LIVING BIOMASS SUPPORTED ON A NATURAL-FIBER BIOFILTER FOR LEAD REMOVAL

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Abstract

Living biomass biofilters constitute an excellent alternative for heavy metal bioremediation. *In situ* biomass and exopolysaccharides production involve a crucial advantage over other bioremediation alternatives such as lignocellulosic biomass-based materials. In this study, a biofilm-forming bacterium was isolated from an ambient exposed to heavy metals. Bacterial biomass was inoculated on a biofilter packed with *Furcraea andina* fibers. The goal was to develop a continuous low-cost biofilter to remove low-to-moderate concentrations of Pb^{2+}. Adsorption equilibrium and kinetics were determined for the fibers and the biofilm developed on the fibers. Biofilm presence had positive effects on the maximum adsorption capacity and the process kinetics. Biofilters packed with 20 g of *F. andina* fibers, with and without living biomass biofilm, were evaluated under continuous inflow of Pb^{2+} (325mg/day) at a concentration of 50 mg/L. The best results were obtained with the biofilm-fiber biofilter where total adsorption on Pb^{2+} were observed for 72 h. Maximum absorption capacity was 48.75 mg/g at pH=7.

Keywords: biofilter; heavy metal; biofilm; bioremediation; natural fiber.

1. Introduction

Metals and metalloids are one of the most relevant environmental problems today. Although attention and proper measures have been implemented in established economies, heavy metal pollution remains an issue (Chon et al., 2011; Wang et al., 2013; Xu et al., 2015). The presence of heavy metals in the environment have diverse origins. Mainly, mining activities and agriculture, and, to a lesser extent, industrial processes, fuels, urban waters or natural sources (e.g. volcanic ashes) (Fu and Wang, 2011). Other industries, such as aquaculture, do not produce metals but are impacted by them and solutions based on biomass have been studied (Aspé et al., 2012; Fernández et al., 2014). The toxicity of some of these elements increases due to their ability to easily bioaccumulate and to be transferred through the food chain. Above certain concentrations, they can cause harmful effects on human health. Lead is one of the metals of greatest concern due to the volume in which it is released to the environment and its toxicity and persistence (Tchounwou et al., 2012). According to the EPA (United States Environmental Protection Agency), the permissible level of Pb in drinking water is 0.015 mg/L. Therefore, treating lead-contaminated water requires highly effective methods.

The mechanisms involved in the removal of contaminants are of three types: physical, chemical and biological. In a bioremediation process several mechanisms can occur simultaneously. Among the alternatives for the removal of heavy metals in aqueous solutions a number of solutions has been proposed: ion exchange, chemical precipitation, reverse osmosis, electrochemical processes and sorption (Elouear et al., 2008; Keleş et al., 2010). However, one of the challenges of the current environmental technology is to incorporate more accessible processes of bioremediation from a technical and economic point of view (Castro et al., 2017; Wang and Chen, 2009).

Several sorbents have been proposed for heavy metal removal so far (Renu et al., 2017). However, bio-adsorption processes involving microorganisms' biomass and treated vegetal material seem the most promising alternatives because of their versatility, availability and efficiency (Demirbas, 2008; Liu et al., 2015; Wang and Chen, 2009). However, despite the fact that many attempts of developing biofilters for remediation of heavy metals have been made, so far there are not commercial examples

of these technologies (Castro et al., 2017; Verma and Sharma, 2017; Wang and Chen, 2009). The more pointed out causes are the relatively unknown fate of the metals in the exhausted biomass and the lack of low-cost retention materials (Wang and Chen, 2009). In recent years, the focus has been put to low-cost materials of vegetal origin which could be used as adsorbent and support (e.g. coconut fiber, wheat fiber, powder snuff, banana peel, etc.). The ability of natural fibers for adsorbing contaminants are linked to their internal structure and composition. For use in bioremediation processes, the specific surface of the fiber (i.e., the surface-volume relation) is paramount. Due to its high surface area (180 $m^2 \cdot L^{-1}$) and, also, its high lignin content, *F. andina* is a good candidate for bioremediation processes. In addition to the use of vegetal by-products, biomass of microbiological origin has been proposed in metal retention processes (Gadd, 2000; Malik, 2004). In general, when active microorganisms are used as adsorbents the process is known as bioaccumulation. Examples of freely-suspended (Chang et al., 1997; Dursun et al., 2003) and immobilized (Diels et al., 1995; Knierim et al., 2015) microorganism have been shown in literature. Treatable concentrations for these processes should be typically lower, although effluents also resulted in lower concentrations (<0.1 mg L^{-1}) (Brierley, 1990; Malik, 2004). The main problem with the use of live microorganisms is the toxic effect on the cells of high concentrations of contaminants. The response to the toxic molecule depends on the species and strain of the microorganism in question and the previous acclimatization. Recent studies have shown that the strains isolated from polluted environments have greater resilience to metals and are more effective in decontamination processes (Malik, 2004). Additional advantages are the *in situ* self-production of adsorbent biomass and better results when real leachates are used (Malik, 2004; Sajjad et al., 2017). On the other hand, biofilms allow for simultaneous decontamination of a wide variety of contaminants, including organic matter, nitrates, sulfates, organic compounds, metals, etc. (Huiliñir et al., 2012).

In this work, we propose a living-biomass biofilter using natural fibers (*Furcraea andina*) as bacterial support and adsorbent for lead bioremediation. The adsorbent biomass was characterized in terms of thermodynamic equilibrium and kinetics. In a previous work, biofilm development of *Pseudomonas*

spp on *Furcraea andina* was shown to occurr in lower times than typical plastic supports (e.g. polyethylene terephthalate) (Gallardo-Rodríguez et al., 2016). By using these natural supports the costs of implementing a biofiltration step in wastewater treatment would be sensibly reduced. Polymeric media usually have a price of around US$1000/m^3 whereas natural support costs are at least ten times lower (Saliling et al., 2007). Thus, the objective was to develop and evaluate a low-cost biofilter for continuous treatment of wastewaters containing medium-to-low concentrations of Pb.

2. Materials and Methods

2.1. Preparation and isolation of Pseudomonas bacteria

About 1 kg of sediments from an aquaculture plant regularly exposed to volcanic ashes (Ecuadorian Andes) were collected in plastic polypropylene bottles. The bottles were previously treated with 0.1 M HCl to remove possible impurities and sterilized at 121°C for 15 minutes. In the laboratory, we proceeded with the isolation and cultivation of *Pseudomonas* strains. Dilutions up to 10^{-6} were made from a sample of 1 mL of slurry after vigorous shaking with a Vortex shaker. Petri dishes with selection agar (DIFCO *Pseudomonas* Isolation Agar, Becton-Dickinson™) were used. Once isolated, they were cultivated at 32ºC under anoxic conditions using nitrate and glycerol as source of C.

2.2. Fibers preparation

Fibers were prepared according to Gallardo-Rodríguez et al. (Gallardo-Rodríguez et al., 2016). Briefly, the fibers were dried in an oven at 75 ° C for 24 hours and weighed. For its use as support, fibers were cut into 4-5 cm length. The width of the fiber ranged from 200 to 250µm. *Furcraea andina* fibers used had a composition of 73% cellulose, 1.90% resins, waxes and fats, 11.30% lignin and 10.50% pentosans.

2.3. Toxicity of Pb for isolated Pseudomonas strain

Tests were performed to determine the resistance of the isolated strain of *Pseudomonas* to different concentrations of lead. Both the resistance of suspended cultures and biofilms were evaluated. For

the studies with bacteria in suspension, 0.5 mL with known concentration (OD = 0.5) were taken and added to culture tubes with different final concentrations of $Pb(NO_3)_2$ (1, 0.5, 0, 2, 0.1, 0.05 $g \cdot L^{-1}$) at pH=7. NaCl was added to reach a final concentration of 100 mM to improve adhesion velocity (Gallardo-Rodríguez et al., 2016). To determine the inhibition of biofilm, the optical density of the suspension (650 nm) was measured after 24 hours. A biofilm was developed on agar (DIFCO *Pseudomonas* Isolation Agar, Becton-Dickinson™) in Petri dishes with the same mass of bacteria (determined previously by optical density measurement) and exposed to the same lead nitrate solutions. After 24 hours, the solution was withdrawn and replaced with another NaCl (100mM). Then, the biofilm was removed, homogenized and the optical density of the solution was measured. The percent inhibition (*I*) was calculated as follows:

$$I(\%) = \frac{OD_{t=0h} - OD_{t=24h}}{OD_{t=0h}} \cdot 100 \qquad (1)$$

Where OD is the optical density measured in the bacterial suspension. $OD_{t=0h}$ and $OD_{t=24h}$ are the values measured of OD for time 0 and 24 hour, respectively.

2.4. Lead Adsorption assays

The lead solution used was obtained by dissolving lead nitrate in distilled water (pH=7). Final Pb^{2+} concentration was 50 $mg \cdot L^{-1}$. Lead retention assays were performed in 10 mL culture tubes in which different amounts (ranging from 1.5 to 3 g/tube) of *F. andina* fibers were introduced. Biosorption tests were performed at 20-27°C. Additionally, adsorption assays (10 mL; 1.5 g of fibers) were carried out at temperatures ranging 20-27°C. Fiber plus bacteria assays were performed using *Pseudomonas* cultures which were added to the fibers 24 hours earlier to allow formation of biofilms. This time was enough for the biofilm to form (Gallardo-Rodríguez et al., 2016). After 24 h, liquid was removed, and the fibers washed with a NaCl solution (100mM). Four tubes were prepared for each condition. Two of them were used to evaluate the bacterial biomass bound to the fiber. The others were used for the lead absorption assays. After wash-up step, the lead solution was added. The determination of Pb was performed using an Atomic Absorption spectrometer (Thermo Scientific, iCE 3300). For lead

determination, the samples were previously centrifuged and filtered (0.45 microns).

A continuous-flow long-term assay (5 days) was carried out with 20 g of fiber and *Pseudomonas* biofilm. Hydraulic PVC tubes were used (40 mm diameter, 200 mm length) to build 4 packed biofilters with a free-liquid volume of 108 mL. Two of them were inoculated with *Pseudomonas* as indicated above. Biofilters were vertically disposed and liquid was pumped upwards. Detached biomass was observed with the exit flow, so it was separated by centrifugation before measuring Pb content in the outlet samples. To maintain bacterial growth water containing lead nitrate (50mg/L) was supplemented with necessary nutrients. The media formulation can be found in Gallardo et. al (2016). Flowrate was fixed a 4.5 mL/min (approx. 6.5 L/day).

2.5. Lead Adsorption studies

Lead adsorption (q_t, mg·g^{-1}) was calculated as follows:

$$q_t = \frac{(C_0 - C_t)V}{m} \quad (2)$$

where C_t is the concentration of lead at time t (mg·L^{-1}). V is the liquid volume. C_0 is the initial lead concentration (mg·L^{-1}). The mass of fiber used (in g) is represented by m. Adsorption mechanism was evaluated using two different kinetic models: pseudo-first-order (Lagergren, 1898) and pseudo-second-order equations (Ho and McKay, 1999). For the models, an equilibrium adsorption of Pb^{2+} was calculated as follows:

$$q_e = \frac{(C_0 - C_e)V}{m} \quad (3)$$

where q_e is the amount of lead adsorbed at equilibrium (mg·g^{-1}). C_e is the equilibrium concentration of lead (mg·L^{-1}).

Four different models were compared to describe adsorption kinetics. The Lagergreen's pseudo-first-order (4), the pseudo-second-order equation (5) (Ho, 2006), Elovich equation (6) and the intraparticle diffusion model (7) (Weber and Morris, 1963). Equations 4 and 6 are transformed from the originals equations according to Wu et al. (Wu et al., 2009).

$$q_t/q_{ref} = 1 - (\exp(-k_1\, t))/(1 - \exp(-k_1\, t_{ref})) \quad (4)$$

$$q_t = {q_e^2\ k_2\ t}/{(1 + q_e\ k_2\ t)} \quad (5)$$

$${q_t}/{q_{ref}} = R_E \ln (t/t_{ref}) + 1 \quad (6)$$

$$q_t = k_p\sqrt{t}\ +\ C \quad (7)$$

where t is time and k_1, k_2 are rate constant for the pseudo-first-order equation and the pseudo-second-order equation, respectively. t_{ref} is the longest time evaluated. q_{ref} is the adsorption a $t = t_{ref}$ (mg·g^{-1}). q_e is the amount of lead adsorbed at equilibrium (mg·g^{-1}). R_E is the approaching equilibrium parameters for the dimensionless Elovich model. From R_E, β (desorption constant in the original Elovich equation (Tran et al., 2017); mg·g^{-1}) can be easily obtained (Wu et al., 2009). In Eq. 7, k_p (mg·g^{-1}·min$^{1/2}$) is the rate constant of the intraparticle diffusion model. C (mg·g^{-1}) is a constant associated with the thickness of the boundary layer.

Kinetic models were compared calculating the standard deviation (%) as:

$$SD(\%) = \sum_{assay}\left[100\ \left(\frac{\sum_N(1 - (\ q_{t,calc}/\mathrm{q_{t,exp}}))^2}{N}\right)^{0.5}\right] \quad (8)$$

where N is the number of data points. $q_{t,calc}$ and $q_{t,exp}$ are, respectively, the calculated and experimental amount of adsorption at time t.

Experimental data were also fitted to Freundlich (Freundlich, 1906) (Eq. 9), Langmuir (Langmuir, 1918) (Eq. 10), Temkin (Eq. 11) (Tempkin and Pyzhev, 1940) and Dubinin (Eq. 12) (Dubinin, 1960) adsorption isotherm models:

$$q = K_f {C_e}^{1/n} \quad (9)$$

$$q = q_{max}\ K_L\ {C_e}/{(1 + K_L\ C_e)} \quad (10)$$

where K_f, n, q_{max} and K_L are empirical constants.

$$q = \frac{RT}{b}\ln (A_T\ C_e) \quad (11)$$

$$q = q_s \exp (-\ K_{ad}\ \varepsilon^{\,2}) \quad (12)$$

where R is the universal gas constant (J·mol^{-1}·K^{-1}) and T is temperature. A_T (L·g^{-1}) and b (J·mg^{-1}) are

the Temkin isotherm constants. In Eq.12, q_s is the theoretical isotherm saturation capacity ($mg \cdot g^{-1}$), K_{ad} ($mol^2 \cdot kJ^2$) and ε ($J \cdot mol^{-1}$) are the Dubinin isotherm constants. The parameter ε can also be calculated with Eq. 13. The model distinguishes between chemical and physical adsorption based on the mean free energy (E) calculated with Eq. 14.

$$\varepsilon = R\,T\,ln(1 + 1/C_e) \quad (13)$$

$$E = 1/(2\,K_{ad})^{0.5} \quad (14)$$

2.6. Thermodynamics

Thermodynamics parameters were calculated using the Van't Hoff equation (15).

$$ln\,K_c = -\frac{\Delta G^0}{R\,T} = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{R\,T} \quad (15)$$

where changes in the equilibrium distribution coefficient (K_c) with temperature allow calculating changes in enthalpy (ΔH^0) and entropy (ΔS^0). R is the universal gas constant ($J \cdot mol^{-1} \cdot K^{-1}$) and T is temperature. K_c was calculated using an accurate basis for adsorption process resulted from the obeyed isotherm (Langmuir isotherms, as was demonstrated in R&D section) (Rahmani-Sani et al., 2017; Tran et al., 2017)

$$K_c = \frac{q_e}{C_e\,q_{max}} = \frac{1}{C_e}\frac{K_L\,C_e}{1+K_L\,C_e} \quad (16)$$

For an ideal diluted solution $K_L \cdot C_e << 1$, then the denominator of Eq. 16 can be simplified (Rahmani-Sani et al., 2017; Tran et al., 2017) and, consequently, $K_c = K_L$. For non-ideal diluted solutions, the Langmuir constant should be divided by the activity coefficient (γ) (Liu, 2009). In the case of charged adsorbates (e.g., multivalent ions), the extended Debye-Huckel law, allows calculating the activity coefficient with Eq. 17 (Liu, 2009).

$$\log\gamma = -A\,z^2\,I_e^{1/2} \quad (17)$$

where A is a constant ($0.509\ mol^{-1/2} \cdot kg^{1/2}$), z is the adsorbate's charge and I_e is the ionic strength (M). Then, K_c can be made dimensionless (Zhou and Zhou, 2014):

$$K_c = 10^3 \cdot 55.5 \cdot M_{Pb} \cdot \frac{K_L}{\gamma} \quad (18)$$

K_L has units of $L \cdot mg^{-1}$ and the equilibrium concentration of lead (C_e) has units of $mg \cdot L^{-1}$. As was

indicated in section 2.4, temperature ranged from 20 to 27°C. M_{Pb} is the molecular weight of lead.

3. Results and Discussion

Figure 1 includes the results of the inhibition assays performed with the isolated *Pseudomonas'* strain. Concentrations of lead tested ranged between 50 and 1000 $mg \cdot L^{-1}$. Although the upper limit of the range cannot be found in urban wastewaters or natural waters, it is close to concentrations measured in industrial wastewaters (Wang et al., 2005). As can be observed, in the case of suspension cultures, significant inhibition was found even at the lowest concentrations assayed. However, the effect of nutrients was slightly positive on bacterial survival up to 100 $mg \cdot L^{-1}$ of Pb^{2+}. Higher concentrations were equally toxic independently of the nutrients. In the case of *Pseudomonas'* biofilm, a very important degree of protection was clearly observed. Up to 200 $mg \cdot L^{-1}$, the percentage of inhibition calculated was less than 20%. Similar results can be found in bibliography (Singh et al., 2006). The protective effect of the EPS (exopolysaccharides) matrix of the cells is the undiscussed cause. Teitzel and Parsek (Teitzel and Parsek, 2003) found that biofilms of *P. aeruginosa* were up to two times more resistant to lead than free cells. In our experiments, virtually all bacteria died with concentrations of Pb^{2+} greater than 500 $mg \cdot L^{-1}$. Although all microorganisms are sensitive to heavy metals, the development and proliferation in contaminated environments can lead to the emergence of more resistant strains. In a similar study, Ceylan and Ugur found for *P. fluorescens* and *P. putida* inhibition concentrations for lead of about 2 and 0.5 $g \cdot L^{-1}$, respectively (Ceylan and Uğur, 2013). Also, Chang et al. showed for *P. aeruginosa* tolerance to Pb^{2+} up to 0.5 $g \cdot L^{-1}$ (Chang et al., 1997). Bacteria of the genus *Pseudomonas* are of great interest for bioremediation processes because of their high resistance to heavy metals and their ability to adapt to different environments and nutritional modes (Pardo et al., 2003). The strain isolated here demonstrated acceptable values of resistance to lead, which makes it suitable for the design of bioremediation processes.

The sorption of Pb^{2+} by the bacteria supported on *F. andina* fibers and the fibers alone was investigated. Lignocellulosic fiber interacts with metals through their negatively charged functional groups. This groups are able to complex metals. Waste materials containing cellulose show better

sorption abilities after treatments including preparation methods (such as used here with *F. andina* fibers) or chemical modifications (Pitsari et al., 2013). Fibers used here also has a considerable percentage of lignin, around 10%, (Mayacela-Rojas et al., 2017) which has been shown to have better metal sorption abilities than cellulose (Pitsari et al., 2013). One of the problems with a high lignin content is that this molecule can inhibit the growth of microorganisms. However, in a previous work, the isolated strain of *Pseudomonas* adhered without problems to this support, showing even higher adhesion speeds than those observed in PET (polyethylene terephthalate) or other natural fibers such as *T. vulgare* (Gallardo-Rodríguez et al., 2016). Figure 2 shows the removal of Pb^{2+} in the solution along time for different amounts of *F. andina* fibers. Assays performed with *Pseudomonas*' biofilm (developed on the fibers) were also included in Fig. 2. The greater Pb^{2+} uptakes were produced in the first hours. More than 90% of the initial concentration of Pb^{2+} (50 $mg \cdot L^{-1}$) was removed in a time of 2 hours in all cases. The amount of fiber and the biofilm had an observable influence in the final Pb^{2+} concentration, as can be observed in subsequent times. The presence of biofilm accelerated the process, reaching a removal of about 90% in the first hour of treatment. Consequently, both the fiber and the biofilm are able to remove Pb^{2+} from contaminated water solutions. As in Figure 2 it is not possible to compare treatments carried out with different quantities of fiber, the maximum uptake has been plotted versus the mass of fiber in Figure 3. Although a tendency is observed, there is not statistic difference between assays carried out with different fiber amounts. Notwithstanding, there is difference between the fibers and the fiber-supported biofilm (P=0.007).

When living microorganisms are used for metal removal the mechanisms are diverse and complex. At least two stages occur: a first phase of faster biosorption (this can also occur with inactivated biomass) and a second, slower, cell metabolism dependent (Garnham et al., 1992; Malik, 2004). Although this second stage is responsible for a smaller percentage of removal, the use of live biofilms presents other important advantages that can result in a better bioremediation system. For example, Chang et. al (Chang et al., 1997), using biomass of *P. aeruginosa*, found almost 40% improvement in lead removal when living biomass was used instead of inactivated. Additional advantages are

obtained when the living cells grow forming a biofilm. EPS's biofilm are anionic due to uronic acids and ketal-linked pyruvates and bind to heavy metals (Vijayaraghavan and Yun, 2008).

Since the rate of adsorption is a crucial operating parameter, kinetic studies were performed. Several previous works have described a two-step sorption of heavy metals by biomass, being the latter sensibly slower (e.g.: (Winters et al., 2016). Kinetic data were modeled using pseudo-first-order (PFO), pseudo-second-order (PSO), Elovich and Intraparticle diffusion (ID) equations. Parameters were gathered in Table 1. According to our results, Pb^{2+} adsorption kinetics were best modelled using the Elovich equation (averaged SD = 2.4%). ID model showed an averaged SD of 8.9%. PFO and PSO averaged SD were 62 and 72%, respectively. In Figure 4, values for Pb^{2+} adsorption (q_t) were plotted versus time for the fiber and the fiber plus bacteria series. It can be observed that the Elovich equation fitted accurately in all conditions assayed. R_E from Elovich equation is known as the approaching equilibrium parameter. According to its value, four zones of adsorption are established. $R_E > 0.3$, zone I; $0.3 > R_E > 0.1$, zone II; $0.1 > R_E > 0.02$ (zone III); $R_E < 0.02$ (zone IV). These zones would correspond to slow, mild, rapid and instant adsorption, respectively. In Figure 5, R_E has been plotted versus the amount of fiber, as can be seen, calculated values were in Zone II and III for fiber and fiber plus bacteria, respectively. For the assays with *F. andina* and *Pseudomonas*' biofilm, greater amount of fiber resulted in a rapider adsorption. The values obtained here were lower than those obtained for formaldehyde or acid treated acorn straw (Örnek et al., 2007) and the presence of the biofilm enhanced the Pb^{2+} removal. ID model also showed an acceptable data fit. This model allows identifying if the adsorption process is controlled by a multi-step mechanism or, on the contrary, completely governed by intraparticle diffusion. For the latter case the parameter C must have values close to zero. C ($mg \cdot g^{-1}$; Table 1) in the ID model is related to the thickness of the boundary layer. Thus, the higher the value of C, the greater effect on the limiting boundary layer. As can be deduced from the calculated values of C, the adsorption process was not governed by intraparticle diffusion. As could be expected, *Pseudomonas'* biofilm increased the boundary layer thickness.

Equilibrium batch biosorption assays were carried out to evaluate the Pb^{2+} removal mechanism. The Langmuir, Freundlich, Temkin and Dubinin isotherm models (See M&M section) were used to analyze biosorption data. Langmuir model considers a mono-layer homogeneous sorption surface. Langmuir fittings were plotted with the experimental results in Figure 6. The Langmuir r^2 values (0.9952 and 0.9971 for fibers and biofilm on fibers, respectively; Table 2) were significantly higher than those obtained with the Freundlich isotherm (0.808 and 0.4849, respectively). Freundlich model considers heterogeneous surfaces. Consequently, from these results it can be concluded that the adsorption surface is homogenous in both cases. Temkin model was also evaluated. This model assumes uniform distribution of binding energies. Our data were not suitable for the analysis since the Temkin model ignores low and large values of concentration. Correlation values (r^2) were 0.753 and 0.491 for fibers and biofilm on fibers, respectively. Model parameters calculation yielded values with no physical sense. Dubinin model considers a mean free energy of adsorption (E) when the ion is transferred to the biosorbent surface. Low values are related to physical adsorption. Between 20 and 40 kJ/mol indicated ion exchange. Finally, values above 40 kJ/mol would imply chemisorption. As with the Temkin model, we found a lack of fitting for the Dubinin isotherm. Correlation values (r^2) were 0.372 and 0.444 for fibers and biofilm on fibers, respectively. Several biosorbent materials did not show good correlations with this model fittings. For example, for rice husk (Dada et al., 2012), Kola nut shell activated carbon (Nwabanne T. et al., 2016), Cashew nut shell (Senthil Kumar, 2014) and passion fruit skin (Gerola et al., 2013). As indicated in Table 2, the Langmuir model returned a maximal adsorption capacity for *F. andina* of 31.5 mg Pb^{2+}/g, whereas the maximal capacity for the biofilm and the fibers was around 35.9 mg Pb^{2+}/g. However, the parameter K_L was higher for the fibers without the biofilm. This parameter is also called the "adsorption equilibrium constant". A lower value indicates lesser affinity. Values found in literature are quite varied since they depend on the specific surface of the adsorbent. Grinded lignocellulosic material such as nut shells were found to have a $K_L \approx 0.3$ L/g (Senthil Kumar, 2014), whereas resting and inactivated cells showed values of 1-3 and 25-43 L/g, respectively (Chang et al., 1997). Values obtained here are more proximate to

those from inactivated cells. In addition, the so-called "dimensionless separation factor" (R_L) was calculated (Table 2). Values equal to or near to zero have been related to irreversible adsorption (Senthil Kumar, 2014). This would indicate that the Pb^{2+} adsorption process on the proposed substrate is highly favorable from a thermodynamic point of view.

The effect of temperature on the adsorption process was studied. Thermodynamic parameters were calculated from the equilibrium distribution coefficient (K_c) at four different temperatures (20-27°C). Values for (Gibbs free energy (ΔG), enthalpy (ΔH^0), and entropy (ΔS^0) are gathered in Table 3. K_c (calculated with Eq. 18) was plotted against 1/T. Acceptable correlation was found (r^2 was 0.9198 and 0.714 for fiber and fiber plus bacteria, respectively). Gibbs free energy revealed that the process is spontaneous. This is logically required for a biosorbent. Calculated values are similar to those found for agriculture by-products (Kamar et al., 2015). *Pseudomonas*' biofilm produced a 30% increase of the Gibbs free energy (Table 3). On the other hand, as $\Delta H > 0$, it is an endothermic reaction. Thus the process qualifies as chemisorption in both cases (fiber and fiber plus bacteria) (Febrianto et al., 2009). Enthalpy was greater for the fiber plus bacteria assay, reaching a value of 120.56 KJ per mol. Entropy values were also positive confirming the disorder increase in the adsorption of Pb^{2+}. Similar results were found for treated passion fruit peels (Gerola et al., 2013) or bacterial biomass (Febrianto et al., 2009). However, previous treatments of biosorbents can alter the nature of the process (Gerola et al., 2013).

A long-term continuous assay was carried out to determine the maximum Pb^{2+} saturation. Evolution of inlet and outlet Pb^{2+} concentrations have been plotted in Figure 7. The *Pseudomonas*' biofilm contribution to the Pb^{2+} adsorption is easily noted. *F. andina* biofilter was able to adsorb the Pb^{2+} inlet for 32 h. As it was packed with 20g, an adsorption capacity of 21.5 mg/g can be calculated. Values calculated in the equilibrium studies were around 31.5 mg/g (Table 2; Langmuir). The biofilm biomass contributed with an additional sorption of 180.5 mg of Pb^{2+} per day. This resulted in a complete retention of Pb^{2+} for 72 h, approximately. If we relativized the Pb^{2+} sorption using the 20 g of fiber, an adsorption capacity of 48.75 mg/g is obtained. This is considerably greater than the

maximum q_e calculated in the equilibrium or kinetic studies. Probably the cause could be attributed to the bacterial EPS produced during the assay. Living biomass can eliminate metal by bioprecipitation, biosorption and metabolic uptake of metals. However, for Pb^{2+}, bioprecipitation and metabolic uptake have not been described. Nutrients were supplied with the inlet flow, so bacterial population were able to proliferate during the assay. During its acclimation process, biofilm-former bacteria produce EPS to create a substrate matrix. The role of the exopolysaccharide matrix is to create an environment conducive to microorganisms. Among other processes, the matrix influences the degradation of particulate matter, adsorption of nutrients, reduction of the oxidation state of molecules and adsorption of metals. Toxic substances such as heavy metals are known to stimulate the EPS production and its chemical composition (Sheng et al., 2005). EPS are crucial elements in bacterial heavy metal bioremediation since it is involved in both degradation and adsorption of heavy metals (Gupta and Diwan, 2017). For lead remediation, reported values can range enormously. For instance, 1 mg/g for corn fiber and luffa peels (Mallampati et al., 2015), 11.7 mg/g for wood powder (Chakravarty et al., 2010), 25.71 mg/g for treated cellulose pulp (Pitsari et al., 2013), 184 mg/g for purified bacterial EPS (Kim et al., 1996), 310 mg/g for mycelial dead biomass (Fourest et al., 1994) and 1103 mg/g for purified polysaccharides solution from *B. firmus* (Salehizadeh and Shojaosadati, 2003). However, the higher adsorption capacity of these materials was obtained after costly processes. Our results are comparable with lignocellulosic material or agriculture by-products. An advantage of lignocellulosic materials is that they can be cyclically regenerated by desorption processes (Senthil Kumar, 2014). Generally, results obtained with these materials are very different since, in many occasions, different degrees of biomass grinding (increasing the specific surface area by reducing the particle size and, therefore, the adsorption velocity) and levels of mechanical mixing are used. In the case of the present study the tests were carried out in static condition and with ungrounded fibers. These are, obviously, the most unfavorable conditions, but also the conditions required for low-cost processes. Other process and design variables that may have influence in the performance will require further research.

4. Conclusions

F. andina fibers and living *Pseudomonas* biomass were able to adsorb Pb^{2+}. Kinetic and thermodynamic studies revealed that the process is chemisorption. Langmuir isotherm and the Elovich equation fitted the experimental data. Compared to the fiber as biosorbent, fibers used as support and adsorbent allowed the biofilm contributing to the adsorption and increasing the maximum adsorption capacity. The results of the present study demonstrated the applicability of *Furcraea andina* fibers together with living *Pseudomonas* biofilms in bioremediation of Pb^{2+} contaminated water in a long-term continuous assay. In contrast to chemical-physical methods, the use of live biofilms allows to simplify the process, since the microorganisms are produced *in situ*.

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Table 1. Parameters of kinetic models for Pb^{2+} adsorption on *F. andina* fibers (F) and *Pseudomonas* biofilm developed on the fibers (F-B).

Kinetic Model	Parameter	Amount of fiber (g)									
		1.5		1.7		2.2		2.6		3	
		F	F-B	F	F-B	F	F-B	F	F-B	F	F-B
Pseudo-first-order	k_1 (h^{-1})	0.32	0.19	0.32	0.25	0.23	0.15	0.31	0.33	0.35	0.69
	SD (%)	50.69	89.70	52.12	67.52	73.40	112.26	53.75	50.49	46.08	25.26
	r^2	0.731	0.994	0.827	0.6365	0.687	0.659	0.999	0.994	0.943	0.995
Pseudo-second-order	k_2 (g mg^{-1} min^{-1})	0.31	0.28	0.34	0.31	0.42	0.39	0.47	0.48	0.56	0.55
	q_e (g mg^{-1})	32.03	34.58	28.86	31.23	23.09	25.19	20.70	20.28	17.62	18.19
	SD (%)	72.55	54.67	167.6	54.50	53.16	54.86	95.70	54.90	53.52	55.50
	r^2	0.828	0.855	0.836	0.836	0.858	0.843	0.856	0.864	0.842	0.831
Elovich	R_E	0.13	0.09	0.12	0.10	0.11	0.08	0.12	0.07	0.13	0.05
	β (mg g^{-1})	0.24	0.33	0.29	0.31	0.40	0.50	0.40	0.67	0.44	1.07
	SD (%)	3.67	2.82	2.56	2.81	2.84	1.05	2.11	3.83	1.85	0.32
	r^2	0.870	0.864	0.926	0.869	0.915	0.978	0.964	0.630	0.969	0.996
Intraparticle diffusion	R_i	0.38	0.22	0.33	0.27	0.28	0.22	0.33	0.17	0.37	0.16
	k_p (mg g^{-1} $min^{1/2}$)	4.94	3.06	3.92	3.51	2.64	2.25	2.75	1.42	2.65	1.18
	C (mg g^{-1})	19.96	26.43	19.18	23.40	16.22	19.57	13.76	16.35	11.10	15.41
	SD (%)	14.46	5.33	12.09	9.05	7.93	7.04	10.55	3.50	13.50	6.07
	r^2	0.839	0.912	0.891	0.833	0.948	0.986	0.985	0.733	0.947	0.976

Table 2. Adsorption isotherms fitting parameters for Pb^{2+} on F. andina fibers and Pseudomonas' biofilm developed on the fibers.

Isotherm	Parameters	*F. andina* fibers	Biofilm on *F. andina*
Freundlich	r^2	0.808	0.485
	K_f $(mg \cdot g^{-1})(L \cdot mg^{-1})^{(1/n)}$	2.70	3.46
	n	0.095	0.1398
Langmuir	r^2	0.995	0.997
	q_{max} $(mg \cdot g^{-1})$	31.5	35.9
	K_L $(L \cdot mg^{-1})$	23.3	25.6
	$R_L=1/(1+ K_L \cdot C_0)$	0.0008	0.0007
Temkin	r^2	0.753	0.491
	A_T $(L \cdot g^{-1})$	399.1	5.40
	b_T $(KJ \cdot mg^{-1})$	1.18	0.61
Dubinin	r^2	0.372	0.449
	q_s $(mg \cdot g^{-1})$	26.47	35.61
	K_{ad} $(mol \cdot KJ)^2$	$2.79 \cdot 10^{-7}$	$9.56 \cdot 10^{-7}$
	E $(kJ \cdot mol^{-1})$	1338.46	723.08

R_L is the dimensionless "separation factor". C_0 is the initial Pb^{2+} concentration.

Table 3. Thermodynamic parameter of Pb^{2+} adsorption on *F. andina* fibers and *Pseudomonas*' biofilm developed on the fibers.

Assay	Temperature (K)	ΔG (KJ mol^{-1})	ΔH^0 (KJ mol^{-1})	ΔS^0 (KJ mol^{-1})	r^2
F. andina fibers	293	-49.3	53.5	0.351	0.919
	295	-50.3			
	297	-50.76			
	300	-51.8			
Pseudomonas' biofilm on F. andina fibers	293	-47.8	120.6	0.577	0.714
	295	-50.4			
	297	-51.4			
	300	-52.0			

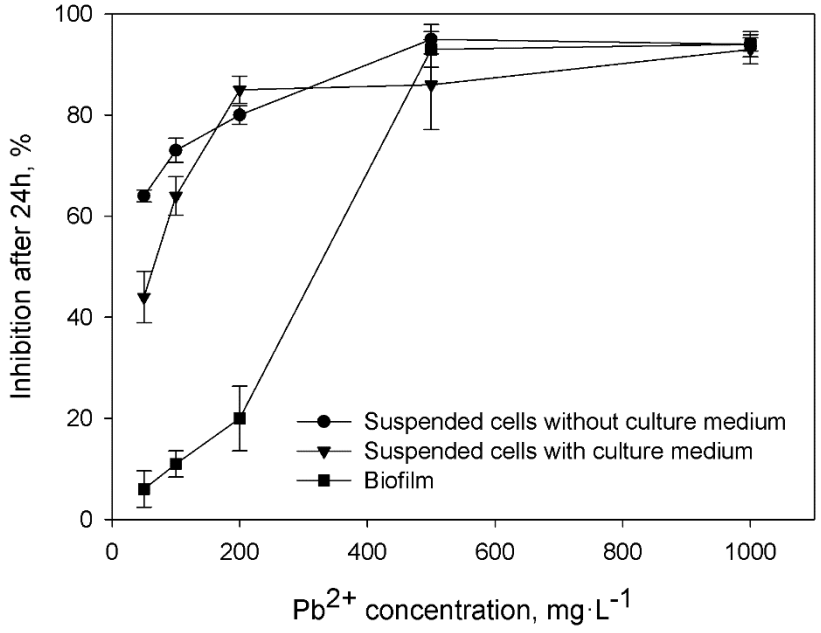

Figure 1. *Pb^{2+} Inhibition (%) after 24h for suspended cultures and Pseudomonas' biofilms.*

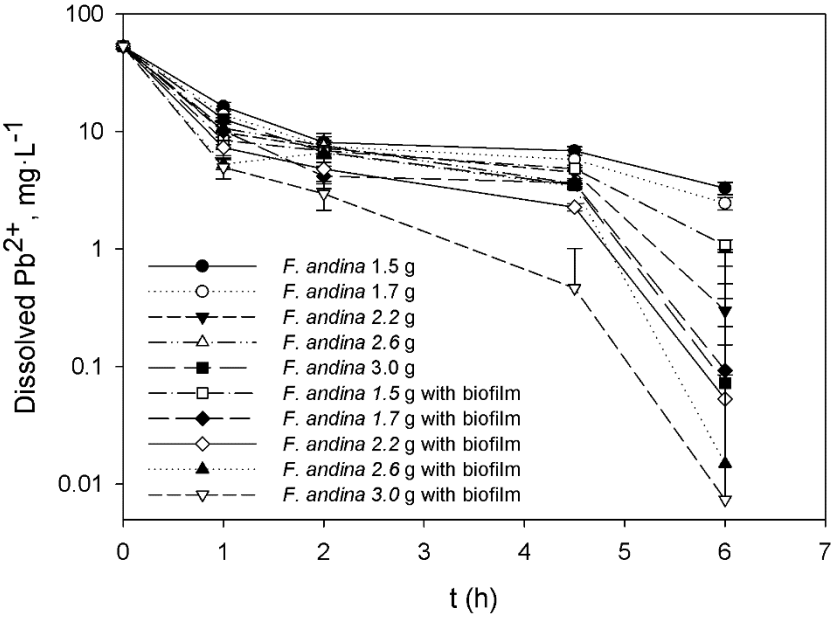

Figure 2. Dissolved *Pb^{2+} versus time for assays performed using F. andina with or without Pseudomonas' biofilm in test tubes (10mL).*

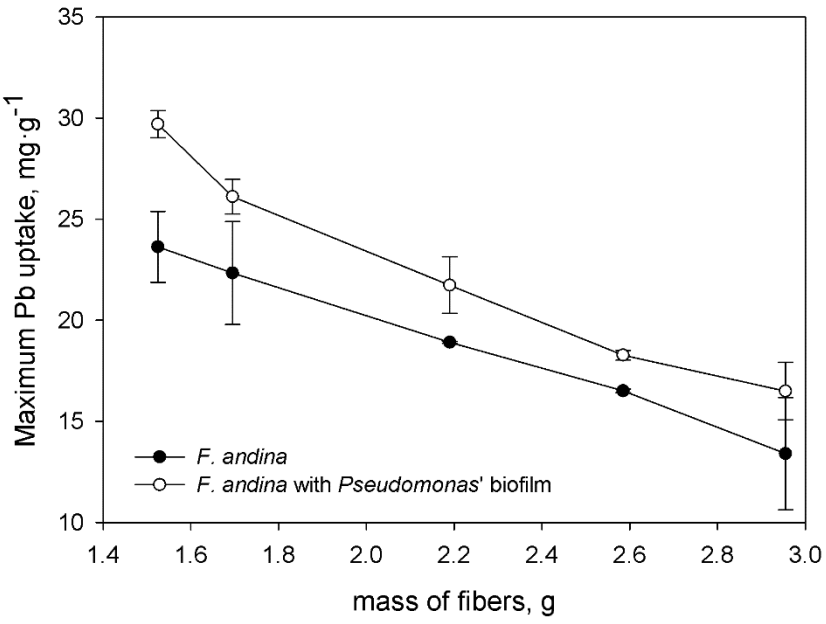

Figure 3. Effect of *the amount of fiber on the maximum lead uptake (t=1h) for assays carried out in test tubes (10mL) with F. andina fibers and with the fiber-bacteria consortium.*

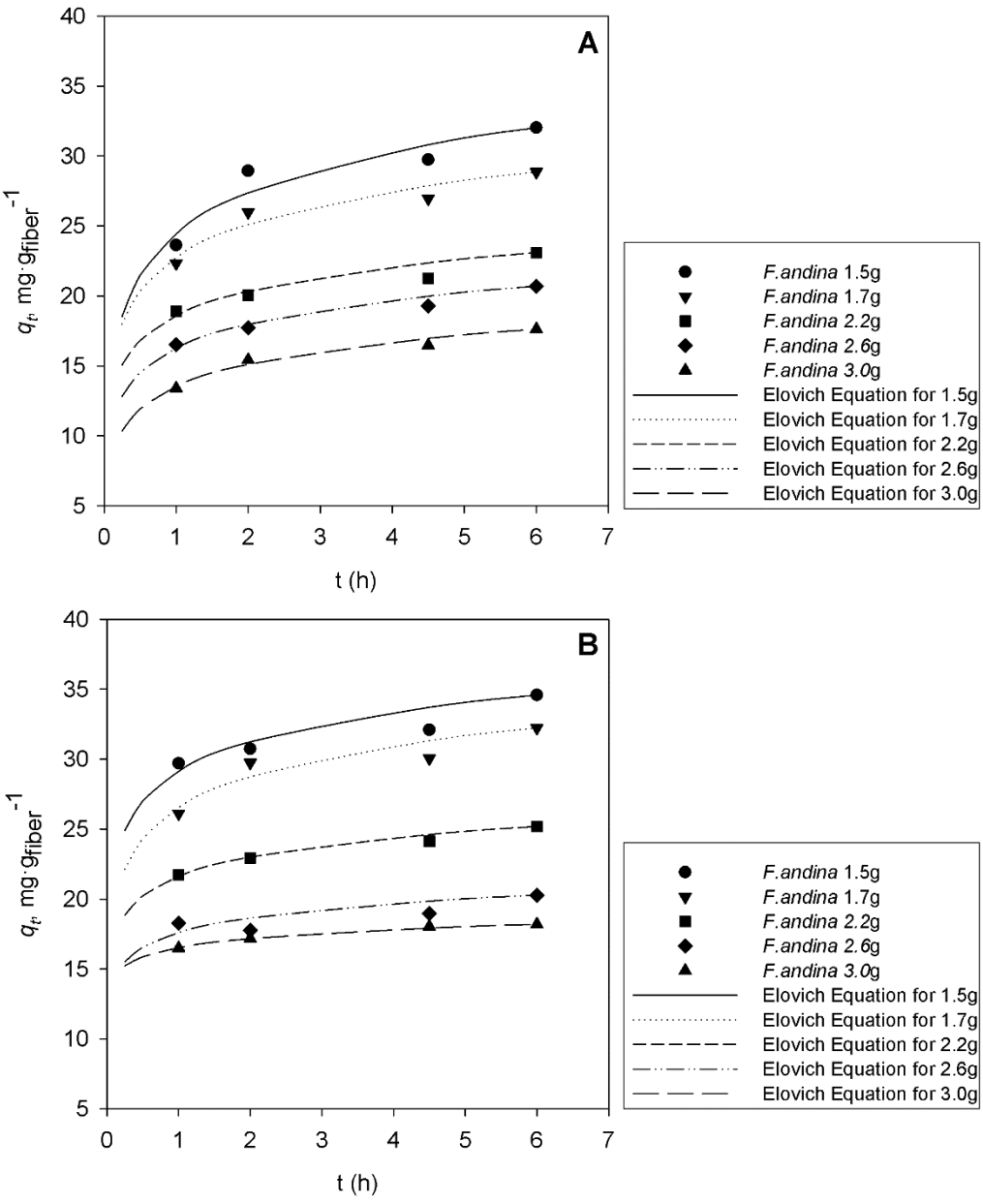

Figure 4. *Pb^{2+} consumption kinetics fitted to the Elovich equation for F. andina fibers (A) and with the fiber-bacteria consortium (B).*

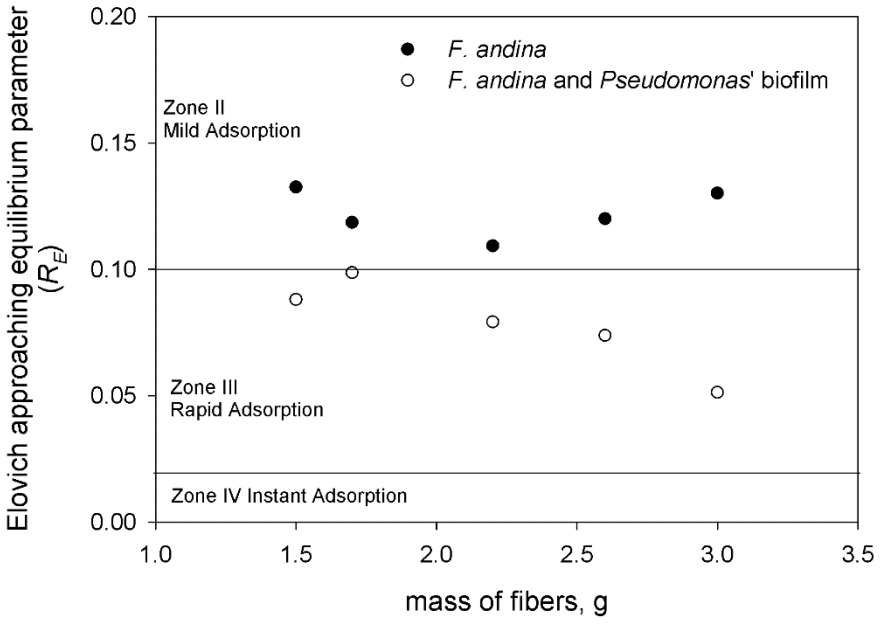

Figure 5. Effect of fiber amount on Elovich approaching equilibrium parameter (R_E) for Pb^{2+} adsorption on *F. andina fibers and the fiber-bacteria consortium.*

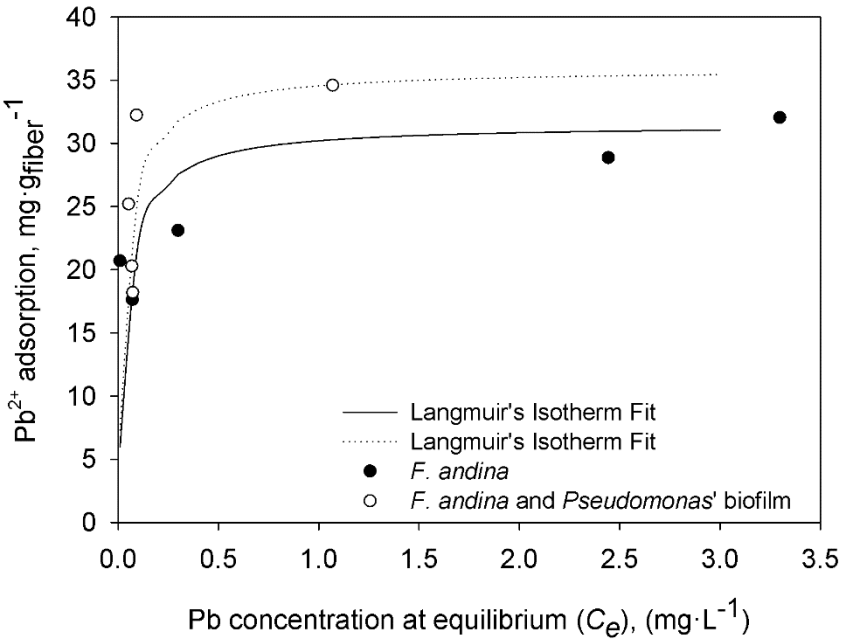

Figure 6. Pb^{2+} adsorption d*ata fitted with Langmuir's isotherms for F. andina fibers and the fiber-bacteria consortium.*

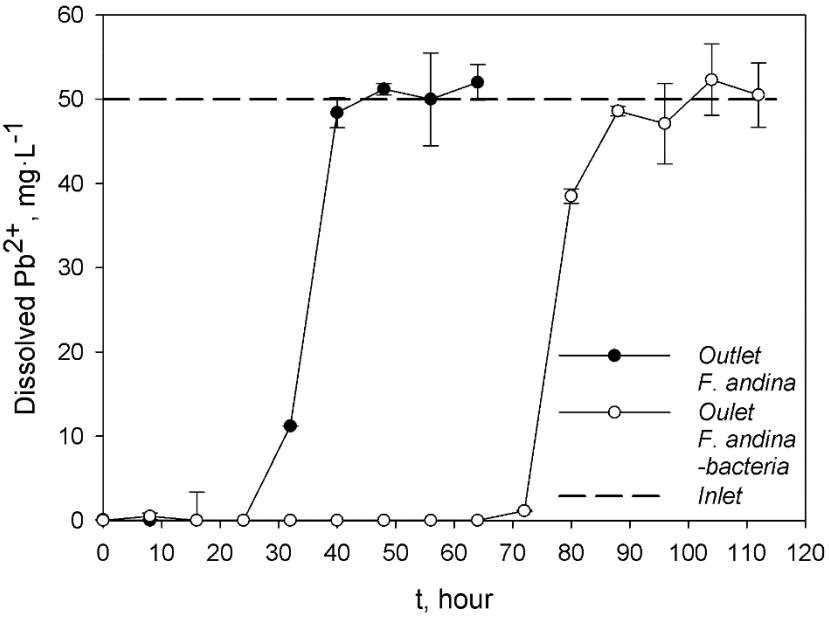

Figure 7. E*volution of Pb^{2+} concentration in lab-scale bioremediation filter for F. andina fibers and with the fiber-bacteria consortium.*