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Autochthonous tropical groundwater bacteria involved in manganese(II) oxidation and removal

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The presence of manganese (Mn) in drinking water causes aesthetic, operational, and health problems. Removal of Mn(II) by the use of Mn oxidizing microorganisms in biofiltration processes has been studied leading to a more economical and environmentally friendly process than conventional treatments with chemical products. There are multiple reports in temperate regions, but little information under tropical conditions, concerning Mn(II) removal by microorganisms. In this study, 13 autochthonous culturable manganese oxidizing bacteria isolated from water of two wells with ~0.6 mg L^{-1} and ~0.2 mg L^{-1} Mn(II), respectively, were evaluated *in vitro* to determine their potential as inoculums for application in biofiltration processes. The oxidation capacity of Mn(II) was evaluated by a qualitative test with Leucoberbelin blue I and the Mn(II) removal was quantified by measurements of the residual Mn(II) in a culture broth with an initial concentration of 5.9 mg L^{-1}. The biofilm formation capacity of the strains was also determined by crystal violet staining and by quantification of polysaccharides of extracellular polymeric substances. Three strains showed the highest percentage of Mn(II) removal in the culture in broth, two belonging to the genus *Aeromonas* (HAC-12 and RUE-1), with removal values of 42.5% and 40.1%, respectively, and one belonging to the genus *Stenotrophomonas* (S-3), with 40.3% removal. Besides, HAC-3B and RUE-6A showed the highest production of exopolysaccharides. These results allowed the selection of an *Aeromonas* strain (HAC-12) and one *Stenotrophomonas* strain (S-3) as possible candidates to be used as inoculums in biofilters for the removal of Mn(II) from groundwater.

Water impact

Removal of manganese from drinking water by biofiltration is a sustainable and environmentally friendly technology applied in temperate regions. This study evaluated the potential of tropical autochthonous bacteria isolated from groundwater to be used as inoculums in biofilters, which could reduce their start-up time. Applications of such inoculums at the pilot and large scale will help to consolidate this technology.

1. Introduction

The presence of manganese (Mn) in water sources for drinking water production can generate aesthetic, organoleptic and operational problems.[1,2] Furthermore, it can cause health effects associated with neurodegenerative diseases[3,4] and disorders in neurological development in children.[5,6] In many countries, conventional manganese treatment involves physical–chemical processes, which include the use of oxidizing chemicals, such as potassium permanganate ($KMnO_4$), ozone (O_3) and chlorine (Cl_2), and removal processes, such as (electro)-coagulation, sedimentation and filtration.[2,7,8] Although these techniques are efficient, they generate large volumes of sludge and have high operating and maintenance costs.[9,10]

For this reason, many studies have focused on Mn removal methods that include the use of microorganisms (biofiltration) at the pilot[11–14] and large scale.[15–23] Biological treatments are more environmentally friendly and less expensive than conventional systems, since they reduce the use of chemical products, the production of sludge, and the associated costs.[8,9,15,16,19] Most of the studies involve the oxidation capacity for Mn(II) of microorganisms,[24–26] and their biofilm formation capacity related to extracellular polymeric substances

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(EPS).[27,28] The biological oxidation of Mn(II) presents a high oxidation rate at neutral pH,[29] increasing the speed of this process up to five orders, compared to abiotic processes with only oxygen in the water.[16,30–32]

In addition, biogenic Mn oxides have a high metal adsorption capacity, due to their negative charge and greater specific surface area compared to synthetic ones.[30,33] Moreover, they are catalytic agents for secondary oxidation reactions of Mn(II) that depend on Mn(II) concentration, temperature and other ions, among others.[32] Furthermore, the biofilm formation capacity of Mn oxidizing bacteria (MOB) immobilizes them in the filter material,[34,35] through their EPS matrix, and thus tolerates the environmental and mechanical conditions of the biofilters[27,28] and contributes to the adsorption of metals.[26,36–39] Biological removal of Mn can be carried out by:[7,32] (a) direct oxidation, through intracellular oxidation of Mn(II) as part of the metabolic pathway of the microorganism; (b) indirect oxidation as a result of a change of the surrounding aqueous environment (pH and redox potential) and/or by the release of metabolic end products that favour the chemical oxidation of Mn(II); (c) extracellular adsorption of Mn on negatively charged EPS, as well as on biogenic oxides formed by bacterial catalytic reactions.

MOB are ubiquitous in nature and can be isolated from different habitats.[32,40] Among the most studied genera are *Leptothrix*, *Hyphomicrobium*, *Crenothrix*, *Siderocapsa*, *Siderocystic*, *Metallogenium*,[16,18,20,32] *Bacillus*,[31,41] *Pseudomonas*[42,43] and *Pedomicrobium*.[44] Therefore, there is a great opportunity for the application of biofiltration for manganese removal; however, the establishment of active biofilms in biofilters can take several months.[9,45] For this reason, the study of MOB for the development of inoculums that contribute to reducing the start-up time of biofilters is of interest.[29,35,46,47] Examples of proactive inoculation methods, listed by Breda *et al.*,[47] include the addition of a concentrated source of microorganisms and/or catalytic surfaces like backwash sludge, matured filter sand, mixed culture bacteria or specific bacterial species. A different approach consists of the use of the indigenous bacteria present in well water containing manganese. Breda *et al.*[48] found that some of the bacteria present in the source water of several biofilters were also found in different filter materials. The study suggests that some of the autochthonous bacteria found in groundwater could act as potential inoculums.

Likewise, it is relevant to study MOB development in tropical regions, where the warm climate and little temperature variation during the year could favour stability in biofiltration processes, and lead to other candidates for inoculums of biofilters. However, to the best of our knowledge, there are only a few studies of MOB in tropical regions; these include *in vitro* assays of MOB isolated from mine water such as *Serratia marcescens*,[49] *Stenotrophomonas* sp. and *Lysinibacillus* sp.[50] in Brazil and the isolation of *Bacillus pumilus* from well water in México.[51] Hence, there is a need for research about MOB in tropical countries like Costa Rica, where this study was performed, especially considering that there are no biofilters in operation to obtain an inoculum for filter start-up. Accordingly, the most suitable option is to isolate MOB from groundwater of existing wells containing Mn and study their suitability as inoculums. By this approach, an inoculum of autochthonous MOB obtained from the water to be treated could more easily adapt to actual biofilters.

Therefore, the aim of this study was to evaluate the potential of naturally occurring, autochthonous bacteria in tropical groundwater to act as an inoculum for the removal of Mn(II) in biofiltration processes, which could reduce the start-up time of biofilters in the tropics. The capacity to remove Mn(II) and to form biofilms of 13 strains of bacteria, isolated from water of two wells in Costa Rica with Mn concentrations of ~0.6 mg L^{-1} and ~0.2 mg L^{-1}, respectively, was evaluated at the *in vitro* level.

2. Materials and methods

2.1 Bacterial strains

For the development of this research, 13 previously isolated bacterial strains from water of two wells located in Cartago, Costa Rica were studied. The physical–chemical parameters of the raw water from these wells are presented in Table 1. These isolations were performed in R2A Agar (OXOID: CM0906) medium with 17 mg L^{-1} $MnSO_4$. Table 2 shows the closest related species identified by 16S rRNA gene sequencing and their sequence homology analysis using the BLAST program (GenBank) and the Database of the National Centre for Biotechnological Information (NCBI) carried out by the Molecular Techniques Laboratory of the University of Costa Rica.

The strains were spread on plates, kept in R2A medium supplemented with 17 mg L^{-1} $MnSO_4$ and incubated at room temperature (26 ± 2 °C) for the tests.

2.2 Mn(II) removal capacity

2.2.1 Qualitative detection of the oxidation capacity of Mn(II) with Leucoberbelin blue I. The presence of Mn oxides was qualitatively confirmed with Leucoberbelin blue I (LBB). This LBB reagent oxidizes when interacting with Mn(III) or Mn(IV), changing from colourless to blue. The intensity of the blue colour of the oxidized LBB is given as a function of the amount of Mn oxides reduced.[52,53] Then, the strains were

Table 1 Physical–chemical characteristics of raw water from two wells

Parameters	Wells	
	HAC[a]	RUE[b]
Mn (mg L^{-1})	0.601 ± 0.001	0.223 ± 0.01
Fe (mg L^{-1})	0.270 ± 0.156	0.190 ± 0.01
OD (mg L^{-1})	1.56 ± 0.19	1.96 ± 0.01
ORP (mV)	−13.8 ± 4.6	41.4 ± 0.1
Temperature (°C)	23.0 ± 0.6	25.4 ± 0.1
pH	7.17 ± 0.14	7.07 ± 0.01

[a] Values with standard deviation of 2 samples taken in April 2018 and September 2019. [b] Values with standard deviation of one sample taken in November 2018.

Table 2 Bacterial strains used in studies of Mn oxidation and biofilm formation

Strain code	Origin	Closest related species	Similarity	No. accession
HAC-3	HAC (−80 °C)	*Enterobacter asburiae*	99%	*KU212142*
HAC-4	HAC (−80 °C)	*Leclercia adecarboxylata*	99%	*MG890203*
HAC-10	HAC (−80 °C)	*Citrobacter freundii*	100%	*CP024677*
HAC-12	HAC (−80 °C)	*Aeromonas taiwanensis*	99%	*KT998825*
HAC-13	HAC (−80 °C)	*Enterobacter roggenkampii*	91%	*CP017184*
RUE-1	RUE (−80 °C)	*Aeromonas hydrophila*	99%	KC800783
RUE-2	RUE (−80 °C)	*Aeromonas sanarellii*	97.3%	*NR_116584.1*
RUE-6A	RUE (−80 °C)	*Stenotrophomonas maltophilia*	100%	MK203000
RUE-7	RUE (−80 °C)	ND	—	—
RUE-18	RUE (−80 °C)	ND	—	—
HAC-3A	HAC[a]	*Microbacterium paraoxydans*	96.56%	*LT629770*
HAC-3B	HAC[a]	*Stenotrophomonas maltophilia*	99.84%	*LT906480*
S-3	HAC[a]	*Stenotrophomonas pavanii*	98.1%	*NR 118008*

ND: not determined. [a] Freshly isolated, not in cryopreservation before the test.

spread on plates with R2A medium supplemented with 17 mg L^{-1} $MnSO_4$ in duplicate and incubated at room temperature (26 ± 2 °C) in the dark for 3–5 weeks. Subsequently, a drop of 0.04% LBB in 45 mM acetic acid was added at 20, 30 and 40 d of growth to detect Mn oxides. The relative oxidation capacity of Mn(II) was reported according to the level of intensities of the blue coloration in the positive samples as follows: (+): slight; (++): moderate; (+++): intense; (++++): very intense. The test was performed in duplicate. Seven strains with positive results and with the highest intensity were selected to carry out the subsequent Mn removal and biofilm formation tests.

2.2.2 Quantification of the removal of Mn(II) in broth culture

Culture. From the plate culture (R2A medium supplemented with 17 mg L^{-1} $MnSO_4$) of the seven positive strains selected, a bacterial suspension in sterile saline (0.85%) was prepared, and the concentration was adjusted to 0.5 McFarland (~1×10^8 cells per mL). One mL of this suspension was inoculated in a 125 mL Erlenmeyer flask with 50 mL of PYM culture medium, consisting of 2 g L^{-1} peptone, 0.5 g L^{-1} yeast extract, 10 mM HEPES, and 0.1 mM $MnSO_4 \cdot H_2O$ (final approximate concentration of 109 μM Mn(II), 5.9 mg Mn L^{-1}). The culture was incubated at room temperature (26 ± 2 °C), 75 rpm and in the dark for 5 d, to form the seed culture or initial inoculum for the assay.

Subsequently, following the method of Vandenabeele *et al.*,[22] 1 mL of the seed culture was inoculated in a 250 mL Erlenmeyer flask with 100 mL of PYM culture medium and incubated at room temperature, 75 rpm and in the dark for 14 d. In addition, a control of the culture medium without an inoculum was prepared, and the entire test was performed in triplicate.

Sampling and analysis of residual Mn. The Mn(II) removal of the evaluated strains was determined by measuring the concentration of residual Mn(II) in the culture broth at different times as recommended by Burger *et al.*[24] and Vandenabeele *et al.*[22] For this, 2 mL aliquots of culture were aseptically collected on days 0, 2, 4, 7, 10, and 14. Each aliquot was centrifuged at 4000 rpm for 15 min and filtered through a 0.45 μm cellulose nitrate filter to separate the dissolved Mn(II) from the suspended species (Mn oxides). The filtrates were acidified with 10% nitric acid and the Mn(II) concentration was determined by atomic absorption spectroscopy using a Perkin Elmer equipment model AAnalyst800, following the standard method 3111-B (APHA-AWWA-WEF, 2017) with a curve of 0.05–2 mg L^{-1} Mn(II) and a detection limit of 0.03 mg L^{-1}. Data were statistically analysed using one-way analysis of variance (ANOVA) ($P < 0.05$).

In addition, the percentage of Mn(II) removal at the end of the experiment (day 14) was calculated considering the control without an inoculum. During each sampling, the pH and culture growth were monitored through counts of CFU mL^{-1}, using 100 μL of the collected sample.

2.3 Biofilm formation capacity

2.3.1 Qualitative test of biofilm formation. Biofilm formation was determined through staining of the adhered cells to the plates using crystal violet (CV) reagent as recommended by Beukes and Schmidt[25] with slight modifications in terms of culture media and quantities of broth, CV and ethanol. The strains were cultured in 100 mL of PYM medium in a 250 mL Erlenmeyer flask and incubated at room temperature for 5 d. Afterward, 10 mL of the culture broth were dispensed into sterile polystyrene Petri dishes and incubated for 24, 48, 72, and 96 h at room temperature. Subsequently, the culture broth was discarded, and two sterile water washes were gently carried out to remove the cells not bound to the surface. The cells that adhered to the plate were then stained with 10 mL of 0.1% crystal violet (CV) stain and incubated for 20 min at room temperature. The CV was discarded and 2 washes with sterile water were performed again to remove the excess of the non-adsorbed reagent. Subsequently, the CV that adhered to the biofilm cells in the plates was extracted with 15 mL of 95% ethanol, incubated at room temperature for 5 min. The collected extract was centrifuged (4000 rpm for 10 min), and its UV absorbance was measured at 540 nm on a Shimadzu UV-visible spectrophotometer, UV-1800, using 95% ethanol as a

blank. Two copies were made for each measurement time of each strain, and a control, consisting of culture medium without an inoculum, was prepared.

2.3.2 Quantification of EPS polysaccharides in broth culture with pumice as a support

Culture. In order to determine the EPS polysaccharides *in vitro*, a culture broth with a filter material (pumice), as a support, was prepared. For that, an initial inoculum of each isolate was prepared in the same way as in section 2.3.1. Then, 1 mL of the seed culture was inoculated in 120 mL bottles with 50 mL of PYM culture medium and 3 g of pumice stone (0.8–1.2 mm). This material was used because of its local origin, high porosity, and large specific surface area,[54] thus having the potential to be used as a filter material in biofiltration. The culture was incubated in the dark at room temperature for 30 d. In addition to the evaluated strains, a control of the culture medium without an inoculum was prepared. The entire test was carried out in triplicate.

Extraction of EPS. The extraction of EPS was performed according to Keithley and Kirisits[55] with some modifications related to centrifugation and the determination of total solids. 2.00 g (wet weight) of the filter material was added to a 50 mL conical tube, followed by 10 mL of the extraction buffer (10 mM Tris, 10 mM EDTA, 2.5% NaCl, pH 8). The tubes were stirred in a vortex (3000 rpm) for 1 min and incubated at 35 °C, then maintained at 200 rpm for 4 h. Subsequently, the samples were centrifuged at 4000 rpm for 15 min at room temperature; the supernatant was filtered through a 0.45 μm polyamide filter and stored at 4 °C until analysis. The precipitate with the pumice was transferred to a crucible and dried at 35 °C for 48 h to determine total solids (TS) which include the weight of the filter material.

Polysaccharide measurement. The polysaccharides, one of the major components of the EPS matrix together with proteins,[27,56,57] were measure by the phenol–sulfuric acid method.[58] Briefly, 2 mL of the EPS extract were added into 40 mL vials, followed by 50 μL of 80% phenol and 5 mL of concentrated H_2SO_4. The mixture was left to stand for 10 min and then vigorously stirred for 30 s, then incubated at room temperature for 20 min. The UV absorbance was measured at 490 nm on a Shimadzu UV-visible spectrophotometer, UV-1800. Reactive grade glucose was used as the standard curve in the range 5–100 mg glucose per L, detection limit 3.35 mg glucose per L. Results were reported in mg of glucose per g of ST. Data were statistically analysed using one-way analysis of variance (ANOVA) ($P < 0.05$).

3. Results and discussion

3.1 Mn removal capacity

3.1.1 Detection of the oxidation capacity of Mn(II) with LBB. Of the 13 evaluated bacterial strains, six presented a positive result in the qualitative test with LBB from the first 20 d of growth (Table 3). After 40 d of incubation, three other strains (HAC-13, RUE-6A and RUE-7) presented a very slight oxidation activity (+).

The results indicate that the HAC-3A strain generated the highest amount of Mn oxides, from the first days of growth, followed by RUE-1, HAC-3B, S-3, HAC-12 and RUE-2. Therefore, these six strains were selected for subsequent tests. In addition, the RUE-6A strain, corresponding to one of the strains with mild activity after 40 d of incubation, was selected.

It was found that the RUE-6A and HAC-3B strains, although being of the same genus (*Stenotrophomonas*) and closest related species according to molecular identification (Table 2), showed different activities (Table 3). This could be related to the fact that the RUE-6A strain was preserved at −80 °C and in a medium without Mn prior to reactivation for the test, while the HAC-3B strain came from a recent isolation in a medium with Mn. It is known that in some cases, the freezing process can cause changes in the enzymatic activity of the microorganism.[59] Furthermore, the oxidation capacity of bacteria isolated from freshwater subcultured in a medium without Mn(II) often loses this activity in the subsequent culturing in a Mn-containing medium.[22] Moreover, the S-3 isolate of the same genus, but different closest related species (Table 2), isolated in a medium with Mn, showed a similar activity to HAC-3B.

Few Mn(II) oxidation studies in moderate and even less tropical climates refer to the genera of bacteria evaluated here. Barboza *et al.*[50] reported the oxidation of Mn(II) in a culture broth by various strains of *Stenotrophomonas*, as found in HAC-3B, RUE-6A and S-3, isolated from a mine in Brazil. In addition, this genus was found during the removal of Fe and Mn at a biological filtration plant,[60] and during the biological removal of Zn and Mn.[61] The actinobacterium of the genus *Microbacterium*, identified in HAC-3A, was also reported by Marcus *et al.*[62] in drinking water systems. The *Aeromonas* genus, found in HAC-12, RUE-1 and RUE-2, has mainly been reported for its ammonium removal activity associated with drinking water[63] and in pilot scale biofilters for ammonium and Mn removal.[14] Finally, Zhang *et al.*[64]

Table 3 Mn(II) oxidation activity measured with LBB reagent in various bacterial strains isolated from wells

Code strain	Genus	Mn(II) oxidation activity on different growth days		
		20	30	40
HAC-3	*Enterobacter*	−	−	−
HAC-4	*Leclercia*	−	−	−
HAC-10	*Citrobacter*	−	−	−
RUE-18	ND	−	−	−
RUE-7	ND	−	−	+
HAC-13	*Enterobacter*	−	−	+
RUE-6A	*Stenotrophomonas*	−	−	+
HAC-12	*Aeromonas*	+	++	++
RUE-2	*Aeromonas*	+	++	++
RUE-1	*Aeromonas*	++	++	++
HAC-3B	*Stenotrophomonas*	++	++	++
S-3	*Stenotrophomonas*	++	++	++
HAC-3A	*Microbacterium*	+++	+++	+++

Blue colour intensity associated with the relative oxidation of Mn(II): −: negative, +: slight, ++: moderate, +++: intense, ++++: very intense.

reported a strain of *A. hydrophila*, isolated from sediments contaminated with Mn from mines, as a new MOB.

3.1.2 Quantification of Mn(II) removal in broth culture. The seven strains selected from the LBB assay showed some degree of Mn(II) removal (Fig. 1A and 2A). The non-inoculated control presented low removal, probably due to some physical–chemical reactions, similar to that reported by Burger *et al.*[24] All strains removed Mn(II) from an initial concentration of 5.9 mg L^{-1} to values between 4.5 and 4.9 mg L^{-1} on the second day of incubation, distinguishing two groups of strains: one that continued removing Mn gradually (Fig. 1A) and another that ceased to remove Mn(II) (Fig. 2A). This initial decrease in Mn(II) in the first two days of cultivation could be linked to an adsorption process, since this is the first step in the bioavailability of metals for many cellular processes.[65]

In the first group, Fig. 1A, the HAC-12, RUE-1, S-3 and RUE-2 strains reduced the Mn(II) concentration in the culture medium by day 14 to 3.2 ± 0.22 mg L^{-1}, 3.3 ± 0.22 mg L^{-1}, 3.8 ± 0.17 mg L^{-1} and 3.3 ± 0.09 mg L^{-1}, respectively. The Mn(II) removal by these strains could be related to the growth of the culture (Fig. 1B). At the beginning, the bacteria were in the lag phase, adapting to the environment and the observed removal could be probably due to adsorption. Similarly Tang *et al.*[66] reported in a broth assay with an MOB isolated from a mine that for the first and second day manganese was removed mainly by extracellular adsorption. After the fourth day (Fig. 1B), the higher manganese removal coincides with the end of the exponential growth phase and the stationary phase of the culture. Unfortunately, the latter cannot be observed in the graph because the sampling and determination (CFU ml^{-1}) in this experiment was performed every two to three days. Therefore, in the stationary phase, estimated between day 4 and 7 for HAC-12, RUE-1 and RUE-2, and between day 7 and 10 for S-3 (Fig. 1B), Mn(II) removal increased probably due to biological oxidation. These results are in agreement with previous findings for different MOB than the ones reported here, suggesting that during the stationary phase biological oxidation of Mn(II) takes place.[25,35,66–72]

In the second group of strains (HAC-3A, HAC-3B and RUE-6A), Fig. 2A, no further Mn(II) removal took place after the second day. Furthermore, the HAC-3A strain even showed metal desorption, after presenting the highest Mn(II) removal on day 2, 4.5 ± 0.05 mg L^{-1}, since on days 4 and 7 the Mn(II) concentration increased again in the medium to 4.9 ± 0.06 mg L^{-1} and 5.3 ± 0.29 mg L^{-1}, respectively. Similarly, Mn desorption was found by Rivera *et al.*[51] in their study with a *Bacillus* strain after 14 d of growth in a culture broth. The results for these strains (HAC-3B, HAC-3A and RUE-6A) suggest that they had limited Mn removal activity under the tested conditions and culture time (Fig. 2B).

HAC-12, RUE 1 and S-3 presented a very similar Mn(II) removal percentage without significant differences ($p > 0.05$), being 42.5 ± 4.0%, 40.1 ± 3.9%, and 40.3 ± 3.5%, respectively (Fig. 3). However, RUE-2 registered only 32.1 ± 3.1% removal, while in the second group of strains, with lower removal activity, the final percentages were 12.7 ± 2.7%, 16.2 ± 2.3% and 16.9 ± 2.5% for HAC-3A, HAC-3B and RUE-6A, respectively. Previous studies, carried out in broth culture (0.55–44 mg L^{-1} Mn) with recognized MOB such as *Bacillus*,[73] *Leptothrix*,[74] and *Pseudomonas*,[35,43] reported Mn(II) removal efficiencies higher than 80%. Meanwhile other studies, using less common genera, reported lower removal percentages, *e.g. Stenotrophomonas* isolated from a mine with 70.9% removal (evaluated in K medium with 50 mg L^{-1} Mn),[50] *Bacillus pumilus* 61%,[51] *Serratia marcescens* 55%[49] and *Aeromonas hydrophila* 89.6% (evaluated in broth with 550 mg L^{-1} Mn), respectively.[64] The lower Mn removal activity found in the present study for the same genera (*Stenotrophomonas* and *Aeromonas*) with respect to the literature could be due to the origin of the strains, the concentration of Mn and bacteria, the type of culture medium, and the specific conditions used in the tests. Interestingly, both genera were found in the water from the two wells studied (Table 2).

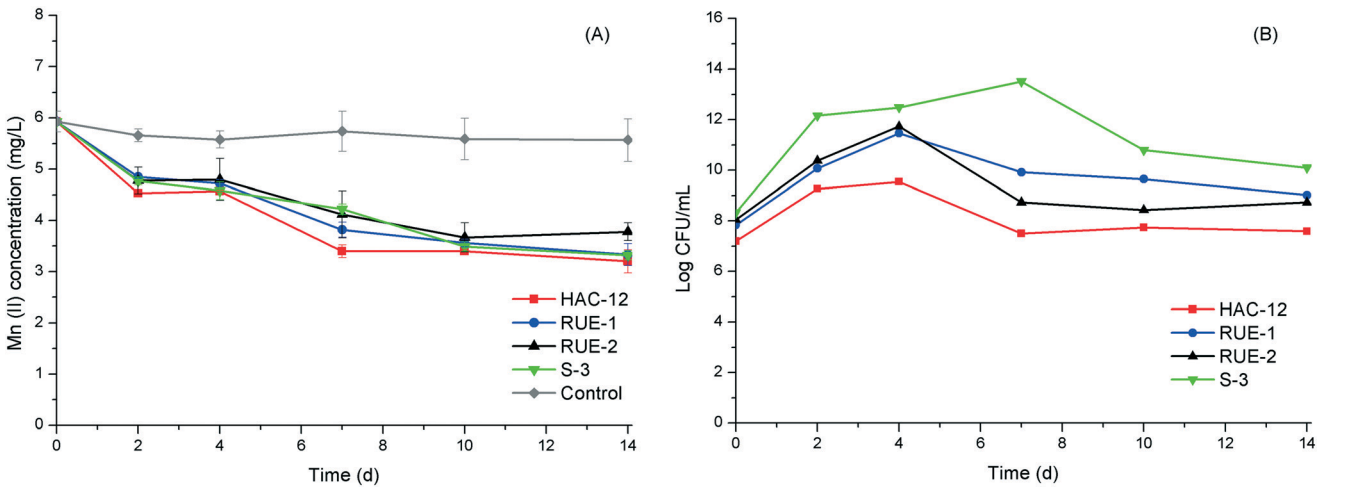

Fig. 1 Culture broth behaviour with bacteria isolated from wells. (A) Mn(II) removal and (B) crop growth. PYM culture medium, initial concentration of 5.9 mg L^{-1} Mn(II) (109 μM). Error bars in (A) show the standard deviation of three replicates.

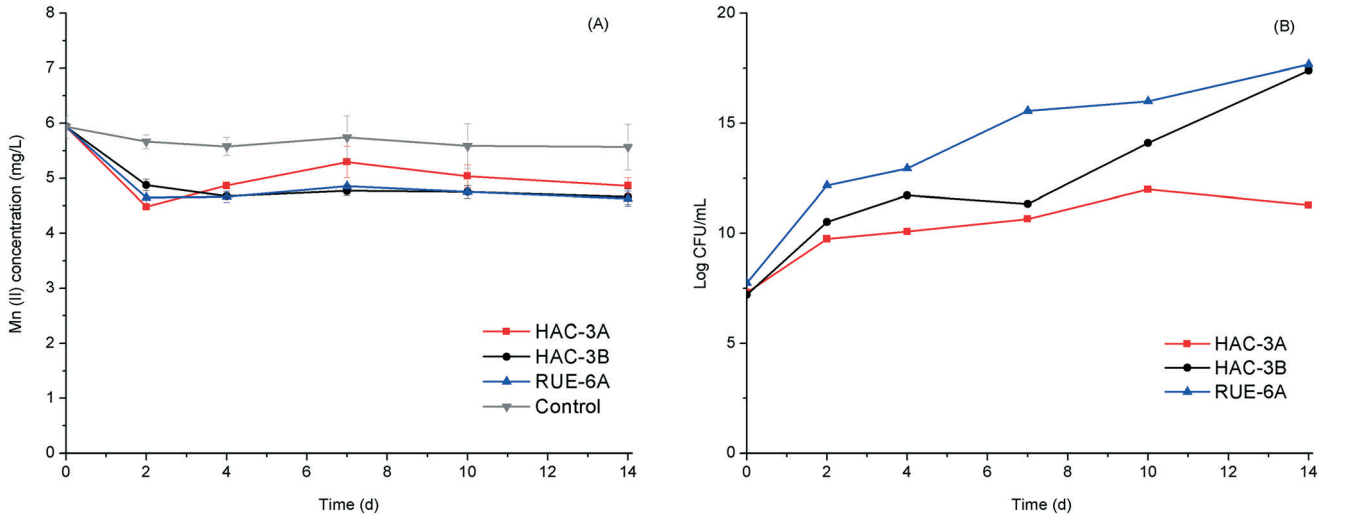

Fig. 2 Behaviour of culture broth with bacteria isolated from wells. (A) Removal of Mn(II). (B) Growth of PYM culture medium, initial concentration of 5.9 mg L^{-1} Mn(II) (109 μM). Error bars in (A) show the standard deviation of three replicates.

The removal of Mn(II) was accompanied by an increase in pH, which was abrupt in the first two days in some strains and gradual in others (Fig. 4A and B, respectively). This increase in pH was probably related to bacterial growth and substance metabolism in a culture.[72] In bacteria with an abrupt increase in pH, the removal of Mn(II) could occur indirectly by favouring chemical oxidation, occurring at a pH above 8.0.[18,22,49] This *e.g.* indicates that the low degree of removal of Mn(II) by the HAC-3B and RUE-6A strains, both *Stenotrophomonas* sp., with the highest pH increase, could be attributed to indirect oxidation (Fig. 4A). This agrees with Barboza *et al.*,[50] who proposed for the same genus that oxidation of Mn(II) occurs indirectly by a non-enzymatic pathway. However, the S-3 isolate (Fig. 4B), also of the *Stenotrophomonas* genus, presented a higher removal of Mn(II) at lower pH, indicating direct biological oxidation, probably because the closest related species is different (Table 2).

For the rest of the evaluated strains (Fig. 4B), which had greater Mn removal activity, the pH remained between 7 and 7.5 during most of the culture, with these values being the most favourable for the biological oxidation of Mn(II).[18,24,72]

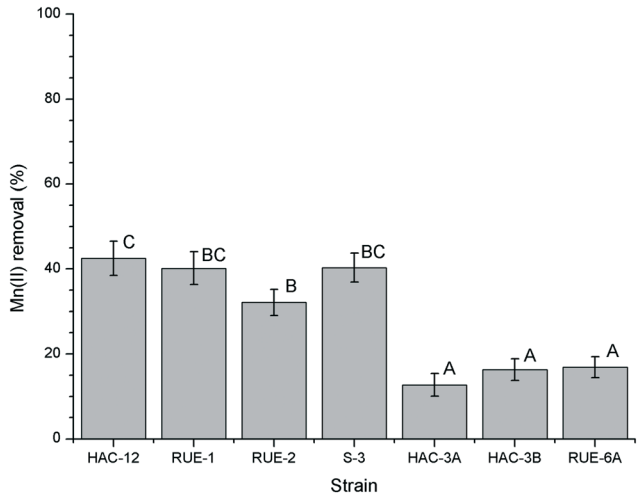

Fig. 3 Percentage of removal of Mn(II) in culture broth with bacteria isolated from wells, at 14 days of incubation and with an initial concentration of 5.9 mg L^{-1} Mn(II) (~109 μM). Error bars show the standard deviation of three replicates. The letters next to each bar (A–C) indicate the groups with similarity or significant differences (α 0.05). Averages with a common letter are not significantly different ($p > 0.05$).

3.2 Biofilm formation capacity

3.2.1 Qualitative biofilm formation test. The biofilm formation capacity was evaluated in the seven strains selected from the results with LBB. The CV test showed that the HAC-12, RUE-6A, HAC-3B and S-3 strains (Fig. 5) were able to form biofilms under the evaluated conditions, since their UV absorbance at 540 nm increased in time, indicating that they have the ability to adhere to solid surfaces, which is relevant for their possible use in biofilters.[35] The HAC-3A, RUE-1 and RUE-2 strains presented very similar values to the control, showing little biofilm formation over time and under the evaluated conditions. However, in the case of RUE-1 and RUE-2, a slight increase in UV absorbance was observed at 72 h and 96 h, indicating that these strains might have required more time to form biofilms.

It was observed that all the strains except for HAC-3A showed some degree of biofilm formation, since the UV absorbance was superior to that of the control, with the cultures of HAC-12, RUE-6A, HAC-3B and S-3 being of particular interest.

3.2.2 Quantification of EPS polysaccharides in broth culture with pumice as a filter material. The EPS present in broth culture with a filter material were extracted from the seven bacterial strains selected in section 3.1 and the polysaccharides were quantified. The filter material used was pumice from a culture broth with one month of incubation,

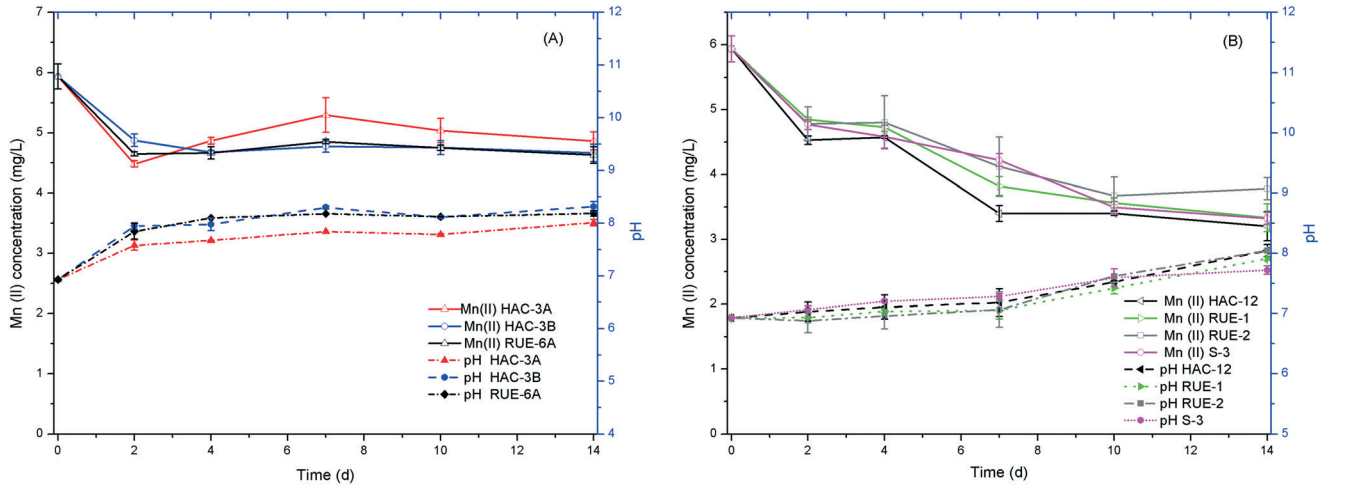

Fig. 4 Effect of pH on the removal of Mn(II) in culture broth with bacteria isolated from wells. (A) Crops with a sudden pH increase and control. (B) Cultures with a gradual increase in pH. Error bars show the standard deviation of three replicates.

intended to simulate the maturation of a real biofilter. The amount of polysaccharides obtained in this experiment was between 0.004 and 0.139 mg D-glucose per g TS of filter material (Fig. 6). Although this type of experiment at the *in vitro* level has not been reported either under temperate or tropical conditions, the values obtained were similar to those reported by EPS studies in mature biofilters in a moderate environment. Keithley and Kirisits,[55] *e.g.*, reported between 0.016 and 0.60 mg glucose per g of TS in EPS obtained from granular activated carbon, anthracite and sand from mature biofilters in eleven full-scale drinking water plants. On the other hand, considering the bulk density of the pumice stone, the amount of polysaccharides obtained in this study was between 0.009 and 0.321 mg D-glucose per cm^3 of filter material. Elhadidy *et al.*[27] recorded a range of 0.011–0.102 mg D-glucose per cm^3 of filter material from a mixture of anthracite and sand from a biological filtration pilot drinking water treatment plant with two months of operation. Markin[75] obtained only 0.056 mg D-glucose per cm^3 on sand and 0.032 mg D-glucose per cm^3 on anthracite of a dual pilot biofilter.

The strains that presented the highest amount of polysaccharides in the extracted EPS (Fig. 6) were the same with the highest biofilm formation in the CV test (HAC-3B, RUE-6A, HAC-12 and S-3) which was expected, since the presence of EPS allows adhesion to surfaces favouring the formation of biofilms.[76]

Although the greater presence of EPS has been related to a higher metal adsorption,[26,36–38] the strains with the highest amount of polysaccharides did not necessarily report the highest Mn(II) removal percentage (Fig. 3 and 6). This indicate that the observed removal would be related to other cellular processes such as biological oxidation rather than adsorption.

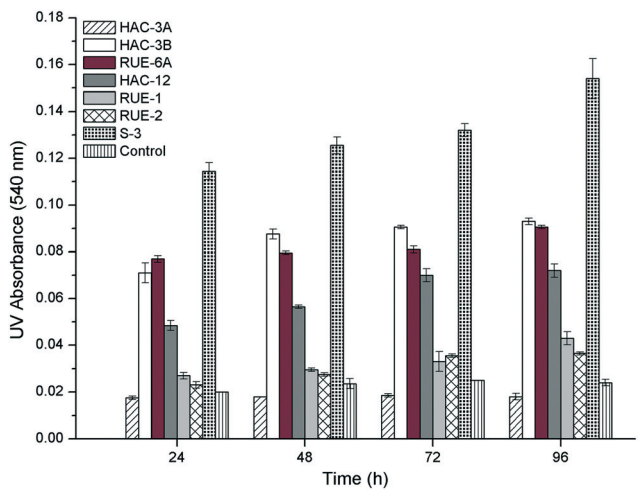

Fig. 5 Biofilm formation of bacterial strains isolated from wells, determined by a CV staining test. Error bars show the standard deviation of two replicates.

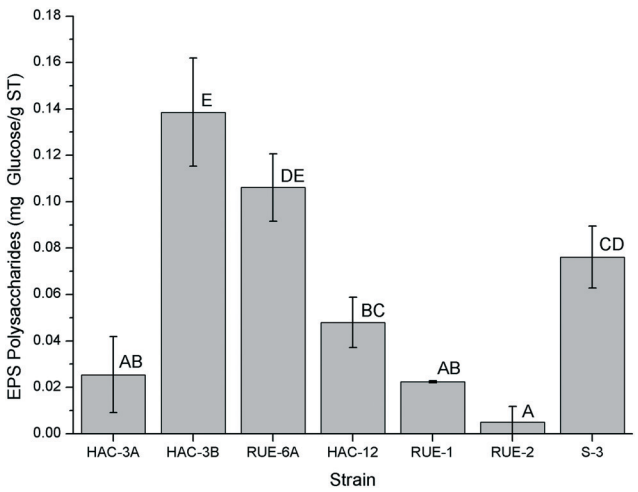

Fig. 6 Polysaccharides present in MOB broth culture with EPS extracts isolated from wells after 30 d of incubation. Error bars show the standard deviation of three replicates. The letters next to each bar (A–E) indicate the groups with similarity or significant differences (α 0.05). Means with a common letter are not significantly different ($p > 0.05$).

In fact, in the cultures with greater Mn removal, the presence of Mn oxides retained in the filters was observed as a brown coloration at the time of processing the sample for its analysis by atomic absorption. Of the strains with the highest percentage of Mn(II) removal (HAC-12, RUE-1, S-3 and RUE-2), only S-3 and HAC-12 showed a higher production of EPS polysaccharides, which makes them candidates for use as inoculums for biofilters with pumice as a filter material.

4. Conclusions

Biofiltration in temperate regions is widely used; however, few studies exist in tropical areas. Therefore, the objective of this research was to evaluate, at the *in vitro* level, the potential of autochthonous bacteria from groundwater in the tropics for the removal of Mn in biofiltration processes, and whether they present potential as inoculums. The results showed that of the 13 strains evaluated in this study, two strains belonging to the genus *Aeromonas* sp. (HAC-12) and *Stenotrophomonas* sp. (S-3) presented a percentage of Mn(II) removal of more than 40% and the highest capacity to form biofilms, making these strains good candidates as inoculums to be used in biofilters, with pumice as a filter material, for the removal of Mn(II) from tropical groundwater. The detection of these promising bacteria indicates the potential of biofiltration, a sustainable free-chemical manganese removal technique, in tropical areas.

Conflicts of interest

There are no conflicts to declare.

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References

1 L. I. Sly, M. C. Hodgkinson and V. Arunpairojana, Deposition of manganese in a drinking water distribution system, *Appl. Environ. Microbiol.*, 1990, **56**, 628–639.
2 A. Postawa, Best Practice Guide on the Control of Iron and Manganese in Water Supply, *Water Intelligence Online*, 2013, vol. 12, p. 131.
3 M. Farina, D. S. Avila, J. B. T. da Rocha and M. Aschner, Metals, oxidative stress and neurodegeneration: A focus on iron, manganese and mercury, *Neurochem. Int.*, 2013, **62**, 575–594.
4 A. B. Santamaria and S. I. Sulsky, Risk Assessment of an Essential Element: Manganese, *J. Toxicol. Environ. Health, Part A*, 2010, **73**, 128–155.
5 G. Bjørklund, M. S. Chartrand and J. Aaseth, Manganese exposure and neurotoxic effects in children, *Environ. Res.*, 2017, **155**, 380–384.
6 M. F. Bouchard, S. Sauvé, B. Barbeau, M. Legrand, M.-È. Brodeur, T. Bouffard, E. Limoges, D. C. Bellinger and D. Mergler, Intellectual Impairment in School-Age Children Exposed to Manganese from Drinking Water, *Environ. Health Perspect.*, 2011, **119**, 138–143.
7 J. E. Tobiason, A. Bazilio, J. Goodwill, X. Mai and C. Nguyen, Manganese Removal from Drinking Water Sources, *Curr. Pollut. Rep.*, 2016, **2**, 168–177.
8 N. Marsidi, H. Abu Hasan and S. R. Sheikh Abdullah, A review of biological aerated filters for iron and manganese ions removal in water treatment, *J. Water Process. Eng.*, 2018, **23**, 1–12.
9 A. G. Tekerlekopoulou, S. Pavlou and D. V. Vayenas, Removal of ammonium, iron and manganese from potable water in biofiltration units: a review, *J. Chem. Technol. Biotechnol.*, 2013, **88**, 751–773.
10 J. H. Bruins, *PhD thesis*, Delft University, 2016.
11 Y. Cai, D. Li, Y. Liang, H. Zeng and J. Zhang, Operational parameters required for the start-up process of a biofilter to remove Fe, Mn, and NH_3-N from low-temperature groundwater, *Desalin. Water Treat.*, 2016, **57**, 3588–3596.
12 L. Yang, X. Li, Z. Chu, Y. Ren and J. Zhang, Distribution and genetic diversity of the microorganisms in the biofilter for the simultaneous removal of arsenic, iron and manganese from simulated groundwater, *Bioresour. Technol.*, 2014, **156**, 384–388.
13 Q. Cheng, L. Nengzi, L. Bao, Y. Huang, S. Liu, X. Cheng, B. Li and J. Zhang, Distribution and genetic diversity of microbial populations in the pilot-scale biofilter for simultaneous removal of ammonia, iron and manganese from real groundwater, *Chemosphere*, 2017, **182**, 450–457.
14 F. Subari, M. A. Kamaruzzaman, S. R. Sheikh Abdullah, H. A. Hasan and A. R. Othman, Simultaneous removal of ammonium and manganese in slow sand biofilter (SSB) by naturally grown bacteria from lake water and its diverse microbial community, *J. Environ. Chem. Eng.*, 2018, **6**, 6351–6358.
15 M. S. Burger, C. A. Krentz, S. S. Mercer and G. A. Gagnon, Manganese removal and occurrence of manganese oxidizing bacteria in full-scale biofilters, *J. Water Supply: Res. Technol.–AQUA*, 2008, **57**, 351–359.
16 I. A. Katsoyiannis and A. I. Zouboulis, Biological treatment of Mn(II) and Fe(II) containing groundwater: kinetic considerations and product characterization, *Water Res.*, 2004, **38**, 1922–1932.
17 X. Li, Z. Chu, Y. Liu, M. Zhu, L. Yang and J. Zhang, Molecular characterization of microbial populations in full-scale biofilters treating iron, manganese and ammonia containing groundwater in Harbin, China, *Bioresour. Technol.*, 2013, **147**, 234–239.
18 P. Mouchet, From Conventional to Biological Removal of Iron and Manganese in France, *J. - Am. Water Works Assoc.*, 1992, **84**, 158–167.
19 V. Pacini, A. M. Ingallinella, R. Vidoni, G. Sanguinetti and R. Fernández, Transformation of an existing physicochemical plant for iron and manganese removal by the application of

biological processes, *J. Water Supply: Res. Technol.–AQUA*, 2014, **63**, 507–517.
20 V. A. Pacini, A. Ingallinella and G. Sanguinetti, Removal of iron and manganese using biological roughing up flow filtration technology, *Water Res.*, 2005, **39**, 4463–4475.
21 A. G. Tekerlekopoulou, I. A. Vasiliadou and D. V. Vayenas, Biological manganese removal from potable water using trickling filters, *Biochem. Eng. J.*, 2008, **38**, 292–301.
22 J. Vandenabeele, D. de Beer, R. Germonpré and W. Verstraete, Manganese oxidation by microbial consortia from sand filters, *Microb. Ecol.*, 1992, **24**, 91–108.
23 H. Zeng, C. Yin, J. Zhang and D. Li, Start-Up of a Biofilter in a Full-Scale Groundwater Treatment Plant for Iron and Manganese Removal, *Int. J. Environ. Res. Public Health*, 2019, **16**, 698.
24 M. S. Burger, S. S. Mercer, G. D. Shupe and G. A. Gagnon, Manganese removal during bench-scale biofiltration, *Water Res.*, 2008, **42**, 4733–4742.
25 L. S. Beukes and S. Schmidt, Isolation and characterization of a manganese-oxidizing bacterium from a biofiltration system for the treatment of borehole water in KwaZulu-Natal (South Africa), *Eng. Life Sci.*, 2012, **12**, 544–552.
26 A. Vázquez and J. B. Fein, Thermodynamic modeling of Mn(II) adsorption onto manganese oxidizing bacteria, *Chem. Geol.*, 2017, **464**, 147–154.
27 A. M. Elhadidy, M. I. Van Dyke, F. Chen, S. Peldszus and P. M. Huck, Development and application of an improved protocol to characterize biofilms in biologically active drinking water filters, *Environ. Sci.: Water Res. Technol.*, 2017, **3**, 249–261.
28 Y.-Q. Liu, Y. Liu and J.-H. Tay, The effects of extracellular polymeric substances on the formation and stability of biogranules, *Appl. Microbiol. Biotechnol.*, 2004, **65**, 143–148.
29 K. P. McKee, C. C. Vance and R. Karthikeyan, Biological manganese oxidation by *Pseudomonas putida* in trickling filters, *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.*, 2016, **51**, 523–535.
30 I. Saratovsky, P. G. Wightman, P. A. Pastén, J.-F. Gaillard and K. R. Poeppelmeier, Manganese Oxides: Parallels between Abiotic and Biotic Structures, *J. Am. Chem. Soc.*, 2006, **128**, 11188–11198.
31 B. M. Tebo, H. A. Johnson, J. K. McCarthy and A. S. Templeton, Geomicrobiology of manganese(II) oxidation, *Trends Microbiol.*, 2005, **13**, 421–428.
32 B. M. Tebo, J. R. Bargar, B. G. Clement, G. J. Dick, K. J. Murray, D. Parker, R. Verity and S. M. Webb, BIOGENIC MANGANESE OXIDES: Properties and Mechanisms of Formation, *Annu. Rev. Earth Planet. Sci.*, 2004, **32**, 287–328.
33 T. Hennebel, B. De Gusseme, N. Boon and W. Verstraete, Biogenic metals in advanced water treatment, *Trends Biotechnol.*, 2009, **27**, 90–98.
34 C. Li, S. Wang, X. Du, X. Cheng, M. Fu, N. Hou and D. Li, Immobilization of iron- and manganese-oxidizing bacteria with a biofilm-forming bacterium for the effective removal of iron and manganese from groundwater, *Bioresour. Technol.*, 2016, **220**, 76–84.
35 A. Piazza, L. Ciancio Casalini, V. A. Pacini, G. Sanguinetti, J. Ottado and N. Gottig, Environmental Bacteria Involved in Manganese(II) Oxidation and Removal From Groundwater, *Front. Microbiol.*, 2019, **10**, 119.
36 M. G. Baker, S. V. Lalonde, K. O. Konhauser and J. M. Foght, Role of Extracellular Polymeric Substances in the Surface Chemical Reactivity of Hymenobacter aerophilus, a Psychrotolerant Bacterium, *Appl. Environ. Microbiol.*, 2010, **76**, 102–109.
37 L. Fang, X. Wei, P. Cai, Q. Huang, H. Chen, W. Liang and X. Rong, Role of extracellular polymeric substances in Cu(II) adsorption on Bacillus subtilis and Pseudomonas putida, *Bioresour. Technol.*, 2011, **102**, 1137–1141.
38 X. Wei, L. Fang, P. Cai, Q. Huang, H. Chen, W. Liang and X. Rong, Influence of extracellular polymeric substances (EPS) on Cd adsorption by bacteria, *Environ. Pollut.*, 2011, **159**, 1369–1374.
39 W.-W. Li and H.-Q. Yu, Insight into the roles of microbial extracellular polymer substances in metal biosorption, *Bioresour. Technol.*, 2014, **160**, 15–23.
40 S. Kitjanukit, K. Takamatsu and N. Okibe, Natural Attenuation of Mn(II) in Metal Refinery Wastewater: Microbial Community Structure Analysis and Isolation of a New Mn(II)-Oxidizing *Bacterium Pseudomonas* sp. SK3, *Water*, 2019, **11**, 507.
41 J. R. Bargar, B. M. Tebo, U. Bergmann, S. M. Webb, P. Glatzel, V. Q. Chiu and M. Villalobos, Biotic and abiotic products of Mn(II) oxidation by spores of the marine *Bacillus* sp. strain SG-1, *Am. Mineral.*, 2005, **90**, 143–154.
42 C. A. Francis and B. M. Tebo, cumA Multicopper Oxidase Genes from Diverse Mn(II)-Oxidizing and Non-Mn(II)-Oxidizing Pseudomonas Strains, *Appl. Environ. Microbiol.*, 2001, **67**, 4272–4278.
43 M. Villalobos, B. Toner, J. Bargar and G. Sposito, Characterization of the manganese oxide produced by *Pseudomonas putida* strain MnB1, *Geochim. Cosmochim. Acta*, 2003, **67**, 2649–2662.
44 E. I. Larsen, L. I. Sly and A. G. McEwan, Manganese(II) adsorption and oxidation by whole cells and a membrane fraction of *Pedomicrobium* sp. ACM 3067, *Arch. Microbiol.*, 1999, **171**, 257–264.
45 T. Štembal, M. Markić, F. Briški and L. Sipos, Rapid start-up of biofilters for removal of ammonium, iron and manganese from ground water, *J. Water Supply: Res. Technol.–AQUA*, 2004, **53**, 509–518.
46 T. Štembal, M. Markić, N. Ribičić, F. Briški and L. Sipos, Removal of ammonia, iron and manganese from groundwaters of northern Croatia—pilot plant studies, *Process Biochem.*, 2005, **40**, 327–335.
47 I. L. Breda, D. A. Søborg, L. Ramsay and P. Roslev, Manganese removal processes during start-up of inoculated and non-inoculated drinking water biofilters, *Water Qual. Res. J.*, 2019, **54**, 47–56.
48 I. L. Breda, L. Ramsay and P. Roslev, Manganese oxidation and bacterial diversity on different filter media coatings during the start-up of drinking water biofilters, *J. Water Supply: Res. Technol.–AQUA*, 2017, **66**, 641–650.

49 N. R. Barboza, M. M. C. A. Morais, P. S. Queiroz, S. S. Amorim, R. Guerra-Sá and V. A. Leão, High Manganese Tolerance and Biooxidation Ability of Serratia marcescens Isolated from Manganese Mine Water in Minas Gerais, Brazil, *Front. Microbiol.*, 2017, **8**, 1946.

50 N. R. Barboza, S. S. Amorim, P. A. Santos, F. D. Reis, M. M. Cordeiro, R. Guerra-Sá and V. A. Leão, Indirect Manganese Removal by *Stenotrophomonas* sp. and *Lysinibacillus* sp. Isolated from Brazilian Mine Water, *BioMed Res. Int.*, 2015, **2015**, 1–14.

51 D. Rivera, A. García, C. Coronel, R. Beltrán, C. Lucho, M. Villanueva, B. Jaramillo and G. Vázquez, in *Ciencias Biológicas y de la Salud Proceedings T-I*, ed. F. R. Trejo-Macotela, ECORFAN-México, S.C., México, Primera, 2017, pp. 21–35.

52 W. E. Krumbein and H. J. Altmann, A new method for the detection and enumeration of manganese oxidizing and reducing microorganisms, *Helgol. Wiss. Meeresunters.*, 1973, **25**, 347–356.

53 K. H. Nealson, *The Prokaryotes*, Springer New York, New York, NY, 2006, pp. 222–231.

54 G. Asgari, B. Roshani and G. Ghanizadeh, The investigation of kinetic and isotherm of fluoride adsorption onto functionalize pumice stone, *J. Hazard. Mater.*, 2012, **217–218**, 123–132.

55 S. E. Keithley and M. J. Kirisits, An improved protocol for extracting extracellular polymeric substances from granular filter media, *Water Res.*, 2018, **129**, 419–427.

56 A. Pal and A. K. Paul, Microbial extracellular polymeric substances: central elements in heavy metal bioremediation, *Indian J. Microbiol.*, 2008, **48**, 49–64.

57 T. T. More, J. S. S. Yadav, S. Yan, R. D. Tyagi and R. Y. Surampalli, Extracellular polymeric substances of bacteria and their potential environmental applications, *J. Environ. Manage.*, 2014, **144**, 1–25.

58 M. DuBois, K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith, Colorimetric Method for Determination of Sugars and Related Substances, *Anal. Chem.*, 1956, **28**, 350–356.

59 L. C. Sánchez and L. C. Corrales, Evaluación de la congelación para conservación de especies autóctonas bacterianas, *Nova*, 2005, **3**, 21–29.

60 R. Thapa Chhetri, I. Suzuki, J. Takezaki, H. Tabusa, M. Takeda and J. Koizumi, Bacterial Diversity in Biological Filtration Plant for the Removal of Iron and Manganese from Groundwater, *J. Water Environ. Nanotechnol.*, 2013, **11**, 33–47.

61 T. Pani, A. Das and J. W. Osborne, Bioremoval of zinc and manganese by bacterial biofilm: A bioreactor-based approach, *J. Photochem. Photobiol., B*, 2017, **175**, 211–218.

62 D. N. Marcus, A. Pinto, K. Anantharaman, S. A. Ruberg, E. L. Kramer, L. Raskin and G. J. Dick, Diverse manganese(II)-oxidizing bacteria are prevalent in drinking water systems, *Environ. Microbiol. Rep.*, 2017, **9**, 120–128.

63 A. Farkas, M. Dragan-Bularda, V. Muntean, D. Ciataras and S. Tigan, Microbial activity in drinking water-associated biofilms, *Open Life Sci.*, 2013, **8**, 201–214.

64 Y. Zhang, Y. Tang, Z. Qin, P. Luo, Z. Ma, M. Tan, H. Kang and Z. Huang, A novel manganese oxidizing bacterium-*Aeromonas hydrophila* strain DS02: Mn(II) oxidization and biogenic Mn oxides generation, *J. Hazard. Mater.*, 2019, **367**, 539–545.

65 J. B. Fein, Advanced biotic ligand models: Using surface complexation modeling to quantify metal bioavailability to bacteria in geologic systems, *Chem. Geol.*, 2017, **464**, 127–136.

66 W. Tang, J. Gong, L. Wu, Y. Li, M. Zhang and X. Zeng, DGGE diversity of manganese mine samples and isolation of a Lysinibacillus sp. efficient in removal of high Mn (II) concentrations, *Chemosphere*, 2016, **165**, 277–283.

67 L. F. Adams and W. C. Ghiorse, Physiology and ultrastructure of *Leptothrix discophora* SS-1, *Arch. Microbiol.*, 1986, **145**, 126–135.

68 C. A. Francis, E.-M. Co and B. M. Tebo, Enzymatic Manganese(II) Oxidation by a Marine α-Proteobacterium, *Appl. Environ. Microbiol.*, 2001, **67**, 4024–4029.

69 B. Toner, S. Fakra, M. Villalobos, T. Warwick and G. Sposito, Spatially Resolved Characterization of Biogenic Manganese Oxide Production within a Bacterial Biofilm, *Appl. Environ. Microbiol.*, 2005, **71**, 1300–1310.

70 J. M. Cerrato, J. O. Falkinham, A. M. Dietrich, W. R. Knocke, C. W. McKinney and A. Pruden, Manganese-oxidizing and -reducing microorganisms isolated from biofilms in chlorinated drinking water systems, *Water Res.*, 2010, **44**, 3935–3945.

71 S. Jiang, D.-G. Kim, J.-H. Kim and S.-O. Ko, Characterization of the Biogenic Manganese Oxides Produced by *Pseudomonas putida* strain MnB1, *Environ. Eng. Res.*, 2010, **15**, 183–190.

72 W. Tang, J. Xia, X. Zeng, L. Wu and G. Ye, Biological characteristics and oxidation mechanism of a new manganese-oxidizing bacteria FM-2, *Bio-Med. Mater. Eng.*, 2014, **24**, 703–709.

73 K. W. Mandernack, J. Post and B. M. Tebo, Manganese mineral formation by bacterial spores of the marine *Bacillus*, strain SG-1: Evidence for the direct oxidation of Mn(II) to Mn(IV), *Geochim. Cosmochim. Acta*, 1995, **59**, 4393–4408.

74 J. Zhang, L. W. Lion, Y. M. Nelson, M. L. Shuler and W. C. Ghiorse, Kinetics of Mn(II) oxidation by *Leptothrix discophora* SS1, *Geochim. Cosmochim. Acta*, 2002, **66**, 773–781.

75 P. Markin, *Master thesis*, University of Waterloo, 2018.

76 G.-P. Sheng, H.-Q. Yu and X.-Y. Li, Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review, *Biotechnol. Adv.*, 2010, **28**, 882–894.