Environmental Science
Water Research & Technology

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DOI: 10.1039/D0EW00777C

Manuscript title: Optimization of bacterial bioaugmentation for groundwater Mn removal using a waste based culture medium and lyophilization

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Water Impact Statement

Bioaugmentation for groundwater Mn removal has been performed with fresh bacterial inoculums. Lyophilized inoculums will solve the problems of keeping bacteria viable and transporting large culture volumes. Growing the inoculum in organic waste offers the possibility to replace expensive culture media and to re-use industrial waste. These new technologies will optimize bioaugmentation processes applicable to Mn groundwater full-scale biofiltration.

Optimization of bacterial bioaugmentation for groundwater Mn removal using a waste-based culture medium and lyophilization

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DOI: 10.1039/D0EW00777C

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DOI: 10.1039/D0EW00777C

Abstract

Biological sand filtration systems are widely used for groundwater Mn removal. Bioaugmentation of sand filters through inoculation with Mn(II)-oxidizing bacteria (MOB) have contributed to the optimization of this biofiltration. However, a challenging aspect of this bioaugmentation process on a large scale is keeping fresh MOB cultures viable and transporting large culture volumes to groundwater treatment plants. In this work, powdered MOB inoculums were prepared by vacuum lyophilization. Bacterial lyophilization was performed in different growth conditions and the best performance was observed in biofilms covered with biogenic Mn oxides. On the other hand, a culture medium to produce the inoculum was developed using crude glycerol waste. Inoculums grown and lyophilized in this glycerol medium were able to be immobilized onto sand filters and to enhance the performance of groundwater Mn removal, reaching the optimal removal efficiency faster than fresh MOB cultures. These results generate new tools to simultaneously, re-use crude glycerol waste and improve large scale bioaugmentation approaches for groundwater Mn removal.

Keywords: Manganese-oxidizing bacteria, Manganese removal, groundwater, freeze-drying, crude glycerol

1. Introduction

In groundwater, a source of drinking water for many populations, the presence of soluble Manganese, Mn(II), is an important concern affecting water quality, interfering with its disinfection process [1, 2] and causing adverse human health effects. The exposure to high levels of Mn may be associated not only with neurological disorders such as Alzheimer's disease, Parkinson's disease and Huntington's disease [3-5], but also, consumption of Mn-contaminated water impacts on children's neurodevelopment triggering changes in the intelligence quotient (IQ) and producing attention-deficit hyperactivity disorders [6, 7]. Furthermore, the overexposure to Mn during pregnancy leads to significant reductions in size, length and weight of newborns [8].

Biological sand filtration, based on bacterial oxidation of Mn(II) to form insoluble oxides (MnOx) that can be filtered out of the water through sand filters, is widely used for groundwater Mn removal [1, 9]. Long start-up periods and low efficiencies of Mn removal frequently occur in this biofiltration and these problems may be solved by bioaugmentation of appropriate Mn(II)-oxidizing bacteria (MOB) through their inoculation on the sand filters [10-13]. However, a full-scale application of bioaugmentation using fresh bacterial cultures requires their storage and maintenance of cell viability. In this context, continuous culturing or cold storage may generate contaminations and bacterial community shifts [14]. In addition, transportation of large volumes of bacterial cultures is challenging and expensive and *ex situ* bacteria cultivation is not applicable since it requires carefully controlled continuous reactor systems and trained staff in water treatment plants [14].

These difficulties may be solved through freeze-drying or lyophilization to obtain a powdered bacterial inoculum for the bioaugmentation process [15]. A shortcoming of lyophilization may be a poor recovery of bacterial activity after rehydration of the powdered inoculum [15]. Also, there are no reports on the functionality of MOB lyophils

to remove Mn from groundwater. Therefore, the principal aims of this work were to determine appropriate conditions to carry out the freeze-drying of MOB and analyze if the lyophils have the ability to remove Mn from groundwater.

In a previous work, a *Pseudomonas sagittaria* strain (named MOB-181) that can only oxidize Mn(II) when grown as a biofilm was isolated [16]. The resulting biofilms covered with self-formed biogenic MnOx, had a high adherence capacity to sands and bioaugmentation of lab-scale sand filters with this biofilms improved groundwater Mn removal performance [17]. Biofilms are sessile high-density communities of bacterial cells, surrounded by a matrix containing exopolysaccharides, proteins and extracellular DNA, that aid in shielding bacteria from external stresses [18]. Therefore, in this work the resistance of MOB-181 biofilm to lyophilization was analyzed.

Moreover, a low-cost culture medium formulated with crude glycerol waste was designed to grow the MOB-181 inoculum. Two main arguments support the use of this waste in this work. Firstly, around 200,000 tons of it are produced as a by-product from biodiesel production process in Argentina per year. Secondly, since producers do not refine glycerol, they give it away for free or even have to pay for its disposal [19]. In Argentina, the typical crude materials for biodiesel production are soybean and sunflower oils [19]. Biodiesel is produced through transesterification of lipids with simple alcohols, such as methanol, generally catalyzed by NaOH and KOH or acid, and crude glycerol is the major by-product of this reaction [20]. Crude glycerol waste may also have varying amounts of methanol, methyl esters, microelements (iron, magnesium, calcium, zinc), nitrogen, phosphorus, fat and proteins, water and alkali soaps and hydroxides if NaOH and KOH are used as catalyst [20]. Despite the presence of these additional components, previous research have shown that crude glycerol waste from biodiesel industry can be used as culture medium for various bacteria, yeast, molds and microalgae [21, 22]. Therefore, MOB-181 was cultured in different media designed with

crude glycerol to obtain an adequate medium to grow and lyophilize this bacterium. Inoculation of a laboratory-scale water purification device with MOB-181 lyophils grown in this glycerol medium showed successful groundwater Mn removal. These results showed that not only it is possible to use a powdered inoculum instead of fresh cultures to improve groundwater Mn removal, but also re-use crude glycerol waste, becoming interesting from a social and environmental standpoint.

2. Methods

2.1 Bacterial strain and culture conditions

A *Pseudomonas sagittaria* strain, named MOB-181 (GenBank accession number MK011867), was used in this work [16]. MOB-181 was grown in the commercial Lept medium [16, 23], supplemented with 100 µM $MnCl_2$ (Lept-Mn) and in culture media designed with residual crude glycerol solution. Crude glycerol waste was obtained from a facility where the storage of the waste from Santa Fe province biodiesel industry is collected (Argentina). The composition of the composite waste used in this work is: 40% glycerol v/v, 13% methanol v/v, 3% inorganic salts w/v and 3% solids w/v. The different assayed media were made diluting this crude glycerol waste in San Lorenzo (SL) natural groundwater [17], to different concentrations (1%, 2% and 5% v/v); the pH was adjusted to a value of 7.5 with a 5 N NaOH solution and the media were autoclaved at 120°C for 30 min, this step contribute to methanol evaporation [21].

2.2 Analysis of MOB-181 growth and Mn oxides quantification

MOB-181 was grown in LB medium [24], with shaking (200 rpm) at 28ºC until an optical density at 600 nm (OD_{600}) of 2.5 was reached. Then, aliquots of 1 mL were centrifuged at 5,000 rpm for 5 min and bacterial pellets were resuspended in 10 mL of each one of the growth assayed media. These bacterial suspensions were grown with agitation (200

rpm in an orbital shaker) or statically at 28ºC during 1 or 6 days, respectively. For static cultures, biofilms formed were disaggregated by gently agitation obtaining homogenous samples. Bacterial growth was quantified by plating serial dilutions of these samples on LB medium-agar and counting the colony forming units (cfu) per milliliter of culture medium (cfu/mL). MnOx present in these samples were quantified with Leucoberbelin Blue (LBB) dye solution, as previously described [16].

2.3 Bacterial freeze-drying and quantification of bacterial survival ratios

Bacteria grown in the different assayed media were harvested by centrifugation for 15 min at 5,000 rpm. Bacterial pellets were frozen at -70ºC and placed in a laboratory scale freeze-dryer (model MPS-86, Operon, Korea). Automatic freeze-drying conditions were used, which consisted of 100 mTorr and drying for 24 h at -96°C. For survival analysis, lyophils were rehydrated in 150 mM NaCl by gentle stirring at 28ºC. Before and after lyophilization cfu/mL were determined and survival ratios (SR) were calculated as:

$$\mathrm{SR} = \frac{\left[\frac{cfu}{mL}\right] after\ lyophilization}{\left[\frac{cfu}{mL}\right] before\ lyophilization} * 100$$

2.4 Quantification of groundwater Mn(II) oxidation performed by sand immobilized MOB-181 lyophils

Static MOB-181 cultures were grown in 10 mL of Glycerol 1% supplemented with 100 µM $MnCl_2$ (Glycerol 1%-Mn), during 6 days at 28°C. Bacterial cells were harvested by centrifugation for 15 min at 5,000 rpm, bacterial pellets were lyophilized and lyophils were rehydrated. Then, 10 mL of rehydrated lyophils, fresh MOB-181 cultures (used as positive controls), and 10 mL of culture media without bacteria (used as negative control), were incubated statically for 4 days with 20 g of sterilized sand at 28ºC to allow bacterial adherence to the sand. Afterward, sands were washed with sterile distilled water to remove non-adhered cells, and incubated at 28ºC with 10 mL of SL

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137 groundwater supplemented with $MnCl_2$ to reach 100 µM final concentration. After 6
138 days of incubation, 1 mL of each groundwater supernatant was mixed with 500 µL of
139 LBB dye and MnOx concentration was determined.
140
141 ***2.5 Analysis of MOB-181 lyophils Mn removal performance***
142 A laboratory-scale water purification device, designed to mimic the actual large-scale
143 sand bed filters was used [17]. SL groundwater was chosen for the assays because of its
144 high Mn concentration (1.0 mg/L) and also because MOB are naturally present
145 suggesting that it may support nutritional MOB requirements. The physico-chemical
146 characteristics of this groundwater were previously determined [17].
147 MOB-181 cultures (200 mL) were grown statically in Glycerol 1%-Mn medium during
148 6 days at 28°C. Bacterial pellets from non-lyophilized (NL) or lyophilized (L) cultures
149 were rehydrated in 50 mL of SL groundwater. Sand columns were inoculated with NL
150 bacteria (IC-NL) or with L bacteria (IC-L) recirculating bacterial suspensions in a
151 down-flow mode at a roughing filtration rate of 0.60 m/h during 24 h with peristaltic
152 pumps (PC 25 Series, Apema). For non-inoculated control columns (CC), SL
153 groundwater was recirculated instead of bacterial inoculums. Then, SL groundwater was
154 pumped from a polyethylene storage tank of 80 L capacity, at a roughing filtration rate
155 of 0.60 m/h. Two IC-NL, IC-L and CC were run in parallel at an average room
156 temperature value of 22ºC. This temperature was chosen since we have previously
157 observed that under summer conditions (lowest average and highest average
158 temperatures of 19ºC and 30ºC, respectively), survival and performance of MOB-181
159 were better than under winter conditions (lowest average and highest average
160 temperatures of 8ºC and 18ºC, respectively). The experiments were performed until the
161 optimal Mn removal efficiency (95%), corresponding to Mn concentrations <0.05 mg/L
162 in the outflow of each column (Effluent water) (Santa Fe Law No. 11,220 Annex A).

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2.6 Determinations of Mn removal efficiencies, dissolved oxygen and pH

Total Mn, pH and dissolved oxygen (DO) in influent and effluent water, were daily measured as previously described [17] and Mn removal efficiency (E) was calculated as:

$$E = \frac{Mn\ content\ (influent) - Mn\ content\ (effluent)}{Mn\ content\ (influent)} * 100$$

2.7 Quantification of MnOx and bacteria retained into the sand filters

After completing the experiments, sands were collected from the top (10 cm), middle (20 cm) and bottom (20 cm) parts of all the columns and each sample was homogeneously mixed. Sand samples (50 g) were washed three times with sterile distilled water and MnOx retained into the sands were quantified as previously described [17]. To quantify bacteria attached to the sands, 50 g of these washed sands were mixed with 100 mL of 150 mM NaCl, gently vortexed for 10 min and serial dilutions were plated out on LB medium-agar to calculate the cfu per gram of sand.

2.8 Statistical analysis

The results showed in the tables and figures are the mean values of triplicate measurements and standard deviation (SD) are also shown. Three independent experiments were performed for all assays. Data were statistically analyzed using one-way analysis of variance (ANOVA) ($p<0.05$).

3. Results and Discussion

3.1 MOB-181 lyophilization and survival rate (SR) quantification in different growth conditions

MOB-181 cultures were grown under agitation (planktonic cultures) or static conditions in Lept and Lept-Mn media, the latter medium was previously used to achieve an

efficient lab-scale bioaugmentation protocol for groundwater Mn removal [17]. Mn oxidation was only observed in static MOB-181 cultures in Lept-Mn (Table 1), in this condition MOB-181 formed ring-shape biofilms covered with biogenic Mn oxides at the liquid-air interface [16]. Bacterial growth was higher in planktonic cultures than in static cultures, and was similar in Lept or Lept-Mn media ($p<0.05$) (Table 1). Lyophilization of planktonic MOB-181 cultures, led to a low number of viable cells; however static MOB-181 cultures showed larger SR than planktonic cultures ($p<0.05$) (Table 1), suggesting that biofilm lifestyle improves MOB-181 resistance to freeze-drying. Although this approach is interesting, this is one of the few studies that assessed the biofilm resistance to lyophilization, highlighting the importance of our results. Until now, this methodology has only been applied to show that *Lactobacillus rhamnosus* encapsulated in alginate-microcapsules were more resistant to freeze-drying when the process of encapsulation is performed with high-density biofilms than with *L. rhamnosus* planktonic cells [25].

Table 1: Quantifications of bacterial growth, lyophilization survival ratios and MnOx production of MOB-181 cultured in Lept and Lept-Mn media in agitation (Planktonic cultures) or static conditions (Static cultures). The mean values of triplicate measurements and SD are shown. Three independent experiments were performed for all assays. Data were statistically analyzed using one-way analysis of variance (ANOVA) ($p<0.05$). *ND: Not detected.

Quantifications	Planktonic cultures		Static cultures	
	Lept	Lept-Mn	Lept	Lept-Mn
Bacteria before lyophilization (cfu/mL)	5.50±0.28 x 10^{11}	5.40±0.25 x 10^{11}	2.21±0.23 x 10^{8}	2.10±0.21 x 10^{8}
Bacteria after lyophilization (cfu/mL)	1.50±0.36 x 10^{4}	1.83±0.32 x 10^{4}	6.17±0.21 x 10^{5}	5.52±0.28 x 10^{6}
SR (%)	2.720 10^{-6}	3.388 10^{-6}	0.279	2.629
MnOx present in the culture (µg/mL)	ND*	ND*	ND*	16.20±0.3

Considering that MOB-181 static cultures showed enhanced lyophilization SR (Table 1) and improved groundwater Mn removal [17], MOB-181 growth ability, lyophilization

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resistant and MnOx production were analyzed in different Glycerol media growing the bacteria statically (Table 2). As observed for Lept (Table 1), in Glycerol media, bacterial growth was similar regardless of the presence or absence of Mn(II) ($p<0.05$). In addition, similar bacterial growth was observed when Glycerol 1% and Lept media were used ($p<0.05$). Growth of MOB-181 was mainly favored by the increase in glycerol concentrations and the highest growth was observed in Glycerol 5% ($p<0.05$). However, Glycerol concentrations higher than 1% negatively affected lyophilization SR and also MnOx production (Table 2). The addition of glycerol, as a cryoprotective agent of proteins and membrane lipids, considerably improves the resistance to freeze-dried [26]. However, our results indicate that an excess of crude glycerol has detrimental effect over the cells during lyophilization and similar results were observed using pure glycerol (data not shown). Therefore, high amounts of glycerol may destabilize bacterial membrane during the drying process, further investigations to understand these results are required. Regarding Mn oxidation, this process occurs in specific minimal media, such as PC media or Lept media [16]. Hence higher amounts of crude glycerol in the media may inhibit Mn oxidation due the high content of nutrients such as we observed for LB rich media [16]. Alternatively, some additional component of the crude glycerol waste could have a detrimental effect on lyophilization and Mn oxidation process. The highest SR value and quantity of MnOx were observed in Glycerol 1%-Mn (Table 2), with values similar to those observed for Lept-Mn medium ($p<0.05$) (Table 1). Negative controls, without MOB-181, did not display any growth or MnOx production (data not shown). These results showed that in Glycerol 1% medium good performances of MOB-181 growth and Mn-oxidation can be achieved and offer the possibility to replace the expensive Lept culture medium with this non-cost medium and to re-use industrial waste.

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Interestingly, either in Lept or Glycerol medium, the higher the content of MnOx, the higher the survival ratio after lyophilization ($p<0.05$) (Table 1 and Table 2), suggesting that biogenic MnOx act as cryoprotective agents that enhance cells resistance to freeze-drying. Such a role for metals was only studied by the addition of Fe_3O_4-pectin nanoparticles to *Lactobacillus plantarum* observing an enhancement of bacterial viability during freeze-drying [27].

Table 2: Quantifications of bacterial growth, lyophilization survival ratios and MnOx production of MOB-181 static cultures in Glycerol media. The mean values of triplicate measurements and SD are shown. Three independent experiments were performed for all assays. Data were statistically analyzed using one-way analysis of variance (ANOVA) ($p<0.05$). *ND: Not detected.

Quantifications	Static cultures					
	Glycerol 1%	Glycerol 1%-Mn	Glycerol 2%	Glycerol 2%-Mn	Glycerol 5%	Glycerol 5%-Mn
Bacteria before lyophilization (cfu/mL)	2.50±0.25 x 10^8	2.40±0.24 x 10^8	3.91±0.25 x 10^8	3.93±0.20 x 10^8	1.45±0.25 x 10^9	1.55±0.24 x 10^9
Bacteria after lyophilization (cfu/mL)	9.30±0.23 x 10^5	5.41±0.21 x 10^6	1.25±0.24 x 10^5	2.55±0.19 x 10^5	2.90±0.23 x 10^4	1.70±0.25 x 10^5
SR (%)	0.372	2.254	0.032	0.065	0.002	0.011
MnOx present in the culture (μg/mL)	ND*	16.10±0.20	ND*	6.80±0.70	ND*	1.00±0.10

3.2 Groundwater Mn(II) oxidation by sand immobilized MOB-181 lyophils

The Mn(II) oxidizing efficiency of static MOB-181 cultures grown in Glycerol 1%-Mn, as fresh cultures and as lyophils, both adhered to sand, was evaluated. MOB-181 lyophils retained the ability to be immobilized on sands and to oxidize Mn(II) present in groundwater, though to a lesser extent than fresh cultures ($p<0.05$). This was probably due to the smaller number of initial viable cells present in the lyophils (Table 3).

Table 3: Groundwater Mn(II) oxidation performed by MOB-181 inoculated sands. The mean values of triplicate measurements and SD are shown. Three independent experiments were performed for all assays. Data were statistically analyzed using one-way analysis of variance (ANOVA) ($p<0.05$).

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Quantifications	Glycerol 1%-Mn Fresh cultures	Glycerol 1%-Mn Lyophils
Initial Bacterial concentration incubated with the sands (cfu/mL)	2.40±0.23 x 10^8	5.32±0.21 x 10^6
MnOx produced by the bacteria adhered to the sands (μg/mL)	104.1±3.4	77.2±2.7

3.3 Mn removal efficiencies of MOB-181 lyophils in lab-scale sand filters

Having established that MOB-181 lyophils obtained from Glycerol 1%-Mn medium can be adhered to sands filter and oxidize Mn from groundwater, the effectiveness of these lyophils to remove groundwater Mn was compared to the effectiveness of MOB-181 fresh cultures in lab-scale sand filters. Columns were inoculated with non-lyophilized (IC-NL), that had 2.20 10^8 cfu/mL, and lyophilized MOB-181 (IC-L), with 5.55 10^6 cfu/mL. The performance of the filters was daily monitored in influent and effluent waters, measuring total Mn concentrations and calculating Mn removal efficiencies (Figure 1). DO and pH were also monitored (Table 4) and values were consistent with those required for the biological Mn(II) oxidation [17].

While control columns (CC) did not exceed 20% of Mn removal efficiencies, with mean values of 13% throughout the assay, IC-NL and IC-L were able to achieve the optimal 95% Mn removal efficiency ($p<0.05$) (Figure 1). The inoculated columns began to remove Mn from the first day of system operation, with a Mn removal efficiency of 40% for the IC-NL and 20% for the IC-L (Figure 1). At the beginning of the assays (4 days), higher Mn removal efficiencies were observed for the fresh cultures than for the lyophils ($p<0.05$), consistent with the higher MOB-181 concentration of fresh cultures compared with lyophils. Except for these initial points, IC-L showed a continuous increase in Mn removal, reaching the optimum Mn removal efficiency of 95%, 6 days earlier than the IC-NL ($p<0.05$) (Figure 1), demonstrating that even though IC-L were inoculated with a lower concentration of viable cells, lyophilized cultures showed a better Mn removal performance than fresh cultures. This may be because lyophilized

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bacteria were in a worse physiological state than fresh cultures that allowed a faster adaptation to the new conditions, such as low organic matter, present in groundwater.

Table 4: Average DO and pH values daily measured in the influent and effluent waters. The average of the mean values of triplicate daily measurements and SD are shown. CC: Control Columns, IC-NL: Columns inoculated with non-lyophilized MOB-181 bacterium, IC-L: Columns inoculated with MOB-181 lyophils.

Quantifications	Influent water (CC)	Effluent water (CC)	Effluent water (IC-NL)	Effluent water (IC-L)
Average DO	7.80±0.32	7.10±0.21	5.55±0.35	5.86±0.32
Average pH	8.21±0.12	8.12±0.16	8.13±0.18	8.15±0.15

Mn oxides accumulation was detected in IC-NL and IC-L by the occurrence of dark brown precipitates that mainly appeared at the top of the columns (Figure 2A). IC-NL and IC-L, showed the highest MnOx accumulation at the top fractions which retained 3.76 and 6 times more oxides than the bottom fractions, respectively ($p<0.05$) (Figure 2B). Also, the amounts of MOB were higher at the top fractions than at the bottom fractions of these columns ($p<0.05$) (Figure 2C). These results indicate that the higher the amount of MOB, the higher the amount of MnOx, suggesting a lead role for MOB in the formation of these MnOx and the removal of this metal. On the other hand, it is reasonable to speculate that some adsorptive Mn removal by the MnOx present in the bacterial cultures may occurs in parallel, as was previously observed [28, 29]. In CC, scarce amounts of MnOx were accumulated in all column fractions (Figure 2), consistent with the low Mn removal efficiencies of CC (Figure 1). In CC, MOB were also detected, mainly at the top fractions, but to a lesser concentration than IC-NL and IC-L, suggesting again that MOB had an important role in Mn removal. Previous results have showed the existence of autochthonous MOB in SL groundwater and the presence of MOB in filter sands of CC, suggesting that biological Mn-removal is possible in this groundwater [17]. In addition, CC may achieve maximal Mn removal efficiency but at longer times than MOB-181 inoculated columns [17]. Overall, the results of this work

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demonstrated that inoculation of the sand filters either with fresh or lyophilized cultures shortens Mn removal start-up periods of SL groundwater compared with non-inoculated CC suggesting an important role for bacterial activity in this process.

4. Conclusions

In this work, progresses in bacterial inoculum preparation were addressed to improve the performance of bioaugmentation approaches for groundwater Mn removal. Our results demonstrated the feasibility of inoculating sand filters with lyophilized MOB inoculums and that crude glycerol waste based medium is appropriate for bacterial inoculums production. Therefore, this proposal simultaneously allows, avoid using large volumes of fresh MOB cultures to enhance the performance of groundwater Mn removal and to re-use crude glycerol waste, becoming an interesting approach from a social and environmental standpoint.

5. Conflicts of interest

There are no conflicts to declare.

6. Acknowledgements

This work was supported by grants from Santa Fe Agency for Science, Technology and Innovation (Project IO-2018-00074 to NG). AP and LCC are fellows and JO and NG are staff members of CONICET. We thank Tec. Hernán Quevedo for the help in physico-chemical analyses

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Figure Legends

Figure 1. Mn removal efficiencies. Mn removal ratios of control columns (CC) and columns inoculated with non-lyophilized (IC-NL) and lyophilized MOB-181 (IC-L) were analyzed. Dash lines indicate 95% removal efficiencies. The mean values of triplicate Mn concentration measured in water samples are shown and error bars are the SD. Two IC-NL, IC-L and CC were run in parallel to take influent and effluent water samples. Data were statistically analyzed using one-way analysis of variance (ANOVA) ($p<0.05$).

Figure 2. Quantification of MnOx and MOB retained into the filter sands. Representative photographs of control columns (CC) and columns inoculated with non-lyophilized (IC-NL) and lyophilized MOB-181 (IC-L) at the end of the assays. (B) Concentrations of Mn oxides (MnOx) in µg per gram of sand and (C) concentrations of MOB in cfu per gram of sand were determined in the top (T), middle (M) and bottom

437 (B) fractions of CC, IC-NL and IC-L. Bars represent mean values of triplicate
438 measurements for control and inoculated columns and error bars are the SD. Data were
439 statistically analyzed using one-way analysis of variance (ANOVA) ($p<0.05$). Asterisks
440 indicate significant difference compared to the CC samples ($p<0.05$).
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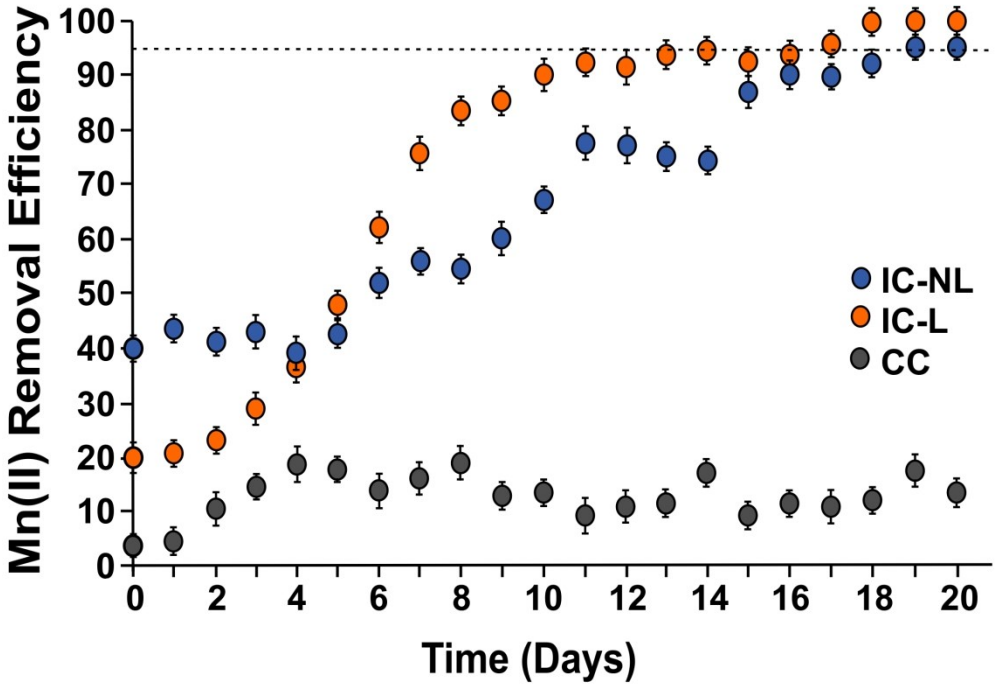

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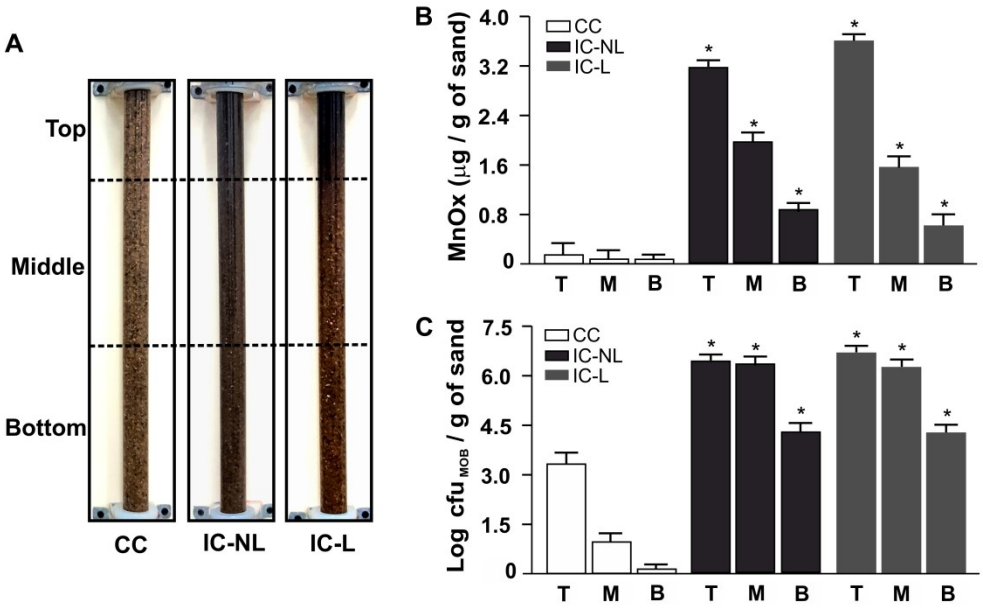

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DOI: 10.1039/D0EW00777C

Manuscript title: Optimization of bacterial bioaugmentation for groundwater Mn removal using a waste based culture medium and lyophilization

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Table of contents entry

Waste based bacterial culture media and inoculum lyophilization to optimize bioaugmentation processes applicable to Mn groundwater full-scale biofiltration.