtually 100%. Biofilm was detached from the sand and tested for presence of *Leptothrix* using real-tme polymerase chain reaction (RT-PCR) DNA amplification. Less specific testing for Mn oxidizing bacteria was done simultaneously using indicative agar plates. Results showed that only one plant contained *Leptothrix* in its filters while three of the four plants tested positive for manganese oxidizing bacteria (MOB) through plating. These results, along with other evidence, suggest that while the mechanism of oxidation observed is likely biological, *Leptothrix* is not necessarily the predominant organism carrying out biooxidation.

Key words | biofiltration, biological treatment, *Leptothrix*, manganese, polymerase chain reaction (PCR)

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INTRODUCTION

Manganese (Mn) in drinking water is subject to Health Canada, US EPA and European Environmental Committee (EEC) guidelines with a maximum concentration of $0.050\,mg\,L^{-1}$ due to aesthetic concerns. Mn oxides can cause episodes of black water and laundry staining at concentrations as low as $0.020\,mg\,L^{-1}$ (MWH 2005). Most Mn treatment methods consist of oxidizing Mn into an insoluble form. In most source waters where pH is lower than 7.5, Mn will be in the dissolved Mn^{2+} form. In these cases the pH and oxidation-reduction potential (ORP) need to be raised to encourage Mn-oxide creation (MWH 2005).

Biological Mn removal by Mn oxidizing bacteria (MOB) has shown much promise as an effective low-cost way to treat Mn contaminated groundwater. The technology has been developed and is commercially available in Europe and North America. However, despite the recent and wider spreading application of this technology, little information is published regarding how this biological Mn filter actually works, or which organisms in the biofilm are responsible for the oxidation of Mn. Over the last century, many MOB species have been identified such as bacteria of the genera *Crenothrix*, *Hyphomicrobium*, *Leptothrix*, *Metallogenium*, *Siderocapsa* and *Sideocystis*, and some species of *Pseudomonas* (e.g. *P. manganoxidans*) (Mouchet 1992; Ehrlich 1996; Katsoyiannis & Zouboulis 2004; Pacini *et al.* 2005).

Among MOB, bacteria of the genus *Leptothrix* have been studied extensively (Ghiorse 1984; Emerson & Ghiorse 1992). After problems retaining natural sheath forming

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ability in laboratory cultures, a strain named *L. discophora SP-6* was isolated and cultured with its sheath-forming ability retained, which is important since *Leptothrix* bacteria rely on the sheath to protect themselves and attach to surfaces in groundwater aquifers, their natural habitat (Emerson & Ghiorse 1992, 1993). Subsequent sequencing of *L. discophora SP-6* and a previously isolated strain named *SS-1* revealed that they are genetically quite different, casting doubt into their nomenclature as two strands of the same species (Siering & Ghiorse 1996). Nevertheless, the genus-wide Mn oxidizing ability of *Leptothrix* species is supported by strong evidence. The presence of *Leptothrix* has been suspected in Mn removing biofilters. In a study of biological Mn (and Fe) removal in Argentina, a microscope slide taken from the filter media counted a large number of sheathed bacteria, assumed to be of the *Leptothrix* genus (Pacini *et al.* 2005). Another study aimed at reducing biofilm maturation times of Mn removing biofilms used *L. discophora* spiked columns under the assumption that *L. discophora* is one of the predominant organisms in Mn removing biofilms (Hope & Bott 2004). Indeed, the long filter maturation time in Mn biofiltration plants has been identified as a deterrent to choosing Mn biofiltration over other processes. Another study of a biofilter allowed to build up an indigenous biofilm found a Mn oxidizing organism present on the filtration media presumed to be *L. ochracea*. Using scanning electron microscopy-energy dispersion spectroscopy (SEM-EDS), it was shown that the "*Leptothrix*" microorganisms had Mn oxides on their surface (Katsoyiannis & Zouboulis 2004).

The 16S ribosomal RNA gene sequences of four *Leptothrix* species, including *L. discophora SP-6*, have been determined in previous research (Siering & Ghiorse 1996). Previous research used fluorescence in-situ hybridization (FISH) to probe environmental samples for the presence or absence of *Leptothrix* species. Thus, two specific probes were made to detect the four *Leptothrix* species whose 16S rRNA gene sequences are known (Siering & Ghiorse 1997). The probes introduced in Siering & Ghiorse (1997) are named PS-1 and PSP-6. The PSP-6 probe can hybridize to *L. cholodnii*, *L. discophora SP-6* and *Leptothrix sp. NC-1* but not *L. discophora SS-1*. The PS-1 can hybridize to all four sequenced *Leptothrix* species, but also hybridizes to *Sphaerotilus natans,* which is a sheathed bacterium very similar to *Leptothrix* but incapable of oxidizing Mn. More traditional methods of detecting MOB through agar plate counting also exist (Tyler & Marshall 1967). By adding manganous sulphate ($MnSO_4$) to agar media during preparation, plated MOB colonies should turn black since they should produce Mn oxides. This practice has been used with MSVP, the medium on which the first sheathed *L. discophora SP-6* colonies were first isolated. Indicative MOB agar can also be made by adding $MnSO_4$ to R2A media (Sly *et al.* 1990).

The first full-scale biological Mn removal water treatment plant was constructed in North America in the town of Woodstock, New Brunswick, Canada in 1999 (Gage *et al.* 2001). The success of this initial plant has led to other towns in the province of New Brunswick adopting the same technology to treat their drinking water. The presence of multiple treatment plants in Eastern Canada provides an opportunity to identify the occurrence of *L. discophora*, or even *Leptothrix* more broadly can be detected on the filter media in full-scale processes.

The objectives of this paper were to identify *Leptothrix* in the full-scale plants using DNA amplification and to compare these molecular-based tools with more traditional MOB plate counting methods.

METHODS

Description and characterization of treatment plants used for study

Samples were obtained from four full-scale biological filtration plants in New Brunswick, Canada. These plants are located in the municipalities of Dorchester, Memramcook, Shediac and Woodstock, all in the province of New Brunswick, Canada. All four plants were built using a similar technology. In all cases, the treatment train consists of water pumped from an aquifer into a downflow filter, which is designed to handle flow rates up to 1,325 L min^{-1}. The Memramcook, Shediac and Woodstock plants operate their filters at this maximum capacity while Dorchester typically operates its filter at a range of 6–9 m h^{-1}. All filter units are circular with an outer diameter of 2.1 m, which results in a surface area of 3.4 m^2. The filter bed depth is

1.8 m at all locations. The filter material is a uniform sand provided by the manufacturer. Compressed air is pumped into the filters to ensure the ORP is high, since the active MOB in the filter are believed to be heterotrophic (Ehrlich 1996). The water passes through the filter only once. No chemicals were added to any of the filters.

There are some differences in how the plants operate relative to one another. Some of these differences and other operational characteristics are highlighted in Table 1. The Dorchester and Woodstock plants operate their filters continuously while the Memramcook and Shediac plants operate part-time to fill water reservoirs. The Shediac plant serves a popular cottage and tourist area which leads to large seasonal population variations, operates its filters as little as 4 hours per day during the winter. All plants backwash their filters at a rate of 20 m h^{-1} (280–300 gallons min^{-1}) for about 5 minutes to ensure optimal performance, although backwash frequency varies between plants. The intermittently operated units in Memramcook and Shediac are backwashed after 300 and 75 hours of operation, respectively. The continuously operated plants in Dorchester and Woodstock are backwashed every 300 to 336 hours, respectively. After backwashing, the filters are ripened for 5 minutes before they enter operation. All filter media samples were obtained during the steady-state filtration phase of operation. The Dorchester plant serves not only the village residents but also a large prison facility. Since the oldest plant described was built in 1999, there are few major operational problems or plant components which need to be repaired or replaced. The few exceptions include the excessive humidity inside the filtration room of the Woodstock plant, which may be contributing to Mn oxides corroding some of the piping. In Memramcook, the reservoir used to hold treated water is old and causes water loss which requires the treatment plant to produce about 40% more water than the town requires. However, as of November 2007, a new reservoir to replace the old one was under construction. The newer plants were aided in their initial inoculation by receiving some sand from inside the filter of another plant. The source of the inoculant is included in Table 1.

Filter media samples were obtained by draining the filter, opening the top service hatch, and collecting sand directly from the top by scooping it into a clean and autoclaved glass jar. This does not compromise the integrity of the filter since a very small amount of sand is lost due to backwashing and must be replenished approximately once per year. The media inside is black in appearance due to the build-up of Mn oxides.

Raw and filtered water was also collected to enable water characterization for each plant. Prior to arriving at each plant, the sample bottles were autoclaved to ensure uncontaminated water samples. The raw water was sampled from the raw inlet before it entered the filter, while the filtered water was sampled immediately after it exited the filter, before any disinfectants were added. Once disinfected, the water would be pumped directly to a reservoir or to the distribution system. Since groundwater is the source for all plants used in this study, natural organic matter (NOM) concentration was low (1.0–1.7 mg L^{-1}).

Chemical analysis

The water samples were characterized by measuring a number of different parameters. All analysis was performed using procedures acceptable by *Standard Methods for the Examination of Water and Wastewater* (2005). A spectrometer (Hach DR 4000) was used to determine colour at

Table 1 | Comparison of selected parameters for 4 biological Mn treatment plants in New Brunswick, Canada

Parameter	Dorchester	Memramcook	Shediac	Woodstock
Year Entered Operation	2005	2006	2001	1999
Average Daily Operation (h d^{-1})	24	12	4 (winter); 11 (summer)	24
Approximate Service Population	1,500	600	5,000 (winter); 25,000 (summer)	5,500
Origin of inoculant	Shediac	Shediac	Woodstock	None
Backwash frequency	Every 300 hours	Every 300 hours of operation	Every 75 hours of operation	Every 336 hours (14 days)

wavelengths of 455 and 254 nm. Concentrations of Mn, iron, magnesium and calcium were determined using Flame Atomic Absorption Spectroscopy (AAS) (PerkinElmer AAnalyst 200). Arsenic concentrations were measured using Graphite Furnace AAS (Perkin Elmer HGA 900 with PerkinElmer AS 80 Autosampler). Alkalinity was determined by titrating a sample of water with sulphuric acid.

Microbiological methods

The procedure to detach biofilm was slightly modified from one found in literature (Gagnon & Slawson 1999). Specifically, 1.5 g of sand filter media from each site was placed inside a stomaching sample bag and suspended in 10 ml of 0.85% NaCl solution. The sample was stomached in a MIX2 Stomacher (AES Laboratoire) for 1 min. The resulting supernatant appeared black, which suggested that biofilm detachment was successful at least to some degree.

A small aliquot (1 ml) of detached biofilm supernatant was used in conjunction with 0.85% NaCl solution to prepare dilution tubes to allow for the plating of detached biofilm. Dilutions of log 1–4 were prepared and plated on two culture media. The first, MSVP, is a low-nutrient media that was used to isolate the first cultures of *Leptothrix discophora SP-6* (Emerson & Ghiorse 1992). The second culture medium used was R2A. 17 mg L^{-1} $MnSO_4$ was added to both media to allow identification of MOB plated on the media (MOB colonies would appear black). The spread plating technique was used to plate 0.1 ml of sample. Plates were left in the dark at room temperature for approximately 4 weeks. This extended incubation time is standard for MOB identification because it sometimes takes time for MOB colonies to appear black (Rygel 2006). At the end of the incubation time, heterotrophic plate counts (HPCs) were performed using an AcoLyte Automatic Plate Counter. Simultaneously, a manual count of black colonies was performed. The plating procedure was carried out in duplicate, approximately one month apart.

For DNA analysis, 1.5 ml of detached biofilm supernatant was transferred into a microcentrifuge tube and spun at 10,000 × g for 10 min. The sample was washed twice with a Phosphate Buffer Solution prior to DNA extraction. Genomic DNA was extracted using an UltraClean™ Microbial DNA Isolation Kit (MoBio Laboratories, Inc).

RT-PCR was performed in a Smart Cycler II (Cepheid). PCR reaction tubes were prepared as follows: 12 μl Power SYBR® Green PCR Master Mix (Applied Biosystems); 10 μl PCR grade water; 1 μl of 10 μM PS-1 primer (5′-ACGGTAGAGGAGCAATC) or PSP-6 primer (5′-CAGTAGTGGGGGATAGCC); 1 μl of 10 μM DSP-6 primer (5′-GATTTCTTCCCTGACAAAAGC) (Sigma Genosys); 1 μl purified DNA. The PS-1 and PSP-6 upstream primers were taken from the sequence used for FISH probes in a previous study (Siering & Ghiorse 1997). The DSP-6 primer was newly developed for this study. The following cycling program was used: 10 min at 94°C; 40 cycles consisting of 0.5 minute at 94°, 0.5 min at 55°; and 1.5 min at 72°. PCR products were run on a 1.7% agarose gel in 1 × TBE buffer. The gel was stained with ethidium bromide and photographed using an ImageMaster VDS-CL (Amersham Pharmacia Biotech). Bands were excised from the agarose gel and purified using a MinElute™ Gel Extraction Kit (Qiagen Sciences). Purified DNA was sequenced using a Beckman CEQ8000 capillary sequencer. Sequencing results were analyzed using BioEdit Sequence Alignment Editor software.

RESULTS

A summary of water characterization results for all four sites can be found in Table 2. Of particular interest are the Mn results. It was found that all four plants, regardless of influent Mn concentration, had effluent Mn concentration below the detection limit. The observed detection limit on the AAS was approximately 0.03 mg L^{-1}, which corroborates Mn analysis performed at the plants themselves indicating that effluent quality virtually always meets guidelines. Thus, all the treatment plants surveyed achieve near 100% removal of Mn, which is consistent with what has been reported in other studies (Pacini *et al.* 2005; Li *et al.* 2006).

Occurrence of MOB with microbiological media

The samples from sand filter media contained a diverse array of bacterial species as indicated by plating on R2A and MSVP media. A graphical summary of plate counting results can be found in Figure 1. The results indicate that the Memramcook and Woodstock filters had a

Table 2 | Characteristics of water treatment plants

Utility	Dorchester		Memramcook		Shediac		Woodstock	
Sample Point	**Raw**	**Filtered**	**Raw**	**Filtered**	**Raw**	**Filtered**	**Raw**	**Filtered**
PH	7.25	7.18	6.46	6.46	7.50	7.63	7.39	7.33
ORP (mV)	295	415	343	370	338	332	368	480
UV-254 (cm^{-1})	0.02	0.01	0.01	0.01	0.01	0.01	0.25	0.03
Colour (455 Pt-Co)	2	0	0	0	0	0	1	0
Conductivity ($\mu s\ cm^{-1}$)	284	295	170	170	361	364	364	356
TOC ($mg\ L^{-1}$)	1.0	1.1	1.0	0.9	0.9	0.9	1.5	1.7
Mn ($mg\ L^{-1}$)	0.93	<0.03	0.86	<0.03	0.86	<0.03	1.39	<0.03
Fe ($mg\ L^{-1}$)	<0.2	<0.2	<0.2	<0.2	0.26	<0.2	<0.2	<0.2
As ($\mu g\ L^{-1}$)	<5	<5	<5	<5	<5	<5	<5	<5
Hardness ($mg\ L^{-1}$ as $CaCO_3$)	144	100	70	69	45	39	65	90
Alkalinity ($mg\ L^{-1}$)	29	23	14	14	30	30	37	36
NH_3 ($mg\ L^{-1}$)	<0.06	<0.06	<0.06	<0.06	0.13	<0.06	<0.06	<0.06

heterotrophic population density in the range of 10^7–10^8 CFU g^{-1}. Contrarily, Dorchester and Shediac had a heterotrophic population density in the range of 10^6–10^7, one order of magnitude lower. The number of heterotrophic bacteria was approximately the same on MSVP and R2A agar for all sites.

Black or dark brown colonies assumed to be MOB were detected at the Memramcook, Woodstock and Shediac plants. As seen in Figure 2, when detected, MOB population density was consistently between 10^3 and 10^4 CFU g^{-1}. For Memramcook and Shediac, the detected MOB were in the same range regardless of the agar media used. MOB were only detected on MSVP media for Woodstock and were not detected on either media for Dorchester samples.

Table 2 shows that the TOC concentration was approximately the same for the Dorchester, Memramcook and Shediac plants (0.9–1.0 $mg\ L^{-1}$) and slightly higher for Woodstock (1.5 $mg\ L^{-1}$). Heterotrophic plate counts show that Memramcook and Woodstock have about 0.5–1 order of magnitude more CFU ml^{-1} than Dorchester and Shediac. Since all of these plants easily achieve 100% Mn removal, there was no indication that a greater or lesser amount of biomass affects removal ability. This is consistent with the findings of other studies performed examining other kinds of biofiltration (Urfer *et al.* 1997).

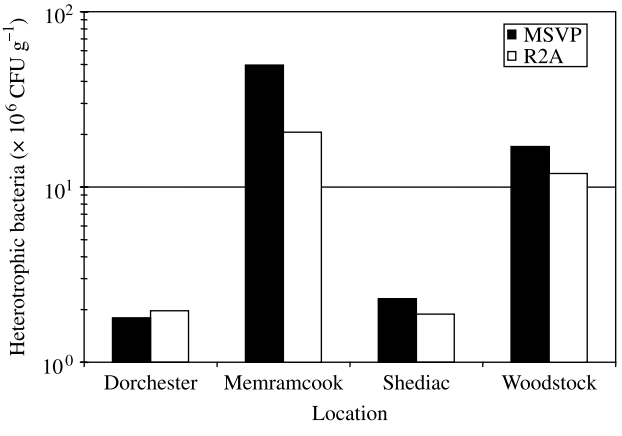

Figure 1 | Number of heterotrophic bacteria for the four sites in New Brunswick, Canada.

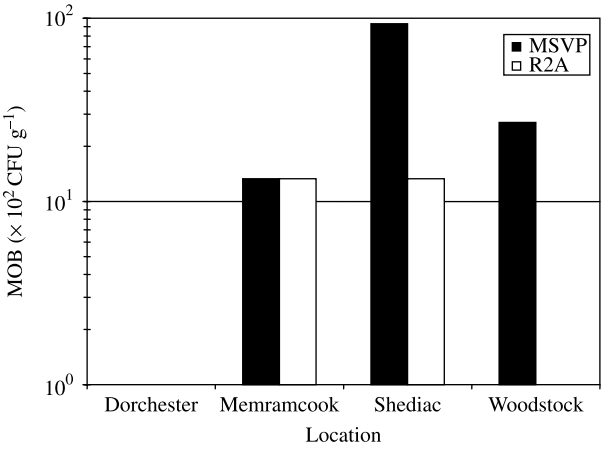

Figure 2 | Number of MOB for four sites in New Brunswick, Canada.

Molecular identification of MOB

DNA was obtained from stomached sand filter samples. The 16s rRNA region of *Leptothrix* genomic DNA was targeted by RT-PCR using two different upstream primers (PSP-6, SP-1) and a downstream primer (DSP-6). PCR products amplified using PSP-6 and DSP-6 primers had an expected size of 320 base pairs (bp). Agarose gel electrophoresis of PCR products from *Leptothrix discophora* strain SP-6 (ATCC 51168) genomic DNA and Memramcook mixed DNA showed that a product of expected size was amplified. This can be seen in wells 2, 3, 8 and 9 in Figure 3. *Escherichia coli* genomic DNA was used as a negative control to monitor primer specificity during the PCR run. The absence of a dark amplified product in well 4 of Figure 2 indicates that the primers used did not amplify any products in the negative control, which is the desired result. Amplified products from Dorchester, Shediac and Woodstock are located in wells 5 through 7 in Figure 2. They show faint bands appearing around the area where PCR products of expected size should be found. However, this was assumed to be the result of non-specific binding of PCR products to the SYBR green fluorescent marker, a known characteristic of the SYBR green dye (Giglio *et al.* 2003). Thus, the results demonstrated that Memramcook was the only site to test positive for the presence of *Leptothrix* bacteria.

Because the 16s rRNA region is highly variable from one species to another, strong similarity of 16s rRNA sequence indicates a close relationship between two species. An optimal global alignment of Leptothrix discophora (ATCC 51168) PSP-6 and Memramcook PSP-6 PCR products showed 95.4% identity. Figure 4 shows the 16s rRNA region of *L. discophora SP-6* and the isolate obtained from Memramcook. The highlighted sections show where the sequences differ. None of the sequence differences are more than 2 bp long and most differences occur in the first half of the 16s rRNA sequence. The sequencing results show that a species closely related to *Leptothrix discophora SP-6* was present in the sand filter media at the Memramcook water treatment facility.

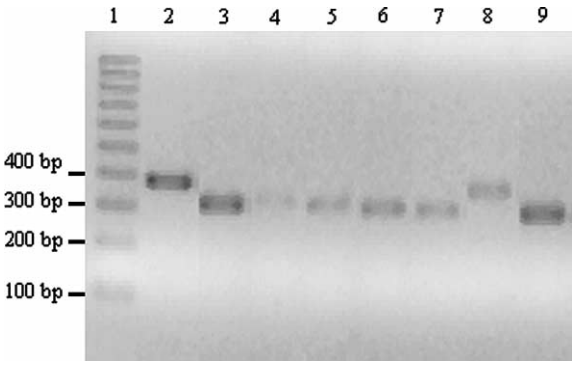

Figure 3 | Agarose gel electrophoresis of RT-PCR products. (1) 100 bp EZ Load Molecular Ruler (BioRad), (2) *L. discophora* SP-6 with PS-1 primer, (3) *L. discophora* SP-6 with PSP-6 primer, (4) *E. coli* with PSP-6 primer, (5) Woodstock with PSP-6 primer, (6) Shediac with PSP-6 primer, (7) Dorchester with PSP-6, (8) Memramcook with PS-1 primer (9) Memramcook with PSP-6 primer.

DISCUSSION

The water quality data demonstrated that all plants provided 100% Mn removal. However, only three of the filters demonstrated positive occurrence of MOB. Further, the plate count results from Woodstock and Shediac showed black colonies but *Leptothrix* spp. were not detected using RT-PCR, which indicates that plate counting will identify some MOB that are not of the genus *Leptothrix*. Thus it is plausible that *Leptothrix* bacteria do not have to be present in biological filters to achieve Mn removal, which contradicts previous claims that *Leptothrix* is the principal constituent in Mn oxidizing biofilms (Hope & Bott 2004).

The test results from Dorchester bring about many important considerations. DNA tests for the supposed predominant organism in Mn oxidizing bacteria and plate counting tests to detect MOB turned out negative in this plant. Other evidence must be taken into consideration to determine whether the Mn removal mechanism in these plants is biological. The mechanisms of biological iron oxidation have been called into question, as iron can demonstrate similar properties to Mn oxidation. Water treatment plants for biological iron removal exist and work under similar operational parameters as biological Mn plants, thus the biological filters are heavily aerated. Since aeration alone is a well accepted treatment for iron in water, it remains dubious whether biological iron removal plants work because of iron bacteria or because they are aerated (Sharma *et al.* 2005).

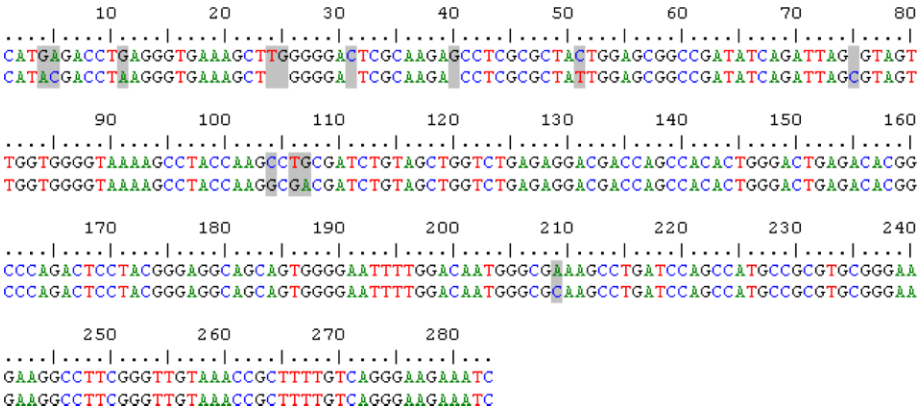

Figure 4 | BioEdit results showing genetic comparison between 16s rRNA region of *L. discophora SP-6* (top) and a Memramcook isolate (bottom) amplified by RT-PCR using PSP-6 primer.

Previous Mn biofiltration studies have listed the pH and ORP as very important operational parameters, just as with more conventional Mn treatment (Mouchet 1992; Katsoyiannis & Zouboulis 2004). It has been suggested in literature that biological Mn oxidation has a specific "field of activity", that is, a specific range of pH and ORP where biological treatment is most effective. Mouchet (1992) recommended biological filters be aerated heavily to give them an ORP between 300 and 400 and that the pH should be around 7.4–7.5. Memramcook was the lowest of all sites tested for pH (Table 2), with raw and filtered pH of 6.5. Memramcook was considerably lower than the other three facilities which had an average pH of 7.4 for both raw and filtered water. To verify that the pH recorded at Memramcook was typical of the plant, the village provided all pH readings made three times weekly at the plant from January through April 2007. The average pH observed was 6.6 and 6.5 for raw and filtered water, respectively. This indicates the plant typically operates at this low pH. It should also be noted that the filtration process itself did not result in pH adjustment of the water at any site, and that the influent and effluent pH (Table 1) of all plants were well below the range at which insoluble Mn carbonates and hydroxides would begin to form.

The ORP was fairly similar for the raw water samples of all plants. However, the ORP of the treated water was about 150 mV higher in Woodstock (480 mV, highest) than Shediac (332 mV, lowest) (Table 2). MOB were detected in both Woodstock and Shediac, these results obtained here indicate that the theoretical "field of activity" for biological Mn oxidation may be much wider than previously thought. Although MOB were not quantified from the Dorchester site, that site was well within the "field of activity" for Mn removal, thus was not limited by oxidation control.

Mn oxides are an effective adsorbent of soluble Mn, but they were not an oxidant by themselves (Drever 1988; Knocke *et al.* 1991). To regenerate a Mn-oxide coated media such as greensand, an oxidant such as potassium permanganate or free chlorine is commonly used (MWH 2005). The filters at all sites achieved excellent Mn removal without any kind of chemical regeneration being applied to the filters. It has been reported that adsorption of Mn^{2+} onto Mn oxides can be autocatalytic in the presence of a strong oxidant such as free chlorine (Knocke *et al.* 1991; Coffey *et al.* 1993). In the filters at the four utilities, air, as a weak oxidant, may have provided some regeneration of the adsorption sites on the Mn oxides. However it is also recognized that Mn removal by aerated filters is very difficult to achieve below a pH of 8.6 without the presence of a catalyst (Graveland & Heertjes 1975). Nevertheless, given the scope of the data collected in the present study, it is difficult to establish which of these two mechanisms was dominant in each filter.

The influent Mn concentration was very similar in the Dorchester, Memramcook and Shediac plants ($\sim 0.9\,mg\,L^{-1}$), while Woodstock's influent Mn was slightly more elevated ($\sim 1.4\,mg\,L^{-1}$). Because all of the plants achieved 100% Mn removal, it was unlikely that influent Mn concentration affected removal under the conditions studied. Hope & Bott (2004) reported that high Mn concentrations

($2-3\,mg\,L^{-1}$) are critical during filter start-up. However, the evidence collected for this study suggests influent Mn plays a less important role during "steady state" operation.

Given that the physical-chemical conditions were not limiting biological oxidation and the fact that chemical adsorption was likely to be minor, it is plausible that other microorganims contribute to the accumulation and removal of Mn in the biological filters. Mn oxidation may be achieved enzymatically and non-enzymatically. *Leptothrix* are enzymatic MOB which are capable of standalone enzymatic oxidation of dissolved Mn^{2+} using O_2 as a terminal electron acceptor (Ehrlich 1996). Some enzymatic MOB gain energy from this reaction while others (including *Leptothrix*) are unable to.

There is also a large group of species capable of non-enzymatic Mn oxidation which use hydrocarboxylic acids produced by biomass as a catalyst. These non-enzymatic reactions seem to be more susceptible to variations in pH and ORP (Ehrlich 1996). The high pH (>8) and high ORP (>500) suggested by Ehrlich (1996) is similar to the "field of activity" reported by Mouchet (1992). In the case of the four plants observed in this study, the Dorchester and Memramcook plants have a pH lower than recommended by either Ehrlich (1996) or Mouchet (1992). Since the plants seem to behave similarly, it is likely that most of the MOB present, whether *Leptothrix* or not, are likely enzymatic MOB.

In addition, the symbiotic effects of a microbial community in biofilms are well documented (Costerton *et al.* 1995). For Mn removal, it has been shown that the presence of nitrifying bacteria (e.g. *Nitrosonomas europa*) played a critical role in the removal of Mn^{2+} (Vandenabeele *et al.* 1995). Utilization of ammonia (NH_3) did not occur at Woodstock and Dorchester, as the NH_3 concentration was below the detection limit ($0.06\,mg\,L^{-1}$). Similarly, in the Memramcook site NH_3 was not detected in the raw water and thus direct enzymatic utilization or Mn was a likely pathway. In Shediac, where NH_3 was removed from $0.13\,mg\,L^{-1}$ to below the detection limit, nitrification may have occurred along with Mn removal.

Balancing the evidence between the fact that Mn does not oxidize as easily as iron by physical or chemical means and the microbiological properties collected at the four plants, it is likely that biological oxidation was the predominant mechanism responsible for removal. However, it is clear from the inconsistencies among the plants that greater effort is needed to quantify the potential microbiological pathways and organisms.

CONCLUSIONS

All four plants tested achieve near-100% Mn removal, regardless of influent water characteristics or the results of tests for *Leptothrix* or MOB. Out of all four plants tested, only one (Memramcook) tested positive for *Leptothrix* and three (Memramcook, Shediac and Woodstock) tested positive for MOB via indicative agar plating. Despite the negative test results in one of the four plants (Dorchester), other evidence overwhelmingly indicates that biological oxidation was likely a significant contributor to the oxidation and ultimate removal of Mn in all plants. Further, much like biological removal of NOM, the number of bacteria was also not a strong indicator of the ability to remove the target contaminant. Nevertheless, physical/chemical mechanisms of Mn removal do take place in the form of adsorption of Mn^{2+} to Mn oxides present in the filter bed. Further research should address the potential pathways and factors affecting biological oxidation or adsorption in these filtration systems, and the potential role that MOB allow the system to achieve autocatalytic operation without the addition of strong oxidants.

This paper demonstrated that while *Leptothrix* is the principal bacterial genus identified in previous studies as an MOB, it is only one of many bacterial genera capable of oxidizing Mn. It is very likely that other microorganisms contributed to the removal of Mn in the biological filters, through non-enzymatic or facultative pathways. It can also be concluded that the MOB detection method of using Mn-oxide infused agar plates may produce some false-negative results by not correctly identifying the presence of some MOB.

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