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Start-up of bench-scale biofilters for manganese removal under tropical conditions: a comparative study using virgin pumice, silica sand, and anthracite filter media

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Biofiltration for Mn removal has not been proven in the tropics (18–29 °C). Also, the use of pumice as an alternative filter medium for Mn removal is still poorly known. In this study, a bench-scale biofiltration experiment, using virgin pumice, silica sand, and anthracite, was conducted for 107 days using tropical groundwater at 22 °C. Characterization of manganese oxide (MnO_x) on filter media was carried out by Raman spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM). The ability of culturable bacteria to oxidize Mn was verified by the leucoberbelin blue dye assay (LBB). The microbial activity on filter media was studied by ATP analyses. Identification of the MOB was performed by 16S rRNA sequencing. Results showed that the ripening time in each column was similar (~80 days); therefore, the filter media and water temperature do not seem to accelerate the start-up period. The MnO_x present on all ripened media was of the birnessite type. The MnO_x morphology, influent water parameters, and microbiological activity suggest a start-up period likely assisted by biological oxidation. Therefore, biofiltration for manganese removal is feasible under tropical conditions. The similarity in the performance of pumice with the other media confirmed its suitability for biotic Mn removal. All materials presented similar known MOB at the genus level; however, different closest related species colonized selectively the filter media. Two strains of *Pseudoxanthomonas* sp., a not recognized genus on matured biofilters, look promising as inoculums in pumice and sand. Proper application of biofiltration in the tropics needs operational and bioaugmentation strategies.

Water impact

Little is known about biofiltration for Mn removal under tropical conditions and the use of pumice as an alternative filter medium. This study demonstrated that this technology is feasible in the tropics and that pumice stone is a potential material for Mn biofiltration. These findings can help to develop the interest in this technology in the tropics, instead of physicochemical ones.

1. Introduction

Biofiltration systems based on aeration followed by rapid filtration are commonly used in Europe, North America, and Argentina for the removal of various types of contaminants including iron and manganese from groundwater.[1,2] Compared to physicochemical systems, they are considered a cost-effective option as they are easy to operate and do not require chemical reagents or commercial adsorbents.[3] Despite this, there is still little experience in the application of this technology in tropical regions.[4,5]

One of the main drawbacks of manganese removal by biofiltration, during groundwater treatment, is the long ripening times of the biofilters. During the start-up period, the virgin filter medium provides the necessary surface for the attachment of the manganese oxidizing bacteria (MOB) present in the raw water, favoring the biological oxidation of Mn.[6] Specifically, MOB remove Mn by intracellular oxidation (enzymatic action), by extracellular adsorption, or by catalysis under the influence of the biopolymers secreted by the bacteria.[7] In addition, the manganese oxides (MnO_x), formed

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by these processes, gradually cover the filter medium, contributing to the removal of Mn through auto-catalytic adsorption and subsequent oxidation of adsorbed Mn by biological and/or physicochemical means.[8,9] At the end of the start-up period, efficient and stable removal of Mn is expected.[10] The length of this period is potentially influenced by the type and amount of manganese oxide coated in the filter medium,[11] the quality of the raw water, and some operational or design aspects such as the volumetric loading of iron, the backwashing strategy, and the type of filter medium.[8,12]

The attachment of bacteria to the filter medium is a physicochemical process that can be influenced by the texture, the properties of the surface, the accessible areas of the support medium,[13] and the water temperature.[14] Microbial colonization would increase as the surface roughness of the filter media increases, as shear forces decrease and the surface area increases.[14] However, this bacteria–filter medium interaction is complex since the precipitates that are deposited on the surface of the virgin media will gradually change the physicochemical properties of the surface of the filter medium. In addition, smaller aggregate sizes can provide greater surface area per unit volume which favors the attachment of bacteria.[15]

Biofiltration has been shown to be effective for Mn removal using various filter media such as silica sand,[16] quartz sand[17] anthracite, and GAC[18] and some other alternative media like polypropylene,[19] lava media,[20] and polystyrene beads,[21] among others. Sand and anthracite are the most used, even though they do not exhibit great Mn adsorption capacities,[17] as they have low costs and are commonly available. GAC can contain three to eight times more biomass than sand and anthracite, mainly due to its large surface area.[13] However, its main disadvantage is investment costs.[5] Additionally, Breda *et al.*[22] showed that, in specific source water, different filter materials influenced not only the start-up period but also the bacterial community formed, suggesting that the latter can be managed using specific media for a specific type of water. Therefore, it is interesting to evaluate different filter media. One of them is pumice, whose use in rapid filters in both drinking water and wastewater treatment is increasing.[23] Pumice is a low-cost, porous, low-density medium, which, according to Farizoglu *et al.*,[24] produces less pressure drop than sand and has a higher specific surface area for stimulating bacterial growth. However, to our knowledge, no previous studies have evaluated the use of pumice in Mn removing biofilters, either at a lab or pilot scale.

Generally, low environmental temperatures (around 7–17 °C) decrease the performance of biofilters.[25,26] Moreover, slower ripening has been reported at water temperatures below 15 °C.[27] Pacini *et al.*[2] identified a decrease in bacterial growth in sand filters, specifically during the winter period (~8–18 °C). Ciancio *et al.*[25] confirmed at a laboratory scale that bacterial diversity in sand filters for Mn removal was influenced by temperature changes and that inoculated sand columns showed the best Mn removal performance under summer conditions (~18–29 °C). Cai *et al.*[26] confirmed that functional oxidizing bacteria could be acclimated in low-temperature groundwater (3–4 °C); however, a long start-up period was required (~240 days) due to the slow growth rate of bacteria. In contrast, an increase in water temperature could benefit the bacteria attachment[14] and reduce the negative effects of low temperature mentioned before. Therefore, it would be expected that a warm climate and stable temperature during the year would benefit the application of biofiltration in the tropics.

Recently, Calderón *et al.*[4] reported autochthonous tropical groundwater MOB at the *in vitro* level and their capacity to form biofilms on pumice, suggesting the potential use of biofiltration for Mn removal. However, this technology has not been proven in the tropics and developing countries that mainly work with chemical treatment for manganese removal. Additionally, it is important to evaluate the use of pumice compared with traditional filter media. Hence, the aim of this study was first to investigate, at the bench scale, the startup period of non-bioaugmented biofilters, for manganese removal under tropical groundwater conditions, and second, to evaluate the use of virgin pumice as an alternative filter medium for Mn removal. Therefore, a comparative study at 22.21 ± 1.14 °C using virgin pumice, silica sand, and anthracite filter media was performed at the bench scale to compare the acclimation on each material. Further, characterization of manganese oxides on the ripened filter medium coating and the presence and growth of culturable MOB were evaluated to identify the start-up mechanism of the manganese removal process at these water temperatures. Moreover, the possible influence of the filter material on the bacterial community was also studied and the potential species to inoculate the respective media were identified.

2. Materials and methods

2.1 Water source used during the experiment

The raw water was taken from a well that supplies 550 people at the La Hacienda Condominium, Cartago, Costa Rica (9°50′28″N83°58′26″W). The groundwater was collected twice a week in 60-liter plastic containers. During the transfer, the water was saturated with oxygen, generating some expected changes as shown in Table 1. The increase of the dissolved oxygen concentration (DO) promotes changes in the oxidation–reduction potential (ORP) and can remove carbon dioxide contained in the groundwater increasing the pH.[28]

2.2 Experimental set up and monitoring

The experimental unit consisted of a feed tank in which the aerated water from the well was stored (Fig. 1). Subsequently, the water was pumped to an elevated tank with a constant water level for distribution by gravity to each of six plastic columns packed, in duplicate, with three types of virgin filter media, previously washed and oven-dried at 110 ± 5 °C, according to the Standard Method C117-17.[29] Each column was 2.8 cm in diameter and had a media height of 10.0 cm.

Table 1 Quality of groundwater and aerated water used in the experiment

Parameter	Unit	Raw water (n = 15)	Laboratory influent (n = 53)
Mn	mg L^{-1}	0.57[a] ± 0.04[b]	0.57 ± 0.06
Fe	mg L^{-1}	0.32 ± 0.15	<0.03[c]
pH	—	7.03 ± 0.11	7.83 ± 0.40
T	°C	22.80 ± 0.48	22.21 ± 1.14
ORP	mV	−35.15 ± 22.50	201.1 ± 55.84
DO	mg L^{-1}	0.42 ± 0.11	6.37 ± 0.95
DO	%	5.46 ± 1.40	87.17 ± 12.33

[a] Average. [b] Standard deviation. [c] Detection limit.

This height was adopted because it has been demonstrated to be appropriate in studying the biological activity and the effectiveness of biofilters for Mn removal at the bench scale.[18,22] The flow rate in each column was 5 mL min^{-1} and was distributed with a contact time of approximately 12 minutes and at a velocity of 0.75 cm min^{-1}.

The physical properties of each of the filter media are presented in Table 2. The BET surface area was determined by nitrogen adsorption at 77 K (Gemini VII 2390p analyzer, Micromeritics). Sieve tests were performed following the Standard Method C136/C136M.[30] The specific gravity was measured using the Standard Method C128-15.[31] The bulk density and medium porosity (voids between particles) were obtained by the Standard Method C29/C29M-17a.[32] In addition, these parameters were validated using the method described by Happiness.[33] Finally, the average particle size was measured by scanning electron microscopy (SEM TM-3000, company Hitachi High-Tech) according to Song *et al.*[34]

The start-up period is defined as the time required for the biofilters to achieve Mn removal efficiencies greater than 90%.[12] Under these conditions and granting a few additional weeks of monitoring, the experiment was carried out in the laboratory for 107 days. Backwashing was performed with filtered water when a 20% flow rate reduction was reached, or filters became clogged. According to hydraulic tests performed during the experiment, a backwashing strategy of 30 s at a loading rate of 144 mL min^{-1} was adopted. In total, backwashing was performed on days 49, 66, 74, 81, 88 and 100 of the experiment.

During the experiment, Mn, and Fe were determined in the inlet and outlet water of the filters. The dissolved oxygen (DO), pH, and oxidation–reduction potential (ORP) were monitored in the influent three times a week. Mn and Fe measurements were done using AAnalyst 800 atomic absorption equipment (Perkin Elmer, Waltham, USA) following the Extraction/Air–Acetylene Flame Method 3111 C.[35] The detection limits for Fe and Mn were 0.10 mg L^{-1} and 0.03 mg L^{-1}, respectively. The pH, dissolved oxygen, and ORP were determined using Hach HQD30 equipment following the methods recommended by the manufacturer (Hach, USA).

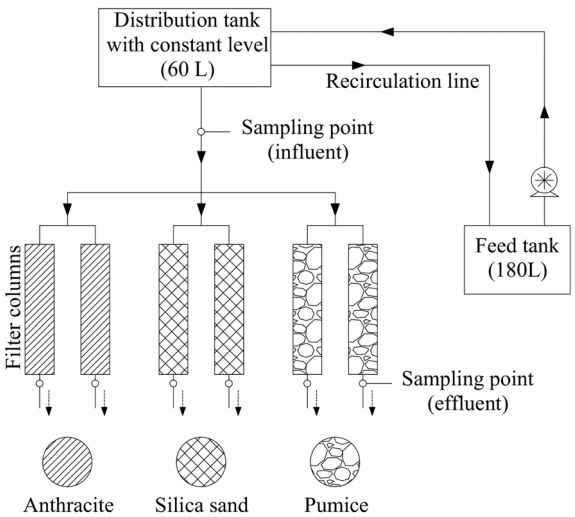

Fig. 1 Configuration of the experimental unit.

2.3 Characterization of the oxides formed (MnO_x)

At the end of the start-up period, the type of manganese oxide (MnO_x) accumulated on each biofilter was identified using Raman spectroscopy and X-ray diffraction (XRD). In addition, electron microscopy (SEM) was used to observe the type of structure of manganese oxide and to verify if the MnO_x was of biological or physicochemical origin, as explained by Bruins *et al.*[17]

2.3.1 Raman spectroscopy. An Alpha 300R spectrometer (company: Witec) was used with the following configuration: objective 100×, laser wavelength λ = 532 nm, output power ~0.8 mW, integration time 20 s and grating 1200. Before analysis, the samples were dried at room temperature to avoid fluorescence due to the presence of water as suggested by Bruins *et al.*[11]

2.3.2 X-Ray diffraction (XRD). XRD analyses were carried out to confirm whether the structure of the MnO_x present on the filter media was crystalline or amorphous. Therefore, a PANalytical diffractometer (Empyrean) was used with the following configuration: CuKα radiation: 1.5418 Å, range 6–80° 2θ and a step size of 0.013° 2θ. Samples of MnO_x deposits (without filter media grains) were collected from the top of the pumice and sand columns. Unfortunately, due to the dark color of both the anthracite grains and the MnO_x deposit, it was impossible

Table 2 Characterization of filter media

Parameter	Unit	Pumice	Anthracite	Silica sand
Bulk density	kg L^{-1}	0.37 ± 0.01	0.78 ± 0.01	1.50 ± 0.02
Particle density	kg L^{-1}	1.02[a] ± 0.03[b]	1.56 ± 0.03	2.63 ± 0.01
Medium porosity	%	84 ± 2	43 ± 2	40 ± 2
Grain size (10–90%)	mm	0.88–1.16	0.85–1.34	0.81–1.31
Uniformity coefficient	[—]	1.20	1.33	1.38
Average particle size	mm	1.03 ± 0.10	1.09 ± 0.19	1.07 ± 0.20
BET surface area	m^2 g^{-1}	2.72	0.38	0.64

[a] Average. [b] Standard deviation.

to obtain the latter in the anthracite columns. Pumice and sand deposits were dried at room temperature and pulverized before analysis.

2.3.3 Electron microscopy (SEM). SEM photographs were taken with a JSM 6390 scanning electron microscope (company: JEOL). Before the observation, a chemical fixation process was carried out to preserve the biological samples similar to the study by Zeng *et al.*[36] A Karnosky (2% glutaraldehyde, 2% paraformaldehyde in 0.5 M phosphate buffer, pH 7.2) fixative was used. The samples were preserved at 4 °C for 48 h. Afterward, each sample was washed in phosphate buffer (0.05 M, pH 7.2) for 10 min. Dehydration was carried out gradually to avoid drastic cell shrinkage using dilutions with 30%, 50%, 70%, and 100% ethanol in distilled water. Finally, before mounting, they were dried at room temperature. Images of samples with and without gold coating were tested, which were placed on aluminum bases on carbon tape for their observation.

2.4 Microbiological analysis

The biofilters were inoculated with the microorganisms present in the raw water. Identification of MOB was carried out by DNA sequencing. At the end of the start-up period, samples of the matured filter media were taken from the top (0–2 cm) and the bottom (8–10 cm) of the columns (to be referred to as "top" and "bottom", respectively) to identify the MOB present in the biofilters. In addition, heterotrophic bacterial counts (CFU g^{-1}) were performed together with adenosine triphosphate (ATP) analysis of the biofilm medium. The ability of isolated bacterial strains to oxidize Mn was verified using the leucoberbelin blue (LBB) dye assay.

2.4.1 Isolation of the MOB present in the filter media. Once the filters were ripened, 10 g of the filter media were taken from the top and the bottom of the columns. The samples were diluted in 90 mL sterile 0.1% (w/v) peptone water isotonic solution. Subsequently, the samples were stomached for 1 min. Serial dilutions were made, up to 10^{-4} and 1 mL of each dilution was plated in duplicate on modified R2A agar with 17 mg L^{-1} $MnSO_4$ and incubated for five days at room temperature (<28 °C). Subsequently, heterotrophic bacteria were counted on days 5 and 20, respectively. The 20-day count corresponded to the MOB strains determined by the LBB method (section 2.4.2).

2.4.2 Leucoberbelin blue (LBB) dye assay. After an incubation period of 20 days, two drops of the LBB reagent (0.04% w/v dissolved in 45 mM acetic acid) were added to the colonies. This qualitative method is based on the LBB oxidation reaction with Mn^{3+} or Mn^{4+} producing a blue color.[37] A strong blue tone implies the highest oxidation capacity and is assigned "++++", light tones are assigned "+++" or "++" and weak tones "+".

2.4.3 Identification of culturable MOB by 16S rRNA sequencing. Genetic material was extracted by the cetyltrimethyl ammonium bromide (CTAB) method from culturable heterotrophic bacteria positive to the LBB assay. The amplification of the region to be sequenced was performed with the universal primers of the 16S rRNA region, specifically 1492 (5′-GGTTACCTTTGTTACGACTT-3′) and 27F (5′-GAGTTTGATCMTGGCTCAG-3′). The results were confirmed by agarose gel electrophoresis (0.8%). Sequencing was performed with the polymerase chain reaction (PCR) product at a concentration of 50 ng μL^{-1} by MACROGEN (Korea), according to the Sanger method using a 3730xl genetic analyzer from Applied Biosystems. 16S rRNA amplicon data were treated as operational taxonomic units (OTUs) and considered as "closest related" species.[25] To have an insight into the most dominant related species present on each filter medium, read abundance percentages of the culturable MOB based on the OTU counts were determined using the ampvis package[38] in the statistical software R.[39]

2.4.4 Medium biofilm adenosine triphosphate (ATP) analyses. A deposit and surface analysis kit (DSA™, LuminUltra Technologies, Canada) was used for ATP measurements. According to the manufacturer's instructions, 1 gram of filter medium was transferred to 5 mL of Ultralize™7 and homogenized for two minutes. Contrary to the original method of vortex homogenization, sonication was used in a Branson 1510 ultrasonic cleaner that allowed increasing ATP concentrations by about 8% compared to the recommended method. After 5 min incubation at room temperature, 1 mL of the solution was diluted in a 9 mL UltraLute™ (dilution) tube. Finally, using 100 µL of the sample together with 100 µL of Luminance™ enzyme, the reading was taken on a PhotonMaster™ luminometer as relative light units (RLUs) which were expressed as ATP according to the manufacturer's indications.

3. Results and discussion

3.1 Start-up period of biofilters

Fig. 2 shows the average Mn removal efficiencies obtained during the bench-scale experiments. Throughout the start-up period, a similar trend in removal efficiencies was observed in each of the filter media. In the first two weeks, the Mn removal efficiencies were less than 10%. This was expected due to the small adsorption capacity of the virgin materials (*e.g.*, the pumice used in this experiment showed 0.3 mg g^{-1} in isotherm data); similar behavior was reported by Bruins *et al.*[8] in sand and manganese oxide-coated sand (MOCS). The increase in removal efficiencies was notable from the third week, reaching values greater than 50% after 60 days (~week 8). Finally, the start-up period was completed in the silica sand and pumice stone columns on day 79 and in the anthracite column on day 84. It is known that the ripening process for Mn removal typically takes between 1 and 4 months, or even more on non-bioaugmented biofilters.[8,9,36] Our results were in this range and there was a minimal difference between the filter media, which indicates that the pumice behaved similarly to the other materials. Furthermore, the possible advantages of pumice, such as greater surface area and roughness, were not decisive, probably because effective MOB did not grow in all the available surfaces due to the competition with other heterotrophic bacteria present in the filter media (section 3.4). Therefore, other parameters such as water quality and filtration velocity might have been more determining factors for ripening.

During the ripening time of the biofilters, in principle, there were favorable conditions for the biological oxidation of Mn. Mouchet[7] has mentioned that to oxidize Mn, MOB require pH > 7.5, dissolved oxygen (DO) > 5 mg L^{-1}, and a redox potential (Eh) of about 300 and 400 mV. As can be seen in Fig. 3, most of the time, the experimental conditions of pH and Eh were within this recommended field of action. As shown in Table 1, the DO, pH, and ORP in the laboratory influent were 6.37 ± 0.95 mg L^{-1}, 7.83 ± 0.40, and 201.1 ± 55.84 mV, respectively. Similarly, other studies in temperate zones have reported, at the bench scale[18,22] and pilot scale,[40]

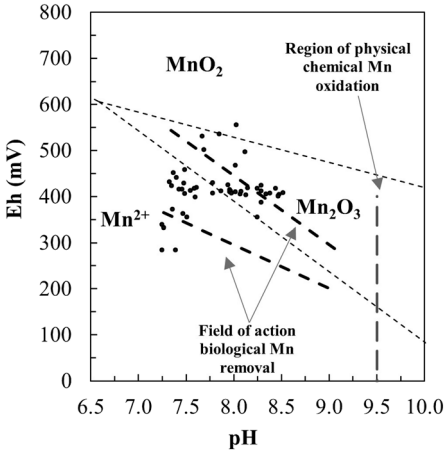

Fig. 3 Field of action of the MOB in the pH–Eh diagram. Adapted from Mouchet.[7]

effective Mn removal within the DO, pH, and Eh ranges suggested by Mouchet.[7]

On the other hand, a reduction in the obtained ripening times would have been expected, as the water temperature was around 22.21 ± 1.14 °C. According to Madigan *et al.*,[41] the optimal growth temperature of mesophilic bacteria, which determines the maximum metabolic capacity, is in the range of 20 to 45 °C. In contrast, low-temperature groundwater (3–18 °C) affects the growth rate of functional oxidizing bacteria and subsequently, the start-up period of biofilters.[26] Moreover, acclimation of new biofilters at surface water temperature below 15 °C has been reported to be slower than that at higher temperature.[27]

Similar to the study by Ramsay *et al.*,[42] inherent inoculation with native MOB, even from the tropics, seemed to be insufficient to achieve a rapid start-up period during the experiments. The filter media and water temperature evaluated in this study did not substantially accelerate the start-up of biofilters. Therefore, similar to temperate zones, the implementation of biofiltration in the tropics should consider bioaugmentation strategies to speed up the ripening

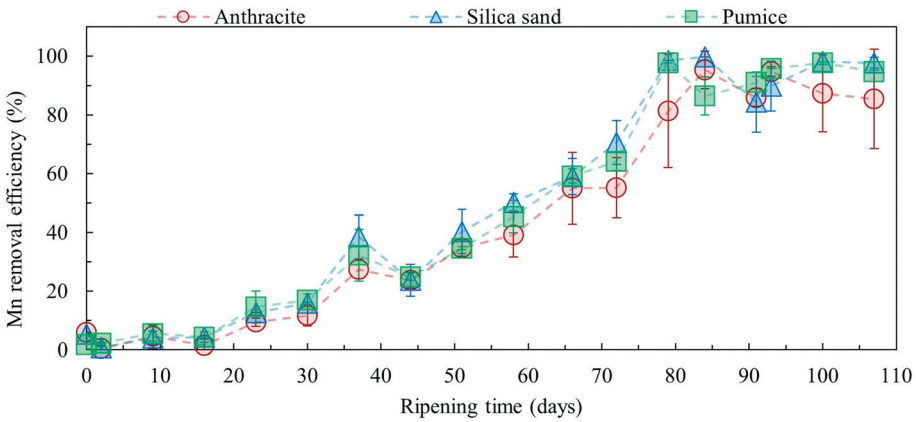

Fig. 2 Mn^{2+} removal efficiencies in anthracite, silica sand, and pumice stone columns (error bars represent standard deviations).

period. Additionally, an increase in flow velocity or nutrient concentration may also equate to increased bacteria attachment to a more rapid association of cells with the surface filter media.[14]

3.2 Characterization of MnO_x

The Raman spectra of the MnO_x deposits obtained in the filter media are shown in Fig. 4. It is observed in all cases that the principal peaks follow a similar pattern between 497–507, 555–560, and 625–655 cm^{-1}, which means that the spectra obtained in each of the materials were similar. The mentioned pattern is similar to the birnessite spectrum reported by Bruins *et al.*[11] in samples of aged manganese oxide-coated sand (MOCS) and manganese oxide-coated anthracite (MOCA), obtained from full-scale groundwater biofilters. The XRD patterns obtained from the MnO_x samples (Fig. 5) show wide and low-intensity peaks, which confirms their amorphous character.[10] In addition, the three peaks at 26.8° 2θ, 36.9° 2θ and 66.2° 2θ in the XRD patterns coincide with the patterns reported by Cheng *et al.*[43] associated with birnessite. In temperate zones, such a type of MnO_x has been reported as responsible for the auto-catalytic action in ripened rapid filters.[11] No XRD spectrum was obtained for MnO_x deposits in ripened anthracite because it was not possible to separate them from the similar dark filter medium.

The morphology of the surface of the materials is shown in Fig. 6. In the virgin pumice (Fig. 6.A1 and A2) a porous skeleton structure is observed, related to an increase in the surface area compared to the virgin anthracite and virgin silica sand (Table 2). The virgin anthracite and the virgin silica sand (Fig. 6.B1, B2, C1 and C2, respectively) are similar to plate structures, with a predominance of smooth regions in the virgin anthracite and a denser, rougher surface with more shallow depressions for the case of the virgin silica sand.

On the other hand, the SEM micrographs of the MnO_x, obtained from the samples of ripened pumice, anthracite and silica sand media (Fig. 6.A3/B3/C3) show great similarities among them. In all cases, a poor crystalline

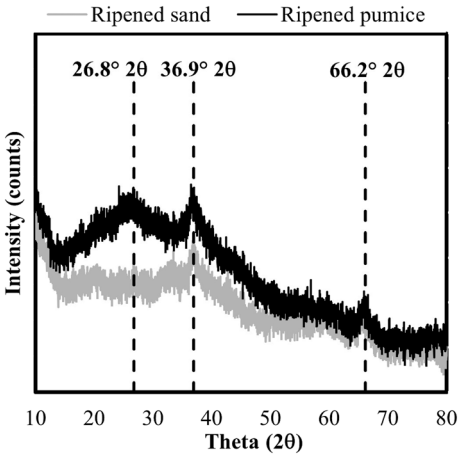

Fig. 5 XRD patterns of MnO_x deposits obtained on the surface of ripened silica sand and ripened pumice.

structure was observed (which confirms the XRD analysis results), with abundant agglomerated structures that form a rough surface with a porous structure which should be favorable for bacterial attachment.[14] Such structure characteristics suggest that probably, in all the materials, the same type of Mn oxide was formed. Furthermore, the same SEM micrographs showed the presence of fluffy plate structures, typical for birnessite of biological origin, as reported by Bruins *et al.*[17] during the ripening of virgin sand biofilters at the pilot scale. Accordingly, during the start-up period, this mineral exhibits surface characteristics of biological origin (*e.g.* fluffy plates) and, over time, birnessite of physical–chemical origin takes place.[17] Probably, the manganese oxides, present on all ripened media of this study, were birnessite of biological origin as reported by Bruins *et al.*[17] in temperate regions with water at a typical temperature of 10–12 °C. Further results in section 3.3 and 3.4 support the possible manganese removal by a biological mechanism that will be summarized in section 3.5.

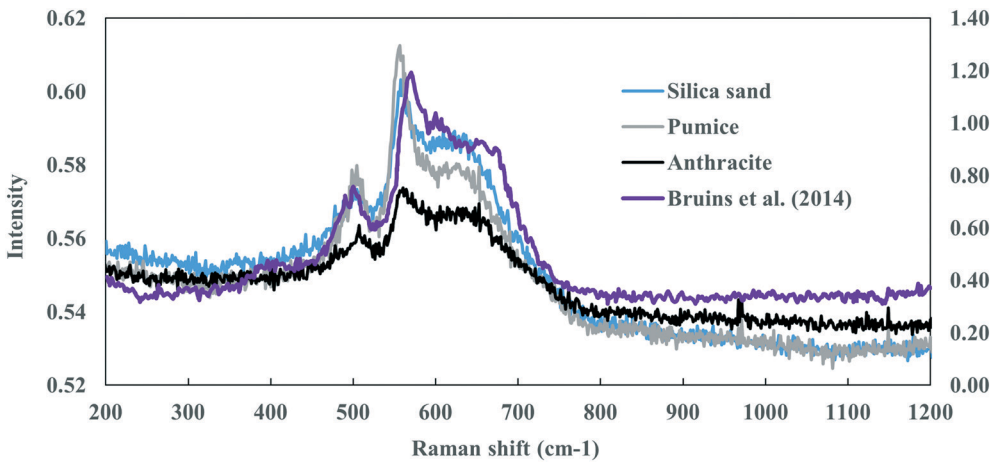

Fig. 4 Raman spectra (at 532 nm) of MnO_x deposits obtained in the ripened materials at 123 days compared to the birnessite reference by Bruins *et al.*[11]

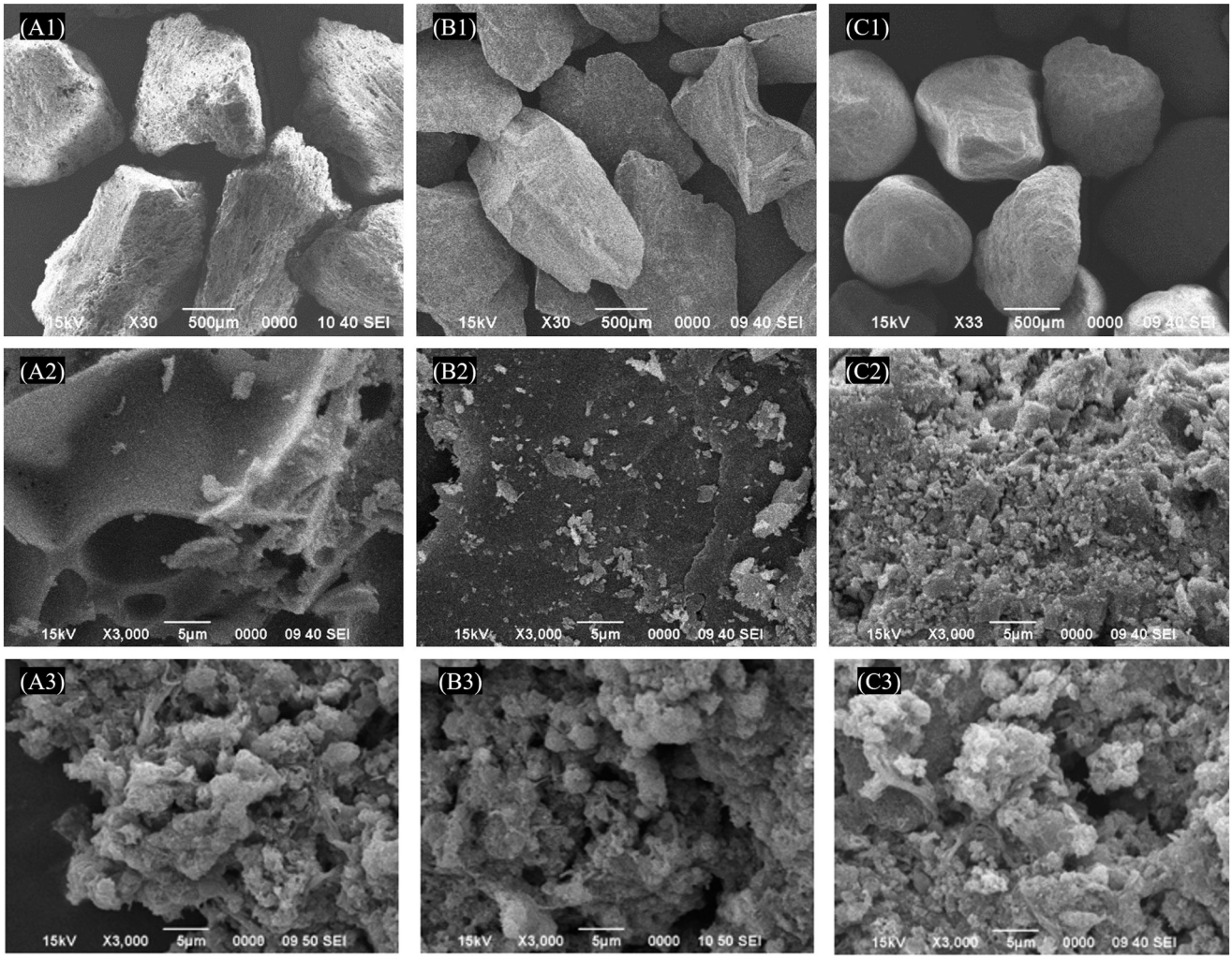

Fig. 6 Scanning electron microscopy (SEM) images with a magnification of 30× and 3000× of (A) pumice stone (B) anthracite, and (C) silica sand.

3.3 Characterization of culturable MOB

Although there is a great variety of MOB that include phyla such as *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria*,[37] still little is known about the diversity of MOB present in groundwater in tropical countries.[44] In the present study, the culturable MOB detected in the water samples belong to the first two phyla and *Proteobacteria* (genus *Stenotrophomonas* and *Aeromonas*) (Table 3). In contrast, isolates on the ripened filter media were taxonomically diverse with a clear dominance of the *Proteobacteria* phylum (*Alpha-* and *Gammaproteobacteria*), as also reported in similar studies[22,37,45] in temperate climates.

Culturable MOB detected in the raw water samples were of the genus: *Stenotrophomonas*, *Aeromonas*, *Microbacterium*, *Cellulosimicrobium*, and *Staphylococcus* (Table 3). The first three genera were already reported by Calderón *et al.*[4] in the same well water of the present research. Those three genera have also been reported elsewhere: *Stenotrophomonas* in Brazilian mine water,[44] *Aeromonas* in simultaneous removal of ammonium and manganese at the pilot scale in slow sand filters,[52] and *Microbacterium* in a water reservoir used for drinking water.[50] *Cellulosimicrobium* sp. is associated with the reduction of Cr(VI) and Cr(III).[53] To the authors' knowledge, there have not been reports of this species as MOB. According to Das *et al.*,[54] *Staphylococcus* has been identified as a MOB in seawater, and no reports in biofiltration were found.

The bacteria found in all the ripened media include the genera *Pseudomonas*, *Sphingopyxis*, and *Pseudoxanthomonas*. Additionally, *Sphingomonas* spp. was observed only in pumice. Compared with similar studies in temperate regions, Breda *et al.*[22] reported the dominance of *Nitrospira* in matured quartz (18%), *Novosphingobium* in virgin quartz (35.2%) and calcium carbonate (20.3%), *Sulfuritalea* in polystyrene (83.4%) and *Pseudomonas* in manganese oxide (38.7%). They also detected *Pseudomonas* at 8.2% in mature quartz and less than 1% in virgin quartz and calcium carbonate. Other studies have reported *Pseudomonas* as a MOB in quartz sand biofilters.[48,55] *Sphingopyxis* was reported by Breda *et al.*[22] in all the filter materials mentioned at a very low percentage (<3%). Piazza *et al.*[37] detected this genus in

Table 3 MOB isolated from raw water and filter media (influent water temperature 22.21 ± 1.14 °C)

Phylum (class)	Genus	Closest related species	Accession number	Similarity (%)	Mn oxidation activity (LBB test)	Reported genus with known MOB
Proteobacteria (*Alphaproteobacteria*)	*Sphingopyxis*	*Sphingopyxis soli*	NR-116739.1	97.30	+ +	22
		Sphingopyxis macrogoltabida	AB675377.1	97.40	+ + + +	
	Sphingomonas	*Sphingomonas koreensis*	NR-113868.1	95.50	+ + +	46
		Sphingomonas colocasiae	NR-159304.1	97.30	+ + +	
Proteobacteria (*Gammaproteobacteria*)	*Pseudoxanthomonas*	*Pseudoxanthomonas mexicana*	KF501482.1	98.79	+ + + +	47
		Pseudoxanthomonas japonensis	NR-113972.1	97.00	+ + + +	
	Pseudomonas	*Pseudomonas stutzeri*	NR-103934-2	80.00	+ + + +	48
		Pseudomonas alcaligenes	NR-113646	97.60	+ + +	
		Pseudomonas aeruginosa	NR-117678	97.00	+ + +	
			LR130537.1	98.69	+ + + +	
		Pseudomonas oleovorans	LC507444.1	98.70	+	
		Pseudomonas entomophila	CP034337.1	98.93	+ + +	
	Stenotrophomonas[a]	*Stenotrophomonas maltophilia*	LR134301.1	99.22	+ + +	4, 44
			CP029773.1	99.59	+ + +	
	Aeromonas[a]	*Aeromonas veronii*	MF111930.1	98.92	+	49
			MF111973.1	98.59	+ +	
Actinobacteria	*Microbacterium*[a]	*Microbacterium* sp.	KX390640.1	98.70	+ + + +	50
	Cellulosimicrobium[a]	*Cellulosimicrobium cellulans*	HG000003.1	97.78	+	Not reported
		Cellulosimicrobium sp.	MN536509.1	98.89	+ + +	
Firmicutes (*bacilli*)	*Staphylococcus*[a]	*Staphylococcus warneri*	MK256311.1	87.17	+ + + +	[51]
			KX454005.1	97.72	+ +	
		Staphylococcus cohnii	MF111620.1	97.00	+ +	

[a] Genera found exclusively in water samples.

the gravel prefilter and the sand filter in two drinking water treatment plants in Argentina. Cai *et al.*[46] reported the presence of *Sphingomonas* sp. in bench-scale gravity quartz sand biofilters inoculated with backwashing sludge containing MOB. With regard to *Pseudoxanthomonas* no reports in the literature were found, and further discussion about this genus species is presented later.

Breda *et al.*[22] found different bacterial compositions between the source water and the biofilm developed in virgin quartz, calcium carbonate, polystyrene and manganese oxide biofilters. The authors suggested that different materials influenced the bacterial composition during the ripening period when using the same raw water. However, in the experiment reported here between the evaluated materials only pumice showed a different genus, *Sphingomonas*. In the present experiment, the characterization of the MOB aimed to detect possible species for future inoculation; therefore, only culturable MOB were analyzed. Knowing that, in an attempt to detect further influence or preference of the bacteria for the type of filter medium, culturable MOB read abundance based on the OTU counts in each ripened filter medium was determined, and the main results are presented next.

Fig. 7 compares the read abundance percentage of isolated MOB in each ripened filter medium obtained from rRNA amplicon data treated as operational taxonomic units (OTUs, section 2.4.3). Some differences were observed in the diversity of species identified in the ripened pumice compared to those in the ripened anthracite and silica sand. Clearly, pumice stone showed a higher MOB species diversity, probably due to its porous structure and higher surface area than anthracite and sand (Table 2). On the other hand, *Sphingopyxis soli* and *Pseudoxanthomonas mexicana* were exclusively found on ripened anthracite and silica sand.

As shown in Fig. 7, *Pseudoxanthomonas japonensis* was one of the most abundant closest related species capable of colonizing in the three-filter media, being the most abundant in pumice (42.9%) and also important in anthracite (30%). The *Pseudoxanthomonas mexicana* was also the most abundant closest related species in silica sand (36.4%). To our knowledge, the *Pseudoxanthomonas* sp. is not commonly related to Mn oxidation on matured biofilter media. This genus was reported as a MOB in the biocorrosion of carbon steel inside the pipelines of sewage treatment plants.[56] However, in this study under tropical conditions, *Pseudoxanthomonas* sp. seems to play an important role in manganese removal by biofiltration and should be considered as a candidate inoculum for sand and pumice medium biofilters. *Pseudomonas aeruginosa* was also detected abundantly in all filter media (Fig. 7) making it interesting as an inoculum. It was reported as an excellent model microorganism for the study of biofilms.[57] Yang *et al.*[55] reported this closest related species in an inoculated pilot quartz sand filter for the simultaneous removal of arsenic, iron, and manganese from groundwater. About the other

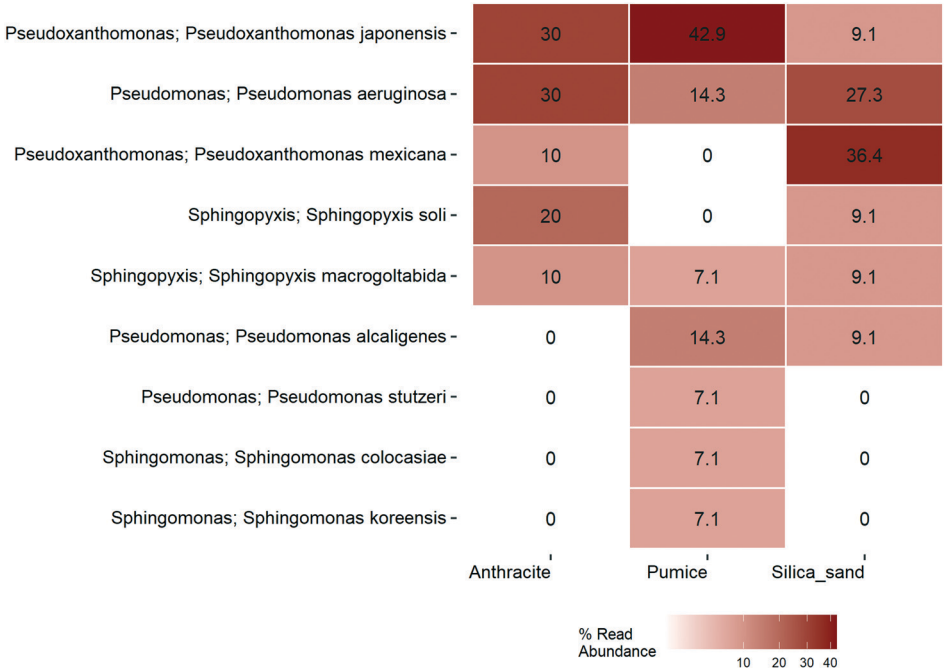

Fig. 7 Read abundance percentage of the culturable MOB based on the OTU counts in each ripened filter medium.

species, they do not seem to be significantly different from those reported in other studies under temperate conditions.

Even though the different filter materials presented no significant difference in the ripening period (section 3.1), similar to the results of Breda *et al.*,[22] they influence the bacterial community developed. Differences in the closest related species on each filter medium were caused probably by the biofilm formation capacity and the ability to attach to the surface media. Bacteria that grow more rapidly colonize first and establish a long-term competitive advantage with the others.[14]

3.4 Microbiological activity

Heterotrophic plate counts confirm the presence of heterotrophic bacteria in similar amounts in each of the filter media. The total of heterotrophic bacteria on the top of the biofilters was in the order of 10^7 CFU g^{-1} but only 10^3 CFU g^{-1} corresponded to culturable heterotrophic MOB (positive to the LBB test). Regarding the bottom of the biofilters, the plate counts were around 10^5 CFU g^{-1} and no strain positive to the LBB test was detected. Also, ATP concentrations, at the top of the biofilters, were around 0.49 ± 0.03 μg g^{-1}, 0.28 ± 0.06 μg g^{-1} and 0.62 ± 0.15 μg g^{-1} for ripened anthracite, ripened sand, and ripened pumice, respectively (Fig. 8). It was found that the ATP values were predominant in the upper part of the columns, which is consistent with the results obtained during the plate counts. Granger *et al.*[18] reported, in bench-scale bioaugmented biofilters with average water temperatures of around 19.2 ± 0.768 °C, similar concentrations of heterotrophic bacteria and MOB of around 10^6–10^7 CFU g^{-1} and 10^2–10^3 CFU g^{-1}, respectively, and ATP measurements in the range of 0.13–0.32 μg g^{-1} on anthracite and 0.28–1.39 μg g^{-1} on GAC media.

3.5 Biological manganese removal process

Several aspects support that Mn was likely removed by biological activity. First, the influent water pH, DO, and ORP were most of the time in the ranges suggested by Mouchet.[7] Moreover, chemical oxidation was not expected as oxygen was the only oxidant present, and Mn oxidation at pH lower than 9 was very slow.[18] Secondly, the MnO_x deposits formed were similar to the birnessite of biological origin previously reported by Bruins *et al.*[17] Thirdly, four genera and nine closest related species capable of oxidizing Mn were detected

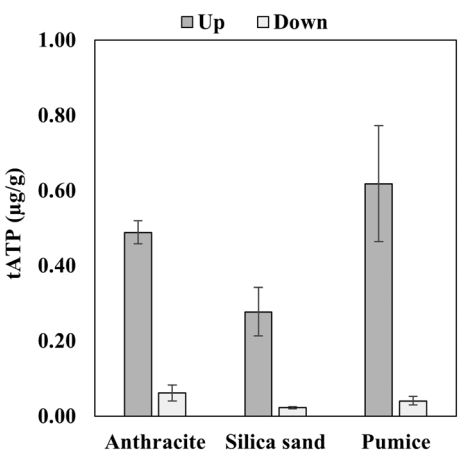

Fig. 8 Concentrations of ATP obtained from filter medium samples (error bars represent standard deviations).

in the filter media. Lastly, the ATP and levels of culturable heterotrophic MOB are in the same range as those in previous studies.[18] Interestingly, all of the previous findings are supported by studies in temperate regions, suggesting similar mechanisms for tropical regions at around 22 °C.

4. Conclusions

Biofiltration for manganese removal is commonly used in temperate regions; however, there are few reports in the tropics. The present research aimed to study the start-up of non-bioaugmented bench-scale biofilters for manganese removal under tropical, Costa Rican, groundwater conditions and to evaluate the use of pumice as an alternative filter medium. Results, with a groundwater temperature of 22.21 ± 1.14 °C, demonstrated that biological removal of Mn with non-bioaugmented biofilters is feasible in the tropics, showing similar start-up periods to reported ones in temperate zones, and consequently the temperature effect was minimum. Pumice stone performed similarly to sand and anthracite, proving that pumice is a potential material for biological Mn removal. These main results indicate good opportunities for biofiltration in the tropics with any of the materials tested.

Key findings include the following:

- There were only small differences in Mn removal efficiencies between the anthracite, silica sand, and pumice during the ripening period of about 80 days.
- The biological removal mechanism in the experiment was supported by the influent water quality characteristics (pH, ORP and DO), the formation of MnO_x similar to birnessite of biological origin, the presence of recognized MOB, and the level of ATP and culturable heterotrophic MOB.
- At the genus level, the bacterial community was not significantly influenced by the filter material, only pumice was specifically influenced by *Sphingomonas* spp. However, different closest related species colonized selectively the filter media. Therefore, a suitable inoculum could be developed depending on the filter media.
- *Pseudoxanthomonas* sp., not reported in Mn biofiltration before, showed high Mn oxidation activities and was abundant in the three media, being the most important one in pumice and silica sand. Therefore, these strains are good candidates for inoculums in these media.

As a final remark, water supply companies in tropical and developing countries that are planning to implement biological treatment technology for Mn removal in groundwater should consider operational (*e.g.*, filtration velocity) and bioaugmentation strategies to speed up the ripening period of the biofilters.

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Conflicts of interest

There are no conflicts to declare.

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